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The Effects of Super-dosing Phytase on Nursery and Grower Pigs

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The Effects of Super-dosing Phytase on Nursery and Grower Pigs

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Abstract

To determine the effects of super-dosing on pigs fed phosphorous and calcium adequate or deficient diets (Experiment 1); the optimum level of Corn express phytase (CEP, Experiment 2); and the effects of xylanase/phytase on growth performance (Experiment 3) in pigs fed reduce nutrient diets, weaned pigs were blocked with initial body weight (BW) and allotted to dietary treatments. Treatments were: Positive control (PC), P/Ca adequate diets (NRC 2012); Negative Control (NC), low P (-0.15%) and Ca (-0.12%), +0, 1000, and 4000 FTU/kg of CEP in both PC and NC (Experiment 1); PC, P/Ca adequate diets; NC, decreased P (0.15%) and Ca (0.1%),+ 500, 1000, and 1500 FTU/kg CEP or NC+500 FTU/kg of Hiphos GT (Experiment 2); PC, P/Ca adequate diet (+500 FTU/kg of phytase); NC, reduced ME and AA of feed; NC+15,000 FTU/kg of phytase, +0, 12,000 or 16,000 DXU/kg of xylanase (Experiment 3). Data were analyzed by MIXED procedures of SAS (SAS inst., Cary, NC) with treatments as fixed effect and initial BW as random effect. In Exp. 1, ADG ($P < 0.05$) and BW ($P < 0.05$) were linearly increased with increasing level of CEP in pigs fed NC diets, but not in those fed PC diets (CEP x P/Ca-level interaction). CEP supplementation improved percentage bone ash ($P < 0.05$) quadratically, independent of P/Ca levels. For Exp. 2, increasing CEP increased ADG ($P<0.01$), G:F ($P < 0.05$), and BW ($P < 0.05$) linearly, and added both phytase at 500 FTU/kg (CEP and HiPhos) restored growth performance phenotypes to PC diets. In Exp. 3, adding the combination of 16,000 DXU/kg xylanase and super-dosing phytase restored BW ($P > 0.10$) similar to the PC when pigs fed reduced ME and AA diets. Pigs fed diets supplement with 16,000 DXU/kg xylanase or super-dosing phytase alone had similar G:F to the PC fed pigs. In addition, pigs fed 16,000 DXU/kg of xylanase diet had similar carcass composition compared to the NC ($P > 0.10$). These experiments suggest phytase in nutrient deficient diets restores growth performance

similar to pigs fed an adequate diet, and corn expressed phytase is as effective as microbial phytase in pigs.

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Table of Contents

List of Tables

Introduction

High concentrations of phytate are found in plant seeds such as corn, canola, and soybean meals, which preserve phosphorus (P) for these seeds. However, phytate bound P is largely indigestible to monogastric animals. Thus, phytate is considered an antinutritional factor in nonruminant livestock production. It is well recognized that dietary supplementation of exogenous microbial phytases in nonruminant diets effectively improves phytate bound phosphorus utilization (Jongbloed et al., 1992, Cromwell et al., 1995, Adeola and Cowieson, 2011). In fact, phytase is the most commonly used enzyme feed additive in monogastric diets; and is used to reduce the antinutritional effect of phytate through improved nutrient digestibility {including phosphorus, calcium (Ca), and potentially amino acids (AA)} while also mitigating the environmental impact from excess nutrient excretion in nonruminant meat production animals (Adeola and Cowieson, 2011). Phytase aids in phytate degradation by liberating phosphate from myo-inositol hexaphosphate through the stepwise removal of phosphorus (Selle et al., 2009). The benefits of feeding phytase can be further seen through the recent estimate that feed enzymes save the global feed market $3 to 5 billion (USD) per year. This large market is currently dominated by phytases, representing a total of 60% of this market (Adeola and Cowieson, 2011).

There are many commercially available phytases that vary in efficacy of phytate dephosphorylation. The activity of phytase is expressed as phytase units or FTU, one FTU is defined as the quantity of enzyme required to hydrolyze 1 mmol of inorganic phosphorus/min, at pH 5.5, from an excess of 1.5 mM sodium phytate at 37°C (International Union of Biochemistry, 1979). To further complicate the efficacy of phytase, Adeola and Cowieson (2011) noted that the

impact of microbial phytase on P release depends on dietary phytate concentration, dietary mineral concentrations, the phytate source, and dosage.

Recently, plants expressing high levels of phytase have been produced through plasmid transformation by *Agrobacterium*-mediated transformation into immature maize embryo as described by Negrotto et al. (2000). These sources seek to serve as a more cost effective means to include phytase in nonruminant diets since feed is 60 to 70% of the production cost for farmers. Several different plants have been created expressing these high levels of phytase, these include tobacco, canola, soybeans, and corn (Bilyeu et al., 2007; Ponstein et al., 2002; Ullah et al., 1999). Since corn is the major ingredient in nonruminant diets and undergoes little post-harvest processing, it is an attractive plant to express transgenic microbial-derived phytase (Nyannor and Adeola, 2008). This suggests that transgenic corn phytase could be a cost effective alternative to microbial phytase, in helping pigs free more inositol P than standard corn supplemented with microbial phytase (Li et al., 2013).

These chapters aim to review the function and absorption of P, phytate's antinutritional factors, the use of phytase and super-dosing effects of phytase, and corn expressed phytase use as an alternative to microbial phytases and its effects on growth performance, bone characteristics, and nutrient digestibility in nursery pigs.

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Chapter 1: Literature Review

Literature Review

Phosphorus

Purpose within plants

Similar to animals, plants need P for normal growth and maturation. Phosphorus plays a role in photosynthesis, respiration, energy storage and transfer, cell division, cell enlargement, and several other processes in plants (Fageria et al., 2017). Additionally, P is needed for the synthesis of nucleic acids and for adenosine triphosphate (ATP). Plants store P in their seeds to serve as the mineral supply during germination. During seed germination the phytate P is liberated by endogenous phytases releasing the P to satisfy the needs of the plant embryo. Therefore, plants need P to complete the normal production cycle.

Purpose within animals

Phosphorus is important in skeletal structure development, but it is also important in soft tissues. Phosphorus plays a key role in acid-base buffering, osmotic balance, metabolic functions such as energy utilization and transport, in diester phosphate bonds such as ATP, phospholipids, fatty acid transport, and amino acid and protein formation (Jongbloed, 1987). Phosphorus is an essential component in energy utilization systems, cell membranes, bone formation, and nucleic acid synthesis. Furthermore, Ca and P are the 2 most abundant minerals in bones, comprising 60 to 80% of the bone at a ratio of 1.75 Ca to P (Eeckhout et al., 1995). Phosphorus plays a key role in skeletal maintenance and development, and enzyme regulation in metabolism and physiological processes (Humer et al., 2015). Additionally, P is a major energy transporter in living organisms, the “energy currency” of the body, ATP, requires 3 P atoms per molecule. Phosphorus also can be found as a component of nucleic acids linking the sugar groups found in

DNA and RNA, and as an activator for many enzymes (Jongbloed, 1987). Overall, P serves a large role in both plants and animals.

Mechanisms of absorption and regulation

The small intestine is the major site for inorganic P absorption in monogastric animals, with absorption being greater in the jejunum compared to the ileum and duodenum, with very little to no absorption in the large intestine (Jongbloed, 1987). Phosphorus is transported across the intestinal wall starting with mucosal uptake and transported to the serosa, then to body fluid; this active transport is stimulated by vitamin D (Kowarski and Schachter, 1969). Phosphorus uptake is regulated by several major compounds, such as parathyroid hormone (PTH), calcitonin, vitamin D, and *fibroblast growth factor (*FGF)-23 (Bergwitz and Jüppner, 2010; Blaine et al., 2014; Breves & Schröder, 1991; Shaker and Deftos, 2014; Graf, 1986; and Quarles, 2012). The hormone, FGF-23 works by inhibiting renal reabsorption of phosphate and subsequently reducing serum P concentrations and vitamin D in plasma (Bergwitz and Jüppner, 2010). While, PTH activates osteoclast and osteoblast activity regulating bone formation and resorption depending on circulating PTH concentrations, which directly impact P absorption and release. This then causes PTH regulation to result in a net increase of serum Ca and a decrease in serum phosphate concentrations, while Vitamin D is related to increased serum Ca and P concentrations. Vitamin D stimulates increased intestinal P uptake (Bergwitz and Jüppner, 2010). Other research has shown that increasing the Ca:P ratio has a negative impact on P absorption in pigs, ducks, and chickens (Reinhart and Mahan, 1986; Xie et al., 2009; Delezie et al., 2015).

Phytate

Phytate is the storage form of P in plants and is found in virtually all seeds (Adeola and Cowieson, 2011). Phytic acid is poorly soluble in the small intestine of swine and poultry and

therefore the phytate P is not digested efficiently. This is because the endogenous phytase in monogastrics is very lowly active and incapable of hydrolyzing sufficient amounts of phytate-bound P (Humer et al., 2015). This is an issue because a large portion of the P found in plant seeds is bound in this indigestible P molecule, and this can impact both the pig and the environment.

Structure, location and function

Phytic acid (myo-inositol-1,2,3,4,5,6-hexadihydrogenphosphate; IP6) is the main storage form of P in plant seeds such as cereal grains and legume meals (Woyengo and Nyachoti, 2013). Over 60% of the P found in corn is bound as part of phytate, making it poorly available to nonruminant animals (Reddy et. al., 1982). Phytic acid is unstable when present in the free acid form (Reddy et al., 1989). The phytic acid is a 6 membered carbon ring which binds 6 phosphate molecules. The phosphate molecules carry a negative charge that allows for the formation of the salts. This causes it to form a variety of salts with positively charged metals including Cu, Ca, Fe, Zn, Mg, and Mn. This mixed salt of phytic acid with various cations is referred to as phytate (Zeller et al., 2015). When these salts form, it reduces the digestibility of these bound minerals along with the already reduced P and can precipitate the polyvalent cations (Graf, 1988).

Roughly 90% of phytate found in corn is located in the germ, however, no specific location has been found in soybeans (O'Dell et al., 1972; Steiner et al., 2007).

Impact

Environment

It is common for diets to be supplemented with phytases to improve P utilization and reduce negative environmental impacts (Ravindran et al., 2000; Leske and Coon, 1999; Onyango et al., 2005; Hart et al., 2004). This supplementation works because pigs fed diets devoid of

phytase supplementation can excrete 50 to 80% of their P intake (Kornegay et al., 1997). Pigs have limited intestinal phytase activity which limits the liberation of phytate P. Without supplemented phytase, pigs are unable to digest the phytate which results in a larger proportion of excreted P. Therefore, supplemental phytase can improve phytate P utilization and subsequently decrease the amount of P excreted into the environment (Adeola, 1999; Nyannor et al., 2007). This reduction in excreted P is environmentally important because swine excrement is often applied to land as a fertilizer. When the excrement is applied with excessive P concentrations over a period of time, the plants and soil cannot absorb enough of the P. With rainfall, this excess P can drain into watersheds and cause the eutrophication of freshwater systems (Sharpley et al., 1995; Sims et al., 1998; Correll, 1998). Eutrophication results in frenzied and excessive growth of algae and vascular plants which leads to the depletion of dissolved oxygen in the water system (Smith et al., 1999). Without dissolved oxygen in the water, much of the aquatic life dies. This explosion of microorganisms and reduction in fish populations can greatly shift species composition at various trophic levels (Correll, 1998). Phytase has been suggested as an effective method to feed pigs with environmental nutrition in mind by reducing the amount of excreted P (Kornegay et al., 1997; Leytem and Thacker, 2010).

Baxter et al. (2003) showed how diet composition can be used to manage P amount and type in pig excreta, and the changes that occur to these excreta types during periods of storage. Phytase reduces undigested and excess P excretion up to 30% in pigs (Simons et al. 1990), and 31% to 38% in poultry litters (Maguire et al. 2004). Nyannor et al. (2007) found that pigs fed a phytase supplemented diet excreted less than half the P as pigs fed the untreated control diet. Nasi et al. (1995) reported that phytase inclusion without P supplementation decreased the amount of P excreted in the feces by one third when compared to no phytase addition.

These studies indicate that phytase can effectively reduce the risks associated with P runoff from manure land application. This is achieved by reducing the total P excretion and the water-soluble P excreted, which greatly reduced the risk of over application of P.

Antinutritional effects of phytate

Phytic acid is a storage form of phosphorus found in plant feedstuffs containing up to 80% of the total P (Kumar et al., 2011). Phytic acid is poorly hydrolyzed by pigs and reduces nutrient digestibility, thus diminishing nutrient utilization and increasing P excretion (Woyengo and Nyachoti, 2013). One way in which phytic acid is capable of reducing nutrient availability is through binding of mineral cations which decreases the available minerals and P in feedstuffs (Angel et al., 2002). The P from phytic acid remains negatively charged in a range of pH conditions, therefore, it can bind the positively charged molecules in the diet and potentially bind endogenous gastrointestinal secretions, thus reducing nutrient digestibility and increasing endogenous secretions (Woyengo and Nyachoti, 2013). However, phytate does not appear to impact the digestibility of monovalent cations such as sodium (Na) and potassium (K). There is a small improvement seen in the Na and K digestibility when phytase is fed at extremely high (15,000 FTU/kg) concentrations (Keis et al., 2006). Furthermore, when phytate binds Ca these complexes potentially form metallic soaps with nonesterified fatty acids in the gut which can inhibit energy being derived from saturated fats (Ravindran et al., 2000).

Woyengo and Nyachoti (2013) listed 4 mechanisms by which phytic acid could increase the endogenous secretion of minerals. To start, phytic acid may interact with dietary and endogenous proteins in the stomach, reducing pepsin's activity. This would prevent the breakdown of protein in the stomach. Second, the digestive metalloenzymes could bind to phytic acid through multivalent cations forming insoluble complexes in the small intestine. Thirdly,

phytic acid could bind to endogenously secreted minerals, preventing their reabsorption and reducing their digestibility; and the final proposed means is that phytic acid may reduce the reabsorption of endogenously secreted minerals by reducing the absorption of nutrients such as sugars and AA. This final mechanism impacts mineral absorption by reducing the minerals absorbed via solvent drag due to the osmotic flow created by the active transport of sugars and AA.

It appears that dietary phytic acid did not affect AA digestibility when fed in a cornstarch and casein diet with supplemented phytic acid (Knuckles et al., 1989; Woyengi et al., 2009). Although it does appear that dietary phytic acid reduced AA digestibility when fed in a corn-soybean meal diet (Bohlke et al, 2005; Liao et al., 2005; Woyengo and Nyanchoti, 2013). This indicates that the effect of dietary phytase on AA digestibility may be impacted by diet composition. When looking at phytate's antinutritional factors further affecting proteins and AA, Woyengo and Nyanchoti (2013) noted that phytic acid increases the endogenous AA loss by increasing the secretion of endogenous enzymes while simultaneously reducing the absorption of endogenous AA. Furthermore, phytate could reduce protein digestibility by binding to the dietary protein and proteolytic enzymes in the stomach and small intestine. Cowieson et al. (2006) observed that phytic acid decreased the true ileal digestibility of amino acids by 8% or more. Onyango et al. (2008) reported a linear decrease in leucine, lysine, and glutamic acid as phytate concentration increased in chicken diets. However, there was no reduction of AA digestibility in pigs fed a cornstarch diet with supplemental phytic acid. It appears that phytic acid is detrimental to AA digestibility or absorption by binding to dietary protein and enzymes in the stomach and small intestine or reducing pepsin and trypsin activity (Liu et al., 2009; Reddy et al., 1989). Woyengo et al. (2010) observed reduced gastric pepsin activity in piglets fed phytic acid. This

reduction of trypsin and pepsin activity is associated with an increased loss of endogenous AA. In addition, this increased loss is associated with the decrease in pepsin, trypsin, and gastrin activity which resulted in an increased secretion of pepsin and mucins. This increased secretion of mucins is thought to protect the gastrointestinal tract from being digested by hydrochloric acid and various enzymes whose secretions were also increased by increased pepsin secretion (Cowieson et al., 2004; Forstner and Forstner, 1994; Munster et al. 1987; Nyachoti et al., 1997; Onyango et al., 2009). Ultimately it appears that phytate increases endogenous AA losses through the increased secretion of digestive enzymes and mucins, and through the reduction of reabsorbed endogenously secreted AA in the small intestine.

Phytase

Introduction

Phytase is the enzyme that catalyzes phytate hydrolysis and frees the inorganic P and phytate-bound nutrients in plant seed (Yin et al., 2007). In order to reduce the antinutritional effects of phytic acid, the salt needs to be quickly hydrolyzed (Dersjant-Li et al., 2015; Graf, 1986). The supplementation of phytase increases P utilization in swine and reduces P excretion by catalyzing the stepwise removal of phosphates from phytic acid (Cromwell et al., 1993, 1995; Liu et al., 1998). When microbial phytases are added to monogastric diets they preferentially hydrolyze the ester bond between carbon-3 or carbon-6 and the associated phosphate group of a fully phosphorylated phytic acid. This frees the phosphate for the animals' use from the phytate molecule converting the myo-inositol hexaphosphate (IP6) to myo-inositol pentaphosphate (IP5). After this 1st hydrolysis, the phytase then sequentially moves around the inositol ring, further liberating phosphates until one of several mechanisms prevents further activity (i.e. kinetics, environmental conditions, substrate solubility). Exogenous phytases are mainly active in the

stomach of pigs where the acidic pH increases the solubility of phytate making it more susceptible to degradation (Campbell and Bedford, 1992). However, in most cases, phytases are not able to degrade phytic acid to the inositol ring and free phosphate (Adeola and Cowieson, 2011; Wyss et al., 1999). The stepwise removal of P reduces the antinutritional factors associated with phytate and increases the digestibility of bound minerals because as phytate is hydrolyzed its binding capabilities with minerals is greatly reduced (Yu et al., 2012; Lutrell, 1993; Persson et al., 1998).

Sources of phytase

The small intestine mucosa generates phytate-degrading phytase and phosphatase, however their activity is considered negligible (Pointillart et al., 1984). Additionally, the gut microflora can hydrolyze phytate in the hindgut digesta; this is considered unimportant in absorption because P is mostly absorbed in the upper parts of the small intestine (Kerr et al., 2000; Seynaeve et al., 2000). Therefore, exogenous phytases are used to reduce the phytate molecules. These phytases can be subdivided based on where they first remove phosphorus from the inositol ring. If the phytase removes the phosphate unit on the third carbon it is a 3-phytase; however, if it first removes the phosphate from the sixth carbon it is a 6-phytase. Generally, 3-phytases are from microbial origin and 6-phytases are of plant origin and both commence dephosphorylation at the 3 or 6 carbons of inositol rings, respectively (Dvořáková, 1998). Most studies have analyzed different types of exogenous microbial phytases. These exogenous microbial phytases are derived from different species of bacteria, yeast, and fungi (Harland and Morris, 1995). The major sources of microbial feed grade phytases have been derived from *Bacillus sp.*, *Aspergillus sp.*, and *Escherichia* coli. (Konietzny and Greiner, 2002). With the first

phytases being from the fungi, yeast, and *Aspergillus niger* (Dersjant-Li et al., 2015; Nelson et al., 1971).

As exogenous phytases are now a common feed additive, industry has been searching for a cost-effective alternative. Microbial phytases are produced through fermentation processes and their successful incorporation into rations requires special attention to feed processing and diet formulation (Gontia et al., 2012). When the temperature of pellets reaches approximately 80°C, the concentration of absorbable P is substantially decreased in feeds that had high levels of phytase activity in both pig and chicken diets (Jongbloed and Kemme, 1990; Kirkpinar and Basmacioğlu, 2011).

More recently an effective corn expressed phytase was developed. There have been several transgenic plants produced that express high levels of phytases including canola, soybeans, tobacco, and corn (Bilyeu et al., 2007; Ponstein et al., 2002; Ullah et al., 1999; Zhang et al., 2000). However, corn is a good system for transgenic phytase destined for use in animal feed, because it is not subject to post-harvest heat processing, which is often used with soybeans and canola. Therefore, corn with transgenic phytase could be incorporated into diets to deliver a similar phytase activity to microbial phytases (Nyannor et al., 2007). This is advantageous because corn is generally fed as the largest portion of a pig's diet.

Superdosing

In 2011 when Cowieson et al. published their review, the majority of reports investigating superdosing phytase showed advantages to using very high doses of phytase; however, the mechanism was not understood. The earliest noted experiment with “superdosing” may be Nelson et al. (1971) where the highest dose fed to chicks was 7,600 FTU/kg. This high level of included phytase resulted in a 94.4% disappearance of phytate-P as compared to 38.9%

disappearance in chicks fed 950 FTU/kg. After the commercialization of phytase as a feed additive in 1991, more phytase superdosing trials have been performed. Between 1990 and 2000, there were several reports with doses of phytase up to 2,500 FTU/kg that showed increased phytase-P disappearance with increasing phytase dose (Simons et al., 1990; Huyghebaert et al., 1992; Zhang et al., 2000). In 2003 in one of the first studies with particularly high doses, Shirley and Edwards (2003) supplemented a corn-based diet with up to 12,000 FTU/kg of microbial phytase and observed a quadratic increase in phytase-P disappearance with increasing phytase dosage. The results of Shirley and Edwards (2003) have been confirmed more recently in several studies where doses up to 10,000 FTU/kg or beyond were used (Augspurger and Baker, 2004; Cowieson et al., 2006; Brana et al., 2006; Kies et al., 2006; Pirgozliev et al. 2007).

Digestibility

Rutherfurd et al. (2014) stated that a large portion of phytate P release occurs in the hindgut and is not absorbed. Ileal estimates were suggested to be a more accurate reflection of P availability because the majority of P absorption appeared to occur in the jejunum. Furthermore, Rutherfurd et al. (2014) suggested that the microbiota of the large intestine of a growing pig plays a significant role in phytate hydrolysis, especially in unsupplemented diets; thereby confounding total tract estimates of phytate. Leytem and Thacker (2010) also suggested that the difference in apparent phytate digestibility coefficient of corn and results from their study indicate that microbial phytate hydrolysis occurs in the lower digestive tract, where it is not absorbed and thus appears as phosphate in the feces. Therefore, a large portion of P made available was not absorbed and was excreted in the feces.

By reducing IP6 concentrations, the antinutritive effects of phytate are reduced. The lower esters have a much-reduced capacity to chelate divalent cations and further allow access to

endogenous phytases (Lutrell, 1993). Brana et al (2006) noted that diets with included phytase can result in an artificial increase of P requirement because of a high Ca:P ratio due to an improved release of Ca. By decreasing the Ca:P ratio from 2 to 1.2 an increase in phytase efficiency can be seen (Brady et al., 2002; Liu et al., 1998). Since phytase supplementation releases P and phytate bound Ca it is common to reduce the concentration of Ca and P in swine diets when feeding phytase by roughly 0.1% and 0.15% respectively. This reduction of fed Ca allows for a better response to phytase supplementation in both growth performance and bone characteristics.

Phosphorus digestibility

Given that around 80% of the P in the body is found in the skeleton and teeth, a deficiency of P will result in impaired bone mineralization, reduced bone strength, and poor growth. To meet the pigs' requirement supplemental inorganic P is added to the diet. More recently phytase is added to increase the digestibility of phytate P. The response seen to including exogenous phytase is greater when pigs are fed low-P diets. The improved digestibility of P is exemplified by the numerous studies showing phytases' improvements. Mroz et al. (1992) showed a 24.1% increase for apparent total tract digestibility (ATTD) of P and a 25.8% increase of apparent ileal digestibility (AID) in pigs fed 800 FTU/kg. Adeola et al. (2004) reported an increased ATTD of P of growing pigs by at least 20% when diets were supplemented with 250 FTU/kg. Radcliffe et al. (2006) reported that AID of P increased by 17% when corn-based diets were supplemented with 500 FTU/kg. Similarly, Kim et al. (2005) reported a 35% increase in ATTD of P for wheat based diets when supplemented with 500 FTU/kg. However, when fed at low levels, Woyengo et al. (2008) reported no significant difference between pigs supplemented with 250 vs. 500 FTU/kg. Jones et al. (2010) evaluated the efficacy of different

commercial phytases largely because there is a wide variation in recommended dosage for similar P release between different phytase sources and noted that the response seen in bone ash is similar to manufacture recommendations, however this may vary when fed a diet with adequate P.

When phytase is supplemented at high levels, it has the potential to replace inorganic P in the diet. Veum et al. (2006) demonstrated that the phytase response, seen in growth performance and bone strength, was greater for pigs fed 2,500 or 12,500 FTU when compared to an unsupplemented diet that met the P requirement. Furthermore, pigs fed low-P phytase diets consumed more feed, absorbed more P, Ca, and Mg, and excreted less P and Ca than the pigs on an unsupplemented control diet. Furthermore, Cromwell et al. (1995) showed that the P requirement of pigs could be met without inorganic P supplementation, in a corn-soybean diet, given that enough of the P in corn and soybeans is degraded by phytase. Cromwell et al. (1993) reported that 1,000 FTU increased P availability from 15% in a phytase free diet to 45% in a phytase supplemented diet.

Calcium digestibility

Woyengo et al. (2008) and Johnston et al. (2004) both reported an increase in AID and ATTD of Ca. Almeida and Stein (2010) noted that one possible mechanism by which Ca absorption is increased with phytase is by phytate hydrolysis reducing phytate esters and therefore reducing the ability of phytate to chelate Ca. As a result, fewer Ca-phytate complexes are formed and Ca availability is increased when phytase is added to the diet (Selle et al., 2009). This may also explain why a lower Ca:P ratio is recommended in diets containing phytase than diets containing no phytase (Bradly et al., 2002; Lei et al., 1994; Liu et al., 2007). Luttrell (1993) noted that the affinity at which Ca^{2+} binds to phytate increases as the number of phosphate

substituents increases on the myo-inositol ring, further showing how phytate degradation can improve Ca digestibility. Stein et al. (2006) described the increased Ca digestibility as due to the Ca retention increasing as more P is absorbed. Additionally, the increased absorption of Ca in a diet containing phytase is the result of some Ca being released from the phytate molecule. Furthermore, exogenous phytases enhance Ca absorption in the small intestine by the partial hydrolysis of phytate to lower phytate esters in more proximal segments of the gut. Lower phytate esters have a reduced capacity to chelate Ca, so the Ca-phytate complex formation is reduced and Ca availability is enhanced.

Amino acid digestibility

Adeola and Cowieson (2011) noted that it seems when titanium dioxide (TiO_2) or acid insoluble ash were used as indigestible markers, there was an improvement in ileal AA digestibility with phytase as compared to when Cr_2O_3 was used as a marker, especially in poultry. There have been several studies that have shown a lack of change in ileal AA digestibility in response to phytase supplementation (Adeola and Sands, 2003; Onyango et al., 2005; Woyengo et al., 2008; Liao et al., 2005). However, Woyengo et al. (2008) reported a numerical increase in AID of AA. More specifically, Kemme et al. (1999) reported that phytase increased the digestibility of lysine, methionine, threonine, and isoleucine along with the majority of the nonessential AA. Furthermore, Mroz et al. (1992) only noted a statistically significant increase in AID of methionine and arginine, and a numerical increase in lysine, cysteine, tryptophan, histidine and proline. Radcliffe et al., (2006) reported an increase in AID of glycine, alanine, valine, isoleucine, threonine, aspartate, glutamate, phenylalanine, lysine, and arginine; and reported that 500 FTU/kg phytase can result in a 0.03% improvement in Lys AID. There is a lot of variation between the studies that report different digestibilities of AA in

phytase supplemented pigs, with improvements in lysine and threonine AID occurring the most frequently within these studies.

Crude protein digestibility

There have been several experiments that have shown that phytase has very little, if any, effect on apparent or true crude protein digestibility (Traylor et al., 2001; Nasi et al., 1995). However, there have also been several articles showing a response to supplemental phytase. Kemme et al. (1999) reported that 900 FTU/kg of phytase in a maize-soybean diet tended to increase crude protein digestibility by 1.6%. Additionally, Mros et al. (1992) noted a numerical increase in AID of crude protein; and Radcliffe et al. (2006) reported a linear increase in crude protein AID with increasing phytase levels, ultimately stating that 500 FTU/kg can replace up to 3.01% of total dietary protein.

Bone bending strength and bone ash

There have been numerous studies that have shown a strong correlation between bone mineral density and breaking strength. There is a similar improvement seen with increased bone ash and bone breaking strength with increasing levels of digestible P in both pigs and chickens (Crenshaw et al., 1981; Cromwell et al., 1972; Qian et al., 1996; Nielsen et al., 2007). Bone tissue synthesis requires both P and Ca. As more P is used, more Ca is needed for bone synthesis, resulting in less excreted Ca (Stein et al., 2006). It has also been demonstrated that phytase supplementation increases bone ash (Brana et al, 2006). Furthermore, a series of studies in growing pigs examined the relationship between bone mineral density and bone strength, these studies demonstrated that as bone mineral density increases, bone strength increases as well (Koo et al., 2001; Van der Meulen et al., 2001). In addition to these studies, Nielsen et al. (2007) showed an increased bone strength with increased mineral concentrations. Subsequently

microbial phytase supplementation improves bone breaking strength by increasing the concentration of minerals in bone (Pagano et al., 2007; Veum et al., 2006).

Scope of research

Phytase allows the swine industry to reduce the environmental impact of swine production while also producing animals more efficiently. With the recent development of corn expressed phytase, the efficacy of the phytase and its super-dosing potential are discussed in the next chapter. To accomplish this two experiments were conducted to determine growth performance and metacarpal bone characteristics when nursery pigs fed phosphorus and calcium adequate or deficient diets in experiment 1, and evaluate optimum level of corn-expressed phytase (CEP) in experiment 2. Further examining the effects of super-dosing phytase an additional chapter has been added, examining the potential synergistic effects of xylanase and super-dosing phytase on swine nursery and grow-finish performance.

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Chapter 2: Effects of a Corn-Expressed Phytase on Growth Performance and Bone Ash of Nursery Pigs

Effects of a Corn-Expressed Phytase on Growth Performance and Bone Ash of Nursery Pigs

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Abstract:

Two experiments were conducted to determine growth performance and metacarpal bone characteristics in nursery pigs fed phosphorus and calcium adequate or deficient diets in Experiment 1; and evaluate optimum level of corn-expressed phytase (CEP) in Experiment 2. In each experiment, a total of 288 pigs were blocked by initial body weight (BW) and allotted to 1 of 6 treatments (8 replicates per treatment). Pens were randomly assigned to dietary treatments. Treatments: Positive control (PC), P/Ca adequate diets devoid of CEP (NRC 2012); Negative Control (NC), low P (-0.15%) and Ca (-0.12%) diets and GraINzyme® Phytase (CEP) was supplemented at 0, 1000, and 4000 FTU/kg in both PC and NC diets fed in all 3 phases (14, 12, 14 d) for Exp. 1. In Exp. 2, treatments consisted of: a) PC, P and Ca adequate diets (NRC 2012); b) NC, decreased P (0.15%) and Ca (0.1%) diets; NC+ c) 500, d) 1000, and e) 1500 FTU/kg CEP; f) NC+ 500 FTU/kg of Ronozyme® Hiphos GT. Pigs were fed a common phase 1 (8 d) diet before initiating treatments in phase 2 (14 d), and phase 3 (14 d). All pigs were euthanized at termination of phase 3 to collect metacarpal bones for ash determination. Data were analyzed by MIXED procedures of SAS (SAS, Cary, NC) with treatments as fixed effect and initial BW block as random effect. Orthogonal contrast was performed to determine the effects of increasing CEP. In Exp. 1, as CEP increased, phase 3 ADG ($P < 0.05$), overall ADG ($P < 0.05$), and end BW ($P < 0.05$) increased linearly in pigs fed NC diets when compared to PC fed counterpart (Linear CEP × P/Ca level interaction). Similarly, G:F increased quadratically with increasing level of CEP in pigs fed the NC diet ($P < 0.05$). Corn expressed phytase supplementation

improved weight ($P < 0.05$) and percentage ($P < 0.05$) of bone ash in a quadratic manner independent to dietary P/Ca levels. In Exp. 2, increasing levels of CEP linearly increased overall ADG ($P<0.01$), G:F ($P < 0.05$), and end BW ($P < 0.05$), which resulted in restored phenotypes in pigs fed at 500 FTU/kg of CEP, and were comparable to pigs fed Hiphos GT. Results suggest that pigs fed diets low in P and Ca supplemented with CEP at 500 FTU/kg performed similar to pigs fed a diet with adequate levels of Ca and P.

Keywords: corn-expressed phytase, growth performance, bone characteristics, nursery pigs

Introduction

Phytate is the main storage form of phosphorus (P) in plant-based feedstuffs (Lavin et al., 2013; Yáñez et al., 2013; Graf, 1983; Chung et al., 2013). Phytate has the ability to bind cationic minerals and potentially proteins, thereby, inhibiting the absorption of these compounds (Cheryan and Rackis, 1980). Phytate-bound P has several anti-nutritional properties that can reduce P use efficiency, increase P excretion into the environment and impact mineral digestibility (Veum et al., 2006; Manobhavan et al., 2016). Supplementation of exogenous phytase has become common in industry and is used to hydrolyze phytate to increase P availability and subsequently reduce the P excreted (Walk et al., 2013). Phytase supplementation has added benefits in that it liberates phytate-bound nutrients such as Ca, Mg, and Zn resulting in an enhanced digestibility of these minerals in addition to the P (Kies et al., 2006). Microbial phytases are the common additive used in industry, however, plant seed including canola, soybeans and corn, have been produced that express high levels of phytase activity (Li et al., 2013; Nyannor and Adeola, 2008). Corn is an attractive alternative to microbial phytases because it does not undergo the heat associated with postharvest processing seen with canola and soybean. This could allow transgenic corn expressing high levels of phytase to be a cost-

effective and attractive replacement for microbial phytases. Therefore, CEP can be directly incorporated into diets. (Nyannor et al., 2007). This also allows for the feeding of unconventionally high doses of phytase to be incorporated into feeds (i.e. ≥ 2,500 FTU/kg of feed), serving as an effective means to maximize P release (Adeola and Cowieson, 2011; Adedokun et al., 2015). These studies were conducted to evaluate the effects of increasing dietary corn-expressed phytase (from 500 to 1,500 FTU/kg) and compare it to commercial phytase (HiPhos) on growth performance and bone characteristics in nursery pigs fed P and Ca deficient diets.

Materials and methods

The institutional Animal Care and Use Committee at the University of Arkansas reviewed and approved the protocols for both experiments.

Sample collection and processing

At the start of each of the studies and at the end of each of the phases, individual pig weights and pen feed disappearance were measured in order to calculate ADG, ADFI, and G:F by phase. At the termination of the study, pigs were euthanized and front feet were removed for isolation of the metacarpal for bone ash and bending strength determination. Feed samples were obtained for each batch of feed mixed. These were accumulated for each phase and subsampled for nutrient analysis.

Bone characteristics

For both studies the front feet were removed and frozen in sealed plastic bags. The right foot samples were later thawed, extraneous tissue removed, and the 4th metacarpal was retained. The length and width of the bones at the narrow and longest dimensions of the bone shaft were measured. Bones were then refrozen in sealed plastic bags for shear force determination as

described by Combs et al. (1991). The sheer force of the right 4th metacarpal was determined using an Instron Universal Testing Machine (model 4466, Instron Corp., Canton, MA). Bones were thawed in sealed plastic bags immediately before testing to prevent desiccation and brought to room temperature. The matching metacarpal from the left foot, was isolated from extraneous tissue and ashed in a muffle furnace at 600°C for 8 h. Bone ash was expressed as a percentage of wet weight.

Experiment 1

Animals and experimental design

A 2 × 3 factorial arrangement of treatments was used to evaluate the response of weanling pigs to three levels of phytase and two levels of apparent P and Ca. A total of 288 weanling pigs were allotted (21 days of age) to one of the six dietary treatments. The pigs were individually weighed and blocked by initial weight and sex. Each treatment contained 8 replicate pens per treatment with 6 pigs per pen. A three-phase feeding program was utilized with pigs fed different diets in each of the three phases. Pigs were housed in 1.49 × 1.20 M^2 pens at the University of Arkansas conventional nursery facility, with *ad libitum* access to feed and water for the duration of the experiment.

Experimental diets

Phase 1 was fed for 14 days, phase 2 was fed for 12 days, and phase 3 was fed for 14 days. Six diets were formulated (Table 1). Three diets were formulated with adequate Ca and P, with increasing levels of CEP (0, 1000, and 4000 FTU/ kg) and formulated to meet nutrient requirements (NRC, 2012). The remaining three diets were formulated with reduced aP and Ca (a reduction of 0.15% and 0.13% respectively), with increasing levels of CEP (0, 1000 and 4000 FTU/kg).

Statistical analysis

The performance and bone ash data were analyzed using the MIXED procedures of SAS. Treatment was the fixed effect. Data was analyzed as a 2 × 3 factorial. Orthogonal contrasts were used to determine the effects of CEP on growth performance and bone ash. Probability values were considered statistically significant at $P < 0.05$, and $0.05 < P < 0.10$ was considered a statistical trend.

Experiment 2

Animals and experimental design

A total of 288 weanling pigs were allotted (21 days of age) to one of the six dietary treatments. The pigs were individually weighed and blocked by initial weight and sex. Each treatment contained 8 replicate pens per treatment with 6 pigs per pen. A four-phase feeding program was utilized with pigs fed different diets in each of the last three phases. Pigs were housed in the University of Arkansas conventional nursery facility, with *ad libitum* access to feed and water for the duration of the experiment.

Experimental diets

A common phase 1 diet was fed for the first 7 days to allow time for acclimation to weaning. Phase 2 was fed for 7 days, phase 3 was fed for 14 days, and phase 4 was fed for 14 days (Table 2). The positive control diet (PC) was formulated with adequate Ca and P, devoid of CEP and to meet nutrient requirements (NRC, 2012). Four diets were formulated with reduced aP and Ca (a reduction of 0.15% and 0.10% respectively), with increasing levels of corn-expressed phytase (0, 500, 1000 and 1500 FTU/kg). The sixth diet was formulated with reduced Ca and P and 500 FTU/kg of HiPhos.

Statistical analysis

The performance and bone ash data were analyzed using the MIXED procedures of SAS. Treatment was the fixed effect. Data were analyzed as a 2 × 3 factorial. Orthogonal contrasts were used to determine the effects of corn-expressed phytase on growth performance and bone characteristics. Probability values were considered statistically significant at $P < 0.05$, and $0.05 < P < 0.10$ considered a statistical trend.

Results

Experiment 1

Growth performance

Main effects of CEP on growth performance are presented in Table 3. During phase 1 and 2, ADG, and G:F were similar among treatments and no significant interactions were observed. However, during phase 3, and for the phase 1 through phase 3 period, increasing CEP in the low P NC diet resulted in increasing ADG, while gain was similar in the pigs fed increasing phytase levels in the adequate PC diet. This resulted in a linear P level × CEP inclusion interaction (Table 4, $P < 0.05$ for phase 3 and overall).

ADFI was not significantly impacted by either P level or CEP level (Tables 3 and 4) although there was an overall increase in feed intake with increasing dietary CEP in phase 3 (Table 3, $P < 0.10$). G:F was also improved in phase 3 with increasing level of CEP in pigs fed the NC low P diet, but G:F was similar among pigs fed the increasing CEP adequate P diet (Quadratic P level × corn-expressed phytase inclusion interaction, $P < 0.05$, Table 4).

Body weight tended to increase with increasing dietary CEP ($P < 0.10$, Table 3). As might be expected based on ADG in the overall study, BW at the end of the study increased with increasing level of CEP in pigs fed the NC, low P diet, but was similar among pigs fed increasing

levels of CEP with adequate P (Linear P level × CEP inclusion interaction, $P < 0.05$, Table 4). Table 4 presents the interactions between P level and CEP. Phase 3 ADG and BW increased with increasing CEP for the low P diets (linear, $P < 0.05$). Similarly, phase 3 feed efficiency also increased with increasing CEP in the low P diets (quadratic, $P < 0.05$, Table 4). However, these responses in ADG, BW and feed efficiency were not seen in the pigs fed the diets with adequate P.

Bone characteristics

Metacarpal bone ash was heavier in pigs fed adequate P diets (1 vs. 0.86 g; $P < 0.001$) (Table 5). A quadratic response in bone ash with increasing phytase addition was observed ($P = 0.049$) regardless of P level in the diets (P level by phytase interaction, $P = 0.230$). Pigs fed 4000 FTU/kg CEP restored the bone ash to a level similar to pigs fed adequate P and Ca without phytase supplementation.

The percentage of metacarpal bone ash was greater in pigs fed adequate P diets (0.26 vs. 0.23%; P level: $P < 0.01$) when compared to pigs fed P deficient diets, and a quadratic increase was observed with increasing level of phytase in the diets fed to pigs (0.24, 0.25, 0.25%, Quad. $P = 0.033$).

Metacarpal bone width tended to have a quadratic increase when pigs were fed increasing levels of phytase (8.55, 8.74, 8.74 mm, Quad. $P = 0.066$). Moreover, increased phytase in pigs fed low P diets increased bone width and length linearly, whereas a quadratic response was observed in the adequate fed counterpart (Linear P level × CEP inclusion interaction, $P = 0.100$ and $P = 0.020$, respectively). Pigs fed the low P diet supplemented with 1000 FTU/kg CEP restored the percentage of bone ash, bone width and length when compared to pigs fed adequate P without adding phytase.

Experiment 2

Growth performance

Note that the pigs were fed a common diet during phase 1 (Table 2) and the study with dietary treatments was initiated at the beginning of phase 2.

In all phases, pigs fed the PC diet had numerically improved ADG when compared to those fed the NC diets although differences were not significant ($P > 0.10$, Table 6). However, ADG increased linearly during phase 2 ($P < 0.05$), phase 4 ($P < 0.01$) and for the overall study ($P < 0.01$) with increasing levels of CEP from 0 to 1,500 FTU/kg of diet (Table 6). The linear improvement in ADG with increasing CEP during the combined phase 2 and phase 3 periods approached a tendency ($P = 0.10$). The ADG in pigs fed the greatest dose of CEP (1,500 FTU/kg of diet) was greater in phase 4 ($P < 0.08$) when compared to those fed the NC diets. Overall ADG tended to be greater ($P = 0.10$) in pigs fed all CEP levels compared to those fed the NC diets. Similarly, ADG in pigs fed the greatest level of CEP was numerically greater than that observed in pigs fed the PC diet in all phases and overall, although differences were not significant ($P > 0.10$). ADG in pigs fed HiPhos at 500 FTU/kg was similar in all phases to ADG observed in pigs fed the PC and CEP at 500 FTU/kg diet ($P > 0.10$) and ADG was not significantly improved over that observed in pigs fed the NC diet ($P > 0.10$). As might be expected based on ADG, BW at study completion increased with increasing dietary level of CEP from 0 to 1,500 FTU ($P < 0.05$). The BW also was improved in pigs fed CEP at 1,500 FTU compared to pigs fed the NC diet although differences were not significant ($P > 0.10$).

The ADFI was similar among all treatments in all phases ($P > 0.35$) with the exception of phase 4 where ADFI increased linearly ($P < 0.01$) with increasing level of CEP from 0 to 1,500

FTU/kg of feed. The ADFI in phase 4 also tended to be greater in pigs fed 1000 or 1500 FTU/kg CEP when compared to those fed the NC diet ($P < 0.10$).

Feed efficiency was similar among pigs fed the NC and PC diets. However, G:F increased linearly with increasing CEP from 0 to 1,500 FTU/kg of diet in phase 3, the combined phase 2 and 3 periods and for the overall study ($P < 0.05$). Efficiency also was numerically greater in pigs fed the greatest level of CEP in all phases when compared to pigs fed the NC diet although differences were not significant ($P > 0.14$). Efficiency was also numerically greater in pigs fed the greatest level of CEP in all phases with the exception of phase 4 when compared to those fed the PC diet.

Bone characteristics

The effects of CEP on metacarpal bone characteristics (Table 7) indicates that bone length, bone ash weight, and maximum load tended to increase linearly with increasing level of CEP from 0 to 1,500 FTU/kg of feed ($P < 0.10$). Percent ash increased both linearly and quadratically ($P < 0.001$) with increasing level of CEP from 0 to 1,500 FTU/kg of feed. In addition, fresh bone weight, ash weight, and percentage ash in pigs fed the greatest dose of CEP (1,500 FTU/kg of diet) was greater than observed in pigs fed the NC diet ($P < 0.01$). Pigs fed the greatest dose of CEP (1,500 FTU/kg of diet) had similar bone ash weight ($P > 0.05$) but lower percent ash ($P < 0.05$) when compared to those fed the PC diet. However, the opposite was observed with pigs fed 1000 FTU/kg CEP, in which percent bone ash was similar ($P > 0.05$) to PC pigs. Weight and percentage of bone ash were greater in pigs fed HiPhos at 500 FTU/kg of diet when compared to those fed the NC diet but lower when compared to pigs fed the PC diet ($P < 0.05$). Pigs fed 500 FTU/kg CEP had greater percent bone ash ($P < 0.05$) than the equivalent dose of HiPhos. For pigs fed all levels of CEP from 500 to 1,500 FTU/kg of feed demonstrated a

heavier maximum metacarpal load than both the NC and PC ($P < 0.05$). Metacarpal maximum load was not significantly different between positive and NC groups. Pigs fed GaiNzyme at 1,000 and 1,500 FTU/kg of feed showed greater maximum load than HiPhos at 500 FTU/kg ($P < 0.05$), while pigs fed CEP at 500 FTU/kg of feed had similar maximum metacarpal bone strength, with CEP being numerically greater. Increasing levels of CEP from 500 to 1,500 FTU/kg of feed increased metacarpal maximum load both linearly and quadratically ($P < .05$).

Discussion

These studies demonstrate that increasing the inclusion rate of CEP in the diet has the potential to improve ADG and G:F and BW. With the exception of phase 3 in the first experiment where feed intake increased with increasing CEP, it appeared that the differences in gain were due to an improved efficiency and not increased intake. The improved efficiency and intake in phase 3 resulted in numerically superior ADG and BW. The CEP was as effective as HiPhos on growth phenotypes when supplemented at the same level. Similar effects of phytase supplementation on growth performance were reported in various other studies, demonstrating that phytase may be able to enhance growth performance (Sands et al., 2001; Veum et al., 2006; Zeng et al., 2015). This improved performance could be associated with the different physiological and biochemical roles in growth and metabolic pathways that P is involved in, including ATP regeneration, DNA repair and recombination, RNA export, and regulation of signal transduction (Raboy, 2003). Kornegay (2001) recommended that phytase be fed between 500 and 1500 FTU/kg in swine feeds to maximize phytase activity. However, there have been several recent studies that show that super-dosing phytase, in this case CEP, in P and Ca deficient diets improves growth performance beyond the recommended phytase or P inclusion rates (Augspurger and Baker, 2004; Cowieson et al., 2006; Braña et al., 2006; Kies et al., 2006;

Pirgozliev et al., 2007; Shirley and Edwards 2003). These studies show that CEP has the ability to restore growth phenotypes in pigs fed a P and Ca deficient diet.

Several previous studies have shown that the inclusion of phytase improves apparent ileal digestibility (AID) of P, Ca and crude protein (Veum et al., 2006; Nyannor et al., 2007; Zeng et al., 2015). Two mechanisms by which super-dosing phytase contributes to faster phytate degradation have been suggested. First, Kies et al. (2006) suggested that the increased rate of phytate degradation allows for additional phytate molecules to be dissolved, however, this is at a rate that is not achieved at phytase doses of 500 FTU/kg. This is often seen by the increase in the estimated phytase release the typical commercial doses of phytases, fed at 500 to 750 FTU/kg, releasing between 0.05 and 0.15% digestible P (Adeola and Cowieson, 2011). To account for this increase, the current study used 0.15% P release. Kies et al. (2006) also suggested that super-dosing phytase helps a large part of the active phytase pass through the stomach to continue working in the small intestine. However, Zeng et al., (2015) noted that more data on phytate in the different regions of the gastrointestinal tract may be required to find the site of the digestive tract that is associated with the additional effect on mineral digestibility.

The current studies showed that adding CEP in Ca and P deficient diets restored bone characteristics; and super-dosing CEP enhanced bone ash percentage and maximum load. Previous studies have shown that phytase supplementation up to 12,000 FTU/kg in low P diets improved tibia ash from 26% to 41%, tibia ash weight, and total P retention in chickens (Shirley and Edwards, 2003). This indicates that increased levels of dietary phytase may enhance bone mineralization by increasing the availability of dietary P and Ca. Increasing overall availability of P and Ca are associated with initial mineralization and further bone complex remodeling (Shirley and Edwards, 2003). Similar results demonstrating the effects of super-dosing phytase

on bone characteristics have been shown in other studies (Kornegay and Qian, 1996; Yáñez et al., 2013; Manobhaven et al., 2016). The current studies suggest that CEP is as effective as microbial phytases and super-dosing CEP can further enhance bone characteristics.

Conclusion

Super-dosing CEP up to 4,000 FTU/kg to P and Ca deficient diets enhanced growth performance, and bone characteristics. Metacarpal bone characteristics such as bone strength and weight of fresh bone, and ash were all enhanced with super-dosing CEP. These studies demonstrate that CEP was as effective as microbial phytase, and super-dosing CEP to P and Ca deficient diet continued to improve performance, and bone characteristics of nursery pigs. Pigs fed P and Ca deficient diets supplemented with CEP above 1,000 FTU/kg performed similarly to pigs fed a diet adequate in P and Ca.

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Appendix

Table 1: Experimental diet composition for Experiment 1

	Nursery Phase 1						Nursery Phase 2						Nursery Phase 3					
Ingredients	PC+0 FTU/kg	PC+1000 FTU/kg	PC+4000 FTU/kg	NC+0 FTU/kg	NC+1000 FTU/kg	NC+4000 FTU/kg	PC+0 FTU/kg	PC+1000 FTU/kg	PC+4000 FTU/kg	NC+0 FTU/kg	NC+1000 FTU/kg	NC+4000 FTU/kg	PC+0 FTU/kg	PC+1000 FTU/kg	PC+4000 FTU/kg	NC+0 FTU/kg	NC+1000 FTU/kg	NC+4000 FTU/kg
Corn, %	38.573	38.547	38.469	39.808	39.782	39.704	44.330	44.330	44.330	45.555	45.555	45.555	47.280	47.254	47.176	48.480	48.454	48.376
Soybean Meal, %	25.000	25.000	25.000	25.000	25.000	25.000	31.600	31.600	31.600	31.600	31.600	31.600	31.600	31.600	31.600	31.600	31.600	31.600
DDGs, %	0.000	0.000	0.000	0.000	0.000	0.000	15.000	15.000	15.000	15.000	15.000	15.000	15.000	15.000	15.000	15.000	15.000	15.000
Poultry Fat, %	3.000	3.000	3.000	2.500	2.500	2.500	3.000	3.000	3.000	2.500	2.500	2.500	3.000	3.000	3.000	2.500	2.500	2.500
Monocalcium P, %	0.800	0.800	0.800	0.065	0.065	0.065	0.750	0.750	0.750	0.025	0.025	0.025	0.700	0.700	0.700	0.000	0.000	0.000
Limestone, %	0.500	0.500	0.500	0.500	0.500	0.500	0.850	0.850	0.850	0.850	0.850	0.850	0.950	0.950	0.950	0.950	0.950	0.950
Salt, %	0.250	0.250	0.250	0.250	0.250	0.250	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500
L-Lysine, %	0.220	0.220	0.220	0.220	0.220	0.220	0.360	0.360	0.360	0.360	0.360	0.360	0.360	0.360	0.360	0.360	0.360	0.360
DL-Methionine, %	0.175	0.175	0.175	0.175	0.175	0.175	0.114	0.114	0.114	0.114	0.114	0.114	0.114	0.114	0.114	0.114	0.114	0.114
L-Threonine, %	0.053	0.053	0.053	0.053	0.053	0.053	0.066	0.066	0.066	0.066	0.066	0.066	0.066	0.066	0.066	0.066	0.066	0.066
Trace Mineral Premix, %	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150
Vitamin Premix, %	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250
Plasma, %	2.500	2.500	2.500	2.500	2.500	2.500	0.500	0.500	0.500	0.500	0.500	0.500	-	-	-	-	-	-
Fish Meal, %	5.000	5.000	5.000	5.000	5.000	5.000	2.500	2.500	2.500	2.500	2.500	2.500	-	-	-	-	-	-
Whey Powder, %	20.000	20.000	20.000	20.000	20.000	20.000	-	-	-	-	-	-	-	-	-	-	-	-
Corn Phytase, %	0.000	0.026	0.104	0.000	0.026	0.104	0.000	0.026	0.104	0.000	0.026	0.104	0.000	0.026	0.104	0.000	0.026	0.104
Ethoxiquin, %	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030
Lactose, %	3.500	3.500	3.500	3.500	3.500	3.500	-	-	-	-	-	-	-	-	-	-	-	-

Table 2: Experimental diet composition for Experiment 2

	Nursery	Nursery Phase 2						Nursery Phase 3						Nursery Phase 4					
	Phase 1			GralNyme			HIPhos			GralNyme			HIPhos			GralNyme			HIPhos
Ingredients		PC	NC	NC+500 FTU/kg	NC+ 1000	NC+150 0	NC+500 FTU/kg	PC	NC	NC+500 FTU/kg	NC+ 1000	NC+150 0	NC+500 FTU/kg	PC	NC	NC+500 FTU/kg	NC+ 1000	NC+150 0	NC+500 FTU/kg
Corn, %	42.100	40.905	42.021	41.997	41.973	41.949	42.017	44.204	45.373	45.349	45.323	45.301	45.369	47.956	49.069	49.045	#####	48.998	49.066
Soybean Meal, %	19.000	27.650	27.650	27.650	27.650	27.650	27.650	31.400	31.400	31.400	31.400	31.400	31.400	30.550	30.550	30.550	#####	30.550	30.550
DDGS, %	0.000	10.000	10.000	10.000	10.000	10.000	10.000	15.000	15.000	15.000	15.000	15.000	15.000	15.000	15.000	15.000	#####	15.000	15.000
Poultry Fat, %	3.000	3.000	2.550	2.550	2.550	2.550	2.550	3.000	2.500	2.500	2.500	2.500	2.500	3.000	2.550	2.550	2.550	2.550	2.550
Monocalcium P, %	0.690	1.175	0.460	0.460	0.460	0.460	0.460	0.715	0.000	0.000	0.000	0.000	0.000	0.710	0.000	0.000	0.000	0.000	0.000
Limestone, %	0.585	0.788	0.845	0.845	0.845	0.845	0.845	0.860	0.910	0.910	0.910	0.910	0.910	0.945	1.000	1.000	1.000	1.000	1.000
Salt, %	0.250	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500
L-Lysine, %	0.220	0.315	0.312	0.312	0.312	0.312	0.312	0.360	0.360	0.360	0.360	0.360	0.360	0.390	0.388	0.388	0.388	0.388	0.388
DL-Methionine, %	0.160	0.152	0.149	0.149	0.149	0.149	0.149	0.135	0.135	0.135	0.135	0.135	0.135	0.123	0.120	0.120	0.120	0.120	0.120
L-Threonine, %	0.038	0.059	0.057	0.057	0.057	0.057	0.057	0.078	0.078	0.078	0.078	0.078	0.078	0.078	0.078	0.078	0.078	0.078	0.078
L-Tryptophan, %	0.028	0.028	0.027	0.027	0.027	0.027	0.027	0.019	0.019	0.019	0.019	0.019	0.019	0.017	0.017	0.017	0.017	0.017	0.017
Trace Mineral Premix, %	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150
Vitamin Premix, %	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250
Plasma, %	5.000	2.500	2.500	2.500	2.500	2.500	2.500	0.500	0.500	0.500	0.500	0.500	0.500	-	-	-	-	-	-
Fish Meal, %	5.000	2.500	2.500	2.500	2.500	2.500	2.500	2.500	2.500	2.500	2.500	2.500	2.500	-	-	-	-	-	-
Milk, Whey Powder, %	20.000	10.000	10.000	10.000	10.000	10.000	10.000	-	-	-	-	-	-	-	-	-	-	-	-
Corn Phytase, %	-	0.000	0.000	0.024	0.048	0.071	0.000	0.000	0.000	0.024	0.048	0.071	0.000	0.000	0.000	0.024	0.048	0.071	0.000
Ethoxiquin, %	-	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030
Milk, Lactose, %	3.500	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TiO2, %	-	-	-	-	-	-	-	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300
HiPhos, %	-	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.003

Table 3: Main effects of corn-expressed phytase on growth performance (Exp 1, LS means)

								P - Value				
	Phosphorus			Phytase, FTU/kg				Main		P level*FTU		
	Adequate	Low	SEM	0	1000	4000	SEM	P level	Phytase	P level	Linear	Quadratic
ADG, kg/d												
Phase 1 ADG	0.052	0.046	0.007	0.047	0.041	0.059	0.008	0.48	0.222	0.48	0.149	0.333
Phase 2 ADG	0.45	0.463	0.023	0.449	0.462	0.459	0.024	0.412	0.768	0.412	0.691	0.546
Phase 3 ADG	0.647^{b}	0.654^{bc}	0.025	0.637^{a}	0.648^{b}	0.666^{c}	0.026	0.559	0.169	0.559	0.064	0.755
Phase 1-2 ADG	0.236	0.239	0.011	0.233	0.235	0.244	0.012	0.762	0.597	0.762	0.314	0.972
Phase 1-3 ADG	0.38	0.384	0.015	0.374	0.38	0.392	0.016	0.618	0.268	0.618	0.108	0.895
Intake, kg/d												
Phase 1 ADFI	0.146	0.135	0.009	0.138	0.13	0.153	0.011	0.292	0.203	0.292	0.143	0.303
Phase 2 ADFI	0.645	0.701	0.046	0.672	0.654	0.693	0.05	0.133	0.68	0.133	0.517	0.557
Phase 3 ADFI	0.974	0.966	0.045	0.963	0.947	1	0.046	0.736	0.156	0.736	0.101	0.304
Phase 1-2 ADFI	0.377	0.396	0.024	0.385	0.372	0.402	0.026	0.318	0.443	0.318	0.324	0.42
Phase 1-3 ADFI	0.586	0.596	0.03	0.587	0.573	0.612	0.032	0.584	0.231	0.584	0.164	0.316
G:F												
Phase 1 G:F	0.337	0.305	0.043	0.32	0.275	0.368	0.048	0.475	0.257	0.475	0.223	0.265
Phase 2 G:F	0.716	0.693	0.035	0.691	0.747	0.676	0.042	0.62	0.432	0.62	0.544	0.254
Phase 3 G:F	0.669	0.68	0.01	0.664	0.688	0.672	0.012	0.434	0.364	0.434	0.93	0.159
Phase 1-2 G:F	0.638	0.621	0.025	0.619	0.656	0.613	0.029	0.594	0.491	0.594	0.646	0.273
Phase 1-4 G:F	0.655	0.65	0.013	0.642	0.67	0.645	0.016	0.732	0.306	0.732	0.787	0.132
BW, kg												
Initial	5.95^{ab}	5.97^{ab}	0.48	5.91^{a}	5.99^{b}	5.99^{b}	0.478	0.587	0.077	0.587	0.117	0.095
End of Phase 1	6.68^{ab}	6.61^{ab}	0.47	6.57^{a}	6.56^{a}	6.81^{b}	0.475	0.596	0.191	0.596	0.081	0.625
End of Phase 2	12.08	12.18	0.71	11.97	12.1	12.32	0.725	0.702	0.525	0.702	0.264	0.882
End of Phase 3	21.14^{b}	21.34^{bc}	1.05	20.88^{a}	21.18^{b}	21.65^{c}	1.069	0.586	0.242	0.586	0.098	0.801

[x.y.z.] LS means with different superscripts tend to be different ($P \leq 0.10$).

[1.] IML procedure of SAS was used to estimate coefficient which then being used in orthogonal contrast analysis for NC, NC+GralNzyme 500 FTU/kg, NC+GralNzyme 1,000 FTU/kg, and NC+GralNzyme 1,500 FTU/kg.

Table 4: P level by phytase interaction effects of corn-expressed phytase on growth performance in nursery pigs (LS means)

	Adequate Phosphorus			Low Phosphorus				*P* - Value		
								P level*FTU		
	0	1000	4000	0	1000	4000	SE	Interaction	Linear	Quadratic
ADG, kg/d										
Phase 1 ADG	0.049	0.039	0.049	0.045	0.043	0.068	0.011	0.538	0.271	0.912
Phase 2 ADG	0.464	0.469	0.457	0.434	0.455	0.462	0.028	0.659	0.375	0.851
Phase 3 ADG	0.665	0.647	0.651	0.609	0.65	0.682	0.028	0.024	0.015	0.2
Phase 1-2 ADG	0.241	0.237	0.238	0.225	0.233	0.25	0.014	0.455	0.22	0.828
Phase 1-3 ADG	0.389	0.381	0.382	0.359	0.379	0.401	0.018	0.089	0.04	0.428
Intake, kg/d										
Phase 1 ADFI	0.136	0.126	0.143	0.141	0.134	0.164	0.014	0.803	0.515	0.922
Phase 2 ADFI	0.713	0.691	0.698	0.632	0.616	0.688	0.059	0.68	0.39	0.885
Phase 3 ADFI	0.959	0.96	0.98	0.968	0.933	1.02	0.05	0.48	0.403	0.382
Phase 1-2 ADFI	0.402	0.387	0.399	0.368	0.356	0.406	0.031	0.638	0.353	0.878
Phase 1-3 ADFI	0.597	0.588	0.602	0.578	0.558	0.621	0.035	0.533	0.315	0.626
G:F										
Phase 1 G:F	0.335	0.252	0.327	0.305	0.298	0.41	0.062	0.591	0.367	0.631
Phase 2 G:F	0.666	0.742	0.672	0.717	0.751	0.68	0.058	0.911	0.758	0.765
Phase 3 G:F	0.695	0.677	0.668	0.633	0.698	0.676	0.017	0.042	0.133	0.039
Phase 1-2 G:F	0.606	0.65	0.607	0.632	0.662	0.62	0.04	0.979	0.888	0.882
Phase 1-4 G:F	0.653	0.658	0.638	0.631	0.682	0.653	0.021	0.456	0.488	0.298
BW, kg										
Initial	5.92	5.99	6	5.9	5.98	5.97	0.479	0.969	0.821	0.916
End of Phase 1	6.6	6.54	6.69	6.53	6.58	6.93	0.488	0.608	0.326	0.896
End of Phase2	12.2	12.17	12.18	11.74	12.04	12.47	0.757	0.486	0.243	0.803
End of Phase 3	21.51	21.23	21.28	20.26	21.13	22.02	1.116	0.103	0.048	0.429

Table 5: Effect of GralNzyme on Metacarpal Bone Characteristics in Nursery Pigs (Experiment 1, LS Means).

	Adequate Phosphorus			Low Phosphorus				*P* - Value		
									Plevel*FTU	
	0	1000	4000	0	1000	4000	SE	P level	Linear	Quadratic
Ash, g	0.92	1.02	1.04	0.77	0.85	0.97	0.06	0.2301	0.1038	0.603
Ash, %	0.25	0.26	0.26	0.22	0.24	0.24	0.01	0.2513	0.163	0.3657
Width, mm	8.57^{a}	8.83^{c}	8.6^{b}	8.53^{a}	8.66^{b}	8.8^{c}	0.17	0.1442	0.1	0.2745
Length, mm	31.36^{b}	31.4^{ab}	30.85^{a}	30.82^{a}	31.29^{b}	31.64^{c}	0.61	0.0641	0.02	0.8478

[a.b.c.] LS means with different superscripts are significantly different (P < 0.10).

[1.] IML procedure of SAS was used to estimate coefficient which then being used in orthogonal contrast analysis for NC, NC+GralNzyme 500 FTU/kg, NC+GralNzyme 1,000 FTU/kg, and NC+GralNzyme 1,500 FTU

Table 6: Effect of GralNzyme on Growth Performance in Nursery Pigs (Experiment 2, LS means)

	PC	NC	NC + GraINzyme			NC + HiPhos		*P* - Value		
FTU/kg			500	1,000	1,500	500	SEM	Treatment	Linear[1]	Quadratic[1]
ADG, kg/d										
Phase 2	0.172	0.143	0.176	0.18	0.187	0.17	0.014	0.3694	0.0412	0.3682
Phase 3	0.419	0.401	0.43	0.438	0.434	0.412	0.019	0.6901	0.2013	0.3596
Phase 4	0.633^{xy}	0.579^{x}	0.640^{xy}	0.634^{xy}	0.682^{y}	0.635^{xy}	0.023	0.0788	0.0044	0.7594
Phase 2&3	0.337	0.315	0.345	0.352	0.351	0.331	0.015	0.5256	0.1016	0.3182
Overall	0.455^{xy}	0.421^{x}	0.463^{y}	0.465^{y}	0.483^{y}	0.453^{xy}	0.015	0.1016	0.0063	0.4157
BW, kg										
Initial	6.67	6.7	6.66	6.73	6.64	6.64	0.28	0.9846	0.8205	0.8112
End of phase 2	7.88	7.7	7.89	7.99	7.95	7.83	0.28	0.8578	0.253	0.49
End of phase 3	13.75	13.32	13.91	14.12	14.02	13.6	0.47	0.693	0.1799	0.3643
End of phase 4	22.61	21.43	22.87	23	23.56	22.49	0.67	0.1846	0.0135	0.4355
ADFI, kg/d										
Phase 2	0.273	0.209	0.256	0.243	0.246	0.24	0.02	0.352	0.2792	0.2747
Phase 3	0.707	0.661	0.697	0.629	0.631	0.646	0.045	0.6582	0.3956	0.6755
Phase 4	0.992^{xyz}	0.913^{x}	0.999^{xyz}	1.018^{yz}	1.056^{z}	0.952^{xy}	0.033	0.0603	0.0041	0.4675
Phase 2&3	0.563	0.511	0.55	0.501	0.503	0.511	0.033	0.6041	0.5999	0.5479
Overall	0.734	0.671	0.73	0.707	0.724	0.687	0.028	0.5198	0.2752	0.4477
G:F										
Phase 2	0.672	0.674	0.685	0.75	0.75	0.722	0.046	0.587	0.1282	0.9011
Phase 3	0.623	0.621	0.631	0.705	0.712	0.653	0.041	0.2419	0.0312	0.9675
Phase 4	0.665	0.638	0.641	0.637	0.654	0.667	0.017	0.6936	0.5962	0.6807
Phase 2&3	0.624	0.627	0.639	0.709	0.717	0.662	0.035	0.1382	0.0172	0.9517
Overall	0.644	0.63	0.638	0.666	0.679	0.663	0.019	0.4122	0.0416	0.9043

[x.y.z.] LS means with different superscripts tend to be different ($P \leq 0.10$).

[1.] IML procedure of SAS was used to estimate coefficient which then being used in orthogonal contrast analysis for NC, NC+GralNzyme 500 FTU/kg, NC+GralNzyme 1,000 FTU/kg, and NC+GralNzyme 1,500 FTU/kg.

Table 7: Effect of GralNzyme on Metacarpal Bone Characteristics in Nursery Pigs (Experiment 2, LS means)

	PC	NC	NC + GraINzyme			NC + HiPhos		*P* - Value		
FTU/kg			500	1,000	1,500	500	SEM	Treatment	Linear[1]	Quadratic[1]
Length, mm	36.87	36.31	36.57	36.75	37	36.39	0.31	0.3862	0.0627	0.9796
Width, mm	9.248	9.113	9.148	9.048	9.365	9.3	4.149	0.451	0.2403	0.2592
Fresh bone, g	4.314^{bc}	3.872^{a}	4.091^{ab}	4.090^{ab}	4.393^{c}	4.134^{b}	0.11	0.003	0.636	0.1876
Ash, g	1.122^{d}	0.840^{a}	0.984^{bc}	1.037^{c}	1.098^{cd}	0.951^{b}	0.029	<0.0001	0.0878	0.3522
Ash, %	26.010^{e}	21.610^{a}	24.070^{c}	25.450^{de}	25.090^{d}	23.080^{b}	0.284	<0.0001	<0.0001	<0.0001
Max Load, kgf	35.355^{a}	32.803^{a}	40.089^{bc}	41.648^{c}	42.410^{c}	37.029^{ab}	1.566	<.0001	<.0001	0.0223

[a.b.c.] LS means with different superscripts are significantly different (P < 0.05).

[1.] IML procedure of SAS was used to estimate coefficient which then being used in orthogonal contrast analysis for NC, NC+GralNzyme 500 FTU/kg, NC+GralNzyme 1,000 FTU/kg, and NC+GralNzyme 1,500 FTU

Chapter 3: Effects of Xylanase and Super-Dosing Phytase on Nursery and Grow-Finish Performance

Effects of Xylanase and Super-Dosing Phytase on Nursery and Grow-Finish Performance

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Abstract

This experiment was conducted to determine the effect of xylanase and super-dosing phytase on nursery and grow-finish performance. A total of 270 pigs were blocked by initial BW and allotted to 1 of 5 treatments (9 replicates per treatment). Pens were assigned randomly to dietary treatments. Treatments were: a) Positive Control (PC), P adequate diet (NRC, 2012) with 500 FTU/kg of feed phytase credit on aP (0.12%) and Ca (0.13%) release; b) Negative Control (NC), 98% of ME as PC and AA deficient diet with 500 FTU/kg of feed; c) NC diet supplemented with 15,000 FTU phytase/kg of diet, and 0, d) 12,000 and e) 16,000 Danisco endo-xylanase units (DXU) of xylanase/kg of diet. Pigs were fed a common phase 1 (14 d) diet before treatments were initiated in phase 2 (14 d) and 3 (14 d). At the end of phase 3, 35 pens (210 pigs) continued on the grower/finisher experiment and were fed a five-phase feeding regime. Data were analyzed by MIXED procedures of SAS (SAS, Cary, NC) with treatment as the fixed effect and initial BW block as a random effect. Orthogonal contrasts were performed to determine the effects of increasing xylanase. Increasing xylanase improved ADG quadratically in the overall grower/finisher period, and for the entire study ($P < 0.05$), which restored final BW in pigs fed superdosing levels of phytase together with 16,000 DXU/kg of xylanase to the level observed in PC. Pigs fed the NC with 16,000 DXU/kg had a greater ADFI in nursery than PC ($P < 0.01$). The G:F was greater in PC than NC in overall nursery and grower ($P < 0.05$). Pigs fed the NC and 12,000 DXU had reduced G:F during overall grower, and grower/finisher periods as well as the entire study when compared to all other treatments ($P < 0.05$). However, pigs fed NC with 16,000 DXU/kg or superdosing phytase alone had a similar G:F as the PC in the overall grower,

overall grower/finisher periods and for the entire study. In addition, pigs fed superdosing level of phytase and 16,000 DXU/kg of diet had similar carcass composition when compared to pigs fed the NC diet ($P < 0.05$). Results of the current study suggests that superdosing phytase and 16,000 DXU/kg xylanase in pigs fed reduced nutrient diets can restore growth and carcass composition to levels observed in pigs fed the PC diet.

Keywords: xylanase, phytase, growth performance

Introduction

Many of the coproducts that are used in swine diets to reduce feed cost contain high levels of phytate and fiber (Jaworski et al., 2015; Rojas et al., 2013). Phytate is the main storage form of phosphorus (P) in plant-based feedstuffs (Lavin et al., 2013; Yáñez et al., 2013; Graf, 1983; Chung et al., 2013). Pigs are unable to break down phytate and xylan hemicellulose due to lack of adequate endogenous enzymes secreted (Pointillart et al., 1987; Nortey et al., 2008; Selle and Ravindran, 2008). This makes the use of exogenous enzymes useful in improving nutrient utilization. The supplementation of exogenous phytase has become common in industry to hydrolyze phytate to increase P availability and subsequently reduce the P excreted (Cromwell et al., 1993, 1995; Liu et al., 1998; Walk et al., 2013). Furthermore, high levels of phytase supplementation have been shown to improve growth rate and feed efficiency in pigs by improving P digestibility (Mroz et al., 1992; Adeola and Cowieson, 2011; Adedokun et al., 2015).

Xylanase supplementation improves fiber digestibility and has been shown to enhance nutrient digestibility (Passos et al., 2015). However, Woyengo et al. (2008) reported that the simultaneous inclusion of xylanase and phytase in wheat-based diets for growing pigs had no synergistic effects for nutrient digestibility, while Ndou et al. (2015) reported that

supplementation of phytase and xylanase improved growth rate, feed efficiency and ATTD of fat. Jang et al. (2017) showed no significant interactions between xylanase and phytase supplementation on growth performance, carcass characteristics and apparent total tract digestibility. However, it appears that the varying results for xylanase supplementation are dependent on different amounts of substrates for xylanase depending on which feed ingredients were used (Diebold et al., 2004). Overall, xylanase has been shown to enhance nutrient digestibility (Atakora et al., 2011). superdosing of phytase at very high dietary levels has been shown to enhance growth performance beyond the expected improvement in performance as a result of release of phytate P (Shirley and Edwards, 2003; Cowieson et al., 2006; Braña et al., 2006; Kies et al., 2006). Therefore, the objective of this study was to evaluate efficacy of superdosing phytase and determine the effect of increasing dietary xylanase on growth performance in pigs fed a superdosing level of phytase.

Materials and Methods

The institutional Animal Care and Use Committee at the University of Arkansas reviewed and approved the protocols for both experiments.

Animals and Experimental Design

Nursery phases

A total 270 crossbred pigs at weaning (21 days of age) were blocked by gender and initial BW into 9 weight blocks; within blocks, pens of pigs (45 pens with 6 pigs/pen) were randomly assigned to 1 of 5 dietary treatments (9 reps/treatment). A 3-phase feeding program was utilized with pigs fed different diets in each of the three phases. All diets were typical nursery diets that were formulated to meet nutrient requirements (NRC, 2012; Table 8). These pigs were fed a common starter diet for 14 days to allow time for acclimation to weaning. Pigs were housed in

1.49 × 1.20 M^2 pens at the University of Arkansas conventional nursery facility, with *ad libitum* access to feed and water for the duration of the experiment.

Grower/Finisher Phases

A total of 35 median-weight-block pens of pigs (210 pigs) continued on the experiment. A four-phase feeding program was utilized and pigs were continued on the same treatments they received during the nursery phase. Pigs were fed different diets in each of the four phases. All diets were typical corn-soy grower and finisher diets (Table 9 and 10). Pigs were housed in 1.49 × 2.99 M^2 pens the University of Arkansas Grow-Finisher facility, with *ad libitum* access to feed and water for the duration of the experiment. Tenth rib backfat measurements and LM area were estimated on all pigs at the end of the study to estimate carcass fat-free lean.

Experimental Diets

A starter diet was fed for the first 14 days to allow time for acclimation to weaning (Table 8). Nursery phase 1 and phase 2 was fed for 14 days (Table 8). For the remainder of the nursery and grower/finisher phases (Tables 9 and 10, respectively). Pigs were fed one of the following treatments: Treatment 1, the positive control diet (PC) was formulated with 500 FTU/kg of phytase accounting for aP (0.12%) and Ca (0.13%) release from phytase, to meet nutrient requirements (NRC, 2012). Treatment 2, the negative control (NC) was formulated with 500 FTU/kg of phytase, with reduced AAs and to meet 98% of the metabolizable energy compared to the PC. Treatments 3, 4 and 5 were the NC with: 3, 15,000 FTU/kg of phytase (0.03%), 4, 12,000 DXU of xylanase/kg (0.008%) and 15,000 FTU of phytase/kg (0.03%), and 5, 16,000 DXU of xylanase/kg (0.01%) of diet and 15,000 FTU phytase/kg (0.03%) of diet respectively.

Sample Collection and Processing

At the start of the study and at the end of each phase, individual pig weights, as well as pen feed disappearance, were measured for each phase to calculate average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G:F). Feed samples were obtained for each batch of feed mixed. These were accumulated for each phase and subsampled for nutrient analysis.

Statistical Analysis

The performance and bone ash data were analyzed using the MIXED procedures of SAS. Treatment was the fixed effect. Orthogonal contrasts were used to determine the effects of phytase and xylanase level on growth performance. Probability values were considered statistically significant at $P < 0.05$, and 0.05 to $P < 0.10$ considered a statistical trend.

Results

Growth Performance

During the overall nursery phase of the study, pigs fed the nutrient deficient diet, with 0.03% phytase and 0.01% xylanase grew more rapidly ($P < 0.06$) than pigs fed the PC diet formulated to normal amino acid specifications and containing 500 FTU phytase/kg of diet (Table 11). During the grower 2 phase and the overall grower phase, pigs fed the nutrients deficient diet with 0.03% phytase and 0.008% xylanase had reduced ADG when compared to those fed the 0.03% phytase diet or the phytase and 0.01% xylanase. This resulted in a significant quadratic phytase × xylanase effect ($P < 0.01$). A similar quadratic effect was observed for the overall grower/finisher phase and for the entire nursery/growing/finishing study ($P < 0.05$).

During nursery phase 2 and for the overall nursery phase, pigs fed the NC diet consumed more feed than those fed the PC diet ($P < 0.10$ and $P < 0.05$, respectively; Table 12). Similarly, pigs fed the nutrient deficient diet with 0.03% phytase and 0.01% xylanase consumed more feed ($P < 0.01$) in nursery phase 2 and for the overall nursery period when compared to pigs fed the positive control diet.

Feed efficiency was greater in pigs fed the PC diet (Table 13) when compared to those fed the NC diet in nursery phase 2 and the overall nursery period as well as during grower phase 2 and the overall grower phase ($P < 0.05$). Similarly, G:F was greater during nursery phase 2 and the overall nursery period in pigs fed the PC diet when compared to those fed the nutrient deficient diet with 0.03% phytase and 0.01% xylanase ($P < 0.10$ and $P < 0.05$, respectively). Additionally, pigs fed the nutrient deficient diet with 0.03% phytase and 0.008% xylanase had reduced G:F during grower phase 2, the overall grower period, the combined growing and finishing period as well as the overall study when compared to all other treatments ($P < 0.05$). In contrast, when pigs were fed 0.03% phytase and 0.01% xylanase or 0.03% phytase alone, G:F was restored to a level similar to the PC in grower 2, grower overall, grower/finisher overall and the overall study. It should be noted that G:F in the grower 2, the overall grower period, the overall grower/finishing phases as well as the overall study tended to decrease with the inclusion of 0.008% xylanase with phytase but improved with the increased inclusion level of 0.01% xylanase ($P < 0.001$, quadratic effect).

As might be expected based on ADG, pigs fed the nutrient deficient diet with 0.03% phytase and 0.01% xylanase tended to be heavier at the end of the nursery phase 2 ($P < 0.10$; Table 14) when compared to pigs fed the PC diet. However, pigs fed the nutrient deficient diet with 0.03% phytase and 0.008% xylanase had reduced BW when compared to all other

treatments at the end of the grower 2 period and were lighter than pigs fed all other treatments with the exception of the pigs fed the NC diet with 0.03% phytase at the end of the finishing 1 period ($P < 0.05$). However, BW was similar among all treatments at study completion.

Carcass Characteristics

The effect phytase on carcass composition are presented in Table 15. Pigs fed the NC diet had increased yield compared to those fed the PC diet ($P < 0.05$). Percent lean was greater in pigs fed the NC diet when compared to those fed the nutrient deficient diet with 0.03% phytase and 0.01% xylanase ($P < 0.05$). Loin muscle depth was greater in pigs fed the PC diet when compared to those fed 0.03% phytase or 0.03% phytase with either 0.008% xylanase or 0.01% xylanase ($P < 0.05$). The 10^{th} rib back fat was greater in pigs fed 0.03% phytase with either 0.008% xylanase or 0.01 % xylanase when compared to those fed the NC diet or the NC diet with 0.03% phytase ($P < 0.05$).

Discussion

This study evaluated the combined effects of super-dosing xylanase and phytase on growth performance and carcass characteristics. There were no differences in weight gain and feed efficiency between the different levels of xylanase supplementation in the nursery phase. However, during the grower phases a quadratic response was observed with increasing xylanase supplementation. The phytase and xylanase supplementation in the pigs fed 0.03% phytase and 0.008% xylanase resulted in a reduced ADG and BW when compared to super-dosing phytase alone or the higher level of xylanase supplementation (0.01%). The pigs fed the diet with 0.03% phytase alone and the 0.03% phytase with 0.01% xylanase had similar grower phase 2 and finisher phase 1 ADG and BW compared to the pigs fed the PC diet.

It has been well documented that phytase addition increases P digestibility (Kerr et al., 2010, Adeola et al., 2004; Cowieson et al., 2011). Phytase dephosphorylates the phytate found in cereal grains to free P while also liberating nutrients that are bound to phytate, thereby, providing more nutrients to pigs fed phytase-containing diets (Selle and Ravindran, 2008). Additionally, super-dosing (feeding high levels) of phytase may remove the antinutritional effects that phytate has on the diets for a more efficient use of minerals and other dietary ingredients (Cowieson et al., 2004; Cowieson et al., 2011; Selle et al., 2012). Previous studies have shown that as phytase supplementation increases, it results in increased ADG and G:F, in low-P or P-deficient diets through increased P digestibility (Braña et al, 2006; Zeng et al., 2014). This is seen in the present study where the inclusion of 0.03% phytase restored the growth phenotypes of the NC to levels similar to that of the PC.

Woyengo et al. (2008) reported no synergistic response with the simultaneous inclusion of xylanase and phytase, for pigs fed a diet with reduced Ca and P, on any of the response criteria measured. While in the present study, increasing the xylanase concentrations resulted in increased back fat thickness and decreased percent lean ($P < 0.05$). However, in the study by Jang et al. (2017) no effect of xylanase supplementation was seen on carcass characteristics in pigs fed a diet with reduced metabolizable energy. Jang et al. (2017) did show an improved carcass leanness that was improved by phytase supplementation.

Several explanations have been proposed to explain the inconsistent effect of xylanase supplementation on growth characteristics. It is thought that digesta flow rate is different for different feeding methods, and possibly *ad libitum* access to feed alters the xylanase response (Dierick et al., 1989). Furthermore, the availability of different substrates for xylanase, depending on which feed ingredients that are included in the diets, could explain the inconsistent

results between studies (Diebold et al., 2004). Finally, the duration of feeding the treatment may impact the inconsistency of the response to xylanase supplementation. Jang et al. (2017) fed xylanase for 100 d before the digestibility assessments were made, where no differences were seen; while other previous studies showed positive responses from xylanase supplementation fed for less than 55 d (Nortey et al., 2008; Woyengo et al., 2008). Longland et al. (1993) reported that non-starch polysaccharide digestibility in wheat increased over time as pigs responded to the dietary fiber from cereal basal diets. Therefore, an increased feeding duration could allow pigs and the gut microbes time to adapt to a high fiber diet, which could result in a decreased effect of xylanase supplementation. Furthermore, Urriola and Stein (2012) showed that the innate fiber digestibility of pigs and total capacity for fiber degradation increases as BW increases, which may result in a diminished xylanase response in heavier pigs when compared to lighter pigs.

Conclusion

Super-dosing phytase in energy deficient diets restored growth performance, while feeding 0.03% phytase and 0.01% xylanase restored growth performance and carcass characteristics similar to that of pigs fed the PC. The effects of phytase and xylanase increased ADG, BW, and feed efficiency in the grower and early finisher phases. These studies demonstrate that phytase and xylanase supplemented in energy deficient diets restored performance, and carcass characteristics in pigs. Pigs fed energy deficient diets supplemented with 0 or 16,000 DXU/kg of xylanase performed similarly to pigs fed a diet with adequate energy.

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Appendix

Table 8: Experimental diet composition nursery phases (as fed basis)

Ingredients	Starter	Nursery Phase 1					Nursery Phase 2				
		PC	NC	NC+1500 FTU/kg	NC+1200 BXU/kg	NC+1600 BXU/kg	PC	NC	NC+1500 FTU/kg	NC+1200 BXU/kg	NC+1600 BXU/kg
Corn, %	32.76	57.24	60.22	60.30	60.29	60.29	61.17	64.22	64.22	64.21	64.21
Soybean Meal, %	11.00	21.00	19.35	19.25	19.25	19.25	23.03	21.30	21.30	21.30	21.30
Soycomil-P, %	5.00	-	-	-	-	-	-	-	-	-	-
Poultry Fat, %	2.50	2.32	1.05	1.05	1.05	1.05	2.35	1.08	1.08	1.08	1.08
Monocalcium P, %	-	0.10	0.10	0.10	0.10	0.10	0.27	0.27	0.27	0.27	0.27
Limestone, %	0.70	0.77	0.77	0.77	0.77	0.77	1.00	1.00	1.00	1.00	1.00
Salt, %	0.25	0.40	0.40	0.40	0.40	0.40	0.55	0.55	0.55	0.55	0.55
L-Lysine, %	0.25	0.41	0.41	0.41	0.41	0.41	0.55	0.55	0.55	0.55	0.55
DL-Methionine, %	0.14	0.15	0.12	0.12	0.12	0.12	0.13	0.11	0.11	0.11	0.11
L-Threonine, %	0.04	0.11	0.10	0.10	0.10	0.10	0.15	0.14	0.14	0.14	0.14
L-Tryptophan, %	0.01	0.04	0.03	0.03	0.03	0.03	0.05	0.04	0.04	0.04	0.04
ZnO, %	0.32	-	-	-	-	-	-	-	-	-	-
Copper Sulfate, %	0.06	-	-	-	-	-	-	-	-	-	-
Trace Mineral Premix, %	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin Premix, %	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Plasma, %	4.00	2.00	2.00	2.00	2.00	2.00	-	-	-	-	-
Fish Meal, %	6.50	3.00	3.00	3.00	3.00	3.00	-	-	-	-	-
Milk, Whey Powder, %	20.00	12.00	12.00	12.00	12.00	12.00	-	-	-	-	-
Quantum Blue, %	0.04	0.01	0.01	0.03	0.03	0.03	0.0068	0.0068	0.0068	0.0068	0.0068
Ethoxiquin, %	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
TiO2, %	-	-	-	-	-	-	0.30	0.30	0.30	0.30	0.30
Milk, Lactose, %	3.50	-	-	-	-	-	-	-	-	-	-
Oat Groat, %	12.50	-	-	-	-	-	-	-	-	-	-
L-valine, %	-	0.03	0.03	0.03	0.03	0.03	0.02	0.01	0.01	0.01	0.01
Econase XT 25, %	-	-	-	-	0.008	0.010	0.00	0.00	0.00	0.008	0.010
Corn DDGS, %	-	-	-	-	-	-	10.00	10.00	10.00	10.00	10.00

Table 9: Experimental diet composition grower phases (as fed basis)

Ingredients	Grower Phase 1					Grower Phase 2				
	PC	NC	NC+1500 FTU/kg	NC+12000 BXU/kg	NC+16000 BXU/kg	PC	NC	NC+1500 FTU/kg	NC+12000 BXU/kg	NC+16000 BXU/kg
Corn, %	63.572	68.035	68.012	68.005	68.002	69.344	72.944	72.921	72.913	68.002
Soybean Meal, %	11.250	8.940	8.940	8.940	8.940	5.750	4.300	4.300	4.300	8.940
Corn DDGS, %	20.000	20.000	20.000	20.000	20.000	20.000	20.000	20.000	20.000	20.000
Fat, %	2.500	0.460	0.460	0.460	0.400	2.500	0.460	0.460	0.460	0.400
Calcium Phosphate, %	0.210	0.210	0.210	0.210	0.210	0.075	0.085	0.085	0.085	0.210
Limestone, %	1.115	1.115	1.115	1.115	1.115	1.020	1.030	1.030	1.030	1.115
Sodium Chloride, %	0.170	0.170	0.170	0.170	0.170	0.100	0.100	0.100	0.100	0.170
L-Lysine, %	0.575	0.600	0.600	0.600	0.600	0.585	0.585	0.585	0.585	0.600
DL-Methionine, %	0.067	0.031	0.031	0.031	0.031	0.044	0.001	0.001	0.001	0.031
L-Threonine, %	0.133	0.112	0.112	0.112	0.112	0.140	0.107	0.107	0.107	0.112
L-Tryptophan, %	0.061	0.048	0.048	0.048	0.048	0.067	0.050	0.050	0.050	0.048
L-Valine, %	0.011	0.003	0.003	0.003	0.003	0.018	0.000	0.000	0.000	0.150
L-Isoleucine, %	-	-	-	-	-	0.022	0.003	0.003	0.003	0.150
Trace Mineral Premix, %	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.030
Vitamin Premix, %	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.030
Quantum Blue, %	0.007	0.007	0.030	0.030	0.030	0.007	0.007	0.030	0.030	0.003
Ethoxiquin, %	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.000
Econase XT 25, %	0.000	0.000	0.000	0.008	0.010	0.000	0.000	0.000	0.008	0.010

Table 10: Experimental diet composition finisher phases (as fed basis)

	Finisher Phase 1					Finisher Phase 2				
Ingredients	PC	NC	NC+1500 FTU/kg	NC+12000 BXU/kg	NC+16000 BXU/kg	PC	NC	NC+1500 FTU/kg	NC+12000 BXU/kg	NC+16000 BXU/kg
Corn, %	73.986	77.512	77.489	77.482	77.479	84.190	87.625	87.602	87.594	87.592
Soybean Meal, %	1.375	0.000	0.000	0.000	0.000	1.300	0.000	0.000	0.000	0.000
Corn DDGS, %	20.000	20.000	20.000	20.000	20.000	10.000	10.000	10.000	10.000	10.000
Fat, %	2.500	0.443	0.443	0.443	0.443	2.500	0.453	0.453	0.453	0.453
Calcium Phosphate, %	0.000	0.015	0.015	0.015	0.015	0.000	0.010	0.010	0.010	0.010
Limestone, %	0.925	0.930	0.930	0.930	0.930	0.880	0.890	0.890	0.890	0.890
Sodium Chloride, %	0.100	0.100	0.100	0.100	0.100	0.150	0.150	0.150	0.150	0.150
L-Lysine, %	0.570	0.570	0.570	0.570	0.570	0.465	0.465	0.465	0.465	0.465
DL-Methionine, %	0.029	0.000	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.000
L-Threonine, %	0.142	0.108	0.108	0.108	0.108	0.134	0.099	0.099	0.099	0.099
L-Tryptophan, %	0.069	0.050	0.050	0.050	0.050	0.055	0.037	0.037	0.037	0.037
L-Valine, %	0.011	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000
L-Isoleucine, %	0.032	0.011	0.011	0.011	0.011	0.033	0.011	0.011	0.011	0.011
Trace Mineral Premix, %	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Vitamin Premix, %	0.125	0.125	0.125	0.125	0.125	0.127	0.125	0.125	0.125	0.125
Quantum Blue, %	0.007	0.000	0.030	0.030	0.030	0.007	0.007	0.030	0.030	0.030
Ethoxiquin, %	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030
Econase XT 25, %	0.000	0.000	0.000	0.008	0.010	0.000	0.000	0.000	0.008	0.010

Table 11: Effect of phytase and xylanase on ADG in wean to finisher pigs (LS means)

	Treatment					SEM	P-Value					
	PC	NC	0.03% QB	0.03% QB+ 0.008% XT	0.03% QB+ 0.01% XT		Trt	Linear QB+XT25	Quad. QB+XT25	PC vs NC	NC vs 0.03% QB+ 0.01% XT	PC vs 0.03% QB+ 0.01% XT
ADG, kg/d												
NP 1	0.443	0.458	0.455	0.471	0.468	0.014	0.6485	0.4145	0.7552	0.4663	0.5973	0.2201
NP 2	0.578	0.587	0.6	0.616	0.635	0.025	0.5144	0.3609	0.7142	0.803	0.176	0.1231
Nursery overall	0.51	0.522	0.528	0.544	0.552	0.014	0.2697	0.2254	0.861	0.5611	0.1431	0.0505
G 1	0.682	0.701	0.669	0.689	0.665	0.031	0.9102	0.9227	0.5479	0.6471	0.3985	0.6956
G 2	0.820^{b}	0.791^{b}	0.812^{b}	0.650^{a}	0.855^{b}	0.026	<0.0001	0.4348	<0.0001	0.433	0.0888	0.3387
G overall	0.792^{b}	0.768^{b}	0.776^{b}	0.661^{a}	0.808^{b}	0.023	0.0009	0.5661	<0.0001	0.4582	0.2197	0.6174
F 1	0.914^{y}	0.890^{y}	0.776^{x}	0.893^{y}	0.860^{xy}	0.038	0.1021	0.0501	0.3012	0.6452	0.5614	0.3018
F 2	0.689	0.632	0.729	0.669	0.696	0.041	0.5387	0.4125	0.5173	0.3247	0.2672	0.8973
F overall	0.82	0.783	0.768	0.811	0.792	0.018	0.2616	0.192	0.3122	0.1451	0.7248	0.2615
G/F overall	0.813^{c}	0.776^{abc}	0.770^{ab}	0.748^{a}	0.799^{bc}	0.015	0.0287	0.5185	0.0194	0.0802	0.2652	0.4991
Overall	0.760^{y}	0.734^{xy}	0.731^{xy}	0.714^{x}	0.756^{y}	0.013	0.0902	0.4669	0.0327	0.1554	0.2158	0.8464

$^{a, b, c}$ Means with a different superscript differ, $P < 0.05$.

$^{x, y}$ Means with a different superscript differ, $P < 0.10$.

Table 12: Effect of phytase and xylanaseon ADFI in wean to finisher pigs (LS means)

	Treatment						P-Value					
	PC	NC	0.03% QB	0.03% QB+ 0.008% XT	0.03% QB+ 0.01% XT	SEM	Trt	Linear QB+XT25	Quad. QB+XT25	PC vs NC	NC vs 0.03% QB+ 0.01% XT	PC vs 0.03% QB+ 0.01% XT
ADFI, kg/d												
NP 1	0.624	0.655	0.648	0.661	0.668	0.017	0.4886	0.416	0.8974	0.2253	0.6072	0.0922
NP 2	0.946[a]	1.034[ab]	1.040[b]	1.058[b]	1.094[b]	0.032	0.0424	0.2817	0.5333	0.0621	0.1842	0.0028
Nursery overall	0.785[a]	0.845[b]	0.844[b]	0.860[b]	0.881[b]	0.019	0.0282	0.2179	0.5735	0.0418	0.1919	0.0018
G 1	1.28	1.375	1.326	1.437	1.305	0.082	0.6774	0.8196	0.2396	0.4178	0.552	0.8269
G 2	1.789	1.904	1.815	1.807	1.884	0.061	0.6056	0.5712	0.4327	0.1972	0.8263	0.2803
G overall	1.664	1.774	1.695	1.716	1.742	0.06	0.7399	0.61	0.8366	0.209	0.7136	0.3668
F 1	2.642	2.652	2.449	2.617	2.505	0.097	0.4889	0.4431	0.3342	0.9412	0.2938	0.3279
F 2	2.589	2.388	2.504	2.6	2.541	0.087	0.442	0.6072	0.5575	0.1144	0.2253	0.6972
F overall	2.62	2.542	2.472	2.61	2.52	0.083	0.6952	0.4591	0.365	0.5154	0.8548	0.4064
G/F overall	2.197	2.203	2.129	2.215	2.176	0.067	0.9016	0.4736	0.5814	0.9574	0.7849	0.8261
Overall	1.945	1.962	1.906	1.973	1.943	0.056	0.9302	0.5018	0.6146	0.8237	0.8112	0.9872

[a, b] Means with a different superscript differ, $P < 0.05$.

Table 13: Effect of phytase and xylanase on feed efficiency in wean to finisher pigs (LS means)

	Treatment					SEM	P-Value					
	PC	NC	0.03% QB	0.03% QB+ 0.008% XT	0.03% QB+ 0.01% XT		Trt	Linear QB+XT25	Quad. QB+XT25	PC vs NC	NC vs 0.03% QB+ 0.01% XT	PC vs 0.03% QB+ 0.01% XT
G:F												
NP 1	0.71	0.698	0.702	0.714	0.694	0.015	0.875	0.971	0.3492	0.589	0.8515	0.4722
NP 2	0.609	0.567	0.576	0.582	0.577	0.012	0.2029	0.8754	0.7437	0.024	0.5804	0.0761
Nursery overall	0.649^{y}	0.619^{x}	0.624^{x}	0.633^{xy}	0.622^{x}	0.008	0.1034	0.8906	0.3292	0.0143	0.7506	0.0292
G 1	0.498	0.468	0.472	0.444	0.472	0.014	0.1505	0.5786	0.1413	0.1386	0.839	0.1971
G 2	0.455^{c}	0.415^{b}	0.444^{bc}	0.348^{a}	0.450^{c}	0.011	<0.0001	0.0506	<0.0001	0.0188	0.0378	0.7513
G overall	0.463^{c}	0.426^{b}	0.450^{bc}	0.367^{a}	0.454^{bc}	0.01	<0.0001	0.0583	<0.0001	0.0138	0.0589	0.5064
F 1	0.347	0.337	0.317	0.337	0.344	0.011	0.3501	0.0806	0.8747	0.5117	0.6292	0.8611
F 2	0.273	0.271	0.293	0.26	0.281	0.014	0.5785	0.3081	0.215	0.9581	0.6267	0.6641
F overall	0.317	0.312	0.308	0.305	0.318	0.007	0.6383	0.4959	0.2524	0.5756	0.519	0.9314
G/F overall	0.367^{b}	0.352^{b}	0.359^{b}	0.328^{a}	0.366^{b}	0.007	0.002	0.496	0.0002	0.1402	0.1554	0.9537
Overall	0.388^{b}	0.374^{b}	0.382^{b}	0.353^{a}	0.388^{b}	0.007	0.0057	0.4674	0.0007	0.1635	0.1717	0.9775

[a, b, c] Means with a different superscript differ, $P < 0.05$.

[x, y] Means with a different superscript differ, $P < 0.10$.

Table 14: Effect of phytase and xylanase on BW in wean to finisher pigs (LS means)

	Treatment						P-Value					
	PC	NC	0.03% QB	0.03% QB+ 0.008% XT	0.03% QB+ 0.01% XT	SEM	Trt	Linear QB+XT25	Quad. QB+XT25	PC vs NC	NC vs 0.03% QB+ 0.01% XT	PC vs 0.03% QB+ 0.01% XT
BW, kg												
Initial	9.34	9.59	9.65	9.49	9.36	0.19	0.7144	0.2945	0.7529	0.3622	0.3866	0.9375
End of NP1	15.54	16	16.02	16.09	15.89	0.31	0.7722	0.8575	0.666	0.3181	0.7957	0.451
End of NP2	23.63	24.21	24.43	24.72	24.78	0.47	0.464	0.5711	0.9892	0.3949	0.3958	0.1007
End of G 1	33.28	34.78	34.57	34.67	34.24	0.79	0.6384	0.8487	0.7123	0.1769	0.6233	0.3803
End of G 2	69.27^{b}	68.67^{b}	69.21^{b}	62.82^{a}	71.08^{b}	1.65	0.014	0.6608	0.0008	0.7901	0.0946	0.4302
End of F 1	95.74^{b}	93.76^{b}	90.52^{ab}	85.77^{a}	95.20^{b}	2.1	0.0111	0.5497	0.0036	0.4991	0.6232	0.852
End of F 2	116.41^{y}	112.97^{xy}	109.35^{x}	108.53^{x}	114.90^{xy}	2.42	0.1048	0.2504	0.095	0.3079	0.5655	0.6504
End of F 3	128.72	125.02	124.43	121.62	128.07	2.1	0.1231	0.5627	0.0398	0.2093	0.2982	0.8226

[a, b] Means with a different superscript differ, $P < 0.05$.

[x, y] Means with a different superscript differ, $P < 0.10$.

Table 15: Effect of phytase and xylanase on carcass composition (LS means)

	Treatment					SEM	P-Value					
	PC	NC	0.03% QB	0.03% QB+ 0.008% XT	0.03% QB+ 0.01% XT		Trt	Linear QB+XT25	Quad. QB+XT25	PC vs NC	NC vs 0.03% QB+ 0.01% XT	PC vs 0.03% QB+ 0.01% XT
HCW, kg	208.9	201.35	198.12	200.8	205.69	3.52	0.2107	0.1743	0.4518	0.1301	0.3766	0.5115
Yield, %	61.68^{a}	62.64^{b}	63.00^{b}	62.42^{ab}	62.36^{ab}	0.28	0.0316	0.0797	0.8638	0.0198	0.4718	0.0903
Lean, %	54.55^{abc}	55.02^{c}	54.74^{b}	53.71^{a}	53.87^{ab}	0.32	0.0244	0.024	0.4055	0.2944	0.0141	0.128
LM depth, mm	67.95^{c}	65.76^{bc}	64.11^{ab}	62.40^{a}	65.15^{b}	0.86	0.0015	0.9104	0.0263	0.0743	0.6061	0.0251
BF, mm	18.20^{ab}	16.85^{a}	17.29^{a}	19.18^{b}	19.35^{b}	0.62	0.0237	0.0133	0.7559	0.1225	0.007	0.1902

$^{a, b, c}$ Means with a different superscript differ, $P < 0.05$.

Appendix

IACUC Approval

UNIVERSITY OF ARKANSAS

Office of Research Compliance

To: Charles Maxwell
FR: Craig Coon
Date: October 7th, 2016
Subject: IACUC Approval
Expiration Date: October 6th, 2017

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # 17027 *Determine the effect of a corn based phytase on growth perfromance in pigs fed an adequate phosphorus diet or a reduced phosphorus diet.*.

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond October 6th, 2017 you can submit a modification to extend project up to 3 years, or submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study: Charles Maxwell, Tsung-Cheng Tsai, Joshua Knapp, Chris Hart, Lensey Watson, Xiaofan Wang, Hayden King, Hannah Crabbe, David Buchanan, Owen Hossack, Elizabeth Bredlow, Brianna Freeze, Anita Maya, and David Price. Please submit personnel additions to this protocol via the modification form prior to their start of work.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/aem

UNIVERSITY OF ARKANSAS

Office of Research Compliance

To:	Charles Maxwell
Fr:	Craig Coon
Date:	April 7th, 2017
Subject:	IACUC Approval
Expiration Date:	October 6th, 2017

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your Modification to protocol # 17027 *Determine the effect of a corn based phytase on growth perfromance in pigs fed an adequate phosphorus diet or a reduced phosphorus diet.* to test different levels of phytase and add another source of phytase.

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond October 6th, 2017 you can submit a modification to extend project up to 3 years, or submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/aem

UNIVERSITY OF ARKANSAS

Office of Research Compliance

To: Charles Maxwell
FR: Craig Coon
Date: January 9th, 2017
Subject: IACUC Approval
Expiration Date: January 8th, 2017

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # **17046:** *Determine the effect of increasing dietary xylanase levels on growth perfromance in nursery and grower/finisher swine fed superdosing levels of phytase..*

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond January 8th, 2017 you can submit a modification to extend project up to 3 years, or submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study: Charles Maxwell, Tsung-Cheng Tsai, Joshua Knapp, Chris Hart, Lensey Watson, Xiaofan Wang, Hannah Crabbe, David Buchanan, Owen Hossack, Brianna Freeze, Anita Maya, David Price, John Gray, and Hayden King. Please submit personnel additions to this protocol via the modification form prior to their start of work.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/aem