Evaluation of a Novel *E. coli*-derived Phytase Fed to Growing Pigs

By

Leann M. McGinnis

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This thesis is approved as a creditable and independent investigation by a candidate for the Master of Animal Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Cody L. Wright, Ph. D.
Major Advisor Date

Hans H. Stein, Ph. D.
Thesis Advisor Date

Douglas C. McFarland, Ph. D.
Animal Science Committee Date

Robert Thaler, Ph. D.
Head, Department of
Animal and Range Sciences Date

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ABSTRACT

Evaluation of a Novel *E. coli*-derived Phytase Fed to Growing Pigs

Leann M. McGinnis

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Five experiments were conducted to determine the effects of adding an *E. coli*-derived phytase (Quantum) to corn soybean meal-based diets fed to growing pigs. Experiments 1 and 2 were designed to determine the optimal inclusion level of Quantum when added to weanling and growing pig diets. Quantum was added at different inclusion levels from 500 to 1500 phytase units/kg (**U**) for weanling and 250 to 650 U for growing pigs. Growing pig performance and bone composition were compared with a fungal derived phytase (Natuphos). Weanling pig ADG increased (linear, $P < 0.10$) as the level of Quantum in the diet increased. The ADG for growing pigs increased (linear, $P < 0.05$) when pigs were fed diets containing increasing concentrations of phytase. Bone ash and mineral concentration was greater for pigs fed Quantum than pigs fed Natuphos. Experiment 3 was designed to determine the site of phytase activity in growing pigs fed Quantum or Natuphos. The apparent duodenal (ADD), ileal (AID), and total tract (ATTD) digestibility of P were calculated for a low-P diet, Quantum diet (500 U), and Natuphos diet (500 U). The concentrations of *myo*inositol hexaphosphate, *myo*inositol pentaphosphate, *myo*inositol tetraphosphate, *myo*inositol triphosphate, and *myo*inositol biphosphate (IP6, IP5, IP4, IP3, and IP2, respectively) in the diet, duodenal digesta, ileal

digesta, and fecal samples were determined. The ATTD of P was similar for Quantum and Natuphos (42.2 and 45.3%, respectively), but both were greater ($P < 0.05$) than for the low-P diet (14.2%). The diets containing phytase had lower ($P < 0.05$) concentrations of IP6 and IP5 in the duodenal digesta compared with the low-P diet. Experiments 4 and 5 used ATTD values obtained from Exp. 3 and 3 diets (adequate-P, low-P, and Quantum) were formulated to determine the effects of feeding Quantum to growing pigs. Pig growth, P absorption, and P retention were calculated. Pigs fed the adequate P diet and the Quantum diet grew faster ($P < 0.05$) than pigs fed the low-P diet. Pigs fed the Quantum diet had a lower ($P < 0.001$) fecal P excretion (7.63 g/5d) and a greater ($P < 0.01$) ATTD of P (62.46%) than pigs fed the adequate P diet (11.57 g/5 d and 56.35%) or low-P diet (11.73 g/5d and 41.85%). The results from these studies show that Quantum can be fed to weanling and growing pigs without reducing pig growth or bone tissue synthesis. Quantum can replace some inorganic P that is added to pig diets and reduce fecal P excretion.

Key words: digestibility, pig, phosphorus, Quantum phytase

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LIST OF ABBREVIATIONS

AA	Amino acid
ADD	Apparent duodenal digestibility
ADF	Acid detergent fiber
ADFI	Average daily feed intake
ADG	Average daily gain
AID	Apparent ileal digestibility
Ala	Alanine
ANOVA	Analysis of variance
AOAC	Association of Analytical Chemists
aP	Available phosphorus
Arg	Arginine
Asp	Asparagine
ATTD	Apparent total tract digestibility
BW	Body weight
C	Carbon
°C	Degrees Celcius
Ca	Calcium
cm	Centimeter
Crf	Chromium content in the dry matter in feed
Crd	Chromium content of the dry matter in digesta
Cu	Copper

Cys	Cysteine
d	Day
DM	Dry matter
Exp.	Experiment
Fe	Iron
g	Gram
G:F	Gain-to-feed ratio
GI	Gastro-intestinal
Glu	Glutamate
Gly	Glycine
h	Hour
HCl	Hydrochloric acid
His	Histine
HPLC	High-performance liquid chromatography
I	Iodine
IL	Illinois
Ile	Isoluecine
IU	International units
IP2	*myo*-inositol biphosphate
IP3	*myo*-inositol triphosphate
IP4	*myo*-inositol tetraphosphate
IP5	*myo*-inositol pentaphosphate

IP6	*myo*-inositol hexaphosphate
K	Potassium
kcal	Kilocalories
kg	Kilogram
Leu	Leucine
Lys	Lysine
m	Meter
ME	Metabolizable energy
Met	Methionine
Mg	Magnesium
mg	Miligram
Mn	Manganese
N	Nitrogen
Na	Sodium
NC	North Carolina
NC	Negative control diet
Nd	Nutrient in digesta
NDF	Neutral detergent fiber
Nf	Nutrient in feces
Nf	Nutrient in feed
Ni	Nutrient intake
NJ	New Jersey

nm	Nanometer
Nr	Nutrient retention
NRC	National Research Council
NP	Natuphos phytase diet
Nu	Nutrient in urine
QP	Quantum phytase diet
P	Phosphorus
PC	Positive control diet
Pi	Inorganic phosphorus
Phe	Phenylalanine
Pro	Proline
PTH	Parathyroid hormone
SAS	Statistical Analysis Software
Se	Selenium
SEM	Standard error of the mean
Ser	Serine
Thr	Threonine
Trp	Tryptophan
Tyr	Tyrosine
Val	Valine
U	Phytase units
USEPA	United States Environmental Protection Agency

wk	Week
wt	Weight
Zn	Zinc

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CHAPTER 1

INTRODUCTION

Phosphorus from agriculture sources is a major contributor to water pollution. For this reason, producers are in need of ways to reduce the amount of P that is excreted in livestock manure. Many cereal grains contain P that is not available to swine because it is bound as phytate and, therefore, producers add inorganic P to the diet to meet the animal's P requirements. Reducing the amount of inorganic P added to the diet will decrease the amount of available P in the diets and will also decrease pig growth and bone tissue synthesis. Swine producers need a way to make the phytate-bound P in cereal grains more available for swine. This will allow them to reduce the amount of inorganic P added to the diet and reduce the amount of P excreted in the feces without decreasing pig growth and bone tissue synthesis. One such method available to reduce the amount of P excreted in the feces is the addition of the enzyme phytase to pig diets. Research on the addition of phytase to pig diets has shown that phytase can improve P utilization from cereal grains by releasing phytate-bound P. Several phytases are commercially available for addition into swine diets.

A novel *Escherichia coli*-derived phytase has been produced using *Pichia pastoris* yeast. This novel phytase, Quantum, has several characteristics that make it different from other commercially available phytases. Quantum phytase has a pH optimum that is closer to stomach pH, thus may be more effective in the stomach at releasing phytate bound P. In addition, Quantum phytase is a heat-stable phytase that can

withstand high temperatures used during feed manufacturing. Research using Quantum phytase in pig diets is limited. Therefore, the effects of feeding Quantum phytase on P digestibility and bone tissue synthesis are unknown. If the optimal inclusion level of Quantum phytase is determined in weanling and growing pig diets, producers will be able to effectively add Quantum phytase to their swine diets.

CHAPTER 2

Evaluation of a novel *E. coli* derived phytase fed to growing pigs

Literature review

INTRODUCTION

As more states begin to require P-based manure applications, producers will need to reduce the total amount of P excreted from livestock and the amount of water soluble P. The reason for the push towards P-based manure application is because P is known to contribute to the pollution of surface water (Smith et al., 2004). In 2000, the United States Environmental Protection Agency published the National Water Quality Inventory, which indicated that nutrients, mainly N and P, are the leading pollutants or stressors of lakes, reservoirs, and ponds. The United States Environmental Protection Agency (2000) also estimates that the largest source of the pollutants or stressors in lakes, reservoirs, and ponds is from agriculture sources. One promising method to reduce the amount of P excreted in pig manure is through the addition of the enzyme phytase to pig diets (Knowlton et al., 2004; Beaulieu et al., 2005).

PHOSPHORUS

Biological Functions

Phosphorus is an essential mineral for monogastric animals. Phosphorus has many functions in the body and is used for bone mineralization, phospholipids, nucleic acids, adenosine tri-phosphate, and acid-base buffering (Crenshaw, 2001).

Second to Ca, P is the most abundant mineral found in the body. Approximately 85% of the P present in the body is deposited in the bones (McDowell, 1992). Bone ash in mammals is comprised of 36% Ca, 17% P, and 0.8% Mg (McDowell, 1992). The remainder of P in the body is stored in the soft tissue, mainly blood cells, muscle, and nerve tissue. The P concentrations of plasma is approximately 6 mg/dL (Stahl et al., 2004) and in whole blood, 35 to 45 mg/dL (McDowell, 1992).

Requirements for Pigs

To achieve optimal growth in pigs, an adequate amount of P needs to be present in the diet. In the growing stage, increasing available P (**aP**) in the diet has increased ADG, G:F, and bone bending moment (Hastad et al., 2004).

Phosphorus requirements are dependent on the physiological status of the pig. As an animal matures, P requirements will decrease if measured as g/kg of diet. When formulating diets for growing pigs, it is recommended that weanling and growing pigs from 5-10 kg receive 0.40% aP; 10-20 kg, 0.32% aP; 20-50 kg, 0.23% aP; 50-80 kg, 0.19% aP; and 80-120 kg, 0.15% aP (NRC, 1998). Mature gestating females, lactating females, and sexually active boars require similar dietary concentrations of aP and similar dietary concentrations of total P. NRC (1998) recommendations are set at 0.60% total P and 0.35% aP for these animals.

Dietary components influence the amount of aP in the diet. The amount of available Ca in the diet can impede P digestion. A wide Ca:P ratio will decrease P absorption. The current recommendations for Ca:total P ratio are 1:1 to 1.25:1 and the

Ca:aP ratio should be between 2:1 and 3:1 (NRC, 1998). The amount of phytate in the feed will also negatively impact P digestibility.

Phosphorus Absorption

Phosphorus absorption occurs in the small intestine. Within the small intestine, P absorption is most active during the proximal half of the small intestine (Moore and Tyler, 1955). Research has shown that the large intestine does not contribute to P absorption in the pig but does contribute to endogenous losses of P (Moore and Tyler, 1955; Crenshaw, 2001; Shen et al., 2002). Phosphorus is absorbed as inorganic P (**P_i**; Crenshaw, 2001).

Regulation of Ca and P absorption and Retention

Calcium and P regulation are closely related. When plasma Ca concentrations are low, the parathyroid releases parathyroid hormone (**PTH**) into the blood to increase plasma Ca concentrations (Crenshaw, 2001). Parathyroid hormone has several functions that influence Ca and P absorption and retention. The PTH will indirectly increase Ca and P reabsorption from the bone and Ca-binding protein synthesis. When PTH is present in the blood, it will activate the synthesis of the active form of vitamin D (1, 25-dihydroxycholecalciferol) in the kidney by activating the enzyme 1α-hydroxylase (Costanza, 1998). Active vitamin D will increase the synthesis of the Ca-binding protein (calbindin) in the enterocytes, thus increasing Ca absorption in the intestines (Costanza, 1998; Crenshaw, 2001). Parathyroid hormone will also cause the osteoclastic cells to increase Ca and P reabsorption from the bone by inhibiting osteoblast cell matrix formation (Crenshaw, 2001).

When plasma Ca concentrations are high, thyroid C cells will release calcitonin (Weaver, 2006). Calcitonin will stimulate Ca uptake into the cytoplasm of cells and renal excretion of Ca (Crenshaw, 2001). In addition, calcitonin will inhibit the osteoclastic reabsorption of Ca and P from the bone allowing the osteoblastic cells to increase bone density (Costanzo, 1998; Crenshaw, 2001).

Several mechanisms are responsible for maintaining P homeostasis. When a low P diet is consumed, serum P concentrations decrease which in turn will increase serum Ca concentrations. The response to this change is a decrease in the release of PTH. The reduction in circulating PTH will cause an increase in P reabsorption from the kidneys and increased 1α-hydroxylase activity in the kidney (Berndt and Kumar, 2007). An increase in 1,25-dihydroxycholecalciferol will stimulate increased P absorption in the intestines (Crenshaw, 2001; Berndt and Kumar, 2007).

An increase in serum P concentrations results in a decrease of serum Ca concentrations. This effect can be caused by feeding a high P diet and results in increased PTH release. In renal tissue, the PTH will inhibit P reabsorption, which increases urinary P excretion and causes a phosphaturic response (Berndt and Kumar, 2007). The kidney will also decrease the synthesis of 1, 25-dihydroxycholecalciferol. The reduced serum level of 1, 25-dihydroxycholecalciferol will decrease P absorption in the small intestine (Berndt and Kumar, 2007).

SOURCES OF PHOSPHORUS

Organic P Sources

Phosphorus from cereal grains and oilseed meals in the diet are organically bound to phytic acid (phytate). In corn, 62% of total P is bound as phytate and 58% of total P in soybean meal is bound as phytate (Nelson et al., 1968; Reddy et al., 1982; Eeckhout and De Paepe, 1994; Godoy et al., 2005). Phytate-P is marginally available to swine because pigs lack the phytase enzyme that is needed to liberate the phytate-bound P from the inositol ring of phytate. Therefore, phytate-P in cereal grains and oilseed meal ingredients has a low digestibility, usually between 20 and 40% (NRC, 1998).

Inorganic P Sources

Inorganic sources of P commonly added to swine diets are dicalcium phosphate and monocalcium phosphate (Crenshaw, 2001; Petersen, 2004). Petersen and Stein (2006) reported that the true total tract digestibility of several P_i sources range from 88.41 to 98.20% indicating that P in P_i is much more digestible than phytate-P in organic sources. Likewise, P in protein sources from animal origin are also inorganic sources of P and, therefore, have a high availability (NRC, 1998).

PHYTASE

Phytate

The phytate molecule or *myo*-inositol hexaphosphate (**IP6**) consists of a 6-C inositol ring with a phosphate group attached to each C when fully phosphorylated (Figure 2.1). Phytate can form several complexes with proteins and minerals such as Zn, Ca, Fe, and Mg (Reddy et al., 1982). Minerals that form complexes with phytate become

unavailable for absorption, but the addition of phytase can increase their availability (Reddy et al., 1982; Kies et al., 2006).

Phytase Sources

Phosphorus availability in cereal grains fed to swine can be improved by the addition of the enzyme phytase. Phytase is found naturally in many of the cereal grains, such as rice, wheat, corn, soybeans, rye, pea, and barley (Dvořáková, 1998). However, with the exception of wheat and barley, phytase concentrations are too low (< 50 phytase units) to increase P availability (NRC, 1998). Phytase is also naturally occurring in bacteria, fungi, and yeast (Liu et al., 1998). Microorganisms found in the rumen of some animals and in the large intestine of some animals can produce phytase; however, no absorption of P occurs in the large intestine and the liberated P is excreted in the feces.

Microbial phytase has been isolated and genetically modified for use in animal feeds (Dvořáková, 1998). Fungal-derived microbial phytases have been developed from *Aspergillus niger* and *Peniophora lycii* (Wyss et al., 1999; Lassen et al., 2001). Natuphos (BASF, Florham Park, NJ) and Ronozyme (DSM Food Specialties, Delft, The Netherlands) are two commercially available phytases derived from *A. niger* and *P. lycii*, respectively. Until recently, fungal-derived phytases were the only commercial phytases available. Novel microbial phytases have now been developed from *E. coli*. Quantum (Syngenta Animal Nutrition, Research Triangle Park, NC), Optiphos (JBS United, INC., Sheridan, IN), and Phyzyme XP (Danisco Animal Nutrition, Marlborough, UK) are now available in many countries for use in swine diets.

Phytase Activity

One unit of phytase activity is defined as the amount of enzyme needed to liberate 1.0 mol of inorganic *ortho*-phosphate per minute under the conditions of the assay (AOAC, 2005). Commercial phytases are categorized as either a 3-phytase or a 6-phytase. A phytase that begins to release the phosphate group attached to C3 is considered a 3-phytase and a phytase that begins to release the phosphate group attached to C6, is considered a 6-phytase (Dvořáková, 1998). Generally, phytases from plants are 6-phytases and those phytases originating from microorganisms are 3-phytases (Liu et al., 1998). However, Greiner et al. (1993) reported that some *E. coli* phytases are 6-phytases.

Phytase functions by releasing P_i from phytate (Figure 2.2). The reaction includes the substrate IP6 and, after 6 cycles, the products P_i and free *myo*-inositol are produced (Liu et al., 1998). As the P is liberated, the phytate complex becomes the intermediates *myo*-inositol pentaphosphate, *myo*-inositol tetraphosphate, *myo*-inositol triphosphate, *myo*-inositol biphosphate, and *myo*-inositol monophosphate. This indicates that, under the ideal conditions, all 6 P_i can be released from the inositol ring of phytate.

The optimal environment for phytases is variable. Phytase derived from *A. niger* is most active around a pH of 2.5 and 5.5 (Liu et al., 1998; Wyss et al., 1999). Phytase from *P. lycii* has a pH optimum of 4.0 to 4.5 and a temperature optimum of 50 to 55°C (Lassen et al., 2001). Studies have indicated that *E. coli* derived phytases have an optimal pH range of 2.0 to 4.5 (Adeola et al., 2004; Onyango et al., 2004).

PHYTASE RESEARCH

Effects of Phytase on Pig Performance

A linear response in ADG and G:F ratio of weanling pigs was observed when feeding increasing levels of Natuphos up to 1,400 phytase units per kg (**U/kg**; Yi et al., 1996). When a low-P diet supplemented with an *E. coli* phytase was fed to nursery pigs, ADG and G:F ratio were improved compared with pigs fed a low-P diet with no phytase (Jendza et al., 2005). Yi et al. (1996) also reported that an increase in the aP level from 0.05% to 0.16% or the addition of increasing levels of Natuphos to the diet enhanced ADG, ADFI, and G:F ratio. Likewise, pig ADG, ADFI, and G:F ratio increased, linear and quadratic, when diets were supplemented with 0, 100, 250, 500, 750, or 1,500 U/kg (Kies et al., 2006). Similar results have been reported in growing and finishing pigs when fed a diet containing an *E. coli* phytase (Adeola et al., 2004; Jendza et al., 2005). It is, therefore, well documented that a low-P diet with added phytase will support pig performance similar to that obtained in high-P diets.

Effect of Phytase on Nutrient Digestibilities

The addition of phytase can improve the digestibilities of Ca, P, Mg, Na, K, and Cu (Kies et al., 2006). The addition of an *E. coli* derived phytase to low-P diets increases Ca and P digestibility and retention (Adeola et al., 2004). The addition of phytase to growing pig diets increases metacarpal, metatarsal, and femur bone strength (Cromwell et al., 1993). In low-P pig diets supplemented with either a fungal or an *E. coli* phytase, fibula ash percent was greater than the low-P diet with no phytase added when fed to pigs (Augspurger et al., 2003a). Some experiments have indicated that the addition of phytase to pig diets improves AA digestibilities; however, these results are inconsistent (Adeola and Sands, 2003). Radcliffe et al. (2006) reported that the addition of 500 U/kg of

Natuphos can replace 0.59 g of P_i in growing and finishing pigs. However, Yi et al. (1996) reported that 0.84 g of P_i can be replaced with the addition of 500 U/kg of Natuphos for weanling pigs. The addition of phytase to pig diets can improve Zn absorption and retention (Adeola et al., 1995). However, if Zn is fed at pharmacological concentrations, the beneficial effects of phytase addition on bone tissiue synthesis is decreased (Augspurger et al., 2003b).

Quantum Phytase Research

Quantum phytase is an *E. coli*-derived phytase that recently was approved for regulatory use in the US. Quantum phytase supplementation to low-P diets fed to chicks can result in increased weight gain and feed intake compared with chicks fed a low-P control diet (Onyango et al., 2005a). The addition of Quantum phytase to diets fed to turkeys can compensate for a 0.14% reduction in aP (Bedford and Wyatt, 2004). Wyatt et al. (2004) reported that a broiler diet with reduced levels of aP, metabolizable energy, and total lysine, but supplemented with 250 U/kg of Quantum phytase, improved broiler performance to a level that was equal to that of a diet adequate in all nutrients. Quantum phytase activity in the digestive tract of broiler chicks was greater compared with a *P. lycii* phytase. Therefore, more phytate was degraded by Quantum phytase than by the *P. lycii* phytase (Onyango et al., 2005b).

Pigs fed a P deficient diet with Quantum phytase inclusion levels of 0, 100, 500, 2500, and 12500 U/kg showed linear and quadratic increases in ADG, ADFI, and G:F ratio (Veum et al., 2006). It also was reported that pigs fed diets containing 2500 or 12500 U/kg of Quantum phytase had greater ADG and ADFI than pigs fed a diet

adequate in aP (Veum et al., 2006). Likewise, Beaulieu et al. (2005) reported that supplementing a diet containing 0.5% total P with 0, 250, 500, 1000, or 2000 U/kg of Quantum phytase resulted in a reduction of total and water soluble P in fecal excretion in weanling pigs, which indicates that more organic P was present when pigs were fed diets supplemented with Quantum phytase. Likewise, supplementing a diet containing 0.40% total P with 0, 100, 500, 2500, and 12500 U/kg of Quantum phytase resulted in an increase in ADG and G:F ratio with pigs fed the diet containing 2500 U/kg being greater than pigs fed a diet adequate in total P (Azain and Bedford, 2004). Feeding high levels of Quantum phytase to low-P diets in the nursery resulted in an overall increase of growth rate between BW of 20 and 130 kg (Tsai et al., 2006).

The addition of Quantum phytase to the diet has been shown to improve nutrient digestibilities in pigs, but not in chicks. Onyango et al. (2005a) reported that apparent ileal digestibilities of DM, energy, N, AA, Ca, and P in chicks were not affected by the addition of Quantum phytase to the diet, but P and Ca retention were improved with the addition of Quantum phytase. Likewise, in pigs there was an increase in Ca and P digestibilities with the addition of Quantum phytase to a low-P diet (Azain and Bedford, 2004). Similarly, Veum et al. (2006) reported that Ca, P, and Mg absorption increased with the addition of Quantum phytase to the diet and pigs excreted less Ca, P, and Mg in their feces. When Quantum phytase was included in low-P diets at 1200 and 12500 U/kg, pigs had greater digestibilities of Ca, P, and Mg than pigs fed a diet adequate in all nutrients (Veum et al., 2006). Azain and Bedford (2004) reported that a Quantum

phytase inclusion level of 2500 U/kg resulted in pigs having greater Ca and P digestibility values than pigs fed a diet adequate in P.

Bone data indicate that the addition of Quantum phytase to the diet can improve several bone characteristics. Several studies have reported that bone weight and bone ash increased when pigs were supplemented with increasing levels of Quantum phytase to low-P diets (Azain and Bedford, 2004; Tsai et al., 2006; Veum et al., 2006). Bone breaking strength also improved with the addition of Quantum phytase to the diet (Azain and Bedford, 2004; Veum et al., 2006; Tsai et al., 2006). Veum et al. (2006) reported that bone length and bone width measurements were increased as pigs were fed diets supplemented with increasing levels of Quantum phytase.

CONCLUSIONS AND PERSPECTIVES

Reducing fecal P excretion in pigs is important for reducing the amount of acres needed for manure application. It is well established that by adding fungal-derived phytases to pig diets, the amount of total P in the diet can be reduced while adequate amounts of aP in the diet can be maintained without reducing pig performance.

Quantum phytase is a novel phytase that is derived from *E. coli* and expressed in *Pichia pastoris* yeast. Research on adding Quantum phytase to pig diets and its implications on Ca and P digestibility and fecal P excretion in pigs is limited. The recommended inclusion level of Quantum phytase in the diets for weanling and finishing pigs has not been established. Likewise, no research has been conducted to investigate the effect of Quantum phytase on apparent duodenal, ileal, and total tract digestibilities

for AA, CP, Ca, and P. In addition, it is unclear how long Quantum phytase stays active in the digestive tract of pigs and how digestive enzymes influence Quantum phytase function. Therefore, there is a need for more research to determine what the optimal inclusion level of Quantum phytase is for weanling and growing pigs. In addition, knowing how phytase is degraded in the digestive tract will indicate its effect on apparent duodenal, ileal, and total tract digestibility. Knowing this information will allow for producers to develop diets for weanling and growing pigs that can improve P utilization in cereal grains and decrease the amount of P excreted in the feces.

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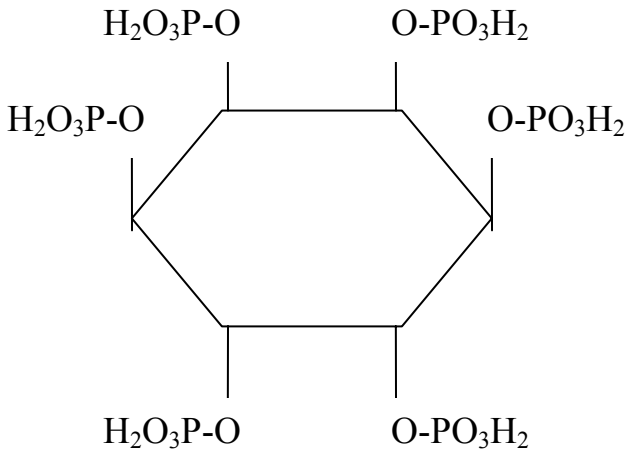

Figure 2.1. Structure of a fully protonated phytic acid. Adapted from Adeola and Sands (2003).

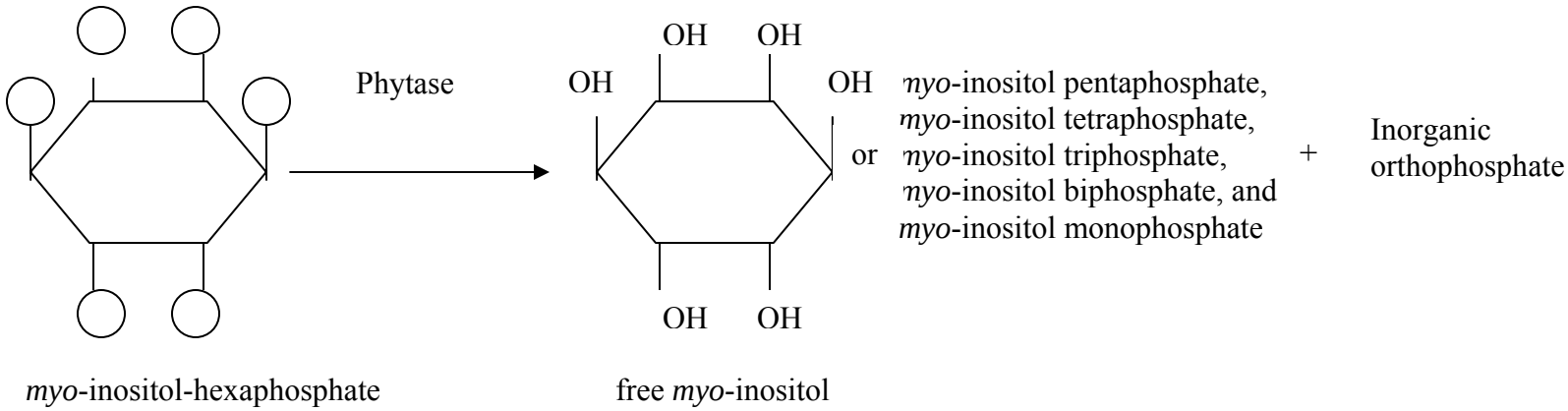

Figure 2.2. General enzymatic reaction of phytase. Adapted from Liu et al. (1998).

CHAPTER 3

Effects of feeding a novel *E. coli*-derived phytase to weanling and growing pigs

ABSTRACT: Two experiments were conducted to determine the effects of including an *Escherichia coli*-derived phytase (Quantum) in diets fed to weanling and growing pigs. In Exp. 1, 120 pigs were weaned at 20 d of age and allocated to 1 of 5 dietary treatment groups with 6 replicates per treatment and 4 pigs per pen. The positive control diet was formulated to meet NRC (1998) requirements and contained 0.81% Ca, 0.60% P and 0.32% digestible P. The negative control diet contained 0.18% digestible P. Three additional diets were formulated by adding 500, 1000, or 1500 Quantum phytase units per kg (**U**) to the negative control diet. Pig BW was recorded at the start of the experiment, after 2 wk, and at the end of the 4 wk experiment. A linear response (tendency, $P < 0.10$) was observed for ADG and G:F. In Exp. 2, 192 growing pigs, initial BW 18.32 ± 0.19 kg, were allotted to 8 treatment groups with 4 pigs per pen (2 barrows and 2 gilts) and 6 replicates per treatment. Diets were fed in 2 phases, with each phase lasting 4 wk. The positive control diet was formulated to contain 0.54% P, 0.64% Ca, and 3,420 kcal ME in phase 1 and 0.62 % Ca, 0.52 % P, and 3437 kcal ME in phase 2. The negative control diet was formulated to contain 0.48% Ca, 0.40% P, and 3,320 kcal ME in phase 1, and 0.46% Ca, 0.39% P, and 3,337 kcal ME in phase 2. Three diets were

formulated by adding 250, 450, or 640 U/kg of Quantum phytase to the negative control diet. An additional 3 diets were formulated by adding 250, 450, or 650 U/kg of Natuphos phytase to the negative control diet. Pig BW was recorded at the start of the experiment, after 4 wk, and at the end of the experiment. At the conclusion of Exp 2, 1 barrow and 1 gilt were selected from each pen and the 3rd and 4th metacarpals were harvested and analyzed for ash, Ca, and P. The results of this experiment showed a linear increase in final BW and ADG in phase 2 and overall for pigs fed diets containing Quantum or Natuphos phytase. Bone data indicate that adding a phytase to a low-P diet will increase (linear, $P < 0.05$) bone weight, total bone ash, total P in bone, percent P in bone, total Ca in bone, and percent of Ca in bone. The conclusion from these experiments is that Quantum phytase is a highly effective phytase source which can be fed to weanling and growing pigs as an alternative to inorganic P.

Key words: bone, phosphorus, phytase, pig

INTRODUCTION

Phosphorus is primarily stored in plants in the form of phytate. Swine are unable to absorb P bound in this form due to the lack of the phytase enzyme in their digestive tract. Addition of a microbial phytase to diets fed to pigs has been shown to improve the availability of dietary P (Crenshaw, 2001). Several types of fungal-derived phytases are available. Phytases derived from microbes, such as *Escherichia coli* are a new type of

phytase that can be added to the diets fed to pigs. Studies have shown that *E. coli*-derived phytases added to diets fed to growing and weanling pigs will improve P absorption (Jendza et al., 2005; Auspurger et al., 2003). Effects of adding a novel *E. coli* phytase, Quantum phytase (Syngenta Animal Nutrition, Research Triangle Park, NC), to diets fed to poultry and pigs has been investigated in a few experiments. Quantum phytase is a 6-phytase derived from a *Pichia pastoris* yeast cell culture (Onyango et al., 2004). The addition of Quantum phytase to low-P corn-soybean meal-based starter diets fed to weanling pigs decreased the excretion of total and water soluble P (Beaulieu et al., 2005). When used in growing pig diets, Quantum phytase improved growth rate, bone mineral concentrations, and Ca and P digestibility compared with pigs fed a P deficient diet (Azain et al., 2004).

The objective of the current experiments were to determine the quantity of Quantum phytase needed to obtain a response similar to diets containing inorganic P if fed to weanling and growing pigs.

MATERIALS AND METHODS

General Procedures

Two experiments were conducted. The South Dakota State University Animal Care and Use Committee approved both experiments. Pigs used in the experiments

originated from the matings of SP-1 boars to Line 13 females (Ausgene Intl. Inc., Gridley, IL).

Experiment 1

Animals, experimental design, and diets. A total of 120 pigs (initial BW 6.22 ± 0.97 kg) were weaned at approximately 20 d of age. Pigs were allowed to consume a standard creep-feed during the last week prior to weaning. Following weaning, pigs were randomly allotted to 5 treatment groups based on ancestry, sex, and BW. Pigs were housed in an environmentally controlled nursery with an initial temperature of 28˚C; during the experiment, the temperature was reduced by 1˚C each week. Six replicate pens were used per treatment group with 4 pigs per pen. Pens were equipped with a metal feeder placed at the front gate and a nipple drinker suspended at one side panel. Pen size measured 1.2 m x 1.2 m and an expanded-metal plastic-coated floor was provided in each pen. Pigs had *ad libitum* access to feed and water throughout the experiment.

Five diets were formulated (Tables 1 and 2). The positive control diet was formulated to meet current estimated nutrient requirements for 5 to 10 kg pigs (NRC, 1998). This diet was formulated to contain 1.20% standardized digestible Lys, 0.81% Ca, 0.60% P, and 0.32% available P. The P in this diet originated from organic sources and from monocalcium phosphate. A negative control diet was also formulated. This diet was similar to the positive control diet with the exception that no monocalcium phosphate

was included in the diet and the concentration of available phosphorus was calculated to be only 0.18%. Three additional diets were formulated. These diets were identical to the negative control diet with the exception that Quantum phytase was included at 500, 1000, and 1500 phytase units per kg (**U**), respectively.

Data collection. Pigs were fed the experimental diets for 4 wk post-weaning. Individual BW was recorded at the start of the experiment, after 2 wk, and at the end of the experiment. The daily allotments of feed were recorded as well. Orts were weighed on d 14 and at the end of the experiment.

Feed samples were analyzed for DM, Ca, P, and phytase activity. Samples were prepared for analysis using a dry ash method (procedure 4.8.02; AOAC, 2000). The concentrations of Ca in the feed were determined using atomic absorption spectrophotometry (procedure 4.8.03; AOAC, 2000) and P concentrations were determined using a spectrophotometer at 650 nm (procedure 3.4.11; AOAC, 2000). Phytase activity in the control diets were determined using procedure 2000.12 (AOAC, 2005). Phytase activity in the Quantum phytase diets were determined using SAN SOP Phytase In-Feed Assay Version 1.4 (Syngenta Animal Nutrition, unpublished).

Calculations. At the conclusion of the experiment, data for pig BW gains within each treatment group were summarized and the ADG for each phase and overall for the experiment were calculated. Likewise, daily feed allotments were summarized and ADFI

for each pen was calculated. By dividing ADG by the ADFI, the average G:F ratios were calculated for each phase and overall.

Statistical analysis. Data were analyzed using PROC MIXED of SAS (SAS Stat Inc. Inst., Cary, NC.). A contrast statement was used to compare the positive and the negative control diets. Likewise, the positive control diet was contrasted against the diets containing Quantum phytase. The linear and quadratic effects of adding Quantum phytase to the negative control diet were also analyzed using a contrast statement. An outlier test was conducted using PROC UNIVARIATE of SAS. No outliers were identified. The pen was the experimental unit for all calculations and an alpha value of 0.05 was used to assess significance among means with a tend being between an alpha value of 0.06 and 0.10.

Experiment 2

Animals, experimental design, and diets. A total of 192 growing pigs (initial BW 18.32 ± 0.19 kg) were randomly allotted to 1 of 8 treatment groups. There were 4 pigs, 2 barrows and 2 gilts, per pen and 6 replicate pens per treatment. Pigs were housed in an environmentally controlled building. Pens were 1.2 x 2.4 m and had fully slatted concrete floors. A nipple drinker and a 1-hole feeder were installed in each pen. Pigs were allowed *ad libitum* access to feed and water during the experiment.

Each treatment group was fed 1 of 8 diets (Tables 3 and 4) in a 2-phase sequence. Phase 1 diets were fed for 4 wk and phase 2 diets were fed during the following 4 wk. A positive control diet (0.64% Ca, 0.54% P, and 3420 kcal ME per kg in phase 1, and 0.62% Ca, 0.52% P, and 3437 kcal ME per kg in phase 2) was formulated. A negative control diet was also formulated. This diet contained 0.48% Ca, 0.40% P, and 3320 kcal ME in phase 1, and 0.46% Ca, 0.39% P, and 3337 kcal ME in phase 2. Three additional diets were formulated by adding 250, 450, and 650 U/kg of Quantum phytase, respectively, to the negative control diet. The remaining 3 diets were formulated by adding 250, 450, and 650 U/kg of Natuphos to the negative control diet. The energy concentration in the negative control diet and in the phytase containing diets was calculated to be lower than in the positive control diet to test the hypothesis that the inclusion of phytase in diets fed to growing pigs may increase the utilization of energy from the diet. All diets were based on corn, soybean meal, vitamins, and minerals and they were formulated to meet or exceed estimated requirements of all nutrients except P (NRC, 1998).

Data collection. The BW of all pigs were recorded at the beginning of the trial, after 4 wk, and at the conclusion of the experiment. Daily feed allotments were recorded as well, and feed intake per pen was summarized for the initial 4 wk, for the final 4 wk, and overall for the entire experiment.

At the conclusion of the experiment, 2 pigs, 1 barrow and 1 gilt, were randomly selected from each pen. The pigs were sacrificed and the third and fourth metacarpals were harvested from the right leg. The bones were labeled and autoclaved to remove soft tissue. Prior to analysis for Ca and P, bones were soaked in ether to remove fat and placed in an oven (100^{o}C) for 24 h to remove moisture. Feed and bone samples were analyzed for ash, Ca, and P as in Exp. 1. Phytase activity in the feed samples for the control diets and the diets containing Natuphos were determined using AOAC procedure 2000.12 (2000). Phytase activity in diets containing Quantum phytase was determined using SAN SOP Phytase In-Feed Assay Version 1.4 (Syngenta Animal Nutrition, unpublished).

Calculations. At the end of the experiment, ADG, ADFI, and G:F was determined for each experimental pen as in Exp. 1. Percent of total ash, Ca, and P in each bone was calculated as was the percentage of Ca and P in the bone ash.

Statistical analysis. Data were analyzed using the PROC MIXED procedure in SAS. A contrast statement was used to analyze differences between the positive and the negative control diet. Linear and quadratic contrasts were used to analyze the effects of adding either Quantum phytase or Natuphos to the negative control diet. In the analysis of bone concentrations of ash, Ca, and P, a contrast statement was used to compare data for the positive control diet with the diets containing either Quantum phytase or Natuphos. The effects of using Quantum phytase were also contrasted against the effects

of using Natuphos. An outlier test was conducted using PROC UNIVARIATE of SAS. No outliers were identified in the growth data; however, 2 outliers were removed in the bone data. The pen was the experimental unit for all performance analyses, and individual bones were the experimental unit for all bone analyses. An alpha value of 0.05 was used to assess significance among means.

RESULTS

Experiment 1

The results from Exp. 1 are summarized in Table 5. The analyzed concentrations of Ca and P were close to expected values. The ADG was lower ($P < 0.05$) in the negative control diet than in the positive control diet from d 15 to 28, but not for the initial 14 d of the experiment or for the overall period. No differences were observed in the ADG between the positive control diet and the phytase supplemented diets during any of the experimental periods, but there was a tendency for a linear increase in ADG with increasing phytase inclusion in the diet ($P = 0.10$, 0.18, and 0.11 for d 0 to 14, d 14 to 28, and d 0 to 28, respectively).

During the initial 2 wk of the experiment, ADFI was similar for all treatment groups. During the following 2 wk, pigs fed the negative control diet tended ($P = 0.12$) to have a lower feed intake than pigs fed the positive control diet. Pigs fed the phytase containing diet had a lower ($P = 0.05$) ADFI from d 15 to 28 than pigs fed the positive

control diet. Likewise, for the entire 4-wk period, ADFI tended to be greater ($P = 0.12$) for the positive control diet compared with the other diets.

When comparing the positive and the negative control diets during the initial 2 wk of the experiment, the G:F ratio was similar. However, as Quantum phytase was added to the negative control diet, the G:F improved (linear, $P < 0.01$). During the final 2 wk of the experiment, G:F was lower ($P < 0.01$) in pigs fed the negative control diets compared with pigs fed the positive control diet, but pigs fed the Quantum phytase supplemented diets had a G:F ratio that was similar to pigs fed the positive control diet. For the entire experimental period, G:F for the negative control diet tended ($P = 0.06$) to be lower than for the positive control diet. However, G:F increased (linear, $P < 0.05$) with increasing inclusion of Quantum phytase to the negative control diet. By adding Quantum phytase to this diet, values for G:F were similar to the G:F for the positive control diet.

Experiment 2

No differences in pig BW, ADG, or ADFI between pigs fed the positive control diet or the negative control diet were observed (Table 6). However, pigs fed the positive control diet tended to have increased final BW, ADG in phase 2, ADG overall, and G:F ratio in phase 1($P = 0.075$). Feeding either Quantum phytase or Natuphos phytase had no effect on pig weights at d 28, but at d 56, pigs fed diets containing either Quantum

phytase or Natuphos had a greater (linear, $P < 0.05$) weight than pigs fed the negative control diet.

The ADG did not differ among treatment groups during the initial 28 d of the experiment, but the addition of Quantum phytase or Natuphos increased (linear, $P < 0.05$) ADG during phase 2 and during the entire experimental period. Average daily feed intake was increased (linear, $P < 0.05$) by the addition of Natuphos to the negative control diet during phase 1. However, in phase 2 and overall, no effects of Natuphos on ADFI were observed. Pigs fed diets containing Quantum phytase did not increase ADFI during phase 1, but in phase 2 and overall, ADFI increased ($P < 0.05$) by the addition of Quantum phytase. Dietary treatments had no effect on G:F for phase 1, phase 2, or overall for the entire period. When comparing performance of pigs fed diets containing Quantum phytase with pigs fed Natuphos, no differences were observed.

Pigs fed the positive control diet had greater ($P < 0.001$) bone weight (8.4 g vs. 6.8 g), ash weight (3.35 g vs. 2.36 g), and percent ash in bones (40.1 vs. 35.2%) than pigs fed the negative control diet (Table 7). When comparing the amount of P present in the bone, the positive control diet contained more ($P < 0.001$) P in the bone (0.567 g vs. 0.399 g) and had a greater ($P < 0.001$) percent of P in the bone (6.76 vs. 5.96%) than pigs fed the negative control diet. Pigs fed the positive control diet also had a greater ($P < 0.001$) content of Ca in the bone (1.24 g vs. 0.88 g). As a percent of ash, P and Ca did not differ between the positive and negative control diets.

Total bone weight was not different among pigs fed the positive control diet and pigs fed the diets containing Quantum phytase. However, bones from pigs fed the positive control diet contained more ash and had a greater percent of bone ash than pigs fed diets containing Quantum phytase ($P < 0.001$). The amount of P and the percent of P present in bones from pigs fed the positive control diet was greater ($P < 0.001$) than in bones from pigs fed diets containing Quantum phytase. However, when expressed as a percent of ash, there was no difference among the positive control diet and the diets containing Quantum phytase. Although there was no difference in the percent of Ca in the ash among the positive control diet and diets containing Quantum phytase, pigs fed the positive control diet had a greater ($P < 0.001$) quantity of total Ca and a greater ($P < 0.001$) percent of Ca in the bone compared with pigs fed diets containing Quantum phytase.

Pigs fed diets containing Natuphos had a lower ($P < 0.01$) total bone weight, total ash weight, percent of ash in the bone, and percent of P in the bone than pigs fed the positive control diet. However, there was no difference among the positive control diet and Natuphos diets when P was determined as a percent of ash. Total Ca in the bone and percent of Ca in the bone was also greater ($P < 0.001$) for the positive control diet than for the diets containing Natuphos. However, percent of Ca in the ash were lower ($P < 0.01$) for pigs fed the positive control diet compared with pigs fed diets containing Natuphos.

Total bone weight and total ash weight increased (linear and quadratic, $P < 0.01$) as increasing levels of Quantum phytase were included in the diets. Pigs fed the negative control diet had an average of 2.36 g of total bone ash while pigs fed diets containing 250, 450, and 650 U/kg of Quantum phytase had an average of 2.88, 3.12, and 2.96 g of total bone ash, respectively. The addition of Quantum phytase to the negative control diet also increased (linear, $P < 0.01$) the percent of bone ash. The amount of P present in the bones increased (linear and quadratic, $P < 0.001$) and the amount of P as a percent of bone increased (linear, $P < 0.05$) as increasing levels of Quantum phytase were included in the diet. Total Ca in bones was 0.88 g for the negative control and increased (linear and quadratic, $P < 0.001$) to 1.15 g in pigs fed 450 U/kg of Quantum phytase. The percent of Ca in the bone increased (linear, $P < 0.01$) from 13.16% in the negative control diet to 14.00% in the diet containing 650 U/kg of Quantum phytase. The percent of P and the percent of Ca present in the ash were not affected by the addition of Quantum phytase.

Pigs fed diets containing Natuphos increased (linear, $P < 0.001$) bone weight from 6.8 g in the negative control to 8.0 g in the diet containing 650 U of Natuphos. Total ash weight and percent of total ash also increased (linear, $P < 0.001$) in pigs fed diets containing Natuphos. Total P in the bone was 0.4 g in pigs fed the negative control diet. This value increased (linear, $P < 0.001$) in pigs fed diets containing Natuphos. Similarly, percent of P in bones increased (linear, $P < 0.001$) for diets containing Natuphos. Pigs

fed the diets containing Natuphos increased total Ca in the bone and percent Ca in the bone increased from 13.16% to 14.66% (linear, $P < 0.001$). No effect of Natuphos was observed for percent of P or Ca in the ash.

Total bone weight and total ash weight were greater ($P < 0.05$) for pigs fed diets containing Quantum phytase than for pigs fed diets containing Natuphos, but there was no difference in the percentage of ash in the bone between pigs fed Natuphos and Quantum phytase. Pigs fed diets containing Quantum phytase tended ($P = 0.053$) to have more P in the bone, but the percent of P in the bone and in the ash did not differ among pigs fed the 2 phytases. Total Ca in the bone did not differ between pigs fed either Quantum phytase or Natuphos, but pigs fed diets containing Natuphos tended to have a greater percent of Ca in the bone than pigs fed diets containing Quantum phytase ($P = 0.054$). Calcium as a percent of bone ash was greater ($P < 0.001$) in pigs fed the Natuphos diets than for pigs fed the Quantum phytase diets.

DISCUSSION

Adding Quantum phytase to weanling pig diets will make more P available for the pigs. The linear effect of Quantum phytase in nursery diets indicates that Quantum phytase will continue to release phytate bound P with increasing levels of phytase. In Exp. 1, the lack of a quadratic response to increasing inclusions of Quantum phytase suggests that the optimal inclusion level of Quantum phytase may be greater than 1500

U/kg. This response concurs with Veum et al. (2006) who reported that when Quantum phytase was fed at 0, 100, 500, 2500, or 12500 U/kg to weaned pigs, linear and quadratic responses in growth performance were observed indicating that the maximal inclusion level in weanling pigs is between 2500 and 12500 U/kg. In the present experiment, it was demonstrated that adding Quantum phytase can be as effective as adding inorganic P. These results concur with Jendza et al. (2005) who reported that pig diets supplemented with 500 or 1,000 U of an *E. coli*-derived phytase (Phyzyme XP, Danisco Animal Nutrition, Marlborough, U.K.) had a linear increase of both ADG, G:F ratio, and final BW in the starter phase.

Quantum phytase added to the diets of growing pigs can also be beneficial. The comparison of the positive control diet to the Quantum phytase diets show that the addition of phytase can restore pig performance by making adequate amounts of P available. The lack of an effect of dietary treatment on G:F indicates that the energy present in the feed is not influenced by the inclusion of phytase to the diet. This concurs with research in weanling pigs and finishing pigs. When Quantum phytase was fed in the weanling phase, apparent energy absorption increased due to increased feed intake (Veum et al., 2006). In the finishing phase, there was no difference in energy digestibility between diets supplemented and not supplemented with Quantum phytase (Azain and Bedford, 2004). The linear response of the addition of Quantum phytase in Exp. 2 indicates that the inclusion level of Quantum phytase to maximize bone tissue

synthesis is greater than 650 U/kg. However, the quadratic response in other bone parameters and pig growth indicates that the optimal inclusion level may be approximately 450 U/kg. Other researchers found that the inclusion of Quantum phytase between 100 and 12,500 U/kg in diets fed to growing pigs improved growth rate and bone mineral characteristics (Azain and Bedford, 2004).

In bone tissue, P and Ca will be deposited in the bone at constant ratios, which was demonstrated by the lack of a treatment response on the percentage of P and Ca present in bone ash. The lack of a negative response in pig growth performance from the removal of inorganic P from the diet may be a result of the pigs fed the negative control diet were able to release P from their bones to maintain P levels in the soft tissue. This is indicated by the lower P concentrations in the bone.

Results of the present experiment are in agreement with data obtained when diets containing Quantum phytase were fed to broilers and turkeys. Supplementing 2500 U/kg of Quantum phytase to low-P diets fed to broilers increased performance to that of the broilers fed a 4 phase positive control diet containing 0.45, 0.39, 0.34, and 0.30% available P (Wyatt et al., 2004). Wyatt et al. (2004) also determined that the inclusion of phytase in diets low in P will restore bone ash levels at or above levels expressed in a positive control diet. Broiler chicks deposited more tibia ash and had a greater bone mineral content and density when supplemented with 1 of 3 different *E. coli*-derived phytases when compared to a low-P diet (Onyango, et al., 2004).

The addition of Natuphos to low-P diets also improved bone mineral concentrations. The linear response of bone mineral concentrations suggests that Natuphos is effective in promoting bone tissue synthesis. Lack of a quadratic response to the inclusion of Natuphos indicates that the maximal inclusion of Natuphos was not determined in this experiment. Although the addition of Natuphos to low-P diets improves bone mineral concentrations, when comparing the Natuphos diets to a diet adequate in P, bone mineral concentrations did not improve.

Results from the present experiment also concur with previous research using Natuphos in low-P diets fed to pigs. Percent of bone ash concentration increased linearly in growing pigs fed diets supplemented with Natuphos (Yi et al**,** 1996). Harper et al. (1997) showed that when low-P diets were supplemented with various levels of Natuphos to growing pigs, pig performance and bone ash concentration increased.

Pigs fed the diets containing Quantum phytase had greater bone weights and contained more bone ash than pigs supplemented with Natuphos indicating that Quantum phytase promoted more bone tissue synthesis. Augspurger et al. (2003) reported similar results when comparing an *E. coli*-derived phytase to Natuphos and Ronozyme phytase fed to young chicks. However, when comparing pigs fed diets with *E. coli*-derived phytases or fungal-derived phytases, the differences in bone ash were smaller (Augspurger et al., 2003).

IMPLICATIONS

The results from this research showed that Quantum phytase can be added to low P diets fed to weanling and growing pigs as an alternative to inorganic P without any negative effects on performance. Based on pig performance, Quantum phytase can be added to grower diets at approximately 450 U/kg for optimal performance. The effect of using Quantum phytase is similar to or better than the effects of using Natuphos. To determine the effects of low-P Quantum supplemented diets on P excretion, additional research is warranted.

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Table 3.1. Composition (%) of experimental diets (as-fed basis) fed to weanling pigs (Exp. 1)

Item Diet:	Positive control	Negative control	Quantum phytase, units/kg		
			500	1000	1500
Ingredients, %					
Corn	47.29	47.29	47.29	47.29	47.29
Whey, dried	15.00	15.00	15.00	15.00	15.00
Soybean meal, 44%	32.00	32.00	32.00	32.00	32.00
Cornstarch	-	0.75	0.74	0.73	0.72
Quantum phytase	-	-	0.01	0.02	0.03
Soy bean oil	3.00	3.00	3.00	3.00	3.00
Limestone	1.00	1.00	1.00	1.00	1.00
Monocalcium phosphate	0.75	-	-	-	-
L-Lysine, HCL	0.23	0.23	0.23	0.23	0.23
DL-Methionine	0.06	0.06	0.06	0.06	0.06
L-Threonine	0.07	0.07	0.07	0.07	0.07
Salt	0.40	0.40	0.40	0.40	0.40
Vitamin premix[1]	0.05	0.05	0.05	0.05	0.05
Micromineral premix[2]	0.15	0.15	0.15	0.15	0.15

Total	100	100	100	100	100

[1] Provided the following quantities of vitamins per kilogram of complete diet: vitamin A, 10,990 IU as vitamin A acetate; vitamin D_3, 1,648 IU as D-activated animal sterol; vitamin E, 55 IU as alpha tocopherol acetate; vitamin K_3, 4.4 mg as menadione dimethylpyrimidinol bisulphite; thiamin, 3.3 mg as thiamine mononitrate; riboflavin, 9.9 mg; pyridoxine, 3.3 mg as pyridoxine hydrochloride; vitamin B_{12}, 0.044 mg; D-pantothenic acid, 33 mg as calcium pantothenate; niacin, 55 mg; folic acid, 1.1 mg; and biotin, 0.17 mg.

[2] Provided the following quantities of minerals per kilogram of complete diet: Cu, 16 mg as copper sulfate; Fe, 165 mg as iron sulfate; I, 0.36 mg as potassium iodate; Mn, 44 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 165 mg as zinc oxide.

Table 3.2. Nutrient composition of experimental diets (as-fed basis)[1] fed to weanling pigs (Exp. 1)

Item Diet:	Positive control	Negative control	Quantum phytase, units/kg		
			500	1000	1500
DM, %	86.45	86.50	86.60	86.73	86.56
Mcal ME/kg	3,365	3,395	3,395	3,395	3,395
CP, %	19.8	19.8	19.8	19.8	19.8
Ca, %	0.80	0.69	0.55	0.56	0.62
P, %	0.60	0.45	0.40	0.40	0.45
Relative available P[2], %	0.32	0.18	0.18	0.18	0.18
Phytase, units/kg	32	37	519	995	1579
Indispensable AA[3]					
Arg	1.15	1.15	1.15	1.15	1.15
His	0.52	0.52	0.52	0.52	0.52
Ile	0.76	0.76	0.76	0.76	0.76
Leu	1.53	1.53	1.53	1.53	1.53
Lys	1.19	1.19	1.19	1.19	1.19
Met	0.33	0.33	0.33	0.33	0.33
Met + cys	0.68	0.68	0.68	0.68	0.68
Phe	0.83	0.83	0.83	0.83	0.83

Thr	0.74	0.74	0.74	0.74	0.74
Trp	0.22	0.22	0.22	0.22	0.22
Val	0.80	0.80	0.80	0.80	0.80

[1] Values for DM, Ca, P, and phytase were analyzed. All other values were calculated (NRC, 1998).

[2] Effects of microbial phytase disregarded in the diets containing Quantum phytase.

[3] Standardized ileal digestible basis.

Table 3.3. Ingredient composition of diets (as-fed basis)[1] fed to growing pigs (Exp. 2)

Phase:	1		2	
Item Diet:	1	2 - 8 [2]	1	2 - 8 [2]
Ingredient, %				
Corn	70.87	74.25	77.34	80.67
Soybean meal, 44%	23.50	23.50	17.00	17.00
Soybean oil	3.00	0.40	3.00	0.45
Limestone	1.05	0.92	1.03	0.90
Monocalcium phosphate	0.85	0.20	0.90	0.25
L-Lysine HCL	0.15	0.15	0.15	0.15
Salt	0.40	0.40	0.40	0.40
Vitamin premix [2]	0.03	0.03	0.03	0.03
Micro mineral premix [3]	0.15	0.15	0.15	0.15
Total	100	100	100	100

[1] Within each phase, diets 3, 4, and 5 were fortified with 250, 450, and 650 units/kg of Quantum phytase while diets 6, 7, and 8 were fortified with 250, 450, and 650 units/kg of Natuphos phytase.

[2] Provided the following quantities of vitamins per kilogram of complete diet: vitamin A, 6,594 IU as vitamin A acetate; vitamin D_3, 989 IU as D-activated animal sterol; vitamin E, 33 IU as alpha tocopherol acetate; vitamin K_3, 2.64 mg as menadione

dimethylpyrimidinol bisulphite; thiamin, 2.0 mg as thiamine mononitrate; riboflavin, 6.0 mg; pyridoxine, 2.0 mg as pyridoxine hydrochloride; vitamin B_{12}, 0.026 mg; D-pantothenic acid, 20 mg as calcium pantothenate; niacin, 33 mg; folic acid, 0.65 mg; and biotin, 0.10 mg.

[3] Provided the following quantities of minerals per kilogram of complete diet: Cu, 16 mg as copper sulfate; Fe, 165 mg as iron sulfate; I, 0.36 mg as potassium iodate; Mn, 44 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 165 mg as zinc oxide.

Table 3.4. Nutrient composition of experimental diets (as-fed basis) [1] fed to growing pigs (Exp. 2)

Phase:	1		2	
Item Diet:	1	2 - 8 [2,3]	1	2 - 8 [2,3]
DM, %[4]	87.87	87.73	87.46	87.40
ME, kcal/kg	3,420	3,320	3,437	3,337
CP, %	16.20	16.40	13.90	14.10
Ca, %[5]	0.64	0.48	0.62	0.46
P, % [6]	0.54	0.40	0.52	0.39
Relative available P, % [7]	0.23	0.11	0.23	0.11
Standardized digestible AA, %				
Arg	0.94	0.94	0.76	0.77
His	0.39	0.39	0.33	0.34
Ile	0.58	0.59	0.48	0.49
Leu	1.35	1.37	1.21	1.24
Lys	0.85	0.85	0.70	0.70
Met	0.24	0.24	0.21	0.21
Met + cys	0.56	0.57	0.44	0.45
Phe	0.70	0.70	0.60	0.61
Thr	0.52	0.52	0.44	0.44

Trp	0.16	0.16	0.13	0.13
Val	0.66	0.66	0.56	0.57

[1] Calculated from NRC (1998).

[2] Within each phase, diets 3, 4, and 5 were fortified with 250, 450, and 650 units/kg of Quantum phytase while diets 6, 7, and 8 were fortified with 250, 450, and 650 units/kg of Natuphos phytase.

[3] Analyzed phytase activity values (FTU/kg) in phase 1 and phase 2 respectively, were: diet 1, 90 and 100; diet 2, 100 and 100; diet 3, 275 and 487; diet 4, 547 and 480; diet 5, 785 and 643; diet 6, 490 and 530; diet 7, 700 and 850; diet 8, 850 and 730.

[4] Analyzed values (%) of moisture in phase 1 and phase 2 respectively, were: diet 1, 87.87 and 87.46; diet 2, 87.11 and 87.50; diet 3, 87.91 and 87.06; diet 4, 87.32 and 87.80; diet 5, 86.99 and 87.19; diet 6, 87.85 and 87.75; diet 7, 88.37 and 87.04; diet 8, 88.58 and 87.47.

[5] Analyzed values (%) of Ca in phase 1 and phase 2 respectively, were: diet 1, 0.64 and 0.64; diet 2, 0.54 and 0.60; diet 3, 0.60 and 0.72; diet 4, 0.57 and 0.56; diet 5, 0.56 and 0.47; diet 6, 0.56 and 0.51; diet 7, 0.45 and 0.47; diet 8, 0.47 and 0.63.

[6] Analyzed values (%) of P in phase 1 and phase 2, respectively, were: diet 1, 0.51 and 0.49; diet 2, 0.38 and 0.36; diet 3, 0.37 and 0.38; diet 4, 0.37 and 0.35; diet 5, 0.38 and 0.36; diet 6, 0.36 and 0.35; diet 7, 0.36 and 0.35; diet 8, 0.35 and 0.35.

[7]Effects of microbial phytase disregarded in the diets containing Quantum or Natuphos phytase.

Table 3.5. Performance of weanling pigs fed diets without or with Quantum phytase (Exp. 1) [1]

Item	Diet:	Positive control	Negative control	Quantum phytase, units/kg			Pooled SEM	*P*-value		*P* - value[2]	
				500	1000	1500		Positive vs. Negative	Positive vs. Phytase	Linear	Quadratic
Initial wt, kg		6.201	6.229	6.246	6.238	6.198	0.428	0.45	0.48	0.46	0.37
Wt after 14 d, kg		8.432	8.169	8.453	8.384	8.634	0.500	0.44	0.81	0.14	0.93
Final wt, kg		16.090	13.978	15.637	15.189	15.188	0.727	0.08	0.31	0.29	0.22
ADG, d 0-14, kg		0.157	0.139	0.158	0.153	0.174	0.014	0.27	0.82	0.10	0.95
ADG, d 15-28, kg		0.521	0.415	0.462	0.486	0.468	0.028	0.03	0.11	0.18	0.28
ADG, d 0-28, kg		0.341	0.277	0.310	0.320	0.321	0.018	0.27	0.23	0.11	0.42
ADFI, d 0-14, kg		0.191	0.178	0.189	0.175	0.188	0.016	0.44	0.68	0.73	0.95
ADFI, d 15-28, kg		0.785	0.669	0.709	0.722	0.697	0.042	0.12	0.05	0.58	0.41
ADFI, d 0-28, kg		0.484	0.423	0.445	0.449	0.443	0.026	0.12	0.12	0.59	0.51
G:F, d 0-14, kg/kg		0.829	0.786	0.836	0.901	0.932	0.052	0.39	0.15	0.004	0.77
G:F, d 15-28, kg/kg		0.696	0.623	0.656	0.673	0.675	0.024	0.01	0.36	0.14	0.53
G:F, d 0-28, kg/kg		0.722	0.658	0.694	0.714	0.728	0.025	0.06	0.72	0.04	0.63

[1] Values are means of six observations per treatment.

[2] Effect of phytase inclusion.

Table 3.6. Performance of growing pigs fed diets without or with Quantum phytase or Natuphos (Exp. 2)[1]

Item Diet:	Positive control	Negative control	Quantum, units/kg			Natuphos, units/kg			Pooled SEM
			250	450	650	250	450	650	
Initial wt, kg	18.243	18.458	18.604	18.083	18.083	18.313	18.250	18.438	0.832
Wt after 28 d, kg	37.542	36.313	38.222	37.833	37.000	37.646	37.625	37.438	1.555
Final wt, kg[abc]	60.924	57.854	62.125	63.521	59.521	62.021	62.042	60.646	2.083
ADG, d 0-28, kg	0.689	0.638	0.701	0.705	0.676	0.690	0.692	0.679	0.031
ADG, d 29-56, kg[abc]	0.835	0.769	0.854	0.917	0.804	0.871	0.872	0.829	0.030
ADG, d 0-56, kg[abc]	0.762	0.703	0.768	0.811	0.740	0.781	0.782	0.754	0.027
ADFI, d 0-28, kg[c]	1.468	1.426	1.514	1.527	1.466	1.507	1.504	1.492	0.067
ADFI, d 29-56, kg[b]	2.219	2.102	2.344	2.461	2.373	2.313	2.371	2.378	0.097
ADFI, d 0-56, kg[b]	1.843	1.764	1.929	1.994	1.919	1.910	1.938	1.935	0.076
G:F, d 0-28, kg/kg[a]	0.471	0.448	0.463	0.462	0.461	0.458	0.460	0.456	0.010
G:F, d 29-56, kg/kg	0.377	0.366	0.365	0.373	0.342	0.378	0.370	0.351	0.011
G:F, d 0-56, kg/kg	0.414	0.399	0.399	0.407	0.387	0.409	0.405	0.391	0.009

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

[a] Tendency for positive control to be greater then negative control ($0.05 < P < 0.10$).

[b] Linear effect of inclusion of Quantum phytase ($P < 0.05$).

[c] Linear effect of inclusion of Natuphos phytase ($P < 0.05$).

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Table 3.7. Effects of Quantum phytase and Natuphos on bone wt, bone ash, bone P, and bone Ca (Exp. 2)

Item	Total bone wt, g	Total ash, g	Total ash, %	Total P in bone, g	P, % of bone	P, % of ash	Total Ca in bone, g	Ca, % of bone	Ca, % of ash
Diet									
Positive control	8.4	3.35	40.1	0.567	6.76	16.85	1.24	14.85	37.06
Negative control	6.8	2.36	35.2	0.399	5.96	16.95	0.88	13.16	37.41
Quantum, 250 units/kg	8.1	2.88	36.1	0.483	6.08	16.80	1.07	13.43	37.14
Quantum, 450 units/kg	8.6	3.12	36.8	0.533	6.30	17.11	1.15	13.62	37.04
Quantum, 650 units/kg	8.0	2.96	37.7	0.500	6.34	16.82	1.10	14.00	37.12
Natuphos, 250 units/kg	7.5	2.71	36.5	0.458	6.17	16.89	1.02	13.76	37.65
Natuphos, 450 units/kg	7.8	2.82	36.8	0.483	6.31	17.15	1.06	13.76	37.48
Natuphos, 650 units/kg	8.0	3.07	38.7	0.518	6.54	16.92	1.16	14.66	37.89
Pooled SEM	0.571	0.173	1.393	0.030	0.255	0.121	0.213	0.065	0.540
P-values									
Positive vs. negative	<0.001	<0.001	<0.001	<0.001	<0.001	0.501	<0.001	<0.001	0.120

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Positive vs. Quantum	0.435	<0.001	<0.001	<0.001	<0.001	0.657	<0.001	<0.001	0.857
Positive vs. Natuphos	0.009	<0.001	<0.001	<0.001	0.002	0.312	<0.001	0.003	0.002
Quantum, linear	<0.001	<0.001	0.001	<0.001	0.010	0.903	<0.001	0.005	0.114
Quantum, quadratic	0.001	<0.001	0.926	<0.001	0.920	0.970	<0.001	0.677	0.254
Natuphos, linear	<0.001	<0.001	<0.001	<0.001	<0.001	0.700	<0.001	<0.001	0.137
Natuphos, quadratic	0.196	0.600	0.459	0.440	0.669	0.486	0.759	0.422	0.669
Quantum vs. Natuphos	0.009	0.034	0.385	0.053	0.308	0.422	0.186	0.054	<0.001

[1]Values are means for 24 observations per diet.

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CHAPTER 4

Identification of the Site of Activity of Quantum Phytase and Natuphos in Growing Pigs

ABSTRACT: An experiment was conducted to investigate the site of activity for an evolved *E. coli*-derived phytase (Quantum) in the intestinal tract of pigs and for a phytase derived from *Aspergillus niger* (Natuphos). Six barrows were fitted with a T-cannula in the duodenum and in the distal ileum. Pigs were allotted to a repeated 3 x 3 Latin square design. Three corn soybean meal-based diets were formulated. The control diet contained no inorganic P. The remaining 2 diets were similar to the control diet with the exception that they contained 500 phytase units per kg of either Quantum phytase or Natuphos. All diets contained 0.5% chromic oxide as an indigestible marker. Pigs were fed the experimental diets during three 9-d periods. The initial 4 d of each period was an adaptation period to the diet. Fecal samples were collected from each pig on d 5. On d 6 and 7, ileal digesta were collected continuously for 12 h. Duodenal digesta were collected on d 8 and 9 during three 2-h periods each day. Samples were stored at -20˚C after collection. Diets, duodenal, ileal, and fecal samples were analyzed for phytase activity, DM, Ca, P, phytate bound P, and concentrations of *myo*inositol hexaphosphate, *myo*inositol pentaphosphate, *myo*inositol tetraphosphate, *myo*inositol triphosphate, and *myo*inositol biphosphate, (IP6, IP5, IP4, IP3, and IP2, respectively). Apparent duodenal (ADD), ileal (AID), and total tract digestibility (ATTD) of Ca and P were calculated for each diet. The AID for AA was calculated as well and the degradation of phytase and

IP6, IP5, IP4, IP3, and IP2 in each segment of the GI-tract. Apparent duodenal digestibility of P was greater ($P < 0.05$) for the Quantum phytase diet compared with the control diet and the Natuphos diet. The AID and ATTD was greater ($P < 0.001$) in the 2 diets containing phytase than in the control diet. The diets containing phytase had lower ($P < 0.05$) concentrations of IP6 and IP5 in the duodenal digesta compared with the control diet indicating that there was phytase activity in the stomach. Total P concentration was lower ($P < 0.01$) in the ileal digesta and feces from pigs fed diets containing phytase than from pigs fed the control diet. The results of this experiment conclude that adding phytase to the diets of pigs can increase P digestibility and decrease total P concentration in the feces. Phytase from Quantum and Natuphos is active in the stomach and upper small intestine, and P-absorption takes place in the small intestine.

Key words: activity site, digestibility, phosphorus, phytase, pig

INTRODUCTION

Most of the P in corn grain and soybean meal fed to livestock is bound as *myo*-inositol hexaphosphate (**IP6**) or phytate (Nelson et al., 1968; O'Dell et al., 1972). Swine cannot utilize this P, and therefore, the P bound in phytate is excreted in the feces and may become a source of environmental pollution. With the addition of the enzyme phytase to the diet, some of the phytate-bound P becomes liberated to a yield free *myo*-inositol ring and inorganic ortho-phosphate. The P is available for the animal to absorb, thus reducing the amount of P excreted in the feces (Adeola et al., 2004, Veum et al., 2006). As the P is liberated, the phytate complex becomes dephosphorylated and the

intermediates *myo*-inositol pentaphosphate (**IP5**), *myo*-inositol tetraphosphate (**IP4**), *myo*-inositol triphosphate (**IP3**), *myo*-inositol biphosphate (**IP2**), *myo*-inositol monophosphate, and *myo*-inositol monophosphate are formed.

An evolved *Escherichia coli*-derived phytase, Quantum phytase (Syngenta Animal Nutrition, Research Triangle Park, NC) was recently developed and is now available to the swine industry in several countries. Quantum phytase is considered a 6-phytase, which implies it begins liberating P from the C6 atom of the inositol ring (Liu et al., 1998). Quantum phytase has been shown to effectively reverse the effects of slowed growth when inorganic P is removed from the diets of weanling and growing pigs (Azain and Bedford, 2004; Beaulieu et al., 2005; Veum et al., 2006). Presumably, this is due to the ability of Quantum phytase to improve the availability of organic P in cereal grains. However, the activity of the phytase and the breakdown of the phytate molecule in the digestive tract of pigs have not been investigated.

The objective of the present research was to identify the site of activity of Quantum phytase in the intestinal tract of pigs and to compare data for Quantum phytase to data obtained using a commercially available *Aspergillus niger* phytase product.

MATERIALS AND METHODS

Animals, Housing, and Experimental Design

Six growing barrows (initial BW 31.1 kg ± 6.4 kg) originating from the matings of SP-1 boars to Line 13 females (Ausgene Intl. Inc., Gridley, IL) were used in the experiment. Each pig was equipped with a simple T-cannula in the duodenum,

approximately 20 cm posterior to the pyloric sphincter, and another T-cannula in the distal ileum, approximately 10 cm anterior to the ileo - cecal valve.

Pigs were housed individually in 1.2 x 1.8 m pens with fully slatted metal floors. A feeder and a nipple drinker were installed in each pen. Pigs were allotted to a repeated 3 x 3 Latin square design. The animal was the experimental unit and each experimental period lasted 9 d.

Diets and Feeding

Quantum phytase and an *Apergillus niger*-derived phytase (Natuphos, BASF, Florham Park, NJ) were used in the experiment. Three diets were prepared (Tables 1 and 2). The control diet was a corn soybean meal-based diet. This diet did not contain any added inorganic P. The Quantum phytase diet was similar to the control diet with the exception that 0.02% of Quantum phytase was added to the diet. The Natuphos diet was also similar to the control diet with the exception that 0.01% of Natuphos was added to the diet. Both phytase products supplied 500 units of phytase to the diets they were used in. Vitamins and minerals were included in the diets to meet or exceed NRC requirements (NRC, 1998) with the exception that no inorganic P was added to the diets. All diets contained 0.5% chromic oxide as an indigestible marker. Diets were mixed in a 225 kg horizontal mixer. The phytase used in the phytase-containing diets was premixed into a corn carrier prior to mixing.

Pigs were fed at a daily level of 3 times their maintenance energy requirement (i.e., 106 kcal ME/$kg^{0.75}$; NRC, 1998) in 2 equal meals at 0800 and 2000. Water was available at all times throughout the experiment.

Data Recording and Sample Collection

Pig BW were recorded at the beginning and at the end of each period and the amount of feed supplied each day was also recorded. The initial 4 d of each period was considered an adaptation period to the diet. On d 5, fecal grab samples were collected from each pig. On d 6 and 7, ileal digesta were collected continuously for 12 h from 0800 to 2000 as described by Stein et al. (1999). A 12-h collection schedule was followed to ensure ileal digesta were collected for the same time duration as duodenal digesta. Duodenal samples were collected in three 2-h periods on d 8 (i.e., from 0800 to 1000, from 1200 to 1400, and from 1600 to 1800) and on d 9 (i.e., from 1000 to 1200, from 1400 to 1600, and from 1800 to 2000). Samples of each diet were collected as well. All duodenal digesta, ileal digesta, and fecal samples were stored at -20^{o}C immediately after collection.

Chemical Analysis

At the conclusion of the experiment, duodenal digesta, ileal digesta, and fecal samples were thawed, mixed within animal and diet, lyophilized, and finely ground prior to chemical analysis. Diet samples were finely ground prior to analysis. Dry matter was determined in all samples (procedure 930.15, AOAC, 2005). Samples were prepared for Ca and P analysis by using a dry ash method (procedure 927.02; AOAC, 2005). The levels of Ca in the samples were determined using atomic absorption spectrophotometry (procedure 968.08; AOAC, 2005) and P levels were analyzed using a spectrophotometer at 650 nm (procedure 931.01; AOAC, 2005). Phytase activity in the diets and digesta samples from the control diet and the diet containing Natuphos were determined using

procedure 2000.12 (AOAC, 2005). Phytase activity in diet and digesta containing Quantum phytase were determined using SAN SOP Phytase In-Feed Assay Version 1.4 (Syngenta Animal Nutrition, unpublished). Total phytate bound P and IP6, IP5, IP4, IP3, and IP2 were determined using a HPLC procedure (Rounds and Nielsen, 1993). Samples were gently agitated for 2 h with HCL and a solution was extracted and filtered before being read on a HPLC (Agilent 1100 series; Agilent Technologies, Inc. Santa Clara, CA). These samples were also analyzed for chromium (Fenton and Fenton, 1979). Ileal digesta and diet samples were analyzed for CP and AA as well. Crude protein was measured according to procedure 984.13 (AOAC, 2005). Amino acids were analyzed using a Beckman Amino Acid Analyzer (Beckman Instrument Corp., Palo Alto, CA). Prior to analysis, samples were hydrolyzed for 24 h at 110˚C with 6 N HCL (procedure 994.12, alternative 3; AOAC, 2005). Methionine and Cys were determined as methionine sulfone and cysteic acid (procedure 994.12, alternative 1; AOAC, 2005). Tryptophan was determined by hydrolysis with NaOH for 22 h at 110˚C (procedure 988.15; AOAC, 2005).

Calculations and Statistical Analysis

The apparent duodenal (**ADD**), ileal (**AID**), and total tract (**ATTD**) digestibility of Ca and total P, and the AID of CP and AA were calculated using the direct approach (Eq. 1) and the changes in chromium concentration as outlined previously (Stein et al., 1999):

$$\text{ADD, AID, and ATTD} = 100 - [(\text{Nd}/\text{Nf}) \times (\text{Crf}/\text{Crd})] \times 100\% \quad [1]$$

where ADD is the apparent duodenal digestibility, AID is the apparent ileal digestibility, and ATTD is the apparent total tract digestibility all in %, Nd is the CP, AA, P, or Ca concentration in the digesta DM, Nf is the CP, AA, P, or Ca concentration in the feed DM, Crf represents the amount of chromium in the feed DM, and Crd represents the amount of chromium present in the digesta DM.

Myo-inositol phosphate (IP6 to IP2) concentrations were measured as a percentage of total P and as a percentage of DM in duodenal digesta, ileal digesta, and feces. The disappearance of *myo*-inositol phosphate (IP6 to IP2) prior to the small intestine, during the small intestine, and in the large intestine gave an indirect measure of the activity of phytase within each of the 3 segments of the GI-tract, while the disappearance of total P indicates where the P was absorbed in the GI-tract.

Data were analyzed using PROC MIXED in SAS (SAS Stat Inc., Cary, NC). Outliers were identified and removed using PROC UNIVARIATE in SAS. An analysis of variance was conducted with diet as the main effect and pig and period as random effects. If differences among means were detected, means were separated using the LSMeans statement and the PDIFF option in SAS. The pig was the experimental unit for all analyses. An alpha level of 0.05 was used to assess significance among means.

RESULTS

Dietary treatment had no effect on AID of CP and AA (Table 3). The ADD of P improved ($P < 0.05$) with the addition of Quantum phytase to the diet compared with the control diet, 14.74 vs. 0.98% (Table 4). However, the ADD of P was similar for pigs fed

the control diet and the diet containing Natuphos (2.28%). Apparent duodenal digestibility of Ca was 22.94, 28.90, and 24.49% for pigs fed the control, Quantum phytase, and Natuphos diets, respectively. These numbers were not different. Pigs fed the diets containing Quantum phytase and Natuphos had a greater ($P < 0.001$) AID for P (41.20 and 36.92%, respectively) than pigs fed the control diet (13.36%). Apparent ileal digestibility of Ca was greater ($P < 0.001$) for pigs fed the diet containing Quantum phytase (73.11%) than for pigs fed the control diet (57.33%) or the diet containing Natuphos (65.78%). Total tract digestibility of P was lower ($P < 0.001$) for pigs fed the control diet than for pigs fed the Quantum phytase diet or Natuphos diet (14.21 vs. 42.18 and 45.32%, respectively). The ATTD for Ca did not differ among diets (59.36, 61.37, and 51.47% for control, Quantum phytase, and Natuphos diets, respectively).

Phosphorus digestibility tended ($P = 0.064$) to increase from the duodenum to the ileum and the feces when pigs were fed the control diet. Calcium digestibility in the duodenum for pigs fed the control diet (22.94%) was lower ($P < 0.001$) than the AID and ATTD for Ca (57.33 and 59.36%, respectively). When pigs were fed the Quantum phytase diet, the AID and ATTD for P (41.20 and 42.18%) were greater ($P < 0.001$) than the ADD (14.74%). Likewise, the AID and ATTD for Ca were greater ($P < 0.01$) than the ADD (73.11 and 61.37 vs. 28.90%). When the Natuphos diet was fed, the AID and ATTD of P was greater ($P < 0.001$) than the ADD of P (36.92 and 45.32 vs. 2.28%, respectively). However, the AID for Ca (65.78%) was greater ($P < 0.01$) than the ATTD (51.47%), and ADD (24.29%).

The *myo*-inositol phosphate profile for each diet as a percent of total P in the diet, duodenal, ileal, and fecal samples are shown in Table 5. In the diets, most P was present as IP6. The percent of IP6 and IP5 in the duodenal digesta was greater ($P < 0.05$) for pigs fed the control diet than for pigs fed the other diets, but there were no differences between pigs fed the 2 phytase containing diets. The percent of IP4, IP3, and IP2 were similar regardless of dietary treatments.

Percent of IP6 and IP5 in the ileal digesta were greater ($P < 0.001$) for pigs fed the control diet than for pigs fed the phytase diets. However, the percent of IP4 was greatest ($P < 0.001$) if the Quantum phytase diet was fed, followed by the Natuphos diet, while the digesta from pigs fed the control diet contained the least amount of IP4. Ileal digesta from pigs fed the control diet and the diet containing Natuphos had a lower ($P < 0.001$) concentration of IP3 compared with the digesta from pigs fed the Quantum phytase diet. Dietary treatment did not affect the percent of IP2 in ileal digesta.

In feces, the percent of IP6 was similar regardless of dietary treatments. Likewise, no treatment effects were observed for the percent of IP5, IP4, or IP3 in the feces. However, the percent of IP2 was greater ($P < 0.05$) if pigs were fed the Quantum phytase diet, than if they were fed the control diet or the Natuphos diet.

Phytase activity, P content, and the *myo*-inositol phosphate concentrations in the diets and in duodenal, ileal, and fecal samples are shown in Table 6. In the diets, P content was similar among all diets and all diets had the same amount of P bound in the IP6 form.

In duodenal digesta, phytase activities were similar for the Quantum phytase and Natuphos diets, while the control diet had the least ($P < 0.001$) amount of phytase activity. Total P in duodenal digesta were similar among all diets. However, IP6 and IP5 contents were greater ($P < 0.05$) in the digesta from pigs fed the control diet compared with the digesta from pigs fed the diets containing phytase. The addition of either phytase had no effects on IP4, IP3, or IP2 contents in the duodenal digesta.

In ileal digesta, phytase activity tended ($P = 0.06$) to be least in the Quantum phytase diet and similar for the control diet and the Natuphos diet. Phosphorus content in the ileum was lower ($P < 0.001$) in ileal digesta from pigs fed the Quantum phytase and Natuphos diets compared with the control diet, and the digesta from these pigs also contained less ($P < 0.001$) IP6 than the digesta from pigs fed the control diet. The digesta from pigs fed the control diet and the Quantum phytase diet had similar IP5 content, but pigs fed the Natuphos diet had ileal digesta that contained more IP5 ($P < 0.01$). The digesta from pigs fed the Quantum phytase diet contained more ($P < 0.001$) IP4 than digesta from pigs fed the Natuphos diet, while pigs fed the control diet contained the least amount of IP4. Pigs fed the control diet and the Natuphos diet had similar IP3 content in the ileal digesta, but the ileal digesta from pigs fed the Quantum phytase diet contained less ($P < 0.001$) IP3 than digesta from pigs fed the other diets. The IP2 content was similar in all ileal digesta samples regardless of dietary treatment.

In the feces, the phytase activity was similar for pigs fed the control diet and the Natuphos diet, but pigs fed the Quantum phytase diet had a lower ($P < 0.001$) phytase activity than if the other 2 diets were fed. Fecal concentration of P was greater ($P =$

0.001) for pigs fed the control diet compared with the Quantum phytase and Natuphos diets. Fecal concentrations of IP6, IP5, IP4, and IP3 were not influenced by dietary treatments. Feces from pigs fed the Quantum phytase diet had a greater ($P < 0.05$) IP2 content compared with feces from pigs fed the control diet and the Natuphos diet.

DISCUSSION

Supplementing diets with phytase does not improve the AID of CP or AA and there was no difference in CP and AA digestibilities among the diets. Liao et al. (2005) reported that the addition of Natuphos improved AID and ATTD of CP and several AA (Arg, His, Phe, Thr, Val, Glu, Gly, and Ser) when added to wheat-soybean meal-canola meal diets, but not when it was added to corn-soybean meal, wheat-soybean meal, or barley-peas-canola meal diets. Currently, there is no published research examining the effects of Quantum phytase on AA digestibility. However, values from this experiment agree with those reported by Liao et al (2005). In a review article by Adeola and Sands (2003), it was concluded that when microbial phytase is added to the diet of growing pigs, AA digestibility does not consistently improve.

The ATTD value of P obtained from the control diet (14.21%) concur with the value of 17.4% that was obtained in a similar diet by Jendza et al. (2006). The addition of phytase improved apparent P digestibility in the stomach as indicated by the greater value for ADD for the Quantum phytase fed pigs. This effect continues in the small intestine where the AID for P is greater for the 2 phytase sources than for the control diet. The addition of Quantum phytase improved AID of P over the control diet by 27.84 percentage units while the addition of Natuphos improved AID of P by 23.56 percentage

units. However, differences in Ca digestibility among diets were only affected in the small intestine. Apparent duodenal digestibility and the ATTD of Ca were not different among treatments, but the addition of Quantum phytase or Natuphos improved the AID of Ca. Yi et al. (1996) reported that dietary phytase increased Ca absorption, which is in agreement with the results obtained for AID of Ca in the present experiment. One explanation for this response is that the addition of phytase increases the level of available P, and thus, the Ca:available P ratio narrows. Another explanation may be that the addition of phytase may release Ca from the phytate complex.

The present study indicates that there is a difference in effectiveness between the 2 sources of phytase. Quantum phytase has a greater AID of Ca and a greater ADD of P than Natuphos. One explanation for this is that Quantum phytase has a pH optimum of 4.5 (Onyango et al., 2005; Veum et al., 2006), whereas Natuphos has a pH optimum of 2.5 and 5.5 (Jongbloed et al., 1992; Augspurger et al., 2003). Therefore, the 2 phytases may elicit different responses.

Phosphorus absorption occurs in the small intestine. Jongbloed et al. (1992) reported there was minimal P absorption in the stomach with a greater digestibility of P for the small intestine. Rapp et al. (2001) reported there was no net absorption of P in the stomach and in the cranial 30 cm of the small intestine when a corn soybean meal-based diet, wheat-based diet, or Natuphos supplemented diet was fed to pigs. Thus, results from this experiment are in agreement with previous results.

The large intestine appears not to be a site of P absorption as indicated by the lack of a difference between AID and ATTD values, regardless of the diet being fed. This is

in agreement with Bohlke et al. (2005) who also reported that there is no difference between AID and ATTD values for P. The low ATTD for P in the control diet is caused by the fact that the majority of P is bound to the phytate complex. However, when phytase is added to the diet, some of the phytate bound P is released and becomes available for absorption in the small intestine. Therefore, it is not surprising that in the control diet, there is a greater concentration of IP6 than IP5, IP4, IP3, or IP2 in the duodenal samples. In the diets containing phytase, a greater percentage of IP2 was present in the duodenum, which indicates that P had been released from the phytate molecule during passage through the stomach. However, in the ileal samples from the control diet and the diet containing Natuphos, a greater proportion of IP6 was present. Conversely, in the diets containing Quantum phytase, a greater percentage of IP4 was found in the ileal samples. This indicates that Quantum phytase is more effective in liberating P from the phytate complex. One explanation for this response is that Quantum phytase is more active in the stomach and small intestine than Natuphos.

The main site of Ca digestibility appears to be the small intestine in all treatment groups, but the large intestine appears to have some influence on Ca digestibility. It is established that Ca is absorbed in the upper small intestine by active transport and to some extent in the lower small intestine and colon by passive absorption (Crenshaw, 2001). In pigs fed the control diet, no differences in Ca digestibility were observed from the small intestine to the large intestine. However, when Natuphos was fed, Ca digestibility decreased in the large intestine. Radcliffe et al. (2006) reported a similar reduction in Ca digestibility in the large intestine when a corn soybean meal based diet

containing 10.2% CP and 500 phytase units/kg of Natuphos was fed to growing pigs. The addition of Quantum phytase to the diet did not influence Ca digestibility in the large intestine.

As expected, Quantum phytase is degraded by the proteolytic enzymes in the stomach and small intestine. Values obtained from the control diet indicate that some phytase may be produced endogenously in the small intestine and in the large intestine. The phytase produced by microbes in the large intestine is capable of cleaving any remaining P from the inositol ring, which is the reason there is no difference in IP6 concentration among diets in the feces.

Previous research has determined that microbial exogenous phytase is most active in the stomach. Yi et al. (1996) determined that when pigs were fed a diet containing Natuphos, the stomach had more phytase activity (40 to 50%) than the upper small intestine (16 to 30%); only negligible amounts of activity was found by the end of the small intestine. The diet containing Quantum phytase showed a similar degradation in the small intestine; however, in the control diet and in the Natuphos diet phytase activity increased from the duodenum to the ileum.

Phytate bound P was degraded throughout the digestive tract. The diets contained a large percentage of P as IP6 and IP2. By adding Quantum phytase or Natuphos to the diet, the concentration of IP2 in duodenal samples increased because some of the phytate bound P was liberated yielding more IP2. At the end of the small intestine, concentrations of total P increased in all dietary treatments indicating that other nutrients have been absorbed. However, there was a difference in the IP profile among treatments

in ileal samples. Of the P in the control diet, 73.21% was bound as IP6. In the diet containing Quantum phytase, 43.74% was bound as IP4 while only 21.23% was bound as IP6. In the diet containing Natuphos, 39.46% was bound as IP6 followed by IP5, 12.98%. This trend is not continued through the large intestine. In the fecal samples, very little P is bound as IP6, IP5, IP4, IP3, or IP2. The reason for this observation is that the microbial phytases in the large intestine degraded any phytate entering the large intestine regardless of the form it was in.

IMPLICATIONS

The addition of phytase to the diet reduces the amount of P bound in the phytate complex and reduces the amount of P excreted in the feces. The addition of phytase also improves apparent Ca and P digestibility. Results from the present study indicate that Quantum phytase is more effective than Natuphos in releasing P in the stomach and upper small intestine, but over the entire GI- tract, no differences between the 2 phytases are observed.

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Table 4.1. Ingredient composition of experimental diets (as-is)

Ingredient	Diet:	Control	Quantum	Natuphos
Corn		70.05	70.05	70.05
Cornstarch		1.00	0.98	0.99
Soybean meal, 44%		24.00	24.00	24.00
Soybean oil		3.00	3.00	3.00
Limestone		0.85	0.85	0.85
Chromium oxide		0.50	0.50	0.50
Salt		0.40	0.40	0.40
Vitamin premix [1]		0.05	0.05	0.05
Micromineral premix [2]		0.15	0.15	0.15
Quantum phytase		-	0.02	-
Natuphos phytase		-	-	0.01
Total		100	100	100

[1] Provided the following quantities of vitamins per kilogram of complete diet: vitamin A, 10,990 IU as vitamin A acetate; vitamin D_3, 1,648 IU as D-activated animal sterol; vitamin E, 55 IU as alpha tocopherol acetate; vitamin K_3, 4.4 mg as menadione dimethylpyrimidinol bisulphite; thiamin, 3.3 mg as thiamine mononitrate; riboflavin, 9.9 mg; pyridoxine, 3.3 mg as pyridoxine hydrochloride; vitamin B_{12}, 0.044 mg; D-pantothenic acid, 33 mg as calcium pantothenate; niacin, 55 mg; folic acid, 1.1 mg; and biotin, 0.17 mg.

[2] Provided the following quantities of minerals per kilogram of complete diet: Cu, 16 mg as copper sulfate; Fe, 165 mg as iron sulfate; I, 0.36 mg as potassium iodate; Mn, 44 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 165 mg as zinc oxide.

Table 4.2. Analyzed composition of experimental diets, as-fed basis

Item	Diet: Control	Quantum	Natuphos
DM, %	87.17	87.45	87.06
Energy, Mcal ME/kg[1]	3,451	3,451	3,451
CP, %	15.67	14.56	14.86
Ca, %	0.49	0.49	0.48
P, %	0.31	0.31	0.31
ADF, %[1]	3.62	3.62	3.62
NDF, %[1]	9.77	9.77	9.77
Phytase, units/kg	50	572	720
Indispensable AA			
Arg	0.88	0.93	0.92
His	0.37	0.39	0.38
Ile	0.58	0.60	0.52
Leu	1.24	1.28	1.23
Lys	0.76	0.80	0.78
Met	0.31	0.32	0.30
Phe	0.68	0.71	0.69
Thr	0.53	0.56	0.56
Trp	0.17	0.16	0.14
Val	0.66	0.68	0.59
Dispensable AA			

Ala	0.73	0.76	0.75
Asp	1.35	1.41	1.41
Cys	0.29	0.27	0.26
Glu	2.44	2.54	2.56
Gly	0.59	0.61	0.61
Pro	0.88	0.87	0.86
Ser	0.64	0.68	0.75
Tyr	0.48	0.50	0.50

[1] Values were calculated (NRC, 1998) rather than analyzed.

Table 4.3. Apparent ileal digestibilities (%) of CP and AA[1]

Item Diet:	Control	Quantum	Natuphos	SEM	*P*-value
CP	74.9	71.7	71.3	1.70	0.301
Indispensible AA					
Arg	87.5	87.8	87.8	0.73	0.928
His	83.1	82.2	81.9	1.13	0.731
Ile	79.0	79.7	75.7	1.49	0.149
Leu	82.1	82.4	80.9	1.15	0.621
Lys	80.5	81.6	79.5	1.30	0.522
Met	84.2	85.8	82.8	0.99	0.123
Phe	81.7	82.2	81.2	1.09	0.792
Thr	67.6	68.8	67.6	1.59	0.805
Trp	81.2	78.1	75.7	1.94	0.181
Val	75.3	75.5	70.8	1.73	0.121
Mean	80.2	80.4	78.4	1.23	0.441
Dispensible AA					
Ala	75.1	75.7	74.4	1.63	0.843
Asp	78.6	79.2	78.5	1.16	0.888
Cys	75.8	72.0	71.6	1.82	0.245
Glu	83.7	84.7	83.1	1.30	0.647
Gly	55.0	57.3	58.8	2.59	0.611
Pro	75.8	68.5	75.2	3.14	0.154

Ser	75.7	76.3	78.2	1.25	0.361
Tyr	81.9	81.8	81.0	1.12	0.832
Mean	75.2	74.4	74.6	1.56	0.943
Mean, all AA	78.0	77.8	76.7	1.35	0.771

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

Table 4.4. Apparent digestibility (%) of P and Ca in duodenal, ileal, and fecal samples from pigs fed experimental diets[1]

Item Diet:	Control	Quantum	Natuphos	SEM	*P* - value
Duodenum					
P, %	0.98^{x}	14.74^{ya}	2.28^{xa}	3.2	0.020
Ca, %	22.94^{a}	28.90^{a}	24.49^{a}	5.3	0.461
Ileum					
P, %	13.36^{x}	41.20^{yb}	36.92^{yb}	3.2	< 0.001
Ca, %	57.33^{xb}	73.11^{zb}	65.78^{yc}	2.2	< 0.001
Total tract					
P, %	14.21^{x}	42.18^{yb}	45.32^{yb}	6.2	< 0.001
Ca, %	59.36^{b}	61.37^{b}	51.47^{b}	7.9	0.666
P					
SEM	4.6	4.0	2.6	-	-
P - value	0.064	< 0.001	< 0.001	-	-
Ca					
SEM	3.8	6.1	6.6	-	-
P - value	< 0.001	0.001	0.002	-	-

$^{x-z}$ Within a row, means without a common superscript letter differ ($P < 0.05$).

$^{a-c}$ Within a column, means without a common superscript letter differ ($P < 0.05$).

[1]Values are means for 6 observations per treatment.

Table 4.5. *Myo*-inositol phosphate profile[1] (% of total P) in experimental diets, duodenal, ileal, and fecal samples[2]

Item	Diet: Control	Quantum	Natuphos	SEM	*P* - value
Diet					
IP6, %	65.812	67.947	65.925	-	-
IP5, %	0.769	1.147	1.113	-	-
IP4, %	0	0	0	-	-
IP3, %	0	0	0	-	-
IP2, %	23.989	24.140	23.421	-	-
Duodenal samples					
IP6, %	23.399^{y}	0.202^{x}	3.241^{x}	5.63	0.021
IP5, %	9.333^{y}	$< 0.001^{x}$	0.918^{x}	2.51	0.042
IP4, %	2.314	< 0.001	< 0.001	1.09	0.254
IP3, %	0	0	0	-	-
IP2, %	15.747	20.968	15.204	3.42	0.463
Ileal samples					
IP6, %	73.207^{y}	21.229^{x}	39.461^{x}	8.52	< 0.001
IP5, %	6.485^{x}	6.677^{x}	12.979^{y}	1.05	< 0.001
IP4, %	0.000^{x}	43.737^{z}	6.946^{y}	1.26	< 0.001
IP3, %	0.000^{x}	5.444^{y}	0.782^{x}	0.38	< 0.001
IP2, %	0.719	1.302	0.360	0.43	0.248

Fecal samples					
IP6, %	1.438	0.535	0.897	0.38	0.273
IP5, %	0	< 0.001	0.062	0.03	0.208
IP4, %	0	0.030	< 0.001	0.02	0.333
IP3, %	0	0	0	-	-
IP2, %	< 0.001[x]	0.357[y]	< 0.001[x]	0.08	0.023

[x-z] Within a row, means without a common superscript letter differ ($P < 0.05$).

[1]*Myo*-inositol phosphate consists of *myo*-inositol hexaphosphate (IP6), *myo*-inositol pentaphosphate (IP5), *myo*-inositol tetraphosphate (IP4), *myo*-inositol triphosphate (IP3), and *myo*-inositol biphosphate (IP2).

[2] Values are means of 6 observations per treatment.

Table 4.6. Phytase activity, P, and *myo*-inositol phosphate[1] concentration (g kg DM^{-1}) in diets and in duodenal, ileal, and fecal samples collected from growing pigs fed a corn-soybean meal diet without phytase or with the addition of 500 units/kg of Quantum phytase or Natuphos[2]

Item Diet:	Control	Quantum	Natuphos	SEM	*P* - value
Diet					
Phytase activity, U/kg	50	572	790	-	-
P, g kg DM^{-1}	3.510	3.488	3.595	-	-
IP6, g kg DM^{-1}	2.650	2.710	2.722	-	-
IP5, g kg DM^{-1}	0.031	0.046	0.046	-	-
IP4, g kg DM^{-1}	0.000	0.000	0.000	-	-
IP3, g kg DM^{-1}	0.000	0.000	0.000	-	-
IP2, g kg DM^{-1}	0.966	0.963	0.967	-	-
Duodenal samples					
Phytase activity, U/kg	136^{x}	376^{y}	319^{y}	39.58	< 0.001
P, g kg DM^{-1}	3.396	3.243	3.333	0.104	0.515
IP6, g kg DM^{-1}	0.812^{y}	0.007^{x}	0.100^{x}	0.195	0.020
IP5, g kg DM^{-1}	0.303^{y}	$< 0.001^{x}$	0.028^{x}	0.082	0.035
IP4, g kg DM^{-1}	0.072	< 0.001	< 0.001	0.032	0.229
IP3, g kg DM^{-1}	0	0	0	-	-
IP2, g kg DM^{-1}	0.536	0.686	0.496	0.116	0.512
Ileal samples					
Phytase activity, U/kg	372	39	555	136	0.060

P, g kg DM^{-1}	10.673^{y}	7.398^{x}	7.836^{x}	0.488	< 0.001
IP6, g kg DM^{-1}	7.980^{y}	1.547^{x}	3.239^{x}	0.941	< 0.001
IP5, g kg DM^{-1}	0.686^{x}	0.484^{x}	1.027^{y}	0.099	0.005
IP4, g kg DM^{-1}	0^{x}	3.163^{z}	0.554^{y}	0.131	< 0.001
IP3, g kg DM^{-1}	0^{x}	0.390^{y}	0.065^{y}	0.030	< 0.001
IP2, g kg DM^{-1}	0.088	0.093	0.030	0.046	0.567
Fecal samples					
Phytase activity, U/kg	187^{y}	0.7^{x}	147^{y}	217	< 0.001
P, g kg DM^{-1}	25.087^{y}	17.695^{x}	20.407^{x}	1.297	0.004
IP6, g kg DM^{-1}	0.344	0.093	0.171	0.080	0.111
IP5, g kg DM^{-1}	0	0	0.012	0.005	0.201
IP4, g kg DM^{-1}	< 0.001	0.005	< 0.001	0.003	0.333
IP3, g kg DM^{-1}	0	0	0	-	-
IP2, g kg DM^{-1}	0^{x}	0.062^{y}	< 0.001^{x}	0.015	0.029

[x-z] Within a row, means without a common superscript letter differ ($P < 0.05$).

[1]*Myo*-inositol phosphate consists of *myo*-inositol hexaphosphate (IP6), *myo*-inositol pentaphosphate (IP5), *myo*-inositol tetraphosphate (IP4), *myo*-inositol triphosphate (IP3), and *myo*-inositol biphosphate (IP2).

[2]Values are means of 6 observations per treatment.

CHAPTER 5

Effects of a *Pichia*-expressed phytase on performance and P excretion of growing pigs

ABSTRACT: Two experiments were conducted to evaluate the effects of feeding a *Pichia*-expressed phytase, Quantum phytase, to growing pigs. In Exp. 1, 60 growing pigs (initial BW: 23 kg) were allotted to 3 treatments with 2 pigs per pen and 10 pen replicates per treatment. The positive control diet was a corn-soybean meal diet containing 1.0% dicalcium phosphate and 0.20% digestible P. The negative control diet and the Quantum phytase diets were similar to the positive control diet with the exception that only 0.32% dicalcium phosphate was used. The Quantum phytase diet contained 500 phytase units per kilogram of Quantum phytase and the concentration of digestible P was calculated at 0.10 and 0.20% in the negative control and the Quantum phytase diets, respectively. The experiment lasted 42 d. Pigs fed the positive control and Quantum phytase diets had greater ($P < 0.05$) ADG (0.92 and 0.91 vs. 0.82 kg/d), G:F ratio (0.41 and 0.43 vs. 0.37 kg/kg), and final BW (62.52 and 61.15 vs. 57.67 kg) than pigs fed the negative control diet. There were no differences between pigs fed the positive control and Quantum phytase diets. In Exp. 2, 9 barrows (initial BW: 22 kg) were placed in metabolism cages and allotted to three 3 x 3 Latin squares with 3 diets and 3 periods in each square. The 3 diets were similar to the diets used in Exp. 1. Urine and feces were collected for 5 d of each period. Pigs fed the Quantum phytase diet had a lower ($P < 0.001$) fecal P excretion

(7.63 g/5d) and a greater ($P < 0.01$) apparent total tract digestibility (ATTD) of P (62.46%) than pigs fed the positive control diet (11.57 g/5 d and 56.35%) or the negative control diet (11.73 g/5d and 41.85%). Fecal Ca output was lower ($P < 0.001$) for pigs fed the Quantum phytase diet than for pigs fed the positive control or negative control diets (6.48 vs. 7.62 and 9.96 g/5d). The ATTD for Ca in pigs fed the Quantum phytase (76.4%) or positive control (75.5%) diets were not different, but they were greater ($P < 0.001$) than the ATTD of Ca in negative control fed pigs (66.0%). The results confirm that a low-P, Quantum phytase containing diet supports pig performance to the same degree as a high-P diet, but pigs fed the Quantum phytase diet have a lower fecal excretion of P and Ca than pigs fed a high-P diet.

Key words: digestibility, performance, pig, phosphorus, phytase

INTRODUCTION

Only a small amount of P is available to pigs in corn-soybean meal-based diets (NRC, 1998) because most of the P in corn and soybean meal is bound to the phytate complex (Nelson et al., 1968; Reddy et al., 1982). Swine are incapable of cleaving the P bound to phytate because they do not produce the enzyme phytase. However, several microbial-derived phytases are available and can be added to diets for growing pigs to improve P digestibility in corn soybean meal-based diets (Azain and Bedford, 2004; Kies et al., 2006; Veum et al., 2006).

The addition of an *Escherichia coli*-derived phytase expressed in *Pichia* yeast (Quantum, Syngenta Animal Nutrition, Research Triangle Park, NC) to the diets for

growing pigs has been shown to be an effective method to lower P excretion in the feces (Beaulieu et al., 2005). Quantum phytase added to the diets of growing pigs has also been shown to improve apparent digestibility of total P in corn soybean meal-based diets deficient in P (Azain and Bedford, 2004).

Previous research completed at South Dakota State University (McGinnis et al., 2007a) has shown that the addition of Quantum phytase to a low-P diet fed to growing pigs can improve growth performance and bone mineral content when compared to the low-P diet without phytase. It has also been shown that the digestibility of P in a corn-soybean meal diet is improved from 14.3 to 42.2% if Quantum phytase is included in the diet (McGinnis et al., 2007b). It is, therefore, possible that the addition of Quantum phytase to the diet fed to growing pigs can reduce fecal excretion of P without impeding animal performance. Based on these results we hypothesized that low-P diets fortified with Quantum phytase will support pig performance to the same degree as high-P diets, but will lower the excretion of P. It was, therefore, the objective of these experiments to test this hypothesis.

MATERIALS AND METHODS

General Procedures

Two experiments were conducted. Pigs used in both experiments originated from the mating of SP-1 boars to Line 13 females (Ausgene Intl., Gridley, IL). Protocols for these experiments were reviewed and approved by the South Dakota State University Animal Care and Use Committee.

Experiment 1

Animals, Experimental Design, and Diets. A total of 60 growing pigs (initial BW 23.0 ± 2.2 kg) were randomly allotted to 3 treatments with 2 pigs per pen and 10 replicate pens per treatment (5 replicates with barrows and 5 replicates with gilts). Pigs were housed in an environmentally controlled building. Pens measured 1.2 x 2.4 m and had fully-slatted concrete floors. Each pen was equipped with a nipple drinker and a 1-hole feeder. Pigs had *ad libitum* access to feed and water throughout the experiment.

The positive control diet was formulated to meet current estimated nutrient requirements for growing pigs (NRC, 1998) and was based on corn, soybean meal, and 1.0% dicalcium phosphate (Table 1). The concentration of total P was 0.47% and the concentration of digestible P in the diet was calculated at 0.20%. Phosphorus in this diet originated from corn, soybean meal, and dicalcium phosphate. The amount of corn and soybean meal was similar to the quantities used by McGinnis et al. (2007b) and the apparent total tract digestibility (**ATTD**) of P in the corn-soybean meal mixture was assumed to be 14.21% as determined by McGinnis et al. (2007b). A digestibility value of 81% was used for dicalcium phosphate (Petersen and Stein, 2006). A negative control diet was also formulated. This diet was similar to the positive control diet with the exception that the inclusion of dicalcium phosphate was reduced to 0.32% and the P in this diet was calculated at 0.10% digestible P, assuming the same digestibility values for P in the corn-soybean meal mixture and in dicalcium phosphate as for the positive control diet. A third diet was formulated to be similar to the negative control diet, but 500 units

per kg of Quantum phytase was added to this diet. The ATTD value for the corn-soybean meal mixture used in the Quantum phytase diet was assumed to be 42.18% (McGinnis et al., 2007b), and the concentration of digestible P in this diet was calculated at 0.20%.

Data Collection and Chemical Analysis. Pigs were fed the experimental diets for 42 d. Individual pig BW was recorded at the start of the experiment, on d 14, d 28, and at the conclusion of the experiment. The daily allotment of feed was recorded as well. Orts were weighed back on d 14, d 28, and at the conclusion of the experiment.

Dry matter was determined in feed samples using procedure 930.15 (AOAC, 2005). Feed samples were prepared for Ca and P analysis using a dry ash method (procedure 968.08; AOAC, 2005). The concentration of Ca in all samples was determined using atomic absorption spectrophotometry (procedure 927.02; AOAC, 2005) and P concentrations were determined using an UV-vis spectrophotometer at 650 nm (procedure 931.01; AOAC, 2005). Phytase activity was analyzed in the positive and negative control diets using procedure 2000.12 (AOAC, 2005). In the diet containing Quantum, phytase activity was analyzed using SAN SOP Phytase In-Feed Assay Version 1.4 (Syngenta Animal Nutrition, unpublished).

Calculations. At the conclusion of the experiment, data for pig BW gains within each treatment group were summarized and the ADG for each phase and overall for the experiment was calculated. Likewise, daily feed allotments were summarized and ADFI for each pen was calculated. By dividing ADG by the ADFI, the average G:F ratios were calculated for each phase and overall.

Statistical Analysis. All data were analyzed using the PROC MIXED procedure of SAS (SAS Stat Inst. Inc., Cary, NC). Outliers were tested using the PROC UNIVARIATE procedure in SAS. No outliers were identified. The class statement included pen, replicate, sex, and diet. The initial model included the effects of diet, sex, and the interaction of diet x sex. However, there was no diet x sex interactions. Therefore, the final model included the main effects of diet and sex, while pen and replicate were used as random effects. Means were separated using the LSMeans statement and the PDIFF option in SAS. The pen was the experimental unit and an alpha value of 0.05 was used to assess significance among means.

Experiment 2

Animals, Experimental Design, and Diets. Nine growing pigs (initial BW: 23.6 ± 1.9 kg) were allocated to three, 3 x 3 Latin squares with 3 pigs and 3 periods in each square. Each period lasted 14 d. Pigs were housed individually in metabolism cages equipped with a feeder and a nipple drinker. Cages had expanded metal slatted floors, a screen based floor for total collection of fecal matter, and a tray for total urine collection. Room temperature was maintained at 22˚C.

The 3 diets used in Exp.1 were also used in this experiment. Feed was supplied to all pigs at a level of 3 times the estimated maintenance requirement for the smallest pig in each square (i.e., 106 kcal of ME/kg of $BW^{0.75}$; NRC, 1998). Daily rations were divided into 2 equal meals and fed at 0800 and 1600. Water was available at all times throughout the experiment.

Data Collection and Chemical Analysis. Pigs were weighed at the beginning and end of each period. The amount of feed supplied was recorded daily for each pig. Orts were collected and weighed before each feeding.

A 7-d adaptation period was used at the start of each period. On d 7 in the afternoon, 7 to 8 g of ferric oxide was added to the meal. Likewise, the afternoon meal on d 12 contained 7 to 8 g of chromic oxide. Appearance of these markers indicated commencement and conclusions of fecal collection, respectively. Urine collection began at the afternoon meal on d 7 and concluded at the afternoon meal on d 12. Urine was collected over a preservative of 50mL of 6 *N* sulfuric acid, which was added to the collection buckets. The buckets were emptied twice daily. For each collection, urine was weighed and mixed, and 20% of the total volume was stored at −20°C. All fecal collections were stored at -20°C. At the end of each period, urine samples were thawed and thoroughly mixed within each pig and a sub sample was taken for further analysis. All fecal samples were dried in a forced air oven and finely ground before chemical analysis. Feed and fecal samples were analyzed for DM, Ca, and P, and urine samples were analyzed for Ca and P. All analyses were conducted as described for Exp. 1.

Calculations. The ATTD of Ca and P was calculated for each diet using the following equation:

$$ATTD = ([Ni - Nf]/Ni) \times 100,$$

where ATTD is the apparent total tract digestibility (%), Ni is the total nutrient-intake from d 7 to d 12 of each experimental period in grams; and Nf is the total fecal output (g) of that nutrient (Petersen and Stein, 2006).

The retention of Ca and P was calculated for each pig and period using the following equation:

$$Nr = ([Ni - \{Nf + Nu\}]/Ni) \times 100,$$

where Nr is the nutrient retention (%), and Nu is the urinary output (g) of the nutrient from d 7 to d 12 (Petersen and Stein, 2006).

Statistical Analysis. All data were analyzed using the PROC MIXED procedure of SAS (SAS Stat Inc. Inst., Cary, NC.). The PROC UNIVARIATE procedure of SAS was used to identify outliers, but no outliers were identified. An analysis of variance was conducted and the main effects of diet were analyzed and pig and period were random variables. Means were separated using the LSMeans statement and the PDIFF option in SAS. The pig was the experimental unit and an alpha value of 0.05 was used to assess significance among means.

RESULTS

Experiment 1

Pigs fed the positive control diet and the Quantum phytase diet had a similar BW after 14 d (Table 3), but pigs fed the negative control diet had a lower ($P < 0.01$) BW than pigs fed the other 2 diets (36.05 for pigs fed the positive control diet and 35.37 for pigs fed the Quantum phytase diet vs. 34.19 kg for pigs fed the negative control diet). Likewise, pig BW after 28 d for pigs fed the positive control diet and the Quantum phytase diet was greater ($P < 0.01$) than for pigs fed the negative control diet (49.41 and 48.78 vs. 45.83 kg, respectively). Final pig BW was lower ($P < 0.05$) when pigs were fed

the negative control diet (57.67 kg) versus the positive or Quantum phytase diet, but there was no difference between pigs fed the positive control diet and the Quantum phytase diet (62.52 and 61.15 kg, respectively).

From d 0 to 14, pigs fed the positive control diet had a greater ($P < 0.001$) ADG (0.87 kg), than pigs fed the Quantum phytase diet (0.82 kg). However pigs fed the negative control diet had the lowest ($P < 0.05$) ADG during this period (0.76 kg). Average daily gain from d 15 to 28 was similar for pigs fed the positive control diet and for pigs fed the Quantum phytase diet, but it was lower ($P < 0.01$) when pigs were fed the negative control diet (0.96 and 0.96 vs. 0.83 kg, respectively). Pigs fed the positive control diet and the Quantum phytase diet also tended ($P = 0.094$) to have a greater ADG for d 29 to 42 than pigs fed the negative control diet (0.94 and 0.96 vs. 0.84 kg, respectively). Overall, pigs fed the positive control diet and the Quantum phytase diet had a greater ($P < 0.01$) ADG than pigs fed the negative control diet (0.92 and 0.91 vs. 0.82 kg).

Throughout the experiment, ADFI was similar for all diets. The G:F ratio for pigs fed the positive control diet and the Quantum phytase diet were similar from d 0 to 14, but greater ($P < 0.05$) than for pigs fed the negative control diet (0.48 and 0.48 vs. 0.44 kg). From d 15 to 28, there was a tendency ($P = 0.084$) for pigs fed the Quantum phytase diet to have a greater G:F ratio, than pigs fed the negative control diet (0.53 vs. 0.48), while pigs fed the positive control diet were intermediate between and not different from the other 2 diets (0.51). The G:F ratio was not different from d 29 to 42 among treatment groups. However, for the entire experiment, G:F was greater ($P < 0.05$) for pigs fed the

positive control diet and the Quantum phytase diet (0.41 and 0.43 kg) than for pigs fed the negative control diet (0.37 kg).

Barrows and gilts had similar final BW (61.67 and 59.23 kg, respectively). Gender did not have an effect on ADG from d 0 to d 14 (0.83 and 0.80 kg/d for barrows and gilts, respectively), but from d 15 to d 28, barrows gained more weight ($P < 0.05$) per day compared with gilts (0.97 vs. 0.85 kg). Likewise, from d 29 to 42 barrows gained 1.01 kg/d, which was greater ($P < 0.001$) than gilts whom gained 0.82 kg/d. Overall ADG from d 0 to d 42 was greater ($P < 0.05$) for barrows (0.94 kg) than for gilts (0.83 kg). Gender did not influence ADFI or G:F for any phase or for the entire experiment.

Experiment 2

Pigs consumed the same amount of feed throughout the experiment regardless of dietary treatment (Table 4). However, P intake was greatest ($P < 0.001$) when the positive control diet was fed (26.66 g/5 d) and similar when the negative control diet and the Quantum phytase diets were fed (20.21 and 20.40 g/5 d, respectively). Fecal P output was greater ($P < 0.001$) for pigs fed the positive control or the negative control diet than for pigs fed the Quantum phytase diet (11.57 and 11.73 vs. 7.63 g/5 d). Phosphorus absorption was similar when the control diet and the Quantum phytase diets were fed (15.09 and 12.78 g/5 d), but these values were greater ($P < 0.001$) than when the negative control diet was fed (8.48 g/5 d). The ATTD of P was greatest ($P < 0.001$) for the Quantum phytase diet (62.46%), intermediate for the control diet (56.35%), and least for the negative control diet (41.85%). Urine P output was similar when the negative control

diet and the Quantum phytase diet were fed (0.081 and 0.064 g/5 d, respectively), but these values were lower ($P < 0.001$) than when the positive control diet was fed (0.418 g/5 d). Phosphorus retention in grams was different ($P < 0.001$) among all 3 diets. It was greatest when pigs were fed the positive control diet, intermediate when pigs were fed the Quantum phytase diet, and least when pigs were fed the negative control diet (14.67, 12.71, and 8.40 g/5 d, respectively). However, when expressed as a percent, P retention was greatest ($P < 0.001$) when pigs were fed the Quantum phytase diet (62.14%), intermediate when pigs were fed the positive control diet (54.80%), and least when pigs were fed the negative control diet (41.45%).

Calcium intake differed ($P < 0.001$) among all treatments (31.38, 29.41, and 27.50 g/5 d for pigs fed positive control, negative control, and Quantum phytase diets, respectively). Fecal Ca output was greatest ($P < 0.001$) when pigs were fed the negative control diet, intermediate when pigs were fed the positive control diet, and least ($P < 0.001$) when pigs were fed the Quantum phytase diet (9.96 vs. 7.62 vs. 6.48 g/5 d, respectively). Calcium absorption was greatest ($P < 0.001$) when pigs were fed the positive control diet (23.76 g/5 d), intermediate when pigs were fed the Quantum phytase diet (21.03 g/5 d), and least ($P < 0.001$) when pigs were fed the negative control diet (19.46 g/5 d). The ATTD of Ca was similar when pigs were fed the positive control diet and the Quantum phytase diet, but lower ($P < 0.001$) when pigs were fed the negative control diet (75.45 and 76.37 vs. 66.00%). Urine Ca output was least ($P < 0.001$) when pigs were fed the positive control diet (1.28 g/5 d), intermediate when pigs were fed the Quantum phytase diet (4.73 g/5 d), and greatest ($P < 0.001$) when pigs were fed the

negative control diet (6.44 g/5 d). Calcium retention in grams was greatest ($P < 0.001$) when pigs were fed the positive control diet (23.48 g/5 d), intermediate when pigs were fed the Quantum phytase diet (16.30 g/5 d), and least ($P < 0.001$) when pigs were fed the negative control diet (13.02 g/5 d). Similarly, Ca retention expressed as a percent was different ($P < 0.001$) among all dietary treatments (71.60 vs. 59.42 vs. 44.12% for pigs fed the positive control, negative control, and Quantum phytase diets, respectively).

DISCUSSION

Experiment 1

The current experiment confirms that the addition of Quantum phytase to growing pig diets can be used to reduce the amount of inorganic P added to the diet without reducing pig performance. The differences obtained between the negative control diet and the Quantum phytase diet indicates that Quantum phytase can replace some of the inorganic P in the diet. With the exception of ADG from d 0 to 14, pigs fed the positive control diet and pigs fed the Quantum phytase diet had similar performance and for the entire 42-d experimental period, no differences between the positive control and Quantum phytase diets were observed. However, pigs fed the negative control diet had reduced performance. The reduction in performance for pigs fed the negative control diet as compared with pigs fed the positive control diet demonstrate that the negative control diet did not provide enough P for maximum performance. However, this situation was ameliorated as Quantum phytase was added to the negative control diet. This indicates that Quantum phytase was able to efficiently release an adequate amount of P from

phytate to maximize pig performance. The G:F obtained in this experiment was similar to the G:F obtained in previous research using Quantum phytase for growing pigs (McGinnis et al., 2007a). The lack of a dietary treatment effect for ADFI indicates that feed intake was not affected by the addition of Quantum phytase or a reduction in total P. However, previous research indicates that ADFI may be reduced in low-P diets and increases when phytase is added to the diet (Adeola et al., 2004; Jendza et al., 2005).

The addition of Quantum phytase to the diet did not influence gender differences in performance. This observation concurs with Augspuger et al. (2007) who reported no diet by sex interaction when phytase was added to the diet and that barrows grew faster and consumed more feed than gilts. Results of the present experiment also agree with this observation.

Results from this experiment concur with other experiments in which Quantum phytase was used. Increasing the level of Quantum phytase in low-P diets resulted in linear and quadratic effects for ADG, ADFI, and G:F (Azain and Bedford, 2004; Veum et al., 2006) Previous research in our lab has shown that pigs fed Quantum phytase had linear increases in final BW, ADG, and ADFI (McGinnis et al., 2007a).

Experiment 2

The addition of Quantum phytase to the diet increased the amount of P that was released from the phytate complex, which in turn increased P-absorption. This is indicated by the reduced amount of P excreted in the feces from pigs fed the Quantum phytase diet compared with pigs fed the positive control diet or the negative control diet.

This observation concurs with Beaulieu et al. (2005) who also reported a reduction in total and water soluble inorganic fecal P when Quantum phytase was added to diets fed to weanling pigs. Pigs fed the Quantum phytase diet also absorbed a similar amount of P as pigs fed the positive control diet despite the reduced P-concentration in the Quantum phytase diet. This observation indicates that it is possible to reduce dietary P-concentration without reducing P-absorption by including Quantum phytase in the diet. Within diets, the ATTD of P and the percent of P retention are similar indicating that all the P absorbed was retained.

Calcium intake was different for all dietary treatments due to the different Ca content in the diets. The ATTD of Ca for the positive control diet and the Quantum phytase diet were greater than for the negative control diet because of the increased absorption and retention of P in these diets. This response is expected because bone synthesis requires both Ca and P, and with less P being absorbed by pigs fed the negative control diet, less Ca was needed for bone synthesis. This observation agrees with recent data from Stein et al. (2006) showing that pigs fed a P-free diet had lower ATTD and retention of Ca than pigs fed a diet that is adequate in P.

Calcium retention and urine output were greater for pigs fed the positive control diet compared with pigs fed the Quantum phytase diet and the negative control diet. Previous research in our lab also indicate that pigs fed a positive control diet had greater concentrations of total Ca and P in their bones compared with pigs fed a diet containing Quantum phytase (McGinnis et al., 2007a). Therefore it can be expected that pigs fed the positive control diet will retain more Ca and P for bone tissue synthesis.

IMPLICATIONS

The addition of Quantum phytase to low-P diets improves P absorption, retention, and ATTD. Quantum phytase also reduces the amount of P excreted in the feces compared with low-P diets and diets supplemented with inorganic P. The low-P diet supplemented with Quantum phytase did not have negative effects on pig performance compared with pigs fed the diet containing adequate amounts of inorganic P. It can, therefore, be concluded that the addition of Quantum phytase to the diet can decrease the amount of inorganic P needed in the diets without impeding maximum pig performance.

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Table 5.1. Ingredient composition of experimental diets (as-fed basis)

Item	Diet:	Positive control	Negative control	Quantum phytase
Ingredients, %				
Corn		70.05	70.05	70.05
Cornstarch		0.60	0.83	0.81
Soybean meal, 44%		24.00	24.00	24.00
Soybean oil		3.00	3.00	3.00
Limestone		0.60	1.05	1.05
Dicalcium phosphate		1.00	0.32	0.32
L-lysine HCL		0.15	0.15	0.15
Salt		0.40	0.40	0.40
Vitamin premix [1]		0.05	0.05	0.05
Micromineral premix [2]		0.15	0.15	0.15
Quantum phytase		-	-	0.02
Total		100	100	100

[1] Provided the following quantities of vitamins per kilogram of complete diet: vitamin A, 10,990 IU as vitamin A acetate; vitamin D_3, 1,648 IU as D-activated animal sterol; vitamin E, 55 IU as alpha tocopherol acetate; vitamin K_3, 4.4 mg as menadione dimethylpyrimidinol bisulphite; thiamin, 3.3 mg as thiamine mononitrate; riboflavin, 9.9 mg; pyridoxine, 3.3 mg as pyridoxine hydrochloride; vitamin B_{12}, 0.044 mg; D-

pantothenic acid, 33 mg as calcium pantothenate; niacin, 55 mg; folic acid, 1.1 mg; and biotin, 0.17 mg.

[2] Provided the following quantities of minerals per kilogram of complete diet: Cu, 16 mg as copper sulfate; Fe, 165 mg as iron sulfate; I, 0.36 mg as potassium iodate; Mn, 44 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 165 mg as zinc oxide.

Table 5.2. Chemical composition of experimental diets (as-fed basis)[1]

Item Diet:	Positive control	Negative control	Quantum phytase
DM, %	88.29	87.94	88.33
Energy, Mcal ME/kg	3479.31	3487.13	3486.45
CP, %	15.43	15.98	16.16
Ca, %	0.55	0.52	0.49
P, %	0.47	0.36	0.37
Digestible P, %	0.20	0.10	0.20
ADF, %	3.26	3.25	3.25
NDF, %	8.86	8.86	8.86
Phytase, units/kg	30	30	501
Amino Acids, %			
Arg	0.90	0.88	0.89
His	0.42	0.41	0.40
Ile	0.68	0.66	0.56
Leu	1.45	1.40	1.38
Lys	1.01	1.03	0.96
Met	0.25	0.23	0.23
Met + cys	0.52	0.47	0.48
Phe	0.77	0.75	0.73
Thr	0.56	0.54	0.57
Trp	0.14	00.13	0.13

Val	0.77	0.76	0.64

[1] Values for ME, digestible P, ADF, and NDF were calculated (NRC, 1998; Petersen and Stein, 2006; McGinnis et al., 2007b). All other values were analyzed.

Table 5.3. Performance of pigs fed experimental diets, Exp. 1[1]

Item	Treatment					Gender			
	Positive control	Negative control	Quantum phytase	SEM	*P* - value	Barrows	Gilts	SEM	*P*- value
Initial wt, kg	23.68	23.20	23.88	0.747	0.492	23.69	23.49	0.947	0.885
Wt after 14 d, kg	36.05^{y}	34.19^{x}	35.37^{y}	1.048	0.007	35.36	35.04	1.420	0.875
Wt after 28 d, kg	49.41^{y}	45.83^{x}	48.78^{y}	1.344	0.003	48.99	47.02	1.734	0.445
Final wt, kg	62.52^{y}	57.67^{x}	61.15^{y}	1.654	0.012	61.67	59.23	2.008	0.415
ADG, d 0-14, kg	0.87^{z}	0.76^{x}	0.82^{y}	0.030	< 0.001	0.83	0.80	0.040	0.505
ADG, d 15-28, kg	0.96^{y}	0.83^{x}	0.96^{y}	0.030	0.005	0.97	0.85	0.028	0.016
ADG, d 29-42, kg	0.94	0.84	0.96	0.037	0.094	1.01	0.82	0.030	< 0.001
ADG, d 0-42, kg	0.92^{y}	0.82^{x}	0.91^{y}	0.024	0.005	0.94	0.83	0.025	0.015
ADFI, d 0-14, kg	1.80	1.73	1.73	0.058	0.336	1.77	1.73	0.069	0.663
ADFI, d 15-28, kg	1.97	1.83	1.93	0.171	0.188	2.02	1.80	0.235	0.528
ADFI, d 29-42, kg	3.08	3.15	3.04	0.202	0.704	3.12	3.05	0.266	0.861

ADFI, d 0-42, kg	2.28	2.24	2.21	0.108	0.147	2.31	2.18	0.150	0.562
G:F, d 0-14, kg/kg	0.48[y]	0.44[x]	0.48[y]	0.011	0.015	0.47	0.46	0.011	0.460
G:F, d 15-28, kg/kg	0.51	0.48	0.53	0.047	0.084	0.51	0.51	0.065	0.968
G:F, d 29-42, kg/kg	0.31	0.28	0.32	0.019	0.100	0.33	0.28	0.022	0.164
G:F, d 0-42, kg/kg	0.41[y]	0.37[x]	0.43[y]	0.023	0.013	0.41	0.39	0.031	0.730

[x-z] Within a row, means without a common superscript letter differ ($P < 0.05$).

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

Table 5.4. Calcium and P balance and digestibility, Exp. 2[1]

Item Diet:	Positive control	Negative control	Quantum phytase	SEM	*P*-value
Feed intake, kg/5 d of DM	4.95	4.94	4.92	0.448	0.836
P intake, g/5 d	26.66^{y}	20.21^{x}	20.40^{x}	2.039	< 0.001
Fecal P output, g/5 d	11.57^{y}	11.73^{y}	7.63^{x}	0.844	< 0.001
P absorption, g/5 d	15.09^{y}	8.48^{x}	12.78^{y}	1.359	< 0.001
ATTD P, %	56.35^{y}	41.85^{x}	62.46^{z}	2.041	< 0.001
Urine P output, g/5 d	0.418^{y}	0.081^{x}	0.064^{x}	0.064	< 0.001
P retention, g/5 d	14.67^{z}	8.40^{x}	12.71^{y}	1.332	< 0.001
P retention, %	54.80^{y}	41.45^{x}	62.14^{z}	2.043	< 0.001
Ca intake, g/5 d	31.38^{z}	29.41^{y}	27.50^{x}	2.675	< 0.001
Fecal Ca output, g/5 d	7.62^{y}	9.96^{z}	6.48^{x}	0.770	< 0.001
Ca absorption, g/5 d	23.76^{z}	19.46^{x}	21.03^{y}	2.288	< 0.001
ATTD Ca, %	75.45^{y}	66.00^{x}	76.37^{y}	2.348	< 0.001
Urine Ca output, g/5 d	1.28^{x}	6.44^{z}	4.73^{y}	0.708	< 0.001
Ca retention, g/5 d	22.48^{z}	13.02^{x}	16.30^{y}	1.731	< 0.001
Ca retention, %	71.60^{z}	44.12^{x}	59.42^{y}	2.167	< 0.001

[x-z] Within a row, means without a common superscript letter differ ($P < 0.05$).

[1]Data are presented as total intake and output over the 5-d collection period. n = 9

CHAPTER 6

CONCLUSION

From the experiments that are reported in this thesis, it is concluded that the addition of Quantum phytase to pig diets can reduce the amount of inorganic P that is needed in the diet. The optimal inclusion level of Quantum phytase to weanling pig diets may be greater than 1500 phytase units/kg. However, based upon pig performance in growing pigs, the optimal inclusion level of Quantum phytase is approximately 450 phytase units/kg. When Quantum phytase is included in low-P growing pig diets, bone tissue synthesis may improve.

It has been confirmed that P is absorbed in the small intestine and no P is absorbed in the large intestine. In addition, Ca is absorbed in the small intestine and adding phytase to the diet will increase Ca absorption in the small intestine. Quantum phytase is more effective in releasing phytate-bound P than Natuphos in the stomach and upper small intestine. The addition of either Quantum phytase or Natuphos to pig diets will improve apparent total tract P digestibility in corn and soybean meal-based diets. This is because adding Quantum phytase or Natuphos to the diet will release P that is bound to the inositol ring. The addition of either Quantum phytase or Natuphos to the diet does not improve apparent ileal digestibilities of CP or AA.

In addition, adding 500 phytase units/kg of Quantum phytase to a low-P diet will improve P absorption, retention, and apparent total tract digestibility. It has also been

confirmed that adding Quantum phytase to low-P diets will decrease the amount of P that is excreted in the feces. Pigs that are fed a low-P diet that is supplemented with Quantum phytase can perform as well as pigs fed a diet that is adequate in P. Quantum phytase can be added to growing pig diets and can decrease the amount of inorganic P that is added to the diets without impeding maximum pig performance. Approximately 0.68 g/kg of diet of inorganic P can be removed in growing pig diets by adding 500 phytase units/kg of Quantum.