EFFECTS OF MICROBIAL PHYTASE ON THE STANDARDIZED TOTAL TRACT DIGESTIBILITY OF PHOSPHORUS IN SOYBEAN MEAL, CORN, AND CORN CO-PRODUCTS

BY

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THESIS

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ABSTRACT

Six experiments were conducted to investigate the effects of microbial phytase on the standardized total tract digestibility (**STTD**) of P in soybean meal (**SBM**), corn, and corn co-products. The objective of Exp. 1 was to measure the STTD of P in SBM, corn, and distillers dried grains with solubles (**DDGS**) without and with the addition of microbial phytase (Optiphos 2000, Enzyvia, Sheridan, IN). Two SBM based diets, 2 corn based diets, and 2 DDGS based diets were formulated to contain microbial phytase at a level of 0 or 500 phytase units (**FTU**) per kg. Soybean meal, corn, and DDGS were the only sources of P in the diets. A P-free diet was also formulated to measure the basal endogenous P losses (**EPL**) from pigs. Addition of phytase improved ($P < 0.01$) the STTD of P in SBM and corn, but did not improve the STTD of P in DDGS. Values measured for the STTD of P in Exp. 1 were used to formulate diets that were used in Exp. 2 and 3. The objectives of Exp. 2 and 3 were to test the hypothesis that pigs fed diets that are equal in STTD of P will perform equally well regardless of the concentration of total P in the diets, and that the addition of microbial phytase, DDGS, or a combination of phytase and DDGS will result in a reduction in P excretion. Four corn-SBM based diets were formulated and used in a 2 × 2 factorial design with 2 levels of phytase (0 or 500 FTU/kg) and 2 levels of DDGS (0 or 20%). All diets contained 0.32% STTD P according to the STTD values that were measured in Exp. 1. Experiment 2 was a growth performance study. Results showed that inclusion of phytase to the diet containing no DDGS tended ($P < 0.10$) to decrease G:F, but inclusion of 20% DDGS to the diets tended ($P < 0.10$) to increase ADG, ADFI, and final BW. Experiment 3 was a P balance study in which the 4 diets from Exp. 2 were used. Phytase and DDGS increased ($P < 0.01$) the apparent total tract digestibility (**ATTD**) of P in the diets. Absorption of P was greater ($P < 0.05$) for pigs fed corn-SBM-DDGS diets than for pigs fed

corn-SBM diets, and phytase, DDGS, or the combination of phytase and DDGS, reduced ($P < 0.01$) P excretion. In Exp. 4 and 5, the objectives were to test the effect of a novel bacterial 6-phytase expressed in a strain of *Aspergillus oryzae* (Ronozyme HiPhos, DSM Nutritional Products, Parsippany, NJ) on the ATTD of P in corn-SBM diets fed to weanling and growing pigs, and to estimate the minimum level of phytase needed to maximize the ATTD of P. For both experiments, 6 diets were formulated (positive control, negative control, and negative control + 4 levels of microbial phytase). Addition of phytase to the diets increased (linear and quadratic, $P < 0.01$) the ATTD of P in corn-SBM diets fed to both weanling and growing pigs. For weanling pigs the breakpoint for ATTD of P (68.4%) was reached at a phytase inclusion level of 1,016 FTU/kg, whereas for growing pigs the breakpoint for the ATTD of P (69.1%) was reached at a phytase inclusion level of 801 FTU/kg. In Exp. 6, the objectives were to measure the effects of graded levels of microbial phytase on the STTD of P in corn, DDGS, high protein distillers dried grains (**HP-DDG**), and corn germ, and to develop regression equations to predict the response of adding phytase to each of these ingredients. Four corn based diets, 4 DDGS based diets, 4 HP-DDG based diets, and 4 corn germ based diets were formulated to contain 0, 500, 1,000, or 1,500 FTU/kg (Optiphos 2000, Enzyvia, Sheridan, IN) within each ingredient. A P-free diet was also formulated to measure the basal endogenous P losses. Addition of phytase increased (linear, $P < 0.01$; quadratic, $P < 0.05$) the STTD of P in corn and HP-DDG, increased (linear and quadratic, $P < 0.01$) the STTD of P in corn germ, and tended to increase (linear, $P = 0.07$) the STTD of P in DDGS. Regression equations for the effect of added microbial phytase on the STTD of P in corn, HP-DDG, and corn germ were developed, and these equations may be used to predict the STTD of P in each ingredient when phytase is supplemented to diets that contain these ingredients.

Keywords: digestibility, endogenous losses, phosphorus, phytase, pig

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Proverb describes the truth for us today: “He who finds a wife finds what is good and receives favor from the Lord (18:22)”.

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CHAPTER 1

INTRODUCTION

Swine manure is rich in P because diets fed to pigs are commonly over supplied with P (Cromwell, 2005) and also because a great portion of P in feed ingredients from plant origin is in the form of phytic acid (**PA**), which is hardly digested by pigs (Cromwell, 2005). Land application of swine manure, therefore, is an issue because of the potential environmental effects of this practice. Because of these effects, many regulations have been created to limit the amount of P that can be applied in the field, and this has led the swine industry to find solutions to reduce P excretion (Cromwell, 2005).

An approach to achieve this objective is the supplementation of diets with exogenous phytase, which has been effective in improving P digestibility and reducing the excretion of this mineral in the manure (Guggenbuhl et al., 2007). Another approach is to formulate diets that precisely meet the requirements of the pigs. Traditionally, diets have been formulated on the basis of the relative bioavailability of P. Relative bioavailability of P can be calculated using the slope-ratio technique (Cromwell, 1992), but these values depend on the standard used to calculate them, and therefore, they are believed not to be additive in mixed diets. Apparent total tract digestibility (**ATTD**) of P has been measured in feed ingredients (Bohlke et al., 2005; Pedersen et al., 2007), but because ATTD values are not corrected for basal endogenous P losses (**EPL**), they are also believed not to be additive in mixed diets. To mitigate this problem, basal EPL can be calculated from pigs fed a P-free diet (Petersen and Stein, 2006) and used to correct ATTD values resulting in standardized total tract digestibility (**STTD**) values that are believed to be additive in mixed diets. The lack of additivity of apparent ileal digestibility values of AA compared with standardized ileal digestibility values of AA have been demonstrated (Stein et al.,

2005). Based on this observation, we believe that values for the STTD of P are also more additive in mixed diets than values for the ATTD of P. There are, however, no data on the values for the STTD of P in feed ingredients.

Increasing ethanol production from corn has yielded many co-products that can be used in diets fed to swine. Distillers dried grains with solubles (**DDGS**) have greater ATTD of P compared with corn and, therefore, is a good source of organic P for pigs (Stein and Shurson, 2009). Other corn co-products such as high protein distillers dried grains (**HP-DDG**) and corn germ are also available to be used in the swine industry. Formulation of diets based on values that better meet the pigs requirements for P, use of exogenous phytase, and use of feed ingredients from the ethanol industry may, therefore, result in reduced P excretion and also in reduced diet costs. The objectives of this thesis are:

1) To calculate STTD of P in commonly used feed ingredients (corn and soybean meal) and in corn co-products,
2) To verify the effectiveness of exogenous phytase in improving the STTD of P in these ingredients,
3) To validate the hypothesis that diets may be formulated on the basis of values for STTD of P without compromising pigs performance, and
4) To test the effects of a novel phytase on improving the ATTD of P in corn-soybean meal diets.

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CHAPTER 2

DETERMINATION OF STANDARDIZED TOTAL TRACT DIGESTIBILITY OF P IN SWINE FEED INGREDIENTS: LITERATURE REVIEW

INTRODUCTION

Concerns about the amounts of P that are excreted into the environment and increasing costs of inorganic P such as monocalcium phosphate (**MCP**) and dicalcium phosphate (**DCP**) have increased the interest in developing strategies to improve the utilization of P by pigs. One of these strategies is to formulate diets that exactly meet the animals` requirement for P. For many years, swine diets have been formulated on the basis of values for the relative bioavailability of P in feed ingredients, which can be measured by the slope-ratio technique (Cromwell, 1992). These values, however, are not always additive in mixed diets and the cost to conduct this kind of research is high. Because of that, apparent total tract digestibility (**ATTD**) of P in some feed ingredients and diets has been reported (Bohlke et al., 2005; Brana et al., 2006), but ATTD values do not account for basal endogenous P losses (**EPL**), and therefore, they are also believed to not always be additive in mixed diets. To mitigate this issue, there is a need for measuring standardized total tract digestibility (**STTD**) of P in various swine feed ingredients because these values are corrected for basal EPL, and therefore, are believed to be additive in mixed diets. If diets are formulated based on values that are additive, it is likely that diets that better meet the animal's requirement of P are produced.

Another strategy that has been extensively investigated is the use of exogenous phytase in swine diets. The effects of exogenous phytase in improving the ATTD of P have been

demonstrated (Cromwell et al., 1995; Brady et al., 2002; Brana et al., 2006), but the effects of phytase on the STTD of P have not been investigated. There is also a need to determine the concentration of phytase that optimizes the response to phytase.

Ethanol production in U. S. has expanded in recent years and many co-products are available to the swine feed industry. Thus, it is important to determine the STTD of P as well as the effects of phytase on the STTD of P in these ingredients so they can be better utilized in the diets. It is believed that a combination of the strategies mentioned above will contribute to reduction of both P excretion and diet costs with simultaneous optimization of pig performance. In this review, the metabolism of P, the concepts of P digestibility, the supplemental use of exogenous phytase, and the use of corn co-products in swine diets will be discussed.

BIOLOGICAL ROLES OF P IN ANIMALS

Phosphorus is an important mineral in the body and it is needed for many biological functions. Approximately 80% of P is located in skeletal tissues while the remaining 20% is located in soft tissues (Breves and Schröder, 1991). In nature, most of the P is combined with oxygen in the form of phosphate (Anderson et al., 2006). Primarily, P is involved in bone mineralization and teeth formation, but P has many other functions within the body. Phosphorus is also an important component of phospholipids in cell membranes (Crenshaw, 2001). Phosphorus also acts as a buffer, a component of ATP (Cashman and Flynn, 1999), and is used in the synthesis of DNA and RNA (Anderson et al., 2006). Another function of P is the phosphorylation of glucose because glucose can undergo glycolysis only if it is phosphorylated (Anderson et al., 2006).

DIGESTION, ABSORPTION, AND REGULATION OF P

Digestion of P is influenced by the form in which it is present in the diet. Pigs cannot secrete enough endogenous phytase to hydrolyze phytate-P, and therefore, organic P of vegetable origin is not well digested by pigs (Nahm, 2004).

Most P absorption takes place in the small intestine, mainly in the jejunum (Metzler and Mosenthin, 2008), and the transport of P from the gut lumen to the enterocyte can be passive or active (Breves and Schröder, 1991). Phosphorus can be absorbed in the small intestines only if it has been hydrolyzed to its inorganic form (phosphate), and absorption occurs through 2 main mechanisms: Na^+- dependent and Na^+- independent absorption (Anderson, 1991).

In the Na^+- dependent mechanism, phosphate maybe transported against a concentration gradient by integral membrane proteins that facilitate the co-transport of Na^+ and phosphate into the cytosol of the enterocyte. After these first steps, phosphate is extruded across the baso lateral membrane by a Na^+ independent mechanism. This final step, however, is not yet fully understood (Breves and Schröder, 1991). The Na+ - independent mechanism occurs by the flow of phosphate through ion channels across the brush border membrane of the enterocytes. In this case, phosphate moves down its electrochemical gradient until it reaches equilibrium (Breves and Schröder, 1991).

Phosphorus homeostasis occurs by the controlled interactions of the intestine, bones, and renal tubules (Taylor and Bushinsky, 2009). The kidney is the most important organ in the regulation of P homeostasis (Anderson et al., 2006). The regulation of P is directly affected by parathyroid hormone (**PTH**), vitamin D, and dietary phosphate level (Marks et al., 2006; Taylor and Bushinsky, 2009). Parathyroid hormone, which is very important in regulation of P homeostasis, is secreted from the cells of the parathyroid glands as a linear protein of 84 AA and

has the cells in bone and kidney as its major target (Bowen, 2003). When serum P levels are low (hypophosphatemia), plasma Ca levels are elevated and that decreases secretion of PTH. As a result, renal inorganic P excretion is reduced. Hypophosphatemia also causes an increase in the concentration of renal 1,25-dihydroxyvitamin D [**$1,25(OH)_2D_3$**] and calcitriol, which results in increased mobilization from bone and soft tissues, and may result in increased intestinal inorganic P and Ca absorption as well. In contrast, when serum P levels are high (hyperphosphatemia), there is a decrease in plasma Ca concentration resulting in increased PTH and increased renal excretion of P in the urine. Concomitantly, calcitriol and renal $1,25(OH)_2D_3$ levels decrease, which leads to decreased intestinal inorganic P and Ca absorption as well as decreased mobilization from bone and soft tissues (Breves and Schröder, 1991). The mechanism by which P is regulated in the intestines, however, was recently questioned by Stein et al. (2008) who fed pigs diets containing P levels that were between 50% below and 50% above the requirement. They observed that absorption of P remained constant across various P levels, which suggests that the intestine may not be a major regulator of P homeostasis. The integrated mechanism of P homeostasis involving PTH and vitamin D is well recognized (Breves and Schröder, 1991; Marks et al., 2006). In a recent review, however, Taylor and Bushinsky (2009) demonstrated the role of other possible regulatory mechanisms. Homeostasis of P may be regulated by phosphatonins, such as fibroblast growth factor 23 (**FGF-23**), which is a phosphaturic peptide that reduces production of $1,25(OH)_2D_3$ and increases the expression of an enzyme (24-hydroxylase) that converts $1,25(OH)_2D_3$ into forms that are less biologically active. This in turn causes increased P excretion from the kidneys and also a decreased absorption of P in the intestines. As a consequence, serum P levels are reduced (Berndt and Kumar, 2007), and P uptake by renal cells is decreased by other phosphatonins such as fibroblast growth factor 7,

frizzled-related protein 4, and matrix extracellular phospho-glycoprotein (Taylor and Bushinsky, 2009). Because regulation of P homeostasis by phosphatonins was recently discovered, further investigations regarding the action and interaction of these phosphatonins are needed.

PHOSPHORUS REQUIREMENTS

There are 2 main techniques to determine P requirements for swine: factorial calculations and empirical measurements. The factorial approach is believed to be more accurate because its measurements account for availability, retention, and also for the obligatory losses of P in the body (Weremko et al., 1997). However, empirical measurements are most common and response criteria such as bone ash or bone breaking strength have been used for many decades to estimate P requirements for pigs (Cromwell, 2009). Requirement values are, however, dependent on the standards used to obtain these values (Crenshaw, 2001) and they vary according to age (NRC, 1998), sex (Fandrejewski and Rymarz, 1986), and other factors. For example, the requirement of total P expressed as a percentage of the diet ranges from 0.7% in a 3 to 5 kg pig to 0.4% in an 80 to 120 kg pig (NRC, 1998), and these values can be also expressed as amount of P required per day. Expressing P requirement on a daily basis or as g per Mcal ME may be an advantage if pigs are fed a high energy diet, because this will likely cause a reduction in feed intake, and therefore, P concentration in this diet need to be increased to compensate for lower feed intake (Knowlton et al., 2004). Additionally, because of the variability among animals and feed ingredients, feed companies have traditionally recommended P allowances for pigs at levels that are above the NRC recommendations (Cromwell, 2005). This practice, however, has led to high amounts of P excretion. In fact, P excretion may increase by 70% when diets are formulated with 0.2% more P

than what is needed by pigs (Cromwell, 2005). Requirements reported by NRC (1998) were measured at least 15 years ago and some of the estimates may be questioned because at that time there was only a limited amount of data available (Knowlton et al., 2004). To mitigate these issues, diets should be formulated based on values that meet the P requirements more precisely. An alternative would be to formulate diets that are based on the STTD of P, but before nutritionists can move in that direction a data base with the STTD of P in all feed ingredients and the P requirements based on STTD P needs to be established.

PHOSPHORUS IN FEED INGREDIENTS

Corn and soybean meal are the major ingredients used in swine diets. The majority of P in these ingredients is in the form of phytic acid (**PA**), which cannot be digested by pigs (Cromwell, 1992). In seeds, up to 80% of P may be stored as PA. Accumulation of PA in seeds occurs from the beginning of seed development until seed maturity (Bohn et al., 2008). The location of PA in the seed, however, varies among plant species. For example, in legume seeds, most PA is located in the cotyledon and in the endosperm, while in corn it is primarily located in the germ (Pallauf and Rimbach, 1997). In plants, PA functions as an antioxidant, as an energy source, and as P storage for the germinating seeds. Phytic acid is a molecule of 6 carbons that binds up to 6 molecules of phosphate, and PA is also known as myo-inositol-1,2,3,4,5,6-hexakisphosphate (Raboy et al., 2001). Phytic acid is considered an anti-nutritional factor in animal diets because the 6 phosphates in PA may bind cations (e.g., Ca, Mg, Fe, Zn, Cu, Mn) due to their negative charges (Rimbach et al., 2008). Pigs and poultry cannot utilize phytate bound P because they lack endogenous phytase. As a consequence, inorganic P has to be

supplemented in the diets to meet the animal's requirement and, therefore, the cost of diets is increased. The PA bound P that cannot be digested by pigs and poultry is excreted in the feces, which may eventually cause eutrophication of lakes and fresh water streams (Bohn et al., 2008). In corn, approximately 66% of total P is bound to PA while in soybean meal 61% of total P is bound to PA (Cromwell, 1992). Phytic acid causes the relative bioavailability of P in corn (14%) and SBM (23 to 31%) to be low (NRC, 1998). In corn co-products such as distillers dried grains with solubles (**DDGS**), PA bound P corresponds to approximately 65% of total P (Noureddini et al., 2009), but the relative bioavailability of P in DDGS (77%) is much greater than in corn (NRC, 1998). There are, however, no reports on the amount of PA in high protein distillers dried grains (**HP-DDG**), and corn germ. Kim et al. (2008) demonstrated that HP-DDG may have similar amount of PA compared with DDGS because both of these co-products have to go through fermentation, while corn germ is expected to have a greater amount of PA compared with DDGS and HP-DDG because most of the PA (90%) in the corn kernel is in the germ fraction (Noureddini et al., 2009). Phytate is also believed to negatively affect the digestibility of AA. Selle and Ravindran (2008), proposed 3 possible mechanisms by which these negative effects may occur. First, phytate may form a binary protein-phytate complex, which would cause protein to be excreted along with the PA bound P. Second, PA may increase endogenous AA flows, which will reduce the apparent ileal digestibility of AA. Third, intestinal absorption of AA may be compromised by the presence of PA in the gut lumen because AA may bind to the PA molecule, which cannot be absorbed. There are, however, controversies on whether or not PA really reduces the digestibility of AA. For example, reduction of AA digestibility in pigs due to PA was reported by Bolhke et al. (2005) and Liao et al. (2005). On the other hand, phytase, regardless of the dietary PA did not improve AA digestibility in studies conducted by Johnston et

al. (2004) and Woyengo et al. (2008). Recently, Woyengo et al. (2009) also reported that dietary PA has little effect on AA digestibility.

Feed ingredients of animal origin are good sources of P. For example, P in meat and bone meal (**MBM**) is approximately 90% bioavailable relative to P in monosodium phosphate (Traylor et al., 2005). Phosphorus is also highly available in other feed ingredients of animal origin. For instance, P is 92% relative bioavailable in blood meal, 94% in fish meal, 91% in skimmed milk and 97% in dried whey (NRC, 1998).

Because pigs are not efficient in digesting the PA present in vegetable feed ingredients, inorganic P has to be supplemented in diets to meet the pigs requirement of P (Cromwell, 1992). The relative bioavailability of P in MCP (102%), and in DCP (107%) has been calculated using the slope-ratio technique (Cromwell, 1992). In this technique, monosodium phosphate (**MSP**) is used as a standard source and considered to be 100% available. Petersen and Stein (2006), however, measured the ATTD and STTD of P in MCP (84 and 91%), DCP (81 and 88%), and MSP (92 and 98%). This indicates that the bioavailability of P in MCP and DCP is less than in MSP, but P in both MCP and DCP is highly digestible by pigs.

AVAILABILITY AND DIGESTIBILITY

Phosphorus availability can be defined as the amount of P in a feed ingredient that is biologically available to be absorbed and metabolically utilized by the pig (Weremko et al., 1997) and is most often expressed as the relative bioavailability of P. The slope ratio technique is usually used to determine relative bioavailability of P in feed ingredients (Cromwell, 1992). In this technique, a basal diet is formulated and graded levels of 1 or more test ingredients are

added to this diet to create diets containing graded levels of P from each test ingredient. A standard source of P is also added to the basal diet at graded levels to provide the same levels of P as those contributed by the test ingredients. Diets that are used in this technique cannot exceed the pigs requirement for P, and the P levels in the diets must be on the linear response curve. These diets are fed to pigs that are euthanized after a period of 4 to 6 weeks. At this time, the third and fourth metatarsal bones are removed and analyzed for ash content or breaking strength. The regression method is used to determine the slopes of the response criteria for pigs fed the test ingredient and the standard diets. The relative bioavailability is calculated by the ratio of the slope of the test ingredient to the slope of the standard ingredient. Monosodium phosphate or DCP or MCP are most often used as the standard source of P. There are 2 main limitations with the use of this procedure. First, the cost to conduct this type of experiment is relatively high because pigs need to be euthanized. Second, because different standards can be used to calculate these values, they are believed not to be additive in mixed diets.

Digestibility of P has been measured as ATTD of P, which is the difference between P intake and P excretion in the feces (Jongbloed et al., 1992; Bruce and Sundstol, 1995). These values, however, present high variability within the same ingredient (Fan et al., 2001). Another disadvantage of ATTD values is the fact that they do not account for EPL, which is believed to result in these values not to be additive in mixed diets (Fan et al., 2001). Endogenous P loss is defined as the amount of P voided in feces that does not originate from the diet. In other words, EPL is P present in the feces that comes from salivary, gastric, and biliary juices, and also from pancreatic secretions and sloughed mucosal cells (Fan et al., 2001). The EPL can be measured indirectly by the regression technique (Fan et al., 2001) or directly by feeding a P-free diet and measuring the amount of P excreted in feces (Petersen and Stein, 2006). Previous data of EPL

using the regression technique has demonstrated great variability with values ranging from 70 to 840 mg/kg DMI (Pettey et al., 2006; Shen et al., 2002). In contrast, estimation of EPL from pigs that are fed a P-free diet seems to be less variable with values ranging from 139 to 211 mg/kg DMI (Petersen and Stein, 2006; Widmer et al., 2007). When EPL is measured using a P-free diet, basal EPL is obtained, which can be used to correct ATTD values to obtain STTD values. These values are believed to be additive in mixed diets. Currently, there are no data on the STTD of P in most commonly used feed ingredients.

PHYTASE

Before pigs can utilize PA bound P or other minerals that are bound in PA complexes, the phytate molecule has to be hydrolyzed. This can be achieved by the enzyme phytase (myo-inositol-1,2,3,4,5,6-hexakisphosphate phosphohydrolase). Phytases can originate from animals, plants, or microbial sources (Pallauf and Rimbach, 1997). Production of mucosal phytase in pigs is minimal, however, these endogenous phytases might complement the use of exogenous phytase (Selle and Ravindran, 2008). In most plants, the presence of phytase is also low resulting in low phytase concentrations in all vegetable feed ingredients, except in wheat and wheat co-products where phytase activity was reported at 9945 (wheat bran) phytase units per kg (Steiner et al., 2007). Many microbial phytases are currently available for commercial use and they are classified into three main categories (3-phytases, 6-phytases, and 5-phytases) according to where they initiate hydrolysis of PA on the inositol ring. For instance, 3-phytases originated from *Aspergillus niger* initiate phytate hydrolysis at the third phosphate group, while 6-phytases (e.g., *Escherichia coli*) initiate phytate hydrolysis at the sixth phosphate, and 5-phytases (e.g., *Pisium*

sativum) initiate phytate hydrolysis at the fifth phosphate group (Rao et al., 2009). Phytases in the same category can also be classified according to different mechanisms of action as well as optimum pH of activity (Bohn et al., 2008). The 3-phytases are the main category of phytases and are mainly present in fungi and bacteria in the form of histidine acid phosphatases. One of the differences between fungal and bacterial phytases is how these organisms produce the enzyme. While fungal phytases are produced extracellularly, bacterial (gram-negative) phytases are produced intracellularly (Rao et al., 2009). Another difference is the substrate specificity of these phytases. Fungal phytases have broad substrate specificity for PA, glucose, fructose, AMP, ADP, and ATP. Bacterial phytases exhibit high substrate specificity for PA (Rao et al., 2009). Despite their differences, similar effectiveness of fungal and bacterial phytase on improving P and Ca digestibility has been demonstrated (Guggenbuhl et al., 2007).

Genetically modified plants and animals have also been developed to express phytase. Transgenic wheat, with the expression of thermostable phytase, improved mineral bioavailability in cereal based diets (Brinch-Pedersen et al., 2006). Transgenic pigs have also been developed to express bacterial phytase in the salivary glands (Golovan et al., 2001; Forsberg et al., 2003). Using these pigs, reductions of up to 75% in phosphorus excretion were achieved. At this point, however, neither the wheat expressing the phytase, nor the transgenic pigs have been approved for use under commercial conditions.

Exogenous phytase improves the ATTD of P (Brana et al., 2006; Akinmusire and Adeola, 2009), and Ca (Lei et al., 1993; Kornegay and Qian, 1996; Radcliffe et al., 1998; Nyannor et al., 2007), and phytase is, therefore, routinely added to diets fed to pigs. The effects of phytase on the STTD of P, however, have not been determined, and it is not proven if phytase has the same effect on the STTD of P as is has on the ATTD of P.

CORN CO-PRODUCTS

Growth in the USA ethanol industry has yielded many corn co-products that can be potentially used in the feeding of livestock. In 2006, 14% of the US corn crop was used for ethanol production. Biofuel production in 2008 represented 2.5% of the fuel usage for transportation in USA (Gibson and Hughes, 2009). With the increasing demand for biofuels, more co-products are expected to be commercially available for the livestock industry in the future. Some of these products include DDGS, HP-DDG, and corn germ, which are produced by the corn ethanol industry.

The use of DDGS in swine diets has become common and levels of up to 30% may be included in diets fed to swine (Stein and Shurson, 2009). Distillers dried grains with solubles contains a relatively great amount of P and this P has a greater ATTD than P in corn (Pedersen et al. 2007; Stein and Shurson, 2009) because the PA is partly destroyed during the production of DDGS (Stein and Shurson, 2009).

New technologies such as Bfrac, which was introduced by the Poet Company (Sioux Falls, SD), have been used to dehull and degerm corn before fermentation. The degerming process yields corn germ. When corn is dehulled and degermed, its fermentation to ethanol yields HP-DDG (Widmer et al., 2007). The ATTD and STTD of P in HP-DDG are similar to that in DDGS, but the ATTD and STTD of P in corn germ is similar to corn (Widmer et al., 2007). There are, however, no data on the effects of phytase on the ATTD and STTD of P in DDGS, HP-DDG, and corn germ, and it is not known if the response to phytase is similar in these co-products.

CONCLUSIONS

Present in every cell in the body, P is an indispensable mineral for pigs. Before absorption in the small intestine, P must be hydrolyzed to phosphate. Most of the P in plant ingredients is in the form of PA, which is biologically unavailable for absorption in the gut. For that reason, phytase is commonly supplemented to swine diets and this improves P utilization by pigs. Relative bioavailability and ATTD of P have been measured in many feed ingredients, but these values cannot always be used to calculate the P requirement of pigs because the standards used to measure values for relative bioavailability vary among experiments and because ATTD values do not account for basal EPL and. Basal EPL can be directly measured by feeding pigs a P-free diet and this value can be used to correct ATTD for EPL to calculate STTD of P. Values for STTD of P are believed to be additive in mixed diets. The ethanol production in U.S. has increased and many co-products are now available to be fed to pigs. Therefore, the STTD of P needs to be measured not only in traditional feed ingredients (corn, SBM), but also in ingredients from the ethanol industry, and the beneficial effects of phytase in improving the STTD of P in these ingredients need to be validated.

SCOPE OF THESIS

The development of a data base with STTD of P values in various ingredients is important to establish a new concept for diet formulation based on STTD values. Currently, the ethanol industry is projected to continue increasing, which will lead to less corn and more corn co-products to be used by the livestock industry. Effects of new phytases also need to be tested in swine diets. The integration of more accurate values of digestible P for diet formulation with the

use of feed ingredients highly available in P, and the continuous supplementation of diets with phytase will likely diminish the negative impacts of P excretion to the environment.

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CHAPTER 3

PERFORMANCE AND PHOSPHORUS BALANCE OF PIGS FED DIETS FORMULATED ON THE BASIS OF VALUES FOR STANDARDIZED TOTAL TRACT DIGESTIBILITY OF PHOSPHORUS

ABSTRACT

Three experiments were conducted to test the hypothesis that pigs fed diets that are equal in digestible P will perform equally well regardless of the concentration of total P in the diets, and that the addition of microbial phytase, distillers dried grains with solubles (DDGS), or a combination of phytase and DDGS will result in a reduction in P excretion. In Exp. 1, a P-free diet and 6 diets containing corn, soybean meal (SBM), or DDGS without or with microbial phytase (500 phytase units per kg, Optiphos 2000, Enzyvia, Sheridan, IN) were formulated. Diets were fed for 12 d to 42 pigs (initial BW: 13.5 ± 3.9 kg) that were housed in metabolism cages that allowed for total collections of feces. Basal endogenous P losses (EPL) were determined to be 199 mg/kg DMI for pigs fed the P-free diet. Addition of phytase increased ($P < 0.01$) the standardized total tract digestibility (STTD) of P in corn (64.4 vs. 26.4%) and SBM (74.9 vs. 48.3%), but there was no effect of the addition of phytase on the STTD of P in DDGS (75.5 vs. 72.9%). In Exp. 2, a total of 160 pigs (initial BW: 11.25 ± 1.95 kg) were allotted to 4 corn-SBM based diets in a 2 × 2 factorial design with 2 levels of phytase (0 or 500 phytase units per kg) and 2 levels of DDGS (0 or 20%). All diets were formulated to be below the requirement for digestible P and they contained 0.32% STTD P according to STTD values determined in Exp. 1. Diets were fed for 21 d and results showed that inclusion of phytase to the diet containing no

DDGS tended ($P < 0.10$) to decrease G:F, but inclusion of 20% DDGS to the diets tended ($P < 0.10$) to increase ADG, ADFI, and final BW. In Exp. 3, diets from Exp. 2 were fed to 24 pigs (initial BW: 14.6 ± 1.4 kg) that were placed in metabolism cages. Feces and urine were collected for 5 d. Phytase and DDGS increased ($P < 0.01$) the apparent total tract digestibility of P in the diets. Absorption of P was greater ($P < 0.05$) for pigs fed corn-SBM-DDGS diets than for pigs fed corn-SBM diets, and phytase, DDGS, or the combination of phytase and DDGS, reduced ($P < 0.01$) P excretion. In conclusion, the addition of phytase increased the STTD of P in corn and SBM, but had no effect on the STTD of P in DDGS. Diets may be formulated based on STTD values without compromising pig performance, and dietary phytase, DDGS, or the combination of phytase and DDGS will reduce P excretion from growing pigs.

Key words: digestibility, endogenous losses, excretion, phosphorus, phytase, pig

INTRODUCTION

Phosphorus excretion from pigs can be reduced if phytase is added to diets based on soybean meal (**SBM**) and corn (Cromwell et al., 1995). It is also believed that P excretion can be reduced if distillers dried grains with solubles (**DDGS**) is used because the digestibility of P is greater in DDGS than in corn and SBM (Stein and Shurson, 2009). Values for the apparent total tract digestibility (**ATTD**) of P in corn, SBM, and DDGS have been reported (Bohlke et al., 2005; Pedersen et al., 2007), but values for ATTD do not account for the endogenous P losses (**EPL**). Endogenous P losses can be measured using the regression procedure (Fan et al., 2001), which is believed to yield total EPL, or by using a P-free diet (Petersen and Stein, 2006), which yields basal EPL. Basal endogenous losses of nutrients are endogenous losses that are related

only to the DMI of the animal and independent of dietary compositon (Hess and Sève, 1999; Stein et al., 2007). If values for ATTD of P are corrected for total EPL, values for true total tract digestibility of P are calculated, whereas values for standardized total tract digestibility (**STTD**) are calculated by correcting ATTD values for basal EPL. For AA, it has been demonstrated that digestibility values based on standardized ileal digestibility of AA and CP are additive in mixed diets, which is not always the case for values based on apparent ileal digestibility (Stein et al., 2005). It is, therefore, believed that values for STTD of P are also additive in mixed diets, but this concept has not been experimentally verified. The STTD of P in corn, SBM, and DDGS has not been reported and the effect of microbial phytase on STTD in those ingredients has not been measured. The objectives of the current experiments, therefore, were to test the following hypotheses: 1) the STTD of P in corn, SBM, and DDGS fed to growing pigs will increase if microbial phytase is used; 2) pigs fed diets that contain equal quantities of STTD P will perform equally well regardless of the concentration of total P in the diets, and 3) the addition of microbial phytase, DDGS, or a combination of phytase and DDGS to diets fed to pigs will result in a reduction in P-excretion.

MATERIALS AND METHODS

Three experiments were conducted. The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocols for all experiments. Pigs used in Exp. 1 were the offspring of line 337 boars that were mated to C-22 females while pigs used in Exp. 2 and 3 were the offspring of Landrace boars mated to Yorkshire-Duroc females (Pig Improvement Company, Hendersonville, TN).

Exp. 1. P-digestibility

Diets, Animals, and Experimental Design. Seven diets were formulated (Tables 3.1 and 3.2). Two diets were based on corn, 2 diets were based on soybean meal, and 2 diets were based on DDGS. Corn, soybean meal, or DDGS were the only source of P in the diets, and 1 of the diets from each ingredient contained no phytase while the other diet contained 500 phytase units (**FTU**) per kg of microbial phytase (Optiphos 2000, Enzyvia, Sheridan, IN). A P-free diet was also formulated.

A total of 42 growing barrows (initial BW: 13.5 ± 3.9 kg) were used. Pigs were placed in metabolism cages equipped with a feeder and a nipple drinker and randomly allotted to the 7 dietary treatments with 6 pigs per treatment.

Feeding and Sample Collection. The quantity of feed provided per pig daily was calculated as 3 times the estimated requirement for maintenance energy (i.e., 106 kcal ME per kg $^{0.75}$; NRC, 1998) and divided into 2 equal meals. At the conclusion of the experiment, the unconsumed feed was mixed within pig and analyzed for DM, P, and Ca and the amount of DM, P, and Ca in the unconsumed feed was subtracted from the quantity of DM, P, and Ca that was provided. Water was available at all times. The initial 5 d were considered an adaptation period to the diet. An indigestible marker was added to the morning meals that were fed on d 6 and 11, and fecal materials originating from the feed provided from d 6 to d 11 were collected according to the marker to marker approach (Adeola, 2001). Chromic oxide was used to mark the beginning of collection while ferric oxide was used to mark the end of collection. Fecal samples were stored at -20^{o}C immediately after collection.

Sample Analysis and Data Processing. Fecal samples were dried at 65^{o}C in a forced air oven and finely ground before analysis. Fecal, diet, and ingredient samples were analyzed in duplicate

for DM by oven drying at 135°C for 2 h (method 930.15; AOAC, 2005) and for P by inductively coupled plasma spectroscopy (method 985.01; AOAC, 2005) after wet ash sample preparation (method 975.03; AOAC, 2005). Diets and ingredients were analyzed for AA (method 982.30 E (a, b, c); AOAC, 2005), ADF (method 973.18; AOAC, 2005), and for NDF (Holst, 1973). Diets were also analyzed for CP by combustion (method 990.03; AOAC, 2005). Corn, SBM, DDGS, and all diets were analyzed for phytase activity (Phytex Method, version 1; Eurofins, Des Moines, IA).

The ATTD (%) of P in each diet was calculated according to the following equation:

$$\text{ATTD (\%)} = [(Pi - Pf)/Pi] \times 100,$$

where Pi is the total P intake (g) from d 6 to 11 and Pf is the total fecal P output (g) originating from the feed that was provided from d 6 to 11.

The basal endogenous P losses (mg/kg of DMI) were measured from pigs fed the P-free diet according to the following equation:

$$\text{EPL (mg/kg DMI)} = ([Pf/Fi] \times 1{,}000 \times 1{,}000),$$

where EPL is the endogenous P loss and Fi is the total feed (g DM) intake from d 6 to 11. The daily EPL loss for pigs fed the P-containing diets was calculated by multiplying the calculated EPL per kg DMI by the DMI of each pig.

The STTD of P was calculated using the following equation:

$$\text{STTD (\%)} = ([Pi - \{Pf - EPL\}/Pi]) \times 100,$$

where STTD (%) is the standardized total tract digestibility of P.

Data were analyzed using the GLM procedure of SAS (SAS Institute Inc., Cary, NC). The UNIVARIATE procedure in SAS was used to confirm that variances were homogenous and also to analyze for outliers, but no outliers were identified. The ATTD and STTD of P in corn,

SBM, and DDGS without and with microbial phytase were compared within each ingredient using an ANOVA. The LSMeans statement was used to calculate mean values and the PDIFF option was used to separate mean values if significant. The pig was the experimental unit and an alpha value of 0.05 was used to assess significance among means.

Exp. 2. Performance

Diets, Animals, and Experimental Design. A total of 160 pigs (initial BW: 11.25 ± 1.95 kg) were weaned at approximately 20 d of age and fed a common phase 1 diet. On d 11 post-weaning, pigs were randomly allotted to 4 diets in a 2 x 2 factorial experiment with 2 levels of DDGS (0 or 20%) and 2 levels of phytase (0 or 500 FTU per kg, Optiphos 2000; Enzyvia, Sheridan, IN). These diets (Tables 3.3 and 3.4) were fed for 3 weeks. All diets were formulated to contain 0.32% STTD P and values for the concentration of STTD of P in corn, soybean meal, and DDGS without and with phytase that were measured in Exp. 1 were used to formulate the diets used in Exp. 2. For dicalcium phosphate, a value for STTD of P of 88% was used (Petersen and Stein, 2006). Pigs were housed in 1.2 x 1.4 m pens with fully slatted floors. There were 4 pigs per pen and 10 replicate pens per treatment. Feed and water were provided on an ad libitum basis throughout the experiment.

Sample Analysis and Data Processing. Individual pig BW was recorded at the start and at the conclusion of the experiment. Daily feed allotments were recorded as well. Diets were analyzed for phytase, AA, ADF, NDF, CP, DM, and P as described for Exp. 1. Diets were also analyzed for Ca by inductively coupled plasma spectroscopy (method 985.01; AOAC, 2005) after wet ash sample preparation (method 975.03; AOAC, 2005). At the conclusion of the experiment, data were summarized to calculate ADG, ADFI, and G:F for each pen and treatment group. Data were analyzed as a 2 x 2 factorial using the Proc Mixed procedure of SAS. The UNIVARIATE

procedure was used to verify the homogeneity of variances and to analyze for outliers, but no outliers were identified. The model included DDGS, phytase, and the interaction between DDGS and phytase as the fixed effects, while block was included as a random effect. The pen was the experimental unit for all calculations and an alpha level of 0.05 was used to assess significance among means.

Exp. 3. Phosphorus Balance

The 4 diets that were used in Exp. 2 were also used in Exp. 3. Twenty four pigs (initial BW: 14.6 ± 1.4 kg) were placed in metabolism cages and allotted to the 4 experimental diets with 6 pigs per diet. The quantity of feed provided per pig daily was calculated as 3 times the estimated requirement for maintenance energy and divided into 2 equal meals. At each feeding, unconsumed feed in the feeders was removed and weighted. Water was available at all times. The initial 5 d were considered an adaptation period to the diet. During the following 5 d, urine was collected in buckets containing 20 mL of sulfuric acid. Fecal samples were also collected over a 5 d period using the marker to marker procedure as described for Exp. 1.

Feces and urine were analyzed for Ca and P as described for Exp. 1 and 2. The ATTD of P and Ca was also calculated as described for Exp. 1. The retention of P was calculated as previously outlined (Petersen and Stein, 2006) using the following equation:

$$Pr = ([Pi - \{Pf + Pu\}]/Pi) \times 100,$$

where Pr is P retention (%), Pi is the intake of P (g), Pf is the fecal output of P, and Pu is the urinary output of P(g) over the collection period. The retention of Ca was also calculated using this equation. Data were analyzed as described for Exp. 2.

RESULTS

Exp. 1. P-digestibility

Pigs remained healthy and readily consumed their diets throughout the experiment. Feed intake, P intake, and fecal output were not affected by the absence or presence of phytase in corn, SBM, or DDGS (Table 3.5). Phosphorus concentration in feces was reduced from 1.98 to 1.15% ($P < 0.001$) in pigs fed corn and from 2.84 to 1.84% ($P < 0.001$) in pigs fed SBM when phytase was used. The daily P output in feces was also reduced from 0.97 to 0.52 g ($P < 0.05$) for corn and from 0.81 to 0.48 g ($P < 0.001$) for SBM when phytase was added to the diets. In contrast, there was no difference in P concentration in feces or in P output in feces when phytase was added to the DDGS diet. The ATTD of P increased ($P < 0.001$) from 19.9 to 57.8% for corn and from 41.5 to 68.4% for SBM when phytase was added to the diets, but the ATTD of P in DDGS was not affected by the addition of phytase. The basal EPL was measured at 199 mg/kg DMI from pigs fed the P-free diet. The calculated daily basal EPL for pigs fed the P-containing diets was not influenced by the presence of phytase in the diet regardless of which ingredient was used. The STTD of P increased ($P < 0.001$) in corn and SBM when phytase was used, (from 26.4 to 64.4% and from 48.3 to 74.9%, respectively), but the STTD of P in DDGS without phytase (72.9%) was not different from the STTD of P in DDGS with phytase (75.5%).

Exp. 2. Performance

The initial BW of pigs was similar across treatments (Table 3.6). After 21 d, final BW was recorded and no difference was detected among treatments although pigs fed the corn-SBM-DDGS diets tended ($P < 0.10$) to have a greater final BW than pigs fed the corn-SBM diets. Likewise, there was a tendency ($P < 0.10$) for pigs fed corn-SBM-DDGS diets to have a greater ADG and ADFI than pigs fed the corn-SBM diets, but there was no effect of phytase on ADG or

ADFI. The interaction between phytase and DDGS was significant ($P < 0.05$) for G:F. Inclusion of phytase to the diet containing no DDGS tended ($P < 0.10$) to decrease G:F from 0.661 to 0.614, while in the diet containing 20% DDGS, G:F was not affected by the addition of phytase.

Exp. 3. Phosphorus Balance

No differences in ADFI were observed among treatments (Table 3.7), but the daily P intake was lower ($P < 0.01$) for pigs fed diets containing phytase than for pigs fed diets containing no phytase. Fecal P output was reduced ($P < 0.01$) for pigs fed diets containing phytase or DDGS compared with pigs fed the diet without phytase or DDGS. The reduction in fecal P output caused by the addition of phytase to the diets was greater for pigs that were fed corn-SBM diets than for pigs that were fed corn-SBM-DDGS diets, resulting in an interaction ($P < 0.01$). The ATTD of P increased ($P < 0.01$) from 56.1 to 71.5% in the corn-SBM diet and from 62.3 to 74.1% in the corn-SBM-DDGS diet when phytase was used. The inclusion of DDGS to the corn-SBM diet also increased ($P < 0.01$) the ATTD of P. Urine P output was reduced ($P < 0.01$) when phytase was added to the diets, but DDGS did not affect urinary P output. Phosphorus absorption was greater ($P < 0.05$) for pigs fed corn-SBM-DDGS diets than for pigs fed corn-SBM diets, but there was no effect of phytase on P absorption. Phytase improved ($P < 0.01$) P retention in the corn-SBM diet from 56.03 to 71.48% and in the corn-SBM-DDGS diet from 62.16 to 74.01%, but when calculated as g/d, P retention was not affected by the addition of phytase to the diet. However, pigs fed diets containing DDGS retained more P ($P < 0.05$) than pigs fed the diets containing no DDGS. Phosphorus excretion was reduced ($P < 0.01$) by phytase and by DDGS, but in the diets containing DDGS, this reduction was less pronounced than in the diets containing no DDGS (interaction, $P < 0.01$).

There was a tendency ($P = 0.07$) for an increase in Ca intake when phytase was added to the diets. Fecal Ca output was reduced ($P < 0.01$) from 1.21 to 0.85 g/d, and from 1.12 to 0.75 g/d when phytase was added to the corn-SBM and corn-SBM-DDGS diets, respectively. The ATTD of Ca increased ($P < 0.01$) in the corn-SBM diet from 69.65 to 80.42% and in the corn-SBM-DDGS diet from 71.22 to 81.04% when phytase was used. Likewise, Ca absorption increased ($P < 0.01$) in the corn-SBM diet from 2.71 to 3.49 g/d and in the corn-SBM-DDGS diet from 2.79 to 3.20 g/d when phytase was used. The inclusion of DDGS to the diets decreased ($P < 0.01$) urine Ca output, but phytase increased ($P < 0.01$) urine Ca output from 0.24 to 0.74 g/d in corn-SBM diets, and from 0.14 to 0.42 g/d in corn-SBM-DDGS diets. When Ca retention was calculated as g/d, no differences among treatments were observed. However, Ca retention measured as a percentage of intake was greater ($P < 0.05$) and Ca excretion was lower ($P < 0.05$) for pigs fed diets containing DDGS compared with pigs fed diets without DDGS. There were, however, no effects of phytase on Ca retention or on Ca excretion.

DISCUSSION

In most diets fed to pigs in the U.S., corn and SBM are the main ingredients. Most of the organic P in these feed ingredients is bound in the form of phytate (Erdman, 1979), which is poorly digested by pigs due to the lack of phytase in the pigs` gastro-intestinal tract (Pointillart et al., 1984). However, addition of microbial phytase to diets fed to pigs increases P digestibility because phytase partially degrades phytate in the stomach and small intestine, which results in release of P that can be absorbed (Maga, 1982).

Values for the ATTD of P in corn and SBM that were measured in the current experiment are in agreement with values reported by Bohlke et al. (2005) and Pedersen et al. (2007). The ATTD of P in DDGS was previously reported with values ranging from 50 to 68% (Pedersen et al., 2007; Stein et al., 2009) and the ATTD of P in DDGS measured in this experiment is similar to the greatest values in this range. This observation confirms that the ATTD of P in DDGS is much greater than in corn.

The total EPL has been measured using the regression method, which resulted in values between 70 mg/kg DMI (Dilger and Adeola, 2006; Pettey et al., 2006) and 670 mg/kg DMI (Shen et al., 2002). In the current experiment the basal EPL was measured by feeding a P-free diet and a value of 199 mg/kg of DMI was obtained. This value is within the range (139 to 211 mg/kg of DMI) that has been reported for pigs fed a P-free diet (Petersen and Stein, 2006; Stein et al., 2006; Widmer et al., 2007).

To our knowledge, the STTD of P in corn, SBM, and DDGS has not been previously reported. However, the true total tract digestibility of P in corn is 59% (Shen et al., 2002), and in SBM, the true total tract digestibility of P has been reported to be between 41 and 59% (Fan et al., 2001; Ajakaiye et al., 2003; Akinmusire and Adeola, 2009). Values for the STTD of P in corn and SBM that were calculated in the present experiment (26 and 48%, respectively), are lower than the values for true total tract digestibility because STTD values are only corrected for basal EPL, whereas values for true total tract digestibility are corrected for total EPL. Therefore, it is expected that values for STTD are lower than values for true total tract digestibility.

Phytase supplementation increased the ATTD and STTD of P in corn and SBM, which was expected because phytate is degraded by phytase (Selle and Ravindran, 2008), and more P is released in the stomach and small intestine when phytase is added to the diet. In the present

experiment, the ATTD of P in SBM increased from 41.5 to 68.4% when phytase was used and these values are in agreement with the ATTD of P in SBM without and with phytase (38.6 and 71.2%, respectively) that were recently reported (Akinmusire and Adeola, 2009). During processing of corn in ethanol plants, a portion of the phytate is hydrolyzed, which is the reason for the greater ATTD of P in DDGS than in corn (Stein and Shurson, 2009). Because of the lower concentration of phytate in DDGS, phytase is not as effective in increasing the digestibility of P in DDGS as in corn and SBM. To our knowledge, no other data for the ATTD or the STTD of P in DDGS with phytase have been reported.

To compensate for the low digestibility of P in corn and SBM, inorganic P in the form of dicalcium phosphate is usually added to diets fed to pigs, but because of the increased digestibility of P in diets containing phytase or DDGS, less inorganic P is needed in the diet if phytase or DDGS are used. We hypothesized that if diets are formulated to contain the same amount of STTD P, no differences in pig performance would be observed, regardless of the level of inorganic P in the diet. In Exp. 2, therefore, all diets were formulated to contain 0.32% STTD P and the inclusion of dicalcium phosphate was reduced in the diets containing phytase or DDGS and no inorganic P was used in the diet containing both phytase and DDGS. The tendency for a reduced G:F for pigs fed the corn-SBM diet containing phytase compared with pigs fed the same diet without phytase was surprising. This response has not been observed in previous experiments with phytase and we did not make a similar observation in the DDGS containing diet with phytase. The fact that P-retention was similar for the diet containing phytase as for the control diet suggests that the tendency for a reduced G:F for the pigs fed the phytase containing diet was not caused by a P-deficiency. It is, however, possible that the Ca:P ratios of the diets may have influenced the G:F. The analyzed Ca and P concentrations of the diets used in Exp. 2

and 3 shows that the concentration of Ca was slightly greater and P concentration was less in the corn-SBM diet containing phytase compared with the diet containing no phytase, which results in differences in the Ca:P ratios between these 2 diets. A Ca:P ratio at or greater than 1.5:1 may reduce feed efficiency of growing pigs (Liu et al., 1998; Brady et al., 2002) compared with pigs fed diets with a narrower Ca:P ratio.

Differences in Ca:P ratios may also influence measured values for the ATTD of P in feed ingredients (Liu et al., 1998). The Ca:P ratios in Exp. 1 did vary among ingredients but the ratio was kept constant within each ingredient regardless of whether or not phytase was included in the diet. It is, therefore, unlikely that the Ca:P ratios influenced the response to phytase that were measured in Exp. 1. The fact that we obtained ATTD values for corn, SBM, and DDGS that agree with previous data also indicates that the ratios used in Exp. 1 did not significantly influence the results. However, effects of the interaction between phytase and Ca:P ratios on measured values for ATTD and STTD of P in feed ingredients is an area that has not been well studied and something that should be addressed in the future.

The fact that we did not observe a significant difference in performance among pigs fed the 4 diets in Exp. 2 shows that values for STTD of P can be used in diet formulation and that no inorganic P is needed in the diet if both phytase and DDGS are used. We are not aware of any other data illustrating the consequences of formulating diets based on the STTD of P and we believe that this is the first time that it has been demonstrated that pigs from 11 kg can be fed diets containing no inorganic P if phytase and DDGS are included in the diet.

As expected, the excretion of P from the pigs was reduced if phytase or DDGS was added to the diet, because the total concentration of P was lower in these diets than in the diet containing no phytase or DDGS. However, the retention of P was not reduced when phytase was

used and pigs fed the diets containing DDGS actually had a greater retention of P than pigs fed the diets without DDGS as demonstrated in Exp. 3. These observations show that the values for the STTD of P that were measured in Exp. 1 did not overestimate the digestibility of P in corn, SBM, or DDGS. The increase in the ATTD of P in the diets used in Exp. 3 as phytase was added to the diets is also in agreement with the values obtained in Exp.1. The small, but significant, reductions in urinary P for both groups of pigs fed phytase-containing diets compared with pigs fed diets containing no phytase might indicate that the STTD of P in the phytase-containing ingredients was slightly overestimated. However, these differences are so small that they are likely of no practical significance. This conclusion is supported by the fact that the small, but not significant, differences in P-retention between pigs fed phytase-containing diets and pigs fed diets containing no phytase were 10 to 20 times greater than the small differences in urine output of P.

Results of Exp. 3 also showed that although the ATTD of P in DDGS is not increased when phytase is added (Exp. 1), the ATTD of P in a corn-SBM-DDGS diet is improved by phytase. The reason for this observation is that corn and SBM contain phytate that can serve as substrate for phytase. Therefore, the improvement in the ATTD of P by microbial phytase in a mixed diet containing corn, SBM, and DDGS is due to the presence of phytate in corn and SBM.

The ATTD of Ca in the diets used in Exp. 3 increased as phytase was added to the diets although almost all the Ca in the diets was inorganic Ca from limestone and dicalcium phosphate. These results are in agreement with data reported by Guggenbuhl et al. (2007) that also showed that phytase increased the ATTD of Ca. One possible mechanism by which Ca absorption is increased by phytase is that phytate hydrolysis reduces phytate esters and, therefore, reduces the ability of phytate to chelate Ca. As a result, the formation of an insoluble

Ca-phytate complex is reduced and Ca availability is increased when phytase is added to the diet (Selle et al., 2009). This may also explain why a lower Ca:P ratio is recommended in diets containing phytase than in diets containing no phytase (Lei et al., 1994; Liu et al., 1998).

The reduction in urinary Ca output for pigs fed the DDGS containing diets compared with pigs fed the diets without DDGS is most likely a consequence of the increased P-retention for pigs fed these diets. Bone tissue synthesis requires both P and Ca and as more P is retained, more Ca is also needed to synthesize bones, which results in less Ca being excreted in the urine (Stein et al., 2006).

The 3 experiments were all relatively short in duration and Ca and P status of pigs may be influenced in the short term by changes in the concentration of Ca and P that are stored in bone tissue of the pigs. The fact that within each combination of diets, no differences in P-retention were observed, however, indicates that results of the 3 experiments were not influenced by changes in bone concentrations, but longer term experiments need to be conducted to verify this hypothesis.

Conclusions

The results of the present experiments showed that diets for pigs may be formulated based on STTD of P. If phytase, DDGS, or the combination of phytase and DDGS is used, P excretion is reduced and diets that contain much less inorganic P than conventional diets may be used without reducing pig performance. More research is needed to determine STTD of P in other feed ingredients and also to measure the requirements of STTD of P. If such data are generated, diets can be formulated based on STTD of P, which may result in more accurate formulation and in a reduction in P-excretion.

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Table 3.1. Ingredient composition of experimental diets (as-fed basis), Exp. 1

Ingredient,%	Corn		Soybean meal		DDGS[1]		P-free
Phytase, FTU/kg[2]:	0	500	0	500	0	500	
Ground corn	97.10	97.10	-	-	-	-	-
Soybean meal (48%)	-	-	40.00	40.00	-	-	-
DDGS[1]	-	-	-	-	50.00	50.00	-
Sugar	-	-	10.00	10.00	20.00	20.00	20.00
Soybean oil	1.00	1.00	3.00	3.00	-	-	4.00
Ground limestone	1.18	1.18	1.20	1.20	1.20	1.20	0.80
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mineral premix[3]	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Phytase premix[4]	-	0.03	-	0.03	-	0.03	-
Cornstarch	0.03	-	45.10	45.08	28.10	28.08	49.22
Potassium carbonate	-	-	-	-	-	-	0.40
Magnesium oxide	-	-	-	-	-	-	0.10
Solka floc[5]	-	-	-	-	-	-	4.00

Table 3.1. (Cont.)

Gelatin[6]	-	-	-	-	-	-	20.00
AA mixture[7]	-	-	-	-	-	-	0.78

[1] DDGS = distillers dried grains with solubles.

[2] FTU = phytase units.

[3] The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A, 11,128 IU; vitamin D_3, 2,204 IU; vitamin E, 66 IU; vitamin K, 1.42 mg; thiamin, 0.24 mg; riboflavin, 6.58 mg; pyridoxine, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid, 23.5 mg; niacin, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

[4] Optiphos 2000, Enzyvia, Sheridan, IN.

[5] Fiber Sales and Development Corp., Urbana, OH.

[6] Pork gelatin obtained from Gelita Gelatine USA Inc., Sioux City, IA.

[7] Provided the following quantities (%) of AA: DL-methionine, 0.27; L-threonine, 0.08; L-tryptophan, 0.14; L-histidine, 0.08; L-isoleucine, 0.16; and L-valine, 0.05.

Table 3.2. Analyzed nutrient composition of diets (as-fed basis), Exp. 1

		Corn		SBM[1]		DDGS[1]		P-free
Nutrient	Phytase, FTU/kg:	0	500	0	500	0	500	
DM, %		90.95	90.91	94.20	93.97	94.16	94.12	95.66
CP, %		8.65	8.50	20.54	18.01	14.61	13.63	21.40
ADF		2.44	2.32	2.23	2.30	4.90	5.05	3.34
NDF		10.16	10.02	6.42	6.14	18.95	23.89	5.75
P, %		0.28	0.28	0.28	0.29	0.43	0.41	0.01
Ca, %[2]		0.45	0.45	0.57	0.57	0.53	0.53	0.28
Phytase, FTU/kg[3]		< 70	630	< 70	680	180	820	-
Indispensable AA, %								
Arg		0.38	0.41	1.35	1.37	0.65	0.64	1.45
His		0.21	0.22	0.50	0.50	0.37	0.36	0.24
Ile		0.26	0.28	0.91	0.89	0.52	0.50	0.40
Leu		0.85	0.91	1.45	1.46	1.53	1.45	0.56
Lys		0.26	0.28	1.23	1.24	0.50	0.49	0.71

Table 3.2 (Cont.)

Met	0.15	0.15	0.25	0.25	0.27	0.26	0.38
Phe	0.36	0.38	0.95	0.95	0.64	0.62	0.39
Thr	0.25	0.27	0.70	0.73	0.48	0.45	0.39
Trp	0.06	0.06	0.35	0.38	0.16	0.16	0.15
Val	0.35	0.37	0.94	0.91	0.69	0.66	0.50
Dispensable AA, %							
Ala	0.53	0.57	0.84	0.84	0.97	0.94	1.61
Asp	0.49	0.53	2.15	2.17	0.89	0.86	1.07
Cys	0.16	0.16	0.27	0.27	0.26	0.25	0.02
Glu	1.29	1.38	3.53	3.59	2.23	2.22	1.88
Gly	0.30	0.32	0.84	0.84	0.57	0.57	4.31
Pro	0.58	0.63	0.88	0.89	0.95	0.90	2.33
Ser	0.31	0.34	0.86	0.92	0.55	0.52	0.55
Tyr	0.24	0.26	0.68	0.69	0.49	0.47	0.13

[1] SBM = soybean meal; DDGS = distillers dried grains with solubles.

[2] Values for Ca were calculated (NRC, 1998) rather than analyzed.

[3] FTU = phytase units per kg, measured by the Phytex Method, version 1; Eurofins, Des Moines, IA.

Table 3.3. Ingredient composition of experimental diets (as-fed basis), Exp. 2 and 3

Diet:	Corn-SBM[1]		Corn-SBM-DDGS[1]	
Ingredient,% Phytase, FTU/kg[2]:	0	500	0	500
Corn	61.76	62.08	47.89	48.15
Soybean meal, 48%	32.00	32.00	26.00	26.00
Distillers dried grains with solubles	-	-	20.00	20.00
Soybean oil	3.00	3.00	3.00	3.00
Limestone	0.85	1.31	1.15	1.52
Dicalcium phosphate	1.15	0.35	0.65	-
L-lysine HCL	0.28	0.28	0.40	0.40
DL-methionine	0.13	0.13	0.08	0.08
L-threonine	0.13	0.13	0.11	0.11
L-tryptophan	-	-	0.02	0.02
Salt	0.40	0.40	0.40	0.40
Vitamin-mineral premix[3]	0.30	0.30	0.30	0.30
Phytase[4]	-	0.03	-	0.03

[1] SBM = soybean meal; DDGS = distillers dried grains with solubles.

[2] FTU = phytase units measured by the Phytex Method, version 1; Eurofins, Des Moines, IA.

[3] The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A, 11,128 IU; vitamin D_3, 2,204 IU; vitamin E, 66 IU; vitamin K, 1.42 mg; thiamin, 0.24 mg; riboflavin, 6.58 mg; pyridoxine, 0.24

Table 3.3. (Cont.)

mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid, 23.5 mg; niacin, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

[4] Optiphos 2000 (2,000 phytase units per gram), Enzyvia, Sheridan, IN.

Table 3.4. Nutrient composition of diets (as-fed basis), Exp. 2 and 3

	Diet:	Corn-SBM[1]		Corn-SBM-DDGS[1]	
Nutrient	Phytase, FTU/kg[2]:	0	500	0	500
DM, %		85.92	85.02	84.31	85.99
CP, %		19.29	17.93	20.55	20.83
ADF		2.69	2.64	4.31	4.57
NDF		10.26	10.68	18.40	16.62
Ca, %		0.62	0.66	0.60	0.60
P, %		0.59	0.44	0.58	0.48
ATTD[3] of P, % of diet P		49.8	66.4	51.7	66.3
STTD[4] of P, % of diet		0.32	0.32	0.32	0.32
Phytase, FTU/kg		190	690	140	680
Indispensable AA, %					
Arg		1.22	1.26	1.27	1.26
His		0.51	0.53	0.57	0.57
Ile		0.81	0.84	0.88	0.88
Leu		1.53	1.59	1.86	1.85
Lys		1.29	1.37	1.37	1.38
Met		0.38	0.43	0.41	0.40
Phe		0.88	0.92	0.98	0.97
Thr		0.75	0.81	0.81	0.82
Trp		0.24	0.22	0.24	0.24

Table 3.4. (Cont.)

Val	0.91	0.94	1.03	1.02
Dispensable AA, %				
Ala	0.89	0.93	1.10	1.09
Asp	1.86	1.95	1.90	1.89
Cys	0.30	0.31	0.34	0.34
Glu	3.13	3.27	3.37	3.34
Gly	0.77	0.81	0.85	0.85
Pro	0.96	1.00	1.16	1.16
Ser	0.69	0.76	0.76	0.75
Tyr	0.59	0.61	0.69	0.66

[1] SBM = soybean meal; DDGS = distillers dried grains with solubles.

[2] FTU = phytase units measured by the Phytex Method, version 1; Eurofins, Des Moines, IA.

[3] Calculated based on values for ATTD in corn, SBM, and DDGS obtained in Exp. 1. For dicalcium phosphate, a value for ATTD of P of 81% was used (Petersen and Stein, 2006).

[4] STTD of P = standardized total tract digestible P; values calculated based on digestibility values measured in Exp. 1. For dicalcium phosphate, a value of 88% was used (Petersen and Stein, 2006).

Table 3.5. Effects of phytase on P-balance, apparent total tract digestibility (ATTD), and standardized total tract digestibility (STTD) of P in corn, soybean meal (SBM), and distillers dried grains with solubles (DDGS), Exp. 1[1]

Ingredient:	Corn				SBM				DDGS			
	Phytase, FTU/kg[2]				Phytase, FTU/kg				Phytase, FTU/kg			
Item	0	500	SEM	*P*-value	0	500	SEM	*P*-value	0	500	SEM	*P*-value
Feed intake, g/d	423	433	34.95	0.885	505	524	10.77	0.383	497	495	15.85	0.943
P intake, g/d	1.19	1.21	0.10	0.887	1.42	1.52	0.03	0.125	2.14	2.03	0.07	0.440
Fecal output, g/d	48.94	44.97	3.80	0.613	28.56	26.01	0.87	0.175	76.74	75.57	2.70	0.833
P in feces, %	1.98	1.15	0.05	0.001	2.84	1.84	0.06	0.001	0.88	0.78	0.04	0.326
P output, g/d	0.97	0.52	0.08	0.013	0.81	0.48	0.02	0.001	0.66	0.59	0.03	0.221
ATTD of P, %	19.90	57.80	2.80	0.001	41.50	68.40	1.78	0.001	68.60	71.00	1.97	0.557
Basal EPL, mg/d[3]	76.67	81.67	0.01	0.682	95.00	98.33	0.002	0.401	95.00	93.33	0.003	0.765
STTD of P, %[4]	26.4	64.4	2.78	0.001	48.3	74.9	1.78	0.001	72.9	75.5	1.97	0.523

[1] Data are means of 6 observations per treatment.

[2] FTU = phytase units measured by the Phytex Method, version 1; Eurofins, Des Moines, IA.

Table 3.5. (Cont.)

[3] EPL = endogenous P loss was measured from pigs fed the P-free diet at 199 mg/kg DMI. The daily basal EPL (mg/d) for each diet was calculated by multiplying the EPL (mg/kg DMI) by the daily DMI of each diet.

[4] Values for STTD were calculated by correcting values of ATTD for basal endogenous losses.

Table 3.6. Effects of distillers dried grains with solubles (DDGS) and phytase[1] on growth performance of weanling pigs, Exp. 2[2]

	Diet:	Corn-SBM[3]		Corn-SBM-DDGS[4]			*P*-value		
Item	Phytase, FTU/kg[5]:	0	500	0	500	SEM	DDGS	Phytase	DDGS×Phytase
Initial BW, kg		11.14	11.16	11.14	11.15	0.365	0.234	0.129	0.606
Final BW, kg		21.79	21.30	21.87	21.96	0.540	0.075	0.334	0.155
ADG, kg		0.507	0.483	0.511	0.515	0.011	0.067	0.303	0.147
ADFI, kg		0.772	0.789	0.811	0.806	0.025	0.065	0.665	0.465
G:F		0.661	0.614	0.634	0.640	0.012	0.952	0.052	0.014

[1] Optiphos 2000, Enzyvia, Sheridan, IN.

[2] Data are means of 10 observations per treatment.

[3] SBM = soybean meal.

[4] Diets contained 20% DDGS.

[5] FTU = phytase units measured by the Phytex Method, version 1; Eurofins, Des Moines, IA.

Table 3.7. Phosphorus and Ca balance, and apparent total tract digestibility (ATTD) of P for pigs fed corn-soybean meal (SBM) diets or corn-SBM-distillers dried grains with solubles (DDGS) diets without or with microbial phytase, Exp. 3[1]

	Diet:	Corn-SBM		Corn-SBM-DDGS[2]			*P*-value		
Item	Phytase, FTU/kg[3]:	0	500	0	500	SEM	DDGS	Phytase	DDGS×Phytase
Feed intake, g/d		633.3	657.8	652.8	658.0	23.368	0.611	0.445	0.618
P intake, g/d		3.74	2.89	3.79	3.16	0.131	0.178	< 0.01	0.351
Fecal P output, g/d		1.68	0.82	1.43	0.82	0.042	< 0.01	< 0.01	< 0.01
ATTD of P, %		56.1	71.5	62.3	74.1	1.240	< 0.01	< 0.01	0.153
Urine P output, mg/d		2.55	1.32	3.44	1.16	0.647	0.559	0.011	0.399
Absorbed P, g/d		2.05	2.07	2.35	2.33	0.125	0.029	0.991	0.829
P retention, g/d		2.05	2.07	2.35	2.33	0.125	0.029	0.991	0.879
P retention, %		56.03	71.48	62.16	74.01	1.234	< 0.01	< 0.01	0.156
P excretion, g/d		1.68	0.82	1.43	0.82	0.042	< 0.01	< 0.01	<0.01
Ca intake, g/d		3.93	4.34	3.92	3.95	0.145	0.108	0.078	0.125
Fecal Ca output, g/d		1.21	0.85	1.12	0.75	0.083	0.230	< 0.01	0.940
ATTD of Ca, %		69.65	80.42	71.22	81.04	2.222	0.613	< 0.01	0.824

Table 3.7. (Cont.)

Urine Ca output, g/d	0.24	0.74	0.14	0.42	0.050	< 0.01	< 0.01	0.045
Absorbed Ca, g/d	2.72	3.49	2.80	3.20	0.170	0.493	<0.01	0.232
Ca retention, g/d	2.48	2.75	2.66	2.78	0.154	0.566	0.231	0.701
Ca retention, %	63.24	63.44	67.70	70.49	2.633	0.041	0.577	0.623
Ca excretion, g/d	1.45	1.59	1.26	1.17	0.101	<0.01	0.823	0.277

[1] Data represent the mean of 6 observations per treatment.

[2] Diets contained 20% DDGS.

[3] FTU = phytase units measured by the Phytex Method, version 1; Eurofins, Des Moines, IA.

CHAPTER 4

EFFECTS OF A NOVEL PHYTASE IN CORN-SOYBEAN MEAL FED TO WEANLING AND GROWING PIGS

ABSTRACT

Two experiments were conducted to evaluate the effects of adding a novel bacterial 6-phytase expressed in a strain of *Aspergillus oryzae* (Ronozyme HiPhos, DSM Nutritional Products, Parsippany, NJ) on the apparent total tract digestibility (ATTD) of P in corn soybean meal diets fed to weanling and growing pigs. In Exp. 1, 6 diets were formulated. The positive control diet was a corn-soybean meal diet that contained dicalcium phosphate to bring the total concentration of P to 0.66%. A negative control diet (0.36% P) without dicalcium phosphate was also formulated. Four additional diets that were similar to the negative control diet with the exception that they contained microbial phytase at levels of 500, 1,000, 2,000, or 4,000 phytase units (FTU) per kg were also formulated. Forty eight weanling pigs (initial BW: 13.5 ± 2.45 kg) were placed in metabolism cages and randomly allotted to the 6 dietary treatments in a randomized complete block design. Feces were collected over a 5-d period. The total P output and the P concentration in feces were reduced (linear and quadratic, $P < 0.01$) as phytase was added to the negative control diet. The ATTD of P was greater ($P < 0.01$) for the positive control diet (60.5%) than for the negative control diet (40.5%), but increased (linear and quadratic, $P < 0.01$) as phytase was added to the negative control diet (40.5 vs. 61.6, 65.1, 68.7, and 68.0%). The breakpoint for the ATTD of P (68.4%) was reached at a phytase inclusion level of 1,016 FTU/kg. In Exp. 2, 6 diets were formulated using the same principles as in Exp. 1. A total of 24 growing

pigs (initial BW: 36.2 ± 4.0 kg) were randomly allotted to the 6 dietary treatments in a balanced 2 period changeover design. The total P output and P concentration in feces were reduced (linear and quadratic, $P < 0.01$) as phytase was added to the negative control diet. The ATTD of P was greater ($P < 0.01$) for the positive control diet (59.4%) than for the negative control diet (39.8%) and increased (linear and quadratic, $P < 0.01$) as phytase was added to the negative control diet (39.8 vs. 58.1, 65.4, 69.1, and 72.8%). The breakpoint for the ATTD of P (69.1%) was reached at a phytase inclusion level of 801 FTU/kg. In conclusion, Ronozyme HiPhos improved the ATTD of P and reduced P excretion in both weanling and growing pigs.

Key words: calcium, digestibility**,** phosphorus, pigs, phytase

INTRODUCTION

Most of the P in cereal grains and oilseeds is bound in phytate (Erdman, 1979). Because pigs lack endogenous phytases, phytate cannot be digested in the small intestine (Selle and Ravindran, 2008). As a consequence, large amounts of P are excreted in the manure, which may potentially cause environmental pollution. Divalent cations such as Ca likely form insoluble phytate complexes, which may reduce the hydrolysis of phytate. Phytases are enzymes capable of hydrolyzing the phytate molecule, which results in the release of the P in phytate as well as Ca, which can then be absorbed and utilized by pigs (Selle and Ravindran, 2008). Addition of microbial phytase to swine diets, therefore, improves P utilization by pigs (Cromwell et al., 1993; Akinmusire and Adeola, 2009). Phytases are classified based on where they initiate the hydrolysis of the phytate molecule (Brana et al., 2006). Most of microbial phytases are classified as 3 or 6-phytases. A 3-phytase initiates the hydrolysis of phytate at the third phosphate ester

group, while a 6-phytase initiates hydrolysis of phytate at the sixth phosphate ester group on the phytate molecule (Brana et al., 2006). Several microbial phytases are commercially available and the inclusion of exogenous phytase to swine diets has become a routine practice, but new and more efficient microbial phytases are constantly being developed. A novel bacterial 6-phytase (Ronozyme HiPhos, DSM Nutritional Products, Parsippany, NJ) produced from *Aspergillus oryzae* was developed, but there is no information on the effectiveness of this phytase when fed to pigs. Therefore, 2 experiments were conducted to test the hypothesis that inclusion of Ronozyme HiPhos in a corn-soybean meal diet will increase the digestibility of P and Ca by weanling and growing pigs.

MATERIALS AND METHODS

Diets, Animals, and Experimental Design, Exp. 1

Six diets were formulated (Tables 4.1 and 4.2). The positive control diet was a corn-soybean meal diet that contained quantities of Ca and P sufficient to meet the requirement of Ca and P for weanling (10-20 kg) pigs (NRC, 1998). Dicalcium phosphate and limestone were used to bring the total concentration of P and Ca in this diet to 0.66 and 0.86%, respectively. A negative control diet that was similar to the positive control diet with the exception that cornstarch replaced dicalcium phosphate was also formulated. This diet contained 0.36% P and 0.48% Ca. Four additional diets that were similar to the negative control diet with the exception that microbial phytase (Ronozyme HiPhos, DSM Nutritional Products, Passipany, NJ) was included in the amounts of 500, 1,000, 2,000, or 4,000 phytase units (**FTU**) were also formulated.

A total of 48 weanling pigs (initial BW: 13.5 ± 2.45 kg) were used in a randomized complete block design. Pigs were the offspring of Landrace boars that were mated to Large White x Duroc sows (Pig Improvement Company, Hendersonville, TN). Pigs were blocked by BW and randomly allotted to the 6 dietary treatments in 8 blocks using the Experimental Animal Allotment Program (Kim and Lindemann, 2007). Pigs were placed in metabolism cages equipped with a feeder and a nipple drinker that allowed for total collection of feces.

Diets, Animals, and Experimental Design, Exp. 2

Six diets were also formulated for Exp. 2 (Tables 4.3 and 4.4). The positive control diet was a corn-soybean meal diet that contained Ca and P in quantities sufficient to meet the requirement of Ca and P for 20-50 kg pigs (NRC, 1998). Dicalcium phosphate and limestone were used to bring the total concentration of P and Ca in this diet to 0.56 and 0.79%, respectively. A negative control diet that was similar to the positive control diet with the exception that cornstarch replaced dicalcium phosphate was also formulated. This diet contained 0.33% P and 0.58% Ca. Four additional diets that were similar to the negative control diet were formulated to contain the same levels of phytase as in the diets used in Exp. 1. A total of 24 growing barrows were used in a 2 period changeover design (Gill and Magee, 1976). Pigs were the offspring of Landrace boars that were mated to Large White × Duroc sows (Pig Improvement Company, Hendersonville, TN). In period 1, pigs (initial BW of 36.2 ± 4.0 kg) were placed individually in metabolism cages and randomly allotted to the 6 dietary treatments in 4 blocks based on BW using the Experimental Animal Allotment Program (Kim and Lindemann, 2007). In period 2, the same pigs (initial BW of 47.3 ± 5.3 kg) used in period 1were allotted in a way that potential residual effects were balanced (i.e., one pig did not receive the same dietary

treatment as in period 1, and one dietary treatment did not follow another dietary treatments more than once). The cages were equipped with a feeder and a nipple drinker.

Feeding and Sample Collection, Exp. 1 and Exp. 2

The amount of feed provided daily was calculated as 3 times the estimated requirement for maintenance energy (i.e., 106 kcal ME per kg $^{0.75}$; NRC, 1998) and divided into 2 equal meals. Water was available at all times. The initial 5 d were considered an adaptation period to the diet. From d 6 to 11, fecal materials were collected according to the marker to marker approach (Adeola, 2001). Chromic oxide and ferric oxide were used to determine the beginning and the conclusion of collections, respectively. Fecal samples were stored at -20˚C immediately after collection.

Sample Analysis and Data Processing, Exp. 1 and Exp. 2

At the conclusion of each experiment, fecal samples were dried in a forced air oven and finely ground. Fecal samples and diets were analyzed for Ca and P by inductively coupled plasma (ICP) spectroscopy (method 985.01; AOAC Int., 2007) after wet ash sample preparation (method 975.03; AOAC Int., 2007). Diets were also analyzed for AA (method 982.30 E (a, b, c); AOAC Int., 2007), ADF (method 973.18; AOAC Int., 2007), NDF (Holst, 1973), DM by oven drying at 135°C for 2 h (method 930.15; AOAC Int., 2007), ash (method 942.05; AOAC Int., 2007), CP (method 990.03; AOAC Int., 2007) using an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ), and for phytase activity (DSM Nutritional Products, Passipany, NJ).

The apparent total tract digestibility (**ATTD**) of P in each diet was calculated according to the following equation:

$$ATTD\ (\%) = [(Pi - Pf)/Pi] \times 100,$$

where Pi = total P intake (g) from d 6 to 11 and Pf = total fecal P output (g) originating from the feed that was provided from d 6 to 11(Petersen and Stein., 2006).

For Exp. 1, data were analyzed as a randomized complete block design using the Proc Mixed Procedure in SAS. The UNIVARIATE procedure was used to verify homogeneity of variances and to identify outliers. The model included diet as the main effect and block as a random effect. For Exp. 2, data were analyzed as a changeover design using the Proc Mixed Procedure in SAS. The UNIVARIATE procedure was used to verify homogeneity of variances and to identify outliers. One outlier was identified and removed from the data set. The model included diet as the main effect while block and period were random effects. The effects of block and period were not significant and, therefore, sequentially removed from the final model. For both experiments, a contrast of the positive control diet vs. the negative control diet was performed to analyze the effects of removing inorganic P from the diets, and orthogonal polynomial contrasts were conducted to test linear and quadratic responses to the inclusion of phytase in the diets. Appropriate coefficients for unequally spaced concentrations of supplemental phytase were obtained using the interactive matrix language procedure (Proc IML) of SAS. The minimum level of phytase that was needed to maximize the ATTD of P and Ca were estimated by subjecting the treatment means to a least squares broken-line analysis as described by Robbins et al. (2006). The pig was the experimental unit for all analyses and an alpha level of 0.05 was used to assess significance among means.

RESULTS

Exp. 1, Weanling Pigs

There was no difference in feed intake and in fecal output among treatments (Table 4.5). Phosphorus intake was greater ($P < 0.01$) for pigs fed the positive control diet than for pigs fed the negative control diet. The concentration of P excreted in the feces was lower ($P < 0.05$) for pigs fed the negative control diet than for pigs fed the positive control diet. Likewise, pigs that were fed phytase containing diets had less (linear and quadratic, $P < 0.01$) concentration of P in feces than pigs fed the negative control diet. The daily P output was also less ($P < 0.01$) for pigs fed the negative control diet than for pigs fed the positive control diet, and the inclusion of increasing levels of phytase to the negative control diet caused linear and quadratic reductions ($P < 0.01$) in P output. The ATTD of P was greater ($P < 0.01$) for pigs fed the positive control diet than for pigs fed the negative control diet (60.5 vs. 40.5%), but, the ATTD of P increased (linear and quadratic, $P < 0.01$) as phytase was added to the negative control diet (61.6, 65.1, 68.7, and 68.0% for pigs fed diets containing 500, 1,000, 2,000, or 4,000 FTU of phytase, respectively). Phosphorus absorption was greater ($P < 0.01$) for pigs fed the positive control diet than for pigs fed the negative control diet (2.6 vs. 0.9 g/d), but the addition of phytase to the negative control diet increased (linear and quadratic, $P < 0.01$) P absorption to 1.4, 1.5, 1.5, and 1.5 g/d. The breakpoint for phytase concentration resulted in an ATTD of P of 68.4%, which was reached when 1,016 FTU/kg of phytase was added to the diet (Figure 4.1).

Calcium intake was greater ($P < 0.01$) for pigs fed the positive control diet than for pigs fed the negative control diet (5.6 vs. 3.0 g/d). Pigs that were fed phytase containing diets tended ($P = 0.06$) to have a greater Ca intake than pigs fed the negative control diet. Concentration of Ca in feces was greater ($P < 0.05$) for pigs fed the positive control diet compared with pigs fed the

negative control diet (2.29 vs. 1.86%), but pigs fed phytase containing diets had less Ca concentration in feces than pigs fed the negative control diet (linear and quadratic, $P < 0.01$). The daily Ca output was also greater ($P < 0.01$) for pigs fed the positive control diet than for pigs fed the negative control diet (1.5 vs. 1.1 g/d), but the addition of 500, 1,000, 2,000, or 4,000 FTU/kg of phytase to the negative control diet reduced (linear and quadratic, $P < 0.01$) Ca output to 0.80, 0.60, 0.52, and 0.50%, respectively. The ATTD of Ca was greater ($P < 0.05$) for pigs fed the positive control diet than for pigs fed the negative control diet (72.5 vs. 63.9%), but pigs fed diets containing 500, 1,000, 2,000, or 4,000 FTU/kg of phytase had greater (linear and quadratic, $P < 0.01$) ATTD of Ca than pigs fed the negative control diet (73.7, 81.7, 84.8, and 84.6%). The absorption of Ca was reduced ($P < 0.01$) from 4.0 to 2.0 g/d for pigs fed the negative control diet rather than the positive control diet, but Ca absorption was increased (linear and quadratic, $P < 0.01$) for pigs fed phytase containing diets compared with pigs fed the negative control diet (2.0 vs. 2.2, 2.7, 3.0, and 2.7 g/d). The breakpoint for phytase concentration was reached when 1,155 FTU/kg of phytase was added to the diet. This inclusion level resulted in an ATTD of Ca of 84.7% (Figure 4. 2).

Exp. 2, Growing Pigs

Throughout the experiment, pigs remained healthy and readily consumed their diets. One of the pigs, however, was removed from the experiment due to coprophagy. No differences in feed intake were observed among treatments (Table 4.6). Phosphorus intake was greater ($P < 0.01$) for pigs fed the positive control diet than for pigs fed the negative control diet (8.5 vs. 4.8 g/d). Fecal output tended ($P = 0.08$) to be greater for pigs fed the positive control diet than for pigs fed the negative control diet. The P concentration in feces was less (linear and quadratic, $P < 0.01$) for pigs fed phytase containing diets than for pigs fed the negative control diet. The daily

P output was less ($P < 0.01$) for pigs fed the negative control diet than for pigs fed the positive control diet (2.9 vs. 3.4 g/d), and the addition of phytase to the negative control diet reduced (linear and quadratic, $P < 0.01$) the daily P output (2.1, 1.8, 1.5, and 1.4 g/d). The ATTD of P was greater ($P < 0.01$) for pigs fed the positive control diet than for pigs fed the negative control diet (59.4 vs. 39.8%). Pigs fed phytase containing diets also had greater (linear and quadratic, $P < 0.01$) ATTD of P than pigs fed the negative control diet (58.1, 65.4, 69.1, and 72.8%). Phosphorus absorption was greater ($P < 0.01$) for pigs fed the positive control diet than for pigs fed the negative control diet (5.1 vs. 1.9 g/d), and the addition of phytase to the negative control diet increased (linear and quadratic, $P < 0.01$) absorption of P to 3.0, 3.3, 3.5, and 3.7 g/d.

Calcium intake was greater ($P < 0.01$) for pigs fed the positive control diet than for pigs fed the negative control diet (12.0 vs. 8.5 g/d). Concentration of Ca in feces was reduced (linear and quadratic, $P < 0.01$) as phytase was added to the negative control diet (2.33 vs. 1.40, 1.29, 1.22, and 0.91%). The daily Ca output tended ($P = 0.07$) to be greater for pigs fed the positive control diet compared with pigs fed the negative control diet (3.2 vs. 2.7 g/d), but addition of phytase to the negative control diet reduced (linear and quadratic, $P < 0.01$) the daily Ca output to 1.6, 1.5, 1.5, and 1.1 g/d. There was also a tendency ($P = 0.07$) for pigs fed the positive control diet to have a greater ATTD of Ca than pigs fed the negative control diet (72.9 vs. 67.3%). As phytase was added to the negative control diet, the ATTD of Ca increased (linear and quadratic, $P < 0.01$) to 81.4, 82.6, 82.4, and 85.6%. Calcium absorption was greater ($P < 0.01$) for pigs fed the positive control diet than for pigs fed the negative control diet (8.8 vs. 5.7 g/d). Likewise, pigs fed phytase containing diets had greater ($P < 0.01$) absorption of Ca than pigs fed the negative control diet.

The breakpoint for phytase concentration resulted in an ATTD of P of 69.1%, which was reached when 801 FTU/kg of phytase was added to the diet (Figure 4.3). For the ATTD of Ca, the breakpoint for phytase concentration was reached when 574 FTU/kg of phytase was added to the diet, which resulted in an ATTD of Ca of 83.5% (Figure 4.4).

DISCUSSION

Phytase supplementation increased P digestibility, which was expected because phytase hydrolyses the phytate molecule in corn and SBM, and therefore, releases some of the P that is bound to the phytate molecule (Cromwell et al., 1993). The beneficial effect of various microbial phytases on the ATTD of P in corn-soybean meal diets has been demonstrated (Kerr et al., 2009). There are, however, no data on the effect of adding the new bacterial 6-phytase, Ronozyme HiPhos to diets for swine. The values for the ATTD of P measured in the present experiment for weanling pigs are in agreement with those measured by Lei et al. (1993) and Qian et al. (1996). In the case of growing pigs, research conducted by Harper et al. (1997) and Johnston et al. (2004) also resulted in P digestibility values that were similar to the P digestibility values measured in the present experiment. The present data also demonstrates that P digestibility reaches a plateau at levels of 1,016 and 801FTU/kg (Exp.1 and Exp. 2, respectively), which is in agreement with previous work showing maximum responses to a fungal phytase at levels around 1,000 FTU/kg in corn-SBM diets (Beers and Jongbloed, 1992; Kornegay and Qian, 1996; Yi et al., 1996). Because P is better utilized by pigs when phytase is added to corn-soybean meal diets, the fecal excretion of P is reduced, which is in agreement with data from Selle and Ravindran (2008). Results from the present experiment showed that

improvements on the ATTD of P by the addition of Ronozyme HiPhos to corn-soybean meal diets are of similar magnitude as other commercially available 6-phytases (Kerr et al., 2009).

The concentration of Ca in the feces was reduced by the addition of phytase to the diets because the ATTD of Ca was increased. These results are in agreement with data reported by Pallauf et al. (1992), Lei et al. (1993), and Guggenbuhl et al. (2007). The Ca digestibility values that were measured in the present experiments are comparable with those measured by Qian et al. (1996) and Harper et al. (1997) for weanling and growing pigs, respectively. To our knowledge, there are no previous data on the effects of graded levels of phytase on Ca digestibility, but results of this experiment demonstrated that Ca digestibility in corn-soybean meal diets is maximized if 1,155 or 574 FTU/kg of phytase are added to diets fed to weanling or growing pigs, respectively. One possible reason for the increase in Ca digestibility with supplemental phytase is that in the process of phytate hydrolysis, phytate esters are reduced and as a consequence, the ability of phytate to chelate Ca is also reduced (Selle et al., 2009). The present data are in agreement with Adeola et al. (1995) who also verified that Ca absorption is increased when phytase is supplemented to corn-soybean meal diets.

Although our objective was not to compare P and Ca digestibility between weanling and growing pigs, it is notable that the digestibility values for P and Ca obtained for weanling and growing pigs were very similar. This observation is in agreement with data from Kemme et al. (1997) who also concluded that there are no differences in the digestibility of P and Ca between weanling pigs and growing-finishing pigs.

Conclusions

Results from the present experiments show that bacterial 6-phytase obtained from *Aspergillus oryzae* (Ronozyme HiPhos) may be used in corn-soybean meal diets to improve the

ATTD of P and Ca. Inclusion levels of 800 to 1,000 FTU/kg of phytase will result in maximum ATTD of P and Ca and no further increases in ATTD of P and Ca are achieved by supplementing Ronozyme HiPhos phytase at levels greater than 1,000 FTU/kg. Supplementation of corn-soybean meal diets with Ronozyme HiPhos phytase also results in a reduction of P excretion in the feces of the pigs.

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

Ingredient, %	Diets					
	Positive Control	Negative Control	Negative control + Phytase (FTU/kg)[1]			
			500	1,000	2,000	4,000
Ground corn	60.60	60.60	60.60	60.60	60.60	60.60
Soybean meal, 48%	32.00	32.00	32.00	32.00	32.00	32.00
Soybean oil	3.00	3.00	3.00	3.00	3.00	3.00
Ground limestone	0.90	0.90	0.90	0.90	0.90	0.90
Dicalcium phosphate	1.65	-	-	-	-	-
Cornstarch	-	1.65	1.625	1.60	1.55	1.45
L-lysine HCL	0.15	0.15	0.15	0.15	0.15	0.15
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Phytase premix [2]	-	-	0.025	0.05	0.10	0.20
Vit. mineral premix [3]	0.30	0.30	0.30	0.30	0.30	0.30
Mecadox premix [4]	1.00	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00	100.00

[1]FTU = phytase units.

Table 4.1 (Cont.)

[2]Ronozyme HiPhos, DSM Nutritional Products, Parsippany, NJ. Produced by mixing 3.4% of concentrated phytase (58,700 units/g) and 96.6% cornstarch.

[3]Provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A, 11,128 IU; vitamin D_3, 2,204 IU; vitamin E, 66 IU; vitamin K, 1.42 mg; thiamin, 0.24 mg; riboflavin, 6.58 mg; pyridoxine, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid, 23.5 mg; niacin, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

[4]The Mecadox premix (Phibro Animal Health, NJ) provided 55 mg per kg of Carbadox to the complete diet.

Table 4.2. Analyzed nutrient composition of diets (as-fed basis), Exp. 1

	Diets					
	Positive	Negative	Negative control + Phytase (FTU/kg)[1]			
Item	control	control	500	1,000	2,000	4,000
ADF, %	2.70	2.98	2.79	3.04	2.95	2.72
NDF, %	8.44	9.50	9.84	10.09	8.91	9.55
P, %	0.66	0.36	0.36	0.36	0.36	0.35
Ca, %	0.86	0.48	0.48	0.51	0.56	0.53
CP, %	18.33	17.96	17.24	18.03	19.24	18.27
DM, %	87.42	88.02	87.97	88.08	88.25	88.15
Ash, %	5.74	4.99	4.43	4.27	4.08	4.10
Phytase, FTU/kg[1]	91	80	440	958	1743	3974
Indispensible AA, %						
Arg	1.26	1.30	1.28	1.19	1.22	1.24
His	0.50	0.53	0.52	0.49	0.50	0.51
Ile	0.80	0.84	0.85	0.81	0.81	0.84
Leu	1.60	1.66	1.64	1.57	1.57	1.60
Lys	1.18	1.21	1.20	1.13	1.15	1.20
Met	0.29	0.32	0.31	0.29	0.29	0.30
Phe	0.92	0.95	0.95	0.90	0.90	0.93
Thr	0.71	0.75	0.71	0.67	0.69	0.69
Trp	0.24	0.24	0.24	0.24	0.23	0.24

Table 4.2 (Cont.)

Val	0.92	0.96	0.97	0.93	0.92	0.96
Dispensible AA, %						
Ala	0.93	0.97	0.94	0.91	0.91	0.93
Asp	1.92	2.03	1.98	1.87	1.91	1.95
Cys	0.31	0.34	0.32	0.29	0.30	0.30
Glu	3.19	3.32	3.26	3.12	3.14	3.20
Gly	0.79	0.83	0.81	0.76	0.77	0.79
Pro	0.94	1.10	1.05	1.02	1.04	1.04
Ser	0.83	0.86	0.79	0.76	0.78	0.76
Tyr	0.63	0.61	0.61	0.56	0.57	0.57

[1]FTU = phytase units (g/kg).

Table 4.3. Composition (as-is basis) of experimental diets, Exp. 2

Ingredient, %	Diets					
	Positive control	Negative control	Negative control + Phytase (FTU/kg)[1]			
			500	1,000	2,000	4,000
Ground corn	65.80	65.80	65.80	65.80	65.80	65.80
Soybean meal, 48%	29.50	29.50	29.50	29.50	29.50	29.50
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00
Ground limestone	0.95	0.95	0.95	0.95	0.95	0.95
Dicalcium phosphate	1.05	-	-	-	-	-
Cornstarch	-	1.05	1.025	1.00	0.975	0.95
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Phytase premix [2]	-	-	0.025	0.05	0.075	0.10
Vit. mineral premix [3]	0.30	0.30	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00	100.00

[1]FTU = phytase units.

[2]Ronozyme HiPhos, DSM Nutritional Products, Parsippany, NJ. Produced by mixing 3.4% of concentrated phytase (58,700 units/g) and 96.6% cornstarch.

Table 4.3 (Cont.)

[3]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A, 11,128 IU; vitamin D_3, 2,204 IU; vitamin E, 66 IU; vitamin K, 1.42 mg; thiamin, 0.24 mg; riboflavin, 6.58 mg; pyridoxine, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid, 23.5 mg; niacin, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

Table 4.4. Analyzed nutrient composition of diets (as-fed basis), Exp. 2

	Diets					
	Positive	Negative	Negative control + Phytase (FTU/kg)[1]			
Item	control	control	500	1,000	2,000	4,000
ADF, %	2.64	2.63	2.61	2.64	2.67	2.84
NDF, %	12.11	8.02	8.81	7.71	7.80	8.52
P, %	0.56	0.33	0.34	0.34	0.34	0.34
Ca, %	0.79	0.58	0.59	0.57	0.56	0.54
CP, %	20.45	21.94	21.29	21.00	20.92	20.45
DM, %	88.30	88.36	88.32	88.19	88.21	88.34
Ash, %	4.73	3.98	3.88	4.02	4.02	4.07
Phytase, FTU/kg[1]	39	41	373	984	1773	3681
Indispensible AA, %						
Arg	1.23	1.22	1.17	1.23	1.23	1.26
His	0.53	0.50	0.50	0.50	0.50	0.51
Ile	0.84	0.81	0.78	0.81	0.82	0.84
Leu	1.63	1.58	1.54	1.60	1.59	1.62
Lys	1.08	1.06	1.02	1.07	1.07	1.09
Met	0.30	0.29	0.28	0.29	0.29	0.29
Phe	0.92	0.90	0.86	0.90	0.90	0.92
Thr	0.70	0.70	0.65	0.70	0.68	0.69
Trp	0.25	0.24	0.25	0.25	0.24	0.25

Table 4.4 (Cont.)

Val	0.95	0.91	0.89	0.91	0.94	0.95
Dispensible AA, %						
Ala	0.94	0.92	0.88	0.92	0.91	0.94
Asp	1.91	1.87	1.79	1.88	1.88	1.92
Cys	0.29	0.30	0.28	0.30	0.30	0.29
Glu	3.39	3.31	3.18	3.32	3.32	3.37
Gly	0.79	0.77	0.74	0.77	0.78	0.80
Pro	1.10	1.10	1.01	1.06	1.03	1.07
Ser	0.79	0.80	0.74	0.82	0.76	0.77
Tyr	0.57	0.57	0.57	0.59	0.57	0.59

[1]FTU=phytase units (g/kg).

Table 4.5. Effects of phytase on apparent total tract digestibility (ATTD) of P and Ca in weanling pigs[1], Exp. 1

	Diets							*P*-value		*P*-value[2]	
	Positive control	Negative control	Negative control + Phytase (FTU/kg)				SEM	Positive vs. Negative	Negative vs. Phytase	L	Q
Item			500	1,000	2,000	4,000					
Feed intake, g/d	645	633	629	646	624	611	18.06	0.646	0.769	0.277	0.685
P intake, g/d	4.3	2.3	2.3	2.3	2.3	2.1	0.08	< 0.01	0.676	0.122	0.494
Fecal output, g/d	66.5	58.8	58.1	56.1	57.1	62.6	3.77	0.157	0.942	0.370	0.361
P in feces, %	2.53	2.30	1.51	1.46	1.22	1.10	0.07	0.023	< 0.01	< 0.01	< 0.01
P output, g/d	1.7	1.4	0.9	0.8	0.7	0.7	0.07	< 0.01	< 0.01	< 0.01	< 0.01
ATTD of P, %	60.5	40.5	61.6	65.1	68.7	68.0	2.34	< 0.01	< 0.01	< 0.01	< 0.01
P absorption, g/d	2.6	0.9	1.4	1.5	1.5	1.5	0.07	< 0.01	< 0.01	< 0.01	< 0.01
Ca intake, g/d	5.6	3.0	3.0	3.3	3.5	3.2	0.10	< 0.01	0.068	0.072	< 0.01
Ca in feces, %	2.29	1.86	1.37	1.11	0.94	0.79	0.13	0.019	< 0.01	< 0.01	< 0.01
Ca output, g/d	1.5	1.1	0.8	0.6	0.5	0.5	0.08	< 0.01	< 0.01	< 0.01	< 0.01
ATTD of Ca, %	72.5	63.9	73.7	81.7	84.8	84.6	2.30	0.012	< 0.01	< 0.01	< 0.01

Table 4.5 (Cont.)

Ca absorption, g/d	4.0	2.0	2.2	2.7	3.0	2.7	0.12	< 0.01	< 0.01	< 0.01	< 0.01

[1]Data are means of 8 observations per treatment.

[2]L = linear contrast; Q = quadratic contrast.

Table 4.6. Effects of phytase on apparent total tract digestibility (ATTD) of P and Ca in growing pigs[1], Exp. 2

	Diets							*P*-value		*P*-value[2]	
	Positive control	Negative control	Negative control + Phytase (FTU/kg)				SEM	Positive vs. Negative	Negative vs. Phytase	L	Q
Item			500	1,000	2,000	4,000					
Feed intake, g/d	1521	1460	1506	1497	1476	1476	58.08	0.471	0.674	0.930	0.808
P intake, g/d	8.5	4.8	5.1	5.1	5.0	5.0	0.23	< 0.01	0.368	0.825	0.582
Fecal output, g/d	132.9	118.2	116.9	116.0	117.5	124.0	5.48	0.076	0.957	0.356	0.529
P in feces, %	2.59	2.44	1.82	1.52	1.31	1.09	0.07	0.170	< 0.01	< 0.01	< 0.01
P output, g/d	3.4	2.9	2.1	1.8	1.5	1.4	0.10	< 0.01	< 0.01	< 0.01	< 0.01
ATTD of P, %	59.4	39.8	58.1	65.4	69.1	72.8	2.25	< 0.01	< 0.01	< 0.01	< 0.01
P absorption, g/d	5.1	1.9	3.0	3.3	3.5	3.7	0.24	< 0.01	< 0.01	< 0.01	< 0.01
Ca intake, g/d	12.0	8.5	8.9	8.5	8.3	8.0	0.36	< 0.01	0.902	0.128	0.838
Ca in feces, %	2.45	2.33	1.40	1.29	1.22	0.91	0.13	0.539	< 0.01	< 0.01	< 0.01
Ca output, g/d	3.2	2.7	1.6	1.5	1.5	1.1	0.16	0.068	< 0.01	< 0.01	< 0.01
ATTD of Ca, %	72.9	67.3	81.4	82.6	82.4	85.6	2.05	0.069	< 0.01	< 0.01	< 0.01

Table 4.6 (Cont.)

Ca absorption, g/d	8.8	5.7	7.3	7.0	6.8	6.8	0.39	< 0.01	< 0.01	0.376	0.122

[1]Data are means of 8 observations per treatment.

[2]L = linear contrast; Q = quadratic contrast.

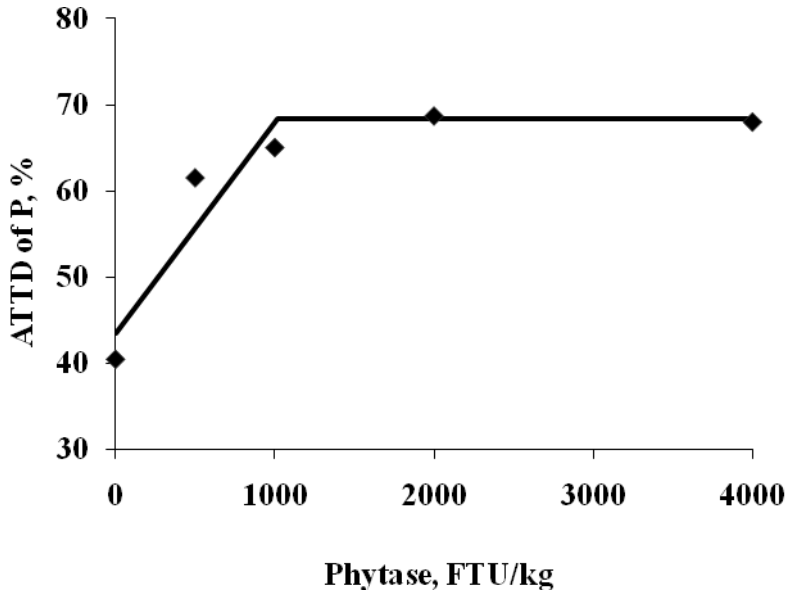

Figure 4.1. Fitted broken-line plot of ATTD of P as a function of dietary phytase level (Exp. 1) with observed treatment mean values (n = 8 observations per treatment mean). The minimal dietary phytase level determined by broken-line analysis using least squares methodology was 1,016 FTU/kg (Y plateau = 68.4; slope below breakpoint = -0.025; Adjusted R^2 = 0.873).

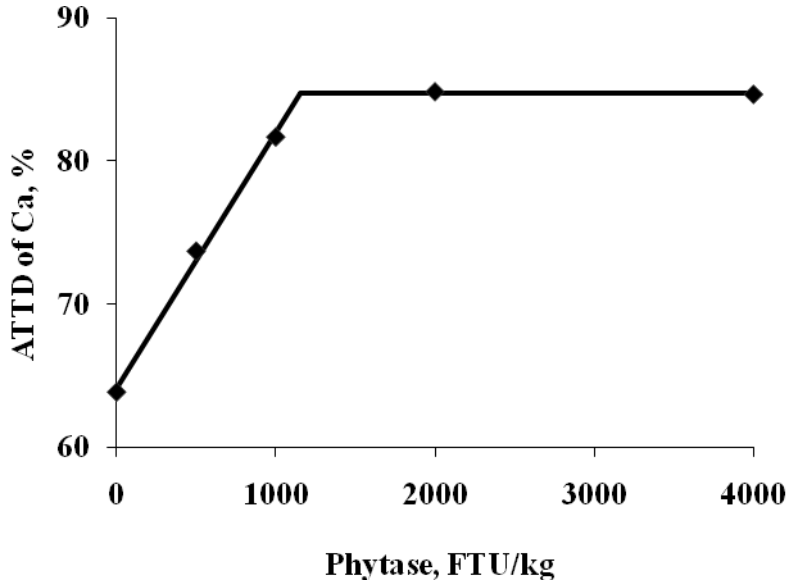

Figure 4.2. Fitted broken-line plot of ATTD of Ca as a function of dietary phytase level (Exp. 1) with observed treatment mean values (n = 8 observations per treatment mean). The minimal dietary phytase level determined by broken-line analysis using least squares methodology was 1,155 FTU/kg (Y plateau = 84.7; slope below breakpoint = -0.0178; Adjusted R^2 = 0.997).

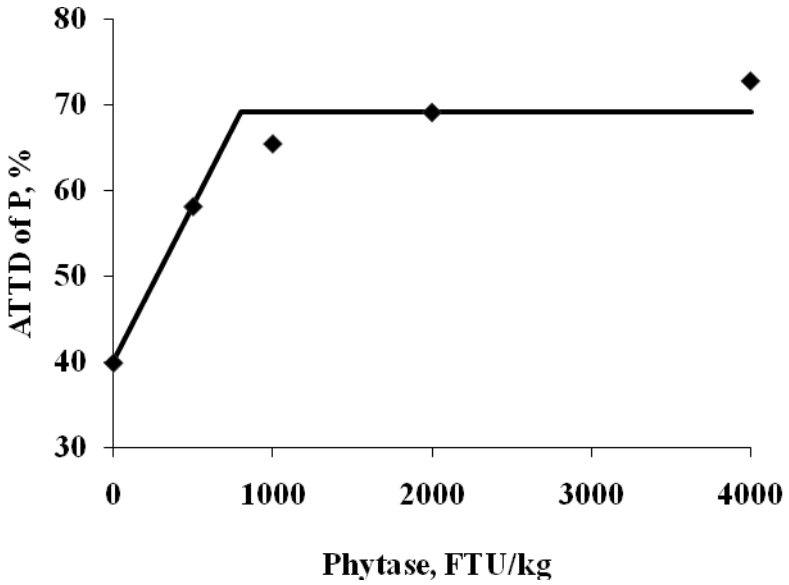

Figure 4.3. Fitted broken-line plot of ATTD of P as a function of dietary phytase level (Exp. 2) with observed treatment mean values (n = 8 observations per treatment mean). The minimal dietary phytase level determined by broken-line analysis using least squares methodology was 801 FTU/kg (Y plateau = 69.1; slope below breakpoint = -0.036; Adjusted R^2 = 0.947).

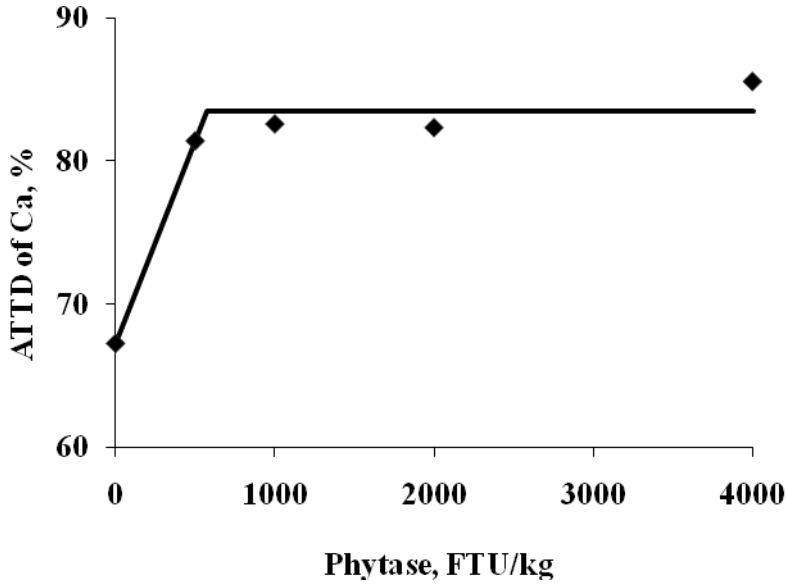

Figure 4.4. Fitted broken-line plot of ATTD of Ca as a function of dietary phytase level (Exp. 2) with observed treatment mean values (n = 8 observations per treatment mean). The minimal dietary phytase level determined by broken-line analysis using least squares methodology was 574 FTU/kg (Y plateau = 83.5; slope below breakpoint = -0.0283; Adjusted R^2 = 0.958).

CHAPTER 5

EFFECTS OF GRADED LEVELS OF PHYTASE ON APPARENT AND STANDARDIZED TOTAL TRACT DIGESTIBILITY OF PHOSPHORUS IN CORN AND CORN CO-PRODUCTS

ABSTRACT

An experiment was conducted to measure the effects of graded levels of microbial phytase on the standardized total tract digestibility (STTD) of P in corn, distillers dried grains with solubles (DDGS), high protein distillers dried grains (HP-DDG), and corn germ. A second objective was to develop regression equations to predict the response of adding phytase to each of these ingredients. Four corn based diets, 4 DDGS based diets, 4 HP-DDG based diets, and 4 corn germ based diets were formulated. The 4 diets with each ingredient contained 0, 500, 1,000, or 1,500 phytase units (FTU) per kg (Optiphos 2000, Enzyvia, Sheridan, IN). A P-free diet was also formulated to measure the basal endogenous losses of P. A total of 102 pigs (initial BW: 18.2 ± 2.1 kg) were individually housed in metabolism cages equipped with a feeder and a nipple drinker and a screen floor that allowed for total collection of feces. Pigs were allotted to the 17 diets in a randomized complete block design with 6 replicates per diet. Supplementation of microbial phytase increased (linear, $P < 0.01$; quadratic, $P < 0.05$) the STTD of P in corn from 40.9 to 67.5, 64.5, and 74.9%, tended to increase (linear, $P = 0.07$) the STTD of P in DDGS from 76.9 to 82.9, 82.5, and 83.0%, increased (linear, $P < 0.01$; quadratic, $P < 0.05$) the STTD of P in HP-DDG from 77.1 to 88.0, 84.1, and 86.9% , and increased (linear and quadratic, $P < 0.01$) the STTD of P in corn germ from 40.7 to 59.0, 64.4, and 63.2% in diets supplemented with 0, 500,

1,000, or 1,500 FTU/kg of phytase, respectively. Regression equations were developed to allow the calculation of the STTD of P with any level of phytase (Optiphos 2000, Enzyvia, Sheridan, IN) for each of the test ingredients. Therefore, results of this experiment allow the prediction of the amount of digestible P in corn and corn germ containing any level of phytase between 0 and 1,500 FTU.

Key words: digestibility, endogenous losses, phosphorus, phytase, pig

INTRODUCTION

Corn contains approximately 0.26% P (NRC, 1998), but the digestibility is low because most of the P in corn is bound to phytate, which is poorly digested by pigs (Selle and Ravindran, 2008). The low digestibility of P in corn is compensated by supplementation of diets with inorganic P. This practice has become expensive because of increasing prices of inorganic P. In an attempt to mitigate this problem, Almeida and Stein (2009) measured standardized total tract digestibility (**STTD**) of P in corn, soybean meal (**SBM**), and distillers dried grains with solubles (**DDGS**) with 0 or 500 phytase units (**FTU**) per kg. It was concluded from this work that the STTD of P is increased in corn and SBM, but not in DDGS if 500 FTU of microbial phytase is added to the diets. There are, however, no data on the effects of adding microbial phytase to other corn co-products such as high protein distillers dried grains (**HP-DDG**) and corn germ. The effects of adding graded levels of exogenous phytase to corn, DDGS, HP-DDG, and corn germ have also not been reported and the inclusion rate of phytase that is needed to maximize the STTD of P in these ingredients is not known. The objectives of this experiment were to measure the effects of graded levels of phytase on the STTD of P in corn, DDGS, HP-DDG, and corn

germ, and to develop regression equations to predict the response of adding different levels of phytase to each ingredient.

MATERIALS AND METHODS

The protocol for the experiment was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois. Pigs used in the experiment were the offspring of Landrace boars that were mated to Large White sows (Genetiporc, Alexandria, MN).

Diets, Animals, and Experimental Design

Distillers dried grains with solubles and corn germ were obtained from Poet Nutrition, Coon Rapids, IA, and HP-DDG was obtained from Poet Nutrition, Corning, IA (Table 5.1). A commercial hybrid of corn was obtained locally. Seventeen diets were formulated (Tables 5.2 and 5.3). There were 4 corn based diets, 4 DDGS based diets, 4 HP-DDG based diets, and 4 corn germ based diets. Phytase (Optiphos 2000, Enzyvia, Sheridan, IN) was added at levels of 0, 500, 1,000, or 1,500 phytase units (**FTU**) per kg to the 4 diets with each ingredient. A P-free diet was used to measure the basal endogenous P loss (**EPL**) from the pigs. A total of 102 growing pigs (initial BW: 18.2 ± 2.1 kg) were housed in metabolism cages equipped with a feeder and a nipple drinker that allowed for total collection of feces. Pigs were allotted to the 17 diets in a randomized complete block design. The Experimental Animal Allotment Program (Kim and Lindemann, 2007) was used to allot pigs to the 17 diets based on BW with 6 replicate pigs per diet.

Feeding and Sample Collection

The daily amount of feed provided to the pigs was calculated as 3 times the estimated requirement for maintenance energy (i.e., 106 kcal ME per kg $^{0.75}$; NRC, 1998) and fed in 2 equal meals. Pigs were allowed ad libitum access to water throughout the experiment. There was a 5 d adaptation period to the diets, which was followed by total collection of feces from d 6 to 11 according to the marker to marker approach (Adeola, 2001). In the morning meal of d 6, chromic oxide was added to the diets to determine the beginning of collections, and on d 11, ferric oxide was added to the diets to determine the end of collections. Fecal samples were collected twice daily and stored at -20˚C immediately after collection.

Sample Analysis and Data Processing

Prior to analysis, fecal samples were dried in a forced air oven and finely ground through a 2 mm screen using a Thomas-Wiley mill (Model 4, Swedesboro, NJ). After wet ash sample preparation (method 975.03; AOAC Int., 2007), fecal samples, ingredients, and diets were analyzed for Ca and P by inductively coupled plasma (ICP) spectroscopy (method 985.01; AOAC Int., 2007) and for DM by oven drying at 135˚C for 2 h (method 930.15; AOAC Int., 2007). Ingredients and diets were also analyzed for CP (method 990.03; AOAC Int., 2007) using an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ), ADF (method 973.18; AOAC Int., 2007), NDF (Holst, 1973), and for phytase activity (Phytex Method, version 1; Eurofins, Des Moines, IA). Ingredients were also analyzed for AA (method 982.30 E (a, b, c); AOAC Int., 2007) and for phytate (Eurofins, Des Moines, IA) using the method of Ellis et al. (1977).

The ATTD (%) of P in each diet was calculated according to the following equation:

$$\text{ATTD (\%)} = [(Pi - Pf)/Pi] \times 100,$$

where Pi is the total P intake (g) from d 6 to 11 and Pf is the total fecal P output (g) originating from the feed that was provided from d 6 to 11 (Petersen and Stein, 2006).

The basal EPL (mg/kg DMI) was measured from pigs fed the P-free diet according to the following equation:

$$\text{EPL (mg/kg DMI)} = ([Pf/Fi] \times 1{,}000 \times 1{,}000),$$

where EPL is the endogenous P loss and Fi is the total feed (g) intake from d 6 to 11 (Petersen and Stein, 2006).

Values for the daily EPL of pigs fed the P containing diets were calculated by multiplying the basal EPL by the average daily DMI of each pig during the 5 d collection period.

The STTD of P was calculated using the following equation:

$$\text{STTD (\%)} = ([Pi - \{Pf - EPL\}/Pi]) \times 100,$$

where STTD (%) is the standardized total tract digestibility of P.

Data were analyzed as a randomized complete block design using the Proc Mixed Procedure in SAS. The UNIVARIATE procedure in SAS was used to confirm that variances were homogenous and also to analyze for outliers, but no outliers were identified. The model included diet as the fixed effect and replicate as a random effect. No effects of replicate were observed, and therefore, replicate was removed from the final model. The effects of adding graded levels of phytase to each ingredient were analyzed by orthogonal polynomial contrasts. Appropriate coefficients for unequally spaced concentrations of supplemental phytase were obtained using the Proc IML of SAS. Estimates for the regression equations between STTD of P and analyzed phytase inclusion level were determined by submitting each treatment observations to Proc GLM of SAS. The pig was the experimental unit and an alpha value of 0.05 was used to assess significance among means.

RESULTS

Throughout the adaptation period pigs remained healthy and readily consumed their diets. During the 5 d collection period, however, 1 pig that was fed the corn germ diet without phytase was diagnosed with pneumonia, and therefore, removed from the experiment. All of the remaining pigs were healthy until the end of the experiment.

The analyzed values for phytase activity of all diets were slightly less than the calculated values (Table 5.3) despite the fact that the phytase activity in the phytase premix was analyzed immediately prior to diet mixing. Because of this discrepancy, analyzed values for phytase activity were used in all statistical analysis. The basal EPL was measured at 206 mg/kg DMI from pigs fed the P-free diet.

Digestibility of P in Corn

No differences in fecal output were detected among treatments (Table 5.4). Phytase increased (linear, $P < 0.05$) ADFI, P intake and EPL. The concentration of P in feces was reduced (linear and quadratic, $P < 0.01$) from 1.94 to 1.25, 1.15, and 0.94% for pigs that were fed diets that were supplemented with 0, 420, 720, or 1,100 FTU of phytase, respectively. Likewise, daily P output was linearly ($P < 0.01$) and quadratically ($P < 0.05$) reduced from 1.0 to 0.6, 0.7, and 0.6 g/d for pigs that were fed diets that were supplemented with 0, 420, 720, or 1,100 FTU of phytase, respectively. The ATTD and STTD of P were linearly ($P < 0.01$) and quadratically ($P < 0.05$) increased from 33.5 to 60.1, 57.0, and 67.4%, and from 40.9 to 67.5, 64.5, and 74.9% for pigs that were fed diets that were supplemented with 0, 420, 720, or 1,100 FTU of phytase, respectively.

Digestibility of P in DDGS

No differences in ADFI, P intake, daily fecal output, or EPL were observed among treatments. Concentration of P in feces decreased (linear, $P < 0.01$) as graded levels of phytase were added to the diets. Likewise, the daily P output was reduced ($P < 0.05$) by the addition of phytase to the diets. Addition of phytase to the diets tended (linear, $P = 0.08$) to increase the ATTD and STTD of P from 72.6 to 78.6, 78.2, and 78.6%, and from 76.9 to 82.9, 82.5, and 83.0% in pigs that were fed diets containing 130, 430, 770, or 1,100 FTU of phytase, respectively.

Digestibility of P in HP-DDG

There were no differences in ADFI, P intake, daily fecal output, or EPL among diets. Addition of phytase to the diets reduced (linear, $P < 0.01$; quadratic, $P < 0.05$) the concentration of P in feces. Phytase also reduced (linear, $P < 0.01$; quadratic, $P < 0.05$) P excretion from 0.4 to 0.3, 0.3, and 0.3 g/d in pigs that were fed diets containing 0, 500, 770, or 1,100 FTU of phytase, respectively. Addition of phytase to the diets increased (linear, $P < 0.01$; quadratic, $P < 0.05$) the ATTD of P from 68.6 to 79.5, 75.6, and 78.4%, and the STTD of P from 77.1 to 88.0, 84.1, and 86.9% in pigs that were fed diets containing 0, 500, 770, or 1,100 FTU of phytase, respectively.

Digestibility of P in Corn Germ

There were no differences in ADFI, P intake, daily fecal output, and EPL among treatments. Addition of phytase to the diets reduced (linear and quadratic, $P < 0.01$) the concentration of P in the feces. Likewise, addition of phytase to the diets reduced (linear and quadratic, $P < 0.01$) the daily P output from 2.2 to 1.6, 1.3, and 1.4 g/d for pigs that were fed diets containing 110, 390, 910, and 1,400 FTU of phytase, respectively. Addition of phytase to the diets increased (linear and quadratic, $P < 0.01$) the ATTD of P from 37.3 to 55.7, 63.0, and

59.8%, and the STTD of P from 40.7 to 59.0, 64.4, and 63.2% in pigs that were fed diets containing 110, 390, 910, and 1,400 FTU of phytase, respectively.

Regression Equations

Regression equations for the STTD of P as affected by microbial phytase in corn, HP-DDG, and corn germ are presented in Table 5.5. Addition of phytase to DDGS did not affect the STTD of P, and therefore, a regression equation for this ingredient is not presented.

DISCUSSION

Phosphorus and Phytate P in Ingredients

The concentration of P that was measured in ingredients used in this experiment is in agreement with the values reported by Widmer et al. (2007) and NRC (1998). The phytic acid data demonstrates that on a percentage basis, corn and corn germ contain similar amounts of phytic acid (72 and 76% of total P, respectively), and that DDGS and HP-DDG also have similar concentrations of phytic acid, but these amounts are much less (30 and 28% of total P, respectively) than in corn and corn germ. The reason for these differences is that during the production of DDGS and HP-DDG, some of the phytic acid is hydrolyzed, which results in a reduced concentration of phytate-bound P.

ATTD and STTD Values of P in Ingredients without Phytase

The value for the ATTD of P in corn measured in the present experiment is in agreement with values reported by Bünzen et al. (2008) and Stein et al. (2009). The ATTD of P in corn germ was greater than the value reported by Widmer et al. (2007). This difference may be due to different processing of corn to obtain corn germ, or differences between corn hybrids that were used to obtain the corn germ. The ATTD of P in DDGS has been reported to vary from 50 to

69% (Pedersen et al., 2007; Almeida and Stein, 2009; Stein et al., 2009). In the present experiment, the value for the ATTD of P in DDGS is close to the greatest value in this range. This observation confirms that the ATTD of P in DDGS is much greater than in corn, which is likely a consequence of the reduced concentration of phytate in DDGS compared with corn. The ATTD of P in HP-DDG measured in this experiment is similar to the ATTD of P in DDGS, and slightly greater than the value reported by Widmer et al. (2007). This observation indicates that the ATTD of P in HP-DDG and DDGS are similar as would be expected from the similar content of phytate in these 2 ingredients. We are not aware of any other experiments in which the ATTD of P in these 2 ingredients has been measured.

The value for the basal EPL that was measured in this experiment is very close to previous values (Stein et al., 2006; Widmer et al., 2007; Almeida and Stein, 2009). Values for the STTD of P are expected to be greater than the ATTD values because they are corrected for basal EPL, and results from the present experiment confirm this hypothesis. If values for the ATTD of P are corrected for total EPL, true total tract digestibility values are obtained and these values are expected to be greater than STTD values. The value calculated for the STTD of P in corn in the present experiment is in agreement with this theory because it is within the range for the ATTD (31.9%) and true total tract digestibility (49.2%) of P in corn reported by Stein et al. (2009) and Wu et al. (2008), respectively. The value for the STTD of P in DDGS is in agreement with the value reported by Almeida and Stein, (2009). Likewise, the STTD of P in HP-DDG and in corn germ measured in the present experiment is in agreement with the STTD of P that was measured in HP-DDG and in corn germ by Widmer et al. (2007).

Effects of Phytase

Supplementation of diets with phytase resulted in increased STTD of P, which was expected because hydrolysis of phytate by microbial phytase liberates P in the intestines of pigs, and therefore, improves the digestibility of P (Selle and Ravindran, 2008). Phytase increases the STTD of P in corn and soybean meal (Almeida and Stein, 2009), but to our knowledge, there are no data on the effects of graded levels of phytase on the STTD of P in corn, DDGS, HP-DDG, and corn germ. Although the objective of this experiment was not to compare the STTD of P among ingredients, our data suggest that the effect of phytase on the STTD of P in corn and corn germ seems to be greater than the effect of phytase on the STTD of P in DDGS and HP-DDG. Corn and corn germ contain greater amounts of phytate than DDGS and HP-DDG. During the processing of corn to obtain ethanol, it is possible that some of the phytate present in corn is hydrolyzed. Therefore, the lack of substrate in DDGS and HP-DDG may inhibit the effectiveness of phytase in improving the STTD of P in these ingredients, which is not the case in corn and corn germ.

Regression equations

The regression equations determined in this experiment allow calculation of the STTD of P with any level of phytase between 0 and 1,500 FTU (Optiphos 2000, Enzyvia, Sheridan, IN). The r^2 value for the regression equation of HP-DDG indicates that the model accounts for only 36% of the variation of the STTD of P, and that the relationship between graded levels of phytase and the STTD of P in HP-DDG is unlikely to be linear or quadratic. As a result, it is possible to predict the amount of digestible P in corn or corn germ containing different levels of phytase, but the regression equation for HP-DDG may not be adequate to predict the effect of

phytase on the STTD of P. This suggests that the effect of phytase on the STTD of P in HP-DDG needs further investigation.

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Table 5.1. Analyzed nutrient composition of ingredients

Item	Ingredient			
	Corn	DDGS[1]	HP-DDG[2]	Corn germ
Ca, %	0.01	0.02	0.01	0.02
Total P, %	0.25	0.85	0.39	1.41
Phytate P, %	0.18	0.26	0.11	1.07
Non-phytate P[3], %	0.07	0.59	0.28	0.33
DM, %	86.67	91.18	91.35	91.45
CP, %	7.27	26.41	38.09	15.36
ADF	3.16	7.30	14.37	8.00
NDF	17.40	28.93	27.68	28.87
Phytase, FTU/kg[4]	< 70	< 70	< 70	180
Indispensable AA, %				
Arg	0.34	1.16	1.37	1.07
His	0.19	0.71	1.00	0.41
Ile	0.24	1.00	1.61	0.45
Leu	0.74	2.84	5.16	1.02
Lys	0.24	0.87	1.05	0.80
Met	0.14	0.51	0.80	0.25
Phe	0.32	1.18	2.08	0.58
Thr	0.23	0.98	1.34	0.51
Trp	0.05	0.20	0.22	0.10

Table 5.1 (Cont.)

Val	0.33	1.32	1.95	0.73
Dispensable AA, %				
Ala	0.47	1.78	2.82	0.88
Asp	0.44	1.67	2.35	1.10
Cys	0.16	0.57	0.76	0.30
Glu	1.09	3.51	6.10	1.79
Gly	0.28	1.05	1.26	0.77
Pro	0.53	1.87	3.23	0.90
Ser	0.29	1.13	1.60	0.58
Tyr	0.21	0.95	1.51	0.40

[1]DDGS = distillers dried grains with solubles.

[2] HP-DDG = high protein distillers dried grains.

[3]Calculated as the difference between phytate-P and total P.

[4]FTU = phytase units per kg.

Table 5.2. Ingredient composition of basal diets (as-fed basis)[1]

Ingredient, %	Corn	DDGS[2]	HP-DDG[2]	Corn germ	P-free
Ground corn	97.00	-	-	-	-
DDGS	-	50.00	-	-	-
HP-DDG	-	-	50.00	-	-
Corn germ	-	-	-	40.00	-
Sugar	-	20.00	20.00	20.00	-
Soybean oil	1.00	-	1.00	-	4.00
Ground limestone	1.20	1.20	1.20	1.20	0.80
Salt	0.40	0.40	0.40	0.40	0.40
Vitamin mineral premix[3]	0.30	0.30	0.30	0.30	0.30
Cornstarch	0.10	28.10	27.10	38.10	49.22
Potassium carbonate	-	-	-	-	0.40
Magnesium oxide	-	-	-	-	0.10
Solka floc[4]	-	-	-	-	4.00
Gelatin[5]	-	-	-	-	20.00
AA mixture[6]	-	-	-	-	0.78

[1]For each ingredient, 3 additional diets were formulated by adding 0.025, 0.050, and 0.075% of phytase (Optiphos 2000, Enzyvia, Sheridan, IN) at the expense of cornstarch. These levels of Optiphos were expected to create diets containing 500, 1,000, or 1,500 phytase units per kg.

[2] DDGS = distillers dried grains with solubles; HP-DDG = high protein distillers dried grains

Table 5.2 (Cont.)

[3] The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A, 11,128 IU; vitamin D_3, 2,204 IU; vitamin E, 66 IU; vitamin K, 1.42 mg; thiamin, 0.24 mg; riboflavin, 6.58 mg; pyridoxine, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid, 23.5 mg; niacin, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

[4] Fiber Sales and Development Corp., Urbana, OH.

[5] Pork gelatin obtained from Gelita Gelatine USA Inc., Sioux City, IA.

[6] Provided the following quantities (%) of AA per kg of complete diet: DL-methionine, 0.27; L-threonine, 0.08; L-tryptophan, 0.14; L-histidine, 0.08; L-isoleucine, 0.16; and L-valine, 0.05.

Table 5.3. Analyzed nutrient composition of diets (as-fed basis)

	Corn				DDGS[1]				HP-DDG[2]				Corn Germ				P-free
Phytase[3]	0	500	1000	1500	0	500	1000	1500	0	500	1000	1500	0	500	1000	1500	
Item																	
Ca, %	0.50	0.50	0.54	0.53	0.37	0.46	0.63	0.67	0.45	0.58	0.71	0.49	0.47	0.50	0.56	0.60	-
P, %	0.24	0.24	0.24	0.24	0.45	0.45	0.45	0.45	0.23	0.23	0.23	0.23	0.56	0.56	0.56	0.56	-
DM, %	86.70	86.85	86.91	86.93	92.57	92.81	93.27	94.08	93.32	93.07	93.43	93.39	93.07	93.21	92.01	92.31	91.59
CP, %	6.89	7.05	6.86	7.20	14.26	14.08	14.50	13.89	20.10	19.89	20.88	20.82	5.85	7.20	6.71	7.21	22.12
ADF	2.78	2.61	2.77	2.77	3.39	3.53	3.69	4.09	6.37	6.17	7.43	6.33	2.89	3.96	3.34	3.51	-
NDF	15.26	17.30	20.22	17.50	13.88	14.92	15.25	15.96	16.49	13.96	14.82	15.02	12.74	16.88	15.27	11.77	-
Phytase[3]	< 70	420	720	1,100	130	430	770	1,100	< 70	500	770	1,100	110	390	910	1,400	-

[1] DDGS = distillers dried grains with solubles.

[2] HP-DDG = high protein distillers dried grains.

[3] Expressed as phytase units per kg.

Table 5.4. Effects of phytase on P-balance, apparent total tract digestibility (ATTD), and standardized total tract digestibility (STTD) of P in corn, distillers dried grains with solubles (DDGS), high protein distillers dried grains (HP-DDG), and corn germ[1]

Item	Feed intake, g/d	P intake, g/d	Fecal output, g/d	P in feces, %	P output, g/d	ATTD of P, %	Endogenous P, mg/d[2]	STTD of P, %
Corn								
Corn + 0 FTU/kg	562	1.56	53.48	1.94	1.0	33.5	115.52	40.9
Corn + 500 FTU/kg	565	1.56	50.26	1.25	0.6	60.1	116.32	67.5
Corn + 1000 FTU/kg	564	1.56	58.94	1.15	0.7	57.0	116.24	64.5
Corn + 1500 FTU/kg	612	1.69	58.59	0.94	0.6	67.4	126.07	74.9
SEM	24.13	0.07	4.14	0.08	0.08	3.68	4.97	3.68
P, Linear	0.03	0.03	0.21	< 0.01	< 0.01	< 0.01	0.03	< 0.01
P, Quadratic	0.12	0.12	0.73	< 0.01	0.03	0.04	0.12	0.04
DDGS								
DDGS + 0 FTU/kg	666	3.20	92.28	0.95	0.88	72.6	137.14	76.9
DDGS + 500 FTU/kg	644	3.09	88.72	0.74	0.66	78.6	132.63	82.9

Table 5.4 (Cont.)

DDGS + 1000 FTU/kg	682	3.25	95.08	0.75	0.72	78.2	140.56	82.5
DDGS + 1500 FTU/kg	680	3.22	94.03	0.71	0.68	78.6	140.21	83.0
SEM	39.02	0.19	4.88	0.06	0.07	2.09	8.03	2.09
P, Linear	0.40	0.64	0.60	< 0.01	0.11	0.08	0.40	0.07
P, Quadratic	0.64	0.72	0.80	0.13	0.21	0.20	0.65	0.20
HP-DDG								
HP-DDG + 0 FTU/kg	536	1.29	53.91	0.75	0.4	68.6	110.39	77.1
HP-DDG + 500 FTU/kg	547	1.32	52.29	0.52	0.3	79.5	112.64	88.0
HP-DDG + 1000 FTU/kg	525	1.27	52.00	0.59	0.3	75.6	108.14	84.1
HP-DDG + 1500 FTU/kg	563	1.36	54.77	0.53	0.3	78.4	115.91	86.9
SEM	11.60	0.03	2.59	0.06	0.03	2.57	2.39	2.57
P, Linear	0.27	0.31	0.84	< 0.01	< 0.01	< 0.01	0.27	< 0.01
P, Quadratic	0.26	0.27	0.41	0.03	0.02	0.04	0.26	0.04
Corn germ								

Table 5.4 (Cont.)

Corn germ + 0 FTU/kg[3]	581	3.48	70.14	3.14	2.2	37.3	119.61	40.7
Corn germ + 500 FTU/kg	586	3.50	61.47	2.57	1.6	55.7	120.68	59.0
Corn germ + 1000 FTU/kg	580	3.52	61.93	2.11	1.3	63.0	119.54	64.4
Corn germ + 1500 FTU/kg	581	3.51	60.26	2.35	1.4	59.8	119.62	63.2
SEM	15.80	0.09	3.67	0.16	0.11	2.37	3.25	2.21
P, Linear	0.91	0.69	0.43	< 0.01	< 0.01	< 0.01	0.91	< 0.01
P, Quadratic	0.81	0.76	0.25	0.02	< 0.01	< 0.01	0.81	< 0.01

[1] Data are means of 6 observations per treatment.

[2] Calculated by multiplying the basal EPL (206 mg/kg DMI) measured from pigs fed P-free diet by the DMI of each individual pig fed P containing diets.

[3] Data are means of 5 observations.

Table 5.5. Regression equations for the STTD of P in corn, HP-DDG, and corn germ against phytase units (FTU) per kilogram of ingredient.

Item	Regression equation	r^2	*P*-value
Corn	$42.34 + 0.059FTU - 0.000028FTU^2$	0.63	< 0.01
HP-DDG	$77.55 + 0.023FTU - 0.000014FTU^2$	0.36	< 0.01
Corn germ	$34.50 + 0.067FTU - 0.000034FTU^2$	0.79	< 0.01

CHAPTER 6

CONCLUSIONS

It is concluded that values for the standardized total tract digestibility (STTD) of P can be measured by correcting apparent total tract digestibility (ATTD) values for basal endogenous P losses (EPL), which can be calculated by feeding pigs a P-free diet. The STTD of P in distillers dried grains with solubles (DDGS) and in high protein distillers dried grains (HP-DDG) is much greater than the STTD of P in corn, soybean meal (SBM), and corn germ because during the ethanol production process most of the phytate present in corn is degraded.

It was also concluded that exogenous phytase improves the STTD of P in corn, SBM, HP-DDG, and in corn germ. In DDGS, however, the effects of phytase on improving P digestibility were not confirmed. The use of linear broken-line equations is a good predictor for the amount of exogenous phytase needed to maximize P utilization by pigs fed corn-SBM diets. Our research demonstrated that at levels greater than 1,000 phytase units per kg, P digestibility reaches a plateau.

Diet costs and P excretion are reduced if diets for pigs are formulated based on values for the STTD of P without compromising pig performance. No inorganic P is needed if the combination of DDGS and phytase is used in the diets.

There is a need for developing a data base with STTD of P in various feed ingredients. If such data base is established, commercial diets may be formulated based on values for the STTD of P and this will likely improve P utilization by pigs resulting in reduced diet costs as well as reduced P excretion in manure.