12-2014

Evaluation of Phytate and Phytase Interactions and Phytase Phase-Feeding on Bird Performance, Bone Characteristics and Meat Quality in Young Growing Broilers

Chance L. Williams
University of Arkansas, Fayetteville

Evaluation of Phytate and Phytase Interactions and Phytase Phase-Feeding on Bird Performance, Bone Characteristics and Meat Quality in Young Growing Broilers

Evaluation of Phytate and Phytase Interactions and Phytase Phase-Feeding on Bird Performance, Bone Characteristics and Meat Quality in Young Growing Broilers

A thesis submitted in partial fulfillment
of the requirements for the degree of
Doctorate of Philosophy in Poultry Science

by

Chance Williams
Texas Tech University
Bachelor of Science in Animal Science, 2009
University of Arkansas
Master of Science in Animal Science, 2011

December 2014
University of Arkansas

This dissertation is approved for recommendation to the Graduate Council.

_____
Dr. Susan Watkins
Dissertation Director

_____
Dr. Michael Kidd
Committee Member

_____
Dr. Craig Coon
Committee Member

_____
Dr. Charles Rosenkrans
Committee Member

ABSTRACT

Two trials were performed using one-day-old male Cobb x Cobb 500 broilers to determine how dietary phytate and phytase levels as well as phytase phase feeding impacted bird performance parameters, tibia characteristics, and malonaldehyde (MDA) content of the liver, breast and thigh tissues. The first experiment consisted of 1,008 birds randomly placed in 48 floor pens within two commercial broiler houses at the Applied Broiler Research Farm (ABRF; 21 birds per pen; 0.76 ft^2 per bird). A 2 X 3 factorial design was used with two levels of dietary phytate (0.21 and 0.31 %) and three levels of phytase supplementation (0, 500 and 1,500 FTU/kg). Main effect phytase improved ($P < 0.05$) feed intake, body weight at 17 d, body weight gain and tibia ash weight and percentage. In addition, phytase and phytate interacted ($P \leq 0.011$) for FCR and FCR corrected to the overall experimental mean for body weight (AFCR).

The second trial consisted of 1,056 total birds randomly placed in 48 floor pens within two commercial broiler houses at ABRF (22 birds per pen; 0.72 ft^2 per bird). Treatments consisted of a positive control, a negative control (NC; less 0.16 % Ca, 0.15 % avP and 0.04 % Na), and four additional treatments based on the negative control. Treatments 3 and 4 consisted of the NC diet supplemented with 500 FTU/kg of phytase in the starter phase that was either continued through the grower diet (treatment 3) or increased to 1,500 FTU/kg (treatment 4). Treatment 5 and 6 were also the NC diet supplemented with 1,500 FTU/kg of phytase for the starter diet and either decreased to 500 FTU/kg in the grower diet (treatment 5) or maintained at 1,500 FTU/kg (treatment 6). A random complete block design was employed and analyzed using SAS GLM. At 35 d of age, phytase regimen did not affect ($P > 0.05$) feed intake, BW gain, FCR, AFCR or mortality. However, increasing phytase concentration from 500 FTU/kg in the starter diet to 1,500 FTU/kg in grower diet increased ($P < 0.05$) proximal and total tibia ash

percentages when compared to broilers fed diets with 500 FTU/kg of phytase for the duration of the study.

ACKNOWLEDGEMENTS

I would like to say a special thanks to the following people that were vital for my program.

Jordan Williams for supporting me through my program and for tolerating living on a poultry farm for an extended period of time.

Kevin McDaniel for leading me in the direction of poultry nutrition and for being a wealth of knowledge.

Dr. Susan Watkins for allowing me into your program, all of the knowledge of poultry production you have shared with me and the “baptism by fire.”

Dr. Kidd, for pushing me to keep my head buried in books and research articles.

Dr. Craig Wyatt

Everyone I worked with at the Applied Broiler Research Farm, thank you for all of your help during flocks and research trials. I made lifelong friends with all of you.

TABLE OF CONTENTS

LIST OF TABLES

Chapter 1: Literature Review

Introduction

Phosphorus (P) is an essential nutrient for poultry, which is needed for bone development, growth, and metabolic functions, however, P rock reserves are finite (Gilbert, 2009) and P is one of the costliest items in poultry feed. However, poultry diets contain a large percentage of plant feedstuffs that contain a large P reserve present as phytate. Phytate offers an abundant source of P, unfortunately, birds are not effective at hydrolyzing phosphate from phytate present in plants and it is excreted from the digestive tract (Ravindran *et al*., 1995). Phytic acid binds P, rendering it indigestible in the gastrointestinal tract of the bird, and then is excreted into the litter. Undigested P can enter the environment and cause ecological effects. For instance, P is known to contribute to eutrophication of freshwater sources due to algal bloom, causing implications for aquatic wildlife and effecting drinking water quality. Chicken litter is considered to be an environmental burden in certain areas. This is due to the high P concentration of litter, and litter is considered to be one of the main sources of P run off (Nahm, 2003). Poultry litter is nutrient rich and a valuable organic fertilizer. Edwards and Daniel (1992) demonstrated that broiler litter has an average P concentration of 1.43 %, with a range of 0.8-2.58 %. Over use of poultry litter in land application can have negative implications on the environment. It is primarily due to these environmental issues with P that exogenous phytase supplementation began in poultry and swine diets.

Poultry research with phytase has resulted in improved P availability by as much as 60 % and decrease P in excreta by as much as 50 % (Simons *et al*., 1990). The improvement in P availability resulted in a reduction in the need to supply inorganic P. Through the hydrolysis of

phytate, phytase has also demonstrated the ability to improve utilization of other divalent cation minerals (e.g. calcium, zinc and copper). It is also thought to enhance protein and energy utilization. These are considered 'extra-phosphoric' effects and are discussed in greater detail later in this review.

As mentioned above, phytase supplementation decreases the phosphate load on the environment; however, the phytase market will continue to expand for other reasons as well. Firstly, the supply of feed-phosphorus supplements, inorganic P rock and meat and bone meals, are supplies are becoming limited or prohibited. Supplementation of inorganic P has become increasingly expensive over the past decade, making it less cost effective for poultry production. In addition, the feed application of inorganic P at the current rate will hasten the exhaustion of the nonrenewable resource (Lei et al., 2013). The EU has also limited the use of meat and bone meal as cheap means for P supplementation. Secondly, the expansion of biofuel and industrial productions will provide large volumes of high-phytate cereal by-products as new feed sources (Liu, 2011). With these continuing pressures and the ability of phytase to enhance efficiency in the bird via growth and feed intake and lower feed costs will most likely continue to push the demand for phytase in the future.

Phytate

Phytate (*myo*-inositol hexakisphosphate with mixed salts, IP6) was first reported in 1855 (Hartig, 1855) and is the mixed calcium-magnesium-potassium salt of phytic acid. Phytate bears six phosphate groups on each of the six carbons comprising a *myo*-inositol ring. Phytate is an abundant plant constituent, 1-5 % by weight in certain plants, and is a rich source of P for the plant during germination (Reddy *et al*., 1982). Approximately 50 -75 % of the total phosphorous found in feed ingredients of plant origin is found as phytate-phosphorous (Mollgaard, 1946).

Phytate has a clear nutritional impact on P availability and phytate-P contains the major portion of total P in plant feed ingredients. Simons *et al*. (1990) demonstrated that two-thirds of P in these ingredients is present in the phytate form. However, the phytate-P concentration in feedstuffs depends upon the plant of origin and meal and cereal by-products typically contain the highest amounts (Table 1; Ravindran *et al*., 1995; Nelson *et al*., 1968). Phytate levels can also have large variations within a single feedstuff. For example, 73 wheat samples from Australia varied from 0.12 to 0.33 % phytate-P (Kim *et al*., 2002; Selle *et al*., 2003). Due to this potentially large variability, there could be altered responses to added phytase.

The location of phytate within feedstuffs varies among cereal grains. For wheat and rice, the largest concentration of phytate is present in the aleurone layers of the kernel and bran, but the endosperm is nearly devoid. Furthermore, about 80 % of the phytate in rice is located in the outer bran (O'Dell, *et al*., 1972). Alternatively, about 90 % of the phytate in corn is concentrated in the germ portion. It appears that many factors can affect phytate concentration in plant materials including maturity, processing, climate and location (Reddy *et al*., 1982).

Phytate and mineral digestion

Phytate, due to negatively charged phosphate groups, has a potent chelating ability for other divalent mineral cations. As phytate passes through the digestive tract of birds, it encounters a gradually increasing level of pH. As pH increases from the acidic stomach/gizzard to the more neutral intestine, phytate becomes more negatively charged due to the dissociation of the bound phosphate groups. At neutral pH, the bound phosphate groups can contain one or two negatively charged oxygen atoms, giving it the ability to chelate strongly between two phosphate groups or weakly with a single phosphate group (Sebastian *et al*., 1998). Consequently, phytate more strongly attracts and binds divalent cations like calcium (Ca), zinc (Zn), iron (Fe) and

copper (Cu) as the pH increases. As a result, stable salts are formed and precipitate out of the solution, creating indigestible complexes. Utilizing titration curves, Vohra *et al*. (1965) reported that phytate forms complexes with cations in the following descending order of strength: Cu^{2+} > Zn^{2+} > Co^{2+} > Mn^{2+} > Fe^{3+} > Ca^{2+}. Maddaiah *et al*. (1964) performed a similar study at a physiological pH, with Zn forming the strongest insoluble salt with phytic acid. Phytate lowers the bioavailability of vital minerals, thus increasing the dietary requirement in animals. Nelson and Kirby (1987) demonstrated that although Ca has the lowest binding efficiency with phytate, the greatest impact of phytate on mineral nutrition is on Ca availability. In a study using White Leghorn chicks, Nelson *et al*. (1968a) demonstrated in a purified diet, with no phytate, the dietary Ca requirement was 0.50 %, but the requirement was increased to 0.95 % in the presence of 1.25 % phytate. These findings were also supported by Harms *et al*. (1962), Nahapetian and Young (1980), Tamim and Angel (2003), and Tamim *et al*. (2004). From this study, it was suggested that Ca requirements for poultry be expressed in terms of available rather that total Ca. Therefore, if a diet contains ingredients with high phytate-P concentrations, the Ca requirement would have to be increased to offset the portion of insoluble Ca-phytate complexes. These findings demonstrate that phytate-P can be digested by poultry if it remains soluble in the small intestine. However, Ca requirements must be met to maintain animal health and efficiency, even though higher dietary Ca puts added pressure on the efficiency of digestible P.

Dietary Ca has also been shown to influence phytate-P utilization (Edwards and Veltman, 1983; Scheirdelerm and Sell, 1987; Mohammed *et al*., 1991). Despite not exhibiting the strongest affinity for chelation with phytate, Ca forms precipitate with phytate in the gastrointestinal tract due to the high concentration in diets. Thus, dietary Ca is important in determining phytase efficacy. In an *in vitro* system, Tamim and Angel (2003) investigated the

impact of Ca on phytate hydrolysis by *A. niger* phytase. These workers found that Ca addition reduced the liberation of P from sodium phytate from 350 to 175 μg P unit^{-1} phytase at pH 6.5. In addition, they also found at pH 2.5 Ca addition caused a similar reduction of P release from 1,250 to 625 μg P unit^{-1} phytase. Ballam *et al*. (1984) demonstrated that chicks hydrolyzed less phytate when fed a diet containing 1.0 % Ca versus those fed a diet containing 0.85 % Ca. Furthermore, about a 15 % increase in phytate-P utilization was seen when dietary Ca was reduced from 1.0 % to 0.5 % (Mohammed *et al*., 1991). There are currently three mechanisms that attempt to explain the interaction between dietary Ca and phytase activity. The first, Ca may form insoluble complexes with phytase (Wise, 1983). The second thought is that Ca negatively affects gastro-intestinal pH, which may decrease microbial phytase activity (Nelson, 1967) or decrease phytate solubility. This mechanism is supported by findings by Shafey *et al*. (1991) where Ca increased ($P < 0.05$) crop pH, the site of the highest exogenous phytase activity (Liebert *et al*., 1993; Takemasa *et al*., 1996). Finally, extra Ca may suppress phytase activity by competing for the active sites on phytate (Qian *et al*., 1996). Consequently, exogenous phytase supplementation is more advantageous in broiler diets with lower dietary Ca concentrations.

The Ca to total P ratio has also been shown to play a role in phytate P utilization (Wise, 1983). A high Ca or Ca:total P ratio of 2:1 has shown to hinder phytate degradation due to insoluble complexes formed in the intestine (Nelson, 1967). Research shows that chicks fed a diet with a Ca:total P ratio of 1:1 performed better than chicks fed a 2:1 ratio (Vandepopuliere *et al*., 1961). Harms *et al*. (1962) also demonstrated that increasing the Ca:P ratio in the diets from 1:1 to 2:1 decreased the availability of phytate-P to a greater extent than inorganic P sources.

At pH 6.0, Zn becomes a limiting mineral in high phytate diets as it forms highly insoluble complexes (Maddaiah *et al*., 1964; Reddy *et al*., 1982). Phytate has been shown to

increase fecal Zn excretion (Savage *et al*., 1964), and due to the affinity of phytate and Zn, it was thought that phytate might be a causative factor of parakeratosis in swine (Oberleas *et al*., 1962). Sebastian *et al*. (1996) observed that exogenous phytase supplementation increased the relative Zn retention in broilers from -27.6 % to 34.7 %. Phytase supplementation was also shown to be effective in improving Zn retention and tibia ash concentration in broilers fed a low Zn corn-soybean meal diet (Yi *et al*., 1996). They determined that approximately 0.9 mg of Zn was released per 100 units of phytase, up to 600 FTU. With the addition of microbial phytase, the ability to reduce dietary Zn is possible, which was demonstrated by Mohanna and Nys (1999) where the use of 800 FTU in a corn-soybean meal diet allowed the lowering of dietary Zn by 14 ppm. Evaluating the sparing effect of phytase on dietary Zn, Jondreville *et al*. (2007) determined that 100 FTU of phytase was equivalent to 1 mg of Zn as Zn sulphate and Zn excretion may be reduced about 10 % in a corn-soybean meal diet with 500 FTU/kg. However, contradicting findings have been reported. Roberson and Edwards (1994) found that phytase supplementation alone had no effect on Zn retention in chicks, but improved retention in conjunction with vitamin D_3.

Phytate and digestive enzymes

In vitro studies have demonstrated the ability of phytate to inhibit the activity of digestive enzymes like pepsin, α-amylase (Deshpande and Cheryan, 1984) and trypsin (Singh and Krikorian, 1982; Caldwell, 1992). Thus, has been suggested that phytate may inhibit proteolysis by altering digestive enzymes (Singh and Krikorian, 1982). It was considered that phytate might bind trypsin via a Ca tertiary complex, therefore inhibiting activity (Singh and Krikorian, 1982). However, phytate inhibition of trypsin may also occur by the chelation of Ca, which is essential

to trypsin and α-amylase activity. Also reported, Cawley and Mitchell (1968) documented that phytate suppressed α-amylase in wheat meal by complexing Ca required for enzyme activity.

Phytase

Phytase is produced by fungi (e.g. *Aspergillus niger, Aspergillus ficcum and Aspergillus oryzae*), bacteria (e.g. *E. coli* and *B. subtilis*) and yeast (e.g. *S.cervisiae*). Wodzinski and Ullah (1996) demonstrated that microbial phytases have a wide optimum pH (2.5-7.5) and temperature range (35-63^{o}C), making them more effective in the gastrointestinal tract than plant phytases. A number of cultured phytase enzyme supplements are available commercially, and with recombinant DNA technology the functional properties of these supplements continue to improve (e. g. thermo-stability). The activity of phytase is measured in terms of inorganic P released from phytate. This is known as Phytase Unit (FYT or FTU). One Phytase Unit is defined as the amount of enzyme needed to release 1 μmol inorganic phosphate per minute from 5.1 mM sodium phytate at pH 5.5 and 37^{o}C (Engelen *et al*., 1994).

From catalytic mechanisms, phytases can be grouped into histidine acid phytases (HisPhy), β-propeller phytases (BPPhy), cysteine phytases (CysPhy) and purple acid phytases (PAPhy; Mullaney and Ullah, 2003; Greiner, 2006). However, the majority of phytases to date belongs to the group HisPhy and do not require a cofactor for optimal activity. Histidine acid phosphatases have been recognized in microorganisms, plants and animals (Wodzinski and Ullah, 1996; Mullaney *et al*., 2000; Konietzny and Greiner, 2002; Lei and Porres, 2003). The structure of histidine acid phytases contains a α/β-domain and a variable α-domain (Kostrewa *et al*., 1997; Lim *et al*. 2000). The active site of HisPhy is located at the interface between the two domains. Differences in substrate binding have been attributed to differences in the α-domain. Histidine acid phytases share a sequence motif of Arginine-Histidine-(Glycine/Glutamine)-X-

Arginine-X-Proline. This site is considered to be the phosphate acceptor near the N-terminus (van Etten *et al*., 1991; Ostanin *et al*., 1992; Lindqvist *et al*., 1994). Furthermore, HisPhy contain a Histidine-Aspartic acid motif near the C-terminus where the aspartic acid is proposed to be the proton donor for the substrate leaving group (Lindqvist *et al*, 1994; Porvari *et al*., 1994). Histidine acid phosphatases have potent inhibitors, including Zn^{2+}, fluoride, molybdate, and the hydrolysis product of orthophosphate (Konietzny and Greiner, 2002). Interestingly, β-propeller phytases are active at neutral and alkaline pH (Greiner *et al*., 2007), making them possibly very useful in the small intestine. In addition, β-propeller phytases also target calcium-phytate complexes (Fu *et al*., 2008). However, there are currently no commercially available β-propeller phytases, despite their potential promise.

Besides phytase addition to feed, there are sources of naturally occurring phytase present in certain feed ingredients, the brush border of the intestinal mucosa and within the gut microflora. Naturally occurring phytase in feed stuffs, especially wheat and wheat by-products, have been reported (Peers, 1953). This intrinsic plant phytase is present to access the abundant storage of phytate bound P present within the plant. Unfortunately, plant phytase may have little impact on P release in feed due to the high heat processing of feed, especially steam pelleting. Plant phytases are heat labile and Konietzny and Greiner (2002) reported that, in purified form, most are destroyed at temperatures above 70°C within minutes. Additionally, a steam pelleted diet of wheat, corn and soybean meal at 80°C reduced wheat phytase activity, reducing total-tract P digestibility by 37 % in pigs.

Endogenous phytase activity in the intestine was first reported by Patwardhan (1937) in rats, and later was also identified in pigs (Hu *et al*., 1996) and poultry (Maenz and Classen, 1998). It appears that mucosal phytase activity is managed by dietary non-phytate P as well as

dietary Ca. Before the two latter studies, it was largely believed that the small intestine of monogastrics had a very limited ability to hydrolyze phytate due to the limited presence of endogenous phytase activity and a low microbial population in the upper digestive tract.

Phytases in animal feed

The bioavailability of phytate phosphorous is considered to be very low for monogastrics, mostly because they lack the ability to efficiently hydrolyze phytate and utilize P in the phytate form. Phytases represent the sub-group of phosphomonoesterases that initiate a stepwise dephosphorylation of phytate. Phytase is a valuable enzyme that is able to free phytate-bound P from the inositol ring and make it readily available to monogastrics. In theory, phytase has the ability to degrade IP6 phytate completely to six phosphate moieties and inositol. However, this depends greatly on feed retention time in the digestive tract and the P moiety at C_2 of the inositol ring is not readily released. Thus, complete breakdown of phytate most likely does not occur in pigs or poultry.

Commercially available phytases require considerably low pH for optimal activity; therefore, the main site of phytate hydrolysis by exogenous phytase is the stomach of pigs and the crop, proventriculus and gizzard in poultry. Liebert *et al*. (1993) found that following a 1,000 FTU/kg phytase activity 45 % of phytase activity was recovered in the crop and 21 % in the proventriculus, but no activity was recovered in the small intestine. Takemasa *et al*. (1996) also concluded that exogenous phytase was mainly active in the crop. Therefore, the limitation of phytase activity in the proximal digestive tract means there is limited time to degrade phytate. In order to reduce the antinutritive effects of phytate, dephosphorylation of higher molecular weight phytate esters (IP6 and IP5) must occur as quickly as possible in the proximal digestive

tract. This allows a release of P from phytate and systematically reduces the IP6/5 concentrations that have lower solubility in the small intestine, thus reducing the antinutritive effect of phytate in the small intestine. The solubility of multiple IP esters in the small and large intestine in pigs was assessed by Schlemmer *et al*. (2001; Table 2), and demonstrated that solubility of phytate decreases as the number of phosphate groups bound to the *myo*-inositol ring increases. The solubility of IP6, IP5, IP4, IP3, and IP2 in the intestinal chyme of pigs (pH 6.6) was 2 %, 7 %, 8 %, 31 % and 75 %, respectively (Schlemmer *et al*., 2001). Additionally, the lower esters of phytate display a reduced capacity to chelate divalent cations. Cowieson *et al*. (2011) states that the primary responsibility of phytase added in feed is not to completely dephosphorylate phytate into inositol and free phosphate, but to reduce the concentrations of high phytate esters released into the duodenum. Thus, endogenous phytases would be able to further hydrolyze the more soluble phytate esters. The ability to increase feed retention time in the crop should enhance phytase efficacy and increase phytate degradation. Intermittent lighting programs have been shown to increase retention time of the feed in the crop (Hooppaw and Goodman, 1976). Hence, phytase and lighting duration she be a focal point for future research.

Crop pH may also have an impact on the efficacy of supplemented phytase. Supplemented glutamic acid and other organic acids have shown the ability to reduce crop pH. Murai *et al*. (2001) found a reduced crop pH (6.0 to 5.4) was associated with enhanced phytase efficacy through femur mineral deposition. Furthermore, citric acid has also been shown to increase phytate-P utilization in broiler chicks (Boling *et al*., 2000; Snow *et al*., 2004). The reduction in pH could increase the solubility of phytate and may be advantageous for phytases with lower optimal pH activity; future research is warranted.

Cowieson *et al*. (2011), report that there is a common misconception that phytate is poorly digested by the chick due to the lack of endogenous phytase. There are supporting studies that indicate that poultry posses an effective endogenous phytase activity in the intestinal mucosa, blood and liver. Therefore, chicks can readily dephosphorylate phytate into inositol and free phosphate (Oshima *et al*., 1964; Maenz and Classen, 1998). It has been shown that a rapid increase in the blood concentration of IP6 was detected in the first 3 weeks post-hatch (Oshima *et al*., 1964), indicating that chicks have the ability to metabolize IP6 to an absorbable phytate ester. In addition, Moore and Veum (1983) showed that IP6 is digestible and the digestibility of IP6 can be inflated when the growing animal is deprived of available P. Other studies have also reported phytase activity in animal tissue (McCollum and Hart, 1908; Nelson, 1967). Ergo, the major problem with phytate digestion within the chick is not due to the complete lack of endogenous enzymes but instead due to poor substrate solubility in the small intestine. It is important to note that individual feed ingredients appear to affect phytase efficiency. Utilizing total tract assessment of phytate degradation, Leske and Coon (1999) demonstrated that degradation ranged from 14.8 % (rice bran) to 39.1 % (barley) at 6,000 FTU/kg phytase activity. Two broiler studies investigating the dephosphorylation of phytate at the ileal level determined that phytate degradation by 500 FTU *A. niger* phytase kg^{-1} does not exceed 35 % (Camden *et al*., 2001; Tamim *et al*., 2004). However, Van der Klis *et al*. (1997) showed a 58 % increase in phytate degradation in laying hens by 500 FTU *A. niger* phytase kg-1. This may be an effect of endogenous phytase or increased retention times in the forestomach.

Commercially available phytases can be divided into 3- and 6-phytases, depending on the initial phosphate group released. It is thought that including a combination of phytases with different hydrolysis initiation sites would create a synergistic, linear additive response regarding

phosphate release. It has been shown that growing pigs feed with intrinsic cereal phytase (rye, wheat) and supplemental *A. niger* phytase exhibited a linear increase on apparent P absorption (Zimmermann *et al*., 2003). However, no synergistic effects have been observed by combining phytases with different initiation sites (Augspurger *et al*., 2003; Stahl *et al*., 2004).

Phytate is well established to reduce the bioavailability of P, Ca, Zn and other divalent cations. However, there are a number of documented factors that can affect phytate degradation. Genotype of birds may have an effect on phytate-P utilization. Edwards *et al*. (1989) demonstrated that the average retention of phytate-P was greater for Leghorn chickens than meat type broilers. There were also differences in phytate-P utilization for different broiler strains (Sebastian *et al*., 1998). The age of the bird also was shown to affect phytate-P utilization because it is generally agreed that endogenous phytase activity in the intestinal tract increases with the age of the birds (Edwards *et al*., 1989). Additionally, Nelson (1967) showed that mature hens utilize phytate-P better than chicks.

Diets marginal or deficient in Vitamin D_3 have been shown to depress phytate-P utilization (Ewing, 1963). Vitamin D_3 and its metabolites (1, 25 $(OH)_2$ D_3) enhance phytate-P utilization in chicks and may be attributed to either increased production or activity of endogenous phytase (Edwards *et al*., 1989; Shafey *et al*., 1991; Biehl *et al*., 1995; Mitchell and Edwards, 1996), increased phytate hydrolysis (Mohammed *et al*., 1991) or enhanced absorption of P (Wasserman and Taylor, 1973).

Nutritional benefits of phytase

Numerous studies have shown that microbial phytase addition to diets increases body weight gain, feed intake and feed efficiency in broilers (Simons *et al.*, 1990; Broz *et al*., 1994; Denbow *et al*., 1995; Mitchell and Edwards, 1996; Sebastian *et al*., 1996; Singh and Khatta,

2002; Singh *et al*., 2003). Nelson *et al*., (1971) were the first to supplement 0.4 % phytase (*A. ficcum*) in corn and soybean meal based broiler diets that contained 0.24 % phytate-P. In this study, there was a 33.3 % improvement in body weight gain. Furthermore, the addition of phytase to low P broiler diets showed significantly improved body weight gain, feed intake and feed efficiency, but this level of improvement was dependent on the level of phytase supplemented (Simons *et al*., 1990). Utilizing graded levels of phytase supplementation (125, 250 and 500 FTU/kg diet) resulted in an increase in growth of broilers by 4.6, 6.4 and 8.5 %, respectively when compared to a control (0 FTU/kg addition; Broz *et al*., 1994).

Employing varying levels of non-phytate P (NPP) in broiler diets, numerous studies have shown that the greatest response was observed in low NPP diets (Denbow *et al*., 1995; Rama Rao *et al*., 1999; Lim *et al*., 2000), mostly attributed to phytate hydrolysis and better nutrient utilization. This response has allowed for the economic replacement of inorganic P supplementation and ultimately reducing feed cost (Singh *et al*., 2003; Singh and Khatta 2003). In semi-purified broiler diets with 0.20, 0.27 and 0.34 % NPP and seven levels of supplemental phytase (0, 200, 400, 600, 800, 1,000 and 1,200 FTU/kg diet), Denbow *et al*. (1995) showed an increase in body weight gain and feed intake at all NPP levels. Alternatively, Lim *et al*. (2000) only found an enhancement in body weight gain in a low NPP diet when utilizing 500 FTU/kg in a corn-soy diet with 0.25, 0.35 and 0.45 % NPP. Further studies have also demonstrated the positive responses of phytase in low NPP wheat-soybean meal diets (Cabahug *et al*., 1999). Improvements seen in growth in broilers fed low NPP diets may be due to the increase in feed intake and improved feed efficiency by the utilization of P from phytate (Qian *et al*., 1996; Sebastian *et al*., 1996), inositol formation and utilization (Simons *et al*., 1990), increased starch

digestibility (Knuckles and Betschart, 1987), increased amino acid utilization (Ravindran *et al*., 2000), or improved overall utilization of nutrients (Miles and Nelson, 1974).

Phytase supplementation has been shown to increase the bioavailability of P and Ca, with positive responses to bone ash. Bone ash is considered to be a more useful criterion for evaluating the availability of dietary P than body weight (Nelson and Walker, 1964). Using a corn-soy diet containing 0.18-0.24 % phytate-P supplemented with phytase, Nelson *et al*. (1971), found an increase in tibia bone ash in broilers. The ash percentage of the tibia and toe was significantly ($P < 0.01$) improved by phytase addition, suggesting the liberation of inorganic phosphate and Ca by phytase improves bone mineralization (Perney *et al*., 1993). Broz *et al*. (1994) also reported 4.17, 4.87 and 7.79 % increases in tibia bone ash for diets supplemented with 125, 250 and 500 FTU/kg feed, respectively, when compared to a control diet without phytase supplementation. Utilizing different dietary Ca levels (0.60, 1.0 and 1.25 %) and phytase supplementation, Sebastian *et al*. (1996) found that phytase supplementation increased tibia ash regardless of Ca level. However, maximum ash content was determined for diets with 1 % Ca. Cabahug *et al*. (1999) also found that toe ash response was the greatest with phytase supplementation in diets with higher phytate-P concentrations, resulting in a significant phytate-P x phytase interaction.

Protein effect of phytate and phytase

Phytase is also considered to impact the availability of protein and amino acids. The possibility that phytate had a negative impact on protein utilization was first suggested by Rojas and Scott (1969). Officer and Batterham (1992), utilizing grower pigs with diets based on linola meal as the major protein source. In this trial, phytase significantly increased ileal digestibility of nitrogen (22.6 %) and lysine (20.3 %). These responses led to the belief that phytase may

release amino acids bound in phytate linkages. Phytate has been established to bind protein to form protein-phytate complexes (Cosgrove, 1966; Anderson, 1985), and with hydrolysis of phytate by phytase in the stomach would reduce the amount of *de novo* protein-phytate complex formation in the higher pH intestine (Selle *et al*., 2000). It was demonstrated that phytate is capable of binding up to ten times its weight of protein under *in vitro* conditions (Kies *et al*., 2006). This would entail that a diet with 10 g phytate kg^{-1} and 250 g protein kg^{-1}, nearly half of the protein in diet could be complexed by phytate. Therefore, the thought that phytase enhances amino acid digestibility in monogastrics through protein-phytate complex formation reduction was conceived.

It appears that pH is the driving force for *de novo* formation of protein-phytate complexes in the digestive tract. Cosgrove (1980) and Anderson (1985) determined that phytate interacts with protein to form two different types of complexes; one in acidic and another in alkaline pH. Binary protein-phytate complexes are present at the acidic pH found in the gastric phase. At extremely low pH (pH 2), phytate carries a strong negative charge whereas proteins are positively charged. Hence, the formation of protein-phytate complexes (Cheryan, 1980). Binary protein-phytate complexes are formed below the isoelectric point of protein (pH < 5-6). At the physiological pH found in the crop (4.5) and gizzard (3), a partial protonation occurs but phytate still maintains a net negative charge (Costello *et al*., 1976). Thus, phytate will interact with α-NH_2 groups and NH_3^+ groups of basic amino acids that include arginine (PI 10.8), histidine (PI 7.6) and lysine (PI 9.7; Cosgrove, 1966). Ultimately, this would affect protein solubility and would cause an increase in the production of pepsinogen, HCl, mucin and $NaHCO_3$ (Selle and Ravindran, 2007). This response can have a detrimental effect on the animal efficiency and increase the nutrient requirements. Due to the refractory nature of these complexes in the

forestomach, the hyper-secretion of HCl must be neutralized once gastric emptying occurs. Thus, extra secretion of mucin and sodium bicarbonate may arise (Munster *et al*., 1987; Allen and Flemstrom, 2005), causing an increase in the presence of endogenous amino acids and sodium in the lumen.

Tertiary protein-phytate complexes are considered to be formed in the small intestine where dietary nutrients encounter a more neutral pH environment. At this higher pH, both phytate and proteins are negatively charged, therefore, the direct electrostatic effect between the two molecules are minimal. Instead, chelated divalent cations are thought to mediate what can be considered phytate-mineral-protein complexes (O'Dell and de Boland, 1976). Either of the complexes that occur between phytate and protein led to decreased protein solubility (Saio *et al*., 1967).

Besides the negative impact on dietary proteins, research has suggested that phytate also has negative implications on endogenous amino acid losses (Cowieson *et al*., 2004; Cowieson and Ravindran, 2007). Endogenous proteins and amino acids originate primarily from digestive secretions, mucoproteins and sloughed epithelial cells. Multiple studies have shown that the amount of endogenous protein recovered in the ileum is increased by numerous anti-nutritive factors, including trypsin inhibitors, tannins, lectins (Nyachoti *et al*., 1997) and phytate (Cowieson *et al*., 2007). It is possible that improvements seen in nutrient digestibility coefficients seen with the addition of exogenous phytase may occur because of a reduction in the loss of endogenous materials (Bedford, 1996; Bedford and Morgan, 1996; Ravindran *et al*., 1999; Cowieson *et al*., 2004). The production and loss of endogenous proteins is nutritionally expensive for animals (Fan *et al*., 1997). Phytate may increase endogenous losses due to interactions with endogenous enzymes or mucin. Furthermore, a positive feedback for extra

endogenous enzyme secretion may occur when a decrease occurs in enzyme activity or availability, similarly seen with protease inhibitors (Clarke and Wiseman, 2003).

Utilizing the peptide alimentation method on 26-day old male broilers (Ross 308), Cowieson *et al*. (2007) demonstrated that phytic acid concentration had a significant ($P < 0.05$) impact on nitrogen and some amino acids (aspartic acid, threonine, serine, glutamic acid, glycine, isoleucine, leucine, cysteine and methionine) ileal endogenous flow, with endogenous loss increasing with increasing phytate concentration. In addition, phytase addition (500 FTU/kg *E. coli* phytase) reduced ($P < 0.05$) nitrogen and most amino acid (aspartic acid, threonine, serine, proline, glycine, valine, isoleucine, leucine, histidine, lysine, arginine, and cysteine) ileal endogenous flow. Furthermore, endogenous flows of Asp, Ser, Thr and Tyr were increased with phytic acid concentration increases, suggesting that phytate may selectively increase the flow of some endogenous proteins more than others. The amino acid composition of mucin and pepsin is highly related to these phytase prompted increases in ileal amino acid flow.

Energy effect of phytate and phytase

Many nutritionists now are utilizing an energy release component in the ingredient matrix for phytase. This is due to research consistently demonstrating an enhancement in metabolizable energy (ME) in broiler diets.

It has been suggested that the positive impact of phytase on energy may arise due to a collective increase in the digestibility of protein, fat and starch. The increase of digestibility of amino acids would increase the energy derived from proteins (discussed above). Furthermore, there is evidence demonstrating the ability of phytate to interact with lipids through complexes of Ca-/Mg-phytate, lipids and proteins (Cosgrove, 1966). The interaction of phytate and lipids likely leads to the formation of metallic soaps in the intestinal lumen, causing decreased energy

derivation from lipids; especially saturated fats (Atteh and Leeson, 1984). A study by Matyka *et al*. (1990) found that beef tallow reduced phytate-P utilization in young chicks and there was a subsequent increase in the percentage of fat excreted as soap fatty acids. If metallic soap formation in the gut is due to Ca-phytate complexes, it seems reasonable that phytase would alleviate some of the metallic soap formation by prior phytate hydrolysis in proximal digestive tract.

Phytate may also impact starch digestion, either by directly binding starch through hydrogen bonds or by binding proteins associated with starch (Thompson, 1988). However, there is limited *in vitro* evidence to support the existence of phytate-starch complexes (Selle *et al*., 2000). Alternatively, phytate may impact starch digestion through another channel. Thompson *et al*. (1987) has demonstrated that phytate reduced the blood glycemic indices in humans. Thus, phytate may depress intestinal uptake of glucose rather than impair starch digestion in the intestinal lumen.

Super-dosing phytase

Super-dosing phytase is defined as the addition of 1,500 FTU/kg or more of microbial phytase with either a partial or no nutrient matrix applied (Cowieson *et al*., 2013). By using 1,500 FTU/ kg inclusion in feed while utilizing a 500 FTU/kg nutrient matrix allows the nutritionists to reduce the nutrient requirements for P and Ca and improve feed conversion and body weight gain. Thus, maximizing profitability through bird performance rather than decreasing diet cost.

The unfortunate reality of phytase feed addition is that all phytases follow a quadratic dose response curve, rather than a linear response. Therefore, by doubling phytase addition in feed, we do not expect to double the amount available P released. Instead, for example, if the

addition of 500 FTU/kg phytase releases 0.10 % available P, then doubling the dose to 1,000 FTU/kg will increase the available P release to 0.13 %. In fact, it may take up to as much as 5,000 FTU/kg to double the effect of that seen with 500 FTU/kg (Cowieson *et al*., 2013).

The majority of poultry diets contain between 0.20 % and 0.30 % phytate-P concentration, but can get as low as 0.15-0.18 % with the use of low phytate grain varieties and/or animal protein meals are utilized. With a diet containing 0.25 % phytate-P and 500 FTU/kg inclusion, the expected available phosphorous release would be 0.13 %, thus, approximately 50 % hydrolysis of phytate-P. Using the laws of log curves we expect that as much as 60-70 % hydrolysis of phytate-P would occur in the first 500-750 FTU/kg. Therefore, super-dosing phytase is only expected to yield small incremental advantages. However, high doses of phytase improve growth performance much beyond that of 500-750 FTU/kg phytase inclusion (Cowieson *et al*., 2013).

The earliest research in phytase super-dosing is most likely Nelson *et al*. (1971), where 950-7,600 FTU/kg of *Aspergillus ficuum* derived phytase was used in a broiler chick experiment to 21 days. Nelson and colleagues found that apparent phytate-P disappearance increased from 38.9 % (950 FTU/kg) to 94.4 % (7600 FTU/kg), with a response to phytase on 21-day weight gain and bone ash percentage. The response found for weight gain and bone ash was log-linear and maximized at 7,600 FTU/kg where gain was increased 131 % compared to the phytase free negative control chicks and the bone ash was also 59 % greater.

It has been exhibited that bacterial phytases prefer to target high molecular weight inositol phosphate esters in the initial reaction phase (IP6/5; Wyss *et al*., 1999; Greiner and Farouk, 2007). Thus, proportionally more IP6 and IP5 molecules are destroyed than that of IP4 and IP3. With this in mind, it has been shown that IP6 and IP5 have a greater chelating capacity

for Ca than seen with IP4 and IP3 (Luttrell, 1992; Persson *et al*., 1998). Demonstrating that Ca release by phytase is most likely not a linear response and the rate of Ca release occurs much more rapid than that of phosphate release (Cowieson *et al*., 2011). It was initially believed that super-dosing phytase may lead to a Ca:P imbalance, potentially causing issues with skeletal growth and wet litter. However, super-dosing may actually benefit in balancing the Ca:P ratio. The Ca:P utilized in most phytase nutrient matrices ranges between 1.1:1 and 1:1. But this is likely inaccurate at some points along the dose response curve. The Ca:P ratio may actually be very high at low-phytase levels and gradually declines with the addition of phytase. In vitro work by Walk *et al*. (2012) demonstrated an initial flurry of Ca release but the Ca:P ratio gradually decreased to less than 1.5:1 over time. Therefore, as increased phytase doses are used, the assumed and actual Ca:P ratios continue to converge. This may be one important reason why super-dose levels of phytase may provide greater responses than that of lower phytase doses.

Myo-Inositol

One of the beneficial effects of exogenous phytase addition in poultry feed is thought to be the generation of *myo*-inositol through complete enzymatic dephosphorylation of dietary phytate (Jozefiak *et al*., 2010; Cowieson *et al*., 2011). The known main function of *myo*-inositol is its involvement in the structure of phospholipids and lipoproteins, as well as phosphatidylinositol, which serves as a cell mediator that regulates metabolism and growth (Michell, 2008).

The first article to demonstrate a growth promoting effect of inositol in chickens was Hegstedt *et al*. (1941). Other studies have also suggested that inositol may alleviate tocopherol deficiency in chicks (Dam, 1944), improve leukocyte number in turkeys (Lance and Hogan, 1948) and depress fatty liver syndrome (Gavin and McHenry, 1941).

Walk *et al*. (2014) measured gizzard phytate, phytate-P esters and inositol concentrations of 21-day old broilers. They found that phytase addition significantly ($P < 0.05$) reduced IP6 and IP5 and increased inositol when compared to a control diet without exogenous phytase. In addition, it they determined that inositol, IP6 and IP5 gizzard concentrations were highly correlated to growth performance. IP6 and IP5 were negatively correlated to body weight gain and positively correlated to feed conversion. Alternatively, inositol concentrations were positively correlated to body weight gain and negatively correlated to feed conversion.

Phytase and phytate effect on antioxidants

Relatively little research has been done in the way of determining the potential impact of phytase supplementation may have on the antioxidant status of varying tissues, however, Karadas *et al*. (2010) demonstrated that phytase inclusion was shown to increase the hepatic tissue levels of ascorbic acid, coenzyme Q10 and β-carotene. Coenzyme Q10 supplementation has been shown to improve liver mitochondrial function, increase anti-reactive oxygen species proficiency and decrease malonaldehyde content (Geng and Guo, 2005). Graf *et al*. (1987) also demonstrated that phytic acid acts as a natural antioxidant, inhibiting the generation of iron-induced radicals and lipid peroxidation. Even though limited research has occurred with the tissue associated phytic acid levels, Sakamoto *et al*. (1993) has shown that increasing tissue phytic acid level within tissues is possible.

The ability to improve the antioxidant capacity of certain tissues could provide an insight on how high phytase levels provide such positive results. Bottje *et al*. (2002) has shown that broilers within the same genetic line have fundamental differences in feed efficiency. Bottje *et al*. (2002) stated that variations in broiler growth performance and phenotypic expression of feed efficiency may be due to differences in mitochondrial function. It was believed that

inadequacies in mitochondrial function may be to blame because mitochondria are responsible for producing the majority of cellular ATP. Through the study of mitochondrial function, it has been demonstrated that a key difference observed between and high and low feed efficient broilers within the same genetic line is oxidative stress (Iqbal *et al*., 2004). In addition, using gene expression, Bottje and Kong (2012) indicated that high feed efficient birds had an increased expression of genes associated with signal transduction pathways, anabolic activities and energy-sensing/coordination activities; all of which would be advantageous for cell growth. On the other hand, low feed efficient birds had an increased expression of genes associated with actin-myosin filaments, cytoskeleton structure and stress-related/responsive genes.

Chapter 2: Dietary phytate and phytase level interactions on bird performance, bone ash and mineral, and antioxidant status of broilers to 18 days of age

Abstract

A total of 1,008 one-day-old male Cobb 500 broilers were randomly placed in 48 floor pens (21 birds per pen; 0.76 ft^2 per bird) within two commercial broiler houses at ABRF (Fayetteville, AR) to evaluate the interactions of dietary phytate and phytase levels related to bird performance, bone ash and mineral content, thiobarbituric acid-reactive substances of the liver, breast and thigh tissues as well as the content of phytate, phytate esters and inositol in the gizzard of young broilers. The floor pens were equipped with a feed pan with a 30-pound feed hopper, a nipple drinker line and a supplemental feeder for the first 10 days. Birds were group weighed prior to placement and at the end of the 18 day evaluation period. At the conclusion of the trial, 3 birds per pen were euthanized via rapid cervical dislocation to obtain the left tibia, gizzard contents and tissue samples of the liver, breast and thigh. A 2 X 3 factorial design in a SAS GLM model was used, two levels of dietary phytate (0.21 and 0.31 %) and three levels of phytase supplementation (0, 500 and 1,500 FTU/kg), to evaluate variables. A significant phytase x phytate interaction was observed for FCR ($P < 0.001$) and BW corrected FC (AFCR; $P = 0.011$). Furthermore, a positive linear effect for phytase level was significant ($P < 0.001$) for feed intake, body weight and body weight gain. Tibia ash mass and percentage for both the proximal and distal tibia was also positively impacted ($P = 0.05$) by phytase level. In the proximal tibia, however, only phytate level was found to affect Mg level. In the distal tibia, phytase level impacted Zn, Mn, Na and S. Phytase also decreased ($P < 0.001$) the level of IP6 and IP5 in gizzard digesta and increased ($P < 0.001$) inositol generation. Finally, phytase level

also decreased (P = 0.020) malonaldehyde content of the thigh. These results support the use of higher doses of phytase in young growing broilers.

Introduction

The anti-nutritive effects of dietary phytate in broiler diets have been well documented, including mineral chelation (Vohra, 1965; Nelson & Kirby, 1987; Maddaiah, 1964; Tamim, 2003; Tamim, 2004), protein binding (Kies *et al*., 2006; Selle *et al*., 2000; Selle *et al*, 2012) and the impact of inefficient overproduction of pepsin, HCl, NaHCO3 and mucin (Cowieson *et al*., 2004). Therefore, the addition of exogenous phytase has been shown to increase nutrient digestibility and growth performance in broilers. Conventionally, phytase is supplemented at concentrations between 300 and 600 FTU/kg to release P from phytate (Cowieson *et al*., 2006). This conventional level eliminates the phytate esters inositol hexa-phosphate (IP6) and inositol penta-phosphate (IP5), thus improving phytate solubility (Schlemmer, 2001) and limiting the anti-nutritive effect of phytate so broilers can utilize more nutrients (Cowieson, 2011). However, recent research has demonstrated inositol tetra-phosphate (IP4) and inositol tri-phosphate (IP3) still maintain a potent chelating ability for nutrients such as Fe3+ as well as limit pepsin catalyzed protein hydrolysis (Yu *et al*., 2012). Therefore, higher levels of exogenous phytase supplementation have been shown to reduce IP4 and IP3 and also generate more inositol, which was positively correlated with BW gain and negatively correlated to FCR. However, these results were not associated with a subsequent increase in tibia ash, demonstrating that Super-dosing benefits may be associated with phytate destruction and inositol generation, rather than excess P and Ca (Walk *et al*., 2014).

Super-dosing phytase is described as the addition of 1,500 FTU/kg or more of microbial phytase with either a partial or no nutrient matrix applied (Cowieson *et a*., 2013). By using 1,500 FTU/ kg phytase inclusion while utilizing a 500 FTU/kg nutrient matrix allows the nutritionists to relax the nutrient requirements and improve feed conversion and body weight gain. Thus, maximizing profitability through bird performance rather than decreasing diet cost. Phytase supplementation to broiler diets produces curvilinear growth performance and nutrient digestibility responses. However, when super-dosing levels are utilized, bird performance is typically better than anticipated, suggesting the impact of extra-phosphoric effects (Cabahug *et al*., 1999; Cowieson *et al*., 2006). Gehring *et al*. (2013), suggests that the magnitude of extra-phosphoric effects may be dependent on the concentrations of substrate and enzyme present. Thus, it would be useful to determine how the phytate profile in the gizzard is affected by differing levels of phytate-P % and how conventional and super-dose levels of phytase affect this phytate ester profile. Therefore, the objectives of this study were to evaluate how dietary phytate-P % impacts early chick performance and how conventional and super-dose levels of exogenous phytase over differing phytate-P % levels alter performance, tibia ash and gizzard phytate ester profile.

Materials and Methods

All procedures relating to the use of live birds were approved by the University of Arkansas Institutional Animal Care and Use Committee through protocol #11056.

Birds and Housing

A total of 1,008 one-day-old Cobb 500 male broiler chicks were obtained from a commercial hatchery and randomly distributed to floor pens (21 birds per pen; 0.76 ft^2 per bird)

within two commercial broiler houses (40’ x 400’) at the University of Arkansas Applied Broiler Research Farm (ABRF; Fayetteville, AR). Broilers were vaccinated for Marek’s disease, infectious bronchitis and Newcastle disease at the hatchery. This study occurred from January, 16 – February, 3 2014. The mini-pens (4’ x 4’) were placed in the brood chamber (half-house) and their position was maintained for the duration of the study. Treatments were blocked from the tunnel inlet to the middle of the house. The two commercial broiler houses used were solid-sided, tunnel-ventilated houses equipped with four 32” side-wall exhaust fans, eight 48” tunnel fans, 18 radiant pancake brooders and two forced air furnaces. Mini-pens were equipped with a Choretime feed pan with a 30-pound feed hopper, a supplemental feeder for the first 10 days of grow-out and a nipple drinker line. Feed and water were available *ad libitum*.

The lighting intensity and lighting curve used in this study were utilized according to a commercial integrator. Light emitting diodes (LED) and compact fluorescent (CFL) bulbs were used in each house on full brightness during the first 7 days of grow-out. On day 8, CFL bulbs were turned off and the LED bulbs remained on at full intensity. On day 16, LED bulbs were dimmed to 0.3 FC and maintained for the remaining duration of the project. During the first week, the birds received 24 hours of light. At day 8, the light:dark period was changed to 18 hours light and 6 hours of dark. Furthermore, on day 16 the light:dark periods were changed to 20 hours light and 4 hours of dark.

The temperature and minimum ventilation curves utilized for this study are summarized in Table 3. Both the temperature and ventilation standards used for this study were run according to a local commercial broiler integrator. The houses were pre-heated two days prior to chick placement. On day -2, the house was pre-heated to 80^{o} F, to begin heating the litter. The day prior to placement (day -1), the house was then heated to 90^{o} F. The 90^{o} F house

temperature was maintained through day 3 of grow-out. After day 3, the temperature began ramping down to 85^{o} F on day 7, 83^{o} F on day 14 and 80.7^{o} F on day 18.

For the minimum ventilation, the on:off time (seconds) for day 1 began at 30:330 using two exhaust fans. The on time continually increased while the off time for the fans gradually declined until a 3 minute timer was used. On day 7 the on:off time was 60:120, which gradually changed to 78:102 and 91:89 on:off times for days 14 and 18, respectively.

Dietary Treatments

All diets (Table 4) were based on corn-soybean meal and fed in crumbled form (77^{o}C conditioning temperature). Dietary treatments consisted of two levels of phytate-P (0.21and 0.31 %) and 3 levels of exogenous phytase supplementation (0, 500 and 1,500 FTU/kg), creating a 2 X 3 factorial design. Energy, protein, amino acids, available Ca and phosphorous, Ca:P ratios, and divalent cation minerals were formulated to be as close to identical in each ration formulation as possible. Each treatment was replicated by 8 pens with 21 chicks/pen. Corn was exchanged with phytase where appropriate to take the diets to 100 %. The phytase was a modified *E. coli* 6-phytase expressed in *Trichoderma reesei* with an expected activity of 5,000 FTU/g (Quantum Blue, AB Vista Feed Ingredients, Marlborough, UK).

All feed ingredients were analyzed prior to mixing for a total mineral analysis and proximate analysis (Table 5). Feed samples were taken at the University of Arkansas Feed Mill post-pelleting. Samples were analyzed for phytate level and phytase activity by AB Vista (Table 6).

Response Variables

Birds were weighed by pen prior to placement and on day 18 to determine BW and calculate average BW gain. Feed intake was also measured from day 1 to day 18 and used to calculate FCR. Mortality was recorded daily, and any mortality was weighed. Thus, FCR was adjusted according to mortality. Furthermore, feed conversion ratios were also corrected to the overall experimental average body weight and adjusted using 27 g = 0.01 FCR (AFCR). Birds for sampling were euthanized via rapid cervical dislocation on day 18 for collection of tibias, liver, breast, thigh and gizzard digesta.

Bone Ash and Mineral Analysis

The left tibia was removed from each of 3 euthanized birds from each pen. All muscle and adhering tissues was removed using cheese cloth. Following bone cleaning, the tibias were divided into approximately a 30/70 division, with the proximal tibia representing the 30 % and the remaining distal bone representing the other 70 %. Tibias were pooled by pen and the proximal and distal sections were kept separate for each step. Bone weights were taken for pooled proximal and distal tibia sections prior to drying.

All bone sections were then dried in an oven at 100^{o} C for 24 hours, along with crucibles. After drying, a dry weight for each crucible and pooled proximal and distal bone sections were recorded. Dried tibias were then ashed in a muffle furnace for 24 hours at 600^{o}C to determine bone ash.

Gizzard Phytate, Phytate Esters and Inositol Analysis

Digesta from the gizzard was obtained from the same 3 birds euthanized for bone ash and pooled per pen. Digesta was stored at -80^{o}C until it was freeze-dried at -55^{o}C and < 100 mTorr.

The freeze-dried digesta was then ground to pass a 1-mm screen. The freeze-dried and ground digesta was then analyzed for phytate [inositol hexa-phosphate (IP6)], phytate esters [inositol penta-phosphate (IP5), inositol tetra-phosphate (IP4) and inositol tri-phosphate (IP3)], and inositol using high-performance ion chromatography and methods of Blaabjerg et al. (2010).

Thiobarbituric Acid-Reactive Substances (TBARS) Assay

Frozen breast, thigh and liver samples were stored at -20^{o} C until the analysis was performed. Samples were removed from the freezer and set on a room temperature surface for 1 hour and covered with a cotton towel. Once samples displayed some slack (not thawed) they were placed in the refrigerator and the analysis was promptly performed.

Duplicates from each pooled liver, breast (*Pectoralis major*) and thigh (*Iliotibialis*) were run and duplicates from each sample were run for absorbance. Two grams from each pooled sample was weighed out and placed in a labeled 50 mL disposable polypropylene centrifuge tube. Next, 8 mL of prepared phosphate buffer (Appendix 1) and 2 mL of TCA reagent (Appendix 2) was added to the tube and the contents were homogenized for 20 to 30 seconds. The homogenate was then filtered using Whatman (No. 4) filter paper into labeled 15 mL disposable polypropylene centrifuge tubes. Next, 2 mL of the sample filtrate was pipette into a labeled 16 x 100 mm borosilicate glass culture tubes in duplicates. Two mL of the prepared TBA reagent (Appendix 3) was added to each sample tube, blanks and standards. The tubes were then covered with aluminum foil and placed into a hot water bath (100^{o}C) for 20 minutes. Afterwards, the sample tubes were removed from the water bath and placed on ice for 15 minutes. Absorbance was then read at 533 nm with a UV-1201 sip spectrophotometer.

Statistical Analysis

The six treatments of the factorial arrangement of two dietary phytate levels and three phytase supplementation level treatments were analyzed by the following model:

$$Y_{ijk} = \mu + Phytate_i + Phytase_j + PhytatePhytase_{ij} + e_{ijk}$$

Where μ is the common mean; $Phytate_i$ is the effect of the i^{th} phytate; $Phytase_j$ is the effect of the j^{th} phytase supplementation level; and e_{ijk} is the random error. This model assesses the main effects and interactions of the factorial arrangement. The pen of broilers served as the experimental unit. In this experiment, treatments were blocked from the tunnel inlet to the middle of the house. Statements of significant difference are based on $\alpha = 0.05$, as obtained from Type III Sums of Squares from the Analysis of Variance generated through the General Linear Models Procedure of SAS (SAS Institute Inc., Cary, NC), means were separated with repeated *t*-test. PROC CORR was also utilized to determine correlations between gizzard phytate esters and other measure variables, statements of significance are based on $P \leq 0.05$, which indicate correlations significantly differ from zero.

Results and Discussion

Analysis confirmed that all diets were within an expected range for phytate-P % and phytase recoveries (Table 6). Analyzed phytate-P % levels in the low-phytate diet were similar to formulation and 16 % higher than formulated for the high phytate-P % diet. The phytate analyses confirm that the low- and high-phytate diets varied in phytate concentration by approximately 44 % and in phytate-P % by approximately 44 %.

Bird Performance

Live performance variables for male broilers fed diets varying in phytate level and phytase level supplementation are summarized in Table 7. Feed intake over the 18-day period

was not significantly impacted by a phytase x phytate interaction ($P = 0.664$) or the main effect phytate level ($P = 0.273$). However, the main effect phytase was significant ($P < 0.001$). Birds fed diets with 1,500 FTU/kg phytase consumed 0.817 kg of feed/bird, which was significantly ($P = 0.015$) greater than the 0.771 kg of feed/ bird consumed by broilers fed diets with 500 FTU/kg of phytase. In addition, birds fed diets with 500 FTU/kg of phytase consumed more ($P = 0.005$) feed/bird than the 0.717 kg of feed/bird consumed by broilers fed diets with 0 FTU/kg of phytase addition. Finally, birds fed diets with 1,500 FTU/kg of phytase consumed significantly ($P < 0.001$) more feed/bird than broilers fed diets with 0 FTU/kg of phytase supplementation.

For BW and BW gain, phytase x phytate interaction and main effect phytate level were insignificant ($P \geq 0.368$), whereas phytase level was significant ($P < 0.001$). Broilers fed diets with 1,500 FTU/kg of phytase gained 0.600 kg, which was greater ($P = 0.010$) than the 0.562 kg of body weight gained by birds fed diets with 500 FTU/kg, leading to a 6.1 % heavier ($P = 0.008$) bird at 18 d. Furthermore, birds fed diets with 500 FTU/kg also gained significantly ($P < 0.001$) more body weight by 18 days of age than the 0.509 kg gained by their counterparts that received diets with 0 FTU/kg, which also led to a 8.4 % increase ($P < 0.001$) in body weight. Birds fed diets with 1,500 FTU/kg of phytase also a more substantial gain ($P < 0.001$) than birds fed diets with 0 FTU/kg, thus a 13.9 % heavier ($P < 0.001$) body weight. Oddly, phytate level was not significant for BW or BW gain ($P = 0.368$), which contradicts other studies, where higher phytate diets were shown to depress bird growth to 28 d of age in Cobb 500 broiler (Liu *et al*., 2008), as well as in 21 d Arbor Acre broilers (dos Santos *et al*., 2014). This would be expected due to phytate's wide antinutritive effects; however, in this study it appears that non-phytase supplemented diets, regardless of phytate level, hindered bird growth. This reinforces the negative impact of phytate on broiler performance.

Increases in feed intake and body weight gain are typically reported in the literature for phytase supplementation; however, improvements in FCR are less commonly reported due to the corresponding parameters unless a BW corrected FC is utilized. Onyango *et al*. (2005) and Rutherford *et al*. (2012) both demonstrated improvements in BW gain and feed intake but feed efficiency was not impacted in broilers fed diets with low avP. Alternatively, Walk *et al*. (2013 and 2014) reported significant improvements in FCR of 49 and 21 d old broilers fed phytase compared with broilers fed a nutrient adequate control diet. In addition, Walk et al. (2014) hypothesized that benefits in FCR associated with super-dosing phytase may be associated with phytate destruction and the subsequent phytate ester profile, rather than excess P and Ca. This thought may provide insight to the observed interaction ($P < 0.001$) between exogenous phytase level and phytate-P % for FCR (Table 8, Figure 1). Of the low phytate-P % diets, the broilers fed diets with 500 FTU/kg phytase supplementation had a lower FCR (1.23; $P \leq 0.036$) than broilers fed diets with 0 or 1,500 FTU/kg of exogenous phytase (1.29 and 1.26, respectively). Alternatively, of the high phytate-P % diets, the broilers fed diets with 1,500 FTU/kg of phytase had a more efficient FCR (1.24; $P \leq 0.015$) than birds fed diets with 0 or 500 FTU/kg (1.28 and 1.29, respectively). The interaction point appears at the 500 FTU/kg of phytase level, which may indicate that the anti-nutritive effect of phytate in the 0.21 % phytate-P diet is overcome by the 500 FTU/kg level, whereas the high phytate-P % diet requires greater doses of phytase to maximize feed efficiency.

A significant interaction ($P = 0.011$) between phytase x phytate levels was also observed for BW adjusted FCR (AFCR). Of the low phytate-P % diets, broilers fed diets with either 500 or 1,500 FTU/kg of phytase had a lower ($P \leq 0.05$) AFCR (1.23 and 1.25, respectively) than birds fed diets with 0 FTU/kg of phytase (1.30), but were not statistically different from one

another. However, of the high phytate-P % diets, the broilers fed diets with 1,500 FTU/kg of phytase were still more efficient (1.23; P) than both other groups, which was similar to the results found before the BW adjustment for FCR.

Bone Ash and Mineral Analysis

The proximal, distal and total tibia ash percentages and weights are summarized in Table 9. The main effect phytase level supplementation was significant for proximal tibia ash weight ($P < 0.001$) and percentage ($P = 0.027$). Broilers fed diets with 1,500 FTU/kg had a greater ($P = 0.044$) proximal tibia ash weight than the birds fed diets with 500 FTU/kg (0.221 vs. 0.204 g, respectively). Furthermore, broilers on diets with 500 FTU/kg were also observed to have greater ($P = 0.002$) proximal tibia ash weight than the 0.177 g ash weight detected in broilers fed diets with 0 FTU/kg. These differences in proximal tibia ash weight led to differences in bone ash percentage. Birds on diets with 1,500 FTU/kg had higher ($P = 0.008$) bone ash percentage (35.26 %) than the 33.70 % observed in broilers fed diets with 0 FTU/kg. Phytase level was significant for distal tibia ash weight ($P < 0.001$) and percentage ($P < 0.001$) where as phytase x phytate interaction and phytate level were insignificant ($P \geq 0.327$) for both measurements. Broilers fed diets with 1,500 FTU/kg had 0.467 g of distal tibia ash which was greater ($P = 0.022$) than the 0.427 g of distal tibia ash observed by birds on diets with 500 FTU/kg. In addition, birds fed diets with 500 FTU/kg of phytase also had a greater ($P < 0.001$) distal tibia ash weight than birds on diets with 0 FTU/kg (0.365 g). Moreover, birds on diets with either 1,500 or 500 FTU/kg had higher ($P \leq 0.003$) distal tibia ash percentages (46.86 and 46.20 %, respectively) than their counterparts fed diets without phytase supplementation (44.54 %).

As expected, phytase level was significant for both whole tibia ash ($P < 0.001$) and tibia ash percentage ($P < 0.001$), but phytase x phytate interaction and phytate level were insignificant ($P \geq 0.349$). Broilers fed diets with 1,500 FTU/kg had 0.688 g of total tibia ash which was greater ($P = 0.022$) than the 0.631 g of total tibia ash detected from birds on diets with 500 FTU/kg. Likewise, birds fed diets with 500 FTU/kg also had greater ($P < 0.001$) total tibia ash than broilers given diets with 0 FTU/kg (0.542 g). Similarly, broilers fed diets with either 1,500 or 500 FTU/kg of phytase supplementation had higher ($P \leq 0.010$) total tibia ash percentages (42.38 and 41.66 %, respectively) than broilers fed diets with 0 FTU/kg of phytase supplementation (40.31 %). These results demonstrating the improvement in tibia ash based on phytase level is in agreement with Nelson *et al*. (1971), Dos Santos *et al*. (2014), and Walk *et al*. (2014) where phytase supplementation improved tibia ash when fed a diet with limiting avP and/or Ca. Interestingly, Cabahug *et al*. (1999) showed that phytate and phytase interacted for toe ash.

Despite differences seen in tibia ash weight and percentages, minimal differences were observed for the mineral content of the ash for the proximal (Table 10) and distal (Table 11) tibia portions. For the proximal tibia ash mineral profile the only statistical difference detected was for the main effect phytate ($P = 0.034$) for Mg level, where birds fed diets with 0.21 % phytate-P had a 0.83 % Mg versus the 0.86 % Mg found in the proximal tibia ash for birds fed diets with 0.31 % calculated phytate-P. In the distal mineral profile, again, the main effect phytate was significant ($P = 0.005$) for Mg, where birds fed diets with 0.21 % phytate-P contained 0.73 % and the birds fed the diets with 0.31 % calculated phytate-P contained 0.76 % Mg. The main effect phytase was also significant for Zn ($P = 0.047$) and Mg ($P = 0.005$). Birds fed diets with 1,500 FTU/kg of phytase had a greater Zn concentration (421.75 mg/kg) than birds fed diets with

500 FTU/kg (407.00 mg/kg) and 0 FTU/kg (404.06 mg/kg). In addition, phytase supplementation increased the Mg concentration of the distal tibia ash above the 0.72 % observed in birds fed 0 FTU/kg of phytase, but a decrease ($P < 0.05$) in Na and S percentages.

The addition of phytase significantly improved tibia ash percentage and weight, which is in agreement with Nelson *et al*. (1971), Perney *et al*. (1993), Broz *et al*. (1994), Walk *et al*. (2014) and Sebastian *et al*. (1996). This would indicate that there is increased availability of minerals due to the breakdown of phytate-mineral complexes (Sebastian *et al*., 1996). The distal portion of the tibia contains the shaft, which is a more rigid state of the bone less susceptible to variations due to availability of minerals. On the other hand, the proximal head is the rapidly growing portion where it is a more active state of change (Sebastian *et al*., 1996). In agreement with Broz *et al*. (1994) and Sebastian *et al*. (1996), our observations did not indicate any significant difference in Ca and P concentrations in the tibia ash.

Thiobarbituric Acid-Reactive Substances (TBARS)

Analysis of thiobarbituric acid-reactive substances in 17 d old broilers is summarized in Table 12. The main effect phytate level was significant ($P < 0.001$) for liver malonaldehyde content. Birds fed diets with 0.21 % phytate-P displayed a reduced level of MDA (1.098 mg/kg) when compared to birds fed diets with 0.31 % phytate-P (1.288 mg/kg). In addition, the main effect phytase was significant ($P = 0.020$) for thigh MDA content, where broilers fed diets with 1,500 FTU/kg of phytase had a reduced level (1.825 mg/kg) when compared to birds fed diets with 0 FTU/kg of phytase. Phytase level also showed a trend ($P = 0.065$) for reducing MDA content of the liver. Karadas *et al*. (2010) demonstrated that phytase inclusion increased the hepatic tissue levels of ascorbic acid, coenzyme Q10 and β-carotene. Coenzyme Q10

supplementation has been shown to improve liver mitochondrial function, increase anti-reactive oxygen species proficiency and decrease MDA content (Geng and Guo, 2005). In addition, ascorbic acid and β-carotene have strong antioxidant capabilities to limit free radicals.

Gizzard Phytate, Phytate Esters and Inositol

Quantification of inositol phosphate esters and inositol in freeze-dried gizzard digesta are summarized in Table 13. The main effect phytase level was significant ($P < 0.001$) for IP6, IP5 IP4 and inositol concentrations (log-transformation of nmol/g of dried weight of digesta). For IP6 concentration, broilers fed diets with 1,500 FTU/kg of phytase had 1.292 log nmol/dry weight, which was significantly less ($P < 0.001$) than the 2.082 log nmol/dry weight observed by birds fed diets with 500 FTU/kg of phytase. In addition, birds fed diets with 500 FTU/kg of phytase had a reduced ($P < 0.001$) IP6 concentration than the 2.611 log nmol/dry weight seen in broilers fed diets without phytase supplementation. A similar progression was seen for IP5 concentrations with the following significant differences: 0 FTU/kg > 500 FTU/kg > 1,500 FTU/kg. Furthermore, broilers on diets with 1,500 FTU/kg of phytase had a reduced IP4 ($P < 0.001$; 0.820 log nmol/dry weight) concentration than bird on diets with 500 and 0 FTU/kg of phytase (1.974 and 1.983 log nmol/dry weight, respectively). Finally, a greater amount ($P < 0.001$) of inositol was generated in the gizzard digesta of birds fed diets with 1,500 FTU/kg (3.281 log nmol/dry weight) when compared to birds fed diets without phytase supplementation (2.794 log nmol/dry weight).

Moreover, in this trial, IP6, IP5, IP4 and inositol concentrations from freeze-dried gizzard digesta was correlated to growth performance, tibia ash and TBARS variables (Table 14). Similar to Walk *et al*. (2014), IP6, IP5 and inositol concentrations were significantly correlated ($P \leq 0.008$) to BW gain, where IP6 and IP5 were negatively correlated and inositol was

positively correlated. In this study, however, no significant correlations were found between inositol-phosphate esters or inositol and FCR, which differs from the study by Walk *et al*. (2014) where IP6 and IP5 were strongly negatively correlated to FCR and inositol was strongly positively correlated. Additionally, IP6, IP5 and inositol were also significantly correlated ($P \leq 0.022$) to proximal, distal and total tibia ash weight, with IP6 and IP5 demonstrating a negative correlation and inositol a positive correlation. Inositol has been shown to have key functions in the central nervous system, phospholipid structure maintenance and lipid metabolism (Holub, 1986; Fisher *et al*., 2002). Supplementing broilers with inositol has been shown to improve BW gain and FCR (Zyla *et al*., 2004; Cowieson *et al*., 2013). In this study, inositol was not added to the feed but was instead generated by phytase from phytate destruction.

Conclusion

Phytate has long been shown to impact mineral digestion and absorption (Vohra, 1965; Nelson, 1987; Maddaiah, 1964; Tamim, 2003; Tamim, 2004), protein digestion (Kies *et al*., 2006; Selle *et al*., 2000; Selle *et al*., 2012) and interfere with enzyme activity (Liu *et al*., 2009), all of which may impede bird performance. Phytase has been shown to target higher weight inositol phosphate esters (IP6 and IP5; Wyss *et al*., 1999), thus proportionally more IP6 and IP5 are destroyed than IP4 and IP3 and the degeneration of IP6 and IP5 leads to the production of more IP4 and IP3. However, at super-dose levels of phytase, the destruction of IP4 and IP3 are also pronounced over a conventional phytase dose and produced more inositol. This study supports the use of super-dose levels of phytase in young growing broilers, positively impacting feed intake, BW, BW gain, tibia ash measurements, MDA content of the liver and thigh, and the gizzard phytate profile.

Chapter 3: Evaluation of phase feeding phytase in broiler starter and grower rations and effect on antioxidant and mineral status

Abstract

A total of 1,056 one-day-old male Cobb 500 broilers were randomly placed in 48 floor pens (22 birds per pen; 0.72 ft^2 per bird) within two commercial broiler houses at ABRF (Fayetteville, AR) to evaluate the effect of phase feeding phytase in the starter (1-17 days) and grower (18-35) rations on bird performance, bone ash and mineral content, and TBARS of the liver, breast and thigh. Treatments consisted of a positive control, a negative control (NC; less 0.16 % Ca, 0.15 % avP and 0.04 % Na), and 4 additional treatments based on the NC supplemented with phytase. Treatments 3 and 4 consisted of the NC diet supplemented with 500 FTU/kg of phytase in the starter phase that was either continued through the grower diet (treatment 3) or increased to 1,500 FTU/kg (treatment 4). Treatment 5 and 6 were also the NC diet supplemented with 1,500 FTU/kg of phytase for the starter diet and either decreased to 500 FTU/kg in the grower diet (treatment 5) or maintained at 1,500 FTU/kg (treatment 6). The floor pens were equipped with a feed pan with a 30-pound feed hopper, a nipple drinker line and a supplemental feeder for the first 10 days. A random complete block design in a SAS GLM model was used to evaluate bird performance, bone ash and mineral content, and TBARS of the liver, breast and thigh. At 35 d of age, phytase regimen did not affect ($P > 0.05$) feed intake, BW gain, FCR or mortality. However, increasing phytase concentration from 500 FTU/kg in the starter diet to 1,500 FTU/kg in grower diet increased ($P < 0.05$) proximal and total tibia ash percentages when compared to broilers fed diets with 500 FTU/kg of phytase for the duration of the study.

Introduction

Exogenous phytase supplementation in broiler diets has been shown to reduced the antinutritive effect of phytate, which includes electrostatic interactions with dietary minerals (Vohra, 1965; Nelson and Kirby, 1987; Tamim and Angel, 2003; Tamim *et al*., 2004) and limiting protein digestion (Kies *et al*., 2006; Selle *et al*., 2000; Selle *et al*, 2012). By mitigating the effect of phytate, phytase has been shown to improve nutrient digestibility and growth performance of broilers and super-dose concentrations typically provide further extra-phosphoric benefits (Cabahug *et al*., 1999; Ravindran *et al*., 2000). Phytase supplementation produces a curvilinear response for growth performance and nutrient digestibility, thus, extra-phosphoric effects and phytase concentrations do not exhibit a 1:1 relationship. However, by altering the exogenous phytase concentration as broilers advance in age, one may be able to limit the cost per unit of gain (Gehring *et al*., 2014).

Nelson (1967) demonstrated that birds may become more capable of utilizing phytate-P with age. Therefore, supplementing higher concentrations of phytase during the starter phase and reducing the level in subsequent diets may lead to savings in enzyme use, given the smaller volume of feed consumed and if the extra-phosphoric effects occur in the starter phase (Gehring *et al*., 2014). Another thought is that high phytase concentrations may become important during the linear portion of the broiler's growth curve (Gous *et al*., 1999). Gehring *et al*. (2014) performed a step-up/step-down regimen for phytase supplementation, demonstrating that increasing phytase concentration after the starter phase (14 d) or decreasing concentration after the grower phase (28 d) increased FCR, but the phytase regimen did not impact carcass variables. Therefore, the objective of this study was to evaluate the phase feeding of phytase in

broiler starter and grower rations and how this would impact bird performance, bone ash and mineralization, as well as MDA content of the breast, thigh and liver tissues of broilers.

Materials and Methods

All procedures relating to the use of live birds were approved by the University of Arkansas Institutional Animal Care and Use Committee through protocol # 11056.

Birds and Housing

A total of 1,056 one-day-old Cobb 500 male broilers were obtained from a commercial hatchery and randomly distributed to floor pens (22 birds per pen; 0.73 ft^2 per bird) within two commercial broiler houses (40' x 400') at the University of Arkansas Applied Broiler Research Farm (ABRF; Fayetteville, AR). Broilers were vaccinated for Marek's disease, infectious bronchitis and Newcastle disease at the hatchery. The mini-pens (4' x 4') were placed in the house (full-house brood) and their position was maintained for the duration of the study. Treatments were blocked from the tunnel inlet to the tunnel fans. The two commercial broiler houses used were solid-sided, tunnel-ventilated houses equipped with four 32" side-wall exhaust fans, eight 48" tunnel fans, 18 radiant pancake brooders and two forced air furnaces. Mini-pens were equipped with a Choretime feed pan with a 30-pound feed hopper, a nipple drinker line and a supplemental feeder for the first 10 days of grow-out. Feed and water were available *ad libitum*.

Light emitting diodes (LED) and compact fluorescents (CFL) bulbs were used in each house on full brightness during the first 7 days of grow-out. On day 8, CFL bulbs were turned off and the LED bulbs remained on at full intensity. On day 15, LED bulbs were dimmed to 0.3 FC and maintained to end of the study. During the first week, the birds received 24 hours of

light. At day 8, the light:dark period was changed to 18 hours light and 6 hours of dark and was maintained for the duration of the study by recommendations from a local commercial broiler integrator.

The temperature and minimum ventilation curves utilized for this study are summarized in Table 3. Both the temperature and ventilation standards used for this study were run according to a local commercial broiler integrator. The houses were pre-heated two days prior to chick placement. On day -2, the house was pre-heated to 80^{o} F, to begin heating the litter. The day prior to placement (day -1), the house was then heated to 90^{o} F. The 90^{o} F house temperature was maintained through day 3 of grow-out. After day 3, the temperature began ramping down to 85^{o} F on day 7, 81^{o} F on day 14, 78^{o} F on day 21, 71^{o}F on day 28 and 68^{o}F on day 35.

For the minimum ventilation, the on:off time for day 1 began at 30:330 using two exhaust fans. The on time continually increased while the off time for the fans gradually declined until a 3 minute timer was used. On day 7 the on:off time was 60:120, which gradually changed to 78:102, 97:83, 107:73 and 125:60 on:off times for days 14, 21, 28 and 35, respectively.

Experimental Treatments

Six dietary treatments consisted of a positive control (treatment 1; PC) diet formulated to be adequate in Ca and nonphytate-P, a negative control (treatment 2; NC) diet formulated with reduced Ca by 0.16 %, nonphytate-P by 0.16 % and Na by 0.03 %; the remaining 4 treatments were based on the NC diet but with varying levels of phytase supplementation (Table 15). Treatments 3 and 4 were the NC diet with 500 FTU/kg exogenous phytase addition in the starter and either continued throughout the study (treatment 3) or increased to 1,500 FTU/kg in the grower diet (treatment 4). Treatments 5 and 6 were also the NC diet, but with 1,500 FTU/kg in

the starter diet and continued for the duration of the study (treatment 6) or reduced to 500 FTU/kg in the grower diet (treatment 5). All diets were created as pellets (77^{o}C conditioning temperature) and fed as crumble form from 1 to 17 days of age and pellet form for the grower diets. Each treatment was replicated by 8 pens with 21 chicks/pen. Corn was exchanged with phytase where appropriate to take the diets to 100 %. The phytase was a modified *E. coli* 6-phytase expressed in *Trichoderma reesei* with an expected activity of 5,000 FTU/g (Quantum Blue, AB Vista Feed Ingredients, Marlborough, UK).

All feed ingredients were analyzed prior to mixing for a total mineral analysis and proximate analysis (Table 5). Feed samples were taken at the University of Arkansas Feed Mill post-pelleting. Samples were analyzed for phytate level and phytase activity by AB Vista (Table 16).

Response Variables

Birds were weighed by pen prior to placement, on day 18 and day 35 to determine body weight (BW) and calculate average body weight gain. Feed intake was also measured from day 1 to day 17 and day 18 to 35 and used to calculate FCR. Mortality was recorded daily, and any mortality was weighed. Thus, FCR was adjusted according to mortality. Furthermore, feed conversion ratios were also adjusted to the overall average body weight of the PC group for the grower and starter phases and adjusted using 27 g = 0.01 FCR (AFCR). Birds for sampling were euthanized via rapid cervical dislocation on days 17 and 35 for collection of tibias, liver, breast, thigh and gizzard digesta.

Bone Ash and Mineral Analysis

The left tibia was removed from birds euthanized from each pen on day 17 and day 35. All muscle and adhering tissues was removed using cheese cloth. Following bone cleaning, the tibias were cut into approximately a 30/70 division, with the proximal tibia representing the 30 % and the remaining distal bone representing the other 70 %. Tibias were pooled by pen and the proximal and distal sections were kept separate for each step. Bone weights were taken for pooled proximal and distal tibia sections prior to drying.

All bone sections were then dried in an oven at 100^{o} C for 24 hours, along with crucibles. After drying, a dry weight for each crucible and pooled proximal and distal bone sections were recorded. Dried tibias were then ashed in a muffle furnace for 24 hours at 600^{o}C to determine bone ash.

Thiobarbituric Acid-Reactive Substances (TBARS) Assay

Frozen breast, thigh and liver samples were removed from the freezer and set on a room temperature surface for 1 hour and covered with a cotton towel. Once samples displayed some slack (not thawed) they were placed in the refrigerator and the analysis was promptly performed.

Duplicates from each pooled liver, breast (*Pectoralis major*) and thigh (*Iliotibialis*) were run and duplicates from each sample were run for absorbance. Two grams of minced meat from each pooled sample was weighed out and placed in a labeled 50 mL disposable polypropylene centrifuge tube. The exact weight was recorded. Next, 8 mL of prepared phosphate buffer (Appendix 1) and 2 mL of TCA reagent (Appendix 2) was added to the tube and the contents were homogenized for 20 to 30 seconds. The homogenate was then filtered using Whatman (No. 4) filter paper into labeled 15 mL disposable polypropylene centrifuge tubes. Next, 2 mL of the sample filtrate was pipette into a labeled 16 x 100 mm borosilicate glass culture tubes in

duplicates. Two mL of the prepared TBA reagent (Appendix 3) was added to each sample tube, blanks and standards. The tubes were then covered with aluminum foil and placed into a hot water bath (100^{o}C) for 20 minutes. Afterwards, the sample tubes were removed from the water bath and placed on ice for 15 minutes. Absorbance was then read at 533 nm with a UV-1201 sip spectrophotometer.

Statistical Analysis

Data were analyzed by a one-way treatment structure with a randomized complete block design. The analysis of variance was analyzed by the following model:

$$Y_{ijk} = \mu + Treatment_i + Block_j + e_{ijk}$$

Where μ is the common mean; $Treatment_i$ is the effect of the i^{th} dietary treatment; $Block_j$ is the effect of the j^{th} block; and e_{ijk} is the random error. Block by treatment interactions were found to be insignificant, therefore, the model was reduced. The pen of broilers served as the experimental unit. In this experiment, treatments were blocked from the tunnel inlet to the tunnel fans with each treatment represented by 8 replicate pens. Least square means were compared using preplanned orthogonal contrasts and statements of significant difference are based on $\alpha = 0.05$, as generated through the General Linear Models Procedure of SAS (SAS Institute Inc., Cary, NC).

Results and Discussion

Analysis of phytase recovery confirmed all diets were within an expected range for phytase activity (Table 16).

Bird Performance

Male broiler performance from 1-17 days is summarized in Table 17. Broilers fed the NC diet did not display reduced performance or feed intake at day 17 when compared to broilers fed the PC diet. From 1 to 17 days of age, birds fed diets with 1,500 FTU/kg displayed a reduced ($P \leq 0.046$) FCR (1.24) and AFCR (1.24) than those fed diets with 500 FTU/kg (1.27 and 1.26, respectively). Furthermore, broilers fed diets with either 500 or 1,500 FTU/kg of phytase had greater ($P = 0.004$) BW gain and subsequently greater ($P = 0.003$) BW than birds fed diets without phytase supplementation. In addition, birds fed diets with phytase supplementation also displayed a reduced ($P \leq 0.017$) FCR and AFCR when compared to broilers not supplemented with phytase. As seen in other studies, reduced FCR with 1,500 FTU/kg of phytase compared with 500 FTU/kg is indicative of extra-phosphoric effects.

Male broiler performance for 1-35 days is summarized in Table 18. The effects of increasing or decreasing exogenous phytase level on growth performance between the starter and grower diets were determined at day 35. A trend for reduced feed intake ($P = 0.059$) and a significant decrease ($P = 0.05$) BW gain and BW was observed by day 35 for birds on the NC diet when compared to broilers on the PC diets. In addition, supplementing phytase improved ($P \leq 0.004$) BW, BW gain, FCR and AFCR when compared to both the PC and NC birds. However, feed intake, BW gain, FCR and AFCR of the broilers was not affected ($P > 0.05$) by increasing or decreasing phytase level between the starter and grower phases.

Similar to Gehring *et al*. (2014) an advantage in FCR was seen in the first phase (starter diet) for the birds fed 1,500 FTU/kg , but by 35 d of age this advantage was no longer statistically significant when compared to birds fed 500 FTU/kg for the duration of the study. In addition, Gehring *et al*. (2014) also demonstrated that increasing phytase concentration after the starter phase (14 d) or decreasing phytase after the grower phase (28 d) increased feed

conversion. The positive impact of higher doses of phytase supplementation through all feeding periods has been well documented. However, it appears that there may be a possible strategy of phase-feeding phytase, if desired. The use of 1,500 FTU/kg during the starter period and reduction to 500 FTU/kg around the inflection point of the growth curve did not have a negative statistical impact on bird performance when compared to birds fed 1,500 FTU/kg to 35 d of age in this study and in the step-up/step-down study performed by Gehring *et al*. (2014). This strategy should be dependent on the economic impact, market prices and phytase efficacy. Nevertheless, additional research should be performed to determine if a more gradual decrease/increase in phytase concentration produces similar effects or alleviates the negative results of changing phytase concentration. It would also be advantageous to determine the impact of changing phytase concentrations in feeding periods for older birds.

Bone Ash and Mineral Analysis

Proximal, distal and total tibia ash weights and percentages for broilers to 17 d of age are summarized in Table 19. Birds fed the NC diet displayed a reduced ($P \leq 0.007$) distal and total tibia ash percentage (51.99 and 48.94 %, respectively) when compared to birds fed the PC diet (53.90 and 50.51 %, respectively). In addition, birds fed diets with 1,500 FTU/kg of phytase had greater ($P \leq 0.008$) proximal, distal and total tibia ash weight and percentage when compared to that of broilers bed diets with 500 FTU/kg. Furthermore, broilers fed diets with phytase supplementation displayed significantly ($P \leq 0.031$) more distal and total tibia ash weight when compared to their counterparts on diets without phytase supplementation.

The effect of increasing or decreasing phytase level on proximal, distal and total tibia ash weights and percentages for broiler to 35 d of age are summarized in Table 20. Birds fed the NC diet had depressed ($P < 0.001$) tibia ash weights and percentages for the proximal, distal and total

tibia when compared to birds fed the PC diet. It was observed that by increasing the phytase level from 500 FTU/kg in the starter diet to 1,500 FTU/kg in grower diet showed a subsequent increase ($P \leq 0.040$) in proximal and total tibia ash percentage (42.45 and 47.33 %, respectively) when compared to birds fed diets with 500 FTU/kg of phytase for the duration of the study (41.34 and 46.11 %, respectively) without a statistically detectable difference ($P > 0.126$) in tibia ash weight. On the other hand, birds fed a diet with 500 FTU/kg in the grower diet after being fed 1,500 FTU/kg of phytase in the starter diet maintained similar ($P \geq 0.130$) tibia ash parameters compared to broilers fed 1,500 FTU/kg of phytase for the duration of the study.

Tibia ash mineral content of male broilers at 17 d of age is summarized by proximal (Table 21) and distal (Table 22) portions. There were no statistically detectable differences observed for the proximal or distal tibia ash mineral content between birds fed the PC and NC diets ($P > 0.05$), however, the 42.05 % Ca and 529.38 ppm Zn observed in proximal tibia portion of the PC birds displayed a trend ($P \leq 0.096$) for increased Ca and Zn when compared to the broilers fed the NC diet (38.15 % and 482.13 ppm, respectively). Oddly, birds fed diets with 1,500 FTU/kg of phytase showed decreased proximal tibia ash concentrations of P, Zn, Cu, Fe and Mg when compared to birds fed diets with 500 FTU/kg of phytase, but when observed on a quantitative basis, no differences exist. However, in the distal tibia ash portion birds on diets with 1,500 FTU/kg of phytase had increased ($P = 0.017$) Mn content (9.95 ppm) when compared to birds on diets with 500 FTU/kg (8.51 ppm). Finally, birds supplemented with phytase showed increased ($P = 0.001$) levels of Mn in the proximal tibia ash portion, as well as increased ($P \leq 0.001$) levels of Zn, Mg and Mn in the distal tibia ash portion when compared to birds that were not supplemented with phytase.

Tibia ash mineral content of male broilers at 35 d of age is summarized by proximal (Table 23) and distal (Table 24) portions. There were minimal statistically detectable differences observed between birds fed the PC and NC diets, with NC birds displaying a greater ($P \leq 0.027$) concentration of distal tibia ash Fe and K (387.13 ppm and 1.08 %, respectively) content when compared to their counterparts on the PC diet (308.38 ppm and 0.86 %, respectively). By increasing the phytase concentration from 500 FTU/kg in the starter diet to 1,500 FTU/kg in the grower diet compared to birds fed 500 FTU/kg of phytase for the duration of the study there was a subsequent increase ($P < 0.05$) in proximal tibia ash P (17.24 % vs. 16.97 %, respectively) and distal tibia ash Mn (6.11 vs. 4.44 ppm, respectively) concentrations. Moreover, by decreasing the phytase concentration from 1,500 FTU/kg in starter diet to 500 FTU/kg in grower diet compared to birds fed 1,500 FTU/kg of phytase for the duration of the study there was a subsequent decrease ($P \leq 0.048$) in proximal and distal tibia ash Mn concentration; as well as a trend for decreased ($P \leq 0.078$) distal tibia ash Zn and Mg concentrations. Finally, birds fed diets with phytase supplementation for the duration of the study had greater ($P < 0.05$) proximal and distal tibia ash Mn and Mg concentrations when compared to birds that were not supplemented with phytase.

Bone tissue is dynamic and can be impacted by numerous factors including nutritional, physiological and physical factors (Rath *et al*., 2000). In addition, growth has been shown to proportionally impact bone mass (Frost, 1997; Seeman, 1999). Nonetheless, phytate has been shown to hinder Ca and P absorption (Vohra, 1965; Nelson and Kirby, 1987; Tamim and Angel, 2003; Tamim *et al*., 2004), which are the primary inorganic nutrients in the bone mineral matrices (Rath *et al*., 2000). Diets considered low in Ca and avP have been shown to negatively affect bone ash, but the addition of phytase alleviates this dilemma by releasing Ca

and P from Ca-phytate complexes. By replacing inorganic Ca and P with phytase is important not only from a feed-cost standpoint, but also from a welfare point-of-view. In fast growing broilers, it has been suggested that bone development cannot fully keep pace with weight accumulation, causing a predisposition to bone deformations. Bone ash content has been used as an index of bone strength (Rath *et al*., 2000), which should limit leg lameness and bone breaks during processing.

Thiobarbituric Acid-Reactive Substances (TBARS)

Thiobarbituric acid-reactive substances analysis for male broilers at 35 d of age is summarized in Table 25. Broilers fed the NC diet actually had a lower (P = 0.003) MDA concentration in the hepatic tissue (1.248 mg/kg) when compared to birds fed the PC diet (1.711 mg/kg). In addition, birds 500 FTU/kg of phytase for the duration of the study had a lower MDA breast content (0.486 mg/kg) when compared to birds fed treatment 4. Finally, phytase supplementation actually increased (P = 0.018) breast MDA content when compared to broilers fed diets without phytase supplementation. These findings contradict the first study where phytase supplementation did not impact (P = 0.520) breast MDA but also reduced thigh MDA.

Thiobarbituric acid-reactive substances analysis is useful method to determine MDA, which is a product of lipid oxidation. Karadas *et al*. (2010) demonstrated that phytase supplementation increased hepatic levels of ascorbic acid, coenzyme Q10 and β-carotene, all of which limit the formation of MDA (Leibovitz *et al*., 1990). For this study, phytase supplementation did not improve MDA concentrations as expected. However, TBARS analysis is also known to react with other sugars and lactones present in the tissue (Raharjo and Sofos, 1993). Further research should be performed to assess phytase supplementation impact on the antioxidant status of tissues. This should include the use of fresh tissue that is flash frozen in

liquid NO2 and utilize a complete oxidative stress model measuring antioxidant capacity and lipid and protein oxidation. There are indications that phytase may improve these levels, which would ultimately impact the efficiency of growing broilers (Iqbal *et al*., 2004; Bottje and Kong, 2012).

Conclusion

The use of high doses of phytase supplementation has shown to positively impact growth performance for broilers. However, limiting feed cost is economically important for integrators and one method of cost-reduction may be reducing enzyme cost. However, it is important to determine periods of broiler grow-out that this would be useful without negatively affecting growth. Therefore, by supplementing higher concentrations of phytase during the starter period then reducing the level in subsequent feeds may lead to saving in enzyme use, given the smaller amount of feed consumed in the starter period and if the extra-phosphoric benefits also occur during the stage (Gehring *et al*., 2014). Alternatively, high phytase concentrations may become important during the linear portion of the growth curve for a broiler (Gous *et al*., 1999). However, from this study and Gehring *et al*. (2014), the most efficacious approach is to supplement a high dose of phytase during the starter period and reduce to 500 FTU/kg of phytase around 14-17 d of age. Birds fed with this method of phase-feeding did not experience a diminished growth performance to 35 d when compared to birds fed diets with 1,500 FTU/kg of phytase. It appears that cost reduction by phytase inclusion may be possible; however, further research should be used to determine if smaller increases and decrease in phytase supplementation create similar results or alleviates some changes seen in phytase supplementation. Furthermore, it would also be advantageous to determine the impact of changing phytase concentrations in feeding periods for older birds.

Table 1: Phytate-P % of various feed ingredients.

Feed Ingredients	Phytate-P (%)		Phytate-P (% of total P)	
Cereals	Ravindran *et al*., (1995)	Nelson *et al*., (1968)	Ravindran *et al*., (1995)	Nelson *et al*., (1968)
Barley (*Hordeum vulgare*)	0.27	0.19	64	56
Corn/Maize (*Zea mays*)	0.23	0.17	74	66
Oats (*Avena sativa*)	0.29	0.19	67	56
Rice (*Oryza sativa*), polished	0.09	-	51	-
Rice (*Oryza sativa*), unpolished	0.27	-	77	-
Broken rice	0.09	-	60	-
Sorghum (*Sorghum vulgare*)	0.24	0.21	66	68
Wheat (*Triticum aestivum*)	0.27	0.20	69	67
Cereal by products				
Rice bran	1.03	1.44	80	86
Rice polish	1.08	-	84	-
Wheat bran	0.81	0.96	73	70
Roots and Tubers				
Cassava (*Mannihot esculenta*) root meal	0.04	-	28	-
Potato (*Solanum tuberosum*) tubers	0.24	-	21	-
Sweet potato (*Ipometa batatas*) tuber meal	0.05	-	24	-
Grain legumes				
Chick peas (*Cicer arieticum*)	0.21	-	51	-
Cowpeas (*Vigna unguiculata*)	0.26	-	79	-
Lentils (*Lens culinaris*)	0.31	-	65	-
Oil seed meals				
Cotton seed (*Gossypium sps.*) meal	0.84	0.75	70	70
Rapeseed meal (*Brassica sps.*) meal	0.70	-	59	-
Soybean (*Glycine max*) meal	0.39	0.37	60	58
Sunflower (*Helianthus annus*) meal	0.89	-	77	-
Other				
Alfalfa (*Medicago sativa*) meal	0.02	< 0.01	12	0
Corn gluten meal	0.41	0.35	59	60
Isolated soy protein	0.48	0.48	60	60

Table 2: Solubility of inositol phosphate esters in the intestinal chyme of pigs (Schlemmer *et al*., 2001)

IP Ester	Small Intestine (pH 6.6)	Large Intestine (pH 6.2)
IP6	2 %	2 %
IP5	7 %	3 %
IP4	8 %	0 %
IP3	31 %	6 %
IP2	75 %	24 %

Table 3: Temperature and ventilation curves utilized during grow-out of Cobb 500 males broilers.

Day	Temperature (F)	Minimum Ventilation (sec.)
-2	92	30:330
1	90	30:330
3	90	45:280
7	85	60:120
14	81	78:102
18	80.7	91:89
21	78	97:83
28	71	107:73
35	68	125:60

Minimum ventilation is depicted as on:off time (seconds).

Table 4: Ingredient and calculated nutrient composition of diets provided to Cobb 500 male broilers from 1 to 18 d of age.

	Low Phytate-P Basal	High Phytate-P Basal
Ingredient (%)		
Corn	62.04	52.10
Soybean Meal	19.55	36.37
DDGS	---	0.34
Meat and Bone Meal	1.93	---
Poultry Meal	5.00	---
Rice Bran	---	5.00
Corn Starch	5.01	---
Isolated Soy Protein	4.00	---
Fat	0.50	3.30
Salt	0.15	0.32
Sodium Bicarbonate	0.10	0.10
DL-Methionine	0.30	0.30
Lysine HCl	0.20	0.15
L-Threonine	0.02	0.03
Limestone	0.95	0.98
Dicalcium Phosphorus	---	0.76
Nicarb[1]	0.04	0.04
Choline Chloride 60	0.05	0.05
Vitamin Premix[2]	0.10	0.10
Mineral Premix[3]	0.10	0.10
Xylanase[4]	0.01	0.01
Calculated nutrient composition		
ME kcal/kg	3095	3085
Crude Protein %	21.44	21.60
Digestible Lys	1.19	1.19
Digestible Met	0.58	0.57
Digestible TSAA	0.89	0.89
Digestible Thr	0.77	0.77
Digestible Val	0.89	0.92
Digestible Trp	0.23	0.23
Calcium %	0.75	0.75
Phosphorus %	0.49	0.62
Available Phosphorus	0.26	0.26
Na	0.17	0.17
Phytate-P %	0.21	0.31
Analyzed		
Ca %	0.81	0.80
P %	0.55	0.68
Na %	0.16	0.17
Phytate-P %	0.21	0.37

[1] Nicarb (Phibro Animal Health, Ridgefield Park, NJ) provided 0.01 % nicarbazin
[2]Vitamin premix provides per kilogram of diet: vitamin A (vitamin A acetate) 7715 IU; cholecalciferol 5511 IU; vitamin E (dl-alpha-tocopheryl acetate) 16.53 IU; vitamin B_{12} 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; menadione (menadionedimethylpyrimidinol) 1.5 mg; folic acid 0.9 mg; choline 1000 mg; thiamin (thiamin mononitrate 1.54 mg; pyridoxine (pyridoxine HCl) 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg.
[3]Mineral premix provides per kilogram of diet: calcium (calcium carbonate) 55.5 mg; manganese (manganese sulfate) 100 mg; magnesium (magnesium oxide) 27 mg; zinc (zinc sulfate) 100 mg; iron (ferrous sulfate) 50 mg; copper (copper sulfate) 10 mg; iodine (calcium iodate) 1 mg.
[4]Econase XT (AB Vista Feed Ingredients, Marlborough, UK) supplied at 8,000 U/kg.

Table 5: Proximate and mineral analyses for ingredients used in experimental diets for Cobb 500 male broilers.

	Corn	Soybean Meal	DDGS	Meat and Bone Meal	Corn Starch	Rice Bran	Poultry Meal	Isolated Soy Protein
Ash %	1.15	6.45	4.49	22.45	0.13	9.50	13.44	3.84
Fat %	3.03	1.23	7.65	12.08	0.05	18.46	12.42	1.90
Moisture %	14.66	13.05	14.77	3.58	11.01	11.59	4.15	4.34
Protein %	6.99	46.5	27.63	58.29	0.10	13.12	66.17	80.41
Calcium %	0.0183	0.4696	0.0329	6.03	< 0.01	0.05	3.03	0.14
Copper ppm	1	14.7	6.86	34	< 1	4	28	12
Iron ppm		167	164	493	< 1	106	268	111
Magnesium ppm	979	3061.5	3255	1800	< 1	0.74	0.13	0.05
Manganese ppm	7.02	40.9	19.4	20	< 1	177	15	13
Phosphorus %	0.293	0.6563	0.92	3.22	0.02	1.60	1.84	0.73
Potassium %	0.3262	2.2062	1.1907	0.54	< 0.01	1.42	0.73	0.15
Sodium %	0.0014	0.00192	0.2533	0.69	0.03	<0.01	0.42	0.90
Zinc ppm	20.9	53.7	72.4	172	< 1	55	100	23

Table 6: Analyzed levels of phytate-P %, total phytate, phytase activity and xylanase activity for finished feeds.

Dietary Variables					
Phytate-P (%)	Phytase (FTU/kg)	Phytate-P (%)	Total Phytate (%)	Phytase (FTU/kg)	Xylanase (BXU/kg)
0.21	0	0.205	0.732	<50	12000
0.21	500	0.205	0.732	788	9500
0.21	1500	0.205	0.732	2340	10800
0.31	0	0.368	1.314	<50	11400
0.31	500	0.368	1.314	600	9500
0.31	1500	0.368	1.314	2320	10000

Table 7: Male broiler performance[1] 1-18 days when fed diets varying in dietary phytate-P %[2] level and phytase[3] level supplementation.

Dietary Variables		Live Performance					
Phytate-P	Phytase	Feed Intake	BW	BW Gain	FCR[3]	AFCR[4]	Mortality
(%)	(FTU/kg)	(kg)	(kg)	(kg)	(kg:kg)	(kg:kg)	(%)
0.21	0	0.719	0.557	0.507	1.29[a]	1.30[a]	8.93
0.21	500	0.755	0.609	0.562	1.23[c]	1.23[c]	3.57
0.21	1500	0.807	0.634	0.586	1.26[ab]	1.25[bc]	4.76
0.31	0	0.716	0.558	0.512	1.28[a]	1.30[a]	2.38
0.31	500	0.787	0.608	0.561	1.29[a]	1.29[ab]	1.78
0.31	1500	0.828	0.661	0.613	1.24[bc]	1.22[c]	4.76
SEM		0.018	0.014	0.014	0.01	0.01	1.73
0.21		0.760	0.600	0.552	1.26	1.26	5.75
0.31		0.777	0.609	0.562	1.27	1.27	2.98
SEM		0.002	0.008	0.008	0.01	0.01	0.10
	0	0.717[c]	0.557[c]	0.509[c]	1.28	1.30	5.65
	500	0.771[b]	0.608[b]	0.562[b]	1.26	1.26	2.68
	1500	0.817[a]	0.647[a]	0.600[a]	1.25	1.24	4.76
	SEM	0.012	0.010	0.010	0.01	0.01	1.22
Probabilities							
Phytase		< 0.001	< 0.001	< 0.001	0.013	< 0.001	0.222
Phytate		0.273	0.425	0.368	0.265	0.414	0.056
Phytase x Phytate		0.624	0.533	0.585	< 0.001	0.011	0.160

[1] Values are least square means of 8 replicate pens with 21 broilers per pen at 1 day of age.
[2]Dietary phytate-P level represents diets formulated to either 0.21 or 0.31 % dietary phytate-P.
[3]Phytase level represents diets containing 0, 500 or 1,500 FTU/kg added Quantum Blue phytase (AB Vista, Marlborough, UK).
[4]FCR represents feed conversion corrected for the weight of the mortality.
[5]AFCR represents feed conversion adjusted to the overall average body weight (27 g = 0.01 FCR)
[a-c] Means with differing superscripts are significantly ($P \leq 0.05$) different.

Table 8: Linear regression slope and intercept values of live performance parameters for male broiler fed diets varying in dietary phytate-P %[1] level and phytase[2] level supplementation.

Regression Variables	Live Performance					
	Feed Intake	BW	BW Gain	FCR[3]	AFCR[4]	Mortality
	(kg)	(kg)	(kg)	(kg:kg)	(kg:kg)	(%)
Phytase 0.21 %						
slope	0.06*	0.04744*	0.0487*	-0.00001	-0.00002	-0.0022
intercept	721.55*	567.98*	519.10*	1.27*	1.27*	7.23*
Phytase 0.31 %						
slope	0.07*	0.06686*	0.0657*	-0.00002*	-0.00005*	0.0018
intercept	730.03*	564.34*	518.33*	1.29*	1.30*	1.79
Equivalent Slope Probabilities	0.615	0.306	0.373	0.254	0.130	0.090

[1]Dietary phytate-P level represents diets formulated to either 0.21 or 0.31 % dietary phytate-P.
[2]Phytase level represents diets containing 0, 500 or 1,500 FTU/kg added Quantum Blue phytase (AB Vista, Marlborough, UK).
[3]FCR represents feed conversion corrected for the weight of the mortality.
[4]AFCR represents feed conversion adjusted to the overall average body weight (27 g = 0.01 FCR)
*Represents slope or intercept that is significantly ($P \leq 0.05$) different from zero.

Table 9: Tibia ash[1] for male broilers fed diets varying in dietary phytate-P %[2] level and phytase[3] level supplementation.

Dietary Variables		Tibia Ash					
Phytate-P	Phytase	Proximal		Distal		Total	
(%)	(FTU/kg)	(g)	(%)	(g)	(%)	(g)	(%)
0.21	0	0.172	34.26	0.357	44.96	0.529	40.82
0.21	500	0.209	35.23	0.428	45.85	0.637	41.72
0.21	1500	0.224	35.39	0.473	46.76	0.697	42.38
0.31	0	0.183	33.13	0.373	44.12	0.555	39.79
0.31	500	0.200	33.90	0.426	46.56	0.625	41.60
0.31	1500	0.218	35.14	0.461	46.95	0.679	42.38
SEM		0.008	0.56	0.017	0.52	0.024	0.50
0.21		0.202	34.96	0.420	45.86	0.621	41.64
0.31		0.200	34.06	0.420	45.88	0.620	41.26
SEM		0.005	0.32	0.010	0.30	0.014	0.29
	0	0.177^{c}	33.70^{b}	0.365^{c}	44.54^{b}	0.542^{c}	40.31^{b}
	500	0.204^{b}	34.57^{ab}	0.427^{b}	46.20^{a}	0.631^{b}	41.66^{a}
	1500	0.221^{a}	35.26^{a}	0.467^{a}	46.86^{a}	0.688^{a}	42.38^{a}
	SEM	0.006	0.40	0.012	0.37	0.017	0.35
Probabilities							
Phytase		< 0.001	0.027	< 0.001	< 0.001	< 0.001	< 0.001
Phytate		0.806	0.056	0.979	0.963	0.948	0.349
Phytase x Phytate		0.394	0.593	0.712	0.327	0.597	0.536

[1]Values are least square means of 3 birds pooled/pen and 8 replicate pens (24 chicks)/treatment.
[2]Dietary phytate-P level represents diets formulated to either 0.21 or 0.31 % dietary phytate-P.
[3]Phytase level represents diets containing 0, 500 or 1,500 FTU/kg added Quantum Blue phytase (AB Vista, Marlborough, UK).
[a-c]Means with differing superscripts are significantly ($P \leq 0.05$) different.

Table 10: Proximal tibia ash mineral analysis[1] for male broilers fed diets varying in dietary phytate-P[2] level and phytase[3] level supplementation.

Dietary Variables		Proximal Tibia Ash Mineral Content									
Phytate-P	Phytase	Ca	P	Zn	Cu	Fe	K	Mg	Mn	Na	S
(%)	(FTU/kg)	%	%	ppm	ppm	ppm	%	%	ppm	%	%
0.21	0	33.17	17.97	441.00	4.94	346.25	3.65	0.81	6.99	1.46	0.84
0.21	500	33.19	18.05	440.25	4.40	341.88	3.80	0.83	5.92	1.47	0.86
0.21	1500	33.31	18.05	436.88	4.20	349.63	3.64	0.84	6.10	1.45	0.85
0.31	0	33.66	18.22	413.50	5.61	385.38	3.89	0.84	5.52	1.54	0.91
0.31	500	32.65	18.05	432.00	5.12	349.38	3.83	0.87	5.66	1.44	0.83
0.31	1500	33.15	18.20	441.75	3.39	342.50	3.85	0.88	6.50	1.41	0.83
SEM		0.35	0.18	10.87	0.68	21.72	0.14	0.02	0.76	0.05	0.03
0.21		33.22	18.02	439.38	4.52	345.92	3.70	0.83[b]	6.34	1.46	0.85
0.31		33.16	18.16	429.08	4.71	359.08	3.86	0.86[a]	5.89	1.47	0.86
SEM		0.20	0.11	6.28	0.39	12.54	0.08	0.01	0.44	0.03	0.02
	0	33.42	18.09	427.25	5.28	365.81	3.77	0.83	6.29	1.50	0.88
	500	32.92	18.05	436.13	4.76	345.63	3.82	0.85	5.79	1.45	0.84
	1500	33.23	18.13	439.31	3.79	346.06	3.74	0.86	6.30	1.43	0.84
	SEM	0.25	0.13	7.69	0.48	15.36	0.10	0.01	0.54	0.03	0.02
Probabilities											
Phytase		0.372	0.919	0.522	0.097	0.573	0.881	0.214	0.405	0.346	0.342
Phytate		0.804	0.371	0.253	0.735	0.462	0.177	0.034	0.200	0.848	0.755
Phytase x Phytate		0.346	0.807	0.336	0.447	0.558	0.750	0.956	0.089	0.389	0.217

[1]Values are least square means of 3 birds pooled/pen and 8 replicate pens (24 chicks)/treatment.
[2]Dietary phytate-P level represents diets formulated to either 0.21 or 0.31 % dietary phytate-P.
[3]Phytase level represents diets containing 0, 500 or 1500 FTU/kg added Quantum Blue phytase (AB Vista, Marlborough, UK).
[a-c]Means with differing superscripts are significantly ($P \leq 0.05$) different.

Table 11: Distal tibia ash mineral analysis[1] for male broilers fed diets varying in dietary phytate-P[2] level and phytase[3] level supplementation.

Dietary Variables		Distal Tibia Ash Mineral Content									
Phytate-P	Phytase	Ca	P	Zn	Cu	Fe	K	Mg	Mn	Na	S
(%)	(FTU/kg)	%	%	ppm	ppm	ppm	%	%	ppm	%	%
0.21	0	34.07	17.32	416.13	2.35	219.25	1.72	0.70	3.17	1.25	0.74
0.21	500	34.04	17.50	405.00	1.76	225.13	1.72	0.73	2.58	1.22	0.70
0.21	1500	34.24	17.59	423.63	1.34	216.38	1.75	0.75	2.70	1.19	0.71
0.31	0	34.33	17.56	392.00	2.57	238.25	1.81	0.73	2.16	1.31	0.78
0.31	500	34.04	17.61	409.00	3.90	202.00	1.69	0.77	2.29	1.21	0.72
0.31	1500	33.91	17.59	419.88	2.05	224.75	1.78	0.78	3.17	1.23	0.73
SEM		0.51	0.13	7.39	1.05	13.68	0.05	0.01	0.38	0.03	0.01
0.21		34.12	17.47	414.92	1.82	220.25	1.73	0.73[b]	2.81	1.22	0.72
0.31		34.10	17.59	406.96	2.84	221.67	1.76	0.76[a]	2.54	1.25	0.74
SEM		0.29	0.08	4.27	0.61	7.90	0.03	0.01	0.22	0.02	0.01
	0	34.20	17.44	404.06[b]	2.46	228.75	1.76	0.72[b]	2.66	1.28[a]	0.76[a]
	500	34.04	17.55	407.00[b]	2.83	213.56	1.70	0.75[a]	2.43	1.21[b]	0.71[b]
	1500	34.08	17.59	421.75[a]	1.69	220.56	1.76	0.77[a]	2.94	1.21[b]	0.72[b]
	SEM	0.36	0.09	5.22	0.74	9.67	0.03	0.01	0.27	0.02	0.01
Probabilities											
Phytase		0.947	0.529	0.047	0.548	0.544	0.348	0.005	0.424	0.024	0.035
Phytate		0.959	0.279	0.194	0.241	0.900	0.437	0.005	0.382	0.157	0.081
Phytase x Phytate		0.847	0.665	0.157	0.640	0.288	0.402	0.978	0.161	0.412	0.867

[1]Values are least square means of 3birds pooled/pen and 8 replicate pens (24 chicks)/treatment.
[2]Dietary phytate-P level represents diets formulated to either 0.21 or 0.31 % dietary phytate-P.
[3]Phytase level represents diets containing 0, 500 or 1500 FTU/kg added Quantum Blue phytase (AB Vista, Marlborough, UK).
[a-c]Means with differing superscripts are significantly ($P \leq 0.05$) different.

Table 12: Thiobarbituric acid-reactive substances (TBARS) analysis for liver, breast and thigh tissues[1] of male broilers fed diets varying in dietary phytate-P[2] level and phytase[3] level supplementation.

Dietary Variables		TBARS		
Phytate-P	Phytase	Liver	Breast	Thigh
(%)	(FTU)	mg malonaldehyde/kg of tissue		
0.21	0	1.209	0.595	2.228
0.21	500	1.059	0.723	2.021
0.21	1500	1.027	0.686	1.632
0.31	0	1.332	0.756	2.094
0.31	500	1.320	0.786	2.094
0.31	1500	1.213	0.683	2.017
SEM		0.063	0.077	0.122
0.21		1.098[b]	0.668	1.960
0.31		1.288[a]	0.742	2.068
SEM		0.037	0.044	0.071
	0	1.271	0.676	2.161[a]
	500	1.190	0.755	2.057[ab]
	1500	1.120	0.684	1.825[b]
	SEM	0.046	0.054	0.086
Probabilities				
Phytase		0.065	0.520	0.020
Phytate		< 0.001	0.241	0.281
Phytase x Phytate		0.557	0.562	0.102

[1]Values are least square means of 3birds pooled/pen and 8 replicate pens (24 chicks)/treatment.
[2]Dietary phytate-P level represents diets containing either 0.21 or 0.31 % dietary phytate-P.
[3]Phytase level represents diets containing 0, 500 or 1500 FTU/kg added Quantum Blue phytase (AB Vista, Marlborough, UK).
[a-c]Means with differing superscripts are significantly ($P \leq 0.05$) different.

Table 13: Gizzard phytate, phytate esters and inositol concentrations[1] of male broilers fed diets varying in dietary phytate-P[2] level and phytase[3] level supplementation.

Dietary Variables					
Phytate	Phytase	IP6	IP5	IP4	Inositol
(%)	(FTU)		nmol/g of dry weight		
0.21	0	2.436	1.771	1.713	2.801
0.21	500	2.229	1.275	2.227	3.075
0.21	1500	1.069	0.203	0.671	3.262
0.31	0	2.786	2.206	2.253	2.788
0.31	500	1.934	0.434	1.721	3.306
0.31	1500	1.514	0.372	0.969	3.300
SEM		0.246	0.273	0.297	0.073
0.21		1.912	1.083	1.537	3.046
0.31		2.078	1.004	1.648	3.131
SEM		0.142	0.158	0.171	0.042
	0	2.611[a]	1.988[a]	1.983[a]	2.794[b]
	500	2.082[b]	0.855[b]	1.974[a]	3.191[ab]
	1500	1.292[c]	0.288[c]	0.820[b]	3.281[a]
	SEM	0.174	0.193	0.210	0.051
Probabilities					
Phytase		< 0.001	< 0.001	< 0.001	< 0.001
Phytate		0.411	0.725	0.650	0.158
Phytase x Phytate		0.272	0.058	0.194	0.221

[1]Values are least square means of 3birds pooled/pen and 8 replicate pens (24 chicks)/treatment.
[2]Dietary phytate-P level represents diets containing either 0.21 or 0.31 % dietary phytate-P.
[3]Phytase level represents diets containing 0, 500 or 1500 FTU/kg added Quantum Blue phytase (AB Vista, Marlborough, UK).
[a-c]Means with differing superscripts are significantly ($P \leq 0.05$) different.

Table 14: Correlation (r) values between gizzard inositol-phosphate ester concentrations and growth performance tibia ash variables.

	Feed Intake (kg)	*P*-Value	BW (kg)	*P*-Value	BW Gain (kg)	*P*-Value	FCR (kg:kg)	*P*-Value	AFCR (kg:kg)	*P*-Value
IP6	-0.326	0.024	-0.377	0.008	-0.378	0.008	0.161	0.275	0.269	0.064
IP5	-0.383	0.007	-0.396	0.005	-0.402	0.005	0.100	0.500	0.184	0.212
IP4	-0.253	0.083	-0.278	0.056	-0.274	0.059	0.031	0.836	0.174	0.236
Inositol	0.320	0.027	0.325	0.240	0.327	0.023	-0.152	0.304	-0.184	0.210

Table 14 (cont.)

	Proximal Ash (g)	*P*-Value	Proximal Ash (%)	*P*-Value	Distal Ash (g)	*P*-Value	Distal Ash (%)	*P*-Value	Total Ash (g)	*P*-Value
IP6	-0.380	0.008	-0.316	0.029	-0.336	0.020	-0.357	0.013	-0.358	0.013
IP5	-0.436	0.002	-0.243	0.096	-0.406	0.004	-0.407	0.004	-0.425	0.003
IP4	-0.217	0.138	-0.138	0.349	-0.229	0.118	-0.165	0.262	-0.230	0.115
Inositol	0.339	0.018	0.119	0.422	0.329	0.022	0.250	0.086	0.340	0.018

Table 14 (cont.)

	Total Ash (%)	*P*-Value
IP6	-0.355	0.013
IP5	-0.352	0.014
IP4	-0.168	0.254
Inositol	0.203	0.167

Table 15: Ingredient and calculated nutrient composition of diets provided to Cobb 500 male broilers from 1 to 35 d of age.

	Starter (1-17 days of age)		Grower (18-35 days of age)	
	Positive Control	Negative Control	Positive Control	Negative Control
Ingredient (%)				
Corn	53.03	54.73	57.20	59.06
Soybean Meal	34.89	34.63	25.65	25.36
Rice Bran	3.00	3.00	4.00	4.00
DDGS	2.50	2.50	5.00	5.00
Poultry Meal	---	---	2.00	2.00
Fat	2.83	2.22	2.91	2.24
Salt	0.33	0.25	0.30	0.21
Sodium Bicarbonate	0.10	0.10	0.10	0.10
DL-Methionine	0.31	0.30	0.23	0.23
Lysine HCl	0.20	0.21	0.23	0.23
L-Threonine	0.04	0.04	0.01	0.01
Limestone	0.81	0.89	0.76	0.86
Dicalcium Phosphorus	1.66	0.84	1.32	0.41
Nicarb[1]	0.04	0.04	---	---
Robenz[2]	---	---	0.05	0.05
BMD[3]	---	---	0.05	0.05
Choline Chloride 60	0.05	0.05	0.05	0.05
Vitamin Premix[4]	0.10	0.10	0.10	0.10
Mineral Premix[5]	0.10	0.10	0.10	0.10
Econase	0.01	0.01	0.01	0.01
Calculated nutrient composition				
ME kcal/kg	3050	3050	3110	3110
Crude Protein %	21.40	21.40	19.50	19.50
Digestible Lys	1.19	1.19	1.06	1.06
Digestible Met	0.57	0.57	0.49	0.49
Digestible TSAA	0.89	0.89	0.78	0.78
Digestible Thr	0.77	0.77	0.68	0.68
Digestible Val	0.89	0.89	0.82	0.82
Digestible Trp	0.23	0.23	0.20	0.20
Calcium %	0.90	0.74	0.80	0.64
Phosphorus %	0.78	0.62	0.71	0.55
Available Phosphorus %	0.43	0.28	0.38	0.23
Na	0.18	0.14	0.18	0.14
Analyzed				
Ca %	0.92	0.77	0.85	0.65
P %	0.81	0.63	0.72	0.57

[1]Nicarb (Phibro Animal Health, Ridgefield Park, NJ) provided 0.01 % nicarbazin
[2]Robenz (Zoetis, Florham Park, NJ) provided robenidine hydrochloride at 30g per ton
[3]BMD (Zoetis, Florham Park, NJ) provided bacitracin methylene disalicylate at 50 g per ton

[4]Vitamin premix provides per kilogram of diet: vitamin A (vitamin A acetate) 7715 IU; cholecalciferol 5511 IU; vitamin E (dl-alpha-tocopheryl acetate) 16.53 IU; vitamin B_{12} 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; menadione (menadionedimethylpyrimidinol) 1.5 mg; folic acid 0.9 mg; choline 1000 mg; thiamin (thiamin mononitrate 1.54 mg; pyridoxine (pyridoxine HCl) 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg.

[5]Mineral premix provides per kilogram of diet: calcium (calcium carbonate) 55.5 mg; manganese (manganese sulfate) 100 mg; magnesium (magnesium oxide) 27 mg; zinc (zinc sulfate) 100 mg; iron (ferrous sulfate) 50 mg; copper (copper sulfate) 10 mg; iodine (calcium iodate) 1 mg.

Table 16: Phytase and xylanase recovery from starter and grower rations provided to Cobb 500 male broilers from 1 to 35 d of age.

	Phytase Activity		Xylanase Activity	
	Starter	Grower	Starter	Grower
PC	<50	<50	14700	13100
NC	<50	<50	12400	10000
500	387	484	10200	11200
1500	1290	1660	15100	10800

Table 17: Male broiler performance when fed diets supplemented with phytase from 1-17 d of age.[1]

	Live Performance 1-17 Days					
Diet	Feed Intake (kg)	BW (kg)	BW Gain (kg)	FCR[2] (kg:kg)	AFCR[3] (kg:kg)	Mortality (%)
1. Positive Control	0.633	0.494	0.454	1.27	1.27	2.9
2. Negative Control	0.654	0.503	0.464	1.29	1.29	4.6
3. 500	0.655	0.516	0.476	1.26	1.25	1.8
4. 500	0.658	0.516	0.476	1.27	1.27	0.0
5. 1500	0.649	0.517	0.477	1.25	1.25	2.3
6. 1500	0.647	0.523	0.483	1.23	1.22	2.3
SEM	0.0108	0.0073	0.0073	0.012	0.013	0.01
CV	4.69	4.04	4.39	2.69	2.82	173.97
Pr > F	0.636	0.067	0.081	0.037	0.014	0.365
Orthogonal Contrast						
1 vs. 2	0.180	0.368	0.357	0.381	0.518	0.400
3 vs. 4	0.876	0.980	0.997	0.499	0.508	0.387
5 vs. 6	0.890	0.529	0.542	0.207	0.177	0.985
3 and 4 vs. 5 and 6	0.450	0.610	0.610	0.046	0.044	0.331
1 and 2 vs. 3, 4, 5 and 6	0.339	0.003	0.004	0.017	0.004	0.084

[1]Values are least square means of 8 replicate pens with 22 broilers per pen at 1 day of age.
[2]FCR represents feed conversion corrected for the weight of the mortality.
[3]AFCR represents feed conversion adjusted to the average body weight of the positive control (27 g = 0.01 FCR)

Table 18: Male broiler performance when fed diets supplemented with phytase from 1 to 35 d of age.[1]

	Live Performance 1-35 Days					
Diet	Feed Intake (kg)	BW (kg)	BW Gain (kg)	FCR[2] (kg:kg)	AFCR[3] (kg:kg)	Mortality (%)
1. Positive Control	3.293	2.088	2.048	1.58	1.58	2.9
2. Negative Control	3.213	2.018	1.979	1.59	1.61	5.2
3. 500/500	3.311	2.123	2.083	1.55	1.53	2.9
4. 500/1500	3.321	2.134	2.094	1.56	1.54	0.6
5. 1500/500	3.259	2.098	2.058	1.54	1.54	2.3
6. 1500 /1500	3.245	2.115	2.075	1.53	1.52	2.9
SEM	0.0291	0.0243	0.0243	0.0098	0.017	0.01
CV	2.51	3.28	3.34	1.77	3.02	151.19
Pr > F	0.097	0.026	0.027	0.001	0.003	0.433
Orthogonal Contrast						
1 vs. 2	0.059	0.051	0.051	0.447	0.130	0.284
3 vs. 4	0.816	0.766	0.761	0.572	0.859	0.276
5 vs. 6	0.735	0.619	0.622	0.330	0.401	0.804
3 and 4 vs. 5 and 6	0.036	0.376	0.375	0.196	0.775	0.577
1 and 2 vs. 3, 4, 5 and 6	0.231	0.004	0.004	< 0.001	< 0.001	0.146

[1]Values are least square means of 8 replicate pens with 22 broilers per pen at 1 day of age.
[2]FCR represents feed conversion corrected for the weight of the mortality.
[3]AFCR represents feed conversion adjusted to the average body weight of the positive control (27 g = 0.01 FCR)

Table 19: Tibia ash of male broilers fed diets supplemented with phytase from 1 to 17 d of age.[1]

Diet	Tibia Ash					
	Proximal		Distal		Total	
	(g)	(%)	(g)	(%)	(g)	(%)
1. Positive Control	0.159	45.14	0.299	53.90	0.458	50.51
2. Negative Control	0.152	44.07	0.285	51.99	0.436	48.94
3. 500	0.150	43.65	0.289	52.49	0.439	49.09
4. 500	0.156	43.84	0.311	53.48	0.467	49.82
5. 1500	0.169	45.70	0.339	54.03	0.508	50.94
6. 1500	0.166	45.76	0.317	54.25	0.483	51.00
SEM	0.0049	0.452	0.0097	0.412	0.0139	0.385
CV	8.82	2.86	8.99	2.19	8.47	2.18
Pr > F	0.053	0.003	0.004	0.002	0.007	< 0.001
Probability						
1 vs. 2	0.300	0.102	0.302	0.002	0.276	0.007
3 vs. 4	0.437	0.765	0.106	0.099	0.159	0.188
5 vs. 6	0.624	0.929	0.128	0.704	0.214	0.909
3 and 4 vs. 5 and 6	0.005	< 0.001	0.007	0.008	0.004	< 0.001
1 and 2 vs. 3, 4, 5 and 6	0.237	0.741	0.014	0.095	0.031	0.150

[1]Values are least square means of 2 birds pooled/pen and 8 replicate pens (16 chicks)/treatment.

Table 20: Tibia ash of male broilers fed diets supplemented with phytase from 1 to 35 d of age.[1]

Diet	Tibia Ash					
	Proximal		Distal		Total	
	(g)	(%)	(g)	(%)	(g)	(%)
1. Positive Control	0.798	43.37	1.542	50.90	2.339	48.05
2. Negative Control	0.615	40.77	1.170	48.17	1.785	45.33
3. 500/500	0.703	41.34	1.395	48.97	2.098	46.11
4. 500/1500	0.760	42.45	1.465	50.31	2.225	47.33
5. 1500/500	0.714	42.18	1.405	49.64	2.119	46.85
6. 1500 /1500	0.771	42.51	1.446	50.04	2.217	47.15
SEM	0.0257	0.353	0.0476	0.490	0.0698	0.404
CV	10.01	2.37	9.60	2.79	9.27	2.44
Pr > F	< 0.001	< 0.001	< 0.001	0.006	< 0.001	< 0.001
Orthogonal Contrast						
1 vs. 2	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
3 vs. 4	0.126	0.034	0.305	0.061	0.207	0.040
5 vs. 6	0.130	0.515	0.546	0.562	0.331	0.593
3 and 4 vs. 5 and 6	0.684	0.215	0.927	0.684	0.930	0.494
1 and 2 vs. 3, 4, 5 and 6	0.178	0.871	0.090	0.634	0.099	0.633

[1]Values are least square means of 3 birds pooled/pen and 8 replicate pens (24 chicks)/treatment.

Table 21: Proximal tibia ash mineral content[1] of male broilers fed diets supplemented with phytase from 1 to 17 d of age.

	Proximal Tibia Ash Mineral Content									
Diet	Ca	P	Zn	Cu	Fe	K	Mg	Mn	Na	S
Response Criteria[2]	%	%	ppm	ppm	ppm	%	%	ppm	%	%
1. Positive Control	42.05	20.81	529.38	5.11	537.25	3.46	0.95	9.46	1.35	1.11
2. Negative Control	38.15	19.50	482.13	5.36	448.25	3.37	0.88	9.61	1.26	1.09
3. 500	40.61	20.71	533.63	6.81	579.13	3.74	0.98	12.24	1.37	1.12
4. 500	38.30	19.61	517.25	5.34	528.00	3.35	0.94	10.91	1.31	1.08
5. 1500	36.52	18.56	483.00	4.82	420.25	3.06	0.86	11.46	1.26	1.07
6. 1500	36.17	18.67	493.00	4.82	438.13	3.03	0.87	11.20	1.22	1.03
SEM	1.609	0.076	17.697	0.602	39.281	0.195	0.040	0.619	0.045	0.039
CV	11.78	10.88	9.88	31.71	22.59	16.58	12.41	16.20	10.00	10.36
Pr > F	0.096	0.178	0.154	0.211	0.036	0.133	0.248	0.021	0.223	0.717
Orthogonal Contrast										
1 vs. 2	0.096	0.227	0.067	0.765	0.118	0.757	0.265	0.863	0.184	0.734
3 vs. 4	0.317	0.309	0.517	0.093	0.364	0.167	0.527	0.137	0.371	0.497
5 vs. 6	0.881	0.921	0.692	0.997	0.750	0.910	0.966	0.763	0.540	0.469
3 and 4 vs. 5 and 6	0.061	0.049	0.042	0.045	0.003	0.015	0.027	0.694	0.048	0.243
1 and 2 vs. 3, 4, 5 and 6	0.123	0.247	0.950	0.688	0.968	0.496	0.899	0.001	0.704	0.556

[1]Values are least square means of 2 birds pooled/pen and 8 replicate pens (16 chicks)/treatment.
[2]Phytase units per kilogram of feed during the starter phase.

Table 22: Distal tibia ash mineral content[1] of male broilers fed diets supplemented with phytase from 1 to 17 d of age.

	Distal Tibia Ash Mineral Content									
Diet	Ca	P	Zn	Cu	Fe	K	Mg	Mn	Na	S
Response Criteria[2]	%	%	ppm	ppm	ppm	%	%	ppm	%	%
1. Positive Control	36.60	17.95	438.88	0.82	214.75	1.10	0.75	6.34	0.95	0.69
2. Negative Control	36.06	17.73	420.00	1.63	214.25	1.15	0.75	7.09	0.96	0.70
3. 500	36.07	18.00	443.88	1.22	240.50	1.25	0.78	8.94	0.99	0.70
4. 500	36.09	17.82	459.63	1.53	218.00	1.10	0.80	8.08	0.93	0.68
5. 1500	36.78	18.13	461.75	2.38	202.13	1.09	0.79	10.67	0.94	0.70
6. 1500	36.24	17.85	462.25	0.74	204.63	1.13	0.79	9.23	0.96	0.70
SEM	0.279	0.015	9.087	0.458	14.087	0.048	0.013	0.573	0.021	0.013
CV	2.17	2.44	5.74	93.77	18.47	11.97	4.61	19.33	6.27	5.14
Pr > F	0.322	0.525	0.013	0.153	0.469	0.173	0.024	< 0.001	0.597	0.838
Orthogonal Contrast										
1 vs. 2	0.185	0.325	0.151	0.222	0.980	0.473	0.967	0.363	0.711	0.816
3 vs. 4	0.948	0.402	0.229	0.644	0.266	0.029	0.393	0.298	0.095	0.334
5 vs. 6	0.184	0.217	0.969	0.016	0.901	0.577	0.787	0.086	0.511	0.720
3 and 4 vs. 5 and 6	0.131	0.624	0.267	0.692	0.075	0.170	0.906	0.017	0.748	0.348
1 and 2 vs. 3, 4, 5 and 6	0.891	0.408	0.001	0.544	0.883	0.604	< 0.001	< 0.001	0.785	0.972

[1]Values are least square means of 2 birds pooled/pen and 8 replicate pens (16 chicks)/treatment.
[2]Phytase units per kilogram of feed during the starter phase.

Table 23: Proximal tibia ash mineral content[1] of male broilers fed diets supplemented with phytase from 1 to 35 d of age.

	Proximal Tibia Ash Mineral Content									
Diet	Ca	P	Zn	Cu	Fe	K	Mg	Mn	Na	S
Response Criteria[2]	%	%	ppm	ppm	ppm	%	%	ppm	%	%
1. Positive Control	33.81	17.14	374.38	2.69	585.38	2.08	0.81	3.34	1.32	0.74
2. Negative Control	34.11	17.26	411.50	2.57	636.38	2.11	0.80	3.74	1.35	0.74
3. 500/500	33.25	16.97	381.38	2.30	684.00	2.16	0.85	4.72	1.40	0.76
4. 500/1500	33.54	17.24	390.88	2.47	645.38	2.16	0.86	5.62	1.32	0.73
5. 1500/500	33.73	17.12	374.13	1.87	659.63	2.08	0.83	5.01	1.36	0.73
6. 1500/1500	33.68	17.29	415.13	2.38	677.13	2.08	0.86	6.02	1.44	0.76
SEM	0.248	0.088	17.969	0.589	37.177	0.092	0.010	0.348	0.031	0.019
CV	2.08	1.46	12.99	70.89	16.23	12.36	3.48	20.74	6.45	7.51
Pr > F	0.270	0.138	0.412	0.948	0.481	0.974	< 0.001	< 0.001	0.086	0.719
Orthogonal Contrast										
1 vs. 2	0.385	0.348	0.153	0.889	0.339	0.812	0.406	0.416	0.542	0.978
3 vs. 4	0.401	0.040	0.711	0.843	0.467	0.999	0.601	0.075	0.076	0.331
5 vs. 6	0.900	0.167	0.116	0.543	0.741	0.999	0.106	0.048	0.105	0.194
3 and 4 vs. 5 and 6	0.220	0.262	0.639	0.661	0.922	0.412	0.418	0.337	0.264	0.833
1 and 2 vs. 3, 4, 5 and 6	0.065	0.607	0.870	0.476	0.093	0.792	< 0.001	< 0.001	0.102	0.758

[1]Values are least square means of 3 birds pooled/pen and 8 replicate pens (24 chicks)/treatment.
[2]Phytase units per kilogram of feed during the starter phase.

Table 24: Distal tibia ash mineral content[1] of male broilers fed diets supplemented with phytase from 1 to 35 d of age.

	Distal Tibia Ash Mineral Content									
Diet	Ca	P	Zn	Cu	Fe	K	Mg	Mn	Na	S
Response Criteria[2]	%	%	ppm	ppm	ppm	%	%	ppm	%	%
1. Positive Control	37.20	17.42	379.75	1.14	308.38	0.86	0.79	3.56	0.99	0.60
2. Negative Control	37.36	17.59	389.63	2.25	387.13	1.08	0.77	3.58	1.03	0.63
3. 500/500	37.16	17.47	394.00	1.46	326.13	0.89	0.83	4.44	0.98	0.58
4. 500/1500	37.13	17.60	402.38	1.26	309.38	0.85	0.83	6.11	0.96	0.59
5. 1500/500	37.17	17.38	372.50	1.81	313.50	0.85	0.80	4.56	0.97	0.58
6. 1500/1500	36.88	17.49	418.25	1.54	343.50	0.86	0.84	5.67	1.01	0.60
SEM	0.332	0.113	17.798	0.550	20.161	0.069	0.012	0.311	0.018	0.012
CV	2.53	1.83	12.82	75.80	17.21	21.76	4.03	18.90	5.04	5.52
Pr > F	0.951	0.683	0.529	0.715	0.069	0.147	< 0.001	< 0.001	0.059	0.083
Orthogonal Contrast										
1 vs. 2	0.726	0.303	0.697	0.145	0.009	0.027	0.405	0.973	0.128	0.194
3 vs. 4	0.953	0.421	0.741	0.786	0.561	0.739	0.892	< 0.001	0.544	0.272
5 vs. 6	0.547	0.466	0.078	0.740	0.300	0.892	0.058	0.017	0.107	0.338
3 and 4 vs. 5 and 6	0.718	0.387	0.875	0.551	0.597	0.824	0.300	0.614	0.158	0.542
1 and 2 vs. 3, 4, 5 and 6	0.495	0.835	0.438	0.691	0.167	0.079	< 0.001	< 0.001	0.047	0.016

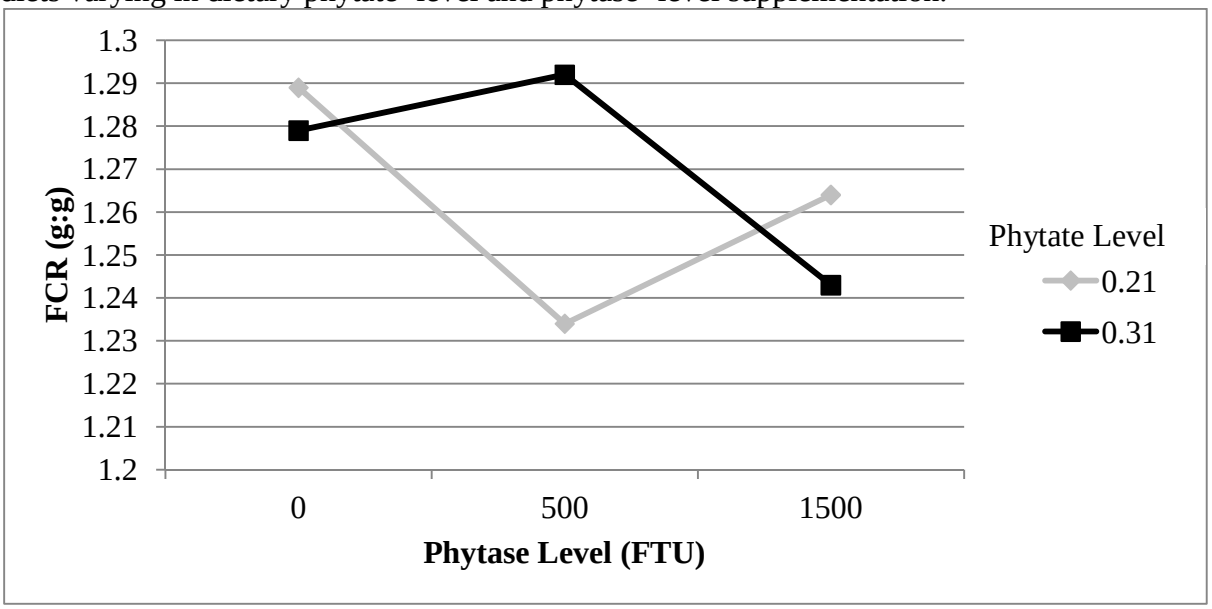
[1]Values are least square means of 3 birds pooled/pen and 8 replicate pens (24 chicks)/treatment.
[2]Phytase units per kilogram of feed during the starter phase.

Table 25: Thiobarbituric acid-reactive substances analysis of male broilers fed diets supplemented with phytase from 1 to 35 days of age.[1]

	TBARS		
Diet	Liver	Breast	Thigh
Response Criteria[2]	mg malonaldehyde/kg of tissue		
1. Positive Control	1.711	0.508	1.163
2. Negative Control	1.248	0.396	0.988
3. 500/500	1.213	0.486	1.125
4. 500/1500	1.379	0.608	1.124
5. 1500/500	1.520	0.555	1.172
6. 1500/1500	1.476	0.506	0.976
SEM	0.1066	0.0422	0.0746
CV	41.929	45.114	38.102
Pr > F	0.014	0.018	0.246
Orthogonal Contrast			
1 vs. 2	0.003	0.060	0.097
3 vs. 4	0.269	0.036	0.991
5 vs. 6	0.769	0.398	0.068
3 and 4 vs. 5 and 6	0.059	0.716	0.502
1 and 2 vs. 3, 4, 5 and 6	0.373	0.018	0.710

[1]Values are least square means of 3birds pooled/pen and 8 replicate pens (24 chicks)/treatment.
[2]Phytase units per kilogram of feed during starter and grower phase.

Figure 1: Interaction plot for phytase x phytate interaction on FCR analysis for male broilers fed diets varying in dietary phytate[1] level and phytase[2] level supplementation.



REFERENCES

Allen, A. and G. Flemstrom. 2005. Gastroduodenal mucus bicarbonate barrier: Protection against acid and pepsin. *Am. J. Phsiol. Cell Physiol.* 288:1-19.

Anderson, P. A. Interactions between proteins and constituents that affect protein quality." Finley, G. W. and Hopkins, D. T. 1985. *Digestibility and Amino Acid Availability in Cereals and Oilseeds*. St. Paul: American Association of Cereal Chemists. 31-36.

Atteh, J. O. and S. Leeson. 1984. Effects of dietary saturated and unsaturated fatty acids and calcium levels on performance and mineral metabolism of broiler chicks. *Poult. Sci.* 63(11):2252-2260.

Augspurger, N. R., D. M. Webel, X. G. Lei and D. H. Baker. 2003. Efficacy of and E. coli phytase expressed in yeast for releasing phytate-bound phosphorus in young chicks and pigs. *J. Anim. Sci.* 81(2):474-483.

Avanzo, J. L., C. X. De Mendoca, S. M. Piccoli and D. E. Cerqueira. 2001. Effect of vitamin E and selenium on resistance to oxidative stress in chicken superficial pectoralis muscle. *Comp. Biochem. Physio.* 129:163-173.

Ballam, G. C., T. S. Nelson and L. K. Kirby. 1984. Effect of fiber and phytate source and of calcium and phosphorus level on phytate hydrolysis in the chick. *Poult. Sci.* 63:333-338.

Bedford, M. R. and A. J. Morgan. 1996. The use of enzymes in poultry diets. *World's Poult. Sci.* 52:61-68.

Bedford, M. R. 1996. The effect of enzymes on digestion. *J App. Poult. Res.* 5:370-378.

Biehl, R. R., D. H. Baker and H. F. Deluca. 1995. Hydroxylated cholecalciferol compounds act additively with microbial phytase to improve phosphorus, zinc and manganese in chicks fed soy-based diets. *J Nutr*. 125:2407-2416.

Blaabjerg, K., J. Hanson-Moller and H. D. Poulsen. 2010. High-performance ion chromatography for separation and quantification of inositol phosphates in diets and digesta. *J. Chrom. B. Analyt. Technol. Biomed. Life Sci.* 878:347-354.

Boling, S. D., D. M. Webel, I. Mavromichalis, C. M. Parson and D. H. Baker. 2000. The effects of citric acid on phytate-phosphorus utilization in young chicks and pigs. *J. Ani. Sci.* 78:682-689.

Bottje, W. and B. -W. Kong. 2012. Cell Biology Symposium: Feed efficiency: Mitochondrial function to global gene expression. *J. Anim. Sci.* 91:1582-1593.

Bottje, W., M. Iqbal, Z. X. Tang, D. Cawthon, R. Okimoto, T. Wing and M. Cooper. 2002. Association of mitochondrial function with feed efficiency within a single genetic line of male broilers. *Poult. Sci.* 81:546-555.

Broz, J., P. Oldane, A. H. Perrin-Voltz, G. Rychen, J. Schulze and C. S. Nuches. 1994. Effect of supplemental phytase on performance and phosphorus utilization in broiler chickens fed low phosphorus diet without addition of inorganic phosphorus. *Brit. Poult. Sci.* 35:273-280.

Cabahug, S., V. Ravindran, W. L. Bryden and P. H. Selle. 1999. Response of broilers to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus levels. I. Effects on broiler performance and toe ash content. *Brit. Poult. Sci.* 40:660-666.

Caldwell, R. A. 1992. Effect of calcium and phytic acid on the activation of trypsinogen and the stability of trypsin. *J Agri.Food Chem.* 40:43-46.

Camden, B. J., P. C. H. Morel, D. V. Thomas, V. Ravindran, and M. R. Bedford. 2001. Effectiveness of exogenous microbial phytase in improving the bioavailabilities of phosphorus and other nutrients in maize-soya-bean mealdiets for broilers. *Anim. Sci.* 73:289-297.

Cawley, R. W. and T. A. Mitchell. 1968. Inhibition of wheat alpha-amylase by bran phytic acid. *J Sci. Food Agri.* 19:106-110.

Cheryan, M. 1980. Phytic acid interactions in food systems. *Critical Reviews in Food Science and Nutrition* 13:297-302.

Clarke, E. and J. Wiseman. 2003. Effect of varying trypsin inhibitor activity of full fat soya on nutritional value for broiler chicks. T. Acamovic, C. S. Stewart and T. W. Pennycott, ed. *Poisonous Plants and Related Toxins.* Wallingfor, UK: CABI Publ., 512-519.

Cosgrove, D. J. *Inositol Phosphates: Their Chemistry, Biochemistry and Physiology*. New York: Elsevier Publication Scientific Publishing Co., n.d.

Costello, A. J. R., T. Glonek and T. C. Myers. 1976. 31P nuclear magnetic resonance-pH titrations of myo-inositol hexaphosphate. *Carbohydr. Res.* 46:159-171.

Cowieson, A. J. and V. Ravindran. 2007. Effect of phytic acid and microbial phytase on the flow and amino acid composition of endogenous protein at the terminal ileum of growing broiler chickens. *Brit. J Nutr.* 98:745-752.

Cowieson, A. J., A. Ptak, P. Mackowiak, M. Sassek, E. Pruszynska-Oszmalek, K. Zyla, S. Swiatkiewicz, S. Kaczmarek and D. Jozefiak. 2013. The effect of microbial phytase and

myo-inositol on performance and blood biochemistry of broiler chickens fed wheat/corn-based diets. *Poult. Sci.* 92:2124-2134.

Cowieson, A. J., P. Wilcock and M. R. Bedford. 2011. Super-dosing effects of phytase in poultry and other monogastrics. *World's Poult. Sci. Ass.* 67:225-235.

Cowieson, A. J., T. Acamovic and M. R. Bedford. 2004. The effects of phytase and phytic acid on the loss of endogenous amino acids and minerals from broiler chickens. *Brit. Poult. Sci.* 45:101-108.

Dam, H. 1944. Ineffectiveness of vitamin E in preventing cholesterol deposition in the aorta. *J. Nutr.* 28(4):289-295.

De Winne, A. and P. Dirinck. 1996. Studies on vitamin E and meat quality. 2. Effect of feeding high vitamin E levels on chicken meat quality. *J. Agri. Food Chem.* 44:1691-1696.

Denbow, D. M., V. Ravindran, E. T. Kornegay, Z. Yi and R. M. Hulet. 1995. Improving phosphorus availability in soyabean meal for broilers by supplemental phytase. *Poult. Sci.* 74:1831-1842.

Deshpande, S. S. and M. Cheryan. 1984. Effects of phytic acid divalent cations, and their interactions on alpha-amylase activity. *J Food Sci.* 49:516-619.

dos Santos, T. T., C. L. Walk and S. Srinongkote. 2014. Influence of phytate level on broiler performance and the efficacy of 2 microbial phytases from 0 to 21 days of age. *J. App. Poult. Res.* 23:181-187.

Edwards, D. R. and T. C. Daniel. 1992. Environmental impacts of on-farm poultry waste disposal- a review. *Bioresource Technol.* 41:9-33.

Edwards, H. M., P. Palo Jr., S. Soonchaerenying and M. A. Elliot. 1989. Factors influencing the bioavailability of phytate phosphorus to chickens. Southgate, D., I. Johnson and G. R. Fenwick. *Nutrient availability: Chemical and Biological Aspects*. Cambridge: The Royal Society of Chemistry, 271-276.

Edwards, Jr., H. M., and J. R. Veltman, Jr. 1983. The role of calcium and phosphorus in the etiology of tibial dyschondroplasia in young chicks. *J Nutr.* 113:1568.

Engelen, A. J., E. C. Van Der Heeft, P. H. G. Randsdorp and E. L. C. Smith. 1994. Simple and rapid determination of phytase activity. *J Assoc. Official Analytical Chemists International.* 77:760-764.

Ewing, W. R. 1963. *Poultry Nutrition. 5th Ed.* Pasadena: The Ray Ewing Company.

Fan, Y. K., J. Croom, V. L. Christensen, B. L. Black, A. R. Bird, L. R. Daniel, B. W. McBride and E. J. Eisen. 1997. Jejunal glucose uptake and oxygen consumption in turkey poults selected for rapid growth. *Poult. Sci.* 76:1738-1745.

Fisher, S. K., J. E. Novak and B. W. Agranoff. 2002. Inositol and higher inositol phosphates in neural tissues: homeostasis, metabolism and functional significance. *J. Neurochem.* 82:736-754.

Frost, H. M. 1997. Obesity and bone strength, and mass: a tutorial based on insight from new paradigm. *Bone.* 21:211-214.

Fu, S., J. Sun and L. Qian. 2008. Effect of Ca2+ on beta-propellor phytases. *Protein Peptide Letters.* 15:39-42.

Gavin, G. and E. W. McHenry. 1941. Insoitol: a lipotropic factor. *J. Biol. Chem.* 139(1):485-485.

Geng, A. L. and Y. M. Guo. 2005. Effects of dietary coenzyme Q10 supplementation on hepatic mitochondrial function and the activities of respiratory chain-related enzymes in ascitic broiler chickens. *Brit. Poult. Sci.* 46:626-634.

Gilbert, N. 2009. The disappearing nutrient. *Nature* 461:716-718.

Goldstein, J. and M. L. Scott. 1956. An elctrophoretic study of exudative diathesis in chicks. *J. Nutr.* 60:349.

Gous, R. M., E. T. Moran Jr., H. R. Stilborn, G. D. Bradford and G. C. Emmans. 1999. Evaluation of the parameters needed to describe the overal growth, the chemical growth and the growth of feathers and breast muscles of broilers. *Poult. Sci.* 78:812-821.

Graf, E., K. L. Empson and J. W. Eaton. 1987. Phytic acid. A natural antioxidant. *J. Biol. Chem.* 262(24):11647-11650.

Greiner, R. and A. Farouk. 2007. Purification and characterisation of bacterial phytase whose properties make it exceptionally useful as a feed supplement. *The Protein Journal.* 26:467-474.

Greiner, R. 2006. Phytate-degrading enzymes: regulation of synthesis in microorganisms and plants. Turner, B. L., Richardson, A. E. and Mullaney, E. J. *Inositol Phosphates: Linking Agriculture and Environment.* Wallingford: CAB Internation, 78-96.

Harland, B. F. and E. R. Morris. 1995. Phytate: A good or bad food component? *Nutrit. Res.* 15:733-754.

Harms, R. H., P. W. Waldroup, R. L. Shirley and C. B. Ammerman. 1962. Availability of phytic acid phosphorus for chicks. *Poult. Sci.* 41:1189-1191.

Hartig, T. 1855. Uber das klebermehl. *Botanische Zeitung.* 13:881-885.

Hegsted, D. M., R. C. Mills, C. A. Elvehjem and E. B. Hart. 1941. Inositol in chick nutrition. *Proc. Soc. Exper. Biol. Med.* 47:376-377.

Holub, B. J. 1986. Metabolism and function of myo-inositol and inositol in phospholipids. *Annual Rev. Nutr.* 6:563-597.

Hooppaw, P. W. and B. L. Goodman. 1976. The influence of intermittent light on growth performance and other traits in young chicks. *Poult. Sci.* 55:2285-2289.

Hu, H. L., A. Wise and C. Henderson. 1996. Hydrolysis of phytate and inositol tri-, tetra-, and penta-phosphates by the intestinal mucosa of the pig. *Nutrit. Res.* 16:781-787.

Iqbal, M., N. R. Pumford, Z. X. Tang, K. Lassiter, T. Wing, M. Cooper and W. Bottje. 2004. Low feed efficient broilers within a single genetic line exhibit higher oxidative stress and protein expression in breast muscle with lower mitochondrial complex acitivity. *Poult. Sci.* 83:474-484.

Jondreville, C., P. Lescoat, M. Magnin, D. Feuerstein, B. Gruenberg and Y. Nys. 2007. Sparing effect of microbial phytase on zinc supplementation in maize-soyabean meal diets for chickens. *Animal* 1:804-811.

Jozefiak, D., A. Ptak, P. Mackowiak, M. Sassek and B. A. Slominski. 2010. Multi-carbohydrase and phytase supplementation improves growth performance and liver insulin receptor sensitivity in broiler chickens fed diets containing full-fat rapeseed. *Poult. Sci.* 89:1939-1946.

Karadas, F., V. Pirgozliev, A. C. Pappas, T. Acamovic and M. R. Bedford. 2010. Effects of different dietary phytase activities on the concentration of antioxidants in the liver of growing broilers. *J. Anim. Phys. Anim. Nutr.* 94(4):519-526.

Karim, A. 2006. Responses of broiler chicks to non-phytate phosphorus levels and phytase supplementation. *Intern. J Poult. Sci.* 5:251-254.

Karimi, A., Y. Min, C. Lu, C. Coto, M. R. Bedford and P. W. Waldroup. 2013. Assessment of potential enhancing effects of a carbohydrase mixture on phyase efficacy in male broiler chicks fed phosphorus-deficient diets from 1 to 18 days of age. *Poult. Sci.* 92:192-198.

Kies, A. K., L. H. De Jonge, P. A. Kemme and A. W. Jongloed. 2006. Interaction between protein, phytate, and microbial phytase. In vitro studies. *J. Agri. Food Chem.* 54(5):1753-1758.

Kim, J. C., B. P. Mullan, P. H. Selle and J. R. Pluske. 2002. Levels of total phosphorus, phytate-phosphorus and phytase activity in three varieties of West Australian wheats in response to growing region, growing season and storage. *Australian J Agri. Res.* 53:1361-1366.

Knuckles, B. E. and A. A. Betschart. 1987. Effect of phytate and other myo-inositol phosphate esters on alpha-amylase digestion of starch. *J Food Sci.* 52:719-721.

Konietzny, U. and R. Greiner. 2002. Molecular and catalytic properties of phytate-degrading enzymes (phytases). *Intern. J Food Sci. and Techn.* 37:791-812.

Kostrewa, D., F. Gruninger-Leitch, A. D'Arey, C. Broger, D. Mitchell and A. P. G. M. van Loon. 1997. Crystal structure of phytase from Aspergillus ficuum at 2.4 A resolution. *Nature Structural Biol.* 4:185-190.

Lance, B. G. and A. G. Hogan. 1948. Vitamin Bc, insotiol, and nicotinic acid in the nutrition of the turkey. *J. Nutr.* 36(3):369-379.

Leeson, S., H. Namkung, M. Cottrill and C. W. Forsberg. 2000. Efficacy of a new bacterial phytase in poultry diets. *Canadian J Anim. Sci.* 80:527-528.

Lei, X. G. and J. Porres. 2003. Phytase enzymology, applications and biotechnology. *Biotechnology Letters.* 25:1787-1794.

Lei, X. G., J. D. Weaver, A. Mullaney, A. H. Ullah and M. J. Azain. 2013. Phytase, a new life for an "old" enzyme. *Annual Rev. Anim. Biosci.* 1:283-309.

Leske, K. L. and C. N. Coon. 1999. A bioassay to determine the effect of phytase on phytate phosphorus hydrolysis and total phosphorus retention of feed ingredients as determined with broilers and laying hens. *Poult. Sci.* 78:1151-1157.

Liebert, F., C. Wecke and P-J. Schoner. 1993. Phytase activities in different gut contents of chickens as dependent on levels of phosphorus and phytase supplementations. *Proceedings of the 1st Symposium Enzymes in Animal Nutrition*. Switzerland: Kartause Ittengen, 202-205.

Lim, D., S. Golovan, C. W. Forsberg and Z. Jia. 2000. Crystal structure of Escherichia coli phytase and its complex with phytate. *Nature Structural Biol.* 7:108-113.

Lim, H. S., H. Namkung, J. S. Um, K. R. Kang, B. S. Kim and I. K. Paik. 2000. The effect of phytase supplementation on the performance of broiler chickens fed diets with different levels of non-phytate phosphorus. *Asian-Australasian J Anim. Sci.* 14:250-257.

Lindqvist, Y., G. Schneider, and P. Vihko. 1994. Crystal structures of rat acid phosphatase complexed with the transition-state analogs vanadate and molybdate. Implications for the reaction mechanisms. *Eur. J. Biochem.* 221:139-142.

Liu, K. Chemical composition of distillers grains, a review. *J Agri. Food Chem.* 59:1508-1526.

Liu, N., Y. J. Ru, A. J. Cowieson, F. D. Li and X. C. Cheng. 2008. Effects of phytate and phtase on the performance and immune function of broilers fed nutritionally marginal diets. *Poult. Sci.* 87:1105-1111.

Luttrell, B. M. 1992. The biological relevance of the binding of calcium ions by inositol phosphates. *J Biol. Chem.* 268:1521-1524.

Maddaiah, V. T., A. A. Kumick, B. J. Hullet and B. L. Reid. 1964. Nature of intestinal phytase activity. *Proc. Soc. Experim. Biol. Med.* 115:1054-1057.

Maenz, D. D. and H. L. Classen. 1998. Phytase activity in the small intestinal brush border membrane of the chicken. *Poult. Sci.* 77:557-563.

Matyka, S., W. Korol and B. Bogusz. 1990. The retention of phytin phosphorus from diets with fat supplements in broiler chicks. *Anim. Feed Sci. Tech.* 31(3):223-230.

McCollum, E. V. and E. B. Hart. 1908. On the occurrence of a phytin-splitting enzyme in animal tissues. *J Biol. Chem.* 4:497-500.

Michell, R. H. 2008. Inositol derivatives: evolution and functions. *Nature Rev. Molec. Cell Biol.* 9:151-161.

Miles, R. D. and T. S. Nelson. 1974. The effect of enzymatic hydrolysis of phytate on the available energy content of feed ingredients for chicken and rats. *Poult. Sci.* 53:1714-1717.

Mitchel, R. D., and H. M. Edwards. 1996. Effect of phytase and 1, 25-dihydroxycholecalciferol on phytate utilization and the quantitative requirements for calcium and phosphorus in young broiler chickens. *Poult. Sci.* 75:95-110.

Mohammed, A., M. J. Gibney and T. G. Taylor. 1991. The effect of dietary level of inorganic phosphorus, calcium and cholecalciferol on the digestibility of phytate phosphorus by the chick. *Brit. J Nutr.* 66:251-259.

Mohanna, C. and N. Nys. 1999. Changes in zinc and manganese availability in broiler chicks induced by vegetable and microbial phytase. *Anim. Feed Sci. Tech.* 77:241-253.

Mollgaard, H. 1946. On phytic acid, its importance in metabolism and its enzymic cleavage in bread supplemented with calcium. *Bioch. J.* 40:589.

Moore, R. J. and T. L. Veum. 1983. Adaptive increase in phytate digestibility by phosphorus-deprived rats and the relationship of intestinal phytase and alkaline phosphatase to phytate utilization. *Brit. J Nutr.* 49:145-152.

Morrisey, P. A., S. Brandon, D. J. Buckley, P. J. A. Sheehy and M. Frigg. 1997. Tissue content of a-tocopherol acetate supplement for various periods per-slaughter. *Brit. Poult. Sci.* 38:84-88.

Mullaney, E. J. and A. H. J. Ullah. 2003. The term phytase comprises several different classes of enzymes. *Biochem. Biophys. Res. Comm.* 312:179-184.

Mullaney, E. J., C. B. Daly and A. H. J. Ullah. 2000. Advances in phytase research. *Advances in Applied Microbiol.* 47:157-199.

Munster, D. J., P. F. Bagshaw and J. G. Wilson. 1987. Peptic erosion of gastric mucus in the rat. *Comp. Bioch. Phys.* 87:509-513.

Murai, A., K. Kita, S. Tsuruta, and J. Okumura. 2001. Interaction of dietary phytase and glutamic acid on bone ash response in chicken thigh. *Jap. Poult. Sci.* 38:146-151.

Nahapetian, A. and V. R. Young. 1980. Metabolism of C14-Phytate in rats: effect of low and high dietary calcium intakes. *J Nutr.* 110:1458-1472.

Nahm, K. H. 2003. Bioavailability of phosphorus in manure. *Avian Poult. Biol. Reviews.* 14:53-62.

Nelson, T. S. and A. C. Walker. 1964. The biological evaluation of phosphorus compounds: A summary. *Poult. Sci.* 43:94-98.

Nelson, T. S. and L. K. Kirby. 1987. The calcium-binding properties of natrual phytate in chick diets. *Nutr. Reports Intern.* 35:949-956.

Nelson, T. S. 1967. The utilization of phytate phosphorus by poultry - a review. *Poult. Sci.* 46:862-871.

Nelson, T. S., J. J. McGillivray, T. R. Shieh, R. J. Wodszinski and J. H. Ware. 1968. Effect of phytate on the calcium requirement of chicks. *Poult. Sci.* 47(6):1985-1989.

Nelson, T. S., T. R. Shieh, R. J. Wodzinski and J. H. Ware. 1971. Effect of supplemental phytase on the utilization of phytate phosphorus by chicks. *J Nutr.* 101:1289-1294.

Noguchi, T., A. H. Cantor and M. L. Scott. 1973. Mode of action of selenium and vitamin E in prevention of exudative diathesis in chicks. *J. Nutr.* 103:1502-1511.

Nyachoti, C. M., J. L. Atkinson and S. Leeson. 1997. Sorghum tannins: A review. *World's Poult. Sci.* 53:5-21.

Oberleas, D., M. E. Muhrer and B. L. O'dell. 1962. Effects of phytic acid on zinc availability and parakeratosis in swine. *J. Anim. Sci.* 21:57.

O'Dell, B. L. and A. R. de Boland. 1976. Complexation of phyate with proteins and cations in corn germ and oilseed meals. *J Agri. Food Chem.* 24:804-808.

O'Dell, B. L., A. R. De Boland and S. R. Koirtyohann. 1972. Distribution of phytate and nutritionally important elements among the morphological componenets of cereal grains. *J Agri. Food Chem.* 20:718-721.

Officer, D. I. and E. S. Batterham. 1992. *Enzyme supplementation of linola meal.* Wollongbar, Australia: Wollongbar Pig Industry Seminar.

Onyango, E. M., M. R. Bedford and O. Adeola. 2005. Efficacy of an evolved Escherichia coli phytase in diets of broiler chicks. *Poult. Sci.* 84:248-255.

Oshima, M., T. G. Taylor and A. Williams. 1964. Variations in the concentration of phytic acid in the blood of the domestic fowl. *Biochem.* 92:42-46.

Ostanin, K., E. H. Harms, P. E. Stevis, R. Kuciel, M. M. Zhou, R. L. van Etten. 1992. Overexpression, site-directed mutagenesis, and mechanism of Escherichia coli acid phosphatase. *J. Biol. Chem.* 267:22830-22836.

Patwardhan, V. N. 1937. The occurrence of a phytin-splitting enzyme in the intestines of albino rats. *Biochem.* 31:560-564.

Pearson, A. M., J. D. Love and F. B. Shorland. 1977. Warmed over-flavour in meat, poultry and fish. *Advances Food Res.* 23:1-74.

Peers, F. G. 1953. The phytase of wheat. *Biochem.* 53:102-110.

Perkins, R. C., A. H. Beth, L. S. Wilkerson, W. Serafin, L. R. Dalton, C. R. Park and J. H. Park. 1980. Enhancement of free radical reduction by elevated concentrations of ascorbic acid in avian dystrophic muscle. *Proceedings of the National Academy of Sciences of the United States of America.* 77:790-794.

Perney, K. M., A. H. Cantor, M. L. Straw and K. L. Herkelman. 1993. The effect of dietary phytase on growth performance and phosphorus utilization of broiler chicks. *Poult. Sci.* 72:2106-2114.

Persson, H., M. Turk, M. Nyman and A. S. Sandberg. 1998. Binding of Cu2+, Zn2+ and Cd2+ to insoitol tri-, tetra-, penta- and hexaphosphates. *J. Agri. Food Chem.* 46(8):3194-3200.

Pillai, P. B., D. T. O'Conner, C. M. Owens and J. L. Emmert. 2006. Efficacy of an Escherichia coli phytase in broilers fed adequate or reduced phosphorus diets and its effect on carcass characteristics. *Poult. Sci.* 85:1737-1745.

Porvari, K. S., A. M. Herrala, R. M. Kurkela, P. A. Taavitsainen, Y. Lindqvist, G. Schneider and P. T. Vihko. 1994. Site-directed mutagenesis of prostatic acid phosphatase. Catalytically important aspartic acid 258, substrate specificity, and oligomerization. *J. Biol. Chem.* 269:22642-22646.

Qian, H., E. T. Kornegay and D. W. Denbow. 1996. Phosphorus equivalence of microbial phytase in turkey diets as influenced by calcium and phosphorus ratios and phosphorus levels. *Poult. Sci.* 75:69-81.

Rama Rao, S. V., V. R. Reddy and R. S. Reddy. 1999. Enhancement of phytate phosphorus availability in the diets of commercial broilers and layers. *Anim. Feed Sci. Tech.* 79:211-222.

Rath, N. C., G. R. Huff, W. E. Huff and J. M. Balog. 2000. Factors regulating bone maturity and strength in poultry. *Poult. Sci.* 79:1024-1032.

Ravindran, V., S. Cabahug G. Ravindran and W. L. Bryden. 1999. Influence of microbial phytase on apparent ileal amino acid digestibility in feedstuffs for broilers. *Poult. Sci.* 78:699-706.

Ravindran, V., S. Carbahug, G. Ravindran, P. H. Selle and W. L. Bryden. 2000. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus levels. II. Effects on apparent metabolisable energy, nutrient digestibility and nutrient retention. *Brit. Poult. Sci.* 41:193-200.

Ravindran, V., W. L. Bryden and E. T. Kornegay. 1995. Phytases: occurrence, bioavailability and implications in poultry nutrition. *Poult. Avian Biol. Rev.* 6:125-143.

Reddy, N. R., S. K. Sathe and D. K. Salunkhe. 1982. Phytates in legumes and cereals. *Advances Food Res.* 28:1-9.

Roberson, K. D. and H. M. Edwards. 1994. Effects of 1, 25-dihydroxycholecalciferol and phytase on zinc utilization in broiler chicks. *Poult. Sci.* 73:1312-1326.

Rojas, S. W. and M. L. Scott. 1969. Factors affecting the nutritive value of cottonseed meal as a protein source in chick diets. *Poult. Sci.* 48:819-835.

Rutherford, S. M., T. K. Chung, D. V. Thomas, M. L. Zou and P. J. Moughan. 2012. Effect of a novel phytase on growth performance, apparent metabolizable energy, and the availability of minerals and amino acids in a low-phosphorus corn-soybean meal diet for broilers. *Poult. Sci.* 92:1118-1127.

Saio, K. E. Koyama and T. Watanabe. 1967. Protein-calcium-phytic acid relationship in soybean. 1. Effects of calcium and phosphorus on solubility characteristics of soybean meal protein. *Agri. Biol. Chem.* 31:110-115.

Sakamoto, K., I. Vucenik and A. M. Shamsuddin. 1993. [3H] phytic acid (inositol hexaphosphate) is absorbed and ditributed to various tissues in rats. *J. Nutr.* 123:713-720.

Savage, J. 1964. Zinc metabolism in the chick. *Nutr. Rev.* 29(10):310.

Scheirdelerm, S. E. and J. L. Sell. 1987. Utilization of phytate phosphorus in laying hens as influenced by dietary phosphorus and calcium. *Nutr. Report Intern.* 35:1073.

Schlemmer, U., K. D. Jany, A. Berk, E. Schulz and G. Rechkemmer. 2001. Degradation of phytate in the gut of pigs - pathway of gastro-intestinal inositol phosphate hydrolysis and enzymes involved. *Anim. Nutr.* 55:255-280.

Sebastian, S., S. P. Touchburn and E. R. Chavez. 1998. Implications of phytic acid and supplemented microbial phytase in poultry nutrition: a review. *World's Poult. Sci.* 54:27-47.

Sebastian, S., S. P. Touchburn, E. R. Chavez and P. C. Lague. 1996. The effects of supplemental microbial phytase on the performance and utilization of dietary calcium, phosphorus, copper and zinc in broiler chicks fed corn-soyabean diets. *Poult. Sci.* 75:729-736.

Seeman, E. 1999. The structural basis of bone fragility in men. *Bone.* 25:143-147.

Selle, P. H., A. R. Walker and W. L. Bryden. 2003. Total and phytate-phosphorus contents and phytsae activity of Australian-sourced feed ingredients for pigs and poultry. *Australian J Exper. Agri.* 45:475-479.

Selle, P. H., V. Ravindran, G. Ravindran and W. L. Bryden. 2007. Effect of dietary lysine and microbial phytase on growth performance and nutrient utilization of broiler chickens. *Asian-Australasian J Anim. Sci.* 20:110-1107.

Selle, P. H., V. Ravindran, R. A. Caldwell and W. L. Bryden. 2000. Phytate and phytase: Consequences for protein utilisation. *Nutr. Res. Rev.* 13:255-278.

Shafey, T. M., M. W. McDoanald and J. G. Dingle. 1991. Effect of dietary calcium and available phosphorus concentration on intestinal pH and on the availability of calcium, iron, magnesium and zinc from the intestinal contents of meat chickens. *Brit. Poult. Sci.* 32:185-194.

Shaw, A. L., J. B. Hess, J. P. Blake and N. E. Ward. 2012. Assessment of an experimental phytase enzyme product on live performance, bone mineralization and phosphorus excretion in broiler chickens. *J App. Poult. Res.* 20:561-566.

Simons, P. C. M., H. A. J. Versteegh, A. W. Jongbloed, P. A. Kemme, P. Slump, K. D. Bos, M. G. E. Wolters, R. F. Beudeker and G. J. Verschoor. 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. *Brit. J Nutr.* 64:525-540.

Singh, J. and S. S. Sikka. 2006. Effect of phytase supplementation at different Ca:P ratios of the growth performance of broiler chicks. *Indian J Poult. Sci.* 41:159-164.

Singh, M. and A. D. Krikorian. 1982. Inhibition of trypsin activity in vitro by phytate. *J Agri. Food Chem.* 30:799-800.

Singh, P. K. and V. K. Khatta. 2003. Effect of phytase supplementation on the broiler production. *Poult. Planner.* 4:13-14.

Singh, P. K. 2002. Phytase supplementation for economic and eco-friendly broiler production. *J Eco-Phys.* 5:117-121.

Singh, P. K. 2008. Significance of phytic acid and supplemental phytase in chicken nutrition: a review. *World's Poult. Sci.* 64:553-580.

Singh, P. K., V. K. Khatta and R. S. Thakur. 2003. Effect of phytase supplementation in maize based diet on growth performance and nutrients utilization of broiler chickens. *Indian J Anim. Sci.* 73:455-458.

Snow, J. L., D. H. Baker and C. M. Parsons. 2004. Phytase, citric acid, and 1a-hydroxycholecalciferol improve phytate phosphorus utilization in chicks fed a corn-soybean meal diet. *Poult. Sci.* 83:1187-1192.

Stahl, C. H., K. R. Roneker, W. G. Pond and X. G. Lei. 2004. Effects of combining three fungal phytases with a bacterial phytase on plasma phosphorus status of weanling pigs fed a corn-soy diet. *J. Anim. Sci.* 82(6):1725-1731.

Takemasa, M., H. Murakami and M. Yamazaki. 1996. Reduction of phosphorus excretion in chicks by addition of yeast phytase. *Japanese Poult. Sci.* 33:104-111.

Tamim, N. M. and R. Angel. 2003. Phytate phosphorus hydrolysis as influenced by dietary calcium and micromineral source in broiler diets. *J Agri. Food Chem.* 51:4687-4693.

Tamim, N. M., R. Angel and M. Christman. 2004. Influence of dietary calcium and phytase on phytate phosphorus hydrolysis in broiler chickens. *Poult. Sci.* 83:1358-1367.

Thompson, L. U. 1988. Antinutrients and blood glucose. *Food Tech.* 42:123-131.

Thompson, L. U., C. L. Button and D. J. Jenkins. 1987. Phytic acid and calcium affect the in vitro rate of navy bean starch digestion and blood glucose response in humans. *Amer. J. Clin. Nutr.* 46(3):467-473.

Van der Klis, J. D., H. A. Versteegh, P. C. Simons and A. K. Kies. 1997. The efficacy of phytase in corn-soybean meal-based diets for laying hens. *Poult. Sci.* 76(11)1535-1542.

van Etten, R. L., R. Davidson, P. E. Stevis, H. MacArthur, D. L. Morre. 1991. Covalent structure, disulfide bonding, and identification of reactive surface and active site residues of human prostatic acid phosphate. *J. Biol. Chem.* 266:2313-2319.

Vandepopuliere, J. M., C. B. Ammerman and R. M. Harms. 1961. The relationship of calcium:phosphorus ratios to the utilization of plant and inorganic phosphorus by the chick. *Poult. Sci.* 40:951-957.

Vohra, P., G. A. Gray and F. H. Kratzer. 1965. Phytic acid-metal complexes. *Proceeding of the Society of Experimental Biology and Medicine.* 120:447.

Walk, C. L., M. R. Bedford and A. P. McElroy. 2012. Influence of diet, phytase, and incubation time on calcium and phosphorus solubility in the gastric and small intestinal phase of an in vitro digestion assay. *J. Anim. Sci.* 90(9):3120-3125.

Walk, C. L., M. R. Bedford, T. T. Santos, D. Paiva, J. R. Bradley, H. Wladecki, C. Honaker, and A. P. McElroy. 2031. Extra-phosphoric effects of superdoses of a novel microbial phytase. *Poult. Sci.* 92:719-725.

Walk, C. L., T. T. Santos and M. R. Bedford. 2014. Influence of superdoses of a novel microbial phytase on growth performance, tibia ash, and gizzard phytate and inositol in young broilers. *Poult. Sci.* 93:1172-1177.

Wasserman, R. H. and A. N. Taylor. 1973. Intestinal absorption of phosphorus in the chicks. Effect of vitamin D and other parameters. *J Nutr.* 103:586-599.

Wise, A. 1983. Dietary factors determining the biological acitivity of phytase. *Nutrition Abstract and Reviews.* 53:791-806.

Wodzinski, R. J. and A. H. J. Ullah. 1996. Phytases. *Advances in Applied Microbiol.* 42:263-302.

Wyss, M., R. Brugger, A. Kronenberger, R. Remy, R. Fimbel, G. Oesterhelt, M. Lehmann and A. P. G. M. Van Loon. 1999. Biochemical characterization of fungal phytases (myo-inositol hexakisphosphate phosphohydrolases): catalytic properties. *Applied Environ. Microbio.* 65:367-373.

Yi, Z., E. T. Kornegay and D. M. Denbow. 1996. Supplemental microbial phytase improves the zinc utilization in broilers. *Poult. Sci.* 75:540-546.

Zimmermann, B. H. J. Lantzsch, R. Mosenthin, H. K. Biesalski and W. Drochner. 2003. Additivity of the effect of cereal and microbial phytases on apparent phosphorus absorption in growing pigs fed diets with marginal P supply. *Anim. Feed Sci. Tech.* 104:143-152.

Zyla, K., M. Mika, B. Stodolak, A. Wikiera, J. Koreleski and S. Swiatkiewicz. 2004. Towards complete dephosphorylation and total conversion of phytate in poultry feeds. *Poult. Sci.* 83(7):1175-1186.

Appendix 1. 50 mM phosphate buffer, pH 7.0 preparation

1. Prepare a 50 mM potassium phosphate monobasic (KH_2PO_4) solution
    a. 3.40 g of KH_2PO_4 was weighed out and transferred to a 500 mL volumetric flask.
    b. Diluted to volume with distilled-deionized water (pH 4.5)
2. Prepare a 50 mM potassium phosphate dibasic (K_2HPO_4) solution
    a. 8.71 g of K_2HPO_4 was weighed out and transferred to a 1 L volumetric flask.
    b. Diluted to volume with distilled-deionized water (pH 8.5)
3. Transfer 100 mL of the 50 mM potassium phosphate monobasic solution and 500 mL of the potassium phosphate dibasic solution to a 2 L beaker. Mix and monitor the pH of the combined solution as continually more of each solution is added until the volume is in excess of 1 L and the pH is near 7.0.
4. Add about 500 mL of mixed solution to a 1000 mL volumetric flask and add 1 g ethylenediamine tetracetic acid (EDTA) and 1 g n-propyl gallate (PG). Allow solution to mix for one hour, or until PG is fully dissolved.
5. Bring to volume.

Appendix 2. 30 % trichloroacetic acid (TCA) reagent preparation

1. Weigh 300 g TCA in a 2 L beaker.
2. Add 1 L of distilled-deionized water and mix until dissolved.

Appendix 3. 0.02 M 2-thiobarbuturic acid (TBA) reagent preparation

1. Weigh 0.7208 g TBA and transfer to a 250 mL volumetric flask.
2. Dilute to volume with distilled-deionized water. Mix for one hour or until fully dissolved.

Appendix 4. IACUC approval

UNIVERSITY OF ARKANSAS

Office of Research Compliance

MEMORANDUM

TO: Susan Watkins

FROM: Craig N. Coon, Chairman
Institutional Animal Care
And Use Committee

DATE: July 12, 2011

SUBJECT: IACUC PROTOCOL **APPROVAL**
Expiration date : **July 11, 2014**

The Institutional Animal Care and Use Committee (IACUC) has **APPROVED** Protocol **#11056- "EVALUATION OF METHODS USED TO IMPROVE THE GROWTH EFFICIENCY AND CARCASS CHARACTERISTICS OF MEAT BIRDS** ". You may begin this study immediately.

The IACUC encourages you to make sure that you are also in compliance with other UAF committees such as Biosafety, Toxic Substances and/or Radiation Safety if your project has components that fall under their purview.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes in the protocol during the research, please notify the IACUC in writing [Modification Request form] **prior** to initiating the changes. If the study period is expected to extend beyond **07-11-2014,** you must submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

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

cnc/car

cc: Animal Welfare Veterinarian

Administration Building 210 • 1 University of Arkansas • Fayetteville, AR 72701-1201 • 479-575-4572
Fax: 479-575-3846 • http://vpred.uark.edu/199