THE EFFECT OF E. COLI PHYTASE ON A SUSTAINED BENEFIT IN SWINE GROWTH PERFORMANCE, BONE STRENGTH, AND NUTRIENT DIGESTIBILITY, AND PHOSPHOROUS BALANCE IN NURSERY PIGS.

by

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(Under the Direction of Michael J. Azain)

ABSTRACT

For this study, two experiments were conducted. Experiment 1 examined the extended benefit at market weight if pigs were supplemented with high level phytase from early nursery. Experiment 2 aimed to clarify varied levels of phytase in P balance in nursery pigs. Exp. 1 showed that pigs fed 12500 U/kg phytase in a deficient P diet (0.13% aP) during both the nursery (Trt. 4) and nursery-growing (Trt. 5) phases had full recovery on overall gain weight from adequate P (0.35% aP), and also can reduced P excretion by 70.79% and 64.60% in the nursery phase when compared to positive controlled ($P<0.0001$). An LP diet with 250, 500, 2500, and 12500 U/kg phytase reduced fecal P (%) by 21.76%, 16.67%, 39.81%, and 49.54% ($P<0.0001$) when compared to HP without phytase supplementation. The results indicated that a high level E. Coli. phytase has a sustained benefit on growth performance, and bone strength in growth-finishing pigs and that 2500 and 12500 U/kg phytase could reduce P and Ca excretion efficiently in nursery pigs.

INDEX WORDS: Phytase, P Digestibility, Bone Strength, Pigs.

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CHAPTER 1

INTRODUCTION

The mineral elements frequently operate in balanced pairs or in small groups. The function of minerals include: maintaining the acid-base balance of body fluid, acting as a component of bone/skeletal structure, maintaining or regulating the colloidal state (osmotic pressure, diffusion, and viscosity), and serving as component and activator of enzymes and other biological systems. The essential macro elements are separated into two groups: The first are the principal cations, such as calcium (Ca), magnesium, sodium, and potassium. The second are the principal anions, such as phosphorous (P), chlorine, and sulfur. The essential micro elements include iron, copper, zinc, manganese, iodine, selenium, chromium, cobalt, molybdenum, flourine, vanadium boron, tin, nickel, silicon, alumium.

Recently, environmentalists have raised concern about phosphorous (P) excretion in livestock. Technologies and feed additive products are available to increase P bioavailability in monogastric animals and reduce excess P impact in the environment. Phytases have been reported to be efficient in helping P utilization. Those studies mainly focused on fungal phytase. Recently a new E. Coli. phytase has became available. We hypothesized that supplementing a high level of E. Coli. phytase to pigs fed in the nursery phase can have extended benefit in growth performance, bone strength, and P digestibility at marketing weight. Moreover, pigs fed with E. Coli. phytase addition in the diets can improve P and Ca utilization, bone strength, and growth performance in weaning pigs.

CHAPTER 2

LITERATURE REVIEW

Calcium and Phosphorous

The importance of calcium and phosphorous in swine nutrition has been reviewed (Crenshaw, 2001). Calcium (Ca) in the body is mostly (99%) in bone. Calcium also serves as cofactors of enzymes, for example pancreatic lipase, acid phosphatase, cholinesterase, myosin atpase, and succinic dehydrogenase, and it is also needed for initiation of blood clot formation. The requirement of Ca for swine is 0.5~0.9% of the diet (NRC. 1998). Young animals retain almost all free Ca in their diet. However, in older animals, endogenous Ca loss increases as the number of intestinal cells are sloughed off and contribute to the endogenous fraction. The factors affecting Ca absorption include Ca level in diet, starch, amino acids, oxalic acid, phosphate, fat, and vitamin D (Vit. D). A deficiency of Ca will cause a decreased in weight gain, rickets, osteomalacia, and muscle spasms. Milk fever is a well-recognized Ca-deficiency disease in dairy cows. An excess of Ca will result in decreased growth rate, decreased protein and energy digestibility, hypersecretion of calcitonin, and arrested bone reabsorbtion. Two-thirds of Ca reabsorption is in the proximal tubule. Ca excretion is positively related to sodium excretion, and is mainly controlled by parathyroid hormone (PTH) (Jones, et al. 1998).

Phosphate is an essential component of bones. Most (85%) of the phosphate in the body is in bones, and most (99%) of the remainder is intracellular and consists of primarily organic phosphates. In plasma, two-thirds of total phosphates are bound, and only one-thirds of phosphates are free phosphates and acid soluble phosphate. Phosphorous (P) is a component of

pepsin, xanthine, and phospholipids, which are cell membrane's primary constructure. Examples of phospholipids are phosphatidylcholine and phosphatidyl-ethanolamine. We can also find P in nucleotides, such as ATP and GTP, as energy sources, and ribo-nucleotide, such as AMP, and UMP.

The NRC requirement of total P in swine requirement is 0.4~0.6% of the diet. Typically plant-based diet contain sufficient total P, but this P is in the form of phytic acid which is biologically unavailable to the Monogastric animals. A deficiency of P causes rickets, decreased weight gain, osteomalacia, and decreased amino acids and protein digestibility. Most animals can not tolerate more than 1% of total P in their diet. The toxicity of P produces urinary calculi, decreased growth rate, decreased feed utilization, loss of bone volume, and stiff joints. Almost all available P is absorbed by the intestines, and re-absorption is related to sodium co-transport. P balance is primarily controlled via PTH.

Calcium : Phosphorous Ratio:

In our body, 99% of Ca and 80% of P are stored in skeleton, and the ratio of Ca : P in bones is 2:1. The remainder of body calcium is mainly extracellular, while the remainder of body P is intracellular and both of them help maintain an acid-base balance. Ca and P absorption from gut is mutually antagonistic. The reasons they are antagonistic may be that P concentration is controlled indirectly by free Ca concentration in serum, which concurrently determines the parathyroid hormone (PTH) level in body. The kidney controls Ca and P excretion, and Vit. D and PTH play a major role (Jones et al. 1998). For example, if the serum Ca level was decreased, it would trigger PTH (parathyroid hormone) to start the hydroxylation of 25-hydroxy vitamin D_3 to 1, 25-dihydroxy vitamin D_3 (calcitriol). Calcitriol would target absorptive proteins in cell

membranes of small intestine, which would synthesize calcium transporter protein, and result in increased Ca and P absorption from the small intestine. However, enhancing PTH receptors would also increase Ca reabsorption by 40% from thick ascending limp of loop of Henle, proximal tubulu and distal nephron, and inhibit P reabsorption in the proximal tubule by stimulating cyclic AMP (Laiken, 1985).

The best ratio of Ca: P in digestive tract, which can maximize retention, is 1:1 (Liu et al. 2000). An imbalance of Ca and P can form insoluble complexes, affecting their absorption. NRC (1998) suggested that the ratio of Ca: total P (tP) in the diet should be between 1.1:1 to 1.25:1. The higher Ca: tP ratio has been linked to lowering P absorption and reducing phytase efficiency (Lei et al. 1994). There have also been suggestion of a benefit to lowering the Ca : P ratio. A ratio of Ca : tP at 1:1 resulted in a linear increase in P absorption from the digestive tract, compared to 1.5:1(Liu et al. 2000).

Environmental Concern

Historically, diets formulated for optimal performance of domestic animals exceed the NRC (National Research Council) recommendations for several nutrients. In the case of P, this results in excess excretion into the environment. Even, following the NRC recommendation for P does contributes to the problem of P output into the environment. Kornegay and Verstegen (2001) presented data from several surveys conducted in the late 1980s through the mid-1990s, indicating that P was being fed at 110 to 155% of its requirement as listed in the most recent NRC (1998). This caused excess P situation to get worse and increased diet cost. One key change in water quality regulations in the past five years is the shift from a primary focus on N to an increasing focus on P contamination of surface water (Knowlton et al. 2004). As people started

to become concerned about the environment, the government was mandated to request the livestock industry to reduce the pollution from manure waste.

In livestock, plants seeds are used as a major diet source. However, plants seeds contain two-thirds of phytin P, which is mostly undigested by monogastric animals. Phytic acid is a sugar molecule (myo-inositol 1, 2, 3, 4, 5, 6,-hexakis dihydrogen) to which six phosphate groups are bound. Phytate is a mineral storage form in plant seed and is important for seeding growth (Raboy. 2003). Phytic acid acts as a chelator and can bind up to 6 moles of di- or trivalent cations at a neutral PH including Ca, Zn, Fe, Mg, Cu, and Co. Potassium and Mg have higher affinity on phytate binding. If phytate binds to Ca, it makes both Ca and P unavailable for animals. Phytate is also closely bound to proteins and starch, and it has been suggested that it interferes with the digestion, preventing the absorption of both the bound components (Kies et al. 2001). This results in an anti-nutritional effect. Although, monogastric animals, such as pigs, have microbes in the digestive tract to break down phytate, it is not sufficient to release a high significant amount of metabolizable P. The bacteria in the hindgut may produce enzymes to release P from grains, but the P that is released just not appears to be absorbed. Thus indigestible phosphorous is inputted into surface water where it stimulates phytoplankton growth, known as algal bloom in the aquatic environment. Algal bloom removes oxygen from the water surface, and causes other organisms not to be able to survive. Moreover, phytoplankton reduces the value of rivers, and lakes and is menace to the safety of drinking water for humans. Phosphorous and nitrogen had been used as a good source of fertilizer for plants. However, since the decline in use of phosphate fertilizer, P excretion in soil has also contaminated the plants. Douymad (1999) indicated that an average of 67% of the N and 66% of the P consumed by the pigs is excreted in feces and urine, resulting in a serious environmental concern.

Technologies Used for Reducing Phosphorous Excretion

To eliminate the overfeeding of P contaminating the environment, we need to investigate what the animal's physiological requirements are and how well the plant P can be utilized by animals. Understanding P digestion and metabolism in livestock will improve the efficiency of P utilization, reducing P excretion and minimizing the imbalance of N and P in manure (Knowlton et al. 2004).

Since the mid-1980s, Netherlands has focused intensively on reducing P excretion. In Denmark, the concern of diminishing P excretion began in the 1990s. The P recommendation changed from total P to digestible P. Up to 75% of the P in feedstuffs of plant origin is present as phytate. Fecal P excretion is about 46% of intake and urine excretion is about 6% of the amount in weaning pigs. Dry or liquid feeding may also affect P digestibility (Poulsen 1999). Diet formulations based on available phosphorous rather than total phosphorous increase P utilization and reduce P excretion. However, P digestibility is still relatively low when only following the NRC's available P recommendations. There are several related technologies that have potential for improving P retention by swine.

Inorganic P Supplementation

Due to monogastric animal's lack of ability to utilize plant source of P source from plant seed, supplementing inorganic P to meet their requirement is practiced. Swine can have 80~98.2% P digestibility of inorganic P sources (Peterson et al. 2006), such as dicalcium phosphorous, monocalcium phosphorous, monosodium phosphorous, and so on. Additions of .04% or .08% more P from the commercial monocalcium phosphate to a low-P control diet resulted in a quadratic improvement (P <0.03) in growth rate, and gain to feed ratio during the grower phase and a linear improvement (P < 0.01) during the finisher phase and for the entire

trial (Haper et al. 1997). Although inorganic P is used more efficiently, it still does not help with environmental impact caused by animal waste.

Low-Phytate Cereal Grains (corn, soybean meal)

Plants have been selected that have low phytin P but similar total P (Raboy, 2002). Pigs fed diets consisting of low-phytate corn-soybean meal with no supplemental P grew as fast and efficiently, had similar bone traits, and excreted 53% less P than pigs fed diets containing conventional corn-soybean meal supplement with sufficient inorganic P (Cromwell et al. 2000b). In the growing and finishing phase, feeding pigs with low-phytate corn-soybean meal diets containing 0.10 to 0.12% less total P than normal results in similar performance and bone mineralization as in pigs fed normal corn-soybean meal diets. Other variation of low phytic acid grains, such as barley, wheat, and rice bran, were also reported to be as efficient in reducing P excretion. Low-phytate cereal grains will be an effective way to deal with P output in the future when the day of reducing crop yields coming. When low-phytate grains are proven, combining phytase with it could have great ability to eliminate P excretion. Lott et al. (2000) reported that low phytic acid mutant grains are somewhat deleterious to plant and seed growth and function, and Raboy et al. (2000) reported that a lighter seed weight added extra cost to handle them.

Phytase

There are several phytases produced by microorganism. Commercial source of phytase are an enzyme produced from genetically altered bacteria. The 3-phytase, which starts dephosphorylation at the 3-position of the myo-inosital ring, is mostly from a microorganism, such as fungi and bacteria. The 6-phytase, starting to dephophorylate phosphate at the 6-position of the myo-inosital ring, is of plant origin. There are four sources of phytases: intestinal secretion

(large intestinal bacteria), endogenous phytase in some feed ingredients (wheat, barley, canola), phytase from residual bacteria (rumen bacteria), and exogenous microorganisms (feed additive). Although plant seeds contain certain phytase, the microorganisms are a more efficient source of the enzyme. The concept of using microbial-derived phytase to improve phytate P utilization by non-ruminant animals was put forth many years ago (Nelson et al. 1971). Supplementation of phytase in swine and poultry diets is a commercially option and is commonly used in many cereal grains of the world. A number of researchers have demonstrated that supplementing phytase is effective at making phytate-bound P nutritionally available to growing pigs (Simons et al. 1990, Jongbloed et al. 1992, Cromwell et al. 1993, Lei et al. 1993a,b, and Kornegay and Qian. 1996). In addition, supplementing low-P diets with phytases has been shown to increase bone strength (Haper et al. 1997). Phytase significantly increased the amount of P digested in the high-P wheat variety diet ($P < 0.01$) (Kim et al. 2005). Addition of 167, 333, and 500 U/kg of phytase to the low-P control diet produced linear improvements in ADG during the grower and finisher phases ($P < .05$) and overall ($P < .01$) (Haper et al. 1997). The use of phytase has also improved total tract apparent digestibility (CTTAD) of protein and starch for pigs (Traylor et al. 2001, Selle et al. 2003). Diet supplementation of phytase increases P utilization, and nutrient digestibility, as well as reducing P excretion.

Endogenous Phytase in Grains

Some grains seeds tend to have less phytic acid because they contain endogenous phytase activity. Cromwell et al. (1993) found a considerably higher bioavailability of P in wheat (50%), wheat middlings (41%), wheat bran (29%), and barley (30%) than that in corn (14%). Feeding grains or feed byproducts that contain less phytic acid can improve the P digestibility, as compared to corn. However, this approach is still not completely effective because has more than

50% of total P not digested, and the availability of these ingredients is limited to certain regional area, and can be more expensive.

Bioengineered Phytase in Plants and Pigs

Alfalfa (Ullah et al. 2002), bioengineered to express in Aspergillus ficuum phytase and wheat engineered to (Henrik et al. 2000), express in Aspergillus niger phytase have been produced and had 17 fold and 56% increased in phytase activity. Denbow et al. (1998) reported that genetically engineered soybean meal (1200 U/kg of A. niger) increased body weight gain, P digestibility and tibia shear force by 11%, 16.6% and 48% in broiler, compared to normal soybean meal.

Golovan et al. (2001) demonstrated the reduction of fecal P 67% in boars and 64% in gilts by transgenic pigs (fungal phytase), which can produced the average of 2000-3000 U/kg phytase activity, without supplementing inorganic P. Forsberg et al. (2003) indicated that 33% less land would be required to spread manure from transgenic pigs, but transgenic animals will require safety quality testing in the country of origin and in countries to which the product is exported to ensure that they do not have a deleterious effect on human health and the environment. Nevertheless, it may be an option in the future as a means to reduce the environmental impact of animal agriculture.

Fungal Phytase

Fungal phytases include Aspergillus fumitagus, Aspergillus niger, and Peniophora lyci. Natuphos, derived from Aspergillus niger, is a 3-position phytase and has been wildly used as phosphatase. The pH optima for A. niger phytase is PH=2.5 and 5.5, and optimum temperature is 50-60 $°C$. The company that produces Natuphos recommends that it be added to swine diets at a level of 500 U/kg. O'Quinn et al. (1997) reported full recovery of growth rate and feed intake

with 300 or 500 U/kg of phytase supplementation (Natuphos) in low-P basal diet (no added inorganic P). The other phytase produced from Peniophora lycii and released several years ago, is called Ronozyme P. Ronozyme P is a 6-position phytase, which hydrolyzes phosphorous-ester linkage from the 6 myo-inosital position. The major advantage of P. lycii is that it is manufactured using a patented coating process that reduces the loss of activity during the high temperature of the pelleting process. The optima pH is from 5.5 to 6.5.

Bacteria Phytase

E. coli. phytase had been reported to have more efficiency than the fungal phytases in improving broiler growth performance, bone characteristics, and retention of P (Onyango et al. 2005). Onyango et al. (2004) indicated that chicks fed the E.coli phytase diet had a consistently higher phytase activity in the crop ($P<0.05$), proventriculus and gizzard ($P<0.05$), jejunum ($P<0.001$), and ileum ($P<0.0001$) than those fed a P. lycii phytase diet. Stahl et al. (2004) showed that no difference in ADG, ADFI, and G : F amount of the same level of Peniophora lycii, Aspergillus fumitagus, Aspergillus niger, and E. coli.(AppA) supplemented in the diet.. The E. Coli. phytases have been expressed in Saccharomyes cerevisiae, Pichia pastoris, Pseudomonas fluorescens and compared the efficiency of growth performance and P digestibility (Silverside 2004). Rodriguez et al. (1999) demonstrated that in vitro E. coli. phytase, r-appA (recombinant protein produced by appA) and r-appA2 (recombinant protein produced by appA2) expressed in Pichia pastoris, released 50% more inorganic P than A. niger phytase (r-phyA, recombinant protein from phy-A). Moreover, there source of phytate are less susceptible to being degraded by proteolytic digestive enzymes, such as r-appA is more resistant to pepsin, but r-phyA is more resistant to trpsin. Pichia pastoris phytase, like Ronozyme P, is a 6-phytase, had better

thermostability than the other two -- bacteria phytase and A. niger phytase (Natuphos) -- above $80\,^{\circ}C$ (Silversides et al. 2004). This is the phytase we used in our experiments.

Phytase activity Site

Phytases are proteins, so temperature and pH are important for phytase activity. Kemme et al. (1998) indicated that the efficacy of phytase was determined by conditions of pH and retention time in the stomach. The pH range of the phytase is between 2 and 6 (Simon and Igbasan. 2002). The site where phytase most active are the upper digestive tract and the stomach (Yi and Kornegay. 1996). The activity of supplemented phytase was detected as 69–86% in the crop and 31–38% in the proventriculus, while phytase activity was not detected in the small intestine of chickens fed a corn-based diet (Liebert et al. 1993).

The Benefit of Supplement Phytase in low-P (LP) Treatment

Growth Performance

The idea of using phytase is to increase P released from phytate mineral-binding complex and reduce P excretion in the environment. To have the maxima benefit on that, adding phytase with lower P content in the diet is mostly considered. The net growth rate improvement over the low-P controls was 17.2% for the 250 U/kg phytase diet, 19.2% for the 500 U/kg phytase diet, and 22.3% for the positive control diet. Numerically, growth rate for the low-P-phytase-supplemented pigs was within 96 to 97% of that observed for the adequate P controls (Haper et al. 1997).

Compromised growth performance of grower-finisher pigs fed low-P corn-soy diets can be restored by supplementing phytase. Cromwell et al. (1995a) reported restoration of grower-finisher pig performance approaching that for typical P diets using a product with

reduced activity added to provide 500 phytase U/kg diet (Allzyme Phytase, Alltech, Nicholasville, KY) or a product similar to the one, Natuphos, at levels of 250, 500 and 1,000 phytase U/kg diet (Cromwell et al., 1995b).

Veum (1996) reported that ADG was increased when 500 or 1,000 U/kg of phytase was added to a low-P corn-soybean meal diet but ADG with the phytase supplemented diet was intermediate between that of pigs fed low-P diets without phytase and those fed adequate P diets.

Digestibility

The other applications of using phytase with low-P diets are to increase P and nutrient retention -- in other words, decreasing excessive nutrients contaminating the environment. Some papers reported that supplementation of phytase can improve P and Ca digestibility (Kim et al. 2005, Beers and Jongbloed 1992, and Lei et al. 1993b). However, improvements in the CTTAD of Ca by the addition of phytase range from no improvement in weaner pigs (Han et al. 1997) to a 41% improvement in 7 kg pigs (Lei et al. 1993a). Higher CTTAD of minerals was seen in pigs fed low-phytate corn (Spencer et al. 2000) and low phytate barley (Veum et al. 2002) compared to that for normal corn and barley, indicating that less minerals were bound to phytic acids in the low-phytate grains. Some studies indicated that phytate was also bound to other nutrients, and caused an anti-nutritional effect. Phytase supplementation did not improve the CTTAD of DM, energy, starch, and CP (Kim et al. 2005, Kemme et al. 1997, and Oryschak et al. 2002). Onyango et al. (2005) showed no differences in apparent ileal digestibilities of DM, energy, N, P or Ca with chicks fed a phytase supplemented low-P inorganic P diet on.

Bone Characteristic Improvement

As P is one of the major bone components, increasing P availability and absorption should also benefit bone development. Cromwell et al. (1995a, b) reported increased

metacarpal-metatarsal ash and greater femur breaking strength with phytase supplementation of a low-p diet. Metacarpal ash and breaking force were also reported by Veum (1996). There was a reference reported that 600 and 800 U/kg produced metacarpal breaking forces equal to those produced by the adequate P diet, and even stronger as supplement with 2500 and 12500 U/kg phytase (Veum et al., 2006).

Calcium and Total Phosphorous

In order to maximize the efficiency of phytase, the ratio of Ca: tP in the diet should be close to 1:1. Lowering the ratio from 2:1 to 1.2:1 increased growth performance, bone characteristics, P digestibility and reduced P excretion in weaning pigs supplemented phytase with dietary requirement (Qian et al. 1996). Liu et al. (1998) also reported that the efficiency of phytase was improved at low Ca: tP ratio (1:1). However, so far the research for the effect of Ca : tP ratio has mainly focused on fungal source of phytase, but effects of new source of derived from E. Coli. phytase remain to be described.

Implication

The current status of the feed enzyme market and future trends have been reported (Sheppy, 2000). Enzymes are available every where from single-celled organisms through plants and insects to human, and have many difference functions. Historically, applications for use of enzymes include food, drink, detergent, paper production and also in animal feeds. Although it is highly influenced by diet component, animals, especially monogastric animals, can’t digest the diet completely. For example, swine only can digest 75-85% of what they eat. The waste from domestic animals is a source point for environmental pollution. As the public have been paying more attention to the environment, the livestock industries started to consider using enzymes to

increase efficiency of nutrient digestibility and eliminate the environment pollution. There are four enzymes commonly added into diets, fiber-degrading enzymes, protein-degrading enzymes, starch-degrading enzymes, and phytic acid-degrading enzymes. Phytases, have exclusively received global acceptance and application, being used in approximately 8% of all monogastric animals' diets, and contributed to total market value in excess 50 million dollars. As world populations are still growth, meat production and consumption will increase as well in the future. More important than the potential profitability of using phytases, the more important benefit of phytases, is increasing availability of the nutrients in swine and poultry diets (minerals and amino acids), to alleviate environmental problem are much more important. Moreover, an additional benefit to realize is to reduce feed costs and produce an economical cheap food for people.

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CHAPTER 3

THE EFFECT OF ADDING HIGH LEVELS OF PHYTASE IN THE NURSERY/ GROWER DIETS ON GROWTH PERFORMANCE, CARCASS CHARACTERISTICS, AND BONE STRENGTH IN GROWER-FINISHING PIGS.

[1]

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Abstract

The objective of this study was to determine the effect of long term feeding high-level E. Coli-derived phytase in growing-finishing pigs on growth performance, phosphorous excretion, bone strength, and carcass characteristics. There were three dietary phases fed: 20-45 kg, 45-90 kg and 90-130 kg during the 16-week study. Treatment (Trt) 1 was the positive control and contained 0.35, 0.30 and 0.25% available (avail.) P in the 3 phases, respectively. Treatment 2-5 had no inorganic P added and contained 0.13, 0.10 and 0.10% avail. P. Treatment 2 was the negative control diet. Treatment 3 was supplemented with 500 U/kg phytase in all phases; Treatment 4 with 12,500 U in phase 1 and 500 U of phase 2 and 3; Treatment 5 had 12,500 U of phase 1 and 2 and 500 U/kg in phase 3. In phase 1, the addition of 500 U/kg phytase (711g/d) increased growth over the negative control (650 g/d), while addition of 12,500 U/kg (863 g/d) normalized weight gain to that of the positive control (873 g/d). Overall, the growth rate was greater in pigs that were fed high levels of phytase in early growth stage than those were fed 500 U for the entire study. The addition of phytase increased final body weight by 19%, 25% and 32%, and improved G : F by 8%, 17% and 15%, compared to none phytase low phosphorous diets. During the nursery phase, pigs had greater efficiency in response to phytase effect in fecal P (%), P and Ca digestibility ($P<0.005$). Metatarsal bone dry weight, ash weight, and percent ash in Trt 4 and 5 were significantly greater than Trt 2 and 3 ($P<0.0001$), and were not different from the positive control in pigs that were fed 12,500 U phytase during phase 1 or 2. Percent ash and bone strength were reduced in the low P diet and recovered with addition of any level of phytase. These results suggest that feeding high levels of phytase in the nursery and or grower phase are sustained in the finisher phase.

Introduction

Phosphorous (P) is an essential nutrient and is particularly critical during times of rapid bone growth in young animals. Corn (0.28%) and soybean meal (0.69%) contain significant amounts of P, but most of this (77-85%) is in the form of phytic acid and is unavailable to monogastric animals. As a result, inorganic sources of P are commonly added to swine and poultry diets, which increase the environmental impact of these industries. There are currently several options to reduce the environmental impact and meet the nutrient requirements. Feeding low phytate corn and SBM can reduce P excretion and still has the same growth performance and bone trait, most importantly reduced 47% P output (Cromwell et al. 2000b). Transgenic plants (Ullah et al. 2002) and transgenic pigs (salivary phytase) (Golovan et al. 2001) can also diminish the P excretion. However, addition of microbial phytase to the diet is most commonly used to improve P utilization (Simons et al. 1990, Jongbloed et al. 1992, Cromwell et al. 1993, Lei et al. 1993a,b, Kornegay and Qian. 1996). Both fungal and bacterial phytases are available. Pichia pastoris phytase(r-appA, r-appA2), which was derived from E.coli, had been reported to have better thermostability than A. niger phytase (Rodriguez et al. 1999). P. pastoris yeast phytase also had better performance in eliminating P excretion, growth rate, and bone trait than fungi phytase in chicken (Onyango et al. 2005), and weaning pigs(Stahl et al. 2000), and also better than Peniophora lycii in young swine (Veum et al. 2006). Phytase addition to the diet results in reduced P excretion, and increased growth rate, feed efficiency, and bone strength. The greatest benefits of phytase are seen in younger pigs. Lei et al. (1993) showed that 1200 PU/g A. niger phytase could maximize phytate-P utilization by weaning pigs. However, Augspurger and Baker et al. (2004) indicated that 5000 and 10000 U/ kg in both fungi and E.coli-derived phytase could

achieve better weight gain than 1000 U/kg.

Veum et al. (2006) demonstrated that feeding high levels of E.coli-derived phytase (2500, 12500 U/kg) in the low P diets resulted greater performance criteria (bone strength, ash weight, and P absorption) than seen in pigs fed the positive control diet. It is not clear whether the benefits obtained in young pigs are sustained or what the long-term benefits of E.coli phytase are for growth performance, carcass characteristics and bone strength. The objective of our study was to determine if the effects of E. Coli. phytase are sustained with continuous feeding through market weight, and whether the benefits on bone strength as a result of early feeding of high levels of phytase are maintained when phytase is reduced to a lower level.

Materials and Method

Animal Handling and Collection

A total of 80 healthy pigs (weaned, at day 21) were randomly selected from the University of Georgia Swine Center and fed a starter for a 4 wk post-weaning period. In a 16-wk study, the pigs were fed in three dietary phases: 20-45kg, 45-90kg, 90-130kg. In phase 1 (4 weeks post weaning), pigs were assigned to one of 20 identical pens (1.8*0.9 m) in an environmentally controlled room. Each pen had four pigs (2 gilts, 2 barrows), and four weight blocks. Pigs were weighed and feed intake was monitored at weekly intervals. Room temperature was controlled at 23 ^{0}C and reduced one degree every other week. In phase 2, pigs were moved to an open-sided barn and in the same grouping that was used in phase 1. Pigs were fed phase 2 diets until approx. 80 kg body weight and phase 3 until the control group reached 130 kg body weight. Body weights and feed intake were recorded every two weeks. Fresh grab fecal samples were collected at day 27, 70, and 104 from each pen and stored at -20 ^{0}C for further analysis.

Carcass Characteristics and Bone Collection

Loin area and subcutaneous fat thickness at 10th rib was determined by ultrasound at 80, 100, and 130kg. Ultrasound images were determined using an ALOKA 500-V ultrasound unit(Corometrics Medical Systems, Wallingford, CT) with a 17.2cm, 3.5 MHz linear probe and interpreted using Beef Information Manager^{TM} software, version 3.0 (Critical Vision, Inc., Atlanta, GA). Pigs were processed in the UGA meat lab. Other carcass characteristics were determined 24hrs post-mortem. The rear legs were removed and frozen for later metatarsal bone isolation and determination of bone ash and bone strength.

Diet

Phytase used in our study was from E. coli (Zymetric Quantum, Inc., a division of Syngenta, Golden Valley, MN) expressed in Pichia pastoris. All diets met or exceeded the NRC recommendation for each phase, except for available phosphorous. Diets 2, 3, 4 and 5 in each phase had reduced available phosphorous. This was achieved by removal of dicalcium phosphate (DCP). The calcium level was maintained at a similar level to that in the positive control diet by additional limestone. Diet 1 in each phase contained 0.35, 0.30, and 0.25% avail. P. Diet 2-5 had no DCP added, so had 0.13, 0.10, and 0.10% avail. P. Diet 3 had 500U/kg phytase in each phase. Diet 4 contained 12,500U/kg phytase in phase 1 and 500U/kg in phases 2 and 3. Diet 5 had 12500U/kg phytase at phase 1 and 2, and 500U/kg in phase 3. The indigestible marker, 0.1% $TiO_{2,}$ was added into the diet and was fed a week before fecal collection. The Ca: Total P ratio was 1.2:1 in HP (0.35% aP) and 1.9:1 in LP (0.13% aP) during three phases. The composition of diets is shown on table 3-1.

Sample Analysis

Fresh grab fecal samples from each pen were thawed at room temperature, and freeze dried.

Fecal freeze dried samples were weighed, and ground for mineral, CP, and energy analysis.

Mineral contents in diet and feces were analyzed by the Agricultural and Environmental Services Laboratories (AESL) of UGA, using inductively coupled atomic emission spectroscopy (ICP-AES). Diets and fecal CP were analyzed by A Leco FP-528 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI). Diets and fecal gross energy were determined by bomb calorimeter (Parr 1261, Parr Instrument Co., Moline, IL).

Two metatarsal bones from each pig were cleaned and weighed. Bones were oven dried at 177 ^{0}C for 72 hrs, and dry weight was recorded for dry matter determination. Bone length and width were measured. One dry metatarsal bone from each pig was used to determine bone strength (Instron Universal Testing Machine, Model 1122 with a 5500R Series system interface), and the other one was ashed at 600 ^{0}C for 72 hrs for ash weight determination.

Statistical Analysis

All data excepted for digestibility were analyzed using the PROC GLM procedure in SAS with main effects of treatment (5 treatments) and blocks (4 weight blocks). Results are presented as least square means for diet treatment effects. The pen was used as an individual unit in growth performance as well as, carcass and bone characteristic determination. In digestibility analysis, data were analyzed using the PROC GLM procedure in SAS with main effects of treatments (5 treatments) and phases (nursery, growth and finishing) as well as the treatments x phase interaction. The pen was used as an experimental unit as well. The results are presented as least square means for treatments x phases effects.

Results

Growth Performance

The result of growth performances are shown in table 3-2.

In the nursery phase, low P (0.13% aP) with no phytase supplementation had significantly lower weight gain and G : F conversion ratio ($P<0.001$) than other groups , as expect. The positive control group had significant better gain weight and feed conversion ratio than no and 500 U/kg phytase of negative control groups. The addition of 12500 U/kg phytase treatments in low-phosphorous (0.13% aP) diets (LP) fully recovered in the average of body weight (BW) at day 28, compared to adequate P (0.35% aP) diets. Pigs fed 500 U/kg phytase in low P diet had numerically higher BW than those were fed no phytase low P diet. Weight gain did not differ between positive control group (0.35% aP + 0 U/kg phytase), and addition of 12500U/kg phytase groups (Trt.4 and Trt. 5) (figure 3-1). 500, 12500, and 12500 U/kg phytase supplementation in the diets had 9.38%, 32.15%, and 33.54% better weight gain than no phytase negative control group ($P<0.0001$). There was no difference in feed intake in the nursery phase between treatments ($P=0.08$). Pigs fed the low P diet with no phytase had poorer G : F ratio than control diet. Addition of 500 U/kg phytase had no effect on G : F, but addition of 12500 U/kg phytase resulted in a normalization of G : F ratio relative to that in control diet.

In the second phase (d28-77), half of the pigs fed 12500 U/kg phytase in the first phase were maintained on this level and half were switched to 500 U/kg phytase in the diet. As in phase 1, pigs fed the no phytase supplemented diet had reduced growth rate compared to those fed the positive control (77 vs 1011 g/d, $P<0.001$). Pigs that were fed 500 U/kg phytase for both phase 1 and 2 had improved growth rate over the negative control (898 g/d). Pigs that had been fed

12500 U/kg phytase and either maintained on that level (999 g/d) or reduced to 500 U/kg during the second phase (992 g/d) had growth rates that were not different than that in the positive control group (1011 g/d).

Intake was affected by diet in phase 2. Pigs fed the negative control diet consumed less feed than any of the other groups. Pigs fed 500 U/kg phytase in phase 2 (treatment 3 and 4) had similar intake that was greater than that in the negative control group. Pigs fed 12500 U/kg throughout phase 1 and 2 had the numerically greatest intake, which was greater than pigs were fed 500 U/kg, but it was not different to the positive control group.

Feed efficiency was poorer in pigs fed the no phytase supplemented diet in phase 2 relative to the positive control. Efficiency was numerically improved in pigs fed 500 U/kg phytase in phase 1 and 2 and was significantly improved and not differ from the positive control in pigs that were fed 12500 U/kg in both phases or that were fed 12500 U/kg in phase 1 and reduced to 500 U/kg in phase 2.

In the last phase (d77-111), pigs fed the no phytase supplemented low phosphorous diet continued to have reduce growth rate and intake relative to the positive control diet. Pigs fed diets supplemented with phytase had growth rates that were not different from the positive control group. There was no different in growth rate due to previous phytase level.

Overall (d0-111), body weight gain in pigs fed the high dose of phytase in phase 1 and 2 was 41% greater than that in pigs fed the negative control diet (721 vs 1014). Gain was also higher in this group than in pigs fed 500 U/kg phytase for the duration of the study (896 vs 1014). The high level of phytase resulted in growth that was not different than that in the pigs fed the positive control diet. Overall, intake of pigs fed any level of phytase was similar to that in the positive control group and greater than that in the negative control. Feed efficiency was

improved by the addition of 500 U/kg phytase of diet fed throughout the study. It was further improved by feeding a higher level of phytase in phase 1 or 2. Efficiency of pigs fed 12500 U/kg phytase at anytime was similar to that in pigs fed the positive control diet.

Phosphorous Excretion and Nutrient Digestibility

Nutrient digestibility and fecal P excretion are shown in table 3 and figure 3-2. The positive control diet had higher fecal P (%) excretion than negative control groups in phase 1 and 2, as expected. The negative control diet showed higher fecal P (%) excretion during the whole study, compared to phytase supplemented groups. The diets supplemented with 500 or 12500 U/kg phytase in 0.13% available (aval.) P diets decreased (8.3%, 64.58%) fecal P (%) excretion when compared to the negative control diet, and reduced (24.4% and 70.79%) P excretion while compared to the positive control (0.35% aP) treatment in nursery phase (figure 3-2). In phase 2 and 3, there was no difference in fecal P excretion in pigs fed 500 U/kg phytase that had been fed higher levels earlier in the study. In general, the response to dietary phytase was greater in the nursery phase than in the growth/finisher phases.

Digestibility

Pigs fed positive control diet showed better phosphorous (P) digestibility than the no phytase supplemented low P diet ($P<0.0001$) in three phases, but had significantly lower P digestibility than high level phytase (12500 U/kg) addition groups in both phase 1 and 2. The negative control diet had the lower P digestibility than other groups ($P<0.0001$) during the study, as expected. Feeding high phytase (Trt. 4 and 5) in the nursery phase increased P digestibility by 69.11% and 54% over that in the no phytase, supplemented low or high P diet. Pigs fed 12500 U/kg phytase in the growth phase had a 27% improvement in P digestibility, compared to the no phytase low P diet. There was no difference in P digestibility in pigs that were previously fed

high dose of phytase in earlier phase and reduced to 500 U/kg in phase 3 relative to pigs fed 500 U/kg the entire time. The high phytase treatments (trt. 4 and 5) had higher Ca digestibility than negative control, no phytase group (13%) and positive control group (21%) in nursery phase. There was no benefit of phytase on Ca digestibility in growther-finishing phase. Phytase did not improve protein or energy digestibility at any time during the study.

Metartarsal Bone Characteristics

The result of metatarsal bone is shown in Table 3-4. The unsupplemented low P group had significantly lower metatarsal bone dry weight, ash weight, and percent ash ($P<0.0001$) relative to the other groups while the positive control group had significant higher metatarsal bone dry and ash weight than none and 500 U/kg phytase supplemented low P groups ($P<0.0001$). The high dose phytase supplemented groups showed no difference or full recovery on dry weight, ash weight, and percent ash, compared to positive control group. There was no phytase effect in bone length. The addition of phytase (500 U/kg in the entire study, 12500 U/kg in phase 1 or 12500 U/kg in phase 1 and 2) had 85%, 69% and 103% improvement in bone strength, compared to negative control group ($P=0.0002$), and were similar to the positive control. Diets supplemented high phytase (Trt. 4 and 5) did not show the benefit in bone strength, compared to pigs fed diet with 500 U/kg phytase during overall periods. Some samples from each treatment were lost for bone strength determination. This may account for some of the lack of difference between treatments.

Carcass Characteristics

The results of carcass characteristics are shown in table 3-5 and figure 3-3. Carcass characteristics reflected the results of the final BW of each treatment, and did not have difference on positive control and high dose phytase treatments. The positive control and low P with

phytase addition groups (treatment 1, 3 , 4 and 5) had significant greater loin area on the first, tenth, and last rib than negative control group ($P<0.05$). Pigs fed high level phytase diets had greater loin area in ultrasound (day 97 and 111) and slaughter than negative control diet ($P<0.001$). There was no phytase effect on muscle score, color, marbling, firmness, and pH. When co-varied to final body weight, all treatment effects were lost, indicating that there was merely a function of change in body weight.

Discussion

The benefits of phytase to reduced P excretion in manure waste were established after people started to focus on P rather than N in livestock manure management. Exogenous phytase supplementation in LP diet is not only increasing P released from phytate but also shows no difference or even fully recovery of growth performance, and bone traits, compared to adequate P diet. The major sources of microbial phytase are divided into two categories: fungal phytase, A. niger (ex. Natuphos), P. lycii (ex. Rozozyme), and bacteria phytase, E.coli. phytase. The most common level of fungi phytase used was 500 U/kg; the same level of E.coli-derived phytase resulted in the same efficacy on growth performance, plasma iP, and plasma alkaline phosphatase (Stahl et al. 2000, 2004). The outstanding thermostability in E.coli-derived phytase had been reported (Roderique et al. 1999), whereas Pichia pastoris of E. coli phytase had even better stability than other E. Coli-derived phytase (Silversides et al. 2004). Addition of 500 U/kg phytase in the diet did not normalize performance to a low P diet, which agrees with Veum et al. 2006. Feeding a higher level of phytase initially and then 500 U for the remaining grow-out period normalized growth performance and bone measures (table 3-4), and resulted in numerically greater weight gain than 500U/kg phytase treatment in the finish phase. Feeding the

high phytase in both nursery and grower phase resulted in a full recovery of growth performance. Phytase supplementation had greater effects on growth performance during the nursery phase, and diminished as pigs became older (Table 3-2, Figure 3-1). This situation was observed both in fungal and E.coli phytase. Harper et al. (1997), and Jendza et al. (2005), showed a linear increased in ADG with phytase addition in the diet during the starter and growth phases, but decreased the benefit in the finishing phase. This is likely accounted for by reduced P requirements in older pigs or by changes in digestive capacity. Addition of 500 U/kg phytase in our study had average ADG at 0.71, 0.90, 1.05, and 0.90 kg/d for nursery, growth, finishing and overall, which is similar to that reported by Harper et al. 1997, Cromwell et al. 1995 (Allzyme and Natuphos expressed in A. niger) and Brana et al. 2006 (Phyzime, E.coli expressed in S. pombe) but is better than 0.48, 0.73, and 0.80 in the nursery-finishing phases (Jendza et al. 2005, Phyzyme), and 0.70 overall (Shelton et al. 2005, Natuphos). Feed consumption in this study increased as phytase level went up, agreeing with the results reported by Cromwell et al. (1995), Harper et al. (1997), Jendza et al. (2005), and pigs got order (O'Quinn et al. 1997, and Pettey et al. 2006).

Supplementation of diets with 500 and 12500 U/kg phytase decreased (8.3%, 64.58%) fecal P (% of DM) excretion when compared to the negative controlled, and reduced (24.4% and 70.79%) excretion when compared to adequate P treatment in phase 1 ($P<0.0001$) (figure 2), which is similar to that reported by Veum et al. (2006) (35% and 61%). Fecal P was reduced as pigs got order, as we expected.

The digestibility of P was improved by adding phytase to the diet. However, feeding high levels of phytase in the first two phases did not show any sustained benefits, which agrees with Cromwell et al. (1995b).The ATTD of Ca increased as 12500 U/kg phytase supplementation in

the first two phases, but was not in the 500 U/kg treatment. The reason might be that high concentration Ca can reduce phytase activity by competitive active site or bound to phosphate to form Ca-P complex when supported by high level phytase. Liu et al. (1998) reported that Ca:tP at 1.1:1 increased linearly ($P<0.06$) in P digestibility, compared to Ca:tP at 1.5:1, and quadratic increased ($P<0.006$) in Ca digestibility when supplemented by 500U/kg of fungi phytase was supplemental in the finishing phase. The Ca:tP ratio in our study was 1.27:1 in the positive control (0.35% aP) and 1.97:1 in Trts. 2, 3, 4, and 5 (0.13% aP) of nursery phase. This may explain why Ca and P digestibility was not improved by addition of the 500U/kg phytase in the nursery phase in our study. There was no difference on ATTD of Ca in finishing phase, agreeing with that what has been reported previously by Kemme et al. (1997), and Harper et al. (1997).

The ATTD of energy and CP was not affected by phytase, which showed agreement with Silversides et al. (2004), Johnston et al. (2004), Kies et al. (2005), Jendza et al. (2005), and Veum et al. (2006). Onyango et al. (2005) reported increased ATTD of N in chicks with phytase. However, the physiological differences between chicks and pigs and diet composition may be factors. In general, the literature suggests that phytase has greater efficiency in chicks than in pigs due to anatomy and physiology of gut and the rate of diet passage (Augspurger et al. 2003). This may explain the difference of ATTD of N in chicks. Radcliff et al. (2006) showed that digestibility of N with or without phytase in LP and low-CP treatment had no difference in total tract digestibility, but showed numerical improvement in apparent ileal digestibility with phytase ($P=0.07$). The reason may be that undigested CP can be utilized by hind gut bacteria or phytase still has activity in the hind gut and bacteria uptake released CP.

While carcass characteristics were different in pigs fed various level of phytase, these effects were related to body weight differences. Greater loin area and slaughter weight in our

study can be explained by BW (figure 3-3). Shelton et al. (2003), and O'Quinn et al. (1997) all reported no carcass merit differences for low-P, phytase-supplemented pigs and those fed adequate P diets -- the same result reported in our study.

Metatarsal bone ash weight increased linearly following the phytase level ($P<0.0001$). Supplementing 12500 U/kg phytase in the nursery phase resulted in significantly better bone ash weight than adequate P treatment and 500 U/kg phytase supplementation in overall ($P<0.05$), agreeing with Veum et al. (2006). Pigs fed the low P diet with no phytase had the lowest bone strength, as we expected ($P=0.0002$). The results of bone strength in our study did not response to phytase level in the diets, as we expected. We had some samples missing from each treatment, which may account for our bone strength not improving linearly.

Implications

Supplementation of a high level of phytase in nursery or nursery-growing phase did not result in a sustained benefit on P or Ca digestibility relative to pigs fed a standard level. However, the data suggests that adding high levels of E.coli-derived phytase (12,500U/kg) in low phosphorous (0.13% avail. P) diet in the nursery and grower phases resulted in sustained effect on growth performance and bone ash weight in the finishing phase.

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Table 3-1. Diet Component

	Phase 1		Phase 2		Phase 3	
	Positive	Negative	Positive	Negative	Positive	Negative
Corn	64.27	64.87	69.24	69.61	75.42	75.80
Soybean meal	29.61	29.51	25.11	25.03	19.18	19.11
Fat	3.00	3.00	3.00	3.00	3.00	3.00
Lysine	0.14	0.14	0.04	0.04	0.05	0.05
Methionine	0	0	0.07	0.07	0.02	0.02
Salt	0.35	0.35	0.35	0.35	0.35	0.35
Limestone	1.08	1.73	0.98	1.49	0.88	1.27
Dicalcium Phosphate	1.15	0	0.91	0	0.69	0
Vitamins[a] / Minerals[b]	0.40	0.40	0.40	0.40	0.40	0.40
TiO2	0.10	0.10	0.10	0.10	0.10	0.10
Calculated Composition						
Crude Protein, %	19.9	19.9	18.3	18.3	16.0	16.0
Lysine, %	1.15	1.15	0.95	0.95	0.80	0.80
Calcium, %	0.75	0.75	0.65	0.65	0.55	0.55
Phosphorous, total %	0.59	0.38	0.52	0.35	0.46	0.33
Available, %	0.35	0.13	0.30	0.10	0.25	0.10
ME[d], kCAL/kg	3442	3459	3460	3470	3471	3481

Phytase (500 or 12,500 U/kg) added to negative control diet in each phase at the expense of corn. Phytase premix contained 2500 U/g.

0.1 % titanium dioxide was added in the diets a week before fecal collection.

a Supplied per kg of premix: vitamin A 4400 IU; vitamin D 660000 IU; vitamine E 17600 IU; vitamin K 1760 IU; riboflavin 3960 mg; niacin 22000 mg; vitamin B12 17600 μg .

b Supplied per kg of premix: iron 110000 mg; copper 11000 mg; manganese 26400 mg; zinc 110000 mg; iodine 198 mg; selenium 198 mg.

c Titanium dioxide used as an indigestible marker.

d Metabolizable energy.

Table 3-2. Growth Performance Response to Phosphorous Deficiency and Phytase Supplementation.

Diet:	1	2	3	4	5		
Nursery Phase: Day 0-28	Pos Control	Neg Control	Neg + 500 U	Neg + 12,500 U	Neg + 12,500 U		
Grower Phase: Day 28 - 77	Pos Control	Neg Control	Neg + 500 U	Neg + 500 U	Neg + 12,500 U	SEM	P Value
Finisher Phase: Day 77-111	Pos Control	Neg Control	Neg + 500 U	Neg + 500 U	Neg + 500 U		
Body Weight, kg							
Day 0	21.0	21.2	21.1	21.1	21.1	0.12	NS
Day 28	45.5[b]	39.4 [a]	41.0 [a]	45.2 [b]	45.4 [b]	0.61	<0.0001
Day 42	57.0[c]	47.2 [a]	50.9 [b]	55.8 [c]	57.1 [c]	1.25	<0.0001
Day 63	78.4[c]	62.1 [a]	70.8 [b]	78.1 [c]	80.5 [c]	1.58	<0.0001
Day 77	95.0[c]	75.4 [a]	85.0 [b]	92.2 [c]	94.4 [c]	1.63	<0.0001
Day 97	114.1[c]	89.0 [a]	105.2 [b]	112.0 [c]	117.3 [c]	2.00	<0.0001
Day 111	130.4[c]	101.2 [a]	120.5 [b]	126.8 [bc]	133.8 [cd]	2.31	<0.0001
Gain, g/d							
Day 0-28	873 [c]	650 [a]	711 [b]	859 [c]	868 [c]	20	<0.0001
Day 28-77	1011 [c]	737 [a]	898 [b]	959 [bc]	999 [bc]	26	<0.0001
Day 77-111	1042 [b]	757 [a]	1045 [b]	1059 [b]	1156 [b]	32	<0.0001
Day 0-111	986 [c]	721 [a]	896 [b]	952 [bc]	1014 [c]	20	<0.0001
Intake, g/d							
Day 0-28	1653	1491	1581	1613	1629	38	0.08
Day 28-77	2540 [bc]	2104 [a]	2425 [b]	2454 [bc]	2629 [c]	33	0.01
Day 77-111	3255 [c]	2756 [a]	3306 [bc]	3100 [b]	3457 [c]	113	0.01
Day 0-111	2535 [bc]	2149 [a]	2482 [b]	2439 [b]	2630 [c]	31	0.001
Gain : Feed							
Day 0-28	0.529 [b]	0.436 [a]	0.450 [a]	0.532 [b]	0.535 [b]	0.0107	<0.0001
Day 28-77	0.399 [b]	0.348 [a]	0.371 [ab]	0.391 [b]	0.381 [b]	0.0105	0.0411
Day 77-111	0.32 [b]	0.275 [a]	0.316 [b]	0.33 [b]	0.335 [b]	0.0085	0.0028
Day 0-111	0.389 [c]	0.335 [a]	0.361 [b]	0.391 [c]	0.386 [c]	0.007	0.0004

Pigs were fed 3 dietary phases over the course of the study. Phase I (d 0-28) was a 1.15% lysine diet, phase II was a 0.95% lysine diet and phase III was a 0.80% lysine. Diets in each phase met or exceeded the NRC requirements for all nutrients excepted available phosphorous in the negative control diets (Diet groups 2-5).
Means within a row lacking a common superscript letter differ (P<0.05).
Results are LS Means for 4 pens of 4 pigs each per diet

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Table 3-3. Apparent Nutrient Digestibility to Phosphorous Deficiency and Phytase Supplementation in Difference Growing Phases.

Treatment:	1	2	3	4	5	P-value			
Nursery Phase: Day 0-28.	Pos Control	Neg Control	Neg + 500U	Neg + 12,500 U	Neg + 12,500 U	SEM	Phase	Treat	Treat x Phase
Grower Phase: Day 28-77.	Pos Control	Neg Control	Neg + 500U	Neg + 500U	Neg + 12,500 U				
Finisher Phase: Day 77-111.	Pos Control	Neg Control	Neg + 500U	Neg + 500U	Neg + 500U				
Fecal avail. P[a], %									
Phase 1	2.91	2.40	2.20	0.85	1.03				
Phase 2	2.47	2.20	1.68	1.76	1.24	0.115	<.0001	0.0017	<.0001
Phase 3	1.79	2.02	1.41	1.52	1.48				
P Digestibility, %									
Phase 1	49.46	44.98	39.44	76.06	72.50				
Phase 2	52.35	43.36	59.94	55.34	66.55	3.345	0.0842	<.0001	<.0001
Phase 3	59.03	40.61	55.38	57.00	55.36				
Ca Digestibility,%									
Phase 1	59.46	63.18	52.33	72.24	69.47				
Phase 2	55.69	68.25	71.63	64.26	60.93	3.156	0.0769	0.0047	0.0035
Phase 3	54.34	62.43	60.85	63.95	61.63				
CP[b] Digestibility,%									
Phase 1	81.79	86.83	82.25	82.15	82.88				
Phase 2	81.98	82.95	81.83	81.51	82.36	1.208	0.0584	0.5754	0.1241
Phase 3	85.00	82.55	83.46	85.43	84.47				
Energy Digestibility, %									
Phase 1	87.81	89.55	86.61	86.46	86.23				
Phase 2	89.11	90.06	89.20	88.18	89.00	0.750	<.0001	0.0857	0.2178
Phase 3	87.38	86.48	86.33	87.67	87.00				

Results are LS means for 4 pens of pigs in each treatment x three growing phases (Nursery, Growing, and Finishing). Apparent digestibility was determined using Titanium dioxide as the marker.

a Phosphorous in % of fecal dry matter.

b Crude protein.

Table 3-4. Effect of Long-term Supplementation Dietary Phytase on Metatarsal Bone Characteristics.

Diet:	1	2	3	4	5		
Nursery Phase: Day 0-28	Pos Control	Neg Control	Neg + 500 U	Neg + 12,500 U	Neg + 12,500 U		
Grower Phase: Day 28 - 77	Pos Control	Neg Control	Neg + 500 U	Neg + 500 U	Neg + 12,500 U	SEM	P Value
Finisher Phase: Day 77-111	Pos Control	Neg Control	Neg + 500 U	Neg + 500 U	Neg + 500 U		
Metatarsal Bone	(n=16)	(n=16)	(n=16)	(n=16)	(n=16)		
Dry Weight, g	17.82^{c}	13.11^{a}	15.31^{b}	16.77^{bc}	18.02^{c}	0.65	<0.0001
Ash weight, g	8.08^{c}	4.89^{a}	6.79^{b}	7.62^{c}	8.30^{c}	0.32	<0.0001
Percent Ash, %	45.5^{b}	37.2^{a}	44.0^{b}	45.4^{b}	45.9^{b}	1.0	<0.0001
Bone length, mm	71.4	65.3	68.2	70.3	70.4	1.7	0.1061
Bone Width, mm	17.1^{bc}	15.3^{a}	16.0^{ab}	17.3^{bc}	17.5^{c}	0.5	0.0151
Bone Strength *	(n=10)	(n=9)	(n=9)	(n=12)	(n=15)		
Displ. @ peak	6.4^{a}	7.1^{b}	7.1^{b}	6.55^{b}	5.54^{a}	0.37	0.0187
Load @ peak	107.3^{a}	54.7^{b}	101.4^{a}	92.7^{a}	110.8^{a}	8.3	0.0002

*Bone strength measures units: displacement @ peak, mm; load @ peak, kgf.
Some samples were missed for bone strength analysis.
Means within a row lacking a common superscript letter differ (P<0.05).
Results are LS Means for 4 pens of 4 pigs each per diet.

Table 3-5. Carcass Characteristics to Phosphorous Deficiency and Phytase Supplementation.

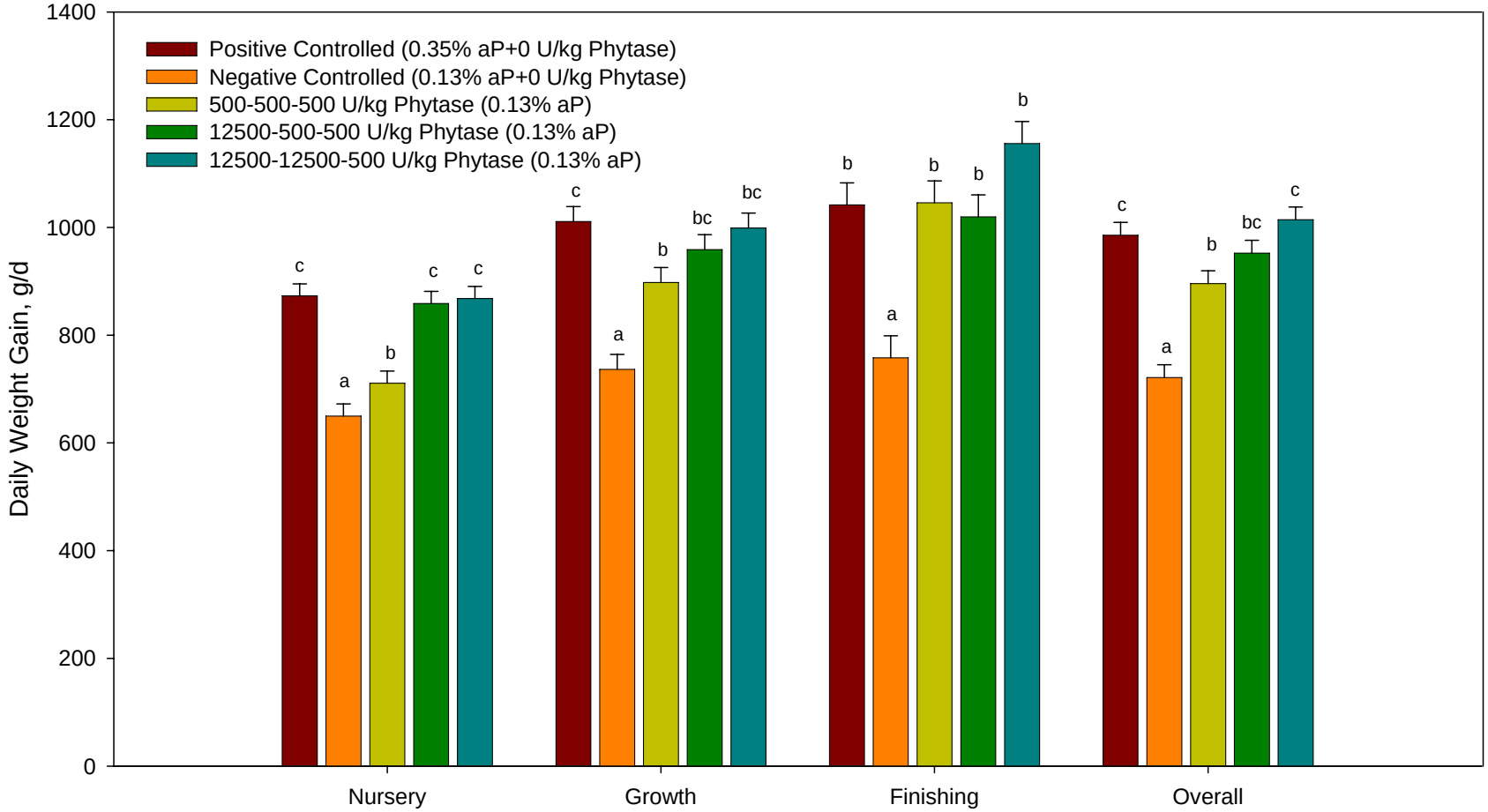
Diet:	1	2	3	4	5		
Nursery Phase: Day 0-28	Pos Control	Neg Control	Neg + 500 U	Neg + 12,500 U	Neg + 12,500 U		
Grower Phase: Day 28 - 77	Pos Control	Neg Control	Neg + 500 U	Neg + 500 U	Neg + 12,500 U	SEM	P Value
Finisher Phase: Day 77-111	Pos Control	Neg Control	Neg + 500 U	Neg + 500 U	Neg + 500 U		
Carcass Weight							
Slaughter wt, kg	130.6[a]	101.5[b]	122.2[c]	127.0[ac]	133.9[ac]	2.7	<0.0001
Hot Carcass, kg	99.2[a]	75.2[b]	91.5[c]	96.0[ac]	101.0[a]	2.1	<0.0001
Yield, %	75.9[a]	74.1[b]	74.9[bc]	75.5[ac]	75.4[ac]	0.3	0.0016
Ultrasound							
Day 97							
Back fat, mm	20.3[a]	16.9[b]	21.4[a]	20.3[a]	21.8[a]	1.1	0.0088
Loin area, cm^2	38.8[a]	31.8[b]	35.3[c]	38.6[a]	38.1[a]	1.0	<0.0001
Day 111							
Back Fat, mm	22.9[a]	18.7[b]	23.0[a]	23.4[a]	24.2[a]	1.2	0.0145
Loin area, cm^2	42.3[a]	35.3[b]	39.0[c]	42.1[ac]	41.3[ac]	1.1	<0.0001
Adjusted BF[a]	19.5	21.7	21.5	20.7	20.2	0.9	0.4566
Adjusted LEA[b]	38.8	37.7	37.7	39.2	37.2	0.9	0.4433
Midline measures							
First rib	43.2[a]	35.6[b]	44.3[a]	41.9[a]	43.2[a]	1.4	<0.0001
Tenth rib	20.9[a]	16.6[b]	21.3[a]	21.4[a]	22.6[a]	1.2	0.0063
Last Rib	24.0[a]	18.9[b]	23.2[a]	23.4[a]	24.1[a]	1.1	0.0038
Last Lumbar	20.0[ac]	18.9[a]	23.1[c]	22.4[c]	22.4[c]	1.1	0.0312
Loin area, cm^2	47.9[a]	38.5[b]	43.4[c]	48.8[a]	47.1[a]	1.1	<0.0001
Muscle score	2.0	1.8	2.0	1.9	2.0	0.04	0.0808
Color	2.38	2.56	2.19	2.56	2.75	0.14	0.0577
Marbling	1.38[a]	1.81[ab]	1.63[ab]	1.38[a]	2.00[b]	0.15	0.023
Firmness	2.25[a]	3.06[bc]	2.38[ac]	2.13[a]	2.87[c]	0.19	0.004
Length, cm	86.7[ac]	80.3[b]	84.0[c]	85.9[c]	87.6[ab]	0.7	<0.0001
pH	5.65	5.67	5.67	5.63	5.69	0.02	0.2934
Hunter L*	56.5[a]	53.6[b]	57.8[a]	57.2[a]	54.4[b]	0.9	0.0046
a*	7.9	6.52	6.5	7.6	8.3	0.66	0.0751
b*	67.82[ac]	54.69[b]	63.15[bc]	68.95[ac]	68.62[ac]	0.2	0.0046

Means within a row lacking a common superscript letter differ (P<0.05).
Results are LS Means for 4 pens of 4 pigs each per diet.

a Adjust backfat (mm).

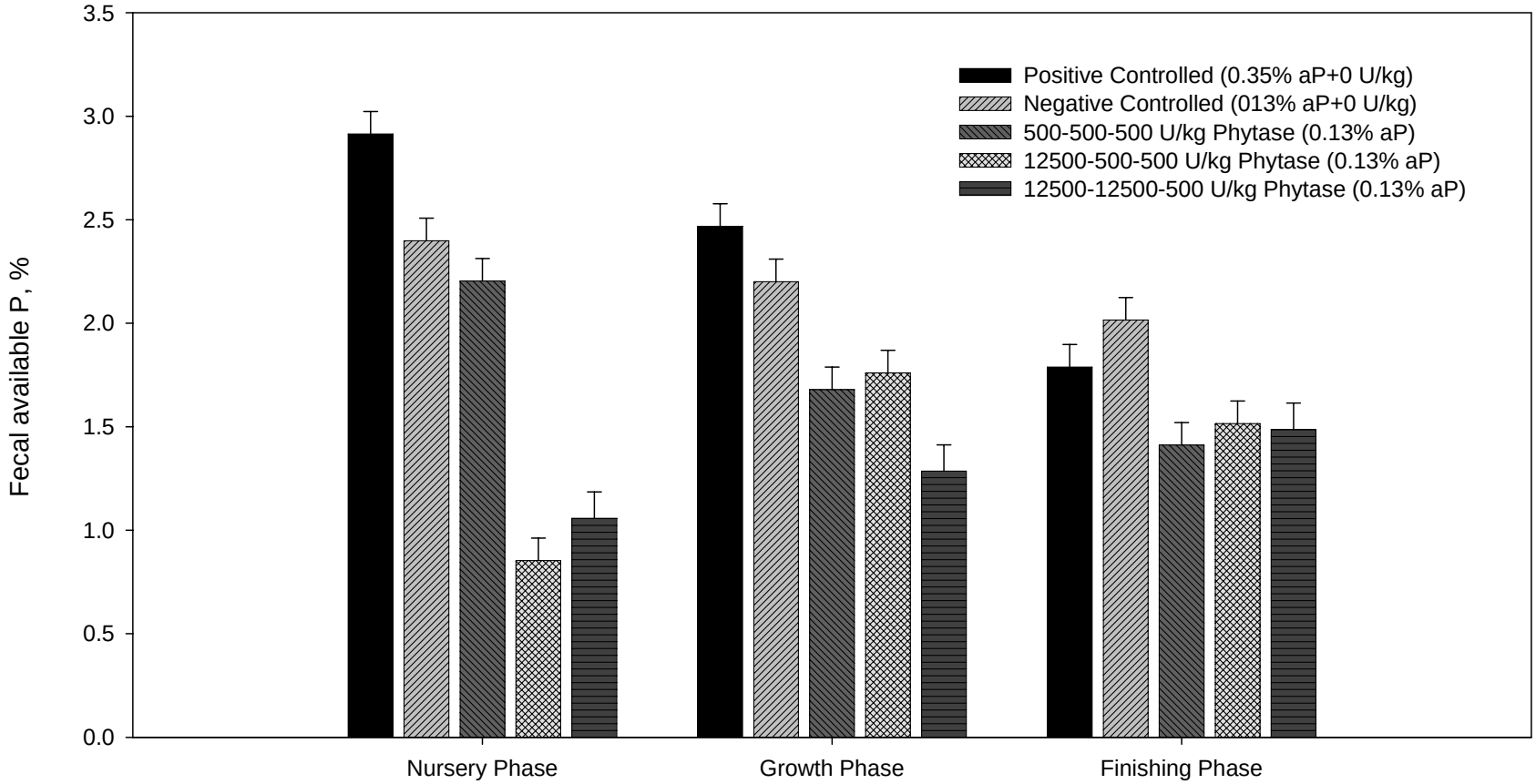
b Adjust loin area (cm^2).

Figure 3-1. The Effect of High Level E. Coli. Phytase Supplementation of Deficient P (0.13% aP) Diet for Daily Weight Gain (kg) in Nursery-Growth-Finishing Phase.



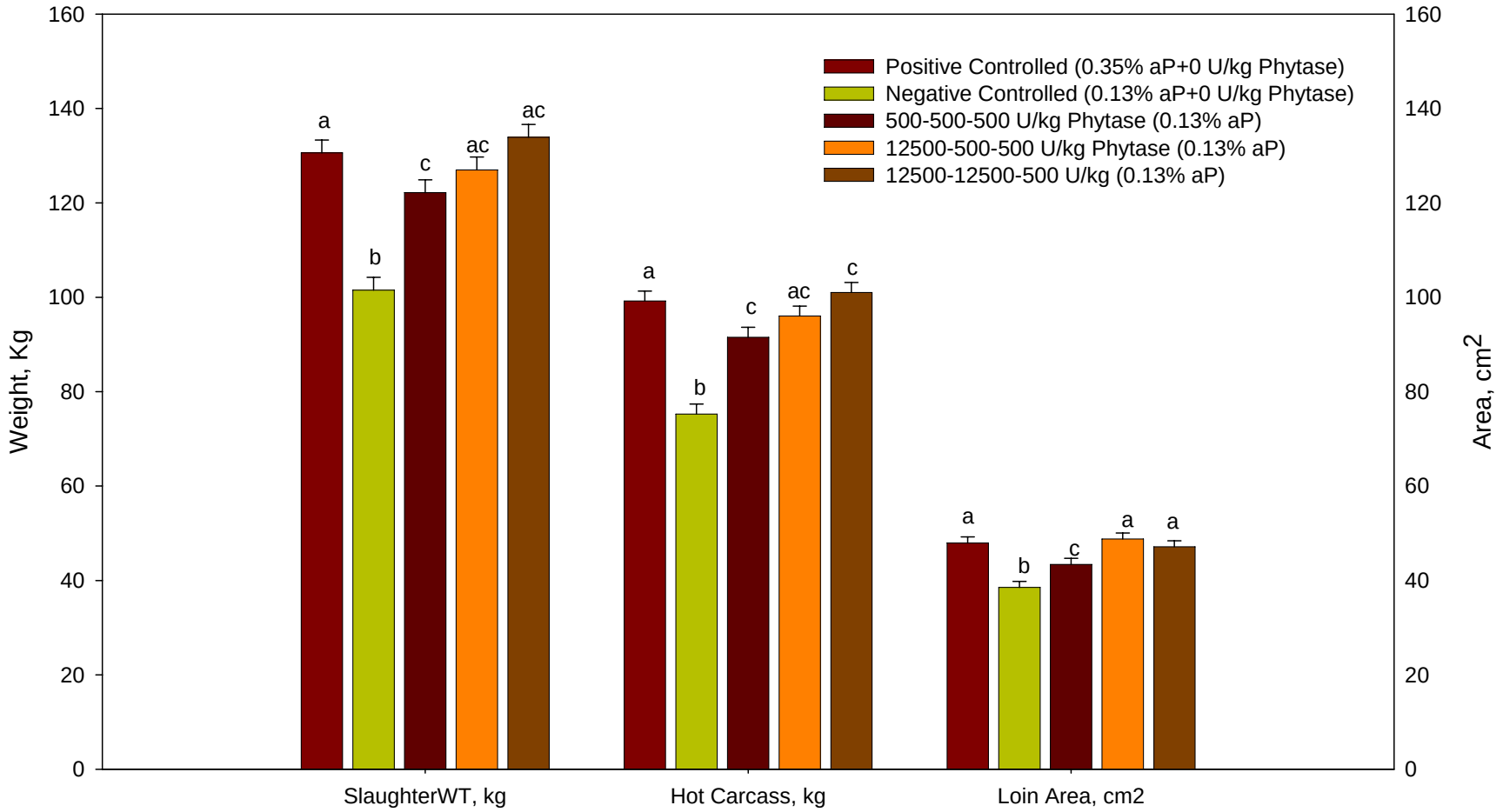
Mean within a bar lacking a common superscript letter differ (P<0.05).
Results are least square means for four pigs of four pens each per treatment.

Figure 3-2. The Effect of Phytase Added in the Diet on Fecal P (%) Excretion During Nursery-Grower-Finishing Phase.



Fecal available P is phosphorous in % of fecal dry matter.
Results are least square means for four pens of pigs in each treatment x phases (Nursery, Growing, and Finishing).
Apparent digestibility was determined by using titanium dioxide as the marker.
Phases (P<0.0001), treatments (P=0.0017), and phase x treatment (P<0.0001) had a significant effect in fecal P percentage of dry matter.

Figure 3-3. The Effect of Long Term Fed High Level Phytase in Pigs' Carcass Characteristics.



Means within a bar lacking a common superscript letter differ (P<0.05).
Results are least square means for 4 pens of 4 pigs each per diet.

CHAPTER 4

THE EFFECT OF VARIED LEVELS OF PHYTASE ON PHOSPHOROUS BALANCE IN WEANING PIGS.

[1]

[1] Tsai, T. C., M. J. Azain, R. Dove, and M. Bedford. To be submitted to The Journal of Animal Science.

Abstract

The objective of this study was to determine the effect of dietary phosphorous (P) and phytase on P balance in weaning pigs. A 2 X 5 fractional arrangement of treatments was used. Two levels of P, low-P (0.13% avail. P) without inorganic P (iP) and adequate-P (HP)(35% avail. P) with 1.15% dicalcium phosphate, were supplemented with 0, 250, 500, 2500, and 12500 U/kg of E. coli-derived phytase. The study involved 20 weaning pigs per replicate and 4 replicates with pigs housed in metabolism cages. In Low-P (LP) treatment, body weight gain had increased linearly as the phytase level went up during the adaptation period and the overall ($P<0.001$). Phytase had more efficiency in LP diets in overall weight gain than in HP diets ($P<0.05$). The overall intake also tended to have more response to phytase in LP than in HP ($P<0.2$). Pigs, fed 2500 and 12500 U/kg phytase, had the better G : F ratio, compared to other treatments within the same P level. Addition of phytase improved G : F ratio more with 0.13% available P than 0.35% available P treatments ($P<0.05$). Fecal P was reduced as the phytase level increased ($P<0.0001$). Phytase supplementation in LP improved P and Ca retention linearly ($P<0.01$). Urinary P was less than 5% of total P excretion in pigs fed the LP diet and was not affected by phytase. Urinary P increased with phytase addition to the HP diet and accounted for 30% of the total P excretion on the HP diet with 12500 U phytase. Calcium retention (%) was improved ($P < 0.001$) by the addition of phytase to both LP and HP diets. Urinary Ca was reduced by phytase ($P < 0.001$) in both diets. Apparent total tract digestibility (ATTD) for P increased 35.53%, 18.09%, 48.97%, and 75.76% in 250, 500, 2500, and 12500 U/kg phytase addition in the diet of LP group. The ATTD for P and Ca showed a greater response to phytase addition in LP diets than HP diets ($P<0.05$). There were no phytase effects for intake, fecal dry matter, digestibility of energy and crude protein. These results suggest that adding phytase to a low-P (0.13% aP) diet had better

efficiently in reducing the P and Ca excretion and increasing body weight gain. Moreover, E. Coli-derived phytase can replace inorganic phosphorous in the diet as well for weaning pigs.

Introduction

The benefit of exogneous phytase from fungi and E. coli has been well established. Aspergillus niger phytase (3-position) in the Natuphos and Allzyme products has been shown to improve growth performance, P release, digestibility of P, Ca and N as well as bone traits (Cromwell et al. 1995, Kemme et al. 1997,1998, Harper 1997, Selle et al. 2003, Kim et al. 2005). The phytase produced in Peniophora lycii (6-position) that is in the Ronozyme product was reported to have a similar benefits (Lassen et al. 2001). E. coli-derived phytase (6-position) has also been shown to improve growth performance at a level of 500 U/kg phytase in the diet (Augspurger et al. 2003, Brana et al. 2006, Veum et al. 2006). Improved thermostability (Rodrique et al. 1999) of the E. Coli. phytase is an advantage, particularly in pelleted diets fed to monogastric animals. The E. Coli. phytase (produced in P. pastoris) that we used in our study has been showed to improve P absorption, bone characteristics, and retention of Ca, N and certain AA (Silverside et al. 2004, Onyango et al. 2005, Veum et al. 2006). E. Coli-derived phytase, expressed in pichia pastoris, was reported to have a greater benefit on nutrient digestibility and reducing P excretion than a fungal phytase in broiler chicks fed deficient P diet (Silversides et al. 2004) and in pigs (Augspurger et al. 2003). A greater understanding of P utilization and balance with phytase supplementation in the diet can maximize the phytase benefit of growth performance and the reduction of environmental contamination. The object was to determine the uppermost benefit in E. Coli. phytase supplementation in young pigs.

Material and Method

A total of 80 weanling barrows (7-week age, average weight 18.50 ± 1 kg) were randomly selected from the University of Georgia Animal & Dairy Science Department Swine Unit. Pigs used for this research were weaned at approximately 21 days of age and fed a common diet for four weeks before the experiment started. There were 20 pigs per replicate and 4 replicates of 14-days trial were conducted. Pigs were housed in an environmentally regulated room in LARU (Large Animal Research Unit). Room temperature was set at 23 ^{0}C, and a 12-hr light/dark (0700/1900) cycle was set. The pigs were trained to meal feeding twice each day at approximately 0800 and 1600hr at the rate of 3-3.5% of body weight per day. Feeding time was 45 minutes. Water was available ad libitum. Feed consumption was recorded daily, and feed refusals and spillage were collected. Pigs were weighed at day 0, and placed into individual stainless steel metabolism cages (0.71mX0.81m), equipped with a water nipple, feeding bowl holder, and plastic-coated expanded metal floors. On day 10, pigs were weighed, and cages were cleaned and set up for a 4-day collection trial. During the 4-day collection period, the total fecal output was collected twice daily from each pig. Urine was collected twice daily into containers with 25ml 3N HCL. The individual pig urine total volume was recorded, and 10% of the total was reserved in a 1L bottle. Fecal and urine samples were stored at -20 $^{\circ}C$ until further analysis. The screens and trays were washed after every collection. At the end of the trial, the pigs were returned to the farm.

All pigs were fed a standard weaning diet for 4 weeks before the trials. During the trial, a corn-soybean basal diet was used, and each treatment met or exceeded the NRC recommendation, except for the P content. The diet contained 1.15% of lysine and 3400 kcal/kg metabolism energy, in both LP and HP. The ratio of calcium and total phosphorous was 1.3:1 in HP and 2:1 in LP.

Treatments 1-5, low-P (0.13% avail. P), were supplemented with 0, 250, 500, 2500, 12500 U/kg phytase at the expense of corn. Treatments 6-10 had adequate P (0.35% avail. P) by adding dicalcium phosphate, and supplementation of 0-12500 U/kg phytase (Table 4-1). Titanium dioxide (0.1%) was added to all diets as an indigestible marker to compare digestibility of total collection and marker. The diet composition is shown in Table 4-2.

Sample Analysis

Total fecal samples collected from each pig were thawed, and mixed well by blender. The total samples weight was recorded. Two sub-samples were prepared. One of the sub-samples was oven dried at 65 ^{0}C for 72 hrs, and the dry matter weight was recorded. The second sub-sample was freeze dried and the freeze-dry matter weight was recorded. Freeze dried samples were ground into mill, and used for determination of mineral, CP, and gross energy. Diet, fecal, and urine CP were analyzed by A Leco FP-528 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI). Diet and fecal gross energy were determined by bomb calorimeter (Parr 1261, Parr Instrument Co., Moline, IL). The sub-samples of urine were thawed and pooled into large measuring containers. After mixing, triplicate 100ml samples were transferred to plastic cups. Fecal freeze-dry matter and urine samples were sent to the Agricultural and Environmental Services Laboratories (AESL) of UGA, and the mineral content was determined by inductively coupled atomic emission spectroscopy (ICP-AES).

Digestibility

Apparent nutrient digestibility was calculated using total collection and the external marker. Apparent total tract digestibility of nutrients was determined by the following equation:

$$\text{ATTD}= \frac{([Nuti.]_{\text{int}\,ake} - [Nuti.]_{feces})}{[Nuti.]_{\text{int}\,ake}} \times 100$$

Apparent total digestibility of nutrient by marker was calculated by following equation:

$$\text{ATTDM}=1-\frac{([mar\,\text{ker}]_{feed}\times[Nuti.]_{feces})}{([mar\,\text{ker}]_{feces}\times[Nuti.]_{feed})}$$

Statistical Analysis

All data were analyzed using the PROC GLM procedure in SAS as a 5X2 design with main effects of level of phytase (0, 250, 500, 2500, 12500) and level of phosphorous (0.13 and 0.35%) as well as replicate trial (1-4) interactions. Results are presented as least square means for the phytase x Plevel effects. Each pig was used as the experimental unit in ANOVA.

Results

Growth Performance

Addition of phytase to both the LP and HP diets resulted in improved body weight gain for day 0-10 ($P<0.0001$. Table 4-3). Pigs fed the HP diet had greater growth rates than those fed the LP diet ($P<0.0001$) (Figure 4-1). There was a trend ($P=0.065$) for an interaction of diet and phytase that was accounted for by the greater response to phytase in pigs fed the LP diet. On the LP diet, addition of 250, 500, 2500 and 12500 U/kg phytase/kg improved gain by 28, 30, 55 and 56% over the unsupplemented control. In the HP diet, addition of phytase improved growth rate by 3, 16, 8 and 14% over the unsupplemented control in this group.

During collection period (day 10-14), the effects of phytase on growth rate were not significant, but pigs fed HP diets had greater gain than those fed the LP ($P<0.0001$). Overall (day 0-14), the main effects of phytase ($P<0.005$) and diet ($P<0.0001$) and their interaction ($P<0.05$) were significant (Figure 4-2). As for day 0-10, the effects of phytase were greater in pigs fed the LP diet than in those fed the HP.

There was no significant effect of phytase on intake. Overall, pigs fed the HP diets had greater intake than those fed the LP diets ($P<0.01$). Feed efficiency was better in pigs fed HP diets than in those fed LP for day 0-10 or day 0-14, and was no effect during the collection period. Overall, there was a significant main effect of phytase on G : F ratio for day 0-10 ($P<0.0001$) and day 0-14 ($P<0.0005$). As with growth rate, there were significant interactions of diet and phytase level on G : F ratio for day 0-10 ($P=0.0652$) and day 0-14 ($P<0.05$). The interactions were accounted for by the greater response to supplemental phytase in pigs fed the LP diets. Efficiency in pigs fed the LP diets with 2500 or 12500 U/kg was not different from that in pigs fed the HP diet.

Phosphorous Excretion and Digestibility

The result of fecal and urine excretion is shown in Table 4-4. P intake was significantly higher in the adequate P (HP) group as expected ($P<0.0001$). No difference was observed in fecal oven-dry and freeze-dry matter between each treatments. Supplementing 250, 2500, and 12500 U/kg phytase in the low-P (LP) diet reduced fecal P excretion by 6.67%, 36%, and 46% while adding the same level phytase in the HP group resulted in 22.07%, 26.76%, 32.86%, and 30.52% less P output. The HP group had higher average P excretion than the LP group in feces ($P<0.0001$). The LP tended to have better phytase response in fecal P excretion than HP ($P<0.15$). The apparent total tract digestibility by marker (ATTDM) of P increased 35.53%, 18.09%, 48.79% and 75.76% ($P<0.0001$) while apparent total tract digestibility (ATTD) of P increased significantly by phytase level (11.44%, 2.98%, 26.67%, 30.37%) in LP ($P<0.0001$). The ATTD of P was increased 14.03%, 14.47%, 21.58%, and 16.37% as 250-12500 U/kg phytase was added in the LP group, compared to non-phytase Trt. in HP. Both ATTDM and ATTD of P showed greater phytase response in LP than in HP ($P<0.3$, $P=0.05$).

Feeding phytase resulted in significant increase in urine P in pigs fed the HP diet ($P<0.0001$). The phytase level and phosphorous level interaction was significant and this due to the lack of change in LP as compared to HP ($P<0.0001$). Total P output was reduced significantly ($P=0.0174$) by increasing the phytase level, similar to feces P output. However, the total P excretion was increased in pigs fed 2500 and 12500 U/kg phytase in HP because urine P excretion dramatically affected the result of the total P output (Figure 4-3). The 0.13% aP treatments had numerically better response to phytase for P retention than 0.35% aP treatments ($P=0.3623$), and showed a greater response to phytase as a percentage of P retention in LP, compared to HP ($P<0.001$). The percentage of P retention increased by 12.81%, 3.73%, 28.16%, and 32.17% as 0-12500 U/kg phytase was supplemented in the LP group, and by 12.89%, 12.05%, 15.93%, and 3.27% in HP.

The percentage of fecal total P (as DM basis) was 15.08%, 9.55%, 34.67%, and 45.23% lower than no phytase treatment in LP group as phytase level increased, and was 18.98%, 24.07%, 26.85% and 21.76% less than 0 phytase treatment in HP group (Figure 4-4).

Calcium Excretion and Digestibility

Calcium intake was not different between each treatment as expected. Phytase did not reduce fecal Ca excretion significantly in either LP or HP diets. The ATTDM and ATTD of Ca was significantly better for phytase in HP than in LP ($P<0.02$, $P<0.05$, Table 4-5). The ATTDM of Ca in pigs fed 2500 and 12500 U/kg phytase in LP diet was increased 31% and 44%, compared to those fed HP with no phytase supplementation in the diet. Urinary Ca excretion was reduced in both LP and HP ($P<0.0001$) whereas LP had numerically greater phytase response than HP ($P=0.3563$). Total Ca output was significantly reduced in both LP and HP ($P<0.0001$) as phytase was applied (Figure 4-4). Calcium retention increased with phytase ($P<0.0005$) and was

greater in the HP diet (P<0.05). The calcium retention showed a trend of more response for phytase in 0.13% aP treatments than 0.35% aP treatments (P<0.06, Figure 4-5).

Energy and Crude Protein Digestibility

The result of energy and crude protein digestibility is shown Table 4-5. There were no significant phytase effect in energy and CP apparent total marker digestibility (ATTDM) observed between each treatment. The apparent total tract digestibility (ATTD) of energy was better in LP than in HP (P<0.005). The ATTD of CP in LP was better than in HP (P<0.005), and also tended to show more phytase response in LP as well (P=0.1426).

Discussion

Recovery of TiO_2 in this study was 76%, but was reported to have perfect recovery by Short et al. (1996), Titgemeyer et al. (2001). This may be caused by limiting intake or abbreviated fecal total collection. Although Kavanagh et al. (2001) observed lower recovery in their data, it was less than that reported in our study. Myers et al. (2004) used a different procedure to analyze TiO_2 recovery from spiked organic matter samples in forage and feces to compare with the dry ash procedure of Short et al. (1996). The result of the dry ash procedure in forage was 74.28, which was similar to our result. So, the procedure of TiO_2 analysis needs to be reviewed in further.

The effect of phytase on growth performance, reducing P excretion, and improving bone strength have been widely discussed (Simons et al. 1990, Jongbloed et al. 1992, Cromwell et al. 1993, Lei et al. 1993a,b, Kornegay and Qian, 1996). Historically, inorganic P (iP) was used in diets to provide sufficient available P (Haper et al. 1997). When dicalcium phosphate is supplemented, BW gain (P<0.0001), intake (P<0.0001), and bone ash (P<0.05) were

significantly higher than LP (0.13% aP) in our study. However, inorganic phosphorous results in significant excess P in manure, and it resulted in environmental impact, which is what we tried to avoid. Compared to dicalcium phosphate addition treatment without phytase, high level phytase supplementation of LP showed a benefit in growth performance, P and Ca digestibility, and bone characteristic (Augspurger et al. 2004, Kies et al, 2006). In our work, phytase addition reduced fecal total P % to 34.67% and 45.23% (Figure 4-3), compared to no phytase in LP, and 40% and 50%, compared to no phytase in HP (0.35% aP). The addition of 2500 U/kg phytase in the diet can replace inorganic phosphate treatment in this study, but the level of E. Coli. phytase needed in the diet still depends on different ingredients used.

The addition of 500 U/kg E. coli phytase in our study increased BW gain by 15% when compared to no phytase treatment (Table 4-3) in LP. This is similar what has been observed with the same level of other phytases (Augspurger et al. 2004, Qian et al. 1996, Stahl et al. 2004, Veum et al. 2006). Moreover, fully recovery of P and Ca digestibility, and bone strength with 2500, 12500 U/kg phytase supplement of LP in our study, compared to HP without phytase, was also in agreement with previous study. Kies et al. (2006) demonstrated that use of 15000 FTU/kg phytase in a deficient P diet improved Ca and P digestibility up to 75.8% and 83.8%; addition of 12500U/ Kg phytase of LP in our study increased Ca and P digestibility up to 82.39% and 81.73%. Moreover, addition of 500, 2500 U/kg phytase of HP in our study resulted in still indicated better BW gain, and bone strength. So, this might mean that the E. Coli. phytase level we used in LP in our study still did not reach maximum performance on growth and nutrient utilization. The result of our study contrasted with those of Augspurger et al. (2003) who reported that 10000 FTU/kg from Natuphos and EcoPhos released approximately 75% to 100% phytate-P, and reached maximum P utilization. However, that was in chicks.

Using genetic selection to lower nutrient loss might be another important option to reduce manure waste. Crocker et al. (2002) indicated that maternal line (WL) genetic group (Landrace x Large white) had the lowest concentration excretion in P, Ca, Zn, and Cu ($P<0.01$) after compared to paternal line (BL) (Hampshire x Duroc) and maternal x paternal cross (F1). Although WL needs more days to reach 105kg, it is necessary to struggle between the benefit of environment impact and disadvantage of money loss in extra days to reach market weight. High-testosterone pigs had greater output of P, Ca, Cu and K than low-testosterone pigs ($P<0.05$). This gives us a different way to reduce environmental impact in the swine industry.

The influence of the ratio of calcium and total phosphorous in the diets in phytase efficiency has been discussed. Maintaining the Ca: tP ratio at 1.2:1 or 1.6:1 with phytase improved growth performance, bone characteristics, and P and Ca digestibility by comparison of to 2:1 (Qian et al., 1996). Most studies discussed the relationship of Ca and tP level effect in pigs by fungal phytase (Liu et al. 2000). There is still a lack of research adequately defining the effect of Ca: tP ratio with E. Coli. phytase in the pig. In this study, calcium and total P for LP was 2.08:1 and 1.29:1 for HP. Pigs supplemented 500 U/kg or lower in LP did have a negative effect on P retention, and Ca and P digestibility. However, high Ca : tP ratio in our diet did not diminish the Ca and P digestibility in 2500 and 12500 U/kg phytase (Table 4-5), compared to Veum et al. (2006). Thus a high level of phytase may interferes with Ca competitive binding to the enzyme active site, and release Ca and P efficiently from phytate.

Total P excretion was mainly related to fecal P excretion in LP (0.13% aP). However, in HP (0.35% aP), urine P, increased dramatically (Figure 4-3), and affected the result of total P excretion. Urine Ca reabsorption had trend to increased linearly in LP ($P<0.06$) as phytase increased (Figure 4-5). The reason for this might be that phytase increases P released from

phytate and iP is absorbed in the small intestine, which increased serum P. Ca: tP must be 2:1 and 1:1 to restore Ca and P into bone, and uptake from digestive tract. So, serum Ca drops or binds to phosphorous or phospholipids, etc. and forms a complex, which trigger PTH (parathyroid hormone) hydroxylation 25-hydroxy vitamin D_3 to calcitriol, which will increase Ca and P absorption from digestive tract, and stimulate cyclic AMP, which will increase Ca reabsorpton in the thick ascending limb of loop of Henle and distal nephron by 40% and inhibit P excretion in the proximal tubule (Laiken, 1985).

Implication

The benefit of E. Coli. phytase in BW gain, P and Ca retention and digestibility, and bone strength was observed. In this study, 2500 U/kg phytase supplementation to a low phosphorous diet (0.13% aP) seems to maximize the phytase efficiency, and most importantly reduced the environmental contamination from excess P excretion. Selecting pigs with better ability to utilize phytate P and supplementing with phytase accompanied by low phytate grain should diminish the P pollution in the environment.

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Table 4-1. The 2x5 Fractional Arrangement Design.

Treatment	Plevel	Phytase level (U/kg)
1	0.13% aP*	0
2		250
3		500
4		2500
5		12500
6	0.35% aP	0
7		250
8		500
9		2500
10		12500

* Available phosphorous requirement for 20-50 kg pigs are 0.23% (NRC1998). 0.35% is marginal value.

Phytase was premixed with cornstarch.

To achieve low phosphorous treatment diet simply removed inorganic phosphorous (dicalcium phosphate).

Table 4-2. Diet Component.

	Low P diet	Adequate P diet
Corn	64.75	64.15
Soybean meal	29.53	29.62
Fat	3.0	3.0
Salt	0.35	0.35
Limestone	1.73	1.08
Dicalcium Phosphate	0.0	1.15
Vitamin Premix[a]	0.25	0.25
Mineral Premix[b]	0.15	0.15
Lysine	0.14	0.14
Marker (TiO_2)[c]	0.1	0.1
Calculated Analysis:		
ME[d], kcal/kg	3420	3400
Crude protein, %	20.16	20.15
Lysine, %	1.15	1.15
Calcium, %	0.75	0.75
Total P, %	0.36	0.58
Available P, %	0.14	0.35

Low P treatment simply removed inorganic P.
Both low P diet and adequate P diet supplemented with 0, 250, 500, 2500, and 12500 U/ kg phytase, formed ten treatments.
Higher limestone in low P diet was to compliment Ca.

a Supplied per kg of premix: vitamin A 4400 IU; vitamin D 660000 IU; vitamine E 17600 IU; vitamin K 1760 IU; riboflavin 3960 mg; niacin 22000 mg; vitamin B12 17600 μg .

b Supplied per kg of premix: iron 110000 mg; copper 11000 mg; manganese 26400 mg; zinc 110000 mg; iodine 198 mg; selenium 198 mg.

c Titanium dioxide used as an indigestible marker.

d Metabolizable energy.

Table 4-3. The Effect of Different Levels Phytases in LP (0.13% aP) and HP (0.35% aP) on Growth Performance.

Diet	1	2	3	4	5	6	7	8	9	10	P Value			
Treatment	LP (13%)					HP (35%)					SEM	Plevel	Phytase	Plevel x Phytase
Phytase	0U	250U	500U	2500U	12500U	0U	250U	500U	2500U	12500U				
Gain, kg														
Day 0-10[a]	3.32	4.24	4.29	5.16	5.20	4.84	4.98	5.61	5.23	5.52	0.288	<.0001	0.0003	0.0647
Day 10-14	1.97	2.11	2.04	2.34	2.32	2.56	2.32	2.54	2.54	2.38	0.167	0.0039	0.6973	0.4725
Day 0-14[a]	5.29	6.35	6.33	7.50	7.35	7.40	7.30	8.21	7.77	7.79	0.353	<.0001	0.0022	0.0348
Intake, g/d														
Day 0-10	879.64	869.59	841.34	919.57	912.59	905.24	894.15	982.18	927.31	906.16	28.004	0.0335	0.5903	0.0859
Day 10-14	988.76	1015.70	965.67	1087.98	1020.17	1086.63	1083.96	1115.23	1107.43	1071.77	44.947	0.0083	0.6583	0.6575
Day 0-14	911.22	911.34	876.86	967.69	943.32	957.07	948.38	1029.34	978.77	953.47	29.734	0.0081	0.6081	0.1211
Feed:Gain														
Day 0-10	0.37	0.48	0.50	0.56	0.57	0.53	0.56	0.57	0.56	0.61	0.027	0.0001	<.0001	0.0652
Day 10-14	0.50	0.51	0.52	0.53	0.61	0.58	0.54	0.57	0.58	0.55	0.034	0.1501	0.5493	0.3625
Day 0-14	0.41	0.49	0.50	0.55	0.55	0.54	0.55	0.57	0.56	0.58	0.020	<.0001	0.0002	0.0242

* One pig in this treatment had F:G=16.64 at day 10-14. After removed that pig, F:G=1.98 for day 10-14, and 2.02 for overall.
a Linear and quadratic improvement in weight gain of low-P at day 0-10 and overall (P<0.005).
One pigs in treatment one was removed during collection because of sick, and it was not caused by treatment effect.
Results are LS Means for 8 pigs each treatment, the interaction between two phosphorous levels (0.13% and 0.35% aP) and five ohytase levels (0, 250, 500, 2500, and 12500 U/kg). Significant was set at P<0.05.

Table 4-4. The Effect of Different Levels Phytases in LP (0.13% aP) and HP (0.35% aP) on P Balance.

Diet	1	2	3	4	5	6	7	8	9	10	P Value			
Treatment	LP (13%)					HP (35%)					SEM	Plevel	Phytase	Plevel x Phytase
Phytase	0U	250U	500U	2500U	12500U	0U	250U	500U	2500U	12500U				
P intake, g/d	4.20	4.66	4.42	4.68	4.47	6.07	6.61	6.07	6.54	6.27	0.267	<.0001	0.2608	0.9859
Fecal P[a], g/d	1.50	1.40	1.54	0.96	0.81	2.13	1.66	1.56	1.43	1.48	0.141	<.0001	<.0001	0.1333
Urine P, g/d	0.066	0.042	0.050	0.050	0.040	0.090	0.189	0.221	0.369	0.655	0.046	<.0001	<.0001	<.0001
Total P[a], g/d	1.57	1.45	1.59	1.01	0.85	2.22	1.85	1.78	1.80	2.13	0.146	<.0001	0.0174	0.005
P retention[a], g/d	2.63	3.21	2.83	3.67	3.61	3.85	4.76	4.29	4.75	4.14	0.262	<.0001	0.0065	0.3623
P retention[a], %	61.14	68.97	63.42	78.36	80.81	62.63	70.70	70.18	72.61	64.48	2.659	0.1395	<.0001	0.0008
Ca intake, g/d	7.99	7.36	6.64	8.17	8.20	7.05	7.51	7.73	7.82	7.91	0.558	0.8574	0.4637	0.4698
Fecal Ca, g/d	1.67	1.88	1.93	1.50	1.47	2.32	1.92	1.80	1.69	2.06	0.189	0.0286	0.2755	0.1748
Urine Ca[b], g/d	2.07	1.80	1.59	1.38	1.12	1.09	0.41	0.47	0.23	0.29	0.142	<.0001	<.0001	0.3563
Total Ca, g/d	3.74	3.69	3.53	2.88	2.59	3.42	2.33	2.27	1.91	2.35	0.251	<.0001	<.0001	0.0865
Ca retention[a], g/d	4.25	3.67	3.12	5.29	5.62	3.64	5.18	5.46	5.91	5.56	0.520	0.0275	0.0039	0.0595
Ca retention[a], %	48.32	49.15	43.04	64.28	68.73	49.02	62.12	69.77	75.28	67.64	4.125	0.0003	<.0001	0.0095
Fecal avail. P[ac], %	1.99	1.69	1.76	1.30	1.09	2.16	1.75	1.64	1.58	1.69	0.134	0.0241	<.0001	0.1071

a Linear and quadratic difference in fecal P excretion, total P excretion, P retention, Ca retention and fecal available P of low-P ($P<0.005$).

b Linear and quadratic difference in urine Ca of high-P ($P<0.0001$).

c Phosphorous in % of fecal dry matter.

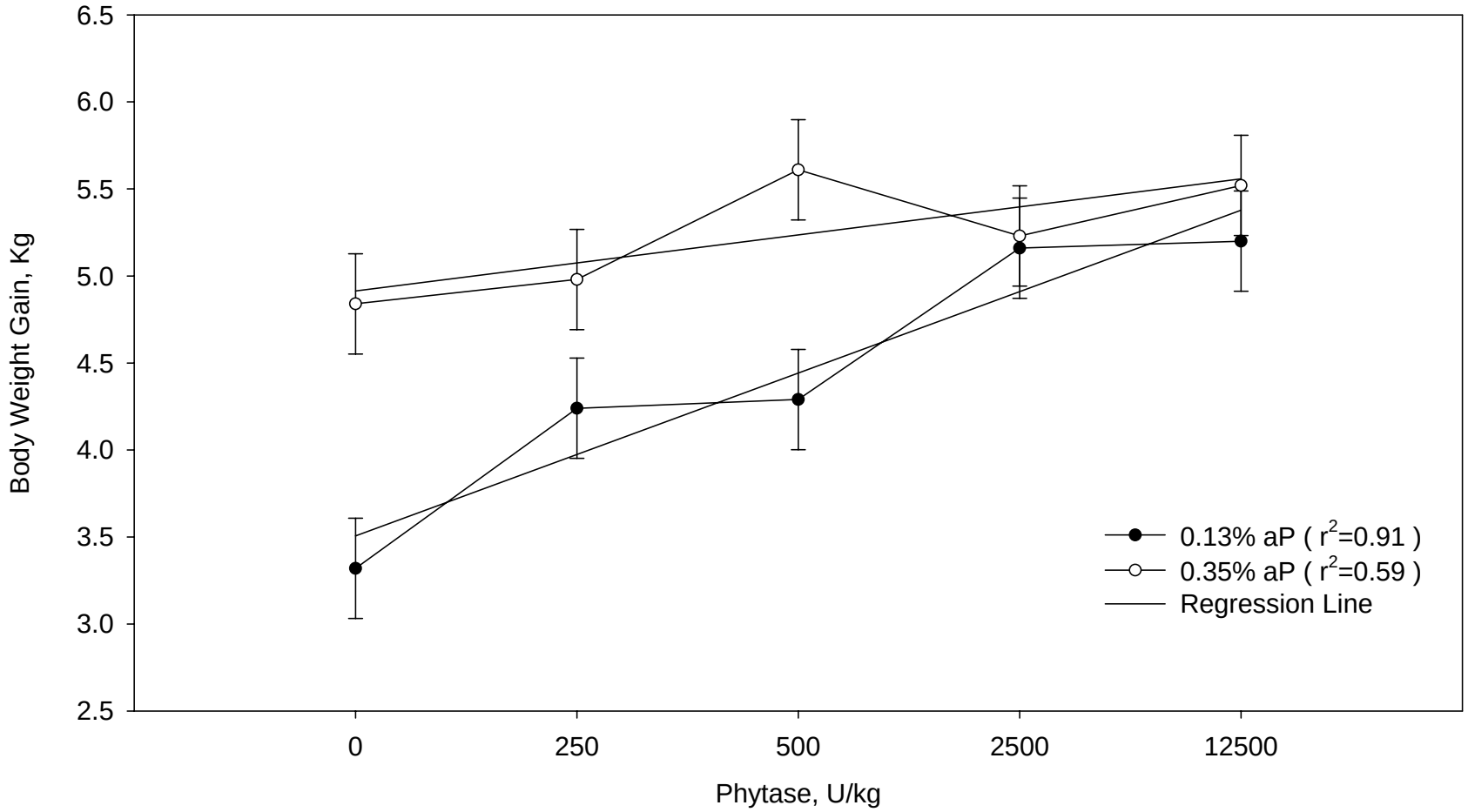
Results are LS Means for 8 pigs each treatment, the interaction between two phosphorous levels (0.13% and 0.35% aP) and five ohytase levels (0, 250, 500, 2500, and 12500 U/kg). Significant was set at $P<0.05$.

Table 4-5. The Effect of Different Levels Phytases in LP (0.13% aP) and HP (0.35% aP) on Apparent Digestibility.

Diet	1	2	3	4	5	6	7	8	9	10	P Value			
Treatment	LP (13%)					HP (35%)					SEM	Plevel	Phytase	Plevel x Phytase
Phytase	0U	250U	500U	2500U	12500U	0U	250U	500U	2500U	12500U				
Fecal oven dry, %	36.44	34.47	35.70	32.81	36.05	34.52	33.10	33.27	32.63	31.75	1.576	0.0449	0.5241	0.7592
Fecal freeze dry, %	39.29	38.04	38.04	34.11	38.54	39.03	38.64	38.96	35.71	37.00	1.691	0.8077	0.118	0.903
Digestibility using marker(Ti2O3), %														
Energy	86	84.58	83.79	83.39	85.221	83.37	85.11	84.4	84.18	85.832	0.865	0.9775	0.2969	0.2547
CP	84.16	83.44	81.49	82.04	82.897	82.08	82.31	83.05	81.02	84.486	1.211	0.7804	0.4536	0.4297
P[a]	37.43	50.73	44.2	55.76	65.787	43.73	62.26	60.67	62.42	63.677	4.255	0.0053	<.0001	0.2659
Ca[a]	59.61	57.59	53.44	60.95	67.427	46.69	59.76	64.51	62.82	58.655	3.727	0.5788	0.0962	0.0169
Apparent Digestibility, %														
Energy	91.63	90.54	89.64	92.07	91.787	89.27	89.39	89.43	90.63	90.305	0.524	0.0011	0.0053	0.3611
CP	90.9	90.08	88.18	91.89	90.826	88.88	87.81	88.77	89.32	89.824	0.688	0.0016	0.0150	0.1708
P[b]	62.69	69.86	65.24	79.41	81.726	64.28	73.3	73.58	78.15	74.801	2.643	0.5407	<.0001	0.0746
Ca[b]	73.98	73.68	68.74	81.09	82.339	64.9	68.4	75.91	77.7	70.689	3.237	0.0585	0.0200	0.0561

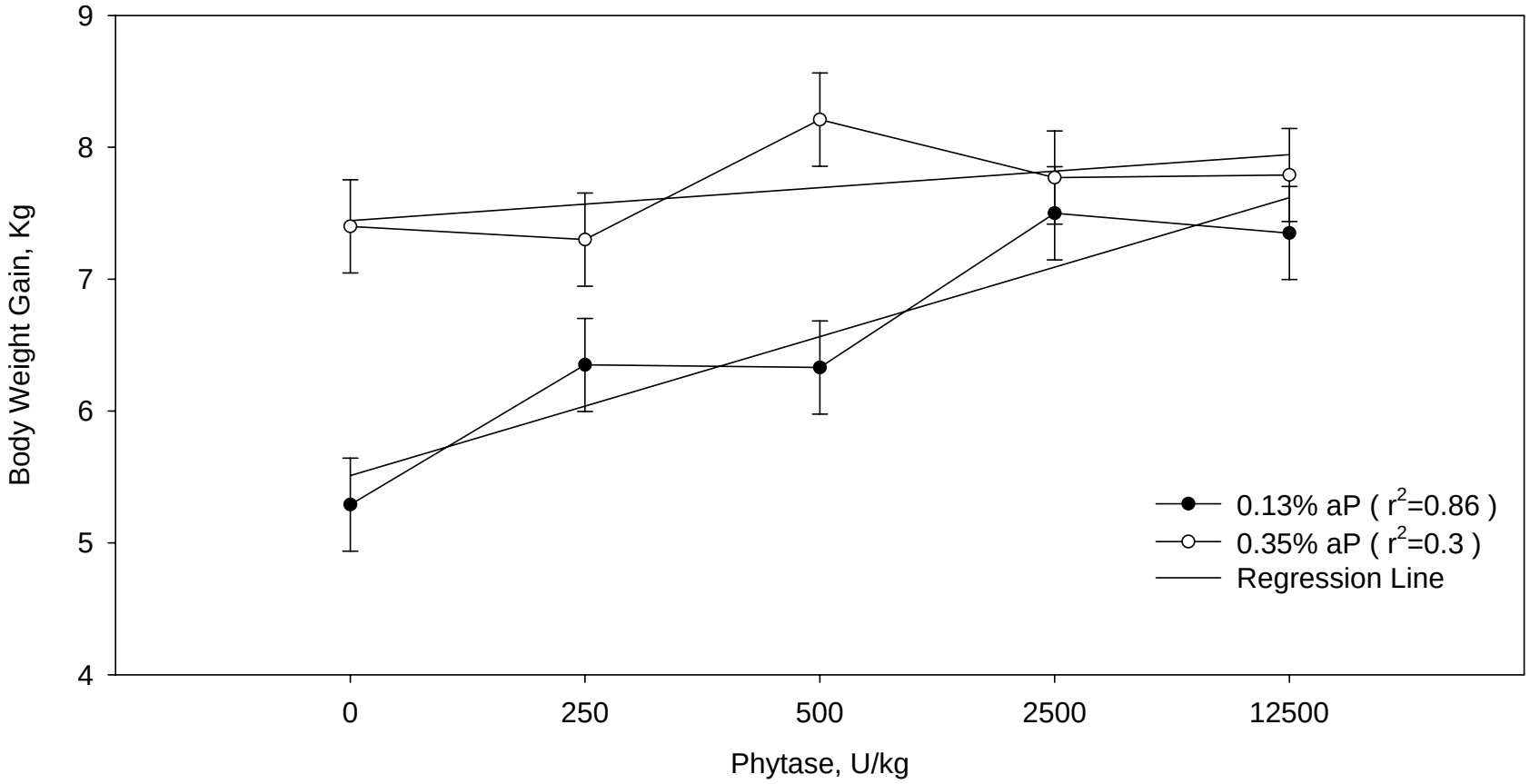
Linear and quadratic improvement in weight gain of low-P at day 0-10 and overall (P<0.005).
Results are LS Means for 8 pigs each treatment, the interaction between two phosphorous levels (0.13% and 0.35% aP) and five ohytase levels (0, 250, 500, 2500, and 12500 U/kg). Significant was set at P<0.05.
a Linear increased in marker digestibility of P and Ca of low-P (P<0.05).
b Linear and quadratic increased in apparent total digestibility of P and Ca of low-P (P<0.05).

Figure 4-1. The Effect of Dietary Phosphorous and Phytase on Growth Rate (D 0-10).



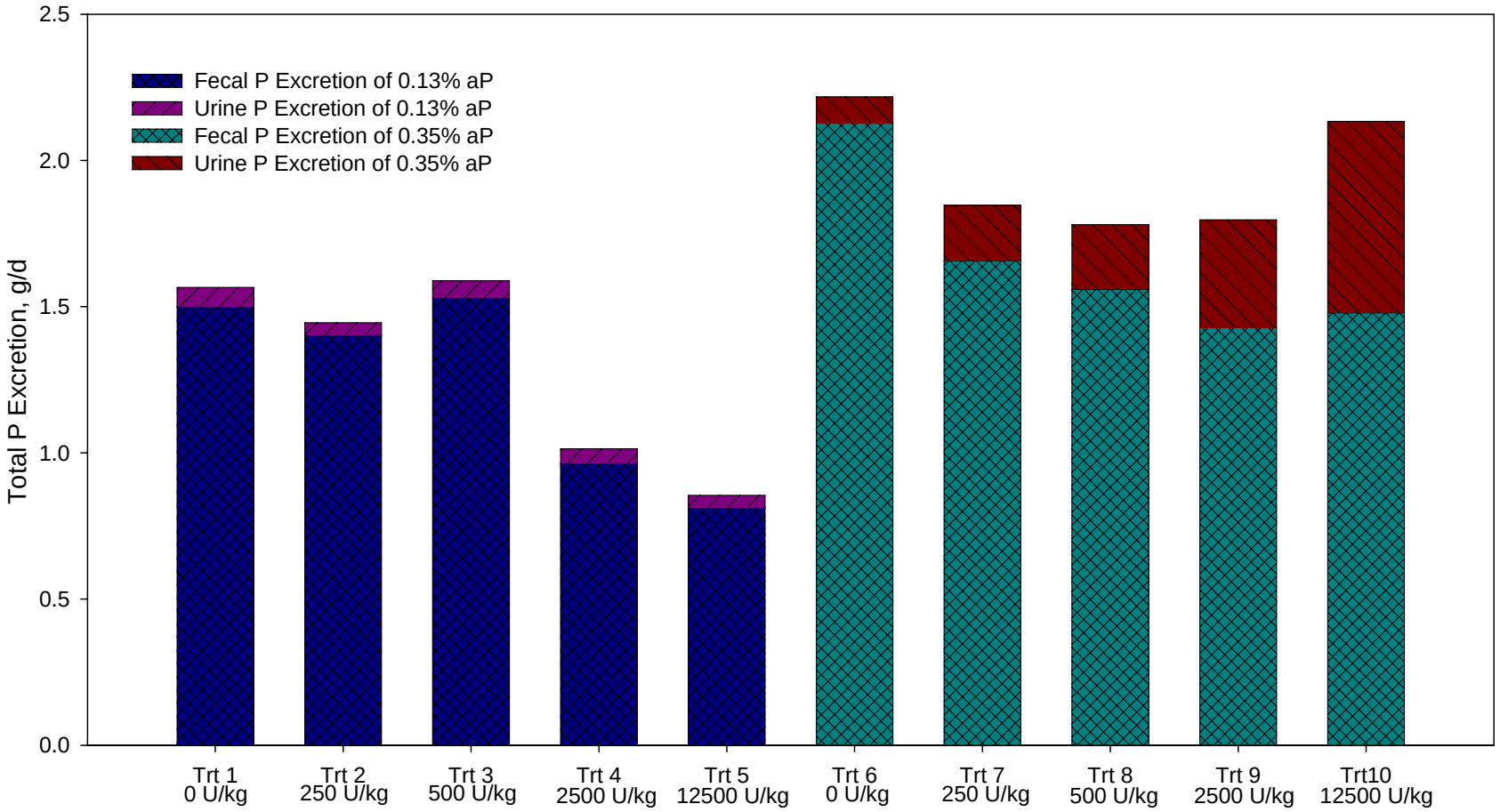
Results are least square means for 8 pigs each treatment, the interaction between two phosphorous levels (0.13% and 0.35% aP) and five ohytase levels (0, 250, 500, 2500, and 12500 U/kg).
Significant was set at P<0.05.

Figure 4-2. The Effect of Dietary Phosphorous and Phytase on Growth Rate (D 0-14).



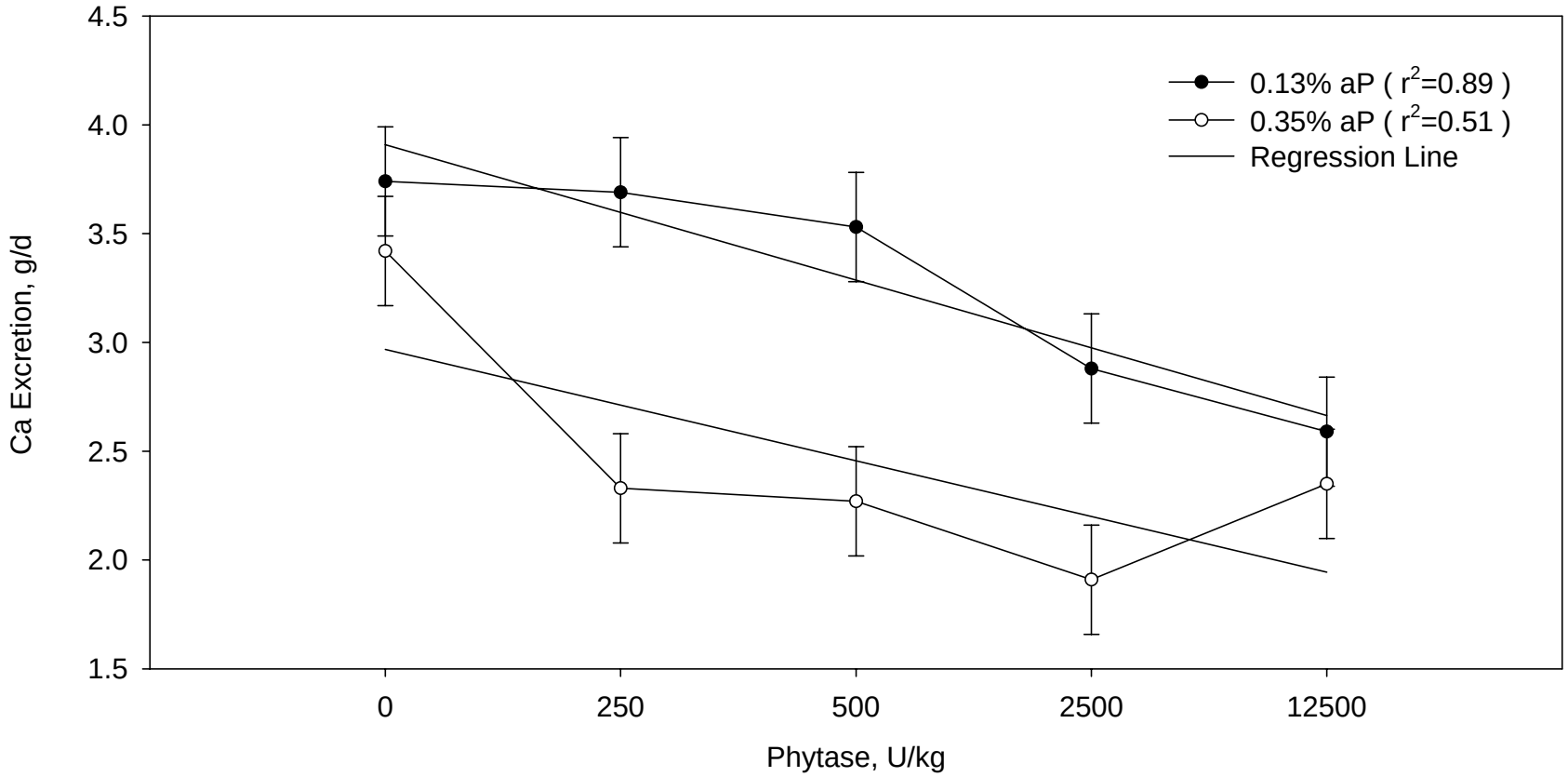
Results are least square means for 8 pigs each treatment, the interaction between two phosphorous levels (0.13% and 0.35% aP) and five ohytase levels (0, 250, 500, 2500, and 12500 U/kg).
Significant was set at P<0.05.

Figure 4-3. The Proportion of P Excretion in Fecal and Urine of Low-P(0.13% aP) and High-P (0.35% aP) as Phytase Addition.



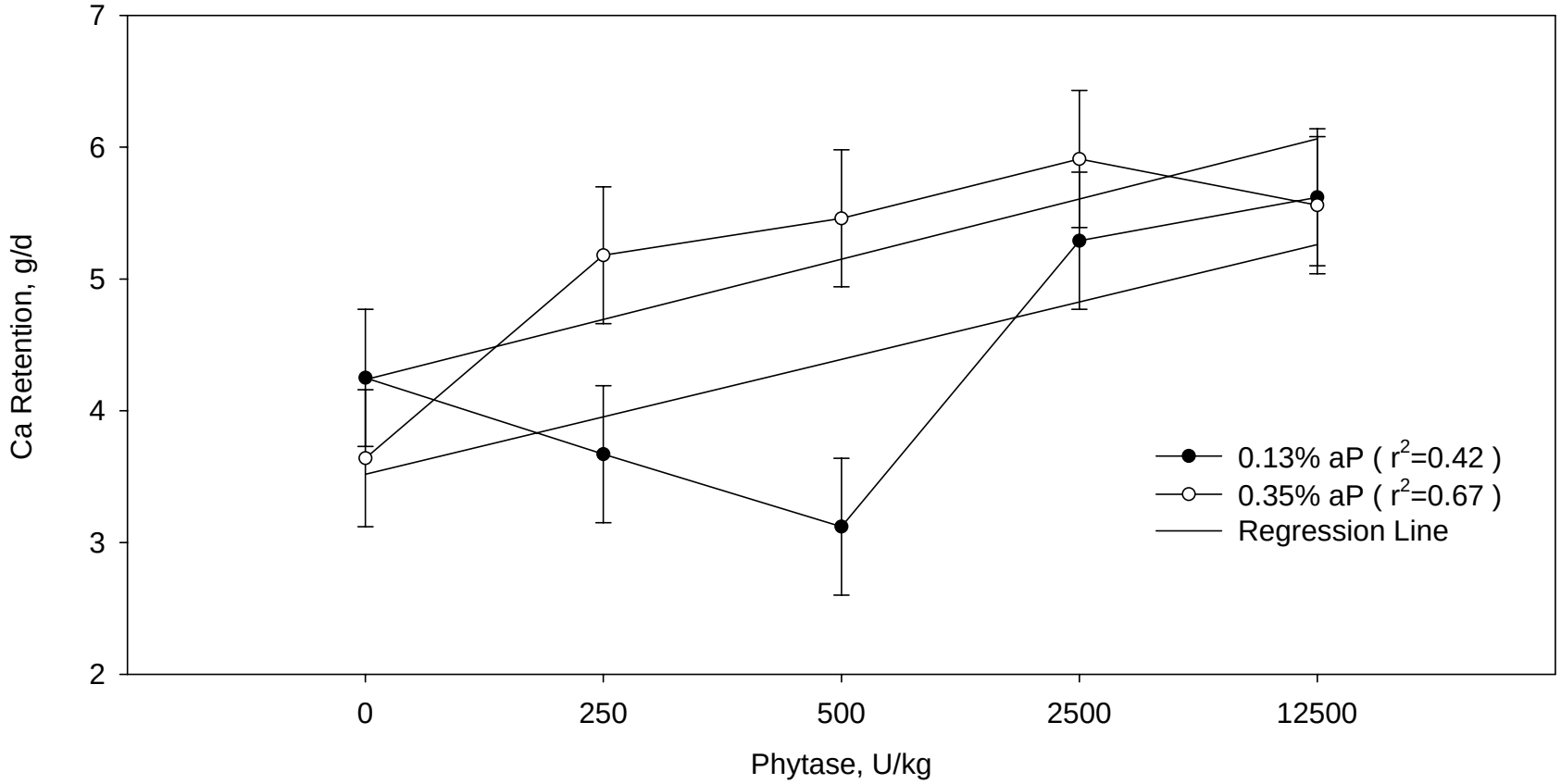
Fecal P decreased linear (P<0.0003) and quadratic (P<0.0194) in low-P (0.13% aP).
Phytase addition had benefit on reducing urine P excretion (P<0.0001) in low-P (0.13% aP).
Urine P increased linearly in high-P (0.35% aP) (P<0.0001).
High-P (0.35% aP) treatments had significant higher total P excretion than low-P (0.13% aP) treatments (P<0.0001).
Results are LS Means for 8 pigs each treatment, the interaction between two phosphorous levels (0.13% and 0.35% aP) and five ohytase levels.

Figure 4-4. The Effect of Dietary Phytase and Phosphorous on Ca Excretion.



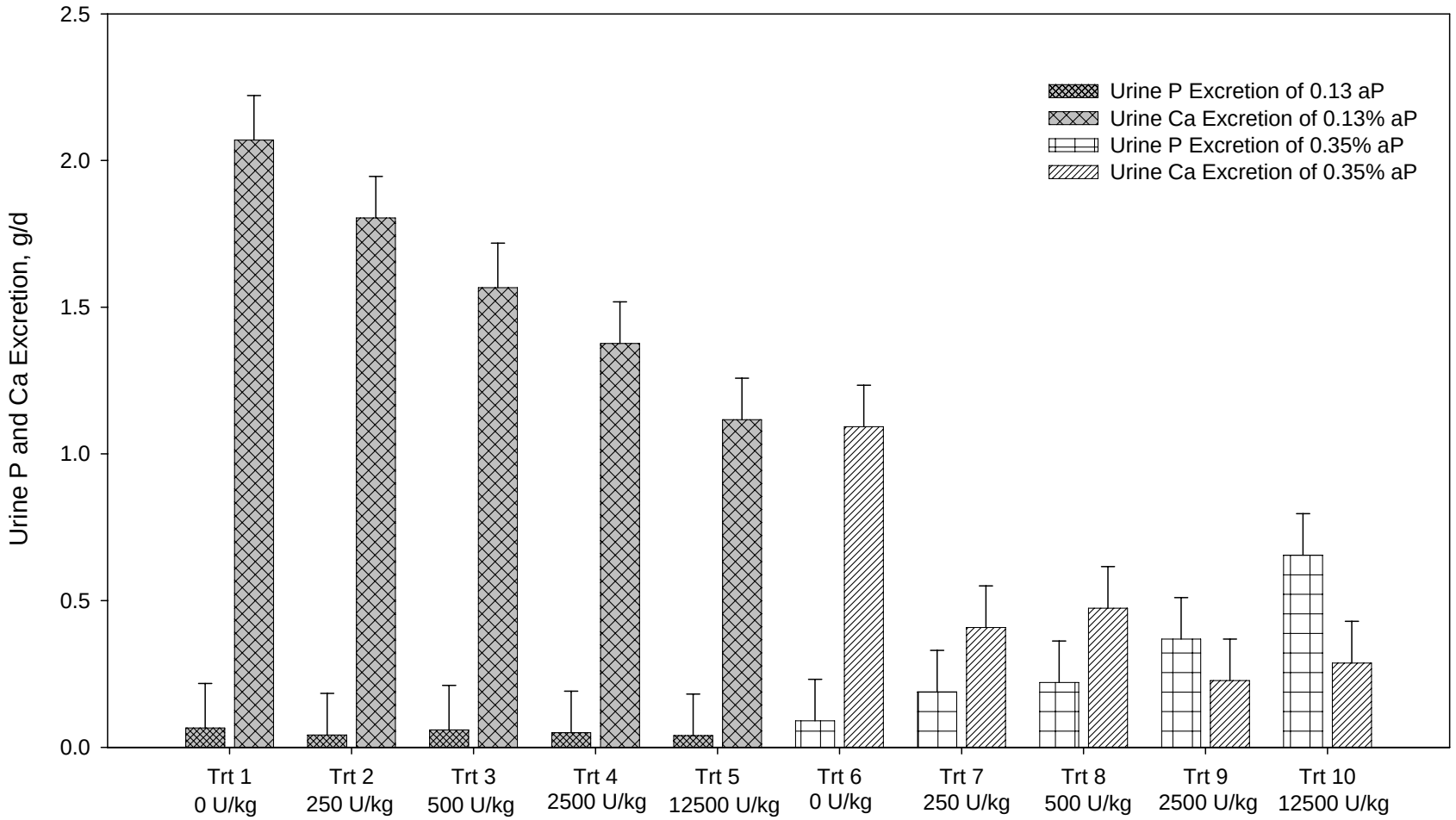
Results are least square means for 8 pigs each treatment, the interaction between two phosphorous levels (0.13% and 0.35% aP) and five ohytase levels (0, 250, 500, 2500, and 12500 U/kg).
Significant was set at P<0.05.

Figure 4-5. The Effect of Dietary Phytase and Phosphorous on Ca Retention.



Results are least square means for 8 pigs each treatment, the interaction between two phosphorous levels (0.13% and 0.35% aP) and five ohytase levels (0, 250, 500, 2500, and 12500 U/kg).
Significant was set at P<0.05.

Figure 4-6. The relationship between urine P and Ca excretion in phytase supplementation of low-P (0.13% aP) and high-P (0.35% aP).



Both urine P and Ca excretion were affected by phytase (P<0.0001).
Urine P increased linearly (P<0.0001), but urine Ca dropped linear and quadratic (P<0.0001) after phytase application in in high-P (0.35% aP).
Results are least square means for 8 pigs each treatment, the interaction between two phosphorous levels (0.13% and 0.35% aP) and five ohytase levels (0, 250, 500, 2500, and 12500 U/kg). Significant was set at P<0.05.

CHAPTER 5

CONCLUSION

Enzyme phytases have been established and suggested to reduce environmental impact, made by P. Both fungal and E.Coli-derived phytases showed efficient on improved gain weight, nutrient digestibility, and bone strength. The benefit of E.Coli. phytase is its better thermoability. The new generation of E. Coli. phytase in our study was able to reduced the day of marketing when fed high level phytase at nursery or nursery and growth phase. Moreover, Bone ash and strength also advantaged from high level phytase. Although P and Ca digestibility of nursery phase increased significantly ($P<0.005$) after phytase supplemented, didn't have sustained effect. Pigs fed 2500 U/Kg phytase in the diet released enough P from phytate in LP (0.13% aP) to result in full recovery of weight gain of HP (0.35%). E. Coli. Phytase not only had magnitude benefit in deficient P, but also had better gain weight and eliminated P excretion in HP, compared to HP with 0 phytase. It indicated that 12500 U/Kg E. Coli. phytase maybe didn't reach the plateau of maximum benefit. Genetic selection the pigs with better nutrient digestibility is the most natural way to solve the manure waste problem, although it takes long time to see the result. Before transgenic pigs were allowed to service people as pork meat consumption, low phytate grains plus phytase seems to be the best combination to diminish extra manure P issue in swine industry. The relationship of Ca : tP ratio in diet as E. Coli. phytase is supplemented is still needed to be distinguished.