ENHANCEMENT OF GROWTH PERFORMANCE AND BONE MINERALIZATION IN MARKET BROILERS THROUGH DIETARY ENZYMES

A Thesis

by

JACOB RYAN COPPEDGE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2010

Major Subject: Poultry Science

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Approved by:

Chair of Committee,	Jason Lee
Committee Members,	Morgan Farnell
	Glenn Holub
Head of Department,	John Carey

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ABSTRACT

Enhancement of Growth Performance and Bone Mineralization in Market Broilers through Dietary Enzymes.

(December 2010)

Jacob Ryan Coppedge, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Jason Lee

Four research experiments were conducted to evaluate the influence of dietary enzyme inclusion (phytase and NSPase) on broiler performance parameters, processing yields, and bone mineralization. In Experiment 1, a 35-day grow out trial was conducted to investigate the effect of three commercially available phytase enzymes on growth performance and bone mineralization in phosphorus deficient corn/soy based diets. Increasing the level of available phosphorus (aP) in the control diets resulted in improved bird performance and bone ash data. The presence of dietary phytase in phosphorus deficient diets resulted in improvements in growth parameters and bone mineralization. Regression analysis confirmed that phytase supplementation can potentially increase the bioavailability of phosphorus in broiler diets up to 0.15 to 0.20%, however, the responses varied according to the enzyme used and inclusion level.

In Experiment 2, a 42-day grow out trial was conducted to analyze the effects of NSPase inclusion on broiler performance and processing parameters when supplemented in diets with varying protein and energy concentrations. Reduced protein and energy

levels reduced bird performance throughout the trial. The inclusion of both NSPase enzymes resulted in improvements in feed conversion throughout the starter and grower periods (day 26 of age). The results from this trial showed that NSPase inclusion can improve broiler performance and processing parameters.

In Experiments 3 and 4, a battery trial and a floor trial were conducted to determine the effects of phytase and NSPase enzyme co-administration on growth and bone ash in low phosphorus diets. Increasing the level of available phosphorus resulted in increased bird performance and bone ash. The inclusion of phytase enhanced bird performance and bone mineralization. NSPase inclusion in diets containing low levels of phytase had improvements in bird performance during early stages of growth. The enhanced effects associated with dual administration of phytase and NSPase were not observed in a full grow out trial during later stages of growth. These four experiments indicate that phytase and NSPase enzyme inclusion in broiler diets have the ability to enhance bird performance, processing yield, and bone mineralization.

DEDICATION

I would like to dedicate this thesis to my parents, brother, grandparents, and girlfriend. Your love, support, and advice have given me the motivation to continue my education. Mom and Steven, you have raised me to be a strong and hard working young man and I am truly blessed to have such loving parents in my life. Both of you expected great things from me and held me to a higher standard. I would also like to dedicate my work to my late father and uncle, James Roland Coppedge and Ricky Moore. Even though both of them only live within me through spirit, I am forever guided by the foundation that both of these men have laid early in my life. My dad is my guardian angel and helps me through the tough roadblocks of life. Both men built a foundation to appreciate the outdoors and to love to hunt and fish. My desire to hunt and fish has kept me out of trouble by occupying my spare money and time.

My grandparents helped me realize that there was always more to gain in life if you were persistent and kept motivated. All my family support was critical for helping me accomplish my goals and reach for more in life.

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Most importantly, I express my love and gratitude to my family and friends, thank you for your continuous love and encouragement.

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CHAPTER I

INTRODUCTION

Feed enzymes have been used in the commercial poultry industry in recent years to increase nutrient digestibility of poultry diets. Enzyme inclusion has been shown to increase bird performance by improving phosphorus and energy utilization from poultry diets. Phosphorus is an essential nutrient for plants and animals and is critically important in the production of poultry. Phosphorus is required for growth, development, and maintenance of the skeleton of chickens. Failure to meet these requirements will decrease performance and increase mortality in broiler flocks (Nelson, 1971). Due to the phosphorus demands of market broilers, many producers over-supplement this nutrient in the diet which has resulted in excessive phosphorus concentrations in poultry waste and litter (Waldroup, 1999). Due to these environmental concerns in addition to elevated prices of inorganic phosphate sources, the use of phytase enzymes in poultry diets have increased. Phytase is an enzyme that is capable of liberating bound phosphorus from phytate for utilization and absorption (Nelson, 1967). Since chickens are limited in their ability to produce endogenous phytase, companies are supplementing diets with exogenous phytase enzymes to improve utilization of phosphorus from the diet (Simmons., 1990; Mitchell and Edwards., 1996). Phosphorus present as phytic acid is unavailable for use by most monogastric animals including commercial broilers. Phytic acid also has anti-nutritive factors associated with the chelating of many nutrients

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This thesis follows the style of Poultry Science.

including calcium and amino acids. Therefore, phytase inclusion can be used to increase growth parameters and bone integrity by releasing phosphorus from phytic acid present in the diet (Powell and Johnston, 2008).

Another anti-nutritive compound found in poultry diets include non-starch polysaccharides (NSPs) which increase intestinal viscosity and decrease the digestibility of nutrients from the diet. Previous published literature supports the data that exogenous enzymes have the ability to improve the nutrient value of broiler diets by degrading NSPs that are commonly found in broiler diets (Cowieson, 2008). NSPase cocktail enzymes, which contain several different types of enzymes, have shown to increase bird performance and digestibility values when fed low energy diets (Zhou, 2009). Recently, published reports indicate that the dual administration of phytase and NSPase enzymes increased apparent metabolizable energy values and digestibility (Ravindran et al. 1999; Juanpere et al. 2005).

Therefore, these experiments were designed to investigate the effects on bird performance and bone mineralization when phytase and NSPase enzymes are supplemented individually or co-administered in broiler diets. The objectives of these experiments include: 1) Evaluate the effectiveness of three commercially available heat stable phytase enzymes at two dosage levels on broiler growth performance and bone ash when fed diets deficient in available phosphorus (aP), 2) Determine the effect of two NSPase cocktail enzymes and diets varying in nutrient profile on broiler performance and processing yield, 3) Determine if NSPase inclusion can enhance phytase activity and improve growth response and bone mineralization during co-administration.

CHAPTER II

LITERATURE REVIEW

Phosphorus's Role in Poultry Production

Due to the high rate of growth of broilers, the demand for adequate skeletal development is critical for proper growth and performance. Calcium and phosphorus (P) are two of the essential minerals required for proper skeletal development during early stages of growth (Nelson, 1971; Waldroup, 1999; Shaw et al., 2010). These minerals must maintain a constant balance to avoid leg abnormalities such as rickets and tibial dyschondroplasia (Waldenstedt, 2006). P is involved in the development of the inorganic matrix of the bone and is present as hydroxyapatite, which provides the skeletal support. P on a dry matter weight constitutes about 170 g/kg of bone ash and represent about 20-30% of bone weight (Ali, 1992; Waldenstedt, 2006). The role of P is more complex than maintaining bone structure as numerous other physiological functions can be affected by a P deficiency. Consequences resulting from the failure to provide adequate levels of P can be detrimental to the physiological well-being of the bird and result in financial losses (Selle, 2007) associated with reduced bird performance, increased condemnations and excessive mortality (Waldroup, 1999). Due to the consequences associated with a P deficiency, it has been a common practice for poultry nutritionists to provide an adequate margin of safety for the inclusion of P in broiler diets (Waldroup, 1999; Waldroup, 2000).

Phytate Phosphorus

There are two forms of P present in poultry diets: organic phosphorus found in cereal grains and inorganic phosphorus in the form of supplemental monocalcium or dicalcium phosphate (Waldroup, 1999; Leske and Coon, 2002). The primary components for poultry diets consist of plant-based ingredients that come primarily from the seeds of the plants (Applegate et al., 2004). In plants, the highest levels of P are typically stored in the seeds as phytin (Applegate et al., 2004).

P in feedstuffs is identified as total phosphorus and nonphytate phosphorus (NPP) (NRC, 1994). The amount of P that is available for utilization by poultry from all cereal grains is not equal to the amount of P present in the feedstuffs (Waldenstedt, 2006; Selle et al., 2007; Applegate et al, 2008). In fact, only about 20-30 % of the P found in most cereal grains is actually available in a usable form for chickens (Simons et a1., 1990; Sebastian, 1998). The lack of equivalency in P levels is due to a large percentage of the P found in cereal grains is bound in the compound, phytate, which is a form of P unavailable for use by chickens (O'Dell et al., 1972).

Phytate, is composed of mixed salts of phytic acid (myo-inositol (1, 2, 3, 4, 5, and 6 hexaphosphate; IP_6) and was first identified by Pfeffer (1872) in the outer layers of rice. Oilseed meals and cereal by-products contain larger amounts of phytate P as compared to legumes. In some cereal grains such as wheat, the phytate is found most predominantly in the bran of the grain (Sebastian, 1998). In oilseeds and legumes, phytate is distributed throughout the kernel in globoids. For many years researchers believed that broilers were unable to utilize the phytate-bound P, but recent literature has

suggested that a small portion of the phytate-bound P can be utilized by birds (Sebastian, 1998; Waldroup; 1999). The phytic acid is a phosphorylated cyclic sugar alcohol that has a chemical description of myoinositol hexakisphosphoric acid and is typically found in plants as an anion called phytate (Applegate et al., 2004; Roa et al., 2009; Kumar, 2010). Phytic acid, in the polyanionic chelating form of phytate can chelate with cations like calcium, magnesium, copper and iron (Rao et al., 2009; Kumar, 2010) forming insoluble salts that can decrease absorption of these nutrients. For this reason, phytate is often considered to be an anti-nutritive compound because the binding of these nutrients results in being partially or completely unavailable for digestion by the chicken (Applegate, 2004; Roa et al., 2009).

Chickens, unlike ruminants, are limited in the amount of endogenous phytase found in the gastrointestinal tract (Waldroup; 2000). Phytase is capable of hydrolyzing phytate into inorganic P and inositol (Selle et al., 2007; Sebastian, 1998). The limited ability of monogastric animals to utilize phytate P presents two problems to animal nutritionists. The first concerns the formulation of diets that satisfy the animal's physiological needs for P, and the second involves the environmental impact of unused dietary P excreted in the feces (Sebastian et al., 1998). Nutritionists have traditionally supplemented diets with inorganic forms of P necessary to meet the bird's demand for available P (Sebastian, 1998; Applegate, 2008). This strategy of inorganic P supplementation has proven to be successful at meeting the bird's P requirements, unfortunately there is an increased cost associated with this usage in addition to excessive levels of P excretion in poultry manure (Sharpley, 1999). In order to

effectively reduce P excretion while also maintaining bird performance, it is necessary to focus on the contributors of the excess P in the feces. The undigested portions of the phytate-bound P and inorganic P in excess of the animals needs tend to comprise a large proportion of the fecal P (Waldroup, 1999).

Phosphorus Requirements for Poultry

There are many factors that control the amount of P required in poultry diets, with probably the most important being the age of the bird. The nutrient requirements as suggested by the NRC (1994) for broilers during the starter phase (0 to 3 weeks) is 0.45% aP, and the requirements decrease (0.35% aP for 3-6 weeks and 0.30% aP for 6-8 weeks) as bird age increases when less bone development is occurring. Considerable research has been conducted investigating P requirements by Waldroup et al. (2000), Angel et al. (2000b) and Powell et al. (2008) which has reported substantial differences in the nonphytate phosphorus (nPP) requirement of broilers as compared to the published requirements by the NRC (1994) (0.45% aP or nPP). Waldroup et al. (2000) studied the effects on broiler growth reared in batteries during the first 3 weeks as affected by different levels of phosphate corn. It was determined that the levels of nonphytate phosphorus required for young broiler's to maximize tibia ash ranged between 0.37% and 0.39% depending on the use of corn with low or high levels of available phosphorus. These levels are considerably lower than the requirements outlined for the starter phase in the NRC of 0.45% nPP.

Similar results were observed by Powell et al. (2008), which reported no differences in broiler performance or bone breaking strength between birds fed diets

with 0.43% and 0.38% nPP reared in floor pens through 14 days of age. Angel et al. (2000b) also reported that similar observations in broilers growth performance and bone strength were achieved when fed a diet containing 0.35% nPP as compared to broilers fed a diet with 0.43% nPP at 17 days of age when reared in batteries. However, bone ash was increased in broilers fed the higher nPP phosphorus (0.43%). These combined reports confirm that lower levels of nPP than the recommendations in the NRC (1994) may be utilized during the starter phase to decrease the concentration of P supplementation and reduce the amount of P in broiler litter. Powell et al. (2008) determined the effects of using a four phase feeding program consisting of a starter period mentioned previously, and a grower, finisher and withdrawal diets. Broilers were fed a control diet in the grower, finisher and withdrawal phase that consisted of (nPP) levels of 0.40, 0.36 and 0.32% and a treatment diet fed for each phase with a 0.05% reduction in nPP resulting in treatment diets of 0.35, 0.31 and 0.27% in the three dietary phases. The control diets had nPP levels similar to NRC recommendations (0.35 and 0.30% nPP). Powell concluded that no negative effects were observed on growth performance when Ross x Ross straight-run broilers were fed diets with a 0.05% reduction in phosphorus. However, bone breaking strength was reduced in the finisher and withdrawal diets with the reduction in dietary nPP while the litter P concentration was reduced by 9% with the decrease in phosphorus. These data indicate that bone strength may be reduced when feeding levels of reduced nPP as compared to NRC (1994) recommendations however growth performance may not suffer. When rearing

larger broilers to 8 weeks of age, Waldroup et al. (1974) suggested that the requirements outlined in the NRC (1994) are adequate for bird growth and health.

Skinner et al. (1992b), Angel et al. (2000a;2000b), Dhandu et al. (2003), and Yan et al. (2001; 2003) suggest that during later stages of growth (grower, finisher and withdrawal phase), nPP requirements in a typical corn-soy based diet can be reduced compared to NRC (1994) recommendations. Skinner et al. (1992b) investigated the effects of feeding diets with 0.12% and 0.24% nPP in broiler diets as compared to a control diet with 0.35% nPP from 42 to 56 days of age in Cobb 500 broilers. Body weight gain, feed consumption, feed conversion, mortality rate, tibia length, and tibia width were not affected with the reductions in nPP. However, tibia strength was reduced in the lowest nPP treatment group (0.12%) as compared to the 0.24% nPP treatment.

Angel et al. (2000a; 2000b) reared Ross 308 broilers in floor and battery pens to determine the necessary P levels required for the grower and finisher phases. In the floor trial conducted, four different nPP levels for the grower and finisher diets were used with concentrations of 0.45, 0.36, 0.32, 0.28% and 0.34, 0.28, 0.24, 0.19%, respectively. The results indicated that the adequate levels of nPP in the grower phase was between 0.32 and 0.28% nPP and in the finisher phase was between 0.24 and 0.19% nPP. The battery trial was designed to analyze the effects of varying P levels of 0.31 and 0.26% nPP in a grower diet. Body weight gain and bone ash were reduced in broilers fed the low nPP diet of 0.26%.

Dhandu and Angel (2003) used regression analysis to determine the (nPP) requirements for Ross 308 broilers reared in battery pens during the finisher (32 to 42

days) and withdrawal diets (42 to 49 days). The evaluated nPP levels during the finisher diet were 0.15, 0.19, 0.26, and 0.31% and nPP levels in the withdrawal diet were 0.10, 0.13, 0.22, and 0.27%. No differences were observed among any of the treatment groups in relation to weight gain, feed intake, and feed efficiency during the two feeding phases. Tibia ash weight was reduced in broilers fed 0.15% nPP during the finisher diet as compared to the rest of the treatment groups. Non-phytin requirement of 0.20% was established by broken line analysis for male broilers weighing ~4 lbs during the finisher phase (32 to 42 days). The nPP requirement of male broilers weighing ~6 lbs was calculated at 0.16% during the withdrawal phase (42 to 49 days).

Yan et al. (2001; 2003) investigated the nPP requirement for male chicks reared in floor pens during the grower (3 to 6 weeks) and finisher phases (6 to 9 weeks). During the grower phase, broilers were fed diets ranging in nPP levels from 0.10 to 0.45% nPP in increments of 0.05%. Results of nonlinear regression analysis estimated the nPP levels of 0.33, 0.186 and 0.163% were required to optimize tibia ash, body weight gain, and feed conversion ratio, respectively. The level of nPP needed for optimum tibia ash (0.33%) was in close agreement with the NRC (1994) nPP requirements during the grower phase (0.35%), yet the requirements for optimum bird performance (0.186 and 0.163%) were much lower than the NRC (1994) requirements. During the finisher phase male broilers were fed diets ranging from 0.10 to 0.35% nPP in 0.05% increments. The lowest level of nPP (0.10%) was sufficient to maintain equivalent broiler performance (i.e. BW gain, feed conversion and livability). The investigators concluded through nonlinear regression the nPP levels for optimum tibia

ash was 0.31% and 0.22% for day 49 and 63, respectively. The results of these reports indicate that the NRC (1994) requirements for broilers during the grower and finisher phase may overestimate the P requirement due to the genetic changes in market broilers since the last revision of the NRC (1994) which has been selected for increased feed consumption and growth rate. Readdressing the current information in the NRC (1994) for nPP requirements could benefit the poultry industry by providing more current and relevant information about the dietary requirements of the current market broiler allowing industry nutritionists to formulate more accurate diets and reduce diet costs and excessive nutrient excretion.

Due to growing concerns about the effects of excreted P on eutrophication, it is rapidly becoming essential for poultry producers to provide levels of dietary P that maximize broiler performance while also minimizing P excretion (Waldroup, 1999; Sharpley, 1999; Waldenstedt, 2006). Significant progress has been made on reducing the amount of P excreted from poultry. The uses of feed additives and alternative strategies such as phytase enzymes, probiotics, low-phytin grains and organic acids have been successful in reducing P excretion (Applegate, 2005; Waldroup, 1999). The financial and ecological concerns stemming from P levels in broiler diets has led to the development of methods aimed at maximizing utilization of P already available in the feedstuffs.

Use of Phytase Enzymes

One of the most popular and effective strategies to increase the amount of P utilized from cereal grains includes the use of supplemental dietary phytase enzymes.

The ability of exogenous phytase to improve the availability of phytate-bound P was demonstrated by Nelson et al. (1968, 1971), who was concerned with the negative effects that phytate could have on calcium and P availability in broiler diets. Even with this early knowledge of phytase enzymes, industry use of these enzymes were not applied until the early 1990's due to inability to produce large quantities at a cost allowable for diet inclusion, additionally, legislation was being passed in certain countries to limit P pollution in the environment (Selle, 2007). The use of phytase is now commonplace in the US poultry industry, and it is estimated that over 50 million tons of feed were treated with phytase in 1999 (Chen, 2000). The increase in demand for phytase was a result of increased costs of inorganic phosphate sources and increasing environmental concerns about P pollution (Ravindran et al., 1995; Waldroup, 1999; Selle, 2007).

Phytase is capable of breaking down phytate bound P by catalyzing the stepwise removal of inorganic orthophosphate from phytic acid within the digestive tract of the chicken (Nayini and Markakis, 1986). Phytases hydrolyze the phosphate ester bond of phytate bound P (Rao et al., 2009) and produce one molecule of inositol and six molecules of inorganic phosphate. Phytase is classified into three categories depending on the site in which the hydrolysis of phytate molecule is initiated, 3-phytases liberate the phosphorus from the C_3 position, 5-phytases initiate phytate hydrolysis at the fifth phosphate group, and the 6-phytase functions at the C_6 position of the hexaphosphate ring (Selle, 2007; Rao, 2009). Phytase is present in most cereal grains. Rye, wheat, and barley were found to be rich in this enzyme, whereas maize, oats, sorghum and oilseeds

contain little to no phytase enzymes (Eeckhout and De Paepe, 1994). Phytase effectiveness in broiler diets has shown to be affected by the dietary calcium and P concentrations. Higher calcium: phosphorus ratios of 2:1 have shown to impair the digestion of phytate (Nelson, 1971) and as the ratio falls closer to 1:1 phytase becomes more effective. Phytase is highly selective for its substrate and functionally sensitive to the environment. In the proventriculus and gizzard of poultry, phytic acid is more soluble and can be readily acted on by phytase enzymes (Applegate, 2004).

Phytase can be produced by fungus *(Saccharomyces cerevisiae* and *Aspergillus* strains), bacteria *(Pseudomonas* and *Bacillus),* yeast, and rumen micro-organisms (Sebastian, 1998). Most of these naturally occurring phytase producing microorganisms operate most efficiently at temperatures below 60°C and a pH of 4-6 (Jongbloed et al., 1992). Due to the fact that most of these naturally occurring enzymes are not heat stable and cannot withstand the high heat treatment of pelleting feeds, research has focused on those microbial phytases that are more heat tolerant. The *Aspergillus* genus *(Aspergillus ficuum* and *Aspergillus niger)* has become a popular phytase due to the fact that these fungal phytases are more heat stable and can withstand the lower pH environment of the GI tract (Jongbloed et al., 1992; Sebastian et al., 1998). *Aspergillus niger* is a 3-phytase and *Peniophora lycii* and *Escherichia coli*, are both 6-phytases which are the three most commonly used phytase enzymes and can be applied to broiler diets as a granule or as a liquid (Selle, 2007).

Extensive research demonstrates microbial phytase supplementation in low P broiler diets can increase body weight gain, feed intake, and bone ash while decreasing

leg deformities in broiler chickens (Simons et al., 1990; Sebastian et al., 1996) and turkeys (Ravindran et al., 1995). These positive effects in broiler performance may be attributed to several mechanisms including: an increase in absorbed P, the release of other minerals from the phytate-mineral complex, increased digestibility, and/or increased availability of amino acids (Sebastian et al., 1998).

Phytases are characterized by the enzyme activity, and one unit of phytase (FTU) is defined as the amount of enzyme required to liberate one μmol of orthophosphate from phytin per minute at a pH of 5.5 at 37°C (Zyla et al., 1995). An extensive amount of research has been performed analyzing the effect of feeding phytase enzymes at varying FTU levels and the effects witnessed on bird performance and bone ash data. Nelson et al. (1968, 1971) was one of the first to describe the possible benefits of phytase inclusion in broiler diets. Nelson (1968) analyzed the effects of feeding a fungal phytase, *Aspergillus ficuum,* to White Leghorn cockerels that were raised in battery brooders through 3 weeks of age. Phytase inclusion at levels of 1, 2, 4 and 8 g/kg of feed resulted in an increase of bone ash as compared to the control diets that had available P levels ranging from 0.12- 0.33%. All levels of phytase inclusion resulted in higher body weights than the control diet with 0.12% available P. The percent phytate P utilized by the varying levels of phytase inclusion was 0.07, 0.10, 0.16 and 0.17% for the four levels of enzyme supplemented. Subsequent experiments conducted by Nelson et al. (1971) yielded similar results with increased weight gain and bone ash when varying levels of phytase was fed.

Further application of phytase enzymes were not reported until Simons et al. (1990) utilized a fungal phytase (Aspergillus *ficuum*) in broiler and pig diets. Male broiler chicks were raised in two-tier battery cages to either 24 days of age or to 4 weeks of age. In the first experiment, phytase inclusion levels investigated were 250, 500, 750, 1000 and 1500 FTU's/kg included in a basal diet that contained 4.5 g/kg of P. Control diets consisted of P levels of 4.5, 6 and 7.5 g/kg of P. Phytase inclusion at the 250 and 500 FTU level increased broiler body weights similar to the control diet with 6 g/kg of P. Phytase inclusion at the higher levels of 750, 1000 and 1500 FTU levels resulted in body weights similar to the growth observed in broilers fed the control diet with 7.5 g/kg P. Feed conversion ratios for all diets with phytase inclusion were similar to the control diet with the 7.5 g/kg P. Due to increased availability of P from the diet, the amount of P in the droppings was significantly lowered by as much as 50%.

The improvements in broiler performance were also witnessed by Ravindran et al. (1999), Waldroup et al. (2000), Yan et al. (2001), Yan et al. (2003), Dilger et al. (2004) and Powell et al. (2008). Ravindran et al. (1999) investigated the effects of using microbial phytases (Natuphos) in different cereal grains and oilseed meals on amino acid digestibility. Male broiler chicks (Inghams TM 70 strain) were raised in battery brooders from day 35 to 42. On day 42, broilers were euthanized and their stomach contents collected for analysis. The supplementation of microbial phytase improved protein and amino acid digestibility of all feed ingredients, yet the greatest increases in digestibility were associated with wheat, sorghum and rice polishing. These results

confirm that the effects of phytase enzymes go beyond increasing P availability and can improve bird performance by enhancing other nutrients digestibility.

Waldroup et al. (2000) evaluated the effects of phytase inclusion in diets composed of normal and high available phosphate corn at the inclusion level of 800 FTU/kg fed to male broilers in battery cages. Phytase supplementation significantly increased body weights in comparison to birds fed diets without phytase supplementation, and the response to body weight due to enzyme inclusion was greater in low phosphate corn. This effect was associated with the increased amount of phytate-bond P present in the diet. Feed conversion was also improved with phytase inclusion. Greater improvements in FCR were observed in diets containing the high available phosphate corn. Phytase supplementation considerably reduced mortality rates associated with the lower levels of nonphytate P. The inclusion of phytase decreased the amount of nonphytate P required in the diet for adequate tibia development, as the nPP% requirement decreased by 0.1% from 0.39 to 0.29% in the low phosphate corn and by 0.05% from 0.37 to 0.32% in diets containing high phosphate corn. The inclusion of phytase liberated approximately 50% of the phytate-bound phosphorus from corn and the fecal phosphorus content decreased by 20% with the addition of phytase in the diets, illustrating the benefits that phytase enzymes can have on decreasing phosphorus excretion.

Yan et al. (2001) investigated the effect of inclusion of 800 FTU/kg of phytase on male broiler performance from 3 to 6 weeks of age in batteries cages. The determined nPP levels required for adequate tibia ash, body weight gain, and FCR were

0.33, 0.186, and 0.163%. Inclusion of phytase reduced the nPP requirements for tibia ash, body weight gain, and FCR to 0.24, 0.151, and 0.109%. The use of phytase resulted in a decrease of nPP requirements of 0.09, 0.035, and 0.054% for the three parameters previously evaluated. The use of phytase enzyme resulted in a release of phytate-bound P from the diet of about 38%. Yan et al. (2003) evaluated the effects of feeding phytase to broilers raised on built up shavings from 6 to 9 weeks of age. Phytase inclusion at a level of 800 FTU was included in diets with varying nPP levels of 0.10 to 0.35% in 0.05% increments. The level of 0.10% nPP with phytase supplementation was sufficient to maximize body weight gain and feed efficiency throughout the trial while the level of nPP needed to maximize tibia ash was 0.15% at day 49. Less than 0.10% nPP was required to maximize tibia ash at day 56 and 63. The level of nPP required without the presence of phytase was 0.31±0.004% at day 49 and 0.22% nPP at day 63. The nPP requirements decreased by 0.16 and 0.12% during different periods of this trial illustrating the positive effects that phytase inclusion can have during later stages of growth.

Dilger et al. (2004) conducted two trials to determine the effects of a microbial phytase (Phyzyme XP) fed to Ross 308 male broilers raised for 14 days in batteries and in floor pens during a 42 day trial. Phytase enzyme was included at 500, 750 and 1,000 FTU/kg and growth parameters, bone ash and amino acid digestibility determined. Phytase inclusion increased body weights, feed consumption, feed efficiency, bone ash, and amino acid digestibility confirming the ability of a microbial phytase derived from

Escherichia coli can improve growth response and P utilization in broilers raised under industry type settings.

Powell et al. (2008) analyzed the effect of using a phytase during a 4 phase feeding program fed to Ross x Ross straight-run broilers reared to 50 days of age in a floor pen trial. Phytase enzyme was supplemented at 600 FTU/kg level throughout the trial while P levels were varying according to dietary phase. Phytase was included in an industry type control diet and also a diet with reduced phosphorus (0.05%) and calcium concentration (0.1%). Phytase inclusion in the two starter diets had no effect on broiler performance, while broiler performance was actually decreased due to phytase inclusion during the grower period. Phytase inclusion decreased average daily gain, feed efficiency and body weight as compared to the two control diets. The decrease in performance parameters was worse for the phytase inclusion in the control diet as compared to the phytase inclusion in the diet with the low level of P and calcium. Similar results were witnessed during the finisher period in that phytase inclusion decreased feed intake and body weight, with the differences observed being greater in the control diet. No effects were witnessed during the withdrawal period in relation to phytase inclusion in the diets. Phytase inclusion in the control diet did result in a 1.6% decrease in final body weight at day 50. Bone-breaking strength was not affected by phytase inclusion in the control diet, but bone-breaking strength was decreased for the low P and calcium diet supplemented with phytase during the grower period (day 14-32). Total P and soluble P levels in the litter were reduced due to phytase inclusion. This data indicated that phytase inclusion of 600FTU/kg negatively affected growth

performance, had no effects on bone breaking-strength, and effectively reduced phosphorus concentrations in poultry litter.

As suggested by the previous literature discussed, phytase enzymes effectively reduce nPP requirements for broilers during all stages of growth. In most cases, broiler performance is enhanced and P levels in litter are reduced with phytase inclusion in broiler diets. Phytase inclusion influences other phytate-bound minerals like calcium; zinc and amino acids, therefore 'extra-phosphoric' benefits do exist that can provide greater benefits of phytase use. Similar benefits of phytase inclusion have been reported in other species of poultry. Ravindran et al. (1995b) illustrated the positive effects of phytase inclusion when fed to young turkeys. Economics, magnitude of the enzyme's effectiveness, product cost and ease of application ultimately helps the producer determine which type of phytase enzyme they will use as a management and cost saving strategy.

Use of NSPase (Energy) Enzymes

Some of the cereal grains in poultry feeds contain anti-nutritive substances like nonstarch polysaccharides (NSP), which can impair feed efficiency and animal performance (Bedford and Morgan, 1996). Non-starch polysaccharides (NSP) enzymes function in similar fashion to phytase enzymes, in that inclusion of NSP cocktail enzymes increase the availability of dietary carbohydrates allowing for nutritionists to reduce the amount of energy in a diet to achieve maximal performance. As with the phytate P, not all the energy found in cereal grains is available for digestion and growth to broiler chickens. Soybean meal (SBM) is the most common vegetable protein source

used in poultry diets in the US. Unfortunately, high concentrations of this vegetable protein have shown to adversely affect growth parameters (Irish and Balnave, 1993).

Most industry broiler diets increase in metabolizable energy (ME) as the bird ages, yet the most recent NRC (1994) has set requirements of 3,200 ME/kg in all diets fed to market broilers. NSPase enzymes function to degrade NSPs (sucrose, raffinose, and galactose) present in feeds. Water-soluble β-glucans and pentosans are major NSP sources that are of concern in broiler diets with high concentrations of rye, wheat and barley. These types of high fiber diets are common in developing countries and Europe, while diets fed in the US are primarily based on corn and soybean meal. The use of NSPase enzymes have shown to be effective in high fiber diets by decreasing intestinal viscosity and increasing feed efficiency, therefore the use of NSPase enzymes have become commonplace when wheat-based diets are fed (Bedford and Classen, 1992; Choct et al., 1999). Arabinoxylans are the main NSP in wheat and can increase the viscosity of the GI tract, resulting in decreased digestion which lowers feed efficiency and suppresses growth (Gao et al., 2007).

The mode of action of NSPase enzymes include the destruction of plant's cell walls which bind many nutrients and possibly stimulate the growth of beneficial bacteria by breaking down the fiber fragments into smaller segments (Wyatt et al., 2001). NSPase enzymes hydrolyze the NSP in the upper GI tract, resulting in decreased viscosity in the small intestine (Bedford and Classen, 1992). Unfortunately, reports on the success of NSPase inclusion in promoting growth are inconsistent in corn-soy based diets which are the primary cereal grains used in diet formulation in the US poultry

industry. Development of enzymes that break down sucrose, raffinose, and galactose which are the most common NSPs found in soybeans, may result in positive responses in corn/soybean meal based diets. Therefore, due to the array of NSPs available in soybeans, the use of enzyme cocktails (containing several different enzymes) has been proposed as a potential method to improve growth performance in commercial broilers. Another direct benefit of using NSPase is the reduction of litter moisture associated with feeding wheat which has a large water holding capacity due to the arabinoxylans. By breaking down the arabinoxylans, the viscosity of the diet is reduced and the water binding capacity is decreased (Bedford and Morgan, 1996).

A significant amount of research has been conducted analyzing the effects of dietary exogenous enzyme supplementation on NSP hydrolyzation and broiler growth (Kocher et al. (2002); Lázaro et al. (2003); Lee et al. (2003); Gracia et al. (2003); Meng et al. (2005a); Meng et al. (2005b); Gao et al. (2007); Boguhn et al. (2010)). Kocher et al. (2002) evaluated the feeding of two enzyme products: Enzyme A (a cocktail enzyme containing multiple enzymes) and Enzyme B (β-galactase) in soybean meal (SBM) and the effects on nutrient value of diets fed to male Cobb broilers from day 24-28. The inclusion level of the enzymes was at the recommended (normal) dose and another treatment at 5 times the normal dose. Enzyme inclusion had no effect on the viscosity of the lower GI tract (jejunum and ileum). Enzyme A improved AME, reduced fecal moisture, and improved protein digestibility at the high inclusion level, but the recommended dose had no effect. Enzyme B improved AME_N and reduced the concentration of soluble NSP, but had no effects on bird performance. Protein

digestibility was reduced by the inclusion of the high level of Enzyme B. This research indicated that enzymes with β-glucanase and galactase have the ability to increase nutrient digestibility resulting in higher metabolizable energy values for a soybean meal based diet.

Lázaro et al. (2003) evaluated the inclusion of a β-glucanase/xylanase enzyme complex on Hy-Line white egg laying hens fed wheat, barley and rye based diets. Parameters evaluated included hen performance, egg quality, GI viscosity and digestibility from 20 to 44 weeks of age. Enzyme inclusion was at three levels (250, 1250 and 2500 mg/kg). Enzyme supplementation increased egg performance throughout the experiment with most improvements observed at the enzyme inclusion level of 250 mg/kg. None of the levels of enzyme resulted in negative effects on egg production. Enzyme supplementation improved feed efficiency per dozen of eggs and increased body weights at the lowest level in the wheat and barley diet. Enzyme supplementation reduced intestinal viscosity in all diets, but the greatest reduction in viscosity was witnessed in the barley based diet. The incidence of dirty eggs decreased linearly with each increase in enzyme concentration. Enzyme supplementation improved AME_N, dry matter digestibility, and NSP digestibility. These data demonstrate that enzyme supplementation could facilitate the use of high fiber diets for layers by increasing parameters related to egg production and nutrient utilization.

Lee et al. (2003) analyzed the effects of β-mannanase inclusion in a guar meal based broiler diet fed to broilers that were raised in battery brooders. Supplementation of *β*-mannanase to feeds containing guar meal resulted in reduced intestinal viscosity

and alleviated the deleterious effects associated with guar meal feeding by increasing the body weights and reducing feed conversion of the diets containing guar hull meal.

Gracia et al. (2003) conducted a 42 day trial raising Cobb male broilers in battery cages to determine the effects of feeding α-amylase on broiler performance and nutrient digestibility of a corn-soybean diet. The enzyme inclusion improved feed conversion, increased feed consumption, and increased body weight at the conclusion of the trial. Enzyme supplementation also improved digestibility of organic matter, starch and AME_n, but no changes were witnessed with crude protein and fat digestibility.

Gao et al. (2007) conducted a study analyzing the effects of feeding a xylanase enzyme in a wheat-based diet fed to broilers from 7-21 days of age. The two diets tested consisted of a wheat-based control diet with and without 0.1% enzyme inclusion. Enzyme inclusion in the diet consisted predominantly of xylanase activity derived from *Aspergillus niger* with small quantities of β-glucanase, cellulase and pectinase. Enzyme inclusion increased body weight, decreased feed conversion ratio and increased total digestibility of dry matter, crude protein and fat. Enzyme supplementation also resulted in decreased viscosity of the jejunum.

Boguhn et al. (2010) analyzed the effects of feeding a endoxylanase and β-glucanase containing enzyme in a wheat, barley and rye based diets to turkeys raised to 22-wk of age. There were no effects witnessed for turkey performance until the final dietary phase in which body weight gain increased and feed conversion was improved. These reports indicate that the addition of an appropriate combination of carbohydrase

enzymes to target cell wall polysaccharide structures can improve bird performance and increase nutrient digestibility.

Co-administration of Phytase and NSP Enzymes

Co-administration of multiple enzymes in an effort to enhance effectiveness of each enzyme is a relatively new concept with little published data. Francesch (2009) demonstrated the benefits of a multi-enzyme complex (RovabioTM Max) containing carbohydrolases and phytase activities on the performance and bone mineralization of broilers fed corn-soybean meal based diets. These results indicated the use of multi-enzyme complexes can allow for a reduction in apparent metabolizable energy (AME), available P, and calcium contents of broiler diets without hindering growth parameters.

Research conducted by Nortey et al. (2007) and Olukosi et al. (2007b) were performed in swine to determine the effects of NSPase and phytase co-administration. Swine have a similar problem to poultry, in that they do not digest feedstuffs with high NSP concentrations well. Pigs are also not capable of producing endogenous phytase (Nortey et al., 2007). Nortey et al. (2007) evaluated the effect of these two enzymes supplemented wheat millrun feed to grower pigs. The xylanase and phytase improved the crude protein utilization and improved P retention. Olukosi (2007b) determined that phytase in the low level nPP diet increased average daily gain (ADG) with the combination of NSPase and phytase also increased ADG in 10 kg pigs, but had no effect on heavy 23 kg pigs.

Published literature by Ravindran et al. (1999), Juanpere et al. (2005), Cowieson et al. (2005), Olukosi et al. (2007a), Leslie et al. (2007), and Ghorbani et al. (2009) has

been done on the effects of feeding a combination of enzymes to broilers. Ravindran et al. (1999) conducted four experiments evaluating the effects of a phytase fed in combination with a xylanase and glucanse enzyme in wheat and barley broiler diets fed to Cobb 500 male broilers. In the first experiment, xylanase and phytase combination slightly increased the AME of the wheat diet ($p>0.05$). In the second experiment, the addition of the two enzymes in a wheat diet did not increase AME values. In the third experiment, the inclusion of phytase and xylanase at increasing levels resulted in a linear increase in body weight and feed efficiency. In the final experiment, the inclusion of glucanase and phytase did not influence the AME values of barley. Juanpere et al. (2005) determined the effect of feeding a 3-phytase and glycosidase enzyme in corn, wheat, and barley-based diets feed to Ross 308 chicks raised to 25 days of age on energy values and nutrient digestibility of NSP rich diets. The enzymes increased the AME values in the corn and barley diets, and the digestibility of the dry matter and starch was increased in the barley and wheat-based diets. Other positive effects included a reduction in P excretion and increased calcium retention.

Leslie et al. (2007) investigated the effect of feeding phytase and glucanase enzymes on energy values of corn and soybean based diets fed to Ross 308 chicks. The use of the combination of enzymes was used in both the corn and soybean diets, and the results indicated numerical increases in digestibility, but the differences were not statistically significant. Similar results were witnessed for the rest of the parameters measured in the trial. These results show that NSP-degrading enzymes and phytase when fed in a complementary fashion can be incorporated in broiler diets to produce

additive beneficial effects. These reports outline a basis for the evaluation and use of other combinations of enzymes.

The objective of these experiments was to evaluate multiple phytase inclusion levels to maximize P release, determine the effect of multiple NSPase enzymes on growth performance and processing yields, and investigate co-administration of phytase and NSPase on bone strength, growth performance, and processing yields. Information obtained from these trials may benefit the industry by providing knowledge about the efficacy of these feed enzymes on providing beneficial effects that can ultimately save the integrators money.

CHAPTER III

EFFECTS OF THREE COMMERCIALLY AVAILABLE PHYTASE ENZYMES ON BROILER PERFORMANCE AND BONE ASH WHEN FED DIETS DEFICIENT IN AVAILABLE PHOSPHORUS

Introduction

Phosphorus (P) makes up 1% of the weight of broilers, with about 80% found in bones and 20% in soft tissue and blood (Waldenstedt, 2006). The bulk of P in the bone is present as hydroxyapatite, which provides important structural support to the skeleton. Because of the need for rapid skeleton development in fast growing broilers, it is essential to meet the P requirement of fast growing broilers. A deficiency of P will result in reduced growth performance, reduced skeletal integrity, and increased mortality. Therefore, a failure to provide adequate P in the diets of broilers can lead to serious problems within the poultry industry (Selle, 2007). Due to these consequences, many integrators have included a safety margin when formulating P concentrations in broiler diets (Waldroup, 1999). The increasing cost of inorganic phosphate sources as well as increased environmental concerns about excessive P concentrations in poultry litter has motivated producers to consider strategies to reduce total P concentrations in broiler diets (Ravindran et al., 1995) without negatively effecting growth performance.

The main strategy currently being used to reduce total dietary P is the inclusion of commercially available phytase, which is an enzyme that hydrolyzes phytate. Phytate is a compound that contains much of the P found within grain seeds which is unavailable for utilization by chickens (Simons et al., 1990). The inclusion of phytase in low P

broiler diets has been shown to increase body weight gain and feed intake in broiler chickens (Simons et al., 1990; Sebastian et al., 1996). To date, many published reports demonstrate that dietary inclusion of phytase allow for reductions of P by approximately 0.1% without resulting in decreased bird performance and health (Simmons et al., 1990; Waldroup et al., 2000; Yan et al., 2003; Dilger et al., 2004). However, limited information is available with respect to increased levels of phytase inclusion focused on increasing the amount of P released from phytate and further reducing total dietary P. Therefore, the current study was conducted to evaluate the effects of three commercially available phytase enzymes with respect to two inclusion rates and the observed effects on broiler performance and bone mineralization.

Materials and Methods

The current project evaluated three commercially available phytase enzymes (A[1], B[2], and C[3]) at two inclusion rates on growth performance and bone mineralization during a 35-day grow out trial in which a starter diet was fed through day 14 and a grower period fed through 35 days of age. The experimental design consisted of a total of 10 treatment groups (Table 3-1). Four of the treatment groups were used to develop a dose response curve with increasing available P levels in increments of 0.05%. The other six treatment groups consisted of supplementation of the three commercial phytase enzymes at two inclusion rates. The low inclusion rate was consistent with inclusion levels to allow for reduced tota1 P by 0.1% while the high inclusion rate of each enzyme

[1] Optiphos 2000 PF- Enzyvia LLC., Sheridan, IN.
[2] Ronozyme 10000 CT- DSM Nutritional Products Ltd., Parsippany, NJ.
[3] Phyzyme 10000 XP- Danisco Animal Nutrition, Marlborough, UK.

Table 3-1. Calculated concentrations of available phosphorus percentage and phytase inclusion rates in broiler starter and grower diets.

Trt #	Phytase Enzyme	Starter AP (%)	Starter (FTU/kg)	Grower AP (%)	Grower (FTU/kg)
1	--	0.22	0	0.17	0
2	--	0.27	0	0.22	0
3	--	0.32	0	0.27	0
4	--	0.37	0	0.32	0
5	A[1]	0.17	250	0.12	250
6	B[2]	0.17	1850	0.12	1850
7	C[3]	0.17	400	0.12	400
8	B[2]	0.14	3700	0.14	3700
9	A[1]	0.12	1000	0.12	750
10	C[3]	0.12	1000	0.12	750

[1] Optiphos 2000 PF- Enzyvia LLC., Sheridan, IN.
[2] Ronozyme 10000 CT- DSM Nutritional Products Ltd., Parsippany, NJ.
[3] Phyzyme 10000 XP- Danisco Animal Nutrition, Marlborough, UK.

was included in an effort to spare between 0.15% and 0.20% total P. Each experimental treatment consisted of six replicate pens of 50 straight-run broilers per replicate; however the lowest available P level (TRT 1) only contained 4 replicate pens. Animal care was provided in accordance with a protocol approved by the Texas A&M University Institutional Animal Care and Use Committee (IACUC) and research was conducted at the Texas A&M Poultry Science Center.

Animals and Management Practices

A total of 2,900 Ross x Ross straight-run broilers were randomly allotted to floor-pens based on body weight and were provided dietary treatment and water *ad libitum*. Chicks were weighed and feed consumption was determined to calculate feed conversion ratio on days 14, 21, and 35. The lowest level of available P without phytase inclusion (TRT 1) was terminated on day 21 due to a high level of observed morbidity and increasing mortality. Fifty straight-run broilers were placed in 6 x 8 broiler rearing pens equipped with tube feeders and nipple drinkers with fresh pine shavings as bedding material.

Experimental Diets

The starter and grower basal diets were corn and soybean meal based and contained a calculated available P level of 0.12% aP and a calculated calcium level of 0.10% (Table 3-2). Monocalcium calcium phosphate, limestone, phytase, and a cornstarch were added to the basal diet to achieve the desired available P level, phytase concentration, and equal calcium concentrations (0.95% in the starter diet and 0.92% in

Table 3-2. Calculated nutrient concentrations and ingredient profile of basal starter (day 1-14) and grower diets (day 15-35). Mono-calcium phosphate, limestone, phytase enzyme, and corn starch were added to achieve the desired available P and enzyme concentration with a calculated calcium concentration of 0.95% for the starter diet and 0.92% for the grower diet.

Ingredient Profile	Starter %	Grower %
Corn	64.67	69.57
Soybean Meal (48%)	33.46	28.51
Sodium chloride	0.51	0.49
A/V Fat Blend	0.60	0.70
L-Lysine HCl	0.18	0.17
DL-Methionine (99%)	0.23	0.19
Vitamins[1]	0.25	0.25
Minerals[2]	0.05	0.05
Coban 60[3]	0.05	0.05
Mono-calcium PO_4	0	0.03
Nutrient Concentration		
Protein (%)	22	20
Metabolizable Energy (kcal/kg)	3050	3100
Methioninine (%)	0.56	0.50
Total Sulfur Amino Acids (%)	0.93	0.84
Lysine (%)	1.30	1.15
Threonine (%)	0.82	0.74
Tryptophan (%)	0.25	0.23
Calcium	0.10	0.10
Sodium	0.22	0.21
Total Phosphorus	0.39	0.38
Available Phosphorus	0.12	0.12

[1] Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

[2] Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, .25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[3] Active drug ingredient monensin sodium, 60 g/lb (90 g/ton inclusion; Elanco Animal Health, Indianapolis, IN). As an aid in the prevention of coccidiosis caused by *Eimeria necarix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, *Eimeria mivati*, and *Eimeria maxima*.

the grower diet). The calculated available P and phytase inclusion rates of the starter and grower diets are described in Table 3-1. Four of the treatments were utilized to develop a dose response curve for observed parameters to calculate increases in bioavailability of P from phytate due to phytase inclusion. The available P concentrations in the starter diet of these treatments were 0.22%, 0.27%, 0.32%, and 0.37% while the available P levels were reduced in the grower diet by 0.05% on day 15. The three phytase enzymes were included in the diets at two dosage levels: the low level of phytase (TRT 5-7) targeted to increase P bioavailability by 0.10%, and a higher phytase inclusion rate (TRT 8-10) targeted to increase P bioavailability between 0.13% and 0.20%. The starter diet was fed as a crumble and the grower diet fed as a pellet. Starter and grower diets included monensin (Coban 90) as a coccidiosis preventative. Diets were pelleted and crumbled at standard conditions for Texas A&M pellet mill with the conditioning and pelleting temperature between 65 and 70C to preserve enzymatic activity. Four feed samples (500 g) of each dietary treatment were collected and sent to an independent laboratory for phytase, total P, and calcium analyses (Table 3-3).

Bone Ash Analysis

On day 35, right tibias were collected from 6 male broilers/ replicate (36 birds/ trt) randomly selected for bone ash determination. Tibias were cleaned of all adhering material, dried for 24 hr at 105°C, and ashed at 600°C for 24 h.

Table 3-3. Analyzed[1] calcium, total phosphorus and phytase activity level of each treatment for the starter and grower diets.

TRT	Starter			Grower		
	Calcium	Phosphorus	Phytase FTU/kg	Calcium	Phosphorus	Phytase FTU/kg
1	1.23	0.48	ND[2]	1.06	0.44	ND
2	1.12	0.51	ND[2]	1.09	0.48	ND
3	1.05	0.60	ND[2]	1.08	0.54	ND
4	1.11	0.63	ND[2]	1.03	0.59	ND
5	0.91	0.42	230	0.94	0.38	240
6	0.90	0.42	1,600	0.99	0.38	1,700
7	1.01	0.43	560	0.97	0.37	530
8	0.98	0.40	2,500	0.99	0.41	2,600
9	1.06	0.39	1,100	0.94	0.37	740
10	1.10	0.37	1,300	1.02	0.38	790

[1] Calcium, total phosphorus, and phytase activity level as analyzed by an independent laboratory from a pooled sample of four 250 gm samples taken during the manufacture of each diet.
[2] Not Detected

Statistical Analysis

Body weight, feed consumption, mortality corrected feed conversion ratio, and bone parameters including ash weight and percentage were analyzed via a one-way ANOVA using the General Linear Model. Mortality data was transformed using an acrsin transformation prior to analysis. Means were deemed significantly different at $p < 0.05$ and were separated using Duncan's Multiple Range Test. Regression analysis was conducted on dose response treatments (TRT 1-4) and determined equation used to calculate increase in bioavailability associated with phytase inclusion.

Results

Throughout the trial, increasing levels of available P concentration in the dose response treatment groups (TRT 1-4) positively influenced growth parameters and bone mineralization with observed increases ($p < 0.05$) in body weight, feed consumption, and bone ash while reducing feed conversion ratio and mortality (Table 3-4, 3-5 and 3-6). Phytase inclusion of all three commercial enzymes positively influenced growth parameters (Table 3-4 and 3-5), as all inclusion levels of each enzyme out performed ($p < 0.05$) the dose response treatment with the lowest level of available P (TRT 1) which contained a higher aP level than each of the phytase treatment groups. Body weights at each observed age (Day 14, 21, and 35) with inclusion of phytase A and B (TRT 5 and 6) at the low level resulted in either increased ($p < 0.05$) or similar body weights to TRT 2 (table 3-4), which contained 0.10% more available P. Phytase C (TRT 7) at the low level of inclusion had similar body weight as compared to TRT 2 at Day 14, but body

weight at the conclusion of the trial was decreased ($p < 0.05$) compared to TRT 2 (Table 3-4). Phytase A and B at the higher inclusion level (TRT 8 and 9) had increased ($p<0.05$) body weights as compared to TRT 2 which contained 0.15% more available P, but did not reach the level of TRT 3 throughout the trial (Table 3-4). Phytase C at the high inclusion rate (TRT 10) yielded similar body weights on day 14 and 35 as compared to TRT 2 (Table 3-4).

Increasing the available P concentration of the dose response diets (TRT 1-4) decreased ($P<0.05$) mortality rates throughout the experiment (Table 3-4). The lowest level of available P in the dose response treatments was terminated on day 21 due to impaired mobility, high rates of morbidity, and the a sudden onset of mortality between day 19 and 21. Inclusion of all phytase enzymes reduced mortality compared to TRT 1 which contained a higher level of available P. Mortality rates were consistent between Phytase A and B with both inclusion levels, while the observed mortality rate for Phytase C was increased (Table 3-4).

Mortality corrected feed conversion ratio (FCR) decreased ($p <0.05$) as the available P concentration increased in the dose response treatment groups during the starter and grower phases (Table 3-5). The lowest level of available P yielded the highest observed FCR during the starter phase. Decreases in FCR were observed as available P increased in TRT 2 and further improvement in FCR was observed with increased available P to TRT 4 in the starter phase.

Table 3-4. Average body weight and mortality rate of market broilers fed varying levels of available phosphorus with the inclusion of three phytase enzymes at two different inclusion rates.

Trt #	Phytase Enzyme	Phytase level Starter/Grower (FTU/kg)	Starter/ Grower aP (%)	Body Weight Day 14 (g)	Body Weight Day 21 (g)	Body Weight Day 35 (kg)	Mortality Day 1-21	Mortality Day 1-35
1	-		0.22/0.17	209.7 ± 6.7^{e}	332.9 ±16.7 ^{g}		25.0 ± 2.5 ^{a}	
2	-		0.27/0.22	272.8 ± 4.1 ^{d}	489.7± 7.3 ^{e}	1.11 ± 0.01 ^{de}	8.3 ± 2.6 ^{cd}	18.0 ± 3.7 ^{bc}
3	-		0.32/0.27	331.1 ± 4.3 ^{b}	606.1± 7.5 ^{b}	1.47 ± 0.03 ^{b}	3.0 ± 0.9 ^{de}	4.3 ± 1.0 ^{ef}
4	-		0.37/0.32	369.5 ± 6.5 ^{a}	687.3 ± 10.6 ^{a}	1.68 ± 0.02 ^{a}	1.7 ± 1.0 ^{e}	1.7 ± 1.0 ^{f}
5	A[1]	250/ 250	0.17/0.12	297.2 ± 5.7^{c}	502.0 ± 8.9 ^{e}	1.09 ± 0.03 ^{e}	6.3 ± 2.0 ^{cde}	15.7 ± 2.8 ^{cd}
6	B[2]	1850/ 1850	0.17/0.12	310.0 ± 1.9 ^{c}	532.1± 5.0 ^{d}	1.18 ± 0.01 ^{d}	4.7 ± 1.2 ^{de}	11.7 ± 2.2 ^{cde}
7	C[3]	400/ 400	0.17/0.12	264.8 ± 3.5 ^{d}	437.7± 9.3 ^{f}	0.95 ± 0.02 ^{f}	11.3 ± 2.5 ^{bc}	24.7 ± 3.6 ^{ab}
8	B[2]	3700/ 3700	0.14/0.14	303.0 ± 3.8 ^{c}	572.2 ± 5.2 ^{c}	1.38 ± 0.03 ^{c}	6.3 ± 1.9 ^{cde}	8.3 ± 2.0 ^{def}
9	A[1]	1000/ 750	0.12/0.12	310.5 ± 3.9 ^{c}	567.7 ± 7.7^{c}	1.37 ± 0.04 ^{c}	4.7 ± 0.7 ^{de}	7.3 ± 1.4 ^{ef}
10	C[3]	1000/ 750	0.12/0.12	268.9 ± 5.8 ^{d}	460.0 ± 9.9 ^{f}	1.05 ± 0.03 ^{e}	14.3 ± 3.1 ^{b}	27.7 ± 2.0 ^{a}

$^{a-g}$ means within columns with different superscripts differ significantly at $P < 0.05$.

[1] Optiphos 2000 PF- Enzyvia LLC., Sheridan, IN.

[2] Ronozyme 10000 CT- DSM Nutritional Products Ltd., Parsippany, NJ.

[3] Phyzyme 10000 XP- Danisco Animal Nutrition, Marlborough, UK.

Table 3-5. Mortality corrected feed conversion ratio and feed consumption of market broilers fed varying levels of available phosphorus with the inclusion of three phytase enzymes at two different inclusion rates.

Trt #	Phytase Enzyme	Phytase level Starter/Grower (FTU/kg)	Starter/ Grower aP (%)	Feed: Gain Day 1-14	Feed: Gain Day 15-35	Feed: Gain Day 1-35	Starter Feed Consumed g/bird/d	Grower Consumed g/bird/day
1	-		0.22/0.17	1.41 ± .02 [a]			16.8 ± 0.39 [g]	
2	-		0.27/0.22	1.36 ± .02 [b]	1.79 ± .03[c]	1.69 ± .01 [b]	22.5 ± 0.23 [e]	67.3 ± 0.79 [e]
3	-		0.32/0.27	1.30 ± .01 [cd]	1.72 ± .02 [d]	1.63 ± .01 [c]	27.0 ± 0.32 [b]	92.6 ± 1.7 [b]
4	-		0.37/0.32	1.29 ± .01 [d]	1.70 ± .01 [d]	1.62 ± .01 [c]	30.5 ± 0.72 [a]	105.9± 1.1 [a]
5	A[1]	250/ 250	0.17/0.12	1.33 ± .02 [bcd]	1.86 ± .03 [b]	1.71 ± .01 [b]	24.3 ± 0.40 [d]	65.2 ± 1.5 [e]
6	B[2]	1850/ 1850	0.17/0.12	1.34 ± .01 [bc]	1.86± .04 [b]	1.72 ± .02[ab]	26.1 ± 0.16 [c]	72.4 ± 0.41[d]
7	C[3]	400/ 400	0.17/0.12	1.36 ± .01 [b]	1.93 ± .03 [a]	1.76 ± .01 [a]	21.6 ± 0.33 [ef]	54.9 ± 1.9 [g]
8	B[2]	3700/ 3700	0.14/0.14	1.35 ± .02 [bc]	1.73 ± .01 [d]	1.64 ± .01 [c]	25.1 ± 0.33 [cd]	86.6 ± 1.5 [c]
9	A[1]	1000/ 750	0.12/0.12	1.32 ± .01 [bcd]	1.74 ± .04 [d]	1.65 ± .01 [c]	25.4 ± 0.18 [c]	86.1 ± 1.9 [c]
10	C[3]	1000/ 750	0.12/0.12	1.32 ± .02 [bcd]	1.85 ± .02 [b]	1.70 ± .02 [b]	21.1 ± 0.27 [f]	60.4 ± 2.0 [f]

[a-g] means within columns with different superscripts differ significantly at $P < 0.05$.

[1] Optiphos 2000 PF- Enzyvia LLC., Sheridan, IN.

[2] Ronozyme 10000 CT- DSM Nutritional Products Ltd., Parsippany, NJ.

[3] Phyzyme 10000 XP- Danisco Animal Nutrition, Marlborough, UK.

Grower phase and cumulative FCR for TRT 2 was increased compared to the two highest levels of available P in the dose response treatment groups. No differences ($p>0.05$) were observed in FCR among any of the three phytase enzymes or inclusion level during the starter phase with all resulting in similar FCR as compared to TRT 2 (Table 3-5). Phytase A and B (TRT 5 and 6) at the low phytase inclusion rate yielded similar FCR during the grower phase of the experiment. Phytase C (TRT 7) exhibited increased ($p < 0.05$) FCR compared to the other two enzymes, however all three enzymes at the low level of inclusion resulted in increased FCR compared to the remaining three dose response treatment groups. Increased levels of phytase inclusion resulted in improve FCR as compared with the low level of inclusion for each enzyme. However, the trend continued that Phytase C exhibited higher FCR as compared to Phytase A and B which had similar FCR compared to the two highest levels of available P. Cumulative FCR followed similar patterns as the grower phase FCR, with TRT 2 yielding the highest ($p < 0.05$) FCR of the dose response treatments. With regard to the low inclusion rates, the observed FCR for Phytase A was reduced ($p < 0.05$) as compared to Phytase C with the Phytase B being intermediate. Similar to the grower phase, increased levels of phytase inclusion improved FCR for all three enzymes, however Phytase C did not perform to the same level of Phytase A and B which was similar to the highest available P concentration in the dose response treatments.

Feed consumption increased linearly as available P concentration increased in the dose response treatment groups (Table 3-5). Increases in feed consumption were observed with each increase in available P level during the starter phase. Inclusion of all

three phytase enzymes at both levels of inclusion resulted in increased ($p < 0.05$) feed consumption compared to the lowest level of available P. Phytase A and B inclusion at the low level increased ($p < 0.05$) feed consumption compared to TRT 2. Phytase B inclusion resulted in the highest feed consumption compared to the two other phytase enzymes at the low level of inclusion during the starter phase. The high level of inclusion of Phytase A and B resulted in increased feed consumption as compared to the high level of inclusion of Phytase C. Feed consumption for each phytase enzyme was similar between both inclusion rates during the starter phase (Table 3-5). Feed consumption during the grower phase resulted in similar observations as the starter phase with increasing available P concentrations increasing ($p < 0.05$) feed consumption in the dose response treatments. Feed consumptions for both levels of inclusion of Phytase A and B met or exceeded the level of consumption observed for TRT 2; however Phytase C inclusion did not reach a similar level. Differences in consumption were observed between the three enzymes with regard to the low level of inclusion, with Phytase B yielding the highest feed consumption and Phytase C yielding the lowest consumption rate. With regard to the high level of inclusion, feed consumption was similar for Phytase A and B with an observed rate between that observed between TRT 2 and TRT 3; however Phytase C inclusion did not achieve a similar response.

The average body weight of sampled males for tibia ash analysis (Table 3-6) was similar to the Day 35 straight-run body weight data (Table 3-4). Each increase in available P in the dose response treatments resulted in increased ($p < 0.05$) weights. With regard to the low and high levels of phytase inclusion, differences were observed

between each of the three enzymes with Phytase B yielding higher body weights and Phytase C yielding the lowest body weights (Table 3-6).

Similar results were witnessed in relation to average tibia weight (grams) and ash weight (mg). The increasing available P in the dose response treatments resulted in increased ($p < 0.05$) tibia and ash weight. The inclusion of Phytase A (TRT 5) and Phytase B (TRT 6) resulted in similar weights to TRT 2. The inclusion of Phytase A (TRT 9) at the high inclusion level resulted in the highest ($p<0.05$) tibia and ash wt. observed among the treatment groups and was slightly ($p<0.05$) higher than Phytase B (TRT 8) at the high inclusion rate. The inclusion of both levels of Phytase C (TRT 7 & 10) resulted in the lowest tibia and ash weight observed in the trial.

Bone ash (%) was increased with each increasing level of available P (TRT 2-4) in the dose response treatment groups (Table 3-6). Phytase A and B at the low level of inclusion were similar, with the inclusion of both enzymes resulting in bone ash percentages similar to the observed ash percentage in TRT 2 which included available P of 0.10% higher in the diet. Phytase C at the low level of inclusion resulted in the lowest observed bone ash. With regard to the high level of phytase inclusion, all enzymes reached a level of bone ash percentage comparable to TRT 2 while Phytase A and B reached a level similar to TRT 3 which included an increased available P concentration of 0.20% in the starter and 0.15% in the grower diet.

Table 3-6. Tibia weight, ash weight, and tibia ash percent of market broilers fed varying levels of available phosphorus with the inclusion of three phytase enzymes at two different inclusion rates.

Trt #	Phytase Enzyme	Phytase level Starter/Grower (FTU/kg)	Starter/ Grower aP (%)	Ave BW of Sampled Males	Ave Tibia Weight (g)	Ash Wt (mg)	Ash (%)
1	-		0.22/0.17				
2	-		0.27/0.22	1229.4 ± 21.1 ^{ef}	8.12 ± 0.16 ^{de}	206 ± 5.9^{de}	40.57 ± .31 ^{c}
3	-		0.32/0.27	1596.0 ± 20.9 ^{b}	9.54 ± 0.21 ^{b}	277.1 ± 8.1^{b}	42.79 ± .46 ^{b}
4	-		0.37/0.32	1830.5 ± 19.4 ^{a}	10.53 ± 0.17 ^{a}	347.9 ± 4.1^{a}	44.20 ± .54 ^{a}
5	A[1]	250/ 250	0.17/0.12	1268.9 ± 25.1 ^{e}	8.32 ± 0.20 ^{d}	217.2 ± 3.5^{de}	41.42 ± .11 ^{c}
6	B[2]	1850/ 1850	0.17/0.12	1341.7 ±23.3 ^{d}	8.58 ± 0.13 ^{cd}	224 ± 2.1^{d}	40.98 ± .24 ^{c}
7	C[3]	400/ 400	0.17/0.12	1083.8 ± 25.6 ^{g}	7.19 ± 0.18 ^{f}	173 ± 8.8^{f}	39.44 ± .21 ^{d}
8	B[2]	3700/ 3700	0.14/0.14	1468.1 ± 25.3 ^{c}	8.92 ± 0.16 ^{c}	258.8 ± 4.5^{c}	42.56 ± .39 ^{b}
9	A[1]	1000/ 750	0.12/0.12	1579.8 ± 27.7 ^{b}	9.77 ± 0.18 ^{b}	293.7 ± 8.0^{b}	42.94 ± .19 ^{b}
10	C[3]	1000/ 750	0.12/0.12	1171.6 ± 25.4 ^{f}	7.75 ± 0.19 ^{e}	201.3 ± 5.5^{e}	41.03 ± .29 ^{c}

$^{a-g}$ means within columns with different superscripts differ significantly at $P < 0.05$.

[1] Optiphos 2000 PF- Enzyvia LLC., Sheridan, IN.

[2] Ronozyme 10000 CT- DSM Nutritional Products Ltd., Parsippany, NJ.

[3] Phyzyme 10000 XP- Danisco Animal Nutrition, Marlborough, UK.

To calculate the increase in bioavailability of P from the inclusion of the three enzymes at both levels of inclusion, regression analyses was performed, calculating equations based on the observed body weight at day 14 (Figure 3-1), day 35 (Figure 3-2), and bone ash (Figure 3-3) percentage as a function of inorganic P consumed. The determined equations for each evaluated parameters were y=188.01x+177.83 (Equation 1, Day 14 BW in grams), y=0.16x+0.85 (Equation 2, Day 35 BW in kg) and y=1.06x+38.75 (Equation 3, bone ash %). Individual data points represent observed parameters for each replicate pen. Following regression analysis, these equations were used to determine the amount of inorganic P that would need to be consumed to achieve the observed effect of each phytase inclusion for each enzyme.

P bioavailability increase was calculated for each enzyme for both inclusion levels following the correction for inorganic P and feed consumption with the results shown in Table 3-7. Inclusion of each of the three enzymes at both inclusion rates increased the bioavailability of P (Table 3-7). Each enzyme reached the level of an increase of 0.10% in P bioavailability in at least one of the evaluated parameters. Phytase A and B inclusion at the low rate exceeded the expected release of 0.10% in multiple parameters reaching the level of 0.14%. High inclusion rates of each enzyme were effective at exceeding the 0.10% increase in P bioavailability with the highest observed increase for each enzyme of 0.20% for Phytase A, 0.17% for Phytase B, and 0.16% for Phytase C (Table 3-7).

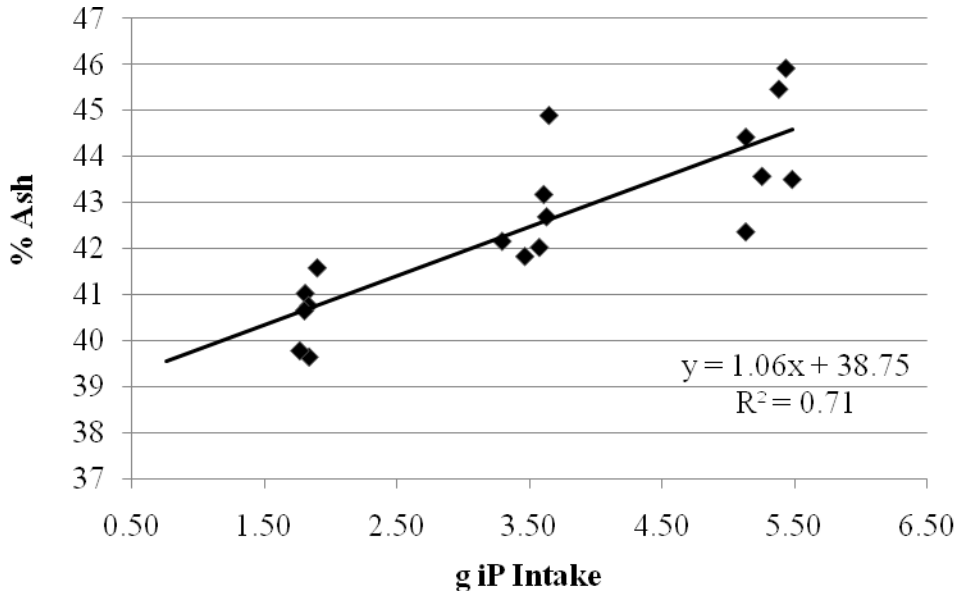

Figure 3-1. Regression analysis depicting the relationship between inorganic phosphorus consumption and the determined tibia ash percentage observed from male broilers at 35 days of age fed diets varying in available phosphorus concentrations.

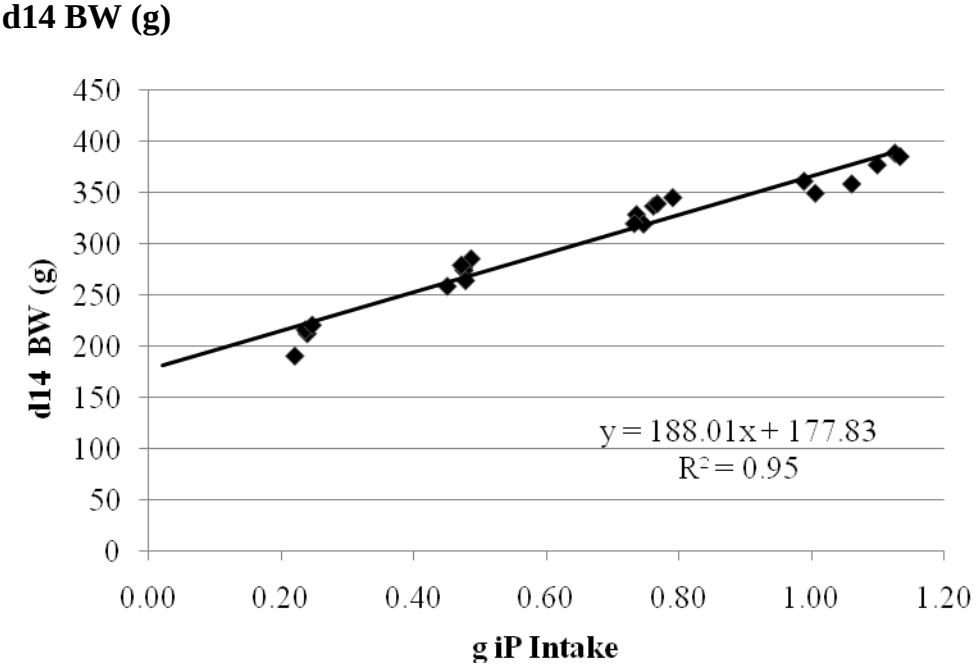

Figure 3-2. Regression analysis depicting the relationship between inorganic phosphorus consumption and the observed average body weight of straight-run broilers at 14 days of age fed diets varying in available phosphorus concentration.

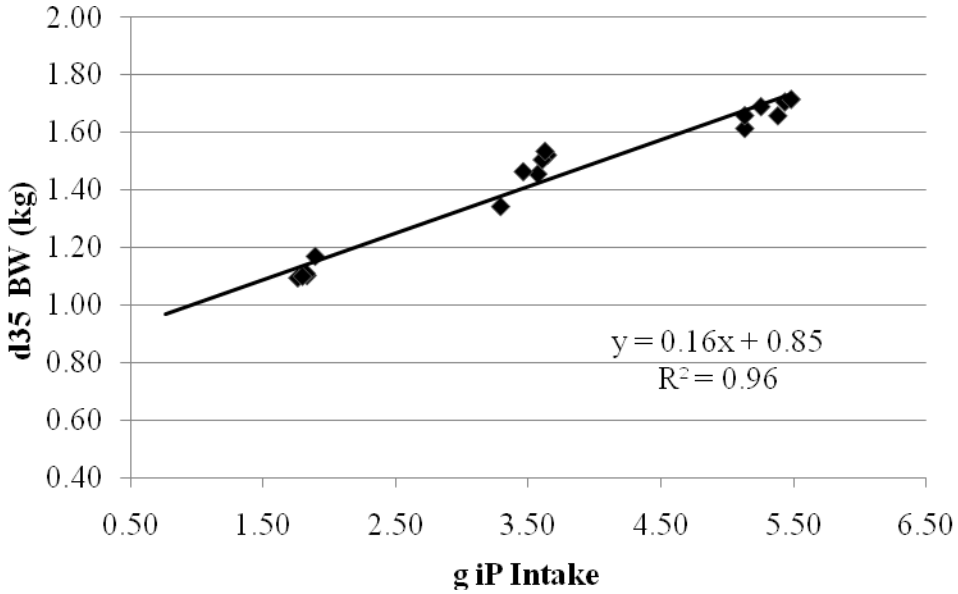

Figure 3-3. Regression analysis depicting the relationship between inorganic phosphorus consumption and the observed average body weight of straight-run broilers at 35 days of age fed diets varying in available phosphorus concentration.

Table 3-7. Calculated increase in phosphorus bio-availability (%) due to inclusion of three phytase enzymes. Calculations were based on regression equations of bone ash percentage (equation 1), day 14 body weight (equation 2), and day 35 body weight (equation 3) of broilers fed selected levels of available phosphorus (aP).

Trt #	Phytase Enzyme	Starter/ Grower aP (%)	Starter/ Grower Phytase FTU/kg	Body Weight Day 14[4]	Body Weight Day 35[5]	Bone Ash[6]
5	A[1]	0.17/0.12	250/250	0.14	0.13	0.08
6	B[2]	0.17/0.12	1850/1850	0.10	0.14	0.10
7	C[3]	0.17/0.12	400/400	0.04	0.10	0.03
8	B[2]	0.14/0.14	3700/3700	0.15	0.17	0.14
9	A[1]	0.12/0.12	1000/750	0.19	0.20	0.15
10	C[3]	0.12/0.12	1000/750	0.14	0.16	0.08

[1] Optiphos 2000 PF- Enzyvia LLC., Sheridan, IN.
[2] Ronozyme 10000 CT- DSM Nutritional Products Ltd., Parsippany, NJ.
[3] Phyzyme 10000 XP- Danisco Animal Nutrition, Marlborough, UK.
[4] Calculated using regression analysis comparing inorganic phosphate consumption and observed body weight at day 14 (g). (y=188.01x+177.83)
[5] Calculated using regression analysis comparing inorganic phosphate consumption and observed body weight at day 35 (kg). (y=0.16x+0.85)
[6] Calculated using regression analysis comparing inorganic phosphate consumption and observed bone ash (%). (y=1.06x+38.75)

Discussion

It is recognized that increasing the levels of available P in phosphorus deficient diets results in improved bird performance and increased bone mineralization (Angel et al., 2000a; Yan et al., 2001; Dhandu et al., 2003; Dilger et al., 2004; Powell et al., 2008). Increasing the available P concentration in the dose response diets (TRT 1-4) resulted in linear improvements in body weight, FCR, feed consumption, bone ash, and mortality with each increase of 0.05%. Similar results were observed by Simons et al. (1990) with observed increases in body weight and decreased feed conversions associated with increasing available P levels in a broiler starter diet. Yan et al. (2001) also observed that increasing the available P level with similar increments of the current study from 3-6 weeks of age resulted in increased tibia ash weight and body weight.

The inclusion of the three phytase enzymes at both inclusion rates resulted in an increase in P bioavailability leading to improvements in broiler performance as compared to the dose response treatments that contained equivalent or higher levels of available P concentrations. These observations of enhanced growth parameters and bone mineralization have been reported with the use of multiple enzyme preparations. Yan et al. (2001) reported increases in body weight and tibia ash and improvements in feed conversion with the inclusion of 800 FTU/kg of Natuphos phytase in broiler diets. Dilger et al. (2004) reported similar observations as seen in the current trial with observed increased body weight gain and feed intake during the starter and grower period with the inclusion of varying levels of Phyzyme XP as compared to a control diet with the same level of Ap. The higher inclusion rate of each phyase enzyme in the

current study (TRT 8-10) resulted in increased ash percentage, day 35 body weight and decreased cumulative FCR as compared to the lower level of inclusion for each enzyme in the experimental design. Simons et al. (1990) also witnessed similar results in that increasing phytase levels from 250-1500 resulted in decreased FCR and increased body weights when included in diets fed through 24 days of age.

During the starter period (Day 1-14) in the current study, the observed FCR of all phytase treatment groups (TRT 5-10) were comparable to TRT 2. The diets with the low level of phytase inclusion (TRT 5-7) had an available P concentration 0.1% less than TRT 2, yet yielded similar if not improved body weights and FCR. The starter period data suggests that in relation to body weight and FCR, the low level of phytase inclusion for all three enzymes was sufficient to make up for the removal of 0.10% available P in young broilers. Simons et al. (1990) witnessed similar results in that 500 FTU inclusion of phytase in a diet containing 4.5 g/kg of P had similar body weights and feed conversion as a diet with 6 g/kg of P feed through day 24. Waldroup et al. (2000) reported the inclusion of 800 FTU/kg of Natuphos with a diet containing 0.15% aP had a body weight (0-3 week) similar to a diet containing 0.30% available P. The same phytase inclusion level in the 0.15% available P diet resulted in a feed conversion similar to a diet containing 0.25% available P. One of the greatest benefits of phytase supplementation in these diets was maintaining livability at extremely low levels of available P, which have been shown to cause excessive mortality (Waldroup et al., 2000). Mortality was highest at the lower levels of aP, and the addition of phytase enzymes to the diets considerably reduced the mortality rates.

Increasing the level of phytase inclusion (TRT 8 and 9) resulted in similar cumulative FCR and ash % as TRT 3 (0.32% available P in the starter diet) suggesting that these levels of inclusion can spare between 0.13% and 0.20% available P. Based on regression analysis, Phytase A and B at the low level of inclusion were able to increased P bioavailability up to or greater than 0.10% for all evaluated parameters from phytate sources present in the diet. Phytase C at the low level had slightly lower releasing values ranging between 0.04% and 0.10%. Increased phytase inclusion for all three enzymes resulted in increases of P bioavailability ranging from a low of 0.08% to a high of 0.20% depending on the enzyme and parameter evaluated. Phytase A achieved the highest increase of P bioavailability with a calculated value of 0.20% while Phytase B and C achieved a 0.17% and 0.16% maximum increase in P bioavailability, respectively. Previous research has estimated varying amounts of phytate-bound P that can be released from the diet, with variations depending on the level of phytase used, the type of diet, and the stage of growth that is evaluated. Nelson et al. (1971) indicated that between 50-100% of phytate-bound P in a corn soy diet is capable of hydrolyzation by use of varying levels of phytase enzymes. Simons et al. (1990) suggested that more than 60% of the P was released due to phytase inclusion in the diet, while Waldroup et al. (2000) suggested that the inclusion of 800 FTU of Natuphos was capable of releasing approximately 50% of the phytate P from the diet.

All evaluated phytase enzymes in the current study resulted in an increase in bioavailability of dietary P from phytate; however the responses varied depending on enzyme and inclusion rate. These data confirm that each of the phytase enzymes have

the ability to increase P bioavailability up to or greater than 0.15% from phytate allowing broiler integrators to use increased levels of phytase to compensate for the use of inorganic phosphates when determined cost effective. This strategy of increased phytase inclusion can be used to decrease the levels of fecal P that is present in poultry litter and reduce the environmental impact associated with land application of litter.

CHAPTER IV

EVALUATION OF ENERGY ENZYMES IN BROILER DIETS VARYING IN NUTRIENT AND ENERGY LEVELS AS MEASURED BY BROILER PERFORMANCE AND PROCESSING PARAMETERS

Introduction

Broiler diets are formulated using cereal grains that contain varying levels of fibrous material called non-starch polysaccharides (NSP). NSP contain anti-nutritive properties leading to increased intestinal viscosity, reduced digestibility of the nutrients, increased feed conversion ratio (FCR), and decreased bird performance (Bedford and Classen, 1992; Bedford and Morgan, 1996; Lazaro et al., 2003, Meng et al., 2005b). Some of the common NSP's found in soybeans and corn includes sucrose, raffinose, and galactose which have shown to negatively affect bird performance by reducing the digestibility of the diet (Kocher et al., 2002). NSP's function to reduce the digestibility of the diets by increasing the intestinal viscosity due to the fiber content and by the cell wall polysaccharides entrapping protein and energy (Meng et al., 2005b). Because chickens have a high rate of food passage and lack the digestive capacity of that of ruminant animals, the presence of NSPs in the diet negatively influence nutrient digestibility. NSPs are indigestible to monogastric animals and high concentrations of NSPs result in a reduction in nutrient utilization (Meng et al., 2005b).

A strategy that has been used to limit the negative impact of NSPs present in poultry diets is the use of a variety of carbohydrase enzymes. These enzymes function to hydrolyze indigestible bonds in the plant's cell wall into smaller fragments allowing

for improved digestibility in poultry. Many of the grains used in poultry diets contain a variety of NSP's including Aribino-xylans, β-mannan, β-glucans, etc.; therefore the use of products containing a cocktail of enzymes ranging in specificity may be the most effective practice in NSP degradation resulting in increased metabolizable energy in poultry diets. The use of energy enzymes have shown to be effective in the high fiber diets by decreasing intestinal viscosity and increasing feed efficiency when fed in wheat-based diets (Bedford and Classen, 1992; Choct et al., 1999; Meng et al., 2005b; Goa et al., 2007). Unfortunately, reports on the success of enzyme inclusion in promoting growth have varied in corn-soy based diets, which are the primary cereal grains used in diet formulation in the US poultry industry (Kocher et al., 2002; Gracia et al., 2003; Meng and Slominski, 2005a). Therefore, the objective of the current experiment was to evaluate the effect of inclusion of two propriety blends of cocktail carbohydrase enzymes (NSPase) in a corn-soy based broiler diets varying in nutrient concentration on broiler growth performance and processing parameters.

Materials and Methods

Experimental Design

To evaluate the effect of NSPase inclusion on broiler growth performance and processing parameters in diets varying in nutrient densities, a 3 (diet) x 3 (NSPase) factorial design was utilized during a 41-day grow out. Three dietary nutrient profiles were used in the experimental design including a control, a diet with reduced energy level, and a diet with a reduced energy and protein level all supplement with one of three enzyme preparations including a control, NSPase A, or NSPase B.

Animals and Management Practices

On day of hatch, 2,025 chicks were randomly allotted to floor-pens and dietary treatments based on body weight. Chicks were provided age appropriate supplement heat and given access to feed and water *ad libitum.* All broilers and feed were weighed on days of dietary changes (day 12, 26 and 41) for calculation of average body weight and feed conversion ratio. Upon completion of the trial (Day 41), 10 male broilers/ replicate were randomly selected and processed to obtain processing yield data.

The experimental design consisted of 9 experimental diets in which there were 5 replicates per diet, and 45 straight run chicks per replicate. Broilers were placed in 6ft x 6ft rearing pens equipped with tube feeders and nipple drinkers with fresh pine shavings as bedding material. Animal care was provided in accordance with a protocol approved by the Texas A&M University Institutional Animal Care and Use Committee (IACUC).

Experimental Diets

The three basal diets were corn and soybean meal based with varying nutrient profiles (Table 4-1). The basal diets consisted of a Positive Control diet which was representative of a local broiler integrator (PC), Negative Control 1 diet (NC1- 4% reduction in ME and protein) and a Negative Control 2 diet (NC2- 4% reduction in ME) (Table 4-2). Each basal diet was divided into thirds and each NSPase enzyme was added to a portion prior to crumbling or pelleting. The two NSPase enzymes (A and B[4]) used in the experiment were included a rate of 0.25 lb/ton and were proprietary blends of Xylanase, β-Glucanase, β-Mannanase, and α-Galactosidase. A starter diet (crumble) was

[4] Enzyvia LLC., Sheridan, IN.

fed from day 1 to day 12, a grower diet (pellet) was fed from day 13 to day 26, and finisher diet (pellet) was fed from day 27 to 41 days of age (termination of the trial). Diets were pelleted and crumbled with the conditioning and pelleting temperature not to exceed 70°C to preserve enzyme activity. Dietary samples (~ 250 grams each) of all diets (5 samples taken at regular intervals) were taken and sent to an independent laboratory for nutrient analysis.

Termination of Trial

All broilers were bulk weighted on the evening of day 41 prior to an eight hour feed withdrawal period for processing on day 42. Ten male broilers from each replicate pen (50 males/ trt) of all treatments containing NSPase B and enzyme control treatments (TRTS 1-3 and 7-9) were removed and individually weighed before processing. Following a 16 hour air chill of the carcasses, breast fillets and tenderloins were removed and weighed to calculate carcass and breast yield. In an effort to increase sample size, only broilers from non-supplemented and NSPase B supplemented treatment groups were processed because performance characteristics of both NSPase A and B were similar at the conclusion of grow out.

Statistical Analysis

Body weights and mortality corrected feed conversion ratios were analyzed via a 3 x 3 factorial ANOVA using the General Linear Model Procedure. Main effect means were deemed significantly different at $p < 0.05$ and were separated using Duncan's Multiple Range Test. Processing data was subjected to a 3 x 2 factorial since data from NSPase A inclusion treatments was not gathered. Means were deemed significantly

Table 4-1. Calculated and analyzed nutrient content and ingredient profile of the three basal diets positive control (PC), negative control 1 (NC1), and negative control 2 (NC2) for the starter (D 1-12), grower (D 13-26), and finisher diets (D 27-42).

Ingredient Profile	Starter %			Grower %			Finisher %		
	PC	NC1	NC2	PC	NC1	NC2	PC	NC1	NC2
Corn	59.19	65.21	62.53	64.39	70.00	67.65	71.14	75.49	74.25
Soybean Meal (48%)	30.29	27.41	29.69	25.19	22.67	24.67	19.27	19.39	19.40
Sodium chloride	0.48	0.48	0.48	0.43	0.43	0.43	0.43	0.45	0.44
A/V Fat Blend	3.65	0.50	0.90	3.93	0.82	1.19	3.5	0.47	0.74
Pork MBM	3.5	3.5	3.5	3.5	3.5	3.5	3.5	1.39	2.78
L-Lysine HCl	0.21	0.23	0.22	0.26	0.27	0.27	0.23	0.24	0.24
DL-Methionine (99%)	0.26	0.24	0.26	0.22	0.21	0.22	0.20	0.18	0.19
Vitamins[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Minerals[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Coban 60[3]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Mono-calcium PO_4	0.97	0.97	0.96	0.76	0.77	0.76	0.56	0.92	0.68
Limestone	1.11	1.12	1.12	0.96	0.98	0.97	0.83	1.12	0.93
Calculated Nutrient Content									
Protein (%)	22	21.1	22	20	19.25	20	17.60	16.90	17.60
Metabolizable Energy (kcal/kg)	3095	2962	2962	3168	3035	3036	3212	3078	3079
Methioninine (%)	0.58	0.55	0.58	0.52	0.50	0.52	0.42	0.45	0.47
TSAA (%)	0.92	0.88	0.92	0.83	0.80	0.83	0.75	0.72	0.75
Lysine (%)	1.3	1.24	1.30	1.20	1.15	1.20	1.02	0.98	1.02
Threonine (%)	0.80	0.76	0.80	0.72	0.69	0.72	0.63	0.60	0.63
Tryptophan (%)	0.24	0.22	0.24	0.21	0.19	0.22	0.17	0.19	0.19
Calcium	0.95	0.95	0.95	0.85	0.85	0.85	0.75	0.75	0.75
Sodium	0.22	0.22	0.22	0.20	0.20	0.20	0.20	0.20	0.20
Total Phosphorus	0.70	0.70	0.70	0.64	0.64	0.64	0.58	0.58	0.58
Available Phosphorus	0.45	0.45	0.45	0.40	0.40	0.40	0.35	0.35	0.35
Analyzed Nutrient content									
Crude Protein (%)	24	21.4	23.3	21.8	20.3	20.7	18.0	17.5	18.1
ME (kcal/kg)	2998	2932	2910	3175	3042	3086	3241	3064	3086
Total Phosphorus	0.90	0.90	0.89	0.80	0.74	0.77	0.73	0.71	0.78

[1] Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

[2] Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[3] Active drug ingredient monensin sodium, 60 g/lb (90 g/ton inclusion; Elanco Animal Health, Indianapolis, IN). As an aid in the prevention of coccidiosis caused by *Eimeria necarix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, *Eimeria mivati*, and *Eimeria maxima*.

Table 4-2. Calculated energy and protein levels of the starter, grower and finisher diets fed to straight-run market broilers through 41 days of age supplemented with NSPase enzyme preparations.

			Starter Diet Day 1-12		Grower Diet Day 13-26		Finisher Diet Day 27-41	
TRT #	Diet	NSPase Enzyme	ME (kcal/kg)	CP (%)	ME (kcal/kg)	CP (%)	ME (kcal/kg)	CP (%)
1	Positive Control	-	3058	22.0	3168	20.0	3212	17.5
2	Negative Control 1	-	2962	21.1	3035	19.2	3078	16.9
3	Negative Control 2	-	2962	22.0	3035	20.0	3078	17.5
4	Positive Control	A[1]	3058	22.0	3168	20.0	3212	17.5
5	Negative Control 1	A[1]	2962	21.1	3035	19.2	3078	16.9
6	Negative Control 2	A[1]	2962	22.0	3035	20.0	3078	17.5
7	Positive Control	B[1]	3058	22.0	3168	20.0	3212	17.5
8	Negative Control 1	B[1]	2962	21.1	3035	19.2	3078	16.9
9	Negative Control 2	B[1]	2962	22.0	3035	20.0	3078	17.5

[1] Both NSPase enzymes are proprietary cocktail blends containing varying concentrations of Xylanase, β-Glucanase,
β-Mannanase, and α-Galactosidase

different at $p < 0.05$ and were separated using Duncan's Multiple Range Test. In instances were a significant interaction was present between diet and NSPase inclusion, data was subjected to a one-way ANOVA with means deemed significantly different at $p < 0.05$ and separated using Duncan's Multiple Range Test.

Results

Reduction of nutrient concentrations in both the NC1 and NC2 diets resulted in decreased performance in multiple parameters at multiple time points. The reduced energy and protein level in the NC1 diet negatively influence ($p < 0.05$) body weight beginning on day 12 as compared to the PC diet and persisted through day 26 (Table 4-3). Body weight was also depressed ($p < 0.05$) in the NC2 as compared to the PC diet on day 26 of age however increased compared to the NC1. Positive influences were not observed ($p > 0.05$) with regard to body weight with inclusion of either NSPase enzyme on days 12 and 26. There was a significant interaction present between diet and NSPase inclusion on Day 41 body weight as body weights were increased when NSPase was included in the PC and NC2 diets but not in the NC1 diet. No significant differences ($p > 0.05$) were observed between the three non-supplemented control diets (PC, NC1, and NC2). The inclusion of NSPase enzymes in the PC diet (TRT 4 and 7) resulted in the highest ending body weights among all treatments. Inclusion of both NSPase enzymes in the NC2 diet resulted in increased final body weight of broilers as compared to NC2 without NSPase with the NSPase B inclusion reaching the level of significance ($p < 0.05$).

Table 4-3. Body weight and mortality corrected feed conversion ratio (FCR) ± SE including main effects means ± SE of diet and NSPase inclusion of broilers fed the diets varying in nutrient profile and supplemented with NSPase enzyme preparations.

		Body Weight			Feed Conversion Ratio (FCR)			
Diet	**NSP**	**Day 12 (g)**	**Day 26 (kg)**	**Day 41 (kg)***	**Starter**	**Grower***	**Day 1-26**	**Day 1-41**
Control	-	284.3 ± 3.0	1.10 ± 0.01	2.40 ± 0.02^{abcd}	1.43 ± 0.04	1.53 ± 0.03^{ab}	Day 1-26	1.68 ± 0.02
NC 1	-	267.7 ± 3.5	1.06 ± 0.03	2.38 ± 0.04^{bcd}	1.49 ± 0.05	1.47 ± 0.01^{cde}	1.51 ± 0.03	1.68 ± 0.01
NC 2	-	289.6 ± 5.7	1.05 ± 0.03	2.36 ± 0.02^{cde}	1.35 ± 0.02	1.54 ± 0.04^{a}	1.47 ± 0.01	1.72 ± 0.03
Control	A	288.9 ± 2.3	1.15 ± 0.03	2.45 ± 0.01^{ab}	1.39 ± 0.04	1.44 ± 0.03^{e}	1.53 ± 0.04	1.68 ± 0.02
NC 1	A	276.0 ± 5.1	1.04 ± 0.01	2.31 ± 0.01^{e}	1.47 ± 0.04	1.49 ± 0.01^{bcd}	1.41 ± 0.03	1.69 ± 0.01
NC 2	A	288.4 ± 1.0	1.10 ± 0.02	2.43 ± 0.03^{abc}	1.33 ± 0.04	1.49 ± 0.02^{bcde}	1.49 ± 0.01	1.68 ± 0.01
Control	B	286.9 ± 6.7	1.11 ± 0.01	2.46 ± 0.01^{a}	1.36 ± 0.03	1.45 ± 0.01^{de}	1.45 ± 0.01	1.67 ± 0.01
NC 1	B	276.2 ± 1.0	1.06 ± 0.02	2.34 ± 0.02^{de}	1.41 ± 0.03	1.47 ± 0.01^{cde}	1.43 ± 0.01	1.69 ± 0.01
NC 2	B	292.4 ± 3.3	1.10 ± 0.01	2.44 ± 0.02^{ab}	1.32 ± 0.01	1.48 ± 0.01^{cde}	1.45 ± 0.01	1.66 ± 0.01
Main Effect Means								
Diet								
Control		286.7 ± 2.2^{a}	1.11 ± 0.01^{a}	2.43 ± 0.01	1.39 ± 0.02^{b}	1.47 ± 0.02	1.45 ± 0.02	1.68 ± 0.01
NC1		273.3 ± 2.2^{b}	1.05 ± 0.01^{c}	2.35 ± 0.01	1.46 ± 0.02^{a}	1.48 ± 0.01	1.47 ± 0.02	1.69 ± 0.01
NC2		290.1 ± 2.1^{a}	1.09 ± 0.01^{b}	2.41 ± 0.01	1.36 ± 0.02^{b}	1.50 ± 0.01	1.47 ± 0.01	1.69 ± 0.01
NSPase inclusion								
	-	280.5 ± 3.4	1.08 ± 0.01	2.38 ± 0.01	1.43 ± 0.03^{a}	1.51 ± 0.01	1.50 ± 0.02^{a}	1.69 ± 0.01
	A	284.4 ± 2.4	1.10 ± 0.01	2.40 ± 0.02	1.40 ± 0.03^{ab}	1.47 ± 0.01	1.45 ± 0.01^{b}	1.68 ± 0.01
	B	285.2 ± 2.8	1.09 ± 0.01	2.41 ± 0.02	1.37 ± 0.02^{b}	1.47 ± 0.01	1.44 ± 0.01^{b}	1.67 ± 0.01

$^{a-e}$ means within columns with different superscripts differ significantly at $P < 0.05$.

*Indicates a significant interaction between diet and NSPase inclusion. Therefore data was analyzed as a one-way ANOVA comparing individual treatment means.

Dietary composition and NSPase inclusion influenced mortality corrected feed conversion ratio (FCR) during the starter and grower phases (Table 4-3). With regard to dietary composition, the NC1 diet increased ($p < 0.05$) FCR as compared to the PC and NC2 diets during the starter phase. The inclusion of NSPase B in the starter diet decreased ($p < 0.05$) FCR compared to the non-supplemented controls while inclusion of NSPase A was intermediate. During the grower phase an interaction was present between NSPase inclusion and dietary composition as improvements were observed in feed conversion due to NSPase inclusion in the PC and NC2 diet but not in the NC1 diet. One way analysis indicates that the FCR in the NC1 diet was reduced ($p < 0.05$) compared to the PC and NC2 diets which were similar. NSPase inclusion in the NC1 diet did not improve FCR. However, improvements in FCR ($p < 0.05$) were observed with NSPase inclusion of both enzymes when included in the PC and NC2 diets during the grower phase. Cumulative FCR through 26 days of age was improved ($p < 0.05$) with the inclusion of both NSPase cocktail enzymes however dietary composition did not influence feed conversion during this period. There were no differences observed in FCR during the finisher phase or cumulatively through day 41.

In an effort to increase sample size only broilers from non-supplemented (TRT 1-3) and NSPase B (TRT 7-9) supplemented treatment groups were processed because performance characteristics of both NSPase A and B were similar at the conclusion of grow out. The live weight of broilers randomly selected for processing in the NC1 diet was lower ($p < 0.05$) than PC broilers while the NC2 was intermediate (Table 4-4). Carcass weight followed a similar trend with the PC and NC2 having increased carcass

weight compared to the NC1 diet while no effect of NSPase was observed. The inclusion of NSPase B resulted in slight increases in live weight and carcass weights when compared to non-supplemented diets however did not reach the level of significance ($p > 0.05$) (Table 4-4). NSPase B inclusion in the PC diet (TRT 7) resulted in the highest processing weights and yields observed among all treatments analyzed. NSPase B inclusion in the PC diet resulted in the highest live weight, carcass weight, breast weight, total breast meat, and carcass yield of any of the treatments and one-way analysis identified increases ($p < 0.05$) in breast weight and total breast meat as compared to the PC non-supplemented diet (Table 4-4). No differences ($p > 0.05$) were observed with regard to tender weight, white meat yield, or breast yield associated with dietary composition and NSPase inclusion. Increased carcass and tender yields were observed with NSPase B inclusion in the PC diet however did not reach the level of significance ($p > 0.05$).

Discussion

Throughout the trial, the reduction in protein and energy levels in NC1 resulted in decreased bird performance and lower male processing weights. Additionally, the reduction in energy level in NC2 diet negatively influenced broiler performance through 26 days of age but was similar to the PC diet at the conclusion of the trial. The similar final performance of NC2 broiler as compared to PC broilers was unexpected as a similar energy reduction in the starter and grower diet did result in reduced performance.

Table 4-4. Live body weight, processing weights (g) and processing yields (%) ± SE of broilers including main effects means ± SE of diet and NSPase inclusion of broilers fed the diets varying in nutrient profile and supplemented with NSPase enzyme preparations.

Diet	NSP	Live Wt	Carcass Wt	Breast Wt	Tender Wt	Tot Breast	Carcass %	Breast %	Tender %
Control	-	2636.9 ± 27.9	1868.5 ± 22.7	433.2 ± 7.0[b]	96.37 ± 1.6	529.6 ± 8.2[b]	70.7 ± 0.3	23.2 ± 0.2	5.2 ± 0.1
NC 1	-	2580.5 ± 30.0	1822.6 ± 23.6	429.1 ± 8.4[b]	96.13 ± 1.7	525.3 ± 9.8[b]	70.5 ± 0.3	23.5 ± 0.2	5.3 ± 0.1
NC 2	-	2662.0 ± 26.1	1884.3 ± 20.1	443.8 ± 6.1[ab]	99.85 ± 1.7	543.7 ± 7.3[ab]	70.8 ± 0.2	23.6 ± 0.2	5.3 ± 0.1
Control	B	2703.7 ± 26.0	1920.7 ± 19.4	457.4 ± 6.3[a]	100.89 ± 1.2	559.3 ± 6.6[a]	71.0 ± 0.2	23.8 ± 0.3	5.3 ± 0.1
NC 1	B	2616.9 ± 27.4	1842.4 ± 20.6	431.7 ± 7.6[b]	94.97 ± 1.5	526.7 ± 8.9[b]	70.2 ± 0.2	23.4 ± 0.3	5.1 ± 0.1
NC 2	B	2641.2 ± 29.1	1871.7 ± 24.7	435.4 ± 8.5[b]	97.95 ± 1.7	533.3 ± 9.8[b]	70.8 ± 0.2	23.2 ± 0.3	5.2 ± 0.1
Main Effect Means									
Diet									
Control		2670.3 ± 19.2[a]	1894.6 ± 15.1[a]	445.3 ± 4.8	98.6 ± 1.0	543.9 ± 5.4	70.8 ± 0.2	23.5 ± 0.2	5.2 ± 0.1
NC 1		2598.7 ± 20.3[b]	1832.6 ± 15.6[b]	430.4 ± 5.6	95.5 ± 1.2	526.0 ± 6.6	70.4 ± 0.2	23.4 ± 0.2	5.2 ± 0.1
NC 2		2651.6 ± 19.5[ab]	1878.1 ± 15.8[a]	439.6 ± 5.2	98.9 ± 1.2	538.6 ± 6.1	70.8 ± 0.2	23.4 ± 0.2	5.3 ± 0.1
NSP inclusion									
	-	2626.5 ± 16.3	1858.7 ± 12.9	435.4 ± 4.2	97.5 ± 1.0	532.9 ± 4.9	70.7 ± 0.1	23.4 ± 0.1	5.2 ± 0.1
	B	2653.9 ± 16.1	1878.0 ± 12.7	441.4 ± 4.4	97.9 ± 0.9	539.3 ± 5.0	70.7 ± 0.1	23.5 ± 0.2	5.2 ± 0.1

[a-b] means within columns with different superscripts differ significantly at $P < 0.05$.

This observation may be related to the loss of statistical power of the experimental design because of the use of the one-way ANOVA due to the interaction present between diet and NSPase inclusion as the differences in body weight were similar at day 41 and day 26. The differences in broiler performance associated with dietary composition are similar to the results reported by Plumstead et al. (2007), in which broiler performance was positively influenced by increasing crude protein, but diets with varying ME values of 3000-3200 kcal/kg did not have an effect on bird performance. Plumstead et al. (2007) adjusted the crude protein level 22%-27% in diets feed to Cobb 500 and Ross 708 broilers raised to 21 days of age in floor pens and witnessed increased bird performance as the levels of CP increased. Sterling et al. (2003) also witnessed similar results in bird performance where body weight and FCR improved as the level of CP increased in the diets feed to Cobb broilers.

NSPase inclusion did not increase body weight through 26 days of age, however, the interaction present between diet and NSPase inclusion for day 41 body weights indicated a significant increased in body weight in NC2 fed broilers when supplemented with NSPase B. Supplementation of NSPase in the PC diet increased body weight by 50 g and 60 g for NSPase A and B, respectively, however did not reach the level of significance. Inclusion of NSPase in the NC1 diet did not result in improvement in performance indicating the dietary composition can influence the potential for a positive impact of NSPase inclusion on body weight. There are conflicting reports in the literature on the ability of NSPase type enzymes to positively influence growth performance. Meng et al. (2005a) fed a mulitcarbohydrase cocktail enzyme containing

1,000 U of xylanase, 400 U of glucanase, 1,000 U of pectinase, and small amounts of cellulase, mannanase, and galactanase to broiler chicks from 5-18 days of age. The inclusion of the multicarbohydrase enzyme in a 69% corn diet resulted in a improvement in FCR, yet the cocktail enzyme in a corn-soy diet resulted in no improvements in bird performance.

Gracia et al. (2003) witnessed improvements when energy enzymes where fed in a corn-soy starter and grower diet containing α-amylase to Cobb 500 chicks to 42 days of age with increasing body weight ($p<0.05$) throughout the trial and increased overall feed intake from day 0-12 and 0-42 with enzyme inclusion. Meng et al. (2005b) fed varying enzyme preparations containing xylanase, glucanase, cellulose, pectinase, and mannanase to broiler chicks grown in batteries from 5-18 days of age and witnessed increased BW with the inclusion of enzymes in diets containing soybean meal, canola meal and wheat. The inclusion of enzyme preparations also increased the AME values of the diet.

The inclusion of energy enzymes in higher fiber diets based from wheat, rye and barley show improvements in FCR with the higher fiber diets (Bedford and Classen, 1992; Meng et al., 2005b; Goa et al., 2007). Lazaro et al. (2003) fed a β-glucanase/ xylanase enzyme in a wheat, barley and rye diets to laying hens and witnessed an improvement in feed efficiency per dozen eggs for those hens fed to supplemented diets. Goa et al. (2007) witnessed an improvement in growth and FCR when a xylanase enzyme was supplemented in a wheat-based diet fed to broilers from 7 to 21 days of age. In the trials conducted by our laboratory, the inclusion of the NSPase enzymes in the

control diets resulted in improvements in FCR during the starter and grower phase, yet no differences were observed in the finisher phase or overall FCR. The improvements in FCR due to the inclusion of NSPase in corn-soy based diets have been witnessed, although some research has shown fewer improvements with enzyme inclusion in the lower NSP content of the corn-soy diets. Gracia et al. (2003) witnessed slight improvements ($p<0.10$) in FCR with the inclusion of a cocktail enzyme in a corn-soy diet fed to broilers raised to 42 days of age. Meng et al. (2005b) witnessed a decreased FCR with the inclusion of varying carbohydrate enzymes in an 18% soy diet fed to broilers from 5-18 days of age.

Processing data appeared to be less sensitive to NSPase inclusion; however, the NSPase inclusion in the PC diet resulted in increased breast weight, and total breast meat weight. Although not significant, the inclusion of NSPase with the positive control diet resulted in the highest yields for all the evaluated parameters (white meat yield, carcass yield, and breast yield). Dietary composition also influenced processing data with the NC1 diet resulting in decreased body weight and carcass weight compared to the PC diet.

These data confirm that performance characteristics are influenced by dietary nutrient profile with decreased early broiler body weight and increased FCR in diets with reduced energy and protein concentrations. Additionally, inclusion of a cocktail enzyme preparation improves broiler performance and processing parameters. These enzyme preparations can be used as a management tool by integrators to reduce dietary costs.

CHAPTER V

EFFECTS OF CO-ADMINISTRATION OF PHYTASE AND ENERGY ENZYMES ON BROILER PERFORMANCE, BONE MINERALIZATION, AND PROCESSING PARAMETERS

Introduction

Phosphorus (P) is an essential nutrient required for proper bone development and for efficient poultry production (Nelson et al., 1971; Waldroup, 1999; Selle et al., 2007), and the failure to meet a bird's requirement of P can lead to many detrimental factors, including reduced bird performance, increased leg disorders and increased bird mortality (Waldroup, 1999; Powell et al., 2008). To avoid these consequences, many integrators have included a safety margin when formulating P concentrations in broiler diets to include a level of P slightly higher than the requirements for bird growth (Waldroup, 1999). This strategy of over supplementing P in poultry diet formulations was utilized until the cost of inorganic phosphate sources increased and awareness of the environmental concerns about excessive P concentrations in poultry litter motivated producers to consider alternative strategies that could reduce the total P concentrations in broiler diets (Ravindran et al., 1995).

The main strategy utilized to decrease the levels of P required in broiler diets is the use of phytase enzymes, which help hydrolyze the phosphate ester bond found in phytate bound P (Rao et al., 2009). As much as 70% of the P found in cereal grains may be unavailable for chickens to utilize as it is bound as phytate. The use of phytase in broiler diets has shown to increase bird performance and improve bone mineralization

(Simons et al., 1990; Sebastian et al., 1996). Phytic acid, in the polyanionic chelating form of phytate can chelate with cations like calcium, magnesium, copper and iron (Rao et al., 2009; Kumar, 2010) forming insoluble salts that can decrease absorption of these nutrients. For this reason, phytate is often considered to be an anti-nutritive compound because the binding of these nutrients results in being partially or completely unavailable for digestion by the chicken (Applegate, 2004; Roa et al., 2009).

Another anti-nutritive factor found in cereal grains used in broiler diets include the presence of non-starch polysaccharides (NSP), which are fibrous material found in plant cell walls. Chickens lack the digestive capacity of ruminant animals and the presence of NSP's in the diets increases intestinal viscosity resulting in decreases in the digestibility of the diet (Bedford and Morgan, 1996). The presence of high levels of NSP's in broiler diets have shown to reduce bird performance by decreasing body weights, increasing feed conversions, increasing litter moisture and increasing the incidence of pasty vents (Bedford and Morgan, 1996; Lazaro et al., 2003; Meng et al., 2005; Goa et al., 2007). To alleviate the negative effects of NSP's, carbohydrase (NSPase) enzymes have been utilized to help increase the digestibility of high fiber broiler diets. These enzymes function to degrade the NSP's found in the diet by breaking the fiber chains in the cell walls into smaller fragments (Wyatt et al., 2001). By breaking down the cell wall of grains, carbohydrase inclusion in diets has shown to decrease intestinal viscosity and increase digestibility (Choct et al., 1999; Kocher et al., 2002; Garcia et al., 2003).

Previous research has been conducted analyzing the effects of dual administration of phytase and carbohydrase enzymes to determine if the effects of each enzyme can be enhanced. Ravindran (1999) and Juanpere et al (2005) witnessed increased apparent metabolizable energy (AME) and digestibility of a diet with the inclusion of phytase and NSPase. Therefore, the objective of these two experiments was to determine if the co-administration of a cocktail enzyme preparation (NSPase) could enhance the activity of phytase when supplemented in low P diets.

Materials and Methods

Two experiments including a 14 day battery trial (Trial 1) and a 41 day grow out floor trial (Trial 2) were conducted to analyze the effects of co-administration of phytase and NSPase enzymes on broiler performance and bone mineralization when included in a low P corn-soy broiler diet. Animal care for both trials was provided in accordance with a protocol approved by the Texas A&M University Institutional Animal Care and Use Committee (IACUC), and research was conducted at the Texas A&M Poultry Science Center. For each of the following experiments, broiler chicks were provided age appropriate supplemental heat and given access to dietary treatment and water *ad libitum*. In Experiment 1, battery pens contained two stainless steel feeders and one water container for each pen of broilers. In Experiment 2, floor pens contained fresh pine shavings for bedding material and were equipped with one 30 lb tube feeder and nipple drinkers.

Experiment 1

The experimental design consisted of ten experimental treatments with 6 replicates per treatment each consisting of 12 male broilers per replicate, for a total of 720 Cobb 500 male chicks raised in battery brooders for a 14 day trial. A corn and soybean meal based basal mash diet was used which contained a total P level of 0.41% aP, available P level of 0.15%, and a calcium level of 0.20% (Table 5-1). Mono-calcium phosphate, limestone, and enzymes were added to the basal diet to achieve the desired available P level, and enzyme inclusion rates required for each individual treatment that is described in Table 5-2. Four treatments (1-4) were utilized to develop a dose response curve for observed parameters to calculate increase in bioavailability of P from phytate associated with phytase inclusion.

The available P concentrations in the diets (TRT 1-4) were 0.15%, 0.20%, 0.25%, and 0.30%. An additional six treatments were evaluated that included three levels of phytase[1] (150, 200, and 250 FTU/kg) with and without NSPase[2] inclusion in a diet containing 0.15% available P. Information from the manufacturer of the phytase enzyme[5] indicated a targeted increase in P bioavailability of 0.06%, 0.08%, and 0.10% with the three selected of inclusion of 150, 200, and 250 FTU/kg. The NSPase[6] was included at an inclusion rate of 0.25 lb/ton.

[5] Optiphos 2000 PF- Enzyvia LLC., Sheridan, IN.
[6] OptiCal- Enzyvia LLC., Sheridan, IN. Contains 300 units/gram Xylanase, 220 units/gram βeta-Glucanase, 22 units/gram βeta-Mannanase and 7 units/gram α-Galactosidase. Inclusion of 0.25 lb/ton.

Table 5-1. Ingredient profile and nutrient concentration of a low phosphorus starter basal diet fed to broilers through 14 d of age. Inorganic phosphorus was added to the basal diet to create four treatments of available phosphorus concentrations between 0.15% and 0.30% (Experiment 1).

Ingredient Profile	%
Corn	63.90
Soybean Meal (48%)	33.57
Sodium chloride	0.52
A/V Fat Blend	0.92
L-Lysine HCl	0.18
DL-Methionine (99%)	0.23
Vitamins[1]	0.25
Minerals[2]	0.05
Coban 60[3]	0.05
Mono-calcium PO_4	0.12
Nutrient Concentration	
Protein (%)	22
Metabolizable Energy (kcal/kg)	3054
Methioninine (%)	0.56
Total Sulfur Amino Acids (%)	0.92
Lysine (%)	1.30
Threonine (%)	0.82
Tryptophan (%)	0.26
Calcium	0.20
Sodium	0.22
Total Phosphorus	0.41
Available Phosphorus	0.15

[1] Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

[2] Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, .25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[3] Active drug ingredient monensin sodium, 60 g/lb (90 g/ton inclusion; Elanco Animal Health, Indianapolis, IN). As an aid in the prevention of coccidiosis caused by *Eimeria necarix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, *Eimeria mivati*, and *Eimeria maxima*.

Table 5-2. Treatment identification for Experiment 1 with calculated available phosphorus percentage, phytase inclusion rates, and NSPase inclusion in a low phosphorus broiler starter diet.

TRT #	Name	Available P[1]	Phytase[2]	NSPase[3]
1	0.15% aP	0.15	-	-
2	0.20% aP	0.20	-	-
3	0.25% aP	0.25	-	-
4	0.30% aP	0.30	-	-
5	Low Phytase	0.15	150	-
6	Mid Phytase	0.15	200	-
7	High Phytase	0.15	250	-
8	Low Phytase + NSP	0.15	150	+
9	Mid Phytase + NSP	0.15	200	+
10	High Phytase + NSP	0.15	250	+

[1] Available P was increased with addition of monocalcium phosphate.
[2] Optiphos 2000 PF- Enzyvia LLC., Sheridan, IN. Low phytase is 150 FTU/kg, Mid is 200 FTU/kg and High is 250 FTU/kg.
[3] OptiCal- Enzyvia LLC., Sheridan, IN. Contains 300 units/gram Xylanase, 220 units/gram βeta-Glucanase, 22 units/gram βeta-Mannanase and 7 units/gram α-Galactosidase. Inclusion of 0.25 lb/ton.

Body weights and feed consumption were determined on days 7 and 14, and following termination of the trial (Day 14), right tibias were removed from all broilers for bone ash determination. The bones were dried for 24 hours at 105°C and ashed in a furnace at 600°C for 24 hours to determine ash weight.

Experiment 2

The experimental design consisted of 8 dietary treatments consisting of 8 replicates per treatment with 48 straight-run chicks/replicate, for a total of 3072 Cobb x Cobb chicks raised in floor pens for a 41 day trial. A corn/soybean based starter, grower and finisher diet was fed with nutrient concentrations listed in Table 5-3 with experimental treatments listed in Table 5-4. The non-enzyme supplemented diets (TRT 1-4) included an industry (IC) control, a low phosphorus (LP) (- 0.10% aP), a low energy (LE) diet (-132 kcal/kg ME) and a low energy and low phosphorus (LEP) diet (-132 kcal/kg of ME and -0.10% aP). Enzyme supplementation treatments included phytase[1] inclusion of 200 or 250 FTU/kg into the low phosphorus (LP) diet. In addition, the low energy and low phosphorus (LEP) diet was supplemented with an NSPase[2] enzyme at a 0.25 lb/ton in addition to the 200 or 250 FTU/kg phytase[1]. A starter diet (crumble) was fed from day 1 to 14, a grower diet (pellet) was fed from day 14 to 26, and finisher diet (pellet) was fed from 27 to 41 days of age. Four feed samples/ treatment were shipped to an independent lab to confirm nutrient analysis and enzyme concentration.

Table 5-3. Calculated nutrient concentrations and ingredient profiles of four dietary treatments varying in energy and phosphorus concentrations and fed to straight run broilers. Dietary treatments include an industry control (IC), low phosphorus (LP), low energy (LE), and a low phosphorus and energy (LEP) diet. The dietary program consisted of 3 dietary phases including a starter (day 1-14), grower (day 15-28), and finisher diet (day 29-41) (Experiment 2).

	Starter %				Grower %				Finisher %			
Ingredient Profile	IC	LP	LE	LEP	IC	LP	LE	LEP	IC	LP	LE	LEP
Corn	59.47	60.10	62.84	63.40	64.49	65.06	67.77	67.84	70.98	70.29	74.31	74.92
Soybean Meal	30.27	30.13	29.65	29.54	25.16	25.08	24.62	24.62	19.47	20.60	19.42	19.34
Fat	3.54	3.31	0.77	0.59	3.92	3.70	1.18	1.10	3.53	3.50	0.74	0.50
Pork MBM	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.38	2.77	2.73
Limestone	1.21	1.41	1.21	1.42	1.01	1.21	1.00	1.01	0.87	1.08	0.97	1.17
Biofos	0.72	0.25	0.72	0.25	0.67	0.19	0.66	0.66	0.46	0.00	0.58	0.12
Sodium Chloride	0.48	0.48	0.48	0.48	0.43	0.43	0.43	0.42	0.43	0.43	0.43	0.43
Vitamins[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Meth.	0.25	0.26	0.25	0.25	0.22	0.22	0.21	0.21	0.20	0.18	0.19	0.19
L-Lysine	0.21	0.21	0.22	0.23	0.26	0.26	0.27	0.27	0.23	0.18	0.25	0.25
Trace Minerals[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Coban 90[3]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Nutrient Conc.												
Protein (%)	22.0	22.0	22.0	22.0	19.98	20.0	20.0	20.0	17.73	18.11	17.60	17.60
Calcium	0.95	0.95	0.95	0.95	0.85	0.85	0.85	0.85	0.75	0.75	0.75	0.75
Total Phosphorus	0.65	0.55	0.65	0.55	0.62	0.52	0.62	0.62	0.56	0.46	0.56	0.46
AP	0.40	0.30	0.40	0.30	0.38	0.28	0.38	0.38	0.33	0.23	0.33	0.23
ME (kcal/kg)	3058	3058	2961	2962	3170	3169	3038	3034	3213	3212	3081	3080
Methionine (%)	0.58	0.58	0.58	0.58	0.52	0.52	0.52	0.52	0.47	0.46	0.47	0.47
TSAA (%)	0.92	0.92	0.92	0.92	0.83	0.83	0.83	0.83	0.75	0.75	0.75	0.75
Lysine (%)	1.30	1.30	1.30	1.30	1.20	1.20	1.20	1.20	1.02	1.02	1.02	1.02
Tryptophan	0.24	0.24	0.23	0.23	0.21	0.21	0.23	0.23	0.17	0.18	0.19	0.19
Threonine	0.80	0.80	0.80	0.80	0.72	0.72	0.72	0.72	0.63	0.65	0.63	0.63
Sodium	0.22	0.22	0.22	0.22	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20

[1] Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

[2] Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, .25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[3] Active drug ingredient monensin sodium, 60 g/lb (90 g/ton inclusion; Elanco Animal Health, Indianapolis, IN). As an aid in the prevention of coccidiosis caused by *Eimeria necarix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, *Eimeria mivati*, and *Eimeria maxima*.

Table 5-4. Treatment identification for Experiment 2 with calculated available phosphorus percentage, phytase inclusion rates, and NSPase inclusion in a low phosphorus and energy diet.

Trt #	Diet[1]	Phytase[2] (FTU/kg)	NSPase[3]	Starter AP (%)	Starter ME (kcal/kg)	Grower AP (%)	Grower ME (kcal/kg)	Finisher AP (%)	Finisher ME (kcal/kg)
1	IC	0	-	0.40	3092	0.38	3170	0.33	3213
2	LP	0	-	0.30	3092	0.28	3170	0.23	3213
3	LE	0	-	0.40	2961	0.38	3038	0.33	3081
4	LEP	0	-	0.30	2961	0.28	3038	0.23	3081
5	LP	200	-	0.30	3092	0.28	3170	0.23	3213
6	LP	250	-	0.30	3092	0.28	3170	0.23	3213
7	LEP	200	+	0.30	2961	0.28	3038	0.23	3081
8	LEP	250	+	0.30	2961	0.28	3038	0.23	3081

[1] Dietary treatments include an industry control (IC), low phosphorus (LP), low energy (LE), and a low phosphorus and energy (LEP) diet.
[2] Optiphos 2000 PF- Enzyvia LLC., Sheridan, IN.
[3] OptiCal- Enzyvia LLC., Sheridan, IN. Contains 300 units/gram Xylanase, 220 units/gram βeta-Glucanase, 22 units/gram βeta-Mannanase and 7 units/gram α-Galactosidase. Inclusion rate of 0.25 lb/ton.

Forty-eight straight-run broilers were placed in 6ft x 6ft broiler rearing pens equipped with tube feeders and nipple drinkers with fresh pine shavings as bedding material. Chicks were weighed and feed consumption determined to calculate feed intake and feed: gain ratio on days of dietary changes (day 14, 26 and 41). On each weigh day, right tibias were collected from 3 broilers/pen (24 birds/ trt/ day) for determination of breaking strength and bone ash analysis. The bones were cleaned of adhering material prior to analysis. Breaking strength was determined by using Instron (Model #1011) machine. Instron settings for each day of sampling were as follows: Day 14 - 50 kg load cell, 10 kg load range, 50 mm/min cross head speed and 3 cm span, Day 26 - 50 kg load cell, 50 kg load range, 50 mm/min cross head speed and 3 cm span, and Day 41 - 500 kg load cell, 100 kg load range, 50 mm/min cross head speed and a 3 cm span. Following breaking strength determination, tibias were dried for 24 hr at 105°C, and ashed at 600°C for 24 hr to calculate ash content.

All broilers were bulk weighed in the evening on day 40, prior to an eight hour feed withdrawal period prior to processing on day 41. Ten male broilers from each pen (80 males/ trt) were randomly selected and individually weighed before processing. Following a 16 hour air chill of the carcasses, breast fillets and tenderloins were removed and weighed to calculate carcass and breast yield.

Statistical Analysis

For both trials, all data (body weight, mortality corrected feed conversion ratio, processing parameters, bone ash and breaking strength) were analyzed via a one-way ANOVA, and means were deemed significantly different at $p < 0.05$. Means were

separated using Duncan's Multiple Range Test. In Experiment 1, regression analysis was conducted on dose response treatments (TRT 1-4) and determined equation used to calculate increase in bioavailability of P associated with phytase inclusion.

Results

Experiment 1

Increasing the level of available P in the dose response treatment groups (TRT 1-4) improved broiler performance parameters (Tale 5-5). Increasing the available P from 0.15% to 0.20% did not improve performance parameters; however the other 0.05% increases in available P improved evaluated parameters. The inclusion of phytase (TRT 5-10) enhanced growth parameters as early as day 7 (Table 5-5) when compared to TRT 1, which had the same level of available P. The medium and high levels (200 and 250 FTU/kg) of phytase inclusion resulted in increased body weight similar to TRT 3, suggesting that these levels of phytase were capable of producing growth response of a diet containing 0.1% more available P. NSPase inclusion (TRT 8-10) did not result in any improvements in day 7 body weight (BW) as compared to the similar levels of phytase alone treatment groups.

Day 14 BW (Table 5-5) was similar to day 7 data, with all phytase treatment groups exhibiting increased BW as compared to TRT 1. The 200 and 250 FTU/kg inclusion levels of phytase resulted in BW similar to TRT 3. The inclusion of NSPase with the low level of phytase (TRT 8) resulted in an increase in BW as compared to the 150 FTU/kg phytase inclusion alone (TRT 5) while no improvements were observed with the inclusion of NSPase in the two higher levels of phytase.

Table 5-5. Body weight, feed consumption, mortality corrected feed conversion, and mortality ± SE of broilers fed selected levels of available phosphorus, multiple phytase inclusion rates, and NSPase inclusion.

TRT	Name	Body Wt		Feed Consumption	Feed Conversion			Mortality
		Day 7 (g)	Day 1-14	Day 1-14 (g)	Day 1-7	Day 8-14	Day 1-14	Day 1-14
1	0.15% aP	110.0 ± 2.16^{d}	214.4 ± 7.56^{e}	17.51 ± 0.69^{f}	1.43 ± 0.03^{ab}	2.02 ± 0.08^{ab}	1.69 ± 0.04^{ab}	37.5 ± 5.1^{a}
2	0.20% aP	109.7 ± 1.72^{d}	222.8 ± 4.67^{e}	19.12 ± 0.51^{f}	1.47 ± 0.05^{a}	2.32 ± 0.35^{a}	1.84 ± 0.13^{a}	26.5 ± 5.5^{ab}
3	0.25% aP	124.1 ± 2.69^{ab}	286.1 ± 4.78^{b}	23.09 ± 0.12^{b}	1.33 ± 0.06^{bc}	1.38 ± 0.05^{c}	1.36 ± 0.04^{cd}	11.2 ± 2.9^{cd}
4	0.30% aP	128.2 ± 1.87^{a}	312.7 ± 5.56^{a}	25.72 ± 0.58^{a}	1.32 ± 0.02^{bc}	1.48 ± 0.08^{c}	1.42 ± 0.06^{cd}	11.2 ± 4.2^{cd}
5	Low Phytase[1]	117.6 ± 1.01^{c}	244.5 ± 8.94^{d}	19.55 ± 0.87^{de}	1.28 ± 0.04^{c}	1.75 ± 0.08^{bc}	1.53 ± 0.04^{bc}	22.2 ± 4.1^{bc}
6	Mid Phytase[1]	123.0 ± 1.80^{bc}	276.6 ± 2.70^{bc}	22.94 ± 0.68^{bc}	1.37 ± 0.04^{abc}	1.37 ± 0.08^{c}	1.37 ± 0.06^{cd}	6.8 ± 2.6^{d}
7	High Phytase[1]	121.4 ± 1.17^{bc}	280.3 ± 6.48^{bc}	22.46 ± 0.57^{bc}	1.32 ± 0.02^{bc}	1.32 ± 0.05^{c}	1.32 ± 0.03^{d}	4.0 ± 1.8^{d}
8	Low Phytase[1] + NSP[2]	117.8 ± 1.23^{c}	264.7 ± 1.67^{c}	21.12 ± 0.52^{cd}	1.36 ± 0.03^{abc}	1.71 ± 0.17^{bc}	1.54 ± 0.07^{bc}	22.1 ± 5.1^{bc}
9	Mid Phytase[1] + NSP[2]	118.5 ± 1.57^{c}	268.6 ± 5.60^{bc}	21.84 ± 0.65^{bc}	1.33 ± 0.02^{bc}	1.57 ± 0.12^{c}	1.47 ± 0.07^{cd}	12.3 ± 4.7^{cd}
10	High Phytase[1] + NSP[2]	121.5 ± 1.57^{bc}	269.3 ± 7.63^{bc}	22.10 ± 0.42^{bc}	1.30 ± 0.03^{c}	1.46 ± 0.07^{c}	1.39 ± 0.05^{cd}	9.8 ± 3.4^{cd}

[a-f] means within columns with different superscripts differ significantly at $P < 0.05$.

[1] Optiphos 2000 PF- Enzyvia LLC., Sheridan, IN. Low phytase is 150 FTU/kg, Mid is 200 FTU/kg and High is 250 FTU/kg.

[2] OptiCal- Enzyvia LLC., Sheridan, IN. Contains 300 units/gram Xylanase, 220 units/gram βeta-Glucanase, 22 units/gram βeta-Mannanase and 7 units/gram α-Galactosidase. Inclusion rate of 0.25 lb/ton.

Similar to BW, feed consumption (g/bird/day) also increased with increasing P levels and the inclusion of phytase in the diet. The low level of phytase inclusion (TRT 5) increased feed consumption as compared to TRT 1 and 2 while increasing the inclusion rate to 200 and 250 FTU/kg resulted in a feed consumption rate similar to TRT 3. The inclusion of NSPase with the low level of phytase (TRT 8) resulted in an increase in feed consumption as compared to the 150 FTU/kg phytase alone treatment (TRT 5). Increases in feed consumption were not observed with NSPase inclusion at the two higher levels of phytase. Mortality corrected feed conversion ratio (FCR) (day 1-14) was improved as the level of available P increased from 0.20% to 0.30%. Similar FCR were observed between the two lowest levels of available P as well as the two highest level of available P (Table 5-5). Phytase inclusion at inclusion rates improved FCR. Additionally, improvements were observed as phytase level increased from 150 FTU/kg to 250 FTU/kg. The inclusion of NSPase with phytase (TRT 8-10) did not result in improvements in FCR when compared to the phytase alone treatment groups.

Bone mineralization data was similar for both parameters evaluated (bone ash weight and bone ash %) (Table 5-6). Increases in bone ash weight and percentage were observed as the level of available P increased in TRT 1-4. The inclusion of phytase increased bone ash weight and percent compared to TRT 1. The low level of phytase (150 FTU/kg) resulted in bone data similar to TRT 2, which had a 0.05% higher level of available P. The inclusion of 200 and 250 FTU/kg of phytase increased bone measurements to a level comparable to TRT 3, suggesting that these levels of phytase inclusion were capable of producing bone ash weight and percent of a diet containing

Table 5-6. Tibia ash weight and percentage ± SE of broilers fed selected levels of available phosphorus, multiple phytase inclusion rates, and NSPase inclusion.

TRT	Name	Ash (mg)	Ash (%)
1	0.15% aP	127.02 ± 7.3^{e}	33.59 ± 0.78^{e}
2	0.20% aP	144.93 ± 6.5^{de}	35.35 ± 0.33^{de}
3	0.25% aP	193.64 ± 9.0^{b}	38.63 ± 0.77^{b}
4	0.30% aP	225.57 ± 11.9^{a}	40.54 ± 0.86^{a}
5	Low Phytase[1]	157.44 ± 7.9^{cd}	35.96 ± 0.80^{cd}
6	Mid Phytase[1]	183.67 ± 3.8^{b}	38.16 ± 0.43^{b}
7	High Phytase[1]	179.68 ± 5.2^{b}	37.79 ± 0.34^{bc}
8	Low Phytase[1] + NSP[2]	171.66 ± 3.7^{bc}	37.21 ± 0.46^{bcd}
9	Mid Phytase[1] + NSP[2]	173.95 ± 4.8^{bc}	36.94 ± 0.58^{bcd}
10	High Phytase[1] + NSP[2]	178.71 ± 3.8^{bc}	37.62 ± 0.73^{bc}

[a-e] means within columns with different superscripts differ significantly at $P < 0.05$.

[1] Optiphos 2000 PF- Enzyvia LLC., Sheridan, IN. Low phytase is 150 FTU/kg, Mid is 200 FTU/kg and High is 250 FTU/kg.

[2] OptiCal- Enzyvia LLC., Sheridan, IN. Contains 300 units/gram Xylanase, 220 units/gram βeta-Glucanase, 22 units/gram βeta-Mannanase and 7 units/gram α-Galactosidase. Inclusion rate of 0.25 lb/ton.

0.1% more available P. The bone mineralization data confirms the manufacturer's recommendations that 250 FTU/kg of phytase is capable of increasing phosphorus bio-availability by 0.10%. The inclusion of NSPase with the low level of phytase (150 FTU/kg) increased observed bone ash and percent to a level comparable to the two higher levels of phytase inclusion. The inclusion of NSPase with the two higher levels of phytase (200 and 250 FTU/kg) did not increased observed bone ash and percent. To calculate the increase in bioavailability of P from the inclusion of the three levels of phytase inclusion with and without NSPase inclusion, regression analysis was performed to develop equations based on the observed body weight at day 14 (Figure 5-1), bone ash weight (Figure 5-2), and bone ash percentage (Figure 5-3) as a function of inorganic P consumed. Individual data points represent observed parameters for each replicate pen. Following regression analysis, the equations were used to determine the amount of inorganic P that would need to be consumed to achieve the observed effect of each phytase inclusion rate. The determined equations for each evaluated parameters were y=187.28x+195.51 (Equation 1, Day 14 BW in grams), y=181.88x+111.13 (Equation 2, Bone Ash in mg) and y=12.552x+32.773 (Equation 3, bone ash %). The calculated increase in bio-availability of P was determined for each of the treatment groups (TRT 5-10) in which the equations previously discussed were utilized to determine the P release resulting from the phytase inclusion rate for the evaluated parameter (Table 5-7). From the calculated values shown in the table, the inclusion of the levels of phytase alone and with NSPase were capable of releasing the 0.06%, 0.09%, and 0.10% aP in

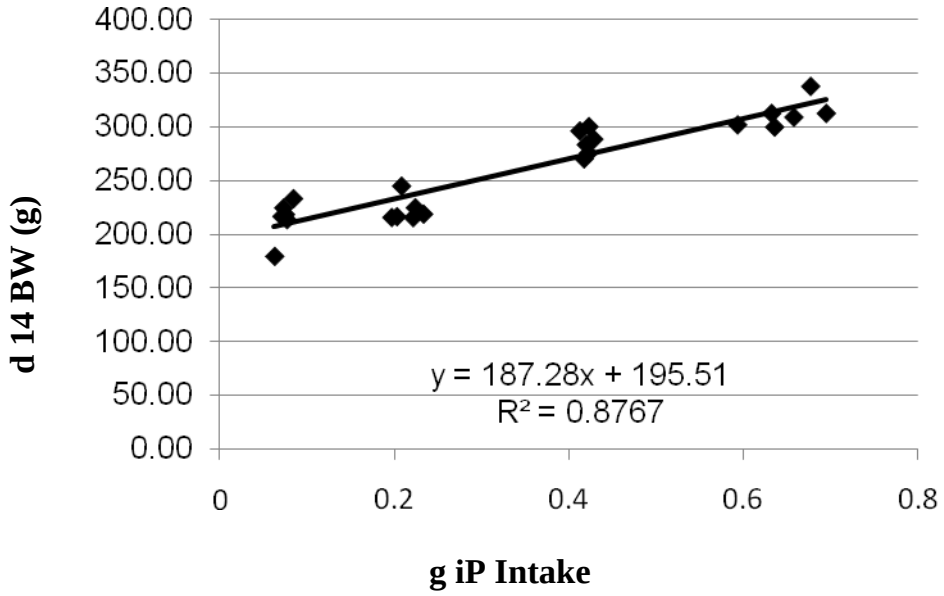

Figure 5-1. Regression analysis depicting the relationship between inorganic phosphorus consumption and the observed average body weight of male broilers at 14 days of age fed diets varying in available phosphorus concentration.

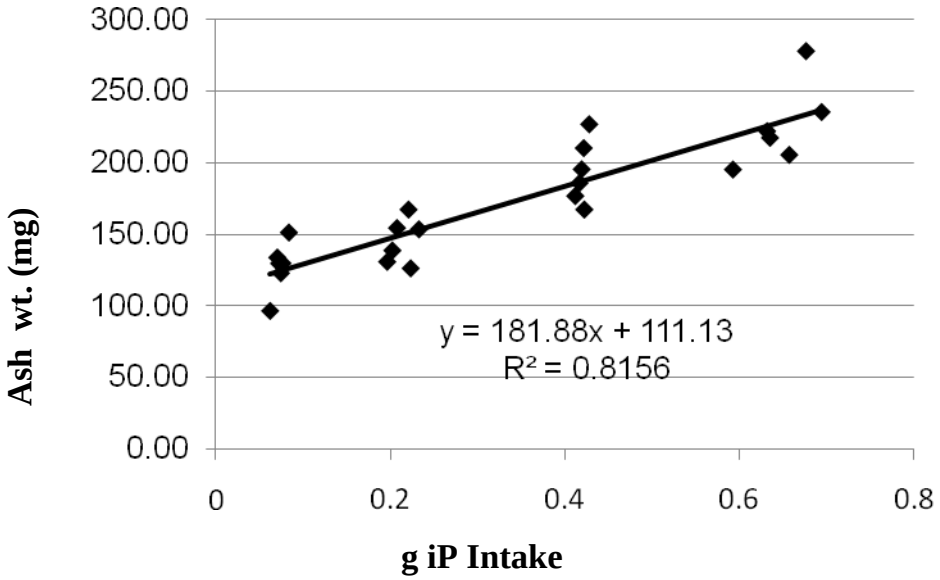

Figure 5-2. Regression analysis depicting the relationship between inorganic phosphorus consumption and the observed bone ash weight (mg) of male broilers fed diets varying in available phosphorus concentration.

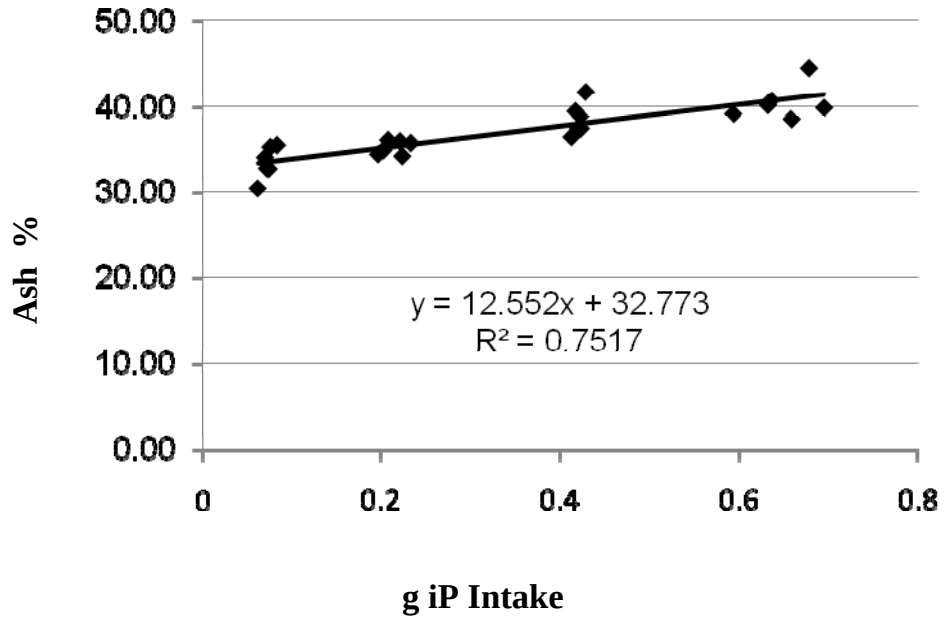

Figure 5-3. Regression analysis depicting the relationship between inorganic phosphorus consumption and the determined tibia ash percentage observed from male broilers fed diets varying in available phosphorus concentrations.

Table 5-7. Calculated increase in P bio-availability (%) due to inclusion of phytase and NSPase enzymes. Calculations were based on regression equations of day 14 body weight (equation 1), bone ash weight (equation 2), and ash percentage (equation 3) of male broilers fed selected levels of available phosphorus (aP).

TRT #	Phytase Level[1]	NSPase[2]	Day 14 BW (g)[3]	Ash (mg)[4]	Ash (%)[5]
5	150	–	0.064	0.063	0.065
6	200	–	0.105	0.095	0.104
7	250	–	0.114	0.091	0.098
8	150	+	0.094	0.082	0.090
9	200	+	0.097	0.083	0.780
10	250	+	0.097	0.091	0.096

[1] Optiphos 2000 PF- Enzyvia LLC., Sheridan, IN.
[2] OptiCal- Enzyvia LLC., Sheridan, IN. Contains 300 units/gram Xylanase, 220 units/gram βeta-Glucanase, 22 units/gram βeta-Mannanase and 7 units/gram α-Galactosidase. Inclusion rate of 0.25 lb/ton.
[3] Calculated using regression analysis comparing inorganic phosphate consumption and observed body weight at day 14 (g). (y=187.28x+195.51)
[4] Calculated using regression analysis comparing inorganic phosphate consumption and observed bone ash weight (mg). (y=181.88x+111.13)
[5] Calculated using regression analysis comparing inorganic phosphate consumption and observed bone ash (%). (y=12.552x+32.773)

the low, mid and high level of phytase inclusion in the diet. Regression analysis determined that NSPase inclusion increased P bio-availability from 0.06% to 0.09% at the 150 FTU/kg inclusion rate of phytase. No other improvements in P release were observed with the inclusion of NSPase in the diets with higher level of phytase (200 and 250 FTU/kg).

Experiment 2

In the non-supplemented diets (TRT 1-4), decreases in bird performance and bone characteristics (Table 5-8) were observed in the Low Phosphorus (LP) and Low Phosphorus + Low Energy (LEP) diet as compared to the Industry Control diet (IC). Throughout the trial, there were no differences observed in bird performance, processing parameters, or bone characteristics in the IC or Low Energy (LE) diets, suggesting that the reduction in energy was not significant enough to negatively affect bird performance. The inclusion of both levels of phytase in TRT's 5 and 6 resulted in increased BW, compared to the Low aP diet (Table 5-8). The inclusion of 250 FTU/kg of phytase improved BW as compared to the low level of phytase, yet the high level of phytase did result in a BW similar to the Industry Control diet. In relation to BW data, the inclusion of the high level of Phytase did not result in a phosphorus release of 0.10%. The inclusion of NSPase with phytase inclusion (TRT 7 & 8) did not result in improvements in BW as compared to the phytase alone inclusion TRT's.

Mortality rates (Table 5-8) were the highest among the TRT 2 and 4, which had the 0.10% reduction in aP. The inclusion of both levels of phytase resulted in mortality rate similar to the mortality rate observed for TRT 1 and 3. The inclusion of NSPase

Table 5-8. Body weight (BW), mortality corrected feed conversion ratio (FCR) and mortality rates ± SE of broilers fed the diets varying in phosphorus and energy concentration containing two phytase inclusion rates and a cocktail carbohydrase preparation.

TRT	Diet[1]	Phytase[2] (FTU/kg)	NSPase[3]	BW Day 14 (g)	BW Day 26 (kg)	BW Day 40 (kg)	Mortality	FCR D1-14	FCR D 15-26	FCR D 1-40
1	IC	0	-	427.9 ± 3.1[a]	1.17 ± 0.01[a]	2.54 ± 0.02[a]	4.3 ± 0.01[c]	1.35 ± 0.03[bc]	1.61 ± 0.01[ab]	1.72 ± 0.01
2	LP	0	-	310.9 ± 1.8[e]	0.81 ± 0.01[e]	1.76 ± 0.03[f]	24.4 ± 0.02[a]	1.44 ± 0.03[a]	1.66 ± 0.02[a]	1.74 ± 0.01
3	LE	0	-	417.8 ± 3.0[b]	1.17 ± 0.01[a]	2.57 ± 0.02[a]	5.3 ± 0.02[bc]	1.34 ± 0.02[bc]	1.60 ± 0.02[ab]	1.71 ± 0.01
4	LEP	0	-	310.3 ± 2.2[e]	0.92 ± 0.02[d]	2.09 ± 0.02[e]	28.0 ± 0.03[a]	1.41 ± 0.04[a]	1.55 ± 0.01[c]	1.73 ± 0.02
5	LP	200	-	392.4 ± 3.1[d]	1.10 ± 0.02[b]	2.33 ± 0.02[cd]	10.4 ± 0.02[bc]	1.34 ± 0.03[bc]	1.61 ± 0.02[ab]	1.72 ± 0.01
6	LP	250	-	404.9 ± 3.3[c]	1.15 ± 0.01[a]	2.44 ± 0.03[b]	9.8 ± 0.02[bc]	1.34 ± 0.01[bc]	1.56 ± 0.01[bc]	1.71 ± 0.01
7	LEP	200	+	389.0 ± 3.5[d]	1.06 ± 0.01[c]	2.27 ± 0.03[d]	11.8 ± 0.02[b]	1.31 ± 0.02[c]	1.65 ± 0.02[a]	1.75± 0.01
8	LEP	250	+	410.9 ± 3.7[bc]	1.11 ± 0.01[b]	2.40 ± 0.03[bc]	7.1 ± 0.01[bc]	1.32 ± 0.03[c]	1.65 ± 0.02[a]	1.74 ± 0.01

[a-f] means within columns with different superscripts differ significantly at $P < 0.05$.

[1] Dietary treatments include an industry control (IC), low phosphorus (LP), low energy (LE), and a low phosphorus and energy (LEP) diet.

[2] Optiphos 2000 PF- Enzyvia LLC., Sheridan, IN.

[3] OptiCal- Enzyvia LLC., Sheridan, IN. Contains 300 units/gram Xylanase, 220 units/gram βeta-Glucanase, 22 units/gram βeta-Mannanase and 7 units/gram α-Galactosidase. Inclusion rate of 0.25 lb/ton.

enzymes with phytase (TRT 7 & 8) did not result in a change in mortality as compared to phytase alone inclusion (TRT 5 & 6). Feed Conversion Ratio (FCR) was highest among the low aP TRT's of 2 and 4 through the starter phase of the trial (Table 5-8). The inclusion of phytase resulted in a decrease in FCR as compared to the low P diet. During the starter phase, the inclusion of NSPase with phytase (TRT 7 & 8) resulted in a slight decrease in FCR as compared to the diet with phytase alone. This effect was not witnessed after the starter phase.

The FCR during the grower phase was similar to the results witnessed in the starter phase, with the inclusion of enzyme resulting in a decrease in FCR. One difference observed was that the low aP and energy diet (TRT 4) had the lowest FCR during this time. This observation with the low aP diet was unexpected, but could have been attributed to the high level of mortality observed with this TRT. The expected results would have been similar to TRT 2, which had the highest FCR. There were no differences observed in FCR during the finisher phase of the diet or the overall FCR throughout the trial. Throughout the trial, the industry control diet and low energy resulted in the lower FCR witnessed. The high phytase alone inclusion (TRT 6) resulted in a lower FCR similar to the industry control.

Tibias were collected at the end of each feed change (days 14, 26 and 40) to analyze for breaking strength and bone ash analysis. Bone parameter data had similar results as the performance data; the low aP level in TRT 2 and 4 resulted in the lowest ash weight, ash percent, and breaking strength of any of the TRT groups following the starter phase (Table 5-9). The inclusion of phytase enzyme improved bone parameters

Table 5-9. Tibia weight (g), ash weight, ash percentage and breaking strength ± SE of broilers fed diets varying in available P level and ME supplemented with two levels of phytase and a cocktail carbohydrase preparation.

	Diet[1]							
	IC	LP	LE	LEP	LP+200 FTU/kg[2]	LP+250 FTU/kg[2]	LEP+200 FTU/kg[2]+NSP[3]	LEP+250 FTU/kg[2]+NSP[3]
Day 14								
Tibia Wt	2.17 ± 0.05[a]	1.68 ± 0.04[c]	2.10 ± 0.06[ab]	1.72 ± 0.05[c]	2.11 ± 0.05[ab]	2.08 ± 0.06[ab]	1.98 ± 0.05[b]	2.11 ± 0.05[ab]
Ash Wt	366.2 ± 9.2[a]	196.4 ± 6.7[d]	341.1 ± 8.8[b]	202.2 ± 6.6[d]	332.5 ± 8.4[b]	326.2 ± 9.1[bc]	303.2 ± 7.9[c]	341.8 ± 10.6[b]
Ash %	47.77 ± 0.27[a]	39.07 ± 0.55[c]	46.94 ± 0.48[ab]	39.28 ± 0.57[c]	46.08 ± 0.38[b]	46.18 ± 0.45[b]	46.11 ± 0.36[b]	46.60 ± 0.60[ab]
BS	6.71 ± 0.19[a]	3.04 ± 0.11[e]	5.91 ± 0.21[b]	3.03 ± 0.12[e]	5.69 ± 0.24[bc]	5.27 ± 0.24[cd]	4.97 ± 0.20[d]	5.49 ± 0.22[bcd]
Day 26								
Tibia Wt	6.40 ± 0.16[a]	4.99 ± 0.20[d]	6.15 ± 0.18[a]	5.51 ± 0.18[bcd]	5.48 ± 0.31[cd]	5.98 ± 0.20[abc]	5.82 ± 0.15[abc]	6.11 ± 0.18[ab]
Ash Wt	1263.1 ± 32.2[a]	744.8 ± 28.1[e]	1192.1 ± 36.1[ab]	1023.9 ± 36.9[cd]	1013.6 ± 40.4[cd]	1120.3 ± 48.1[bc]	992.5 ± 27.3[d]	1083.3 ± 38.1[cd]
Ash %	47.71 ± 0.43[a]	42.63 ± 0.46[d]	47.44 ± 0.35[a]	47.05 ± 0.37[a]	45.18 ± 0.41[bc]	46.27 ± 0.46[ab]	44.94 ± 0.36[c]	45.44 ± 0.37[bc]
BS	22.81 ± 1.24[a]	11.07 ± 0.73[c]	17.86 ± 1.18[b]	16.89 ± 1.12[b]	14.66 ± 0.93[b]	17.99 ± 1.20[b]	15.36 ± 1.23[b]	16.45 ± 1.05[b]
Day 41								
Tibia Wt	14.13 ± 0.29[a]	11.64 ± 0.29[b]	14.77 ± 0.25[a]	12.33 ± 0.32[b]	14.05 ± 0.22[a]	14.70 ± 0.27[a]	14.19 ± 0.24[a]	14.25 ± 0.31[a]
Ash Wt	2923.8 ± 53.7[ab]	1928.5 ± 60.3[e]	3020.6 ± 52.5[a]	2290.4 ± 69.2[d]	2699.1 ± 40.6[c]	2917.9 ± 57.3[ab]	2735.2 ± 59.6[c]	2806.0 ± 61.9[bc]
Ash %	43.46 ± 0.30[a]	39.93 ± 0.46[d]	43.09 ± 0.35[ab]	42.03 ± 0.48[bc]	41.27 ± 0.42[c]	41.72 ± 0.47[c]	42.42 ± 0.43[abc]	42.03 ± 0.39[bc]
BS	40.74 ± 2.18 [a]	28.38 ± 1.56 [c]	42.31 ± 2.35 [a]	33.50 ± 1.77 [bc]	36.88 ± 1.27 [ab]	38.48 ± 2.02 [ab]	37.56 ± 1.93 [ab]	39.30 ± 1.84[ab]

[a-e] means within rows with different superscripts differ significantly at $P < 0.05$.

[1] Dietary treatments include an industry control (IC), low phosphorus (LP), low energy (LE), and a low phosphorus and energy (LEP) diet.

[2] Optiphos 2000 PF- Enzyvia LLC., Sheridan, IN.

[3] OptiCal- Enzyvia LLC., Sheridan, IN. Contains 300 units/gram Xylanase, 220 units/gram βeta-Glucanase, 22 units/gram βeta-Mannanase and 7 units/gram α-Galactosidase. Inclusion rate of 0.25 lb/ton.

as compared to the low aP groups, but the inclusion of phytase did not improve bone parameters to the level observed in the industry control diet. The inclusion of NSPase did not enhance the effects observed with phytase alone, and the inclusion of NSPase with the low level of phytase did result in a reduction in ash weight and breaking strength when fed in the starter diet. The day 26 tibia (Table 5-9) collection had similar results as mentioned previously with the day 14 tibia collection, with the low aP groups (TRT 2 and 4) resulting in decreased ash weight. The low aP and energy (TRT 4) did not result in a reduction in ash % and breaking strength as we would have expected. The inclusion of phytase improved bone parameters compared to the low aP diets, and the inclusion of the high phytase inclusion (250 FTU/kg) numerically improved ash weight, ash % and bone strength. For the day 41 tibia collection (Table 5-9), the reduction in aP in TRT 2 and 4 resulted in decreased bone parameters compared to the rest of the treatments. The phytase inclusion improved bone weights and breaking strength data with the higher level of phytase inclusion (250 FTU/kg) resulting in increased weights as compared to the low level of phytase inclusion (200 FTU/kg). The inclusion of NSPase with phytase did slightly ($p>0.05$) improve the ash %.

Following day 41, ten male broilers were processed from all pens except TRT's 2 and 4 to determine processing weights and yields (Table 5-10). The whole bird carcass (WOG) weights were the lowest among TRT 5 and 7 (low phytase) and highest WOG weight with TRT 6 (high phytase). The inclusion of NSPase did not enhance the carcass weight. The other processing weights (i.e. breast, tender and fat pad) were similar to the results witnessed in WOG weights. The low phytase inclusion groups (TRT 5 and 7)

Table 5-10. Processing weights and yields ± S.E. of male broilers fed diets varying in available phosphorus level and ME supplemented with multiple levels of phytase and a cocktail carbohydrase preparation.

TRT	Diet[1]	Phytase[2] (FTU/kg)	NSPase[3]	Processing Weights				Processing Yields			
				WOG	Breast	Tender	Fat Pad	Carcass	Breast yield	Tender	Fat Pad
1	IC	0	-	1822.1 ± 26.4^{a}	437.3 ± 9.2^{ab}	96.4 ± 1.8^{ab}	36.8 ± 2.1^{a}	69.48 ± 0.13^{a}	23.98 ± 0.18^{ab}	5.29 ± 0.08	2.02 ± 0.07^{a}
3	LE	0	-	1852.4 ± 23.5^{a}	447.0 ± 11.6^{a}	97.6 ± 1.6^{a}	33.5 ± 2.3^{ab}	69.72 ± 0.19^{a}	24.10 ± 0.20^{a}	5.27 ± 0.05	1.81 ± 0.06^{b}
5	LP	200	-	1650.3 ± 26.1^{c}	384.2 ± 9.1^{d}	86.0 ± 2.4^{c}	29.0 ± 1.6^{c}	68.75 ± 0.18^{b}	23.23 ± 0.18^{c}	5.21 ± 0.05	1.75 ± 0.06^{b}
6	LP	250	-	1798.3 ± 20.7^{a}	427.0 ± 7.5^{bc}	94.4 ± 1.8^{ab}	34.3 ± 2.2^{ab}	69.50 ± 0.16^{a}	23.71 ± 0.18^{abc}	5.25 ± 0.05	1.91 ± 0.07^{ab}
7	LEP	200	+	1646.9 ± 30.2^{c}	388.1 ± 8.3^{d}	85.8 ± 1.9^{c}	29.0± 2.7^{c}	68.72 ± 0.18^{b}	23.49 ± 0.19^{bc}	5.22 ± 0.06	1.74 ± 0.07^{b}
8	LEP	250	+	1736.2 ± 26.4^{b}	417.2 ± 9.0^{c}	92.5 ± 2.1^{b}	31.5 ± 2.0^{bc}	69.40 ± 0.21^{a}	23.97 ± 0.21^{ab}	5.32 ± 0.06	1.80 ± 0.06^{b}

$^{a-d}$ means within columns with different superscripts differ significantly at $P < 0.05$.

[1] Dietary treatments include an industry control (IC), low phosphorus (LP), low energy (LE), and a low phosphorus and energy (LEP) diet.

[2] Optiphos 2000 PF- Enzyvia LLC., Sheridan, IN.

[3] OptiCal- Enzyvia LLC., Sheridan, IN. Contains 300 units/gram Xylanase, 220 units/gram βeta-Glucanase, 22 units/gram βeta-Mannanase and 7 units/gram α-Galactosidase. Inclusion rate of 0.25 lb/ton.

had the lowest weights, and the high phytase inclusion in TRT 6 showed the greatest increase in weight. The processing yield data for carcass, breast, tender and fat pad had similar trends as the results discussed for the processing weights. The low phytase inclusion TRT's (5 and 7) had significantly lower carcass yield than all other treatments. There were no significant differences observed in tender yield, and the Industry Control diet (TRT 1) had a significantly higher fat pad weight as compared to the phytase inclusion groups.

Discussion

Reducing levels of available P in the diet reduced growth performance and bone mineralization in both experiments. It is recognized that decreasing the level of available P in diets will result in decreases in bird performance and reduced bone mineralization. Simons et al. (1990) witnessed lower bird weights, increased FCR and increased mortality when broilers were fed lower levels of available P. Yan et al. (2001) also observed that increasing the available P level resulted in increased tibia ash weight and body weight.

The inclusion of dietary phytase enzymes can assist in increasing the bioavailability of P and improve broiler performance while also decreasing the level of inorganic phosphorus required in the diet (Simons et al., 1990; Waldroup, 1999; Waldroup et al., 2000; Yan et al., 2001; Powell et al., 2008). In trial 1, the inclusion of the three different phytase levels resulted in an increase in P bioavailability leading to improvements in broiler performance and bone mineralization as compared to the dose response treatments (TRT 1-4) that contained equivalent or higher levels of available P

concentrations. In Experiment 2, the inclusion of the two levels of phytase resulted in increased broiler performance and bone mineralization as compared to TRT 2 and 4.

These results from the two experiments illustrate that phytase enzyme inclusion in P deficient diets have the ability to improve broiler performance and bone ash. Enhanced growth parameters and bone mineralization due to phytase inclusion in diets have been previously reported. Yan et al. (2001) reported increases in body weight and tibia ash and improvements in feed conversion with the inclusion of Natuphos in broiler diets. Dilger et al. (2004) observed increased body weight gain and feed intake during the starter and grower period with the inclusion of varying levels of Phyzyme XP as compared to a control diet with the same level of available P. The higher inclusion rates of phyase enzyme in these trials resulted in increased ash percentage, body weight and decreased FCR as compared to the lower level of inclusion for each enzyme in the experimental design. Simons et al. (1990) also witnessed similar results in that increasing phytase levels from 250-1500 resulted in decreased FCR and increased body weights when included in diets fed through 24 days of age.

In Experiment 1, FCR was decreased in treatment groups with including phytase than FCR for TRT 1, which had the same level of available P. The two higher levels of phytase inclusion (200 and 250 FTU/kg) had a similar FCR as treatments containing 0.10% more available P or more suggesting that phytase inclusion was capable of increasing P bioavailability by up to 0.15%. In Experiment 2, the inclusion of the two levels of phytase was capable of improving FCR during the starter and grower period to a level observed in the control diet. Simons et al. (1990) witnessed similar results with

inclusion of 500 FTU of phytase in a diet containing 4.5 g/kg of P had similar body weights and feed conversion as a diet with 6 g/kg of P feed through day 24 of age. Waldroup et al. (2000) reported the inclusion of 800 FTU/kg of Natuphos with a diet containing 0.15% aP resulted in a feed conversion similar to a diet containing 0.25% available P. One of the greatest benefits of phytase supplementation in these diets was maintaining livability at extremely low levels of available P, which have been shown to cause excessive mortality (Waldroup et al., 2000). In the current experiments, mortality was highest at the lowest levels of available P and the addition of phytase significantly reduced observed mortality rates.

Regression analysis was conducted on observed parameters in Experiment 1 to quantify the increase in P bioavailability from the inclusion of the three levels of phytase with and without NSPase inclusion. Equations were determined based on the observed body weight at day 14 (Figure 1), bone ash (mg) (Figure 2), and bone ash percentage (Figure 3) as a function of inorganic P consumption. The inclusions of the three levels of phytase (TRT 5-7) were capable of sparing 0.06%, 0.08%, and 0.10% available P with 150, 200, and 250 FTU/kg inclusion rates, respectively. Previous research has estimated varying amounts of phytate-bound P that can be released from the diet, with variations depending on the level of phytase used, the type of diet, and the stage of growth that is evaluated. Nelson et al. (1971) indicated that between 50-100% of phytate-bound P in a corn soy diet is capable of hydrolyzation by use of varying levels of phytase. Waldroup et al. (2000) suggested that the inclusion of 800 FTU of Natuphos was capable of releasing approximately 50% of the phytate P from the diet.

In Experiment 2, a low energy diet with a 132 kcal/kg reduction in ME was fed, however this reduction in energy did not negatively influence performance parameters which was unexpected, however did reduce fat pad yield following processing. Ravindran et al. (1999) witnessed that the energy content of a 2,980 AME wheat diet may not be limiting performance resulting in no effects when a xylanase enzyme was added which was observed during Experiment 2. Delezie et al. (2009) witnessed reduced body weight, feed intake and slaughter weights with birds fed a diet with a 10% reduction in energy as compared to the control diet, this was the expected outcome of the current trial prior to termination.

In Experiment 1, the addition of NSPase to a diet containing the low level of phytase (150 FTU/kg) increased broiler performance parameters and bone ash to a level comparable higher phytase treatment groups (200 and 250 FTU/kg) phytase inclusion. Regression analysis determined that NSPase inclusion increased P bio-availability from 0.06% to 0.09% with 150 FTU/kg phytase inclusion rate indicating that reduced levels of phytase in conjunction with a cocktail carbohydrase preparation can be used to achieve desired effects in young broilers as compared to higher levels when fed alone. Unfortunately, when the experimental design for Experiment 1 was expanded to a 41 day grow-out trial, the inclusion of NSPase with varying levels of phytase did not result in significant improvements in broiler growth, processing parameters or bone mineralization. This observation has been previously reported by Ravindran et al. (1999), who witnessed that the inclusion of xylanase with phytase did not result in an improvement in bird performance. Ghorbani et al. (2009) also witnessed no significant

improvements resulting from co-administration of enzymes on carcass composition. The improvements in bird performance resulting from co-administration of phytase and NSPase witnessed in Experiment 1 agreed with Cowieson et al. (2005) in that the inclusion of both a phytase and cocktail energy enzyme could enhance growth as compared to diets that were individually supplemented with enzymes. Francesch et al. (2009) observed an increase in feed consumption and a reduction in FCR in male broilers through 43 days of age, when a enzyme complex containing carbohydrases and phytase was added to reduced energy diets. The magnitude of the enzyme effect in providing beneficial effects was greatest in the diets with the lowest levels of available P and calcium. Tiwari et al. (2010) determined that the improvements in bird performance associated with the co-administration seemed to be largely due to phytase and broilers benefited most from the enzymes at an early age. However, Olukosi et al. (2010) reported the inclusion of phytase and carbohydrase did not produce greater benefit than the use of phytase alone.

These data indicate that inclusion of cocktail carbohydrase preparation can improve phytase activity in young broilers allowing for reduction in phytase inclusion rates. However, in totality, the data from these two experiments add to the recently published literature that report inconsistent results of co-administration of phytase and carbohydrases in corn-soy based diets. Therefore, further research is needed to accurately determine the effectiveness of different cocktail carbohydrases on a variety of phytase enzyme under multiple inclusion levels to determine the timeline allowable for reduced phytase inclusion rates while achieving the desired effects.

CHAPTER VI

CONCLUSION

Previous research has shown that enzyme supplementation in broiler diets has proven to be a practical management practice to help integrators increase digestibility and reduce nutrient output. The four research experiments discussed confirm and validate the use and effectiveness of multiple enzymes in poultry production. Additionally, these projects evaluated new enzyme preparations that could financially benefit poultry producers. In the first experiment, three commercially available phytase enzymes were used to determine the effect of multiple inclusion levels on increases in phosphorus bio-availability from dietary phytic acid. The effectiveness of each of the enzymes at the two inclusion levels varied with in terms of phosphorus release determined through regression analysis to be between 0.05-0.20percent. Typically, researchers evaluate the effects of phytase on broiler performance and bone mineralization by comparing supplemented diets to control diets with 0.10% higher available phosphorus levels to determine phosphorus released. In this current trial, regression analysis was used to calculate the increase in bioavailability of P from the inclusion of these three commercially available phytase enzymes. Elevated levels of phytase enzymes were included in the diets that were capable of releasing between 0.15-0.20% aP. The ability of commercially available phytase enzymes to release up to 0.20% aP can allow broiler integrators to use increased levels of phytase to compensate for the use of inorganic phosphates when determined cost effective.

The inclusion of a two proprietary cocktail carbohydrase preparations were evaluated in the second experiment, with a 41-day grow out trial conducted to analyze the effects on broiler performance and processing parameters when NSPase enzymes are included in corn-soy based diets. The inclusion of NSPase resulted in a decrease in FCR as compared to the control diet during the starter and grower phases. Additionally, the inclusion of NSPase in the control diet resulted in the highest processing weights and significantly increased breast weight, and total breast meat weight from processed broiler carcasses compared to control broilers. Previous research conducted analyzing the effect of NSPase inclusions in corn-soy based diets have shown minimal improvement resulting from enzyme inclusion, with most improvements of energy enzymes resulting in high fiber diets. The improvements in FCR and processing parameters resulting from the inclusion of these two blends of NSPase in corn soybean meal based diets suggest that energy enzymes can be utilized by integrators to reduce diet costs.

The co-administration of phytase and NSPase enzymes in corn-soy based diets was evaluated to determine if dual administration would enhance enzyme functionality. Variable results with similar trials have previously been reported when co-administering these two types of enzymes in a corn-soy based diet. NSPase inclusion was capable of enhancing the effects of phytase when fed to broilers during early stages of growth raised in brooder batteries. When the trial was replicated in an industry grow out trial, the inclusion of NSPase with phytase did not enhance broiler growth, processing parameters or bone mineralization. The data generated through this series of

experiments validate that feed enzymes, specifically phytase and cocktail carbohydrases, can be used to reduced dietary costs through the removal of inorganic phosphate at levels higher than previously reported and the removal of energy from the diet without sacrificing broiler performance.

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