EVALUATION OF THE EFFICACY OF HIGH LEVELS OF MICROBIAL PHYTASE IN BROILERS

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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled

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And hereby certify that in their opinion it is worthy of acceptance.

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ABSTRACT

Three experiments were conducted to evaluate the efficacy of high levels of microbial phytase in broiler diets. In experiment one (EXP 1), phytase was included in the diets at 0, 250, 500, 10,000, and 20,000 PU/kg diet. In experiments two and three (EXP 2 and 3), phytase was included in the diets at 0, 500, 2,500, 12,500, and 62,500 PU/kg diet. In all three experiments, dietary Ca and P were reduced to fit industry recommendations. In EXP 1, phytase supplementation improved ($P < 0.05$) feed intake (FI), body weight gain (BWG), feed:gain (F:G), bone ash (BA), P digestibility (PD), and reduced ($P < 0.05$) total litter P (TLP) by 53%. In EXP 2, phytase supplementation decreased ($P < 0.05$) FI, had no effect ($P > 0.05$) on BWG, and improved ($P < 0.05$) F:G, BA, and PD in EXP 2. In EXP 3, phytase supplementation decreased ($P < 0.05$) FI, improved ($P < 0.05$) BWG, F:G, BA, PD, and reduced ($P < 0.05$) TLP by 33%. In young broilers (hatch to 21 days), high levels of dietary phytase improved BWG above the NRC and positive control diets (EXP 1 and 3). However, as broilers reached market weight there were no differences ($P > 0.05$) in BWG. Results of this research indicate that microbial phytase was efficacious in broiler diets. However, there were no added benefits to feeding high levels of dietary phytase to broilers raised to market weight.

CHAPTER ONE

LITERATURE REVIEW

PHYTIC ACID

Phytic acid (myo-inositol hexa phosphate) is a common constituent of plants. It is primarily found in the seeds of plants and serves as a storage form of phosphorus (Tamim and Angel, 2003). Phytic acid is made up of six negatively charged phosphate groups bound to 12 hydrogens in an inositol ring. These negatively charged sites can bind cations such as Ca^{+}, K^{+}, Mg^{++}, Zn^{+}, Fe^{+}, and Mn^{++}. Phytic acid can have a negative effect on mineral absorption. O'Dell and Savage (1960) first reported low absorption of Zn in chicks fed phytate. Morris (1986) documented the negative effects of phytic acid on the absorption of Zn, Fe, Cu, Mn, and Ca.

Poultry diets are composed primarily of plant materials and about two-thirds of the P in cereal grains and oilseed meals is bound in the phytic acid structure (Viveros et al., 2000) and is poorly available to poultry. Phytic acid in seeds may interact with other food components in the digestive tract. These interactions are complex and depend on many factors such as the type of digestive tract, the pH of the digestive tract, and the presence of other food items that compete with phytic acid for mineral binding (Thompson, 1993). Other

factors involved in phytate/food interaction include, the presence of intestinal or bacterial phytase enzymes, the nature of the processing of the foods, the type of phytic acid ingested, and the amount of phytate ingested (Thompson, 1993).

Phytate is a term used synonymously with phytic acid. Phytate is a mixed cation salt of phytic acid also known as IP_6 (myo-inositol hexa dihydrogen phosphate). Phytate is located in the seeds of plants in varying concentrations and locations. In corn, phytate is primarily located in the germ (Wyatt et al., 2004). In wheat and barley, phytate is located in the aleurone layer, and in soybean meal, phytate is associated with protein bodies (Wyatt et al., 2004). Phytate in seeds acts as a store of phosphate, K, Mg, Ca, Mn, Fe, and Zn for use by the seedling (Lott et al., 2000). Phytate is also used to control the inorganic phosphate levels in both developing seeds and seedlings (Lott et al., 2000).

In mature seeds, phytate is found as a complex salt of Ca, Mg, and K, and in some cases, it is bound to proteins and starches (Angel et al., 2002). The complexed molecule of phytate, Ca, Mg, and K is called phytin, and most of the phosphorus in plant material is referred to as phytate- or phytin-phosphorus. Any phosphorus not bound to the phytate molecule is known as nonphytate or nonphytin phosphorus (npP).

Phytate is synthesized in the plant cells in which it is stored. The location of phytate within the seed and its chemical associations with other nutrients influence its availability (Angel et al., 2002). Nonruminant animals such as poultry and swine lack the endogenous enzymes capable of breaking apart the phytate molecule. Eighty-two percent of the phytate consumed in poultry diets is

recovered in the excreta (Cowieson et al., 2004a). To meet phosphorus requirements inorganic sources of phosphorus are often added to poultry diets. Passage of phosphorus bound to phytate and excess inorganic phosphorus in poultry excreta can create environmental concerns such as soil saturation, soil run-off, and eutrophication.

Aside from environmental concerns and phosphorus unavailability, phytate may also bind other nutrients such as amino acids, starch, and mineral cations creating an anti-nutritive effect. Ravindran et al. (2006) reported that increasing dietary concentrations of phytic acid resulted in reductions in apparent metabolizable energy (AME) and ileal phosphorus digestibility. Most phytate-mineral chelates are soluble at a low pH (< 3.5). Unfortunately, the broiler small intestine is a neutral environment (Simon and Igbasan, 2002). The neutral pH allows for the formation of insoluble phytate-mineral chelates and decreases phytate phosphorus and mineral cation availability, thus increasing the excretion of endogenous minerals and amino acids (Cowieson et al., 2004a).

PHYTASE

Phytases (myo-inositol hexaphosphate hydrolases) are enzymes capable of catalyzing the hydrolysis of one or more phosphate groups from IP_6, thus yielding inorganic P (P_i). Phytases are the only known enzymes with the ability to initiate dephosphorylation of phytate phosphorus (Applegate and Angel, 2005). Phytases are widespread in nature and can be found in plants, animals, and

microorganisms (Haefner et al., 2005). Micro flora or the intestinal mucosal membrane in an animal's digestive tract can produce phytase. Feed ingredients such as wheat contain phytase and it is common practice to supplement broiler diets with exogenous microbial phytase (Angel et al., 2002).

Sources for producing phytase range from fungi to yeast to bacteria (Simon and Igbasan, 2002). Lan et al. (2002) performed an experiment utilizing a phytase-producing bacteria found in the rumen of cattle in Malaysia called active *Mitsuokella jalalundinii* culture (AMJC). Supplementing low npP broiler diets with AMJC increased weight gain, feed intake, AME value, digestibility of DM and CP, bone mineralization, and the retention of P, Ca, and Cu (Lan et al., 2002).

The first commercial phytases were marketed in 1991 (Haefner et al., 2005). The International Union of Pure and Applied Chemistry and the International Union of Biochemistry (IUPAC-IUB) recognize two major classes of phytase enzymes: three-phytases and six-phytases. The three-phytases, such as the fungal phytases *Aspergillus ficuum* and *Aspergillus niger*, initiate dephosphorylation of the inositol ring at the three position. The six-phytases, such as the fungal phytase *Peniophora lycii* or the microbial phytase *Escherichia coli*, initiate dephosphorylation at the sixth position of the inositol ring. After the initial dephosphorylation the other five phosphate groups are released in sequential order.

Two phytase products are marketed, one derived from submerged liquid fermentation that uses genetically manipulated organisms to achieve maximum

enzyme production. The other phytase product uses solid-state fermentation which uses normal organisms for enzyme production (Wu et al., 2004a). Phytases produced utilizing solid-substrate fermentation may also have several side enzyme activities (Wu et al., 2004a). Some commercial phytases are being produced using recombinant DNA technology. Novel phytases are created by inserting a bacterial phytase gene into yeast. The use of recombinant DNA technology has greatly improved the use of phytases by improving thermo-stability, pH specificity, and resistance to proteolysis (Applegate and Angel, 2005).

Currently, there are three major types of commercially available phytases (Remus, 2005). The first two commercially available phytases were Natuphos® (BASF Corporation, Mount Olive, NJ) and Ronozyme® P (DSM, Parsippany, NJ). Natuphos® is derived from the fungi *Aspergillus niger* and is a three-phytase (BASF Corporation, Mount Olive, NJ, 2001). Ronozyme® P is derived from the fungi *Peniophora lycii*. Ronozyme® P and its advanced forms release phosphorus from the sixth phosphate group first (DSM, Parsippany, NJ). There are new phytases derived from the bacteria *Escherichia coli*, but only one is available in the US, Phyzyme® XP (Danisco Animal Nutrition, Wiltshire, UK, 2005; Remus, 2005).

Much of the literature to date relates to *Aspergillus* and *Peniophora* derived phytases, however there is recent interest in *Escherichia coli* phytases (Wyatt et al., 2004). Unfortunately, the response to feeding phytases, especially fungal phytases, is not consistent (Wyatt et al., 2004). Some three-phytases and

six-phytases have limitations in the digestive tract of broilers partly due to solubility in different parts of the intestinal tract, and a short retention time in the bird's intestine (Zyla et al., 2004).

Phytate is soluble at low pH, and in poultry diets, phytate is most susceptible to attack by phytases in the crop, proventriculus, and/or gizzard (Wyatt et al., 2004; Applegate and Angel, 2005). However, phytase enzymes are proteins and are vulnerable to proteolysis by digestive enzymes in the crop, proventriculus, and/or gizzard. This environment also has high concentrations of minerals of dietary and endogenous origin (Wyatt et al., 2004) thus creating a harsh environment for phytase enzymes.

The production system in which phytase enzymes are expressed may alter the characteristics of the enzyme, such as pH optima, thermo stability, efficacy, and resistance to degradation (Wyss et al., 1999). For example, plant phytases have optima conditions at 45 to 60°C, whereas microbial phytases prefer temperatures ranging from 35 to 63°C and 95 to 145°C (Wodzinski and Ullah, 1996).

Plant phytases initiate dephosphorylation at the six position of the phytate ring (Applegate and Angel, 2005). Although six-phytases are normally found in plant seeds, they are commercially available as microbial (*Peniophora lycii)* phytases (Zyla et al., 2004). Zyla and others (2004) reported three-phytases released significantly more phosphorus than six-phytases in an in vitro study. Although the two enzymes belong to the same family of histidine acid phosphatases, the *Phy A* gene on *Aspergillus niger* and *Peniophora lycii* differs

in structure, physiochemical, and catalytic properties (Zyla et al., 2004). The enzyme from *Peniophora lycii* has a narrower pH and is less stable than *Aspergillus ficuum* phytase (Zyla et al., 2004). However, findings from the in vitro study were not reflected in the in vivo study. Broilers fed *Aspergillus ficuum* and *Peniophora lycii* performed comparably (Zyla et al., 2004).

Any new phytase preparation requires rigorous evaluation of its efficacy in hydrolyzing phytic acid (Adeola et al., 2004). To evaluate the efficacy of phytase as a feed additive, temperature optimum and stability, pH optimum and profile, and proteolytic stability are very important considerations (Simon and Igbasan, 2002). The site and action of phytase in the digestive tract depends on the luminal pH and the pH activity of the enzyme (Simon and Igbasan, 2002). In poultry the pH of the crop ranges between four and five. However, in the stomach the pH is much lower (2 to 5) thus, the pH optima and range is very important when determining phytase activity. Proteolytic degradation is important when evaluating phytases because the rate and site of inactivation are determined by their ability to withstand proteolysis (Simon and Igbasan, 2002).

Most phytases act at low pH optima, thus most phytase activity occurs in the crop and proventriculus. In vitro experiments conducted by Simons et al. (1990) determined that the microbial phytase *Aspergillus ficuum* had two pH optima, 5.5 and 2.5. Adeola and others (2004) reported that a six-phytase cloned from E. coli in pig intestines had an optimum pH range of 2 to 4.5. A three-phytase produced from *Aspergillus niger* had optima pH at 2.5 and 5.5 and a three-phytase produced from *Aspergillus ficuum* had optima pH at 2.5 and 5.0

(Kies et al., 2001). Six-phytases such as *Peniophora lycii* have only one pH optima between 4.5 and 5.0 (Kies et al., 2001). Only one phytase, produced from *Bacillus*, had a neutral pH optimum (Simon and Igbasan, 2002). The optima pH of the current phytases are narrow and activity past the proventriculus or stomach is minimal.

Research suggests bacterial phytases exhibit a greater efficiency than fungal phytases (Augspurger et al., 2003; Silversides et al., 2004). Augspurger and others (2003) reported *E. coli* phytases may have an advantage over commercial phytases in young chicks. At 500 PU/kg diet, the novel *E. coli* phytase resulted in superior body weight gain and tibia ash values compared to Natuphos®. In a second experiment, phosphorus-release values were higher for the novel *E. coli* phytase compared to Natuphos® and Ronozyme® (Augspurger et al., 2003). Onyango and others (2004) reported no difference in body weight gain, feed intake, and feed efficiency among birds fed diets containing 1,000 units of three microbial phytases produced in different yeast systems: *Pichia pastoris, Schizosaccharomyces pombe,* and *Saccharomyces cerevisiae,* however, the microbial phytase expressed in *Schizosaccharmyces pombe* improved bone strength above a diet adequate in phosphorus (Onyango et al., 2004).

Thermo stability is important for phytase enzymes since many feed companies pellet poultry feed through dies at temperatures exceeding 80°C. Silversides et al. (2004) found novel phytases derived from an *Escherichia coli* gene and produced using *Saccharomyces cerevisiae* and *Pseudomonas*

fluorescens, and the commercial phytase Natuphos® were not able to withstand pelleting temperatures above 80°C. However, a novel *Escherichia coli* phytase produced using *Pichia pastoris* was able to withstand pelleting temperatures above 80°C (Silversides et al., 2004).

To prevent phytase denaturation due to high pelleting temperatures, liquid phytases are used in broiler diets post-pelleting. However, equipment costs for liquid phytase application are expensive (Ward, 2002). In a comparative study utilizing Ronozyme™ P (CT) a dry phytase with a patented thin, lipid coating to protect against moisture and heat, and two liquid phytases applied post-pelleting, (Ronozyme™ P (L) and Natuphos® 5000L), all three phytases performed similarly for body weight, feed conversion, and bone ash (Ward, 2002).

PHYTASE IN MONOGASTRIC NUTRITION

Approximately two-thirds of the phosphorus in plant materials, such as corn and soybean meal, is bound to phytate (Viveros et al., 2000). Poultry possess an intestinal phytase, but it is ineffective (Cowieson et al., 2004b) and chicks do not have the ability to utilize phytate phosphorus in the absence of exogenous phytase (Nelson et al., 1971). To meet the nutritional requirements of monogastric animals, inorganic sources of phosphorus are added to the diets. Consequently, much of the phytate phosphorus and inorganic phosphorus in the diet is passed out in the excreta. When monogastric excreta high in phosphorus

is applied to fields as fertilizer, environmental problems such as soil saturation, soil run off, and eutrophication can result.

Microbial phytases have been in practical use for over a decade, and their acceptance in the poultry and swine industries as a means of reducing phosphorus excretion in manure continues to grow (Ravindran et al., 2006). The first reports of phytase and its ability to liberate phytate phosphorus started in 1968 and 1971 when Nelson et al. reported phytate phosphorus in soybean meal and corn was made available to chicks by the addition of an *Aspergillus ficuum* phytase. Since 1971, numerous experiments have been conducted to evaluate the efficacy of phytase addition to poultry (Simons et al., 1990; Ravindran et al., 1999; Augspurger et al., 2003; Onyango et al., 2004), swine (Simons et al., 1990; Harper et al., 1997; Augspurger et al., 2003; Adeola et al., 2004), and fish diets (Liebert and Portz, 2005). For the purpose of this review, I will only discuss phytase in broiler nutrition.

PERFORMANCE

Many researchers report the benefits of supplementing phytase in low phosphorus poultry diets. Simons et al. (1990) concluded that an addition of 1,000 PU/kg diet of microbial phytase to broiler diets provides levels of performance which are as good as or better than broilers fed diets supplemented with phosphate. Supplemental phytase in low phosphorus broiler diets improved body weight gain (Denbow et al. 1995; Qian et al.,1996; Sebastian et al., 1996a; Yi et al., 1996b; Qian et al., 1997; Sebastian et al., 1997; Lan et al., 2002; Namkung and Leeson, 1999; Sohail and Roland, 1999; Zyla et al., 2000; Viveros

et al., 2002; Augspurger et al., 2003; Shirley and Edwards, 2003; Wu et al., 2003; Augspurger and Baker, 2004; Dilger et al., 2004; Silversides et al., 2004; Wu et al., 2004a; 2004c; Zyla et al., 2004; Onyango et al., 2005), feed intake (Denbow et al. 1995; Qian et al.,1996; Sebastian et al., 1996a; Yi et al., 1996b; Qian et al., 1997; Sebastian et al., 1997; Namkung and Leeson, 1999; Zyla et al., 2000; Lan et al., 2002; Viveros et al., 2002; Shirley and Edwards, 2003; Dilger et al., 2004; Silversides et al., 2004; Zyla et al., 2004; Onyango et al., 2005), and feed efficiency (Sebastian et al., 1996a; Namkung and Leeson, 1999; Lan et al., 2002; Shirley and Edwards, 2003; Dilger et al., 2004; Wu et al., 2004a; 2004c). The improvement in body weight gain, feed intake, and feed efficiency from phytase supplementation to low phosphorus broiler diets indicates phytate phosphorus is more available when phytase is supplemented.

Nelson et al. (1968) and Simons and Versteegh (1990) suggest a reduction in the level of calcium below NRC requirements when phytase is added to the diet. Calcium levels greater than 0.75% were found to decrease bird performance when dietary phytase was supplemented in the diet (Perney et al., 1993). Perney et al. (1993) reported the addition of dietary phytase at 25, 50, 150, 250, 500, and 750 units per kg to diets containing 1.0% calcium did not significantly improve weight gain, feed intake, or feed conversion.

Sebastian et al. (1996a) conducted a 21 day experiment to determine the efficacy of supplemental phytase at three levels of dietary calcium (0.60, 1.00, and 1.25%) in broiler diets. The diets contained 0.30% available phosphorus and 0 or 600 phytase units per kilogram. The optimum levels of feed intake, body

weight gain, and feed efficiency were obtained with the low (0.60%) calcium plus phytase treatment diet. Sebastian et al. (1996a) and Tamim wet al. (2004) concluded that dietary calcium levels have a significant effect on the response of supplemental phytase.

Dietary calcium to total phosphorus ratios of 1.1:1 to 1.4:1 appear critical to the efficient use of supplemental phytase for improving the utilization of phytate phosphorus and calcium in broilers (Qian et al., 1997). When supplementing dietary phytase, wide calcium to total phosphorus ratios negatively influence body weight gain, feed intake, bone mineralization, and calcium and phosphorus retention (Qian et al., 1997). However, Driver et al. (2005) reported that at higher calcium levels (0.86%) and lower non-phytate phosphorus levels (0.20%) dietary phytase elicits a greater response.

Many experiments suggest bird performance plateaus at dietary phytase levels around 500 to 1,000 PU/kg diet (Simons et al., 1990; Denbow et al., 1995). Denbow et al. (1995) reported that at 0.20% non-phytate phosphorus (npP), gains and feed intake of broilers improved linearly up to 800 PU/kg diet and then reached a plateau. When diets contained 0.27% and 0.34% npP the response variables reached a plateau between 400 and 600 PU/kg diet, and “no marked improvements were evident beyond these added levels of phytase”. Sohail and Roland (1999) reported no additional benefit to broiler chicks from increasing phytase levels past 300 PU/kg diet. In a lysine-deficient diet, Ravindran et al. (2001) reported broiler response to weight gain reached a plateau at 500 PU/kg diet.

In 2003, Shirley and Edwards reported that birds consuming up to 12,000 PU/kg diet can achieve maximum performance. Body weight gain, feed intake, feed efficiency, tibia ash, plasma phosphorus, phosphors and nitrogen retention, and AME were significantly increased as dietary phytase levels increased from 0 to 12,000 PU/kg diet. Ledoux et al. (2005) reported turkeys grown to market weight fed diets containing 10,000 PU/kg diet performed better than birds fed the positive control diet. Wu et al. (2004a) fed supplemental phytase at inclusion levels up to 2,000 PU/kg diet and reported that feed efficiency for broilers fed diets supplemented with phytase were superior to those fed diets adequate in phosphorus without phytase supplementation.

Augspurger and Baker (2004) conducted four experiments to evaluate high levels (10,000 PU/kg diet) of three phytase enzymes. The enzymes used included a fungal phytase derived from *Aspergillus niger*, a fungal phytase derived from *Peniophora* and an *Escherichia coli* phytase. *Escherichia coli* phytase inclusion levels of 1,000 PU/kg diet maximized growth and bone responses whereas addition of both fungal phytases at 5,000 and 10,000 PU/kg diet resulted in increasing responses. These results demonstrate that high levels of dietary phytase can release most of the phytate phosphorus, 70 to 100% according to Augspurger and Baker (2004) and 95% according to Shirley and Edwards (2003).

BONE ASH

Eighty to eighty-five percent of the phosphorus in animals is located in the bones (McDowell, 1992). Abnormal bone development is one of the most

obvious signs of a phosphorus deficiency (Qian et al., 1996). Nelson et al. (1971) reported that birds consuming phytase had an increase in percent bone ash resulting from an improvement in phytate phosphorus hydrolysis.

Qian and others (1996) reported dietary supplementation of phytase along with inorganic phosphorus increased the length, shear force, shear stress, and ash content of tibias in 21-day old broiler chicks. Phytase supplementation to low phosphorus diets also increased the width of cartilaginous and proliferative zones in tibias, increased tibial bone density, and improved the development and mineralization of cartilage and bone cells (Qian et al., 1996). Phytase supplementation improved bone mineral content (Sebastian et al., 1996b; Sohail and Roland, 1999; Onyango et al., 2004), bone density, and bone breaking strength (Sohail and Roland, 1999; Onyango et al., 2004). Phytase supplementation to low phosphorus broiler diets improved toe ash (Perney et al., 1993; Yi et al., 1996b; Qian et al., 1997; Wu et al., 2003; Dilger et al., 2004; Wu et al., 2004a; Zyla et al., 2004) and tibia ash (Perney et al., 1993; Lan et al., 2002; Viveros et al., 2002; ; Augspurger et al., 2003; Shirley and Edwards, 2003; Augspurger and Baker, 2004; Dilger et al., 2004; Onyango et al., 2004; 2005).

The need to quantify bone mineralization has long been recognized by researchers in poultry nutrition (Garcia and Dale, 2006). The use of tibia ash is recommended as a means of evaluating bone mineralization by the AOAC (1990). The tibia is the fastest growing bone in the body and has thus been used as a valid marker of bone mineralization for many years. The tibia is also considered the most sensitive to calcium and phosphorus deficiencies.

Unfortunately, determination of tibia ash is labor intensive, expensive and time consuming, and variations in the collection process may change the results (Orban et al., 1993).

Numerous experiments have been conducted to evaluate the variances between tibia ash collection parameters. In 2003, Hall et al. ran an experiment to estimate the variances of two methods of determining tibia ash with bones containing a range of ash contents from treatments and birds of different ages. The objective of that study was to compare the results from the AOAC (1955) approved boiling/extraction method to the autoclaving method (Boling-Frankenbach et al., 2001). Hall et al. (2003) concluded each method had a range of benefits. Autoclaving minimizes the time required to clean the bones, and the exposure to potentially dangerous chemicals such as anhydrous ether and ethanol. However, in order to find comparable differences, autoclaving requires more samples and thus more birds.

Various other parameters can be used to measure tibia mineralization including, bone ash content, bone mineral content, bone density, and bone breaking strength. In 2004, Kim et al. conducted an experiment to evaluate various bone parameters from different bone preparation methods (fresh, dry, and fat-free dry). Kim et al. (2004) reported bone breaking strength was greatly influenced by bone preparation methods. However, there was no significant difference in ash weight or ash concentration among the different bone preparations (fresh, dry, and fat-free dry).

Toe ash is another method used to evaluate bone mineralization and is thought to be equivalent to tibia ash in sensitivity to calcium and phosphorus concentrations. In many experiments only the middle toes are used and the sample size is quite small compared to tibias at the same age. Ravindran et al. (1995) reported toe ash percentages are an equally or a more sensitive criteria for assessment for phosphorus availability than tibia ash.

Dale and Garcia (2004) suggested the use of foot ash as an alternative method for evaluating bone mineralization. Foot ash provides a larger sample size than toe ash and may reduce the time required to obtain results. Preliminary results show foot ash to have great promise as a rapid means of monitoring bone development in broiler chickens (Mendez and Dale, 1998). Mendez et al. (1998) reported a high degree of relationship between toe and tibia ash ($R^2 = 0.82$) and tibia and foot ash ($R^2 = 0.85$) in 18-day old broilers.

In 2005, Yan et al. ran an experiment comparing alternative methods of evaluating response to phosphorus concentrations in diets of young male broilers, including fat-extracted tibias, unextracted tibias, toe ash, and foot ash. Yan et al. (2005) reported a high degree of relationship between extracted tibias and unextracted tibias ($R^2 = 0.95$), extracted tibias and foot ash ($R^2 = 0.92$), and extracted tibias and toe ash ($R^2 = 0.88$). Garcia and Dale (2006) hypothesized the entire foot, with 17 individual bones, might be a better indicator of bone mineralization as opposed to toes and tibias. In 2006, Garcia and Dale ran a series of experiments to evaluate foot ash. From these experiments is was concluded that foot ash accurately reflected a response in bone mineralization

when birds were fed different levels of dietary phosphorus, and that fat extraction did not affect the sensitivity of the tibia or foot ash essay.

Many considerations exist when using alternative methods to tibia ash. When evaluating bone mineralization using foot ash it is important to remember the use of foot ash may not be suitable for use in older chickens (Yan et al., 2005). Studies on foot ash to date have utilized only young chicks. Also it is important to remember situations that alter lipid metabolism may not accurately reflect bone mineralization when using unextracted toe, foot, or tibias to determine bone ash (Yan et al., 2005). Also, there may be bone growth differences between broilers reared in cages versus floor pens and between male and female broiler chickens (Bond et al., 1991).

Bird response to supplemental phytase also depends on the concentrations of phosphorus and calcium in the diet (Driver et al., 2005). Calcium binds to the phytate molecule making phytate phosphorus less soluble. Sebastian et al. (1996a) reported the optimum level of bone ash was observed in birds fed diets containing 0.60% calcium and supplemental phytase. An increase in bone ash suggests an improvement in bone mineralization due to an increase in calcium and phosphorus utilization (Perney et al., 1993). Increasing the amount of phytate in the diet increases the amount of calcium needed to produce a given level of bone ash in growing broilers (Farkvam et al., 1989).

MINERAL DIGESTIBILITY/RETENTION

Simons et al. (1990) reported that the availability of phosphorus and calcium can be improved by adding different levels of microbial phytase.

Phytase liberates phosphorus bound to phytate thus improving the amount of non-phytate phosphorus released. An increase in non-phytate phosphorus improves ileal digestibility and retention of phosphorus (Wu et al., 2004b). Many researchers have reported the benefits of phytase supplementation on improved phosphorus digestibility in broiler diets (Ravindran et al., 2000; Rutherfurd et al., 2002; Wu et al., 2003; Dilger et al., 2004; Rutherfurd et al., 2004; Silversides et al., 2004; Wu et al., 2004b; Ravindran et al., 2006).

The addition of microbial phytases has been shown to improve digestibility of other nutrients such as calcium (Silversides et al., 2004), nitrogen (Ravindran et al., 2000; 2001; Wu et al., 2003; 2004b), and zinc (Yi et al., 1996a) in broilers. According to Yi et al. (1996a), phytase supplementation of broiler diets released approximately 0.9 mg of Zn per 100 units of phytase. Phytase supplementation of broiler diets also improved the retention of certain nutrients, such as phosphorus (Sebastian et al., 1996b; Yi et al., 1996b; Qian et al., 1997; Ravindran et al., 2000; Zyla et al., 2000; Lan et al., 2002; Viveros et al., 2002; Shirley and Edwards, 2003; Dilger et al., 2004; Onyango et al., 2004; Zyla et al., 2004; Onyango et al., 2005), calcium (Sebastian et al., 1996b; Yi et al., 1996b; Qian et al., 1997; Lan et al., 2002; Viveros et al., 2002; Onyango et al., 2004; 2005), nitrogen (Yi et al., 1996b; Ravindran et al., 2000; 2001; Shirley and Edwards, 2003; Wu et al., 2003; Cowieson et al., 2004b; Dilger et al., 2004; Wu et al., 2004b; Onyango et al., 2005), copper (Sebastian et al., 1996b; Lan et al., 2002), magnesium (Viveros et al., 2002), and zinc (Sebastian et al., 1996b; Viveros et al., 2002).

MINERAL EXCRETION

Eutrophication is the over-enrichment of receiving waters with mineral nutrients leading to over production of algae (Correll, 1998). This high productivity of algae leads to low levels of oxygen in the water, ultimately killing aquatic life (Correll, 1998). Phosphorus in runoff from agricultural land is an important component of water pollution (Daniel et al., 1998). Long-term application of phosphorus as fertilizer or animal manure has resulted in elevated levels of phosphorus in the soil (Daniel et al., 1998). Many states have passed legislation that regulates agricultural phosphorus applications based on soil phosphorus levels in an attempt to protect receiving waters (Penn et al., 2004).

Phytase enzymes and low non-phytate phosphorus diets are potential remedies to the high levels of phosphorus in animal manure. The presence of phytate and phytase in the diet may alter endogenous excretions and improve the availability of minerals from the diet (Cowieson et al., 2004a). An improvement in phosphorus digestibility also decreased the amount of phosphorus in the excreta (Wu et al., 2004b). Supplementation of phytase lowered the phosphorus content in the excreta (Simons et al., 1990; Perney et al., 1993; Yi et al., 1996b; Wu et al., 2003; Dilger et al., 2004; Wu et al., 2004b; 2006) and litter (Applegate et al., 2003; Maguire et al., 2004; Penn et al., 2004; Shelton et al., 2004; Angel et al., 2005) of poultry fed low phosphorus diets. Supplemental phytase has also been reported to lower the excretion of calcium and sodium (Cowieson et al., 2004a).

Substantial controversy exists around the issue of feeding practices to improve phosphorus retention and the possibility of increasing the proportion of water soluble phosphorus relative to total litter phosphorus (Applegate et al., 2003). However, supplemental phytase inclusion in poultry diets decreased or had no effect on the solubility of phosphorus in litter (Applegate et al., 2003; Maguire et al., 2004; Penn et al., 2004; Shelton et al., 2004; Angel et al., 2005).

PROTEIN AND AMINO ACID DIGESTIBILITY/RETENTION

It has been theorized that phytate in plants not only binds mineral cations such as Ca, P, Mg, and Zn, but may also bind nutrients such as protein and carbohydrates. In 1999, Ravindran et al. reported that supplementation of microbial phytase improved protein and amino acid digestibility in corn, sorghum, wheat, soybean meal, canola meal, cottonseed meal, sunflower meal, wheat middlings, and rice polishings. However, the level of improvement in amino acid and protein digestibility was variable among plant ingredients (Ravindran et al., 1999).

Rutherfurd et al. (2002) reported that phytase improves amino acid digestibility for certain plant-based feed ingredients. They found that amino acid digestibility was significantly higher in wheat based diets supplemented with microbial phytase compared to diets made predominately of corn, rapeseed meal, rice bran, and soybean meal. These responses may be due to side enzyme activities such as proteases, amylases, xylanases, and cellulases (Wu et al., 2003), which are present from solid-state fermentation of the phytase

enzyme. It is also possible that microbial phytases act on the cell wall of wheat, since phytate is an integral part of wheat cell wall.

When phytase hydrolyzes phytate phosphorus, phosphorus is released along with the phytate-bound protein (Kies et al., 2006), which may increase the digestion and absorption of protein and amino acids (Ravindran et al., 2000). Many studies suggest microbial phytases improve the utilization of protein (Ravindran et al., 1999; Selle et al., 2000; Lan et al., 2002; Silversides et al., 2004; Ravindran et al., 2006) and certain amino acids (Namkung and Leeson, 1999; Ravindran et al., 1999; 2000; Selle et al., 2000; Ravindran et al., 2001; Rutherfurd et al, 2002; Cowieson et al., 2004b; Dilger et al., 2004; Silversides et al., 2004; Rutherfurd et al., 2004; Onyango et al., 2005; Ravindran et al., 2006). An improvement in protein utilization would increase the cost effectiveness of using phytase (Peter and Baker, 2001).

There are conflicting data in the literature in regards to phytase improving amino acid digestibility (Rutherfurd et al., 2004). Sebastian et al. (1997) reported that supplemental phytase had no effect on the digestibility of protein or any amino acid, except Met and Phe in male broilers. Peter and Baker (2001) also reported that dietary phytase did not improve amino acid utilization in soybean meal. Augspurger and Baker (2004) fed high levels of dietary phytase and reported no improvements in protein utilization. Zhang et al. (1999) reported no significant differences in protein and amino acids digestibilities in broilers fed diets supplemented with phytase. Data may vary due to the concentration of phytate in the diet, the level of phytase added, and the intrinsic properties of the

phytase enzyme used (Ravindran et al., 2006). Data are also variable between and among laboratories (Rutherfurd et al., 2002; 2004), and some of this variation may be due to differences in bird age (Rutherfurd et al., 2004) and diet composition.

CHROMIC OXIDE AND TITANIUM OXIDE

Digestibility studies are commonly used in nutrition research to evaluate mineral availability from the use of enzymes such as phytase and protease. Digestibility studies are also conducted in ruminants to determine flow rate through the rumen and nutrient utilization. Indigestible markers such as chromic oxide (Cr_2O_3) or titanium dioxide (TiO_2) are often added to diets in digestibility studies. Such markers are essential when examining nutrient uptake at a specific site along the gastrointestinal tract (Short et al., 1996).

An ideal marker should be totally indigestible, inactive, uniform, and easy to determine. Unfortunately, the markers used do not always accurately represent digestibility. Chromic oxide (Cr_2O_3) is a marker commonly used in digestibility studies with ruminants and poultry. Despite the popularity of Cr_2O_3, there are several problems associated with the use of this dietary marker. Chromic oxide is not approved by the Food and Drug Administration as a dietary additive, and may be considered a health hazard when inhaled (Titgemeyer, 1997). Researchers also report low recovery rates associated with Cr_2O_3 when used in studies with poultry and ruminants (Sales and Janssens, 2003;

Titgemeyer, 1997). It is also difficult to get repeatability between laboratories when Cr_2O_3 is used as a dietary marker, namely due to the assay (Sales and Janssens, 2003).

Titanium dioxide (TiO_2) has recently been evaluated as an alternative marker for digestibility studies in ruminants and poultry. Titanium dioxide has many benefits over the use of Cr_2O_3. Titanium dioxide does not have the carcinogenic properties associated with Cr_2O_3, and is approved for use as a feed additive by the Food and Drug Administration. Plus TiO_2 is reported to be a reliable digestibility marker in studies utilizing beef steers (Titgemeyer et al., 2001), poultry (Short et al., 1996), and ewes (Meyers et al., 2003a).

Titgemeyer et al. (2001) reported fecal recoveries of TiO_2 from 90 to 95%. Meyers et al. (2003a) concluded that TiO_2 is an acceptable alternative to Cr_2O_3 as a digestibility marker in studies utilizing ruminants. However, fewer studies have been conducted to evaluate TiO_2 as a dietary marker in poultry in comparison to other species (Sales and Janssens, 2003).

The analysis for TiO_2 is a work in progress. Short et al. (1996) developed a TiO_2 assay using poultry digesta. This assay is reported to be accurate, simple, and requires only a small sample size (0.1 g; Short et al., 1996). However, Meyers et al. (2003b) could not obtain consistent and accurate results within their laboratory utilizing the Short et al. (1996) method. Therefore, Meyers et al. (2003b) developed a rapid and accurate analytical procedure for determining TiO_2 in ruminants. Comparison of the two methods yielded acceptable recoveries of TiO_2 utilizing the Meyers et al. (2003b) method, and

lower, more variable recoveries of TiO_2 utilizing the Short et al. (1996) method (Meyers et al. 2003b).

The recovery of a marker is an important indication of its efficacy (Jagger et al., 1992). There are many reports of higher recoveries of TiO_2 than of Cr_2O_3 in pigs (Jagger et al., 1992), ewes (Meyers et al., 2003a), and cattle (Meyers et al., 2004). The marker with the lowest recovery, Cr_2O_3, was associated with the greatest difference between the two methods (Jagger et al., 1992) and led to lower estimates provided by Cr_2O_3 (Meyers et al., 2003a). The recovery rates for some species of poultry varied between 88% and 110% (Sales and Janssens, 2003).

Chromic oxide does not associate with either the particulate- or fluid-phase during digestion (Titgemeyer, 1997). The lower recoveries associated with Cr_2O_3 may be a result of the incomplete mixing properties or uneven flow rate of Cr_2O_3 (Titgemeyer, 1997; Sales and Janssens, 2003). Changes in intake rate may also influence the quantity of endogenous material secreted and may change the digestibility values obtained (Jagger et al., 1992).

Despite its problems, Cr_2O_3 is still the most widely used of all digesta markers because it is inexpensive, easy to mix into the diets, and analyzed with ease (Titgemeyer, 1997). Studies on the use of markers to determine nutrient digestibility with poultry are limited in comparison to research with other animals (Sales and Janssens, 2003). The only dietary marker intensively studied in poultry is Cr_2O_3 (Sales and Janssens, 2003). The lack of standardization of

analytical assays could partly explain the huge variation among and between laboratories. It is evident that an accurate and reliable method for determining nutrient digestibility is required.

CHAPTER TWO – EXPERIMENT ONE

EVALUATION OF THE EFFICACY OF HIGH LEVELS OF MICROBIAL PHYTASE ON BROILER PERFORMANCE, BONE ASH, ILEAL DIGESTIBILITY, AND LITTER PHOSPHORUS

ABSTRACT

An experiment was conducted to evaluate the efficacy of high levels of microbial phytase on broiler performance, bone ash, and litter phosphorus. Seven hundred one day-old male broilers were weighed, wing-banded, and randomly assigned to dietary treatments in floor pens. Dietary treatments for the first three weeks consisted of a positive control NRC diet (NRC; 0.45% npP and 1.00% Ca), an industry standard diet with 500 PU/kg phytase (IND; 0.35% npP and 0.90% Ca), a negative control basal diet (NEG; 0.20% npP and 0.85% Ca), NEG + 250 PU/kg phytase, NEG + 500 PU/kg phytase, NEG + 10,000 PU/kg phytase, and NEG + 20,000 PU/kg phytase. From four to six weeks, the calcium and non-phytate phosphorus (npP) levels were reduced by 0.1% in the NRC and IND diets, and calcium was decreased by 0.05% in the NEG diets. Supplemental phytase levels were identical to the starter period. Chicks fed the NRC and IND diets had similar ($P > 0.05$) growth performance, whereas chicks fed the NEG diet had lower ($P < 0.05$) feed intake (FI) and body weight gain (BWG) compared

to all other dietary treatments. Feed intake, BWG, and tibia ash increased ($P < 0.05$) with phytase supplementation and birds fed the two highest levels of phytase (NEG + 10,000 and NEG + 20,000) had FI, BWG, and tibia ash equivalent to the positive control (NRC) and industry standard diets (IND). Phosphorus digestibility at day 21 and 42 was improved ($P < 0.05$) due to phytase supplementation. Calcium digestibility was improved ($P < 0.05$) at day 21 due to phytase supplementation. Litter phosphorus was significantly lower ($P < 0.05$) for all treatments, compared to the NRC treatment. Birds fed the NEG, NEG + 10,000, and NEG + 20,000 treatments had significantly lower litter phosphorus ($P < 0.05$) compared to all other diets, with NEG + 10,000 and NEG + 20,000 having a litter phosphorus of 0.73% and 0.74%, respectively, compared to 1.56% for the NRC treatment. Soluble litter phosphorus was not affected ($P > 0.05$) by phytase supplementation. Litter calcium was decreased ($P < 0.05$) by phytase supplementation. Phytase addition to the industry standard diet led to the same growth performance as the positive control diet. Phytase was effective in improving phytate phosphorus utilization, and this improvement occurred even at the lowest level (250 PP/kg) of supplemental phytase.

Key Words: Phytase, Broilers, Floor Pens, Litter Phosphorus

INTRODUCTION

Most of the phosphorus in plants is located in the seeds, and approximately two-thirds of that phosphorus is bound in the storage form of a molecule called phytate (Viveros et al., 2000). Non-ruminant animals such as pigs and poultry have insufficient phytase in their GI tract to effectively digest phytate (Nelson et al., 1971), making the phosphorus in plants unavailable. This unavailability of phosphorus increases the need to add inorganic forms of phosphorus such as dicalcium phosphate to the diet. The combination of unavailable phosphorus and unused inorganic phosphorus increases the cost of the diet and causes an excess amount of phosphorus to be excreted in the manure (Viveros et al., 2002). The excess phosphorus in the manure creates environmental concerns such as soil saturation, run-off, and eutrophication when manure is applied as fertilizer.

Commercial phytases are available and are effective in improving phytate phosphorus utilization in poultry diets (Wu et al., 2003; Dilger et al., 2004). However, enzyme producers are continuously developing new phytases for improved efficacy and thermo stability. Previous research suggests bird performance plateaus at phytase inclusion levels of 500 to 1,000 PU/kg diet (Simons et al., 1990; Ravindran et al., 2000; Dilger et al., 2004). Recent research suggests bird performance continues to improve as dietary phytase levels increase above current industry recommendations (Shirley and Edwards, 2003; Wyatt et al., 2004; Ledoux et al., 2005). Augspurger and Baker (2004)

conducted an experiment with a phytase inclusion rate of 10,000 PU/kg and concluded that the fungal phytase levels currently being used by the poultry industry may need to be revised.

There were four objectives of this experiment. 1) To evaluate the efficacy of a novel microbial six-phytase on broilers grown to market weights. 2) To determine if bird performance will continue to improve with increasing concentrations of dietary phytase up to 20,000 PU/kg feed. 3) To determine if the improved body weight gain and feed conversion is caused by improvements in nutrient utilization i.e. ileal digestibility. 4) To determine if three week ileal phosphorus digestibility data will give the same relative statistical results as ileal phosphorus digestibility data collected at six weeks.

MATERIALS AND METHODS

In March 2005, 700 male Cornish Cross broilers were purchased from a commercial hatchery, weighed, wing-banded, and randomly assigned to dietary treatments in floor pens (5 ft x 4 ft). Each pen was covered with approximately three inches of pine shavings. Feed and water were provided *ad libitum.* Chicks were housed under constant lighting in a commercial-type facility with thermostatically controlled natural gas heaters and a ventilation fan. The animal care and use protocol was reviewed and approved by the University of Missouri Animal Care and Use Committee.

Five replicate pens of 20 chicks were randomly assigned to one of seven dietary treatments from day 1 to 21, and 10 chicks from day 22 to 42. Dietary treatments consisted of an NRC (1994) positive control diet (NRC; 1.0% Ca and 0.45% npP), an industry standard diet (IND; 0.90% Ca and 0.35% npP) plus 500 PU/kg diet, a negative control diet (NEG; 0.85% Ca and 0.20% npP), and NEG + 250, 500, 10,000, and 20,000 PU/kg. All experimental diets were formulated to meet or exceed NRC (1994) requirements, except for calcium and phosphorus. The phytase used was a bacterial six-phytase expressed in the *trichoderma reesei* fungus. Diets were fed in mash form. A coccidiostat (Diclaurizl) was added at 0.2% per ton. Chromic oxide was added at 0.1% and was used as a dietary marker. Dietary treatments are outlined in Table 2.1. Phytase analysis revealed phytase levels in the diets were adequate for this experiment (Tables 2.2 and 2.3).

The birds were monitored daily for morbidity and mortality. Any birds removed were weighed and feed intake and feed conversion were adjusted according to the number of bird days. Body weights were measured on a pen basis on days one, 21, and 42. Feed intake was also determined on days 21 and 42, and feed conversion calculated.

On day 21, 40 birds (five replicates of eight birds each) from each treatment were euthanized by lethal injection with 4% sodium pentobarbital (Pentobarb 300, Chemstock Animal Health Ltd., Christchurch, New Zealand), after which the body cavity was opened and digesta samples were collected from the lower half of the ileum (Meckel's diverticulum to the ileocecal junction).

Digesta samples from birds housed in the same pen were pooled, weighed for dry matter determination, and dried in a forced air oven at 55°C for 72 hours.

Middle toes and right tibias were collected from three birds per pen and pooled (15 birds per treatment) for determination of bone ash. Toes were dried for 24 hours at 100°C and then ashed for 24 hours in a muffle furnace at 600°C. Tibias were boiled, stripped of adhering tissue, wrapped in cheese cloth and dried overnight at 100°C. Fat was extracted from the tibias using a cold extraction procedure with 90% ethyl ether and 10% methanol. Fat-extracted tibias were then dried for 24 hours at 100°C and ashed in a muffle furnace for 24 hours at 600°C. Two additional birds were removed from each pen on day 21, leaving 10 birds per pen for the 22 to 42 day period.

On day 42, four birds from each pen were euthanized by lethal injection with 4% sodium pentobarbital and digesta samples were collected from the ileum as previously described. Middle toes and right tibias were taken from 15 birds per treatment for bone ash determination. Litter samples were collected from four sections of each pen, pooled, weighed for dry matter determination, and dried in a forced air oven at 55°C for 72 hours.

Feed, litter, and ileal digesta samples were ground to pass a one millimeter screen. Duplicate samples were digested by nitric-perchloric acid wet ash digestion, which was validated by including standard reference material 1547 (peach leaves) from the National Institute of Standards and Technology. Total phosphorus content in feed, ileal digesta, and litter was determined colorimetrically by the molybdo-vandate method (AOAC, 1970). Chromium and

calcium concentrations were determined by flame atomic absorption spectrophotometry. Soluble litter phosphorus was determined using a modification of the Self-Davis and Moore (2000) method.

The data were analyzed as a completely random design (CRD), with seven treatments as mentioned previously, using the General Linear Models (GLM) procedure of SAS (SAS Institute, 2005). Mean differences were determined using Fisher's Least Significant Difference (LSD). Linear, quadratic, and cubic orthogonal polynomial contrasts were performed on treatments NEG, NEG + 250, NEG + 500, NEG + 10,000, and NEG + 20,000. Statistical significance was accepted at $P < 0.05$.

RESULTS

PERFORMANCE

Performance results for days 0 to 21 and 0 to 42 are presented in Tables 2.2 and 2.3, respectively. At 21 days, there were no significant differences ($P > 0.05$) in feed intake (FI) and body weight gain (BWG) between the NRC and IND birds. Feed intake and BWG were significantly lower ($P < 0.05$) for the NEG treatment compared to all other treatments. Inclusion of phytase at 250 and 500 PU/kg increased ($P < 0.05$) FI and BWG above the NEG treatment. Inclusion of phytase at 10,000 and 20,000 PU/kg increased ($P < 0.05$) FI to that comparable with the NRC and IND treatments. In fact, phytase included in the diet at 10,000 PU/kg significantly increased ($P < 0.05$) BWG above all other treatment diets

including the NRC and IND diets. However, the NEG + 10,000 treatment was not statistically different ($P > 0.05$) than the NEG + 20,000 treatment. Feed intake and BWG plateaued at 10,000 PU/kg diet. There was no significant difference ($P > 0.05$) in FI and BWG among the NRC, IND, and NEG + 20,000 treatments.

Feed to gain (F:G) for the NRC treatment was significantly lower ($P < 0.05$) than the IND, NEG, and NEG + 250 treatments, but comparable to the NEG + 500, 10,000, and 20,000 treatments. Feed to gain was significantly lower ($P < 0.05$) for the NEG + 10,000 and NEG + 20,000 treatments compared to all other treatments, except the NRC treatment. Feed conversion among the IND and the NEG treatments, and the two lowest levels of phytase was not different ($P > 0.05$). There was a linear, quadratic, and cubic increase ($P < 0.05$) in FI, a linear and cubic increase ($P < 0.05$) in BWG, and a linear improvement ($P < 0.05$) in F:G due to phytase addition over 21 days. Mortality was low and there were no significant differences ($P > 0.05$) among treatments.

At day 42, there were no significant differences ($P > 0.05$) in FI, BWG, and F:G between the NRC and IND treatments. Feed intake and BWG were lower ($P < 0.05$) for the NEG treatment at 2,910 and 1,959 grams, respectively, compared to all other treatments. Inclusion of phytase at 250 and 500 PU/kg increased ($P < 0.05$) FI and BWG above the NEG treatment. Inclusion of phytase at 10,000 and 20,000 PU/kg increased ($P < 0.05$) FI and BWG to that comparable with the NRC and IND treatments.

Feed conversion for the NEG treatment was lower ($P < 0.05$) than all other treatments, except the NEG + 500 treatment. Feed conversion was not

statistically different (P > 0.05) among any other treatments. There was a linear increase (P < 0.05) in FI, linear and cubic increase (P < 0.05) in BWG, and a linear and quadratic improvement (P < 0.05) in F:G due to phytase addition over 42 days. The negative control treatment had significantly higher (P < 0.05) mortality compared to all other treatments. Mortality was low and not statistically different (P > 0.05) among any other treatments.

BONE ASH

Bone ash results for day 21 are summarized in Table 2.4. Toe ash percent and toe ash weight (mg) were not different (P > 0.05) at 21 days for broilers fed the NRC and IND diets. Birds receiving the negative control diet without added phytase had significantly lower (P < 0.05) toe ash weight (mg) compared to all other treatments, and significantly lower (P < 0.05) percent toe ash compared to all other treatments, except the NEG + 250 treatment. Toe ash percent and toe ash weight increased (P < 0.05) linearly and quadratically as dietary phytase supplementation increased. Broilers consuming phytase at 250 and 500 PU/kg diet had statistically similar (P > 0.05) toe ash percent and weight. Broilers receiving the two highest levels of phytase (10,000 and 20,000 PU/kg) had toe ash percent and weight statistically similar (P > 0.05) to the NRC and IND treatments.

There were no significant differences (P > 0.05) in tibia ash percent and weight (mg) between the NRC and IND treatment. The negative control had a lower (P < 0.05) tibia ash percent and weight compared to all other treatments. The supplementation of the negative control with phytase at 250 and 500 PU/kg

diet significantly improved ($P < 0.05$) tibia ash percent and weight above the negative control. However, tibia ash percent was not statistically similar ($P > 0.05$) to the NRC and IND treatment until the addition of phytase at 10,000 and 20,000 PU/kg diet. Supplementation of the NEG treatment with phytase at 10,000 and 20,000 PU/kg diet significantly improved ($P < 0.05$) tibia ash weight above the IND treatment, and numerically improved tibia ash weight above the NRC treatment, although the difference was not significant. Tibia ash percent and weight increased ($P < 0.05$) linearly, quadratically, and cubically due to phytase supplementation.

Forty two day toe ash and tibia ash data are presented in Table 2.5. There were no significant differences ($P > 0.05$) in the percent toe ash among the NRC and IND treatments, and the two highest levels of phytase (10,000 and 20,000 PU/kg). The negative control, NEG + 250, and NEG + 500 had a lower ($P < 0.05$) toe ash percent compared to all other treatments. Toe ash weight was significantly lower ($P < 0.05$) for the NEG treatment compared to all other treatments. Addition of phytase at 250 and 500 PU/kg diet improved ($P < 0.05$) toe ash weight with the NEG + 500 treatment having values statistically similar ($P > 0.05$) to the IND and NRC treatments. Addition of phytase at 10,000 and 20,000 PU/kg diet significantly improved ($P < 0.05$) toe ash weight above the IND treatment. Toe ash percent increased ($P < 0.05$) linearly and quadratically with phytase supplementation. Toe ash weight increased ($P < 0.05$) linearly and cubically with phytase supplementation.

There were no significant differences (P > 0.05) in tibia ash percent and tibia ash weight among the NRC, IND, NEG + 10,000, and NEG + 20,000 treatments. The negative control diet without phytase supplementation had a lower (P < 0.05) tibia ash percent and weight compared to all other treatments. Tibia ash percent and weight increased (P < 0.05) linearly and cubically as dietary phytase supplementation increased. The addition of phytase at 250 and 500 PU/kg diet significantly improved (P < 0.05) tibia ash percent and tibia ash weight above the negative control.

ILEAL DIGESTIBILITY

Phosphorus and calcium digestibility at day 21 and 42 are presented in Table 2.6. Decreasing calcium and phosphorus by 0.10% and supplementing microbial phytase at 500 PU/kg diet improved (P < 0.05) phosphorus and calcium digestibility above the NRC treatment at day 21. The NRC treatment had the lowest (P < 0.05) phosphorus and calcium digestibility at day 21. Addition of phytase at 250 PU/kg diet to did not improve (P > 0.05) phosphorus and calcium digestibility above the NRC treatment. The negative control had higher (P < 0.05) phosphorus and calcium digestibility than the NEG + 250 treatment. However, phosphorus digestibility in the NEG treatment was lower (P < 0.05) than the NEG + 500, 10,000, and 20,000 treatments. Phytase supplementation at 10,000 and 20,000 PU/kg diet had the highest (P < 0.05) phosphorus digestibility (80.98 and 84.32%, respectively) and these numbers were not different (P > 0.05). Phytase supplementation (P < 0.05) linearly, quadratically, and cubically affected phosphorus digestibility. Calcium digestibility at day 21

was not different (P > 0.05) among the IND, NEG, NEG + 500, NEG + 10,000, or NEG + 20,000 treatments. Supplementing low calcium and phosphorus diets with phytase over 250 PU/kg diet improved (P < 0.05) calcium digestibility above the NRC treatment, however, no specific trends were observed using regression analysis.

At day 42, phytase supplementation (P < 0.05) linearly and quadratically improved phosphorus digestibility and (P < 0.05) linearly decreased calcium digestibility. Phosphorus digestibility was significantly lower (P < 0.05) in the NRC treatment compared to all other treatments, except the negative control. Phytase supplementation to the negative control improved (P < 0.05) phosphorus digestibility. However, supplementation of phytase at 250 PU/kg diet improved (P < 0.05) phosphorus digestibility over 500 PU/kg diet. Further phytase supplementation of the negative control with 10,000 and 20,000 PU/kg diet significantly increased (P < 0.05) phosphorus digestibility above all other treatments. Ileal calcium digestibility was significantly higher (P < 0.05) in the NEG and NEG + 500 treatments, compared to all other treatments, except the NEG + 250 treatment. Supplementation of phytase at 500 PU/kg diet to the IND diet and supplementation of phytase at 250, 10,000 and 20,000 PU/kg diet to the negative control appears to have significantly decreased (P < 0.05) calcium digestibility, and these values were comparable to the NRC treatment.

LITTER PHOSPHORUS AND CALCIUM

Total litter phosphorus (TP), litter soluble phosphorus (SP), and total litter calcium data are presented in Table 2.7. Phytase supplementation to the

negative control decreased ($P < 0.05$) total litter phosphorus quadratically and cubically. Total litter phosphorus was significantly lower ($P < 0.05$) in the IND treatment, compared to the NRC treatment (1.17 versus 1.56%, respectively). The NEG, NEG + 10,000, and NEG + 20,000 treatments had the lowest ($P < 0.05$) total litter phosphorus compared to all other treatments. The NEG + 250 treatment had significantly lower ($P < 0.05$) total litter phosphorus compared to the NRC and IND treatments. The NEG + 500 treatment had significantly lower ($P < 0.05$) total litter phosphorus compared to the NRC, IND, and NEG + 250 treatments.

Phytase supplementation linearly and cubically increased ($P < 0.05$) soluble litter phosphorus. Soluble litter phosphorus was significantly lower ($P < 0.05$) in the negative control compared to all other treatments. There were no significant differences ($P > 0.05$) in soluble litter phosphorus among the NRC, IND, NEG + 250, NEG + 500, and NEG + 10,000 treatments. In fact, as phytase increased to 10,000 and 20,000 PU/kg diet soluble litter phosphorus increased. Soluble litter phosphorus in the NEG + 20,000 treatment was significantly higher ($P < 0.05$) than the IND, NEG + 500, and NEG + 10,000 treatments.

Total litter calcium was linearly and quadratically decreased ($P < 0.05$) by supplementation of dietary phytase. The NRC treatment had significantly higher ($P < 0.05$) total litter calcium compared to all other treatments, except the NEG + 250 treatment. Total litter calcium was significantly lower ($P < 0.05$) in the negative control compared to all other treatments. Phytase addition of 500 and 10,000 PU/kg to NEG significantly increased ($P < 0.05$) total litter calcium

compared to the NEG treatment. Further phytase supplementation of 20,000 PU/kg diet significantly decreased ($P < 0.05$) total litter calcium compared to the NRC, NEG + 250, NEG + 500, and NEG + 10,000 treatments.

DISCUSSION

Many researchers have reported the benefits of supplementing low phosphorus diets with microbial phytase (Viveros et al., 2002; Wu et al., 2003; Rutherfurd et al., 2004). The first objective of the current study was to evaluate the efficacy of a novel microbial six-phytase. The statistically similar performance and bone ash data of birds fed the NRC and IND diet suggest that this phytase was efficacious in utilizing phytate phosphorus when calcium and phosphorus levels were reduced by 0.10% and phytase was included in the diet at 500 PU/kg. The results from this experiment are similar to results from a series of experiments conducted by Augspurger et al. (2003) and an experiment by Wu et al. (2004a). Both researchers reported the benefits of supplementing a low phosphorus diet with 500 PU/kg diet of microbial phytase.

At 21 days, birds fed the NRC diet had a better feed conversion than birds fed the IND diet. However, feed efficiency was not different at 42 days thus any improvements in feed efficiency from the NRC were negated as the birds aged. These results were similar to results by Simons et al. (1990), Perney et al. (1993), Sebastian et al. (1996b), and Viveros et al. (2002). In contrast, Wu et al.

(2004a) reported improved feed efficiency in diets supplemented with 500 PU/kg diet of microbial phytase.

The improvement in phosphorus digestibility between the NRC and IND diet is indicative of an improvement in the release of phytate-phosphorus. A 25% reduction in total litter phosphorus is consistent with the improvement in phosphorus digestibility. These results are consistent with a previous study in our lab utilizing the same microbial six-phytase in market weight turkeys (Ledoux et al., 2005). The decrease in litter phosphorus was lower than previous reports by Wu et al. (2004b) who observed a 35% decrease in excreta P when low P diets were supplemented with 500 PU/kg of phytase.

Supplementation of 500 PU/kg of phytase to low phosphorus diets improved calcium digestibility at day 21 and reduced litter calcium. However, there was no difference in calcium digestibility between NRC and IND at day 42. In fact, calcium digestibility was numerically lower for IND than NRC at day 42. The high variability in calcium digestibility may be due to the age of the birds and the difference in the calcium requirement. Dilger et al. (2004) reported no response in calcium digestibility from phytase supplementation of broiler diets even when there was a growth response. Phytase supplementation of 500 PU/kg phytase to a low phosphorus and low calcium diet appeared to have no significant effect on soluble litter phosphorus and these results are comparable to previous reports by Applegate et al. (2003) and Maguire et al. (2004).

Phytase supplementation up to 20,000 PU/kg in low calcium and phosphorus diets linearly improved broiler performance, toe ash, tibia ash, and

phosphorus digestibility at 21 and 42 days. Yi et al. (1996b) reported linear improvements in FI, BWG, and toe ash weight in broilers fed increasing levels of supplemental phytase. Ravindran et al. (2006) reported that increasing phytase supplementation improved ileal P digestibility in young broilers.

At 21 days, FI also showed a quadratic and cubic response to phytase supplementation, and intake reached a plateau at 10,000 PU/kg diet. Body weight gain at 21 and 42 days responded cubically to phytase supplementation. At 21 days, birds fed 10,000 PU/kg diet achieved a significant improvement in body weight gain above those fed the NRC diet, however this improvement was not maintained to 42 days. In fact, at 42 days, bird performance and bone ash plateaued as enzyme levels reached 10,000 PU/kg diet. This disproves our hypothesis, which suggested that bird performance will continue to improve as phytase levels increased above industry standards. The results from this study are also different from a previous experiment in our lab (Ledoux et al., 2005) utilizing market weight turkeys, where birds fed diets containing 10,000 PU/kg diet outperformed birds fed the positive control diet. It is speculated that the calcium and phosphorus levels were too low in the basal diet to sufficiently improve bird performance above the NRC diet in treatment diets containing the highest levels of enzyme past 21 days.

Ileal digestibility is a good indicator of the enzymatic digestion of a diet for broiler chicks (Jallier et al., 2003). In the present study phosphorus digestibility was linearly improved with phytase supplementation at both 21 and 42 days. High levels of microbial phytase (10,000 and 20,000 PU/kg) significantly

increased ileal phosphorus digestibility to 80.98 and 84.32%, respectively, at 21 days. Wu et al. (2003) and Rutherfurd et al. (2004) reported improvements in ileal phosphorus digestibility due to microbial phytase supplementation in broiler diets. However, Wu et al. (2003) and Rutherfurd et al. (2004) reported phosphorus digestibility values between 49 and 58% and 53 and 65%, respectively.

Augspurger and Baker (2004) reported that the phosphorus-releasing efficacy of a microbial phytase was approximately threefold greater than fungal phytases. Shirley and Edwards (2003) reported an increase in plasma phosphorus and total phosphorus retention in broilers fed 12,000 PU/kg diet, and addition of a fungal phytase to 12,000 PU/kg diet made approximately 95% of the phytate phosphorus available for utilization in the chick. The high phosphorus digestibility reported in our study at 21 days may be a result of an increase in phosphorus release since no data exists to compare ileal phosphorus digestibility utilizing extremely high levels of microbial phytase.

At day 42, phytase supplementation linearly and quadratically improved phosphorus digestibility, and these results are slightly higher but more similar to 21 day results obtained by Wu et al. (2003) and Rutherford et al. (2004). When comparing ileal phosphorus digestibility results it is important to remember the phytase used in this experiment is new and may contain benefits not yet known over other phytase enzymes. This particular enzyme may have side enzyme activities that reach effective concentrations when higher levels are supplemented in the diet. Another factor contributing to the high digestibility data

may be the low calcium and phosphorus levels in the NEG diet, which results in a more efficient use of dietary nutrients by the bird.

Phytase supplementation at 42 days linearly decreased calcium digestibility. These results cannot be explained, but it is important to note that at 21 days there were no effects of phytase supplementation on calcium digestibility. Our results are different from Silversides et al. (2004) who reported improved calcium digestibility when broilers were fed microbial phytase for 21 days. The values for calcium digestibility in the Silversides et al. (2004) experiment are similar to that reported in this experiment, however, the range between diets in this experiment was higher. The higher calcium digestibility may, like the high phosphorus digestibility, be a result of the efficacy of the novel microbial phytase, the low levels of calcium and phosphorus in the NEG diet, an interaction between calcium and phosphorus, or the high levels of phytase added to the diet.

It is important to note that decreasing the calcium and phosphorus in the diet by .10% and supplementing 500 PU/kg microbial phytase improved ileal phosphorus and calcium digestibility during the first 21 days. Phosphorus digestibility was improved by 19% and calcium digestibility was improved by 27%. During the last 22 to 42 days phosphorus digestibility improved by 20%, and calcium digestibility was not significantly affected, although it numerically decreased. Silversides et al. (2004) reported lower calcium digestibility in a low phosphorus diet compared to a diet adequate in phosphorus. The improved ileal

phosphorus digestibility suggests that this enzyme is efficacious at improving phytate phosphorus utilization.

Total litter phosphorus was lowest in the NEG, NEG + 10,000, and NEG + 20,000 diets compared to all other treatments. The higher total litter phosphorus at 250 and 500 PU/kg microbial phytase compared to NEG, NEG + 10,000, and NEG + 20,000 PU/kg microbial phytase may be due to the lack of sufficient enzyme to break down the phytate molecules resulting in greater phosphorus excretion. Adding phytase to the diet at 10,000 and 20,000 PU/kg decreased the total litter phosphorus by 53% compared to the NRC diet (0.73 and 0.74, compared to 1.56%, respectively).

Soluble litter phosphorus was significantly lower in the NEG diet without phytase supplementation. These data follow the same trend as total litter phosphorus. It is evident from the poor performance, bone ash results, and high mortality that the NEG control diet was too low in calcium and phosphorus, and it was expected to have the lowest levels of total litter and soluble phosphorus. Enzyme supplementation in the NEG diet linearly and cubically increased soluble litter phosphorus. However, data were not significantly different from the NRC diet. Phytase supplementation appeared to have no significant effect on soluble litter phosphorus and these results are comparable to previous reports by Applegate et al. (2003) and Maguire et al. (2004).

Total litter calcium was significantly decreased between the NRC and IND, suggesting a 0.10% decrease in calcium and phosphorus and 500 PU/kg microbial phytase improve calcium utilization. The negative control diet had a

significantly lower percent litter calcium compared to all other treatments creating a quadratic effect from phytase supplementation. Increasing phytase supplementation of NEG resulted in a linear and quadratic decrease in total litter calcium. Other researchers have also reported a decrease in calcium excreted due to phytase supplementation (Cowieson et al., 2004a).

Phytase addition to industry standards led to the same growth performance and bone ash as the positive control diet. Phytase was effective in improving phytate phophorus utilization, and this improvement occurred even at the lowest level (250 PPU/kg) of supplemental phytase. These results are similar to other experiments utilizing microbial phytases in broilers (Augspurger et al., 2003; Ravindran et al., 2006), turkeys (Applegate et al., 2003), and pigs (Augspurger et al., 2003).

Table 2.1. Composition and calculated analysis of the experimental diets.

Ingredients	------- (0 to 21 day) -------		----- (22 to 42 day) ------	
	NEG[1] (%)	NRC[2] (%)	NEG[1] (%)	NRC[2] (%)
Corn	52.063	50.267	61.660	60.528
Soybean Meal	38.738	39.055	30.928	31.128
Corn Oil	5.803	6.399	4.315	4.691
Limestone	1.609	1.165	1.495	1.255
Salt	0.464	0.463	0.335	0.334
Sand	0.400	0.400	0.400	0.400
Dicalcium Phosphate	0.387	1.714	0.437	1.233
DL-Methionine	0.178	0.180	0.070	0.071
Trace Mineral	0.100	0.100	0.100	0.100
Chromic Oxide	0.100	0.100	0.100	0.100
Selenium Premix	0.054	0.054	0.056	0.056
Vitamin Mix	0.050	0.050	0.050	0.050
Clinicox*	0.050	0.050	0.050	0.050
CuSO4	0.004	0.004	0.004	0.004
Nutrients:				
ME, kcal/kg	3200	3200	3200	3200
Crude Protein, %	23.00	23.00	20.00	20.00
Available P, %	0.20	0.45	0.20	0.35
Analyzed tP, %	0.50	0.76	0.45	0.59
Calculated Ca, %	0.85	1.00	0.80	0.90
Analyzed Ca, %	0.95	1.23	0.91	0.94

* Coccidiostat (Diclazuril 0.91 g/ton diet).
[1] Basal diets supplemented with 0, 250, 500, 10,000, or 20,000 PU/kg phytase.
[2] Basal diets supplemented with 0 or 500 PU/kg phytase. IND = NRC diet supplemented with 500 PU/kg phytase and had calcium and phosphorus levels decreased to 0.90 and 0.35%, respectively from day one to day 21, and 0.80 and 0.20%, respectively from day 22 to 42.

Table 2.2. Efficacy of a microbial phytase on growth performance[1] and mortality of 21-day old male broilers[2]

Diet	Phytase[3] (PU/kg)	Phytase[4] (PU/kg)	Ca (%)	P (%)	FI[5] (g)	BWG[5,7] (g)	F:G[5,6] (g:g)	Mortality[7] (%)
NRC +	0	0	1.00	0.45	1,072[a]	859[b]	1.25[bc]	5.0
IND +	500	512	0.90	0.35	1,108[a]	851[b]	1.30[a]	4.0
NEG +	0	0	0.85	0.20	666[d]	496[e]	1.35[a]	9.0
NEG +	250	215	0.85	0.20	927[c]	704[d]	1.32[a]	2.0
NEG +	500	593	0.85	0.20	1,020[b]	785[b]	1.29[ab]	3.0
NEG +	10,000	8,790	0.85	0.20	1,110[a]	918[a]	1.21[c]	4.0
NEG +	20,000	20,500	0.85	0.20	1,097[a]	887[ab]	1.24[c]	4.0
SEM					14	13	0.02	0.06

[1] FI = feed intake; BWG = body weight gain; F:G = feed conversion.
[2] Data are means of five replicate pens of 20 chicks each.
[3] Calculated phytase values.
[4] Analyzed phytase values.
[5] Linear response to graded levels of a microbial phytase ($P < 0.05$).
[6] Quadratic response to graded levels of a microbial phytase ($P < 0.05$).
[7] Cubic response to graded levels of a microbial phytase ($P < 0.05$).
[8] Mortality data analyzed using the ARSIN procedure of SAS.
[a-e] Values within columns with no common superscript differ significantly ($P < 0.05$).

Table 2.3. Efficacy of a microbial phytase on growth performance[1] and mortality of 42-day old male broilers[2]

Diet	Phytase[3] (PU/kg)	Phytase[4] (PU/kg)	Ca[5] (%)	P[6] (%)	FI[7] (g)	BWG[7,9] (g)	F:G[7,8] (g:g)	Mortality[10] (%)
NRC +	0	0	0.90	0.35	4,819[a]	3,021[a]	1.60[a]	9.0[b]
IND +	500	601	0.80	0.25	4,868[a]	2,997[a]	1.62[a]	6.0[b]
NEG +	0	0	0.80	0.20	2,910[d]	1,959[d]	1.49[b]	24[a]
NEG +	250	337	0.80	0.20	3,901[c]	2,421[c]	1.61[a]	6.0[b]
NEG +	500	612	0.80	0.20	4,438[b]	2,747[b]	1.62[a]	6.0[b]
NEG +	10,000	11,700	0.80	0.20	4,771[a]	3,012[a]	1.58[a]	5.0[b]
NEG +	20,000	22,800	0.80	0.20	4,908[a]	3,017[a]	1.63[a]	6.0[b]
SEM					90	56	0.02	0.06

[1] FI = feed intake; BWG = body weight gain; F:G = feed conversion.
[2] Data are means of five replicate pens of 20 chicks each (days 0 to 21) and 10 chicks each (days 22 to 42).
[3] Calculated phytase values.
[4] Analyzed phytase values.
[5] Dietary calcium levels from days 0 to 21 were: 1.00, 0.90, 0.85, 0.85, 0.85, 0.85, and 0.85%, respectively.
[6] Dietary phosphorus levels from days 0 to 21 were: 0.45, 0.35, 0.20, 0.20, 0.20, 0.20, and 0.20%, respectively.
[7] Linear response to graded levels of a microbial phytase ($P < 0.05$).
[8] Quadratic response to graded levels of a microbial phytase ($P < 0.05$).
[9] Cubic response to graded levels of a microbial phytase ($P < 0.05$).
[10] Mortality data analyzed using the ARSIN procedure of SAS.
[a-d] Values within columns with no common superscript differ significantly ($P < 0.05$).

Table 2.4. Efficacy of a microbial phytase on bone ash of 21-day old broilers[1]

Diet	Phytase (PU/kg)	Ca (%)	P (%)	---- Toe Ash [2,3] ---- (%)	(mg)	---- Tibia Ash [2,3,4] ---- (%)	(mg)
NRC +	0	1.00	0.45	13.25[a]	130[ab]	45.06[a]	1,168[ab]
IND +	500	0.90	0.35	12.36[a]	119[b]	42.82[a]	1,070[b]
NEG +	0	0.85	0.20	8.75[c]	58[d]	29.95[d]	460[e]
NEG +	250	0.85	0.20	9.43[bc]	83[c]	33.15[c]	664[d]
NEG +	500	0.85	0.20	10.29[b]	92[c]	37.51[b]	825[c]
NEG +	10,000	0.85	0.20	12.39[a]	137[a]	44.22[a]	1,260[a]
NEG +	20,000	0.85	0.20	12.90[a]	138[a]	44.26[a]	1,241[a]
SEM				0.34	4.9	0.88	52

[1] Data are means of five replicate pens of three chicks each.
[2] Linear response to graded levels of a microbial phytase ($P < 0.05$).
[3] Quadratic response to graded levels of a microbial phytase ($P < 0.05$).
[4] Cubic response to graded levels of a microbial phytase ($P < 0.05$).
[a-e] Values within columns with no common superscript differ significantly ($P < 0.05$).

Table 2.5. Efficacy of a microbial phytase on bone ash of 42-day old broilers[1]

Diet	Phytase (PU/kg)	Ca[2] (%)	P[3] (%)	Toe Ash (%)[4,5]	Toe Ash (mg)[4,6]	Tibia Ash (%)[4,6]	Tibia Ash (mg)[4,6]
NRC +	0	0.90	0.35	12.05[a]	408[ab]	45.32[a]	4,279[a]
IND +	500	0.80	0.25	11.82[a]	393[b]	44.52[ab]	4,044[a]
NEG +	0	0.80	0.20	10.38[b]	241[d]	38.75[d]	2,185[d]
NEG +	250	0.80	0.20	10.94[b]	348[c]	40.90[c]	3,199[c]
NEG +	500	0.80	0.20	10.65[b]	379[bc]	43.55[b]	3,513[b]
NEG +	10,000	0.80	0.20	12.09[a]	435[a]	45.71[a]	4,329[a]
NEG +	20,000	0.80	0.20	12.19[a]	411[a]	45.15[a]	4,157[a]
SEM				0.27	12	0.54	104

[1] Data are means of 5 replicate pens of 3 chicks each.
[2] Dietary calcium levels from days 0 to 21 were: 1.00, 0.90, 0.85, 0.85, 0.85, 0.85, and 0.85%, respectively.
[3] Dietary phosphorus levels from days 0 to 21 were: 0.45, 0.35, 0.20, 0.20, 0.20, 0.20, and 0.20%, respectively.
[4] Linear response to graded levels of a microbial phytase ($P < 0.05$).
[5] Quadratic response to graded levels of a microbial phytase ($P < 0.05$).
[6] Cubic response to graded levels of a microbial phytase ($P < 0.05$).
[a-d] Values within columns with no common superscript differ significantly ($P < 0.05$).

Table 2.6. Efficacy of a microbial phytase on ileal P and Ca digestibility in 21- and 42-day old broilers[1]

Diet	Phytase (PU/kg)	Ca[2] (%)	P[3] (%)	P[4,5,6] (%)	Ca (%)	P[4,5] (%)	Ca[4] (%)
				------ (day 21) ------		-------- (day 42) -------	
NRC +	0	0.90	0.35	28.31^{e}	31.80^{c}	31.47^{e}	29.17^{bc}
IND +	500	0.80	0.25	34.88^{d}	43.56^{ab}	38.92^{c}	21.33^{c}
NEG +	0	0.80	0.20	40.09^{c}	50.93^{a}	31.96^{de}	54.83^{a}
NEG +	250	0.80	0.20	26.77^{e}	38.38^{bc}	47.11^{b}	38.65^{ab}
NEG +	500	0.80	0.20	47.69^{b}	51.08^{a}	38.47^{cd}	51.43^{a}
NEG +	10,000	0.80	0.20	80.98^{a}	45.60^{ab}	59.05^{a}	29.17^{bc}
NEG +	20,000	0.80	0.20	84.32^{a}	51.37^{a}	63.66^{a}	30.03^{bc}
SEM				1.7	3.7	2.4	5.9

[1] Data are means of five replicates of eight chicks each (days 0 to 21) and four chicks each (days 22 to 42).
[2] Dietary calcium levels from days 0 to 21 were: 1.00, 0.90, 0.85, 0.85, 0.85, 0.85, and 0.85%, respectively.
[3] Dietary phosphorus levels from days 0 to 21 were: 0.45, 0.35, 0.20, 0.20, 0.20, 0.20, and 0.20%, respectively.
[4] Linear response to graded levels of a microbial phytase ($P < 0.05$).
[5] Quadratic response to graded levels of a microbial phytase ($P < 0.05$).
[6] Cubic response to graded levels of a microbial phytase ($P < 0.05$).
[a-e] Values within columns with no common superscript differ significantly ($P < 0.05$).

Table 2.7. Efficacy of a microbial phytase on total and soluble litter P and total litter Ca of 42-day old broilers[1]

Diet	Phytase (PU/kg)	Ca[2] (%)	P[3] (%)	Litter TP[4,7,8] (%)	Litter SP[5,6,8] (%)	Litter Ca[6,7] (%)
NRC +	0	0.90	0.35	1.56[a]	0.22[ab]	2.69[a]
IND +	500	0.80	0.25	1.17[b]	0.19[b]	2.26[bc]
NEG +	0	0.80	0.20	0.70[e]	0.11[c]	1.69[d]
NEG +	250	0.80	0.20	0.92[c]	0.20[ab]	2.67[a]
NEG +	500	0.80	0.20	0.86[d]	0.18[b]	2.34[b]
NEG +	10,000	0.80	0.20	0.73[e]	0.19[b]	2.33[b]
NEG +	20,000	0.80	0.20	0.74[e]	0.24[a]	2.14[c]
SEM				0.03	0.01	0.09

[1] Data are means of five replicate pens of 20 chicks each (days 0 to 21) and 10 chicks each (days 22 to 42).
[2] Dietary calcium levels were:1.00, 0.90, 0.85, 0.85, 0.85, 0.85, and 0.85%, respectively, for days 0 to 21.
[3] Dietary phosphorus levels were: 0.45, 0.35, 0.20, 0.20, 0.20, 0.20, and 0.20%, respectively, for days 0 to 21.
[4] TP = total litter P.
[5] SP = soluble litter P.
[6] Linear response to graded levels of a microbial phytase ($P < 0.05$).
[7] Quadratic response to graded levels of a microbial phytase ($P < 0.05$).
[8] Cubic response to graded levels of a microbial phytase ($P < 0.05$).
[a-e] Values within columns with no common superscript differ significantly ($P < 0.05$).

CHAPTER THREE – EXPERIMENT TWO

EVALUATION OF THE EFFICACY OF HIGH LEVELS OF A DIETARY PHYTASE ON 21-DAY BROILER PERFORMANCE, BONE ASH, AND ILEAL DIGESTIBILITY

ABSTRACT

An experiment was conducted to evaluate the efficacy of high levels of a dietary phytase on 21-day broiler performance, bone ash, and ileal digestibility. One-day old male broilers were fed an NRC diet adequate in all nutrients for six days. On day seven, 900 chicks were weighed, wing-banded, and randomly assigned to dietary treatments in floor pens. Dietary treatments consisted of a positive control diet (POS; 0.85% Ca and 0.42% npP), a negative control diet (NEG; 0.85% Ca and 0.25% npP), NEG + 500 PU/kg diet, NEG + 2,500 PU/kg diet, NEG + 12,500 PU/kg diet, and NEG + 62,500 PU/kg diet. Phytase supplementation did not affect ($P > 0.05$) body weight gain (BWG) but linearly decreased ($P < 0.05$) feed intake (FI). Phytase supplementation linearly and quadratically improved ($P < 0.05$) feed conversion. Percent toe ash was not affected ($P > 0.05$) by phytase supplementation. Toe ash weight (mg) increased linearly ($P < 0.05$) due to phytase supplementation. Phytase supplementation linearly increased ($P < 0.05$) tibia ash percent and tibia ash weight (mg). Phytase

supplementation linearly, quadratically, and cubically affected ($P < 0.05$) phosphorus and calcium digestibility. Dietary markers, chromic oxide and titanium dioxide yielded similar statistical results for ileal digestibility, however data were numerically different, with titanium having higher digestibility coefficients. Results indicate that phytase was efficacious in improving phytate phosphorus utilization.

Key Words: Phytase, Broilers, Ileal digestibility

INTRODUCTION

Primarily found in the seeds of plants, phytic acid serves as a storage form of phosphorus (Tamim and Angel, 2003). Unfortunately, nonruminant animals lack the enzymes necessary to degrade phytic acid. Phytases are the only known enzymes with the ability to initiate dephosphorylation of phytate phosphorus (Applegate and Angel, 2005). Commercial phytases are available and are effective in improving phytate phosphorus utilization in poultry diets (Wu et al., 2003; Dilger et al., 2004).

The development of new phytase products for improved thermo stability and efficacy require re-evaluation of the current recommendation for inclusion of phytase in poultry diets. Previous research suggests that bird performance plateaus at phytase inclusion levels of 500 to 1,000 PU/kg diet (Simons et al., 1990; Ravindran et al., 2000; Dilger et al., 2004). Recent research suggests bird performance continues to improve as dietary phytase levels increase above

current industry recommendations (Shirley and Edwards, 2003; Wyatt et al., 2004; Ledoux et al., 2005). Augspurger and Baker (2004) conducted an experiment with a fungal phytase at 10,000 PU/kg diet and concluded that the current phytase levels utilized in the poultry industry may need revision.

Indigestible markers such as chromic oxide (Cr_2O_3) and titanium dioxide (TiO_2) are included in diets in digestibility studies to examine nutrient uptake at a specific site along the gastrointestinal tract (Short et al., 1996). Unfortunately, there are many problems associated with chromic oxide as a digestibility marker such as, incomplete recovery, variable results, and difficulty with the assay (Sales and Janssens, 2003). Titanium dioxide is an alternative to chromic oxide as an indigestible marker. The analysis for titanium dioxide is accurate and simple, and uses a small sample size (Short et al., 1996). However, studies utilizing titanium dioxide as a dietary marker in poultry diets are few compared to chromic oxide.

There were two objectives of this research. 1) To determine if bird performance continues to improve with increasing concentrations of dietary phytase up to 62,500 PU/kg feed. 2) To determine the efficacy of chromic oxide and titanium dioxide as ileal digestibility markers in 21-day old broilers.

MATERIALS AND METHODS

In December 2005, 1,000 male Cobb x Cobb broilers were purchased from a commercial hatchery and fed an NRC (1994) corn-soybean meal diet

adequate in all nutrients for six days. On day seven, 900 chicks were weighed, wing-banded, and randomly assigned to dietary treatments in floor pens (8 ft x 6 ft) in a commercial-type facility with thermostatically controlled curtain sides, gas heaters, and overhead ventilation fans. Each pen was covered with approximately two inches of pine shavings. Feed and water were provided *ad libitum*. Chicks were reared for the first six days on a 23 hour light and one hour dark regime. Thereafter the lighting regime consisted of 16 hours of light and eight hours of dark. The animal care and use protocol was reviewed and approved by the University of Missouri Animal Care and Use Committee.

Five replicate pens of 30 chicks were randomly assigned to one of six dietary treatments from day seven to 21. Dietary treatments consisted of a positive control diet (POS; 0.85% Ca and 0.42% npP), a negative control diet (NEG; 0.85% Ca and 0.25% npP), and four experimental diets (0.85% Ca and 0.25% npP) containing 500, 2,500, 12,500, and 62,500 PU/kg phytase. All birds received diets in crumbled form from day one to 21. A coccidiostat (Monensin) was added to the diet at 90 g/ton. A growth promotant (BMD) was also added to the diet at 50 g/ton. Chromic oxide and titanium dioxide were used as dietary markers and added to each diet at 0.3%. Dietary treatments are presented in Table 3.1.

The birds were monitored daily for morbidity and mortality, and any birds removed were weighed and the feed intakes and the feed conversion were adjusted according to the number of bird days. Body weights were measured on

a pen basis on days seven and 21. Feed intake was also determined on day 21 and feed conversion calculated.

On day 21, 50 birds (five replicates of 10 birds each) from each treatment were euthanized by lethal injection with 4% sodium pentobarbital, after which the body cavity was opened and digesta samples were collected, pooled, and processed from the lower half of the ileum in the same manner as in experiment one. Middle toes and right tibias were collected from 3 birds per pen and pooled (15 birds per treatment) for determination of bone ash similarly to experiment one.

Feed and ileal digesta samples were processed similarly to experiment one for determination of total phosphorus, calcium, and chromium. Titanium dioxide in the feed and ileal digesta was determined using the Short et al. (1996) method. The data were analyzed as a completely random design (CRD), with six treatments as described previously, using the General Linear Models (GLM) procedure of SAS (SAS Institute, 2005). Mean differences were determined using Fisher's Least Significant Difference (LSD). Linear, quadratic, and cubic orthogonal polynomial contrasts were performed on treatments NEG, NEG + 500, NEG + 2,500, NEG + 12,500, and NEG + 62,500. Statistical significance was accepted at $P < 0.05$.

RESULTS

PERFORMANCE

Performance results for days seven to 21 are presented in Table 3.2. At 21 days, phytase supplementation up to 62,000 PU/kg diet did not affect ($P > 0.05$) body weight gain. Feed intake linearly decreased ($P < 0.05$) due to phytase supplementation. There were no significant differences ($P > 0.05$) in FI between birds fed POS and NEG control diets (1,076 and 1,052 grams, respectively). Feed intake was significantly higher ($P < 0.05$) in the POS, NEG, and NEG + 2,500 birds compared to birds fed NEG + 62,500. Supplementing 500, 2,500, and 12,500 PU/kg phytase did not affect ($P > 0.05$) feed intake compared to the NEG diet.

Feed conversion linearly and quadratically improved ($P < 0.05$) due to phytase supplementation. Feed conversion was significantly higher ($P < 0.05$) in the POS treatment compared to all other treatments, except the NEG and NEG + 2,500 treatments. There were no differences ($P > 0.05$) in F:G among the POS, NEG, and NEG + 2,500 birds. Phytase supplementation at 12,500 PU/ kg diet resulted in a significant ($P < 0.05$) improvement in feed conversion compared to the POS, NEG, and NEG + 2,500 treatments. Phytase supplementation at 62,500 PU/kg diet resulted in a significant ($P < 0.05$) improvement in feed conversion compared to the POS, NEG, NEG + 500, and NEG + 2,500 treatments. Mortality was low in this experiment and was not treatment related.

BONE ASH

Bone ash results for days seven to 21 are summarized in Table 3.3. Percent toe ash was not affected ($P > 0.05$) by phytase supplementation. Toe ash weight (mg) linearly improved ($P < 0.05$) due to phytase supplementation. Toe ash weight (mg) was significantly lower ($P < 0.05$) in the NEG birds compared to the POS, NEG + 500, NEG + 12,500, and NEG + 62,500 birds. Phytase supplementation of the NEG diet at 12,500 and 62,500 PU/kg diet improved toe ash weight, but this improvement was not ($P > 0.05$) higher than the POS birds (147 and 153 vs. 145 mg, respectively). Phytase supplementation linearly improved tibia ash percent and weight. Tibia ash (percent and weight) was significantly lower ($P < 0.05$) in the NEG birds compared to all other treatments. Percent tibia ash and milligrams tibia ash were numerically higher in the NEG + 12,500 and NEG + 62,500 birds than the POS birds however, this improvement was not statistically significant ($P > 0.05$).

ILEAL DIGESTIBILITY

Phosphorus and calcium digestibility for day 21 are presented in Table 3.4. Phytase supplementation linearly, quadratically, and cubically affected ($P < 0.05$) phosphorus digestibility. Phosphorus digestibility was highly correlated between chromic oxide and titanium dioxide ($R = 0.97$). However, the data were numerically different. Both chromic oxide and titanium dioxide indicate that phosphorus digestibility for the POS treatment is significantly lower ($P < 0.05$) than all other treatments. The decrease in dietary calcium and phosphorus in the NEG treatment significantly improved ($P < 0.05$) phosphorus digestibility above

the POS treatment. However, addition of 500 PU/kg diet to the NEG diet decreased ($P < 0.05$) phosphorus digestibility compared to the NEG treatment. According to the dietary marker titanium dioxide, supplementation of phytase in the NEG diet above 500 PU/kg significantly improved ($P < 0.05$) phosphorus digestibility above the POS, NEG, and NEG + 500 treatments. However, according to the dietary marker chromic oxide, phosphorus digestibility in the NEG + 2,500 treatment and the NEG + 62,500 were not statistically different ($P > 0.05$) whereas the NEG + 12,500 treatment was significantly higher ($P < 0.05$) than all other treatments.

Phytase supplementation linearly, quadratically, and cubically affected ($P < 0 .05$) calcium digestibility. Calcium digestibility was highly correlated between dietary markers ($R = 0.94$). However, the data were numerically different. According to both digestibility markers, calcium digestibility was significantly lower ($P < 0.05$) in the POS treatment compared to all other treatments. A decrease in dietary calcium and phosphorus in the NEG treatment improved ($P < 0.05$) calcium digestibility above all other treatments.

According to the dietary marker chromic oxide, addition of phytase in the NEG diet at 500 PU/kg decreased ($P < 0.05$) calcium digestibility compared to all other treatments, except the POS and NEG + 62,500 treatments. Supplementation of phytase at 2,500 and 12,500 PU/kg diet improved ($P < 0.05$) calcium digestibility above all other treatments, except the treatment NEG + 62,500. According to the dietary marker titanium dioxide, phytase supplementation in the NEG diet at 500 PU/kg decreased ($P < 0.05$) calcium

digestibility below all other treatments, except the POS treatment. Supplementation of dietary phytase above 500 PU/kg diet improved ($P < 0.05$) calcium digestibility above all other treatments.

DISCUSSION

In 2003, Shirley and Edwards reported that birds consuming up to 12,000 PU/kg diet can achieve maximum performance. Body weight gain, feed intake, feed efficiency, tibia ash, plasma phosphorus, phosphorus and nitrogen retention, and AME were significantly increased as dietary phytase levels increased from 0 to 12,000 PU/kg diet. Ledoux et al. (2005) reported turkeys grown to market weight fed diets containing 10,000 PU/kg diet outperformed turkeys fed the positive control diet. Wu et al. (2004a) fed supplemental phytase at inclusion levels up to 2,000 PU/kg diet and reported that feed efficiency for broilers fed diets supplemented with phytase were superior to those fed diets adequate in phosphorus without phytase supplementation.

In this experiment, phytase supplementation to low calcium and phosphorus diets had no significant effect on body weight gain. However, the birds fed the two highest levels of enzyme (12,500 and 62,500 PU/kg diet) were numerically heavier. The lack of a significant effect on body weight gain could be a result of feeding an NRC (1994) diet for the first six days and/or a result of feeding higher than expected calcium and phosphorus levels. Many researchers report the importance of calcium and phosphorus levels in the feed and its effect

on an adequate response from phytase (Sebastian et al., 1996a; Qian et al., 1997; Tamim et al., 2003; Driver et al., 2005). Phytase supplementation linearly decreased feed intake. The decrease may be a result of the bird's dietary requirement being met with less feed due to high levels of phytase.

The performance results from this experiment are different from experiment one. In the first experiment, the 21-day old broilers consuming 10,000 PU/kg phytase had body weight gains above the NRC diet. Ledoux et al. (2005) reported improved body weight gain in turkeys consuming the highest level of enzyme. Phytase supplementation also increased feed intake in experiment one and in many other experiments with phytase supplementation (Qian et al., 1997; Shirley and Edwards, 2003; Dilger et al., 2004).

In this experiment, phytase supplementation improved feed conversion, tibia ash, and phosphorus and calcium digestibility. These improvements suggest that high phytase supplementation may be beneficial in broiler diets. Shirley and Edwards (2003) and Dilger et al. (2004) reported phytase supplementation improved in feed conversion, tibia ash, and phosphorus digestibility. Silversides et al. (2004) reported improvements in calcium digestibility due to phytase supplementation. Augspurger and Baker (2004) reported high levels of dietary phytase were very efficacious for improving phytate phosphorus utilization. Shirley and Edwards (2003) suggest high levels of dietary phytase are necessary to make almost all of the phytate phosphorus in corn and soybean meal available for utilization by young broiler chicks.

High levels of a dietary phytase were beneficial in 21-day old broiler diets. Feed efficiency, tibia ash, and calcium and phosphorus digestibility were improved due to phytase supplementation, suggesting high levels of phytase are necessary to utilize all the phytate phosphorus in corn and soybean meal. This theory is supported in research by Shirley and Edwards (2003) and Augspurger and Baker (2004).

Digestibility studies are commonly used in nutrition research to evaluate mineral availability from the use of enzymes. Indigestible markers such as chromic oxide (Cr_2O_3) or titanium dioxide (TiO_2) are often added to diets in digestibility studies. Few studies exist that evaluate titanium dioxide as a digestibility marker for poultry. In this experiment, there were significant differences within each treatment between the types of marker used. However, digestibility data were highly correlated between both markers, R = 0.97 and 0.94, for phosphorus and calcium digestibility, respectively. Jagger and others (1992) reported significant differences between marker type when analyzing digestibility of nitrogen and amino acids. Numerically, titanium dioxide yielded higher digestibility values than chromic oxide. The analysis of dietary markers in ileal samples yielded lower values for chromic oxide than titanium dioxide (1.03 versus 1.17%, respectively). The recovery of a marker is an important indication of its efficacy (Jagger et al., 1992). Low recovery rates of chromic oxide can lead to lower estimates provided by chromium (Meyers et al., 2003a). There are many reports of higher recoveries of titanium dioxide than chromic oxide in pigs (Jagger et al., 1992), ewes (Meyers et al., 2003a), poultry (Sales and Janssens,

2003), and cattle (Meyers et al., 2004). The significant difference in marker type further demonstrates the need to find better and more reliable markers for use in digestibility studies.

Table 3.1. Composition and calculated analysis of the experimental diets.

Ingredients	------- (7 to 21 days) ------- NEG[1] (%)	POS (%)
Corn	59.549	63.855
Soybean Meal	21.982	21.391
Poultry By-product	5.000	5.000
Fish Meal	3.400	3.300
Corn Oil	4.767	3.325
Sand	2.500	--
Dicalcium Phosphate	0.297	1.200
Limestone	0.974	0.419
Salt	0.361	0.360
DL-Methionine	0.160	0.155
Chromic Oxide	0.300	0.300
Titanium Dioxide	0.300	0.300
L-Lysine	--	--
Trace Mineral	0.100	0.100
L-Leucine	0.083	0.066
Coban60*	0.076	0.076
Vitamin Mix	0.050	0.050
BMD+	0.042	0.042
L-Threonine	0.045	0.046
Selenium Premix	0.010	0.010
CuSO4	0.004	0.004
Nutrients:		
ME, kcal/kg	3200	3200
Crude Protein, %	21.00	21.00
Available P, %	0.25	0.42
Analyzed tP, %	0.64	0.81
Calculated Ca, %	0.85	0.85
Analyzed Ca, %	1.00	1.22

* Coccidiostat (Monensin at 0.90 g/ton diet); + Growth promotant (BMD at 50 g/ton diet).
[1] NEG = negative control diets supplemented with 0, 500, 2,500, 12,500, or 62,500 PU/kg phytase.

Table 3.2. Efficacy of high levels of dietary phytase on growth performance[1] and mortality of 21-day old male broilers[2]

Diet	Phytase (PU/kg)	Ca (%)	P (%)	FI[3] (g)	BWG (g)	F:G[3,4] (g:g)	Mortality[5] (%)
POS +	0	0.85	0.42	1,076[a]	759	1.42[a]	5.0
NEG +	0	0.85	0.25	1,052[ab]	754	1.39[ab]	3.0
NEG +	500	0.85	0.25	1,036[bc]	760	1.36[bc]	1.0
NEG +	2,500	0.85	0.25	1,048[b]	758	1.38[ab]	6.0
NEG +	12,500	0.85	0.25	1,041[bc]	784	1.33[cd]	5.0
NEG +	62,500	0.85	0.25	1,017[c]	784	1.30[d]	7.0
SEM				9	12	0.02	0.06

[1] FI = feed intake; BWG = body weight gain; F:G = feed conversion.
[2] Data are means of five replicate pens of 30 chicks each.
[3] Linear response to graded levels of a microbial phytase ($P < 0.05$).
[4] Quadratic response to graded levels of a microbial phytase ($P < 0.05$).
[5] Mortality data analyzed using the ARSIN procedure of SAS.
[a-d] Values within columns with no common superscript differ significantly ($P < 0.05$).

Table 3.3. Efficacy of high levels of dietary phytase on bone ash of 21-day old male broilers[1]

Diet	Phytase (PU/kg)	Ca (%)	P (%)	------- Toe Ash ------- (%)	(mg)[2]	------- Tibia Ash ------ (%)[2]	(mg)[2]
POS +	0	0.85	0.42	14.28	145[ab]	44.83[a]	1,251[a]
NEG +	0	0.85	0.25	13.37	132[c]	42.74[b]	1,102[b]
NEG +	500	0.85	0.25	13.90	143[ab]	44.27[a]	1,212[a]
NEG +	2,500	0.85	0.25	14.21	142[bc]	44.75[a]	1,231[a]
NEG +	12,500	0.85	0.25	13.74	147[ab]	45.03[a]	1,311[a]
NEG +	62,500	0.85	0.25	13.88	153[a]	45.12[a]	1,301[a]
SEM				0.41	3.7	0.35	37

[1] Data are means of five replicate pens of three chicks each.
[2] Linear response to graded levels of a microbial phytase ($P < 0.05$).
[a-b] Values within columns with no common superscript differ significantly ($P < 0.05$).

Table 3.4. Efficacy of high levels of dietary phytase on ileal P and Ca digestibility in 21-day old broilers[1]

Diet	Phytase (PU/kg)	Ca (%)	P (%)	$P^{2,3,4}$ (%)	$Ca^{2,3,4}$ (%)	$P^{2,3,4}$ (%)	$Ca^{2,3,4}$ (%)
				------ (Cr_2O_3) ------		------- (TiO_2) -------	
POS +	0	0.85	0.42	37.75[e]	30.31[d]	46.64[d]	40.14[c]
NEG +	0	0.85	0.25	51.75[c]	60.83[a]	62.36[b]	69.46[a]
NEG +	500	0.85	0.25	45.86[d]	35.47[cd]	55.49[c]	47.03[c]
NEG +	2,500	0.85	0.25	64.37[b]	47.19[b]	72.01[a]	58.51[b]
NEG +	12,500	0.85	0.25	70.23[a]	51.83[b]	73.45[a]	56.99[b]
NEG +	62,500	0.85	0.25	65.46[b]	43.57[bc]	75.85[a]	60.48[b]
SEM				1.5	2.9	1.7	2.9

[1] Data are means of five replicate pens of 10 chicks each.
[2] Linear response to graded levels of a microbial phytase ($P < 0.05$).
[3] Quadratic response to graded levels of a microbial phytase ($P < 0.05$).
[4] Cubic response to graded levels of a microbial phytase ($P < 0.05$).
[a-e] Values within columns with no common superscript differ significantly ($P < 0.05$).

Table 3.5. Efficacy of high levels of dietary phytase on ileal P and Ca digestibility utilizing Cr_2O_3 and TiO_2 in 21-day old broilers[1]

Diet	Phytase (PU/kg)	Ca (%)	P (%)	P (%)[2] Cr_2O_3	P (%)[2] TiO_2	P = †	Ca (%)[4] Cr_2O_3	Ca (%)[3] TiO_2	P =‡
POS +	0	0.85	0.42	37.75	46.64	0.0001	30.31	40.14	0.0001
NEG +	0	0.85	0.25	51.75	62.36	0.0001	60.83	69.46	0.0001
NEG +	500	0.85	0.25	45.86	55.49	0.0001	35.47	47.03	0.0001
NEG +	2,500	0.85	0.25	64.37	72.01	0.0001	47.19	58.51	0.0001
NEG +	12,500	0.85	0.25	70.23	73.45	0.0007	51.83	56.99	0.0001
NEG +	62,500	0.85	0.25	65.46	75.85	0.0001	43.57	60.48	0.0001
SEM				1.5	1.7		2.9	2.9	

[1] Data are means of five replicate pens of 10 chicks each.
[2] Linear response to graded levels of phytase ($P < 0.05$).
[3] Quadratic response to graded levels of phytase ($P < 0.05$).
[4] Cubic response to graded levels of phytase ($P < 0.05$).
† Probability of the difference between dietary markers.
‡ Probability of the difference between dietary markers.

CHAPTER FOUR – EXPERIMENT THREE

EVALUATION OF THE EFFICACY OF HIGH LEVELS OF A DIETARY PHYTASE ON BROILER PERFORMANCE, BONE ASH, ILEAL DIGESTIBILITY, AND LITTER PHOSPHORUS

ABSTRACT

An experiment was conducted to evaluate the efficacy of high levels of microbial phytase on broiler performance, bone ash, ileal digestibility, and litter phosphorus. Six hundred and fifty five-day old male broilers were weighed, wing-banded, and randomly assigned to dietary treatments in floor pens. Dietary treatments for the first three weeks consisted of a positive control NRC diet (POS; 0.42% npP and 0.85% Ca), a negative control basal diet (NEG; 0.25% npP and 0.85% Ca), NEG + 500 PU/kg phytase, NEG + 2,500 PU/kg phytase, NEG + 12,500 PU/kg phytase, and NEG + 62,500 PU/kg phytase. From four to six weeks, the calcium and non-phytase phosphorus (npP) levels were reduced to 0.76% and 0.35% in the POS diet, and 0.76% and 0.22% in the NEG diet. Supplemental phytase levels were identical to the starter period. Phytase addition to the negative control diet for 21 days improved growth performance. Chicks fed the three highest levels of phytase had the highest ($P < 0.05$) body weight gain (BWG) and were more ($P < 0.05$) efficient at day 21. At 42 days,

there were no differences in performance, except among the POS, NEG, and NEG + 2,500 treatments. At 21 days, phytase supplementation linearly increased ($P < 0.05$) bone ash. Bone ash was not affected by phytase supplementation at 42 days. Phosphorus digestibility at day 21 and 42 was linearly improved ($P < 0.05$) due to phytase supplementation. Calcium digestibility was cubically affected ($P < 0.05$) due to phytase supplementation. Litter phosphorus was significantly lower ($P < 0.05$) for all treatments, compared to the POS treatment. Soluble litter phosphorus and litter calcium was not affected ($P > 0.05$) by phytase supplementation. Phytase was effective in improving phytate phosphorus utilization, and this improvement occurred even at the lowest level (500 PU/kg) of supplemental phytase.
Key Words: Phytase, Broilers, Floor Pens, Litter Phosphorus

INTRODUCTION

Phytic acid is a common constituent of plant seeds and it primarily serves as a storage form of phosphorus (Tamim and Angel, 2003). Phytate is a term used synonymously with phytic acid. Phytate is a mixed cation salt of phytic acid often complexed with Ca, Mg, and K (Angel et al., 2002). Phytate binds over two-thirds of the phosphorus in plants.

Phytases are enzymes capable of breaking apart the phytate molecule. Unfortunately, nonruminant animals such as poultry lack endogenous phytase. To meet dietary phosphorus requirements, poultry diets are supplemented with

inorganic sources of phosphorus. Consequently, some of the inorganic phosphorus and most of the phytate-bound phosphorus is excreted in the manure. The high concentration of phosphorus in poultry excreta can create environmental problems such as soil saturation, soil run-off, and eutrophication.

Commercial phytases are available and are effective in improving phytate phosphorus utilization in poultry diets (Dilger et al., 2004; Ledoux et al., 2005; Wu et al., 2003). Enzyme producers are continuously developing new phytase products for improved thermo stability and efficacy. Recent research suggests bird performance continues to improve as novel dietary phytase levels increase above current industry recommendations (Augspurger and Baker, 2004; Shirley and Edwards, 2003; Ledoux et al., 2005; Wyatt et al., 2004).

The need to quantify bone mineralization has long been recognized by researchers in poultry nutrition (Garcia and Dale, 2006). The use of tibia ash is recommended as a means of evaluating bone mineralization by the AOAC (1990). Unfortunately, determination of tibia ash is labor intensive, expensive, and time consuming. Garcia and Dale (2006) hypothesized that the entire foot, with 17 individual bones, might be a better indicator of bone mineralization as opposed to toes and tibias.

Indigestible markers such as chromic oxide (Cr_2O_3) and titanium dioxide (TiO_2) are included in diets in digestibility studies to determine nutrient uptake at specific sites along the gastrointestinal tract (Short et al., 1996). Unfortunately, there are many problems associated with chromic oxide as a digestibility marker such as, incomplete recovery, variable results, and difficulty with the assay

(Sales and Janssens, 2003). Titanium dioxide is an alternative to chromic oxide as an indigestible marker. The analysis for titanium dioxide is accurate and simple, and requires a small sample size (Short et al., 1996). However, studies utilizing titanium dioxide as a dietary marker in poultry diets are rare compared to chromic oxide.

There were three objectives of this research. 1) To determine if bird performance continues to improve with increasing concentrations of dietary phytase up to 62,500 PU/kg feed in market weight broilers. 2) To compare bone ash parameters (toe, foot, and tibia ash) in 21- and 42-day old chicks. 3) To determine the efficacy of chromic oxide and titanium dioxide as ileal digestibility markers in 21- and 42-day old broiler chicks.

MATERIALS AND METHODS

In March 2006, 650 male Ross x Ross 308 broilers were purchased from a commercial hatchery and fed an NRC (1994) corn-soybean meal diet, adequate in all nutrients, for four days. On day five, 600 chicks were weighed, wing-banded, and randomly assigned to dietary treatments in floor pens (5 ft x 4 ft) in a commercial-type facility with thermostatically controlled natural gas heaters and a ventilation fan. Each pen was covered with approximately two inches of pine shavings. Feed and water were provided *ad libitum*. Chicks were reared for the first ten days on a 23 hour light and one hour dark regime. Thereafter the lighting regime consisted of 16 hours of light and eight hours of dark. The animal care

and use protocol was reviewed and approved by the University of Missouri Animal Care and Use Committee.

Five replicate pens of 20 chicks were randomly assigned to one of six dietary treatments from day five to 21, and 10 chicks from day 22 to 42. Dietary treatments consisted of a positive control diet (POS; 0.85% Ca and 0.42% npP), a negative control diet (NEG; 0.85% Ca and 0.25% npP), NEG + 500 PU/kg, NEG + 2,500 PU/kg, NEG + 12,500 PU/kg, and NEG + 62,500 PU/kg. The phytase used was Quantum 2500D, a bacterial six-phytase expressed in the *Pichia pastoris* fungus. All birds received diets in crumbled form from day one to 21 and pelleted form from day 22 to 42. A coccidiostat (Monensin) was added to the diet at 90 g/ton. A growth promotant (BMD) was also added to the diet at 50 g/ton. The dietary markers chromic oxide and titanium dioxide were added to each diet at 0.1%. Dietary treatments are presented in Table 4.1. Phytase analysis revealed phytase levels were adequate for this experiment (Tables 4.2 and 4.3).

The birds were monitored daily for morbidity and mortality, and any birds removed were weighed, and the feed intakes and the feed conversion were adjusted according to the number of bird days. Body weights were measured on a pen basis on days five, 21, and 42. Feed intake was also determined on days 21 and 42, and feed conversion calculated.

On day 21, 50 birds (five replicates of 10 birds each) from each treatment were euthanized by lethal injection with 4% sodium pentobarbital, after which the body cavity was opened and digesta samples were collected, pooled, and

processed from the lower half of the ileum in the same manner as experiment one and two. Right legs of 15 birds per treatment (five replicates of three birds each) were removed along with all four toes. Right tibias were used for determination of tibia ash, and all four toes were used to determine toe ash similarly to experiments one and two. The left foot of the same 15 birds per treatment was removed at the tibiotarsal junction for determination of foot ash. Feet were dried for 48 hours at 100°C and then ashed for 24 hours in a muffle furnace at 600°C (Garcia and Dale, 2006).

On day 42, five birds from each pen were euthanized by lethal injection with 4% sodium pentobarbital and digesta samples were collected from the lower half of the ileum as previously described. On the right leg all four toes and tibia were taken from 15 birds per treatment for bone ash determination. Left feet on the same 15 birds (from the tibiotarsal junction) were also collected for determination of foot ash. Litter samples were collected from four sections of each pen, pooled, weighed for dry matter determination, and dried in a forced air oven at 55°C for 72 hours.

Feed, litter, and ileal digesta were processed similarly to experiments one and two for determination of total phosphorus, calcium, titanium dioxide, and chromic oxide. Litter samples were also processed similarly to experiment one for soluble phosphorus. The data were analyzed as a completely random design (CRD), with six treatments as described previously, using the General Linear Models (GLM) procedure of SAS (SAS Institute, 2005). Mean differences were determined using Fisher's Least Significant Difference (LSD). Linear, quadratic,

and cubic orthogonal polynomial contrasts were performed on treatments NEG, NEG + 500, NEG + 2,500, NEG + 12,500, and NEG + 62.500. Statistical significance was accepted at $P < 0.05$.

RESULTS

PERFORMANCE

Performance results for days five to 21 are presented in Table 4.2. At 21 days, feed intake was significantly higher ($P < 0.05$) in birds consuming 2,500 PU/kg diet compared to birds consuming 62,500 PU/kg diet. Feed intake (FI) was not statistically different ($P > 0.05$) among any other treatments. Phytase supplementation improved ($P < 0.05$) body weight gain (BWG) linearly and cubically. Body weight gain was significantly lower ($P < 0.05$) in the POS birds compared to the NEG + 2,500, NEG + 12,500, and NEG + 62,500 birds. Body weight gain was not statistically different ($P > 0.05$) among the POS, NEG, and NEG + 500 birds. There were no differences ($P > 0.05$) in BWG among the NEG, NEG + 500, and NEG + 62,500 birds. There were no significant differences ($P > 0.05$) in BWG among the three highest levels of phytase (2,500, 12,500, and 62,500 PU/kg).

Phytase supplementation linearly and quadratically improved ($P < 0.05$) feed conversion (F:G). Feed to gain was improved significantly ($P < 0.05$) in treatments containing the three highest levels of phytase, compared to the POS treatment. Feed conversion in POS was significantly higher ($P < 0.05$) than any

other treatment, except the NEG + 500 treatment. There were no significant differences ($P > 0.05$) in feed conversion between the NEG and NEG + 500 treatments, or the NEG and NEG + 2,500 treatments. Mortality in the NEG + 500 treatment was significantly lower ($P < 0.05$) than mortality in the NEG + 12,500 treatment. However, mortality for the first 21 days was low and no significant differences ($P > 0.05$) were observed among other treatments.

Performance results for days five to 42 are presented in Table 4.3. Feed intake was significantly higher ($P < 0.05$) in the NEG birds compared to the POS and NEG + 62,500 birds. There were no significant differences ($P > 0.05$) in feed intake among the POS, NEG + 500, NEG + 12,500, and NEG + 62,500 birds. There were no significant differences ($P > 0.05$) in feed intake among the NEG, NEG + 500, NEG + 2,500, and NEG + 12,500 birds. Body weight gain was significantly higher ($P < 0.05$) in the NEG and NEG + 2,500 birds compared to the POS birds. However, the POS birds were not statistically different ($P > 0.05$) from the NEG + 500, NEG + 12,500, or NEG + 62,500 birds. There were no differences ($P > 0.05$) in BWG among the NEG, NEG + 500, NEG + 2,500, NEG + 12,500, or NEG + 62,500 birds. Feed conversion was not statistically different ($P > 0.05$) among treatments. Mortality was significantly lower ($P < 0.05$) for the NEG + 500 treatment compared to the NEG + 12,500 treatment. However, mortality for 42 days was low and there were no significant differences ($P > 0.05$) among other treatments.

BONE ASH

Bone ash results for day 21 are presented in Table 4.4. Phytase supplementation linearly improved ($P < 0.05$) toe ash percent and weight (mg). Toe ash percent was significantly lower ($P < 0.05$) in the NEG treatment compared to the POS treatments and the treatments with the two highest levels of phytase (NEG + 12,500 and NEG + 62,500). Toe ash weight was significantly lower ($P < 0.05$) in the NEG treatment compared to the POS and NEG + 12,500 treatments. There were no significant differences ($P > 0.05$) in percent toe ash among the NEG, NEG + 500, and NEG + 2,500 treatments. There were no significant differences ($P > 0.05$) in toe ash weight among the NEG, NEG + 500, NEG + 2,500, and NEG + 62,500 treatments.

Phytase supplementation linearly improved ($P < 0.05$) tibia ash percent. Tibia ash percent was significantly lower ($P < 0.05$) for the NEG treatment compared to all other treatments. Phytase supplementation of 500 and 2,500 PU/kg diet significantly improved ($P < 0.05$) tibia ash percent above the NEG treatment, and this improvement was not statistically different ($P > 0.05$) from the POS treatment. Phytase supplementation at 12,500 PU/kg diet significantly improved ($P < 0.05$) tibia ash percent above the POS, NEG, NEG + 500, and NEG + 2,500 treatments. Tibia ash percent was significantly higher ($P < 0.05$) in the NEG + 62,500 treatment compared to the NEG, NEG + 500, and NEG + 2,500 treatments. There was no significant difference ($P > 0.05$) in tibia ash percent between the two highest levels of phytase (12,500 and 62,500 PU/kg diet).

Phytase supplementation linearly and cubically improved ($P < 0.05$) tibia ash weight (mg). Tibia ash weight was significantly lower ($P < 0.05$) in the NEG treatment compared to the NEG + 2,500 and NEG + 12,500 treatments. There were no differences ($P > 0.05$) in tibia ash weight among the POS, NEG, NEG + 500, and NEG + 62,500 treatments. Tibia ash weight was significantly higher ($P < 0.05$) in the NEG + 12,500 treatment compared to the NEG, NEG + 500, and NEG + 62,500 treatments. The positive control treatment was not statistically different ($P > 0.05$) from any other treatment.

Phytase supplementation linearly improved ($P < 0.05$) foot ash percent. Foot ash percent for the NEG treatment was significantly lower ($P < 0.05$) than the POS and NEG + 62,500 treatments. There were no significant differences ($P > 0.05$) in foot ash percent among the NEG, NEG + 500, NEG + 2,500, or NEG + 12,500 treatments. There were no significant differences ($P > 0.05$) in foot ash percent among the POS, NEG + 12,500, or NEG + 62,500 treatments. Foot ash weight (mg) was significantly lower ($P < 0.05$) in the NEG treatment compared to the NEG + 2,500 and NEG + 12,500 treatments. There were no significant differences ($P > 0.05$) in foot ash weight among other treatments.

Bone ash results for day 42 are presented in Table 4.5. Toe ash percent was significantly lower ($P < 0.05$) in the NEG + 12,500 treatment compared to the POS treatment. There were no significant differences ($P > 0.05$) in toe ash percent among other treatments. Toe ash weight (mg) was significantly lower ($P < 0.05$) in the NEG + 500 treatment compared to the NEG and NEG + 62,500 treatments. There were no significant differences ($P > 0.05$) in toe ash weight

among other treatments. Phytase supplementation did not significantly affect ($P > 0.05$) tibia ash percent or weight. Foot ash percent was significantly lower ($P < 0.05$) for the NEG + 12,500 treatment compared to the POS and NEG + 62,500 treatments. There were no significant differences ($P > 0.05$) in foot ash percent among other treatments. Foot ash weight was significantly lower ($P < 0.05$) in the POS, NEG + 500, and NEG + 12,500 treatments compared to the NEG treatment. There were no significant differences ($P > 0.05$) in foot ash weight among the NEG, NEG + 2,500, and NEG + 62,500 treatments.

Relationships between toe and foot, toe and tibia, and foot and tibia ash for day 21 are presented in Table 4.6. There was a high degree of relationship between percent toe ash and percent foot ash ($R^2 = 0.76$), mg toe ash and mg foot ash ($R^2 = 0.84$), mg toe ash and mg tibia ash ($R^2 = 0.72$), mg foot ash and mg tibia ash ($R^2 = 0.85$), and mg tibia ash and percent tibia ash ($R^2 = 0.40$). There were relationships between percent toe ash, mg toe ash, mg foot ash, percent tibia ash, and mg tibia ash. However, the coefficient of determination (R^2) values were lower than 0.40.

Relationships between toe and foot, toe and tibia, and foot and tibia ash for day 42 are presented in Table 4.7. There was a high degree of relationship between percent toe ash and percent foot ash ($R^2 = 0.74$), mg toe ash and mg foot ash ($R^2 = 0.77$), mg toe and mg tibia ash ($R^2 = 0.57$), and mg foot ash and mg tibia ash ($R^2 = 0.63$). There were relationships between percent toe ash and mg toe ash and percent foot ash and mg foot ash. However, the coefficient of

determination (R^2) values were lower than 0.40. Percent tibia ash demonstrated no relationship with any other bone ash response variable.

ILEAL DIGESTIBILITY – CHROMIC OXIDE (Cr_2O_3)

Phosphorus and calcium digestibility utilizing chromic oxide (Cr_2O_3) are presented in Table 4.8. Phytase supplementation linearly improved ($P < 0.05$) phosphorus digestibility at day 21. Phosphorus digestibility was significantly lower ($P < 0.05$) in the NEG and NEG + 500 treatments compared to the POS and NEG + 62,500 treatments. There were no significant differences ($P > 0.05$) in phosphorus digestibility among the NEG, NEG + 500, NEG + 2,500, or NEG + 12,500 treatments. There were no significant differences ($P > 0.05$) in phosphorus digestibility among the POS treatment and the treatments with the three highest levels of supplemental phytase (2,500, 12,500, and 62,500 PU/kg diet).

Phytase supplementation cubically affected ($P < 0.05$) calcium digestibility at day 21. Calcium digestibility was significantly higher ($P < 0.05$) in the NEG + 500 treatment compared to the NEG + 2,500 and NEG + 12,500 treatments. There were no differences ($P > 0.05$) in calcium digestibility among the NEG, NEG + 2,500, NEG + 12,500, or NEG + 62,500 treatments. There were no other significant differences ($P > 0.05$) in calcium digestibility at day 21 among any other treatments.

At day 42, phytase supplementation linearly and quadratically improved ($P < 0.05$) phosphorus digestibility. Phosphorus digestibility in the NEG treatment was significantly lower ($P < 0.05$) than phosphorus digestibility in the treatments

containing the two highest levels of supplemental phytase (12,500 and 62,500 PU/kg diet). Phosphorus digestibility in the NEG + 62,500 treatment was significantly higher ($P < 0.05$) than all other treatments, except the NEG + 12,500 treatment. There were no significant differences ($P > 0.05$) in phosphorus digestibility among the POS, NEG, NEG + 500, or NEG + 2,500 treatments. Phosphorus digestibility was similar ($P > 0.05$) among the POS, NEG + 500, and NEG + 12,500 treatments. Phytase supplementation cubically affected ($P < 0.05$) calcium digestibility at day 42. Calcium digestibility was significantly lower ($P < 0.05$) in the NEG + 2,500 treatment compared to all other treatments, except the NEG + 12,500 treatment. Calcium digestibility in the POS, NEG, and NEG + 62,500 treatments was significantly higher ($P < 0.05$) than the NEG + 2,500 and NEG + 12,500 treatments. There was no significant difference ($P > 0.05$) in calcium digestibility between the NEG + 500 and NEG + 12,500 treatments.

ILEAL DIGESTIBILITY – TITANIUM DIOXIDE (TiO_2)

Calcium and phosphorus digestibility utilizing titanium dioxide (TiO_2) as a dietary marker are presented in Table 4.9. Phytase supplementation linearly improved ($P < 0.05$) phosphorus digestibility at day 21. Phosphorus digestibility was significantly lower ($P < 0.05$) in the NEG treatment compared to all other treatments, except the NEG + 500 treatment. There were no significant differences ($P > 0.05$) in phosphorus digestibility among other treatments.

Phytase supplementation quadratically affected ($P < 0.05$) calcium digestibility at day 21. Calcium digestibility was higher ($P < 0.05$) in the NEG + 500 treatment compared to all other treatments, except the POS treatment.

There were no differences (P > 0.05) in calcium digestibility between the POS and NEG + 12,500 treatments. There were no differences (P > 0.05) in calcium digestibility among the NEG, NEG + 2,500, NEG + 12,500, or NEG + 62,500 treatments.

At day 42, phytase supplementation linearly improved (P < 0.05) phosphorus digestibility. Phosphorus digestibility in the NEG treatment was significantly lower (P < 0.05) than all other treatments. Phosphorus digestibility in treatments containing the two highest levels of phytase (12,500 and 62,500 PU/kg diet) was higher (P < 0.05) than phosphorus digestibility in the POS treatment. There were no significant differences (P > 0.05) in phosphorus digestibility among the POS, NEG + 500, and NEG + 2,500 treatments. Phosphorus digestibility in the NEG + 62,500 treatment was statistically similar (P > 0.05) to phosphorus digestibility in the NEG + 500 treatment. Calcium digestibility was lower (P < 0.05) in the NEG treatment compared to the NEG + 500 and NEG + 12,500 treatments. There were no differences (P > 0.05) in calcium digestibility among other treatments.

LITTER PHOSPHORUS AND CALCIUM

Total litter phosphorus (TP), soluble litter phosphorus (SP), and litter calcium are presented in Table 4.12. Total litter phosphorus was significantly higher (P < 0.05) in the POS treatment compared to all other treatments. There was no significant difference (P > 0.05) in total litter phosphorus among any other treatment.

Soluble litter phosphorus was linearly, quadratically, and cubically affected ($P < 0.05$) by phytase supplementation. Soluble litter phosphorus was significantly lower ($P < 0.05$) in the NEG treatment compared to all other treatments. Soluble litter phosphorus in the NEG + 500 treatment was significantly lower ($P < 0.05$) than all other treatments, except the negative control. Soluble litter phosphorus in the NEG + 2,500 treatment was significantly lower ($P < 0.05$) than all other treatments, except the NEG and NEG + 500 treatments. There was no difference ($P > 0.05$) in soluble litter phosphorus among the POS, NEG + 12,500, and NEG + 62,500 treatments. Litter calcium was not affected ($P > 0.05$) by phytase supplementation.

DISCUSSION

Microbial phytases have been in practical use for over a decade, and their acceptance in the poultry industry, as a means of reducing manure phosphorus, continues to grow (Ravindran et al., 2006). Dietary phytase at inclusion levels above industry recommendations are reported to improve bird performance above that of control birds (Ledoux et al., 2005). In this experiment, there were no differences in body weight gain at market weight between the positive control diet and the highest levels of phytase. These results are similar to both experiment one and experiment two. Experiment one reported improved BWG at 21 days, but BWG was similar to the positive control at 42 days. At 21 days in the current experiment, BWG was significantly higher in the treatments

containing the three highest levels of phytase compared to the positive diet. However, this improvement in BWG was not sustained to 42 days.

At 21 days, phytase supplementation linearly and quadratically improved feed efficiency. However, feed efficiency was not affected by phytase supplementation at 42 days. Wu et al. (2004a) reported improved feed efficiency in market weight broilers due to phytase supplementation. In contrast, Viveros et al. (2002) reported phytase supplementation improved weight gain but had no effect feed efficiency in broilers raised to market weight.

The negative control diet without phytase supplementation had performance data similar, if not better, than the positive control diet and some treatment diets. These results were not expected and could be a result of higher than expected calcium and phosphorus levels in the diet. Research suggests that bird growth rate influences the non-phytate phosphorus (npP) requirement of poultry (Persia and Saylor, 2006). Modern broilers grow faster and heavier than broilers from 10 to 20 years ago. The Ross 308 broiler is used for multipurpose production and has a fast growth rate (Persia and Saylor, 2006). Because of differences in growth rate, it is important to know if the npP requirement is affected by genetic strain (Persia and Saylor, 2006). Persia and Saylor (2006) determined the npP requirements for body weight gain, feed intake, and tibia ash percent in eight- to 22-day old Ross 308 broilers were 0.32 to 0.35, 0.33 to 0.39, and 0.35 to 0.39%, respectively. The calculated total phosphorus levels the Persia and Saylor (2006) experiment were approximately 0.61%. In our experiment, the negative control diet without phytase supplementation contained

0.66% total phosphorus from five to 21 days and 0.61% total P from 22 to 42 days. The total phosphorus levels in our experiment are similar to the calculated total phosphorus requirement for body weight gain, feed intake, and tibia ash in Ross 308 broilers (Persia and Saylor, 2006).

The lower BWG of birds consuming the positive control diet was also not expected. However, it is important to note that all the birds in this experiment performed exceptionally well, with an average body weight of approximately 2,969 grams (6.5 lbs). According to the NRC (1994), six week male broilers weigh approximately 2,088 grams. And according to NASS, as of May 2006, young chickens slaughtered averaged 5.49 pounds per bird. It is also important to remember only phosphorus levels were reduced in the negative control basal diet. Calcium levels were similar among all treatments.

Bone ash data at 21 days suggests phytase was effective in improving bone mineralization. These results are similar to the bone ash data from experiments one and two. Dilger et al. (2004) and Sebastian et al. (1996b) reported improvements in bone mineralization due to phytase supplementation. Phytase supplementation at 42 days had no effect on bone ash. The lack of effect was most likely due to an increase in body weight of the negative control birds compared to the positive control birds.

Phytase supplementation linearly improved phosphorus digestibility and significantly reduced total litter phosphorus. Rutherford et al. (2002), Wu et al. (2003), and Ravindran et al. (2006) reported improvements in phosphorus digestibility due to phytase supplementation. Wu et al. (2004a), Yi et al. (1996b),

Simons et al. (1990), and Ledoux et al. (2005) reported a decrease in phosphorus excretion due to phytase supplementation. High levels of dietary phytase did not increase soluble litter phosphorus above the positive control. These results are similar to soluble litter phosphorus results from experiment one, Maguire et al. (2004) and Applegate et al. (2003).

Phytase supplementation did not greatly improve calcium digestibility. In fact, there were no differences in calcium digestibility or calcium digestibility was lower in the treatment diets containing phytase compared to the positive control. This data is similar to 42 day calcium digestibility data in experiment one. In experiment one, calcium digestibility linearly decreased as phytase supplementation increased, and there was no difference in calcium digestibility between the NRC diet and the diet with the highest level of phytase (20,000 PU/kg diet). The variable calcium digestibility in this experiment may be a result of the efficacy of high levels of phytase, the high levels of calcium in the diet, or an interaction between calcium and phosphorus. Dilger et al. (2004) reported phytase supplementation of broiler diets improved growth rate, but had no effect on calcium digestibility. However, other researchers have reported that phytase supplementation improves calcium digestibility (Silversides et al., 2004; Driver et al., 2005).

Litter calcium was not affected by phytase supplementation. These results are different than experiment one. In experiment one, litter calcium decreased linearly due to phytase supplementation. Other researchers have also reported a decrease in calcium excreted due to phytase supplementation

(Cowieson et al., 2004a). When comparing the results of experiments one and three it is important to remember the calcium and phosphorus levels within each treatment. In experiment one, the calcium and phosphorus levels of the diets supplemented with phytase were reduced by 0.15 to 0.25% compared to the positive control diet. In this experiment only the phosphorus levels were reduced, calcium levels remained the same throughout the treatments.

Bird response to supplemental phytase depends on the concentrations of calcium and phosphorus in the diet (Driver et al., 2005). The lack of significant response in performance, bone ash, and calcium digestibility between the positive control and negative control diet may be a result of the high calcium level in the diet. Sebastian et al. (1996a) reported that the optimum level of bone ash was observed in birds fed diets containing 0.60% calcium and supplemental phytase. Calcium levels greater than 0.75% were found to decrease bird performance when dietary phytase was added (Perney et al., 1993). Extremely high levels of dietary phytase could increase the negative effects of calcium on bird performance. Tamim et al. (2004) reported phytase efficacy was affected by calcium level in the diet. However, Driver et al. (2005) reported higher calcium levels (0.86%) and lower phosphorus levels (0.20%) elicit a greater response due to phytase supplementation. In this case, the combination of calcium and phosphorus in this experiment was too high to elicit a great response from added phytase.

Dale and Garcia (2004) and Garcia and Dale (2006) reported a high degree of agreement between tibia and foot ash data. Foot ash provides a larger

sample size than toe ash and may reduce the time required to obtain results. Preliminary results show foot ash to have great promise as a rapid means of monitoring bone development in broiler chickens (Mendez and Dale, 1998). Yan et al. (2005) reported high relationships between percent foot ash and percent tibia ash (R^2 = 0.92), and percent toe ash and percent tibia ash (R^2 = 0.88).

In this experiment, we reported relationships between percent toe ash and percent foot ash (R^2 = 0.74 and 0.76), toe ash weight and foot ash weight (R^2 = 0.77 and 0.84), foot ash weight and tibia ash weight (R^2 = 0.85), and toe ash weight and tibia ash weight (R^2 = 0.72). There were other significant relationships but the coefficient of determination (R^2) value was below 0.70. The lack of relationship between tibia ash and the other bone ash parameters at day 42 may be a result of a lack of response of the tibia to phytase supplementation.

Digestibility studies are commonly used in nutrition research to evaluate mineral availability from the use of enzymes. Indigestible markers such as chromic oxide (Cr_2O_3) or titanium dioxide (TiO_2) are often added to diets in digestibility studies. Despite the popularity of chromic oxide, there are several problems associated with the use of this dietary marker. Chromic oxide is not approved by the Food and Drug Administration as a dietary additive, and may be considered a health hazard when inhaled (Titgemeyer, 1997). Researchers also report low recovery rates associated with chromic oxide when used in studies with poultry and ruminants (Sales and Janssens, 2003; Titgemeyer, 1997). It is also difficult to get repeatability between laboratories when chromic oxide is used

as a dietary marker, namely due to the assay for chromic oxide (Sales and Janssens, 2003).

Titanium dioxide (TiO_2) has recently been evaluated as an alternative marker for digestibility studies in ruminants and poultry. Titanium dioxide has many benefits over the use of chromic oxide. Titanium dioxide does not have the carcinogenic properties associated with chromic oxide, and is approved for use as a feed additive by the Food and Drug Administration. Plus titanium dioxide is reported to be a reliable digestibility marker in studies utilizing beef steers (Titgemeyer et al., 2001), poultry (Short et al., 1996), and ewes (Meyers et al., 2003a).

In this experiment, there were significant differences within some of the treatments between the types of marker used. However, there was a high correlation among phosphorus and calcium digestibility values and the marker used (R = 0.92 and 0.85 for 21 and 42 day phosphorus digestibility, respectively; R = 0.86 and 0.41 for 21 and 42 day calcium digestibility, respectively). Jagger and others (1992) reported significant differences between marker type when analyzing digestibility of nitrogen and amino acids. Ruminal dry matter digestibilities in ewes were not different between titanium dioxide and chromic oxide in one experiment, however in a second experiment with ewes, ruminal dry matter digestibilities were higher for chromic oxide than titanium dioxide (Meyers et al., 2003a). The results from the second Meyers et al. (2003a) experiment are similar to experiment two (Chapter 3). However, in our second experiment, there were significant differences within each treatment between the type of marker

used. Also in the second experiment, titanium dioxide yielded higher values than chromic oxide however, the relationship between the two markers was highly correlated. In our third experiment (Chapter 4), neither marker yielded consistently higher values. The numeric values were also more closely related in this experiment compared to experiment two. The better matched results from this experiment in comparison to experiment two may be a result of better assay practices. This experiment along with experiment two, demonstrate the need to find a consistently reliable digestibility marker and assay.

Table 4.1. Composition and calculated analysis of experimental diets

Ingredients	------(5 to 21 days)------ NEG[1] (%)	POS (%)	-----(22 to 42 days)----- NEG[1] (%)	POS (%)
Corn	60.325	64.715	68.130	72.723
Soybean Meal	21.903	21.173	17.079	16.241
Poultry By – Product	5.000	5.000	5.000	5.000
Fish Meal	3.360	3.360	--	--
Corn Oil	4.506	3.040	4.444	2.922
Sand	2.500	--	2.500	--
Limestone	0.938	0.379	0.946	0.521
Salt	0.362	0.359	0.287	0.285
Dicalcium Phosphate	0.300	1.190	0.677	1.355
DL-Methionine	0.159	0.153	0.197	0.194
L-Lysine	--	--	0.143	0.159
Trace Mineral	0.100	0.100	0.100	0.100
Chromic Oxide	0.100	0.100	0.100	0.100
Titanium Oxide	0.100	0.100	0.100	0.100
Coban60*	0.076	0.076	0.076	0.076
L-Leucine	0.079	0.063	--	--
Selenium Premix	0.050	0.050	0.050	0.050
Vitamin Premix	0.050	0.050	0.050	0.050
L-Threonine	0.045	0.046	0.076	0.078
BMD[+]	0.042	0.042	0.042	0.042
CuSO4	0.004	0.004	0.004	0.004
Nutrients:				
ME, kcal/kg	3,200	3,200	3,250	3,250
Crude Protein, %	21.00	21.00	17.40	17.40
Available P, %	0.25	0.42	0.22	0.35
Analyzed tP, %	0.66	0.80	0.61	0.72
Calculated Ca, %	0.85	0.85	0.76	0.76
Analyzed Ca, %	0.95	0.97	0.93	0.89

[1] NEG = negative control diet supplemented with 0, 500, 2,500, 12,500, or 62,500 PU/kg diet.
* Coban 60 supplies Monensin at 90 g/ton; [+] BMD = bacitracin methylene disalicylate at 50 g/ton.

Table 4.2. Efficacy of high levels of dietary phytase on growth performance[1] and mortality of 21-day old male broilers[2]

Diet	Phytase[3] (PU/kg)	Phytase[4] (PU/kg)	Ca (%)	P (%)	FI (g)	BWG[5,7] (g)	F:G[5,6] (g:g)	Mortality[8] (%)
POS +	0	0	0.85	0.42	1,049^{ab}	785^{c}	1.34^{a}	4.0^{ab}
NEG +	0	0	0.85	0.25	1,048^{ab}	808^{bc}	1.29^{bc}	4.0^{ab}
NEG +	500	547	0.85	0.25	1,051^{ab}	808^{bc}	1.30^{ab}	1.0^{b}
NEG +	2,500	2,943	0.85	0.25	1,086^{a}	861^{a}	1.26^{cd}	4.0^{ab}
NEG +	12,500	13,173	0.85	0.25	1,065^{ab}	852^{a}	1.25^{d}	6.0^{a}
NEG +	62,500	62,426	0.85	0.25	1,034^{b}	840^{ab}	1.23^{d}	4.0^{ab}
SEM					14	12	0.07	0.06

[1] FI = feed intake; BWG = body weight gain; F:G = feed conversion.
[2] Data are means of five replicate pens of 20 chicks each.
[3] Calculated phytase values.
[4] Analyzed phytase values.
[5] Linear response to graded levels of phytase supplementation ($P < 0.05$).
[6] Quadratic response to graded levels of phytase supplementation ($P < 0.05$).
[7] Cubic response to graded levels of phytase supplementation ($P < 0.05$).
[8] Mortality data analyzed using the ARSIN procedure of SAS.
[a-d] Values within columns with no common superscript differ significantly ($P < 0.05$).

Table 4.3. Efficacy of high levels of dietary phytase on growth performance[1] and mortality of 42-day old male broilers[2]

Diet	Phytase[3] (PU/kg)	Phytase[4] (PU/kg)	Ca[5] (%)	P[6] (%)	FI (g)	BWG (g)	F:G (g:g)	Mortality[7] (%)
POS +	0	0	0.76	0.35	4,604[b]	2,879[b]	1.60	6.0[ab]
NEG +	0	0	0.76	0.22	4,819[a]	3,017[a]	1.60	6.0[ab]
NEG +	500	699	0.76	0.22	4,718[ab]	2,958[ab]	1.60	3.0[b]
NEG +	2,500	2,689	0.76	0.22	4,838[a]	3,024[a]	1.60	6.0[ab]
NEG +	12,500	13,847	0.76	0.22	4,779[ab]	2,990[ab]	1.60	12.0[a]
NEG +	62,500	56,872	0.76	0.22	4,594[b]	2,928[ab]	1.57	4.0[ab]
SEM					69	41	0.02	0.06

[1] FI = feed intake; BWG = body weight gain; F:G = feed conversion.
[2] Data are means of five replicate pens of 20 chicks each (days 0 to 21) and 10 chicks each (days 22 to 42).
[3] Calculated phytase values.
[4] Analyzed phytase values.
[5] Dietary calcium levels from days 0 to 21 were: 0.85, 0.85, 0.85, 0.85, 0.85, and 0.85%, respectively.
[6] Dietary phosphorus levels from days 0 to 21 were: 0.42, 0.25, 0.25, 0.25, 0.25, and 0.25%, respectively.
[7] Mortality data analyzed using the ARSIN procedure of SAS.
[a-b] Values within columns with no common superscript differ significantly ($P < 0.05$).

Table 4.4. Efficacy of high levels of dietary phytase on bone ash of 21-day old male broilers[1]

Diet	Phytase (PU/kg)	Ca (%)	P (%)	Toe Ash (%)[2]	Toe Ash (mg)[2]	Tibia Ash (%)[2]	Tibia Ash (mg)[2,3]	Foot Ash (%)[2]	Foot Ash (mg)
POS +	0	0.85	0.42	13.24[a]	156[a]	44.27[bc]	1,185[abc]	16.81[a]	1,091[ab]
NEG +	0	0.85	0.25	12.18[b]	143[b]	43.20[d]	1,119[c]	15.51[c]	1,029[b]
NEG +	500	0.85	0.25	12.84[ab]	152[ab]	43.84[c]	1,134[bc]	15.90[bc]	1,044[ab]
NEG +	2,500	0.85	0.25	12.86[ab]	153[ab]	44.21[c]	1,190[ab]	15.69[bc]	1,107[a]
NEG +	12,500	0.85	0.25	13.44[a]	158[a]	45.00[a]	1,237[a]	16.13[abc]	1,105[a]
NEG +	62,500	0.85	0.25	13.24[a]	153[ab]	44.80[ab]	1,164[bc]	16.45[ab]	1,081[ab]
SEM				0.29	4.5	0.20	24	0.29	26

[1] Data are means of five replicate pens of three chicks each.
[2] Linear response to graded levels of phytase ($P < 0.05$).
[3] Cubic response to graded levels of phytase ($P < 0.05$).
[a-d] Values within columns with no common superscript differ significantly ($P < 0.05$).

Table 4.5. Efficacy of high levels of dietary phytase on bone ash of 42-day old male broilers[1]

Diet	Phytase (PU/kg)	Ca[2] (%)	P[3] (%)	Toe Ash (%)	Toe Ash (mg)[5]	Tibia Ash (%)	Tibia Ash (mg)	Foot Ash (%)	Foot Ash (mg)
POS +	0	0.76	0.35	12.60[a]	454[ab]	44.90	4,003	14.89[a]	3,148[b]
NEG +	0	0.76	0.22	11.83[ab]	482[a]	44.83	4,009	14.38[ab]	3,417[a]
NEG +	500	0.76	0.22	12.06[ab]	434[b]	44.80	3,730	14.43[ab]	3,060[b]
NEG +	2,500	0.76	0.22	11.88[ab]	467[ab]	44.87	4,046	14.43[ab]	3,258[ab]
NEG +	12,500	0.76	0.22	11.81[b]	460[ab]	44.08	4,014	13.85[b]	3,153[b]
NEG +	62,500	0.76	0.22	12.04[ab]	474[a]	44.07	4,011	14.67[a]	3,274[ab]
SEM				0.27	12	0.43	157	0.25	86

[1] Data are means of five replicate pens of three chicks each.
[2] Dietary calcium levels from days 0 to 21 were: 0.85, 0.85, 0.85, 0.85, 0.85, and 0.85%, respectively.
[3] Dietary phosphorus levels from days 0 to 21 were: 0.42, 0.25, 0.25, 0.25, 0.25, and 0.25%, respectively.
[4] Linear response to graded levels of phytase ($P < 0.05$).
[5] Quadratic response to graded levels of phytase ($P < 0.05$).
[6] Cubic response to graded levels of phytase ($P < 0.05$).
[a-b] Values within columns with no common superscript differ significantly ($P < 0.05$).

Table 4.6. Relationship (R^2) between toe, foot, and tibia ash in 21-day old broilers[1]

	Toe Ash (%)	Toe Ash (mg)	Foot Ash (%)	Foot Ash (mg)	Tibia Ash (%)	Tibia Ash (mg)
Toe Ash (%)	1.00	0.23*	0.76****	0.21*	0.39***	0.21*
Toe Ash (mg)	0.23*	1.00	0.25*	0.84****	0.23*	0.72****
Foot Ash (%)	0.76****	0.25*	1.00	0.32**	0.21*	0.24*
Foot Ash (mg)	0.21*	0.84****	0.32**	1.00	0.26*	0.85****
Tibia Ash (%)	0.39***	0.23*	0.21*	0.26*	1.00	0.40****
Tibia Ash (mg)	0.21*	0.72****	0.24*	0.85****	0.40****	1.00

* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$
**** $P < 0.0001$

Table 4.7. Relationship (R^2) between toe, foot, and tibia ash in 42-day old broilers

	Toe Ash (%)	Toe Ash (mg)	Foot Ash (%)	Foot Ash (mg)	Tibia Ash (%)	Tibia Ash (mg)
Toe Ash (%)	1.00	0.22*	0.74****	0.11	0.03	0.08
Toe Ash (mg)	0.22*	1.00	0.18	0.77****	-0.08	0.57****
Foot Ash (%)	0.74****	0.18	1.00	0.36***	0.12	0.11
Foot Ash (mg)	0.11	0.77****	0.36***	1.00	0.08	0.63****
Tibia Ash (%)	0.03	-0.08	0.12	0.08	1.00	0.18
Tibia Ash (mg)	0.08	0.57****	0.11	0.63****	0.18	1.00

* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$
**** $P < 0.0001$

Table 4.8. Efficacy of high levels of dietary phytase on ileal P and Ca digestibility utilizing Cr_2O_3 in 21- and 42-day old broilers[1]

Diet	Phytase (PU/kg)	Ca[2] (%)	P[3] (%)	P[4] (%)	Ca[6] (%)	P[4,5] (%)	Ca[6] (%)
				------ (day 21) ------		------ (day 42) ------	
POS +	0	0.76	0.35	69.24[a]	45.30[ab]	54.26[bc]	38.08[a]
NEG +	0	0.76	0.22	52.75[b]	39.22[abc]	49.13[c]	37.45[a]
NEG +	500	0.76	0.22	54.31[b]	46.92[a]	54.17[bc]	37.17[ab]
NEG +	2,500	0.76	0.22	59.11[ab]	31.90[c]	52.64[c]	27.72[c]
NEG +	12,500	0.76	0.22	59.15[ab]	36.39[bc]	59.47[ab]	30.26[bc]
NEG +	62,500	0.76	0.22	69.95[a]	38.56[abc]	64.23[a]	37.77[a]
SEM				3.7	3.5	1.9	2.5

[1] Data are means of five replicate pens of 10 chicks each (day 21) and five chicks each (day 42).
[2] Dietary calcium levels from days 0 to 21 were: 0.85, 0.85, 0.85, 0.85, 0.85, and 0.85%, respectively.
[3] Dietary phosphorus levels from days 0 to 21 were: 0.42, 0.25, 0.25, 0.25, 0.25, and 0.25%, respectively.
[4] Linear response to graded levels of phytase ($P < 0.05$).
[5] Quadratic response to graded levels of phytase ($P < 0.05$).
[6] Cubic response to graded levels of phytase ($P < 0.05$).
[a-c] Values within columns with no common superscript differ significantly ($P < 0.05$).

Table 4.9. Efficacy of high levels of dietary phytase on ileal P and Ca digestibility utilizing TiO_2 in 21- and 42-day old broilers[1]

Diet	Phytase (PU/kg)	Ca[2] (%)	P[3] (%)	P[4] (%)	Ca[5] (%)	P[4] (%)	Ca (%)
				------ (day 21) ------		------ (day 42) ------	
POS +	0	0.76	0.35	69.77[a]	46.16[ab]	50.46[c]	32.96[ab]
NEG +	0	0.76	0.22	46.98[b]	31.88[c]	40.29[d]	26.53[b]
NEG +	500	0.76	0.22	58.46[ab]	51.77[a]	55.02[bc]	38.55[a]
NEG +	2,500	0.76	0.22	59.83[a]	33.49[c]	56.04[abc]	32.76[ab]
NEG +	12,500	0.76	0.22	59.88[a]	37.14[bc]	62.42[a]	35.73[a]
NEG +	62,500	0.76	0.22	64.43[a]	27.56[c]	61.28[ab]	32.83[ab]
SEM				4.1	4.1	2.2	2.7

[1] Data are means of five replicate pens of 10 chicks each (day 21) and five chicks each (day 42).
[2] Dietary calcium levels from days 0 to 21 were: 0.85, 0.85, 0.85, 0.85, 0.85, and 0.85%, respectively.
[3] Dietary phosphorus levels from days 0 to 21 were: 0.42, 0.25, 0.25, 0.25, 0.25, and 0.25%, respectively.
[4] Linear response to graded levels of phytase ($P < 0.05$).
[5] Quadratic response to graded levels of phytase ($P < 0.05$).
[a-d] Values within columns with no common superscript differ significantly ($P < 0.05$).

Table 4.10. Efficacy of high levels of dietary phytase on ileal P and Ca digestibility utilizing Cr_2O_3 and TiO_2 in 21-day old broilers[1]

Diet	Phytase (PU/kg)	Ca (%)	P (%)	P (%)[2] Cr_2O_3	P (%)[2] TiO_2	P = †	Ca (%)[4] Cr_2O_3	Ca (%)[3] TiO_2	P =‡
POS +	0	0.85	0.42	69.24	69.77	0.5866	45.30	46.16	0.5282
NEG +	0	0.85	0.25	52.75	46.98	0.0001	39.22	31.88	0.0001
NEG +	500	0.85	0.25	54.31	58.46	0.0003	46.92	51.77	0.0014
NEG +	2,500	0.85	0.25	59.11	59.83	0.4635	31.90	33.49	0.2473
NEG +	12,500	0.85	0.25	59.15	59.88	0.4591	36.39	37.14	0.5790
NEG +	62,500	0.85	0.25	69.95	64.43	0.0001	38.56	27.56	0.0001
SEM				3.7	4.1		3.5	4.1	

[1] Data are means of five replicate pens of 10 chicks each.
[2] Linear response to graded levels of phytase ($P < 0.05$).
[3] Quadratic response to graded levels of phytase ($P < 0.05$).
[4] Cubic response to graded levels of phytase ($P < 0.05$).
[a-c] Values within columns with no common superscript differ significantly ($P < 0.05$).
† Probability of the difference between dietary markers.
‡ Probability of the difference between dietary markers.

Table 4.11. Efficacy of high levels of dietary phytase on ileal P and Ca digestibility utilizing Cr_2O_3 and TiO_2 in 42-day old broilers[1]

Diet	Phytase (PU/kg)	Ca (%)	P (%)	P (%)[2,3] Cr_2O_3	P (%)[2] TiO_2	P = †	Ca (%)[4] Cr_2O_3	Ca (%) TiO_2	P =‡
POS +	0	0.76	0.35	54.26	50.46	0.0040	38.08	32.96	0.0112
NEG +	0	0.76	0.22	49.13	40.29	0.0001	37.45	26.53	0.0001
NEG +	500	0.76	0.22	54.17	55.02	0.5507	37.17	38.55	0.4671
NEG +	2,500	0.76	0.22	52.64	56.04	0.0091	27.72	32.76	0.0123
NEG +	12,500	0.76	0.22	59.47	62.42	0.0214	30.26	35.73	0.0072
NEG +	62,500	0.76	0.22	64.23	61.28	0.0211	37.77	32.83	0.0139
SEM				1.9	2.2		2.5	2.7	

[1] Data are means of five replicate pens of five chicks each.
[2] Linear response to graded levels of phytase ($P < 0.05$).
[3] Quadratic response to graded levels of phytase ($P < 0.05$).
[4] Cubic response to graded levels of phytase ($P < 0.05$).
[a-d] Values within columns with no common superscript differ significantly ($P < 0.05$).
† Probability of the difference between dietary markers.
‡ Probability of the difference between dietary markers.

Table 4.12. Efficacy of high levels of dietary phytase on total and soluble litter P and total litter Ca of 42-day old broilers[1]

Diet	Phytase (PU/kg)	Ca[2] (%)	P[3] (%)	Litter TP[4] (%)	Litter SP[5,6,7,8] (%)	Litter Ca (%)
POS +	0	0.76	0.35	1.58[a]	0.30[a]	2.04
NEG +	0	0.76	0.22	1.17[b]	0.17[d]	2.12
NEG +	500	0.76	0.22	1.14[b]	0.22[c]	2.01
NEG +	2,500	0.76	0.22	1.06[b]	0.26[b]	1.97
NEG +	12,500	0.76	0.22	1.13[b]	0.34[a]	2.09
NEG +	62,500	0.76	0.22	1.06[b]	0.32[a]	1.97
SEM				0.07	0.02	0.15

[1] Data are means of five replicate pens of 20 chicks each (days 0 to 21) and 10 chicks each (days 22 to 42).
[2] Dietary calcium levels from days 0 to 21 were: 0.85, 0.85, 0.85, 0.85, 0.85, and 0.85%, respectively.
[3] Dietary phosphorus levels from days 0 to 21 were: 0.42, 0.25, 0.25, 0.25, 0.25, and 0.25%, respectively.
[4] TP = total litter P.
[5] SP = soluble litter P.
[6] Linear response to graded levels of a microbial phytase ($P < 0.05$).
[7] Quadratic response to graded levels of a microbial phytase ($P < 0.05$).
[8] Cubic response to graded levels of a microbial phytase ($P < 0.05$).
[a-d] Values within columns with no comon superscript differ significantly ($P < 0.05$).

CHAPTER FIVE – CONCLUSION

Research presented in Chapters Two through Four, suggests that high levels of dietary phytase are efficacious in broiler diets. At 21 days, broilers fed high levels of a microbial phytase had better body weight gains and feed efficiency than broilers fed industry recommended levels of phytase and birds fed NRC diets (Chapters Two and Four). However, at 42 days, birds fed low phosphorus diets and high levels of microbial phytase performed similarly to birds fed the positive control diets.

Microbial phytase was efficacious at improving phytate phosphorus utilization. Phytase supplementation improved performance, bone ash, and phosphorus digestibility in broilers. Phytase supplementation at levels above industry recommendations improved calcium digestibility in young broilers, but decreased calcium digestibility in market weight broilers (Chapter Two). In Chapter Three, phytase supplementation above industry recommendations improved calcium digestibility above the positive control diet. In Chapter Four, phytase supplementation above industry recommendations reduced calcium digestibility. The variable calcium digestibility data may be a result of calcium levels in the diet and/or an excess of phosphorus release due to high levels of phytase, which may interfere with calcium digestibility.

Phytase supplementation decreased total litter phosphorus. Phytase supplementation at levels above industry recommendations did not affect soluble

litter phosphorus. Phytase supplementation decreased litter calcium in Chapter Two. However, litter calcium was not affected by phytase supplementation in Chapter Four.

Dietary markers such as chromic oxide and titanium dioxide are often used in digestibility studies. However, there are some problems associated with dietary markers and finding a reliable marker is important. The research presented in Chapters Three and Four, suggests that an accurate marker is still needed. In Chapter Three, titanium dioxide values were higher than chromic oxide, however they were highly correlated, and there were significant differences in digestibility values between the two markers. In Chapter Four, chromic oxide and titanium dioxide values were highly correlated, however there were still significant differences between the two markers.

Tibia ash is widely used to determine bone ash. Unfortunately, the analysis is time consuming and may require the use of hazardous materials such as ethyl ether. Foot ash has been suggested as a simpler means to evaluate bone ash. The research presented in Chapter Four suggests foot ash may be a reliable alternative to tibia ash. There were significant relationships between foot and toe ash (R^2 = 0.74, 0.76, 0.77, and 0.84) and tibia ash and foot ash (R^2 = 0.63 and 0.85).

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