INFLUENCE OF AGE AND FEEDING LENGTH ON PHYTASE EFFICACY IN BROILER CHICKENS

by

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Dedicated to God, my wife, and my family

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NOMENCLATURE

SYMBOL	DESCRIPTION
ATP	Adenosine triphosphate
AA	Amino acid
AID	Apparent ileal digestibility
BW	Body weight
BWG	Body weight gain
Ca	Calcium
Cr	Chromium
CP	Crude protein
d	Day
DNA	Deoxyribonucleic acid
DM	Dry matter
FI	Feed intake
FTU	Phytate unit
G:F	Gain to feed ratio
h	Hour
IP	Inorganic phosphorus
IP6	Myo-inositol hexaphosphoric acid
Lys	Lysine
ME	Metabolizable energy
MSP	Monosodium Phosphate
NC	Negative control
nPP	Non-phytate phosphorus
NSP	Non-starch polysaccharides
P	Phosphorus
PP	Phytate phosphorus

PC	Positive control
RNA	Ribonucleic acid
SI	Small intestine
TTR	Total tract retention
wk	Week

ABSTRACT

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Title: Influence of Age and Feeding Length on Phytase Efficacy in Broiler Chickens
Major Professor: Dr. Layi Adeola.

The objective of this thesis was to investigate the effect of age and feeding length on phytase efficacy in broiler chickens during the starter phase. Two studies were carried out to evaluate this objective.

Study 1 was a randomized complete block design with 4 × 5 factorial arrangements of treatments. There were four diets; a positive control (PC), negative control (NC) and two phytase supplemented diets with inclusion levels of 1,000 and 2,000 phytase units/kg. There were five age and duration of feeding groups; Three 2-d feeding lengths terminated at d 8, 14, and 22 (d 6 to 8, d 12 to 14, and d 20 to 22), a 5-d feeding length terminated at d 14 (d 9 to 14) and a 16-d feeding length terminated on d 22 (d 6 to 22). Growth performance and sample collections were collected at the end of each phase i.e. d 8, 14 and 22. There was a difference ($P < 0.01$) in weight gain, feed intake, and feed efficiency between birds fed the PC diets and birds fed the NC diets across all groups as birds on the NC diets had lower performance ($P < 0.05$) than birds on the PC diet. However, birds fed the phytase supplemented diets had higher ($P < 0.05$) growth performance compared with birds fed the NC diet across all groups. Similarly, phosphorus (P) and calcium (Ca) digestibility and retention of birds fed the NC were lower ($P < 0.05$) as compared with birds fed the PC diet while birds fed the phytase supplemented diets had higher mineral digestibility and retention ($P < 0.05$) compared with birds on the NC diet. Age effect was evaluated by comparing the performance of birds fed the experimental diets for 2 d until d 8, 14, and 22. Birds fed until d 14 had the highest impact of the NC diet on mineral utilization, and the largest improvement of phytase on mineral utilization as compared with birds fed until d 8 and 22. Similarly, when feeding length effect was considered, birds fed for a shorter period had greater response to phytase ($P < 0.05$) on nutrient utilization than birds fed for a longer period at d 14 and 22. Tibia ash was higher ($P < 0.05$) in birds fed phytase supplemented diets for a longer period (i.e. 16 d) compared with birds fed or 2 or 5 d. The results from this study observed that age and duration of feeding

influenced phytase efficacy especially in younger birds fed for a short period. However, it could not be determined if feeding birds for a short period at different ages in the starter phase would have a similar effect.

In study 2, the effects of age and feeding low P diets to birds for a short period of time on phytase efficacy and super dosing were evaluated at two critical points in the starter phase. This study had 3 × 2 × 2 factorial arrangements of treatments comprising 3 diets; a PC, NC, and a NC with phytase supplemented at 2,000 phytase units/kg; and 2 ages (i.e d 14 and 22) and 2 feeding lengths (i.e 2-d and 5-d). Thus, birds were fed the experimental diets from d 12 to 14, 9 to 14, 20 to 22, and 17 to 22 respectively. Results observed were similar to the first study. Birds fed the NC diet had lower ($P < 0.01$) performance as compared with birds fed the PC diets across all age and feeding length groups. Similarly, birds fed diets with the super dose level of phytase had greater growth performance ($P < 0.01$) compared with birds fed the NC diets. When age effect was considered, birds fed for 2 or 5 d until d 14 had the greatest improvements of phytase on nutrient utilization and bone mineralization compared with birds fed for both periods until d 22. When effect of feeding was considered, birds fed for 2-d at both ages had greater responses to phytase in performance and nutrient utilization compared with birds fed for 5-d at both ages. Plasma myo-inositol was higher ($P < 0.01$) in birds fed the super dose level of phytase compared with birds fed the NC diet.

In summary, we could conclude that the efficacy of phytase both at 1,000 and 2,000 FTU/kg was higher in birds fed for 2 d until d 14 as compared with the other groups. This could potentially help in designing studies to evaluate new phytase products or for comparing the efficacy of phytase from various sources. Feeding broiler chickens during the suggested time phase would potentially reveal the maximum efficacy of the phytase product.

CHAPTER 1. LITERATURE REVIEW

1.1 Introduction

Chickens are a variety of poultry that serve as a major source of protein for human consumption. They can be categorized into egg-laying and meat producing birds (broilers). Broilers are a major source of meat in the world with the US being one of the largest producers of broiler chickens. In 2017, about 41 billion pounds of chicken products were marketed in the US (National Chicken Council, 2018). Scientists have continuously conducted research in management of broiler chickens with focus on their nutrition, because approximately 70% of broiler chicken production costs are attributed to feed with energy, protein and phosphorus serving as its most expensive components. The ability for broilers to optimize nutrients available for growth, development and production has been attributed to the type of feed given, age, genetics, duration of feeding, sex and environmental factors.

Phosphorus (P) is one of the most abundant minerals on earth and is important for the sustenance of life both in plant and animals. P is necessary for the support and development of the animal through skeletal formation. P is also involved in various biochemical reactions in the body including being a component of the energy currency (adenosine triphosphate). P is widely found in plants but in a form generally unavailable to non-ruminant animals like chickens and pigs. P is bound as phytin in cereal grains and oil seeds such as corn and soybean, commonly used as feed ingredients.

Phytin or phytate has been regarded as an anti-nutritional factor because of its ability to bind with other nutrients including proteins, starch and salivary amylase, and minerals such as calcium, magnesium, iron and copper in the digestive tract of animals thus making them

unavailable for use by the animals (Singh et al., 2003). This has encouraged the supplementation of corn-soy based diets with inorganic P (IP) sources and with exogenous enzymes to make P available for use by broilers and pigs.

Phytase is a phosphatase enzyme capable of breaking phytate bonds systematically, thus releasing P for use. Phytase exists naturally in plants and animals or can be synthesized by microbes such as bacteria or fungus. Phytase is administered as an exogenous enzyme in diets. Studies have found that supplementing corn and soybean-based diets with phytase in the presence of little or no addition of inorganic P, improves growth performance, mineral utilization and bone mineralization by hydrolyzing phytate bonds and releasing P (Dilger et al., 2004, Woyengo et al., 2010; Rutherfurd et al., 2012)

The efficacy of phytase has been measured in numerous studies using different methods. Some studies have used a regression model and a P bio-equivalency value to measure the amount of P released from the diet by phytase using phenotypic responses such as growth performance, P and calcium (Ca) digestibility and retention, plasma P and Ca, and tibia ash as parameters for evaluation (Adeola, 2010; Vieira et al., 2015; Ribeiro et al., 2016). Others have compared sources of inorganic P in a low P negative control diet with P derived from inclusion of phytase, responses recorded from birds given phytase were subtracted from values obtained in birds fed the low P control diet (Adedokun et al., 2004; Han et al., 2009).

These methods have helped researchers and companies to develop new phytase products (Onyango et al., 2005), compare the value of various phytase sources from bacteria and fungi (Ribeiro et al., 2016) and determine the true value of commercial phytase available to the feed industry (Adedokun et al., 2004). However, the efficacy of phytase in broiler chickens can be

affected by the source of inorganic P used or the innate characteristics of the phytase product, as well as, the age or physiological state of the bird or the duration of feeding low P diet.

The objective of this literature review is to describe P and phytate in non-ruminant animal nutrition, present results from previous studies reporting the effects of phytase on phenotypic attributes of broiler chickens including growth performance, nutrient utilization and bone mineralization. Lastly, this literature will cover the measurements of phytase efficacy and factors that could possibly affect the efficacy of phytase in broiler chickens.

1.2. Broiler Chickens

Broiler chickens are a major source of protein for humans worldwide. They are defined as the meat-type chickens (Krutchen, 2002). According to the USDA (2018), 87 million tons of broiler meat was produced in the United States in 2014 with approximately 4 million tons being exported. They postulated that in 2018, export of broiler meat from the US would rise to a record 19 million tons. With this staggering increase in the consumption of broiler meat, there has been a rise in research to further improve the production of broiler meat at an effective cost and with increased productivity to meet the demand of the growing world population for quality protein.

1.2.1. Nutrient Requirement of Broilers

Some studies have reported that the nutrient requirement and utilization of broilers can be affected by age, genotype, sex and environmental factors (Doeschate et al., 1993; Huang et al., 2005).

1.2.2. Influence of Age on Nutrient Utilization in Broiler Chickens

Tarvid (1995) reported that age has an influence on the ability of poultry to digest and absorb nutrients, especially crude protein. His observation has been supported by numerous

studies, although, they differ in the direction of the influence. Some studies report that younger birds have increased digestibility of nutrients, particularly crude protein and amino acids, when compared with older birds (Hakansson and Eriksson, 1974; Fonolla et al., 1981; Carre et al., 1991; Zuprizal et al., 1992; Mahagna et al., 1995; Batal and Parsons, 2002; Huang et al., 2005). In agreement with these studies, Huang et al. (2005) reported that digestibility of amino acids in birds fed wheat and sorghum diets where higher at d 14 when compared with birds at d 28. These studies reasoned that the observed increase in nutrient utilization in younger birds could be due to high nutrient uptake associated with a rapid growth rate, or an increased intestinal surface area through increased villus height and width (Nitsan et al., 1991; Uni et al., 1995; Mahagna et al., 1995). Birds have been known to have rapid intestinal growth during the first 7 d of life. This growth is characterized by a transition from enterocyte proliferation in the villus during the late embryonic phase to localized proliferation within the crypt after hatching (Geyra et al., 2001a). Iji et al. (2001a) reported that in comparison with body weight, the weight of most organs including the gizzard, small intestine (SI), pancreas and yolk sac reduced with age. However, the length of the SI including jejunum and ileum increased with age. They also observed a rapid increase in cell size, proliferation, migration and an increase in protein: DNA ratio during the first 7 d post-hatching. This indicates that a large amount of nutrients are required within the first few days to support the rapid cell proliferation in the crypt and villus of intestines. This sets the stage for further intestinal absorption as birds mature and SI length increases. Obst and Diamond (1992) observed that nutrient utilization in broilers peaked at 2 weeks post-hatching, declined between 3-5 weeks, and then peaked again at 6 weeks post-hatching, thus, supporting theories that younger birds had an increased nutrient uptake capacity when compared with older birds. Wallis and Balnave (1984), and Noy and Sklan (1995) on the other hand reported an increase in digestibility of nitrogen and

amino acids with age. Duckworth et al. (1950) and Carew et al. (1972) reported an increase in digestion of fat with increasing age while Zelenka (1968) and Sell (1996) reported an increase in metabolizable energy of diets with increasing age of birds. These could be due to an increase in enzymatic activity as with increasing age of birds. Iji et al. (2001b) observed an increase in the expression of maltase, sucrase, N-aminopeptidase and alkaline phosphatase in all regions of the SI of birds at 21 d of age as compared with birds at 7 d. They attributed this observation to the general increase in surface area of the SI (i.e., increased villus height and intestinal length) over which the enzymes were expressed. They also observed an increase in enterocyte lifespan which allowed longer enzyme secretions on the villus of older birds. This may explain the ability of older birds to digest copious quantities of nutrients. Therefore, it is clear that age plays a key role in the utilization of nutrients by broiler chickens.

1.2.3. Influence of Duration of Feeding on Nutrient Utilization in Broiler Chickens

Birds require a period of time when diets are introduced before the effect of the diets are observed, especially when switching from a nutrient sufficient diet to a nutrient deficient diet or when a feed additive or enzyme is added to the diet. This is because of the time required for proper digestion and absorption of ingested feed in the gastro-intestinal tract. The required time varies depending on the age of the bird as older birds have longer residence time in the gut than younger birds. It may also take a day or several days of consuming the diet before effects can be observed in the growth performance or nutrient utilization of the bird. The number of days allocated to the experimental period differs among researchers. It is dependent on the objective of the study, type of diets used, age of the birds, cost of running the study and several other factors, and can range from 1 d (Plumstead et al., 2008; Anwar et al., 2018) or through the entire lifecycle of the bird (Qian et al., 1996a; Huang et al., 2005) before parameters are measured or samples collected.

Typically, 5 – 7 d are more common for testing experimental diets although, some studies allow for an adaptation period to the diets. Invariably, duration of feeding could affect nutrient utilization in chickens depending on the age of the bird or the type of diet fed.

1.3. Phosphorus

Phosphorus (P) with a relative atomic mass of 30.97 is one of the most abundant minerals on earth. However, P does not exist in a free form because of its highly reactive nature. P comprises about 1% of an animals body weight and is essential to life through its functions in growth and metabolic development (Boling et al., 2000).

1.3.1. Functions of Phosphorus

Phosphorus forms an integral part of various metabolic systems in the body including being a component of Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA). Phosphorus is necessary in the formation of phospholipids, which are component of the cell membrane present in every cell of the body. Phosphorus also plays a role in energy metabolism through adenosine triphosphate (ATP) synthesis and is important in the synthesis of proteins for growth, maintenance, and repair of worn out tissues (Humer et al., 2015). Phosphorus works with vitamins to control muscle contractions, cardiac rhythms, nerve signaling and kidney functions. Phosphorus and calcium (Ca) form the major component of the skeletal system necessary for the support and stability of the animal. About 80% of P and 99% of Ca are found in the bones and teeth through deposition of hydroxyapatite throughout the lifetime of the animal (Crenshaw, 2001). Phosphorus is the third most economically important nutrient to the poultry industry after energy and protein (Boling et al., 2000).

1.3.2. Sources of Phosphorus

Phosphorus is abundantly found in plant seeds, especially in cereal grains and oilseed plants (Trotter and Allee, 1979a, b). The availability of P in cereals are variable and range from 46% in wheat to as low as 15% in corn, and this is due to the presence of naturally occurring phytase enzyme in wheat, thus increasing the availability of P when compared with corn (Better Crops, 1999). In oilseeds, there is a slightly greater concentration of total P, ranging from 0.45 to 0.75% (Eeckhout and De Paepe, 1994). Conversely, animal derived feed ingredients such as meat and bone meal and fish meal have a much higher digestibility of P (Traylor et al., 2005). Due to the variability in the bioavailability of P in plant sources, inorganic sources including mono and di-calcium phosphates, monosodium phosphate, and defluorinated phosphate, which are highly digestible P sources, have been widely used in the animal feed industry.

1.3.3. Phosphorus Bioavailability

It has been of importance to researchers to estimate the bioavailability of P to animals in feedstuffs and ingredients. Some studies calculate this bioavailability using a slope ratio method with a reference ingredient. This method has been useful in ranking the availability of P in feed ingredients. However, this method cannot be used to determine the digestibility of P in that ingredient. This has led to other studies measuring the digestibility of P in animals to estimate the availability of P in an ingredient (Adeola, 2001). Some criteria used includes performance parameters like weight gain or feed efficiency (Potter et al., 1995), toe ash (Potter, 1988), tibia ash (Pillai et al, 2006), apparent digestibility of P (Viveros et al, 2002), total tract retention of P (Adedokun et al, 2004), and plasma concentration of P (Cowieson et al, 2017). Apparent ileal digestibility and total tract retention of P are more commonly used in broiler studies and involve measuring the feed intake and output which could be ileal digesta or excreta. The total collection

method may be used to measure the digestibility of P in broilers and this involves recording the feed intake of the birds over a determined period and the total collection of excreta during the same period (Adeola, 2001). Indigestible markers such as chromium or titanium can be used in the index method to measure the apparent ileal digestibility (AID) or total tract retention (TTR) of P in broilers. This involves determining the concentration of the marker in the diet and in the output, which could be the ileal digesta or excreta. These methods have thus made it easier to determine the bioavailability of P in common ingredients used in formulating diets for chickens or pigs.

1.3.4. Phosphorus Digestion, Absorption and Excretion.

Animals maintain homeostasis of P in the body by controlling absorption of P in the small intestine (SI) and excretion through the fecal matter and urine (combined as excreta in poultry). The bones also serve as a medium of maintaining this balance by the release and reabsorption of P depending on the status of the animal. Studies have shown that P is digested in the upper SI in chickens specifically the proventriculus, gizzard and crop due to the optimal acidic environment of the region (Brejnholt et al., 2011; Gao et al., 2013; Li et al., 2016, 2018). Phosphorus is absorbed as inorganic P anion (PO_4^-, HPO_4^-, or $H_2PO_4^-$) in the lumen of the SI by the epithelial cells in the brush-border membrane. The epithelial cells absorb these P anions via active transport or paracellular transport (passive diffusion) into the blood stream (Anderson, 1991). Sodium coupled phosphate transport is the major active transport pathway for P in the SI. This Na^+-inorganic P (Pi) cotransporter can be categorized into type I, II, and III. Type II can be further divided into two with type IIa more commonly found in the kidney and IIb found in the SI (Bai et al., 2000). Type IIb and III have been shown to be of importance in the transport of P across the basal membrane of the SI and their expression are regulated by various hormones, vitamin D_3 and dietary P (Olukosi et al., 2011; Sabbagh et al., 2011). In the blood, P is transported as phospholipids or attached to

serum proteins in its inorganic form before being utilized by the animal for its metabolic processes or stored in the bones (Pond et al, 2005). The metabolic functions and excretion of P are regulated by the endocrine system through the parathyroid hormone and calcitonin. This regulation also involves interactions between vitamin D, circulating and stored P, and various hormones (Jongbloed and Kemme, 1990). The kidneys play an important role in the excretion of P to maintain the homeostasis required by the body. Deficiency of P results in reduced appetite, feed conversion, growth and deformation of bones and teeth (Reinhart and Mahan, 1986; Singh et al., 2003). Toxicity of P due to excessive intake is not common but over a prolonged period may lead to renal failure due to the pressure of excretion on the kidneys.

1.3.5. Phosphorus and Calcium Interactions

The interaction between P and Ca have been extensively investigated due to the close interrelation in their metabolic processes and biological pathway. Phosphorus and Ca also share a common form of storage in the body (Pond et al., 2005). Adequate metabolism of both minerals usually requires the consideration of the following factors: 1) Sufficient supply of each nutrient, 2). suitable ratio between both minerals and 3) presence of vitamin D. (Better Crops, 1999). The desirable total Ca:P ratio in poultry or swine nutrition when feeding a corn-soy based diet is between 1:1 and 2:1 (NRC, 1994,1998). An imbalance in this ratio causes a deviation in the metabolism of one or both minerals (Bethke and Edgington, 1928). Mahan (1982) reported that an increase in the Ca:P ratio could reduce P absorption, circulating P and bone mineralization. However, this effect is mitigated in the presence of excessive dietary P or addition of exogenous phytase (Cromwell et al., 1995). Elevated levels of Ca could reduce the bioavailability of P by increasing the pH of the gut, thus reducing the effectiveness of enzymes or microbe action (Sandberg et al 1993). Calcium could also bind with phytate-P to form an insoluble phytate

complex, thus making P unavailable to the animal (Selle et al., 2009). A deficiency in vitamin D decreases Ca utilization causing a possible distortion of the ration while, an excess may lead to mobilization of P and Ca from the bones (Jongbloed, 1987; Better Crops, 1999). Vitamin D plays a role in P absorption in the SI.

1.4. Phytate

Most P (60-80%) found in plant-based feed ingredients is bound as phytin, (i.e. a complex of P with magnesium, potassium and Ca). Phytin exists more commonly as the salts of phytic acid, also called phytate (Denbow et al., 1995; Boling et al., 2000; Adeola, 2018); or as myo-inositol-1,2,3,4,5,6-hexakis dihydrogen phosphate (IP6) which is the unstable free acid form (Kornegay, 2001) (Figure 1-1). Phytin is located within the germ portion of the corn kennel whereas, phytin is found in the outermost region of the kernel and in the bran of rice and wheat (O'Dell et al., 1976). In oilseeds, phytin is bound with proteins and distributed within the kernel (Ravindran et al., 1995). Phytate content in seeds can be influenced by climatic conditions, genetics, location, soil type and even fertilizer application. Reddy et al. (1989b) observed an increase in the proportion of phytate P (PP) when high dose of P in the form of fertilizer was supplied to plants. Phytate serves as a major storage of P in plants, during germination they are hydrolyzed by the young seedlings, thus releasing P and other minerals such as Ca and Mg necessary to support the growth of the plant. Myo-inositol released by phytate plays a role in cell wall formation (Eckardt, 2010). Phytate also serves as an energy reserve during the germination processes, and a regulator of available P in the seeds (Cosgrove and Irving, 1980). When phytate is hydrolyzed, phytate can be a major source of P to animals. However, non-ruminants, including pigs and chickens, are unable to adequately break down phytate due to a limited amount of endogenous enzymes capable of breaking the phytate bonds (Nelson, 1967; Raboy, 1990; Yan et al., 2003). This reduces the

bioavailability of P in corn and soybean diets and promotes the use of supplemental IP sources and the release of unused P into the environment, thereby polluting the environment and allowing eutrophication of aquatic bodies (Nelson, 1967; Cromwell, 1992; Adeola, 1999).

1.4.1. Binding Ability of Phytate

Phytate in feed has been referred to as an antinutritional factor because of its ability to bind nutrients and make them unavailable for use by animals (Pallauf and Rimbach, 1997). Studies have shown that phytate has the ability to form strong chelates with positively charged metallic ions like Zn^{2+}, Ca^{2+}, $Cu^{2+}$and Fe^{2+} due to its high negatively charged density in the intestines thus, forming insoluble complexes with P making it unavailable for animal use (Cosgrove and Irving, 1980; Barrientos and Murphy, 1996; Humer et al., 2015). The pH of the SI is a crucial factor in the solubility of these complexes. Selle et al. (2009) observed that the solubility of the phytate complex is higher in an acidic environment and that precipitation of these complexes occurs under neutral pH conditions thus, its passage into the alkaline environment of the SI reduces its solubility and hinders the absorption of the minerals bound in the complex. The effect of phytate on mineral availability in animals may be affected by the pH of the intestines, solubility of phytate and the content of minerals and phytate in the SI (Humer et al., 2015). Phytate has also been reported to decrease protein and amino acid utilization through the formation of insoluble phytate-protein complexes (Okubo et al., 1976; Rajendran and Prakash, 1993; Li et al., 2015) thereby negatively impacting protein solubility and digestibility as well as reducing the activity of enzymes in the gut (Deshpande and Damodaran, 1989; Urbano et al., 2000; Cowieson and Bedford, 2009). Other studies have found that phytate may not have an effect on amino acids digestibility depending on the feed ingredients used, type of marker, amino acid being considered or even the age of the animal (Batal and Parsons, 2002; Kim and Corzo, 2012; Selle et al., 2012; Li et al., 2015). Some

reports show a negative interaction between phytate and energy utilization. Inagawa et al. (1987), observed a reduction in the activity of β-galactosidase by phytate through interactions with the enzyme, although it is not proven that starch-phytate complexes are formed. Thompson et al. (1987) also suggested that an indirect binding of phytate to starch may occur through the proteins associated with starch causing a negative impact on energy metabolism.

1.4.2. Non-phytate Phosphorus

When diets for chickens are formulated, the total P concentration of the diet is usually considered to ensure that the birds meet their P requirement. More recently, diets have been formulated based on the available P concentration, which is also referred to as the non-phytate P (nPP), thus total P is divided into the relatively unavailable PP and the readily available nPP. NRC (1994) recommends 0.45% nPP at the starter phase for broilers (0-3 weeks) and 0.35% at the finisher phase (3-6 weeks) to meet the chickens P requirement. Due to the unavailability of PP for chickens when feeding corn-soybean based diets, these requirements are achieved by supplementing diets with inorganic P sources including monocalcium P and dicalcium P which contain 21% and 18.7% of P respectively (NRC, 1994) and are approximately 80% digestible. Other sources of supplemental P include bone meal, meat and bone meals, and fish meal (Lima et al., 1997). A study has suggested that a range of 0.30-0.39% nPP is adequate for broiler chickens during the starter phase when body weight gain and bone mineralization responses were observed (Waldroup et al., 2000). However, several studies have reported high body weight, mineral utilization and increased bone mineralization when birds were fed diets containing 0.45% nPP. Panda et al. (2007) reported the highest responses for birds in BWG, feed intake, tibia ash and breaking strength in birds fed 0.45% nPP as compared with birds fed lower nPP concentrations, this corroborated with reports from other studies (Qian et al., 1996b; Singh et al., 2003; Manangi

and Coon, 2008; Jiang et al., 2013). Subsequently, feeding diets deficient in nPP, referred to as low P diets, had negative impacts on growth performance, nutrient utilization and bone mineralization of birds. Kornegay et al. (1996) reported that broilers fed corn-soybean based diets with 0.20% nPP had a significantly lowered weight gain (380g) as compared with birds fed the required 0.45% nPP (613g). A similar observation was recorded in feed intake (625 and 963 g/bird), gain to feed ratio (584 and 636 g/kg) and toe ash (8.2 and 13.3%) responses respectively, thus, proving that chickens were not able to utilize P present in plant-based ingredients without the supplementation of IP (Nelson et al., 1976). Other studies have recorded similar observation in chickens and pigs (Waldroup, 1999; Leeson et al., 2000; Manangi and Coon, 2008; Cowieson et al., 2013; Yáñez et al., 2013).

1.4.3. Environmental Issues with Phosphorus release in Excreta

Pollution of the environment is a major concern to researchers all over the world. Major focus is on the swine and poultry industry due to excessive P wastage that has found its way into the environment and aquatic bodies. Since diets are formulated based on available P, a lot of PP from corn and soybean are underutilized by pigs and chickens and are passed through excreta. When these excreta are used as manure on crop fields, the excess P contained in them move into the soils where they accumulate (Panda et al., 2007). P not used up by roots of crops can be leeched or eroded, hence finding their way into bodies of water. This causes eutrophication, which is associated with the abnormal increase in algae and bacteria present in the water due to presence of excess nutrients in the water (Waldroup, 1999). This algae bloom causes oxygen present in the water to be used up and become unavailable for other marine life, subsequently killing them and causing an imbalance of the aquatic ecosystem (Corell, 1999, Dilger et al., 2004). The need to prevent the loss of P in the environment, attaining the P requirement of the animals while

simultaneously reducing the cost of feed production due to relatively high cost of IP sources has encouraged the use of exogenous enzymes such as phytase that is capable of breaking PP bonds and releasing P to animals (Simons et al., 1990).

1.5. Phytase; Enzyme Activity and mode of Action

The enzyme phytase (myo-inositol hexaphosphate hydrolase) is a phosphatase capable of systematically hydrolyzing the phosphate ester bonds in IP6 and releasing P (Nayni and Markakis, 1986). Phytase is widely found in microorganisms, plants and animals (Angel et al., 2002) and can be classified into 2 groups (3-phytase and 6-phytase). Grouping depends on the position on the IP6 ring where dephosphorylation is initiated (i.e., position 3 and 6 respectively) (Selle and Ravindran, 2007; Kumar et al., 2010). Phytase produced by microorganisms such as fungi (Aspergillus sp.) and bacteria are referred to as 3-phytase because it initiates the hydrolysis of the phosphate group on carbon 3 (Kornegay, 2001). Phytase from plant origin are usually 6-phytase because they initiate hydrolysis of the phosphate group on carbon 6 (Pallauf and Rimbach, 1995). Although, exceptions have been known to occur with bacteria such as *Escherichia coli* producing 6-phytase (Greiner et al., 1993) and soybean producing 3-phytase (Sandberg and Andlid, 2002). Phytase from fungi or bacteria have been known to differ in their capacity to hydrolyze phytate, their pH stability, and their resistance to digestive enzymes present in the SI (Igbasan et al., 2000). Phytase can be categorized into 2 classes based on their optimum pH. These include acidic phytases with a pH optimum of 3.0-5.5 and alkaline phytases with an optimum of 7.0-8.0 (Vijayaraghavan et al., 2013). The presence of phytase in the gut of animals may be attributed to exogenous phytase added to the diet of the animal, the intrinsic phytase produced by the plant in the feed ingredient or the phytase produced by the resident microbes in the gut. Plants have been known to produce phytase capable of hydrolyzing phytate, however, they vary in their activity levels. High protein plants

such as maize, sorghum and soybean have negligible phytase activity and cereals such as rye, wheat, and barley have relatively high phytase activity (Viveros et al., 2000; Steiner et al., 2007). Phytase activity in plants could also be affected by temperature, pH, and moisture content of the plant seeds (Haraldsson et al., 2004). Phytase produced by plants are not reliable sources of the enzyme in diets because of the possibility of enzyme denaturation. In the production of feed ingredients and diets, high temperatures are often used during heat treatments and pelleting processes, thus, causing a breakdown of phytase, which are proteins in nature (Nys et al., 1996; Brady et al., 2002; Slominski et al., 2007). Exogenous phytases have been isolated from bacteria, fungi, and yeast and were first investigated in the 1970's. They were not fully studied until the 1980's, however. This was because of its high cost, and the relatively low awareness on the pressure of P waste on the environment (Nelson et al., 1970; Harland and Morris, 1995). Technological advances and increased attention to environmental issues have led to the commercialization of phytase enzymes in poultry and pig diets to combat the impact of P loss in the environment through the genetic modification of phytase producing micro-organisms (Kornegay, 2001; Humer et al., 2015).

Exogenous and endogenous phytase could be subject to destruction by the acidic pH of the upper SI or by action of proteolytic enzymes present in the crop or proventriculus. However, this region has been suggested as the optimum environment for phytase action as phytase from Aspergillus have a pH optima of 2.0 – 5.5 (Tamim et al., 2004). Phytase is lost when bound to other minerals or nutrients in the lower less acidic SI due to their strong chelating properties. Endogenous phytase could be affected by standard dietary Ca levels, which form complexes and inhibit their solubility in the SI, reducing their efficiency. Therefore, the efficacy of phytase is usually of importance because it can be easily affected by heat stability, handling and storage

procedures, and the short window of activity on digesta in the crop and proventriculus (Jacela et al., 2010). Numerous studies have shown phytase to be efficient in making P bioavailable to chickens and pigs when fed corn-soy diets or diets deficient in inorganic P and reducing its excretion into the environment (Nelson et al 1968; Schoner et al., 1991; Mroz et al., 1994; Singh et al., 2003; Yanez et al., 2013). Phytase is therefore measured by the quantity of inorganic P released from the diet. Thus, 1 phytase unit (FTU) is described as the amount of enzyme required to release 1 μmol of inorganic P/min, at pH 5.5, from sodium phytate at 37°C (International Union of Biochemistry, 1979; Dilger et al., 2004)

1.5.1. Effect of Phytase on Growth Performance of Broiler Chickens

It is well documented that phytase improves the growth performance of non-ruminants especially when fed diets deficient in P. Broz et al. (1994) described a 6.5% increase in mean BW of broiler birds fed low P diets supplemented with 500 FTU/kg of phytase during the starter phase and a 13.1% increase at the finisher phase. Broz et al. (1994) also recorded a 9.2% increase in feed intake, which explained the difference in the weight gain and an improvement in feed to gain ratio within the first 21 d. Similarly, Kornegay et al. (1996) reported improvements in BW, weight gain (by 28-48%), feed intake (30-45%), and feed efficiency (2.5-11%) in birds fed graded levels of phytase (200-1200 FTU/kg). Lei et al. (1993b), Kornegay and Qian, (1996), and Sands et al. (2001) reported improvements in average daily gain and average daily feed intake of pigs fed diets supplemented with phytase. Similar linear improvements in growth performance were observed in Pekin ducks (Orban et al., 1999; Adeola, 2010, 2018) and turkeys (Qian et al., 1996a; Atia et al., 2000) fed low-P diets supplemented with phytase.

1.5.2. Effect of Phytase on Utilization of Other Nutrients

Studies have found out that addition of phytase to P deficient diets have a positive impact on mineral utilization in birds. Reports from Olukosi et al. (2013) found that phytase elicited a linear improvement in ileal P and Ca digestibility by 26% and 15% respectively as compared with birds fed the low P control diet. Olukosi et al. (2013) reported a significant quadratic improvement on total tract retention of P and Ca, correlating with work from previous studies (Denbow et al., 1995; Kornegay et al., 1996; Tamim et al., 2004; Selle and Ravindran, 2007; Adeola, 2010; Walk et al., 2012; Coweison et al., 2014; Adeola, 2018). Similar observation was observed in pigs (Yáñez et al., 2013). Zinc is known to be affected by phytate. Contrary to Roberson and Edward (1994), supplementation of phytase improved the digestibility and retention of zinc in broilers (Biehl et al., 1995) and pigs (Nasi and Helander, 1994). Some published work report that phytase supplementation up to 1000 FTU/kg improved protein and amino acid (AA) digestibility but that further increase of phytase dosage did not elicit any further response in AA digestibility (Ravindran et al., 1999b; Ravindran et al., 2001; Dilger et al 2004; Bryden et al., 2009; Walk et al., 2012; Amerah et al., 2014). Phytase improved the apparent metabolizable energy of diets (Selle et al., 1999; Ravindran et al., 2000, 2001) further supporting that phytase mitigates some of the anti-nutritional effect of phytate in plant-based diets.

1.5.3. Effect of Phytase on Plasma Calcium and Phosphorus

Plasma P has been regarded as a good indicator of P availability in diets. Reports from various studies have reported that plasma P was increased in birds fed low P diets and supplemented with phytase. This could be due to the increased P digested and absorbed in the gastro intestinal tract with increased activity of phytase (Han et al., 2009). Plasma Ca has been documented to decrease with addition of phytase in low nPP diets. This could be due to the

increasing deposition of Ca on the bones, although results have not been consistent across studies (Sands et al., 2001; Han et al., 2009; Rousseau et al., 2012).

1.5.4. Effect of Phytase on Bone Mineralization

The bones are almost completely comprised of P and Ca, and thus serve as good sensors of their bioavailability in the animal. Therefore, it is not farfetched that with increasing release of digestible P and Ca in the tract of broilers with phytase supplementation, there is consequent increase in the deposition of hydroxyapatite on the bones. Bone strength, ash weight and percentage, and mineral concentration particularly in the tibia or toe bone are common parameters measured when phytase is supplemented in low P diets fed to birds. Studies have established that there is consistent increase in bone mineralization with increasing phytase supplementation (Waldroup et al., 2000; Pillai et al., 2006; Adeola, 2010; Rousseau et al., 2012; Walk et al., 2012)

1.5.5. Super Dosing Phytase

In theory, when phytase completely hydrolyses phytate, phytase is expected to liberate all the P bound in it; however, this is not the case for all quantities of phytase added into the diet. Industry standards suggest an inclusion level of 500 FTU/kg into the diets of chickens and pigs. This level has been established in several studies as being sufficient to induce the improvement of P utilization in broiler chickens. However, it has been shown that there is incomplete hydrolysis of phytate at that level. Increased BWG, feed intake, P digestibility and tibia ash have been consistently reported when phytase was supplemented in broiler chicken and pig diets up to 1,000 FTU/kg (Dilger et al., 2004; Walk et al., 2013), 2,000 FTU/kg (Rutherfurd et al., 2012), 4,000 FTU/kg (Han et al., 2009; Taheri and Taherkhani, 2015), 12,000 FTU/kg (Shirley and Edwards, 2003), and even 20,000 FTU/kg (Zeng et al., 2014). This suggests that if there is soluble phytate

present in the diets, phytase may continue to hydrolyze the phytate bonds and release P for use by the animal.

Cowieson et al. (2011) postulated that there could be 3 possible mechanisms responsible for the beneficial effects of super dosing observed in chickens and pigs. These include: 1. Increased destruction of phytate, which serves as an antinutritional factor. With the adverse effects of phytate on mineral, energy and protein utilization in monogastric nutrition discussed earlier, it is believed that the continuous destruction of phytate by the excess phytase included in the diet will reduce any anti nutritional effect on the animal and induce the beneficial results observed in those studies. 2. Increased liberation of phosphate and the restoration of the P and Ca ratio. 3. Release of myo-inositol with vitamin-like or lipotropic effects. Inositol has been known to prevent fatty liver syndrome and possess growth promoting factors (Lance and Hogan, 1948; Katayama, 1999). It is therefore possible that with the increased hydrolysis of phytate and the subsequent release of inositol, these ameliorative effects may be bestowed on the animals thus eliciting the responses observed with super dosing phytase. However, it is unknown if the positive response of broiler chickens to super doses of phytase may be affected by the age of the birds or other environmental factors.

1.6. Phytase Efficacy

Phytase efficacy refers to the ability of phytase to make P bioavailable and synonymous with inorganic P sources. Efficacy is usually compared with other sources of inorganic P in diets low in nPP (Augspurger, 2003), and is a good measurement of new phytase products from either bacterial or fungal sources. Many studies measure efficacy by deriving a P equivalency value, defined as the amount of inorganic P that is released by a certain amount of phytase (Kornegay,

2001). Denbow et al. (1995) describes a procedure for determining the equivalency value using regression models generated from responses of birds fed a low P diet with or without phytase supplementation. More common responses measured by researchers are P digestibility and retention (Demyr and Sekerodlu, 2002; Applegate et al., 2003), BWG and feed intake (Leeson et al., 2000; Onyango et al., 2005), plasma P and bone mineralization (Driver et al., 2005). This could be due to the high sensitivity of these parameters to the availability of P in the bird.

1.6.1. Effect of Source of Inorganic Phosphorus on Phytase Efficacy

Previous studies have reported that the source of inorganic P could influence the efficacy of phytase. Adedokun et al. (2004) observed that the P equivalency values of phytase was lower when monosodium phosphate (MSP) was used as the source of IP with 1000 FTU/kg phytase releasing 1097mg of P in the form of MSP when BW gain was considered, compared with the 1,274mg of P released by same amount of phytase when defluorinated phosphate was used by Yi et al. (1996). This could be attributed to the fact that approximately 100% of P is readily released from monosodium phosphate relative to the 90% P that is available in defluorinated phosphate. Thus, more phytase would be required to release similar quantities of P from MSP as compared with defluorinated phosphate (Soares, 1995). Similarly, Ribeiro et al. (2016) reported a P equivalency value of 2,437mg of P from a similar level of phytase when dicalcium phosphate was used as the source of IP and weight gain was considered. Although their values were higher than other reports, they attributed the possible variation to the high level of Ca from their IP source.

1.6.2. Effect of Calcium on Phytase Efficacy

Calcium has been known to influence the efficacy of phytase because high concentrations of Ca in the diet can bind to phytate to form insoluble phytate-Ca complex in the SI, reducing the hydrolyzing capacity of phytase. Some studies have reported as high as a 12% reduction in the

hydrolyzing ability of phytase when Ca levels in the diet were about 9.0g/kg (Applegate et al., 2003; Selle et al., 2009). A high Ca:P ratio can also reduce the solubility of phytase, thereby hindering its hydrolyses by phytase (Lei and Stahl, 2000; Ribeiro et al., 2016). Other studies, however, have reported negative or no impact of Ca on phytase efficacy (Manangi and Coon, 2008; Adeola and Walk, 2012).

1.6.3. Effect of other Anti-nutritional Factors on Phytase Efficacy

Some anti-nutritional factors present in diets, such as non-starch polysaccharides (NSP), may affect the break-down of phytate complex by phytase, Although, NSP concentration is higher in wheat and rye-based diets, NSP is also present in soybean and corn and can hinder the utilization of nutrients in broilers due to its viscous inducing nature in the gastro intestinal tract (Marsman et al., 1997). In the absence of carbohydrases when diets rich in NSP are fed, cell wall of the plant materials fed may prevent the access of phytase to the phytic bonds and reducing its hydrolyzing ability and its efficacy (Olukosi et al., 2007). It has been suggested that phytase efficacy may be improved if it is combined as a cocktail with other enzymes such as pectinase, protease, and carbohydrases that could break down anti-nutritional factors such as NSP or pectin, thus, facilitating its contact with phytate (Zyla et al., 1996).

1.6.4. Effect of Age on Phytase Efficacy

Studies have reported that the age of broiler chickens could affect the efficiency of exogenous enzymes, particularly in the first 2 weeks of life when rapid growth and development occurs. Due to the limited absorptive capacity and low levels of intestinal enzymes in chicks during this period, addition of exogenous enzymes in broiler diets could support the nutrient absorbing capacity of the chick, which may lower the requirement of the enzyme by the chick thus making available more energy and nutrients that can be channeled into further development of the chick

(Olukosi et al., 2007). Although limited work has been done on how age affects phytase efficacy, we can infer from the results reported by Olukosi et al. (2007) that younger birds had increased efficacy of phytase as birds in week 1 had increased P retention with phytase addition and were able to extract up to 120% more P from a P deficient diet as compared with birds in weeks 2 or 3 with lowered ability to use the enzyme. More recently, Li et al. (2018) examined the impact of age and Ca on phytase efficacy comparing the digestibilities of Ca and P of birds at d 9 and 21. They reported minor difference in the impact of phytase on P digestibility between younger and older birds but observed an age effect on Ca digestibility with younger birds having increased phytase efficacy when compared with older birds. However, because of their varying levels of Ca, it was more difficult to clearly identify the effect of age on phytase efficacy. Therefore, it is pertinent that more research be carried out to observe the true effect of age on phytase efficacy using a broader range of parameters associated with P bioavailability in broiler chickens.

1.6.5. Effect of Duration of Feeding low Phosphorus Diet on Phytase Efficacy

Limited studies have examined the effect of duration of feeding on phytase efficacy using parameters like P utilization and tibia ash. However, Li et al. (2018) suggested that feeding birds beyond 2 d could impact the digestibility of Ca and P under imbalance conditions due to homeostatic changes that could occur in the digestive and absorptive capacity of the birds. Although, no other study was found in chickens to corroborate this theory, Proszkowiec-weglarz and Angel (2013) suggested that a short term exposure to dietary P changes would involve intestinal factors that would regulate the absorption or excretion of P as compared with the hormonal factors that would be involved in the regulation of P when long term exposure to dietary P occurred. However, this observation was made in human trials and is unknown if this observation translates into broiler chickens. Therefore, more studies are required to test the effect of duration

of feeding low P diets on phytase efficacy using parameters such as growth performance, mineral utilization, and tibia ash.

1.7. Summary

In summary, this literature review describes nutrient utilization in broiler chickens, outlining some impacting factors. Phosphorus availability and utilization in chickens were discussed. An outline was also provided on phytate and its relationship with phosphorus and the effect of phytase on some parameters in non-ruminant animal nutrition, particularly in broiler chickens by reviewing results from several studies. Finally, this literature details phytase efficacy and factors that could influence this efficacy.

1.8. Objectives

The objective of this study was to evaluate the impact of age and duration of feeding low P diet on phytase efficacy in broiler chickens during the starter phase. Two studies were designed to achieve the objective. A broad age and duration of feeding groups were used in the first study to evaluate this impact using growth performance, nutrient utilization, bone mineralization and blood parameters. Based on results from the first study, the age/ duration of feeding groups was narrowed down for examination in the second study.

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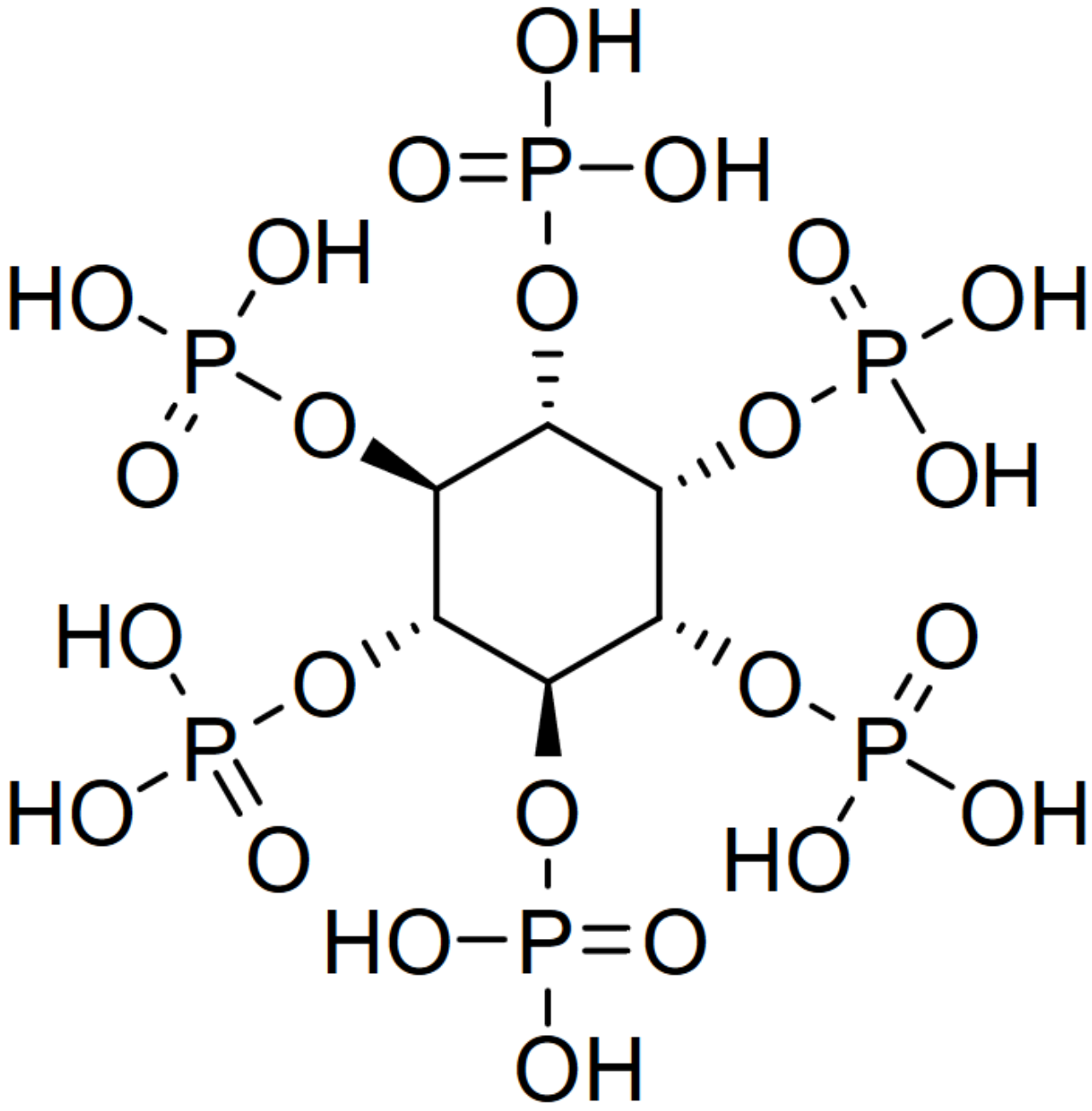

Figure 1-1 Structure of Phytate

Source: Wikipedia (https://upload.wikimedia.org/wikipedia/commons/4/45/Phytic_acid.svg).

CHAPTER 2. INFLUENCE OF AGE AND DURATIONOF FEEDING ON PHYTASE EFFICACY IN BROILER CHICKENS DURING THE STARTER PHASE

2.1. Abstract

A total of 1,408 male broiler chickens were used to evaluate the impact of age and duration of feeding low phosphorus (P) diet on the efficacy of phytase using growth performance, nutrient utilization, tibia ash and plasma indices. Diets were formulated with 2 non-phytate P (nPP) concentrations (i.e., 0.20 and 0.40%) and two phytase concentrations (i.e., 1,000 and 2,000 FTU/kg) added to the 0.20% nPP diet. Four dietary treatments with 8 replicate cages each were fed to broiler chicks at different ages and for different durations. Specifically, these were d 6 to 8 (12 birds per replicate), d 12 to 14, d 9 to 14, d 20 to 22 or d 6 to 22 (8 birds per replicate). Measurements were taken on the last day of each period. Effect of duration of feeding was examined by comparing responses of birds fed for 2 or 5 d at d 14 and for 2 or 16 d at d 22. Age effect was determined by comparing responses of birds fed for 2 d at age 8, 14 and 22 d post hatching. Body weight gain and gain-to-feed ratio were increased ($P < 0.01$) in birds fed diets supplemented with phytase. However, an increase in duration of feeding improved ($P < 0.01$) gain-to-feed ratio with birds fed for 16 d performing better than birds fed for 2 d and 5 d. In addition, phytase supplementation improved ($P < 0.01$) apparent P and calcium (Ca) digestibility and retention but the age effect on phytase efficacy was more apparent at d 14 and the duration of feeding effect was evident in birds fed for 2 d due to the increased levels of mineral utilization at that age/duration of feeding as compared with the other groups. Results of this study observed that phytase efficacy was at optimum in birds fed low P diet for 2 d at d 14. This period can be recommended for further bio-efficacy studies of phytase.

Key words: age, calcium, duration of feeding, phosphorus, phytase efficacy

2.2. Introduction

Phytate is a natural form of P storage found in many plant seeds including those used in animal nutrition, but it is poorly digested by non-ruminants without supplemental phytase (Pallauf and Rimbach, 1997). Phytate forms strong chelates with cations such as Ca^{2+} or Cu^{2+} in the small intestines, thus, forming insoluble complexes that hinder the efficiency of endogenous phytase present in the gut of poultry and pigs and reduce their ability to release P from plants sources (Orban et al., 1999; Selle et al., 2009; Li et al., 2016). Microbial phytase as a dietary additive was first evaluated by Nelson et al. (1971), during that time, the production and practical use of this enzyme was cost prohibitive to compete against inorganic phosphates. Following, an increasing awareness regarding environmental pollution from undigested P in the manure of animals (Orban et al., 1999), studies on phytase, especially in poultry species, has been carried out to improve nutrient efficiency and reduce mineral and nitrogen wastes through animal manure (Orban et al., 1999; Angel et al., 2002; Selle and Ravindran, 2007; Adeola and Cowieson, 2011). The addition of phytase to diets containing grains and oilseed meals releases P from the phytate complex, thus increasing P availability in non-ruminant animals (Mohammed et al., 1991; Coelho and Kornegay, 1996; Akinmusire and Adeola, 2009).

Previous studies have shown that age might have an impact on the utilization of nutrients in chicks especially during the first 2 wk because rapid development and growth of organs and tissues occur during this period (Nitsan et al., 1991; Batal and Parsons, 2002). Duration of feeding has been known to affect true ileal digestibility of P (Perryman et al., 2017), as longer feeding duration may result in broilers adapting to low P diets to maintain P homeostasis resulting in higher digestibility of P (Yan et al., 2005). Limited work has been done to observe how the age of birds

and duration of feeding may affect data obtained from the supplementation of phytase to broilers during the starter phase. Thus, the objective of this study was to examine the influence of both factors on phytase efficacy.

2.3. Materials and Methods

Protocols of animal experiments were reviewed and approved by the Purdue University Animal Care and Use Committee.

2.3.1. Birds, Diets, and Experimental Design

A total of 1,408 male broiler chicks (Cobb 500, Siloam Springs, AR.) were obtained from a commercial hatchery. The mean BW at d 0 after hatching was 44 g. Each bird was weighed individually, tagged with identification numbers, and raised in heated battery brooders (model SB 4 T; Alternative Design Manufacturing, Siloam Springs, AR) with temperatures maintained as previously described by Park et al. (2017). They had unlimited access to water via nipple drinkers, and were fed a commercial starter diet formulated with 225g/kg CP, 3,028 kcal/kg ME, 14g/kg digestible lysine (Lys), 10g/kg Ca, and 4.8g/kg non-phytate P (nPP), that met or exceeded the nutrient requirements of growing broiler chicks (NRC, 1994) before introduction of the experimental diets. On d 6 post-hatching, birds were weighed and divided into five groups comprising 5 different age and duration of feeding combinations. Four diets, which comprised a positive control (**PC**) diet (0.40% nPP), and three negative control (**NC**) diets (0.20% nPP) with phytase concentrations of 0, 1,000, or 2,000 phytase units (FTU)/kg were formulated (Table 2-1). Ingredient composition of diets were similar between PC and NC diets except for limestone and monocalcium phosphate levels which were adjusted to obtain high and low concentrations of P. A phytase premix was prepared with phytase (RONOZYME® HiPhos, DSM Nutritional Products,

Switzerland) and ground corn to contain 50 FTU/g of corn and added at 20 or 40 g/kg to the NC diets. One FTU is defined as the activity that releases 1 µmol of inorganic phosphate from 5.0mM sodium phytate per minute at pH 5.5 and 37˚C (Olukosi et al., 2013). Chromic oxide was incorporated at 5g/kg into the diets as an indigestible marker to determine ileal digestibility and total tract retention by index method as described by Iyayi et al. (2013). Two groups were placed on the experimental diets on d 6 post-hatching and were fed until d 8 and d 22, respectively. Three other groups were fed the starter diet until the beginning of their experimental periods at d 9, d 12, and d 20 respectively and were fed until d 14, 14, and 22 respectively. A randomized complete block design was used for this study with birds individually weighed and blocked for BW to ensure average weight across the diets. The initial BW served as the blocking factor for the entire 20 treatments with 8 replicate cages per treatment, 12 birds per cage for one group and 8 birds per cage for the other groups.

2.3.2. Collection of Samples and Chemical Analyses

Feed consumption by cage and individual BW was recorded at the end of each feeding period. Excreta were collected twice daily before the end of each experimental period from pans placed under the cages and dried in a forced air oven at 56˚C for 7 d. On d 8, 14, and d 22 post-hatching, birds were euthanized by CO_2 asphyxiation and dissected to collect ileal digesta from the distal two-thirds of the ileum which is located between the Meckel's diverticulum and the ileocecal junction (Li et al., 2015). Ileal digesta samples were obtained by flushing the ileal content with distilled water into plastic containers, pooled by cage, and stored at −20˚C until they were lyophilized (Adeola and Walk, 2012). Blood samples were collected from the median weight bird in each cage by cardiac puncture using EDTA tubes, and plasma was obtained by centrifugation at 3,000 x g for 15 min at 4˚C (Jiang et al., 2013) and stored at −80˚C until further analyses. The

left tibia from 4 birds which had the closest BW to median per cage were collected. Collected bones were defatted using a Soxhlet extractor, weighed, and ashed in a muffle furnace at 600˚C for 24 h to determine bone ash (Ogunwole et al., 2017). Dried ileal digesta and excreta samples were ground to pass through a 0.5mm screen (Retsch ZM 100, GmbH, Haan, Germany). Dry matter was determined by placing the samples in a drying oven for 24 h at 105˚C (The Precision Scientific Co., Chicago, IL; method 934.01; AOAC, 2006). Calcium, P, and Cr concentrations were determined in the diets, digesta and excreta samples following nitric and perchloric acid wet-ash digestion (Fenton and Fenton, 1979). Cr and P concentrations were estimated by spectrophotometry and the absorbance read at 450 nm (Spectronic 21D; Milton Roy Co., Rochester, NY). Calcium concentrations in the digested samples were determined by flame atomic absorption spectroscopy using a Varian Spectr.AA 220FS (Varian Australia Pty Ltd., Victoria, Australia; Iyayi et al., 2013). Phytase activity was determined using methods described by Engelen et al. (1994). Plasma P, Ca, glucose, alkaline phosphatase, uric acid, urea and myo-inositol were analyzed by spectrophotometry using an ADVIA 1650 chemistry system (Bayer diagnostic, Puteaux, France).

2.3.3. Calculation and Statistical Analysis

The index method was used to calculate the apparent ileal digestibility (AID) and retention of P in the diets by the following equation:

$$\text{AID or retention (\%)} = 100 - [(Cr_I/Cr_O) \times (N_O/N_I) \times 100]$$

Cr_I is Cr concentration in the diets, Cr_O is the Cr concentration in the excreta or ileal digesta, N_O is the concentration of a nutrient in the ileal digesta or excreta, and N_I is the concentration of a nutrient in the diets. The concentration of Cr and nutrients in this equation was expressed as grams per kilogram of DM.

Data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC.) as a 4 × 5 factorial treatment arrangements with 4 diets, 5 ages with duration of feeding, their interaction and block as independent variables. The cage of birds was used as the experimental unit for all analyses. Responses of graded level of phytase at 0, 1,000, and 2,000 were examined using contrasts of PC vs. NC diets, and linear and quadratic contrast. Contrast within duration of feeding among birds fed from d 6 to 8, 12 to 14, and 20 to 22 post-hatching (i.e., 2 d duration of feeding) was used to test the effect of age regardless of duration of feeding. Birds fed from d 12 to 14 and 9 to 14 post-hatching regardless of diets were compared with to analyze the duration of feeding effect at d 14 post-hatching and birds fed from d 20 to 22 and 6 to 22 post-hatching were also compared with to analyze the duration of feeding effect at d 22 post-hatching.

Phytase efficacy was calculated by subtracting the nutrient digestibility of birds fed the negative control diet from the digestibility of birds on the 1,000 and 2,000 FTU/kg phytase supplemented diets. Statistical significance set at $P \leq 0.05$.

2.4. Results

Analyzed nutrients and phytase activity in the diets were within acceptable ranges when sampling and assay variations were considered except for dietary Ca, which was approximately 44% greater than calculated concentration and it may be due to addition of limestone as a flow agent in soybean meal and as a carrier in the vitamin and mineral premix (Table 2-1). Diets supplemented with phytase were formulated to contain 1,000, or 2,000 units/kg and analyzed at 1,266 and 2693 units/kg respectively.

Growth performance responses of broiler chickens to diets are presented in Table 2-2. Birds that received the NC diet for longer durations had lower weight gain and feed intake than those that received the NC diet for shorter durations whereas the opposite was the case for groups that

received the PC diet, resulting in a diet*duration interaction ($P < 0.01$). Birds fed the phytase supplemented NC diets at earlier ages and for shorter periods had improved feed efficiency, feed intake and weight gain. In birds fed the diets for 2 d, the decrease in nPP concentrations decreased ($P < 0.01$) BW (5.4, 5, and 0.6%), feed intake (18.9, 20.7 and 3.8%), and feed efficiency (11.8, 10.7 and 2.3%) in birds at d 8, 14 and 22 respectively. Addition of 1,000 and 2,000 FTU/kg of phytase, linearly improved ($P < 0.01$) BW at d 8, d 14, and d 22 respectively. A similar observation was recorded in BW gain (Figure 2-1 A) and feed intake of birds. At d 14, the decrease in nPP concentrations reduced ($P < 0.01$) BW gain (20.7 and 14.1%) and feed efficiency (10.7 and 2.9%) of birds exposed to the diets for 2 d and 5 d respectively. With addition of phytase at 1,000 and 2,000 FTU/kg, BW gain of birds was improved at 2 d and 5 d duration of feeding respectively (Figure 2-1 B). Birds fed phytase supplemented diets for 2 d had increased feed efficiency than birds fed for 5 d. However, the reverse was the case with feed intake, as improvements were decreased in 2 d duration as compared with 5 d with addition of phytase. At d 22, the negative impact of decreased nPP concentration on BW gain was higher in birds fed diet for 16 d (28%; $P < 0.01$) as compared with birds on the diet for 2 d (3.8%). A similar observation was recorded for feed intake and feed efficiency. Birds that recieved phytase-supplemented low nPP diets for 16 d had improved ($P < 0.01$) weight gain (Figure 2-1 C), feed consumption and feed efficiency as compared with birds exposed for 2 d.

Birds that received low-P, phytase-supplemented diets over a longer duration had greater ($P < 0.01$) tibia ash than birds fed the diets for a shorter period. There was a reduction in tibia ash of birds fed the NC diet regardless of age and duration of feeding when compared with birds fed the PC diet and a linear and quadratic increase in tibia ash ($P < 0.01$) with inclusion of phytase (Table 2-2). Birds exposed to the NC diets for 2 d had a reduction in tibia ash by (4.1, 2.7 and 2%)

at d 8, 14 and 22 respectively when compared with birds fed the PC diet. However, the addition of 1,000 and 2,000 FTU/kg of phytase to the NC diets improved the tibia ash by at least 3% ($P < 0.01$) across the ages when compared with birds fed the NC diet. At d 14, birds fed the NC diet for 5 d had an 8% reduction in tibia ash when compared with birds fed the PC diets, as compared with the 2.7% reduction in birds fed for 2 d. Thus, the impact of the low nPP concentration was observed in birds fed for a longer duration. The addition of phytase increased the tibia ash of birds fed for both 2 d and 5 d. A similar result was observed at d 22 with birds fed the low P diet for 16 d having an increased negative impact on tibia ash as compared with birds fed for 2 d, however, addition of phytase at both concentrations improved the tibia ash in both groups of birds but more so in birds fed for 16 d.

There was a decrease ($P < 0.01$) in the apparent ileal digestibility (AID) of P and Ca in birds fed the NC diet as compared with birds fed the PC diet across all the age and duration of feeding periods (Table 2-3). However, the difference in the AID of P in birds fed the PC and NC for 2 d was 20% higher at d 14, 12% higher at d 8 or 7.6% higher at or 22 (Figure 2-2 A). At d 14 and 22, the difference in AID of P in birds on the PC and NC diets was higher in birds fed for 2 d (20%, 7.6%) as compared with birds on the diets for 5 d (10.6%) or 16 d (4.7%) respectively (Figure 2-2 B, and 2 C). Supplementation of the NC diet with both levels of phytase linearly improved ($P < 0.01$) the AID of P and Ca across all exposure periods. Phytase efficacy was best at d 14 because the greatest improvement on AID of P and Ca was observed in birds exposed to phytase supplemented NC diet for 2 d and 5 d as compared with birds fed only the NC diet (Figure 2-3 A to 3 C). Birds fed for 2 d had increased Ca digestibility as compared with birds fed for 5 d when phytase was supplemented in the NC diet (Table 2-4). This however was not the case at d 22, as birds exposed to the phytase-supplemented diet at both levels for a longer duration (16 d)

had better Ca digestibility than birds fed for a shorter duration (2 d). A similar trend occurred with P retention across all duration of feeding period, there was an interaction effect between diet and duration of feeding on the phytase efficacy in P retention. Birds exposed to phytase supplementation for 2 d at d 14 resulted in the highest improvement in P retention and birds on phytase supplementation for 16 d at d 22 had the least improvement (Table 2-4). Total tract retention of Ca in birds exposed for 2 d to diets supplemented with phytase at d 8 had increased phytase efficacy as compared with birds at 14 d and 22 d. However, birds fed the phytase supplemented diets for 5 d at d 14 had increased Ca retention when compared with birds on the diets for 2 d at d 14. The reverse was the case at d 22 as birds fed the diets for a shorter period (2 d) had increased Ca retention than birds fed for a longer period of 16 d.

Plasma metabolite concentration responses of birds fed the experimental diet are presented in Table 5. There was a linear increase ($P < 0.01$) in myo-inositol levels among the phytase supplemented diets within each group but there was little to no difference among the different duration of feeding groups (Figure 2-4 A to 4 C). Plasma P levels were lower ($P < 0.01$) in birds fed the NC diet than in birds fed the PC diet but greater ($P < 0.01$) for Ca levels in NC diet than PC diet. There was a linear and quadratic increase ($P < 0.05$) in plasma P as phytase was supplemented to the NC diet up to 2,000 units/kg diet. A higher plasma P ($P < 0.01$) was however observed in birds at d 22 exposed to the phytase supplemented diet for 2 d as compared with those exposed to the diets for 16 d. There was an interaction effect ($P < 0.01$) of diet and duration of feeding in plasma Ca levels as well as a quadratic decrease in Ca levels as NC diet was supplemented with phytase. There was no effect of diets on plasma alkaline phosphatase, glucose, uric acid, and urea. However, there was an effect of duration of feeding on these parameters as

birds fed diets for a shorter period (2 or 5 d) had increased average responses than birds fed for a longer period (16 d).

2.5. Discussion

The influence of phytase on growth performance, nutrient utilization, bone mineralization and plasma indices when birds were fed with P deficient diets have been established and results from the current study is in agreement with other studies (Orban et al., 1999; Dilger et al., 2004; Onyango et al., 2005; Paiva et al., 2014) regardless of the age of birds and duration of feeding. The age effect on phytase efficacy was determined by comparing the responses of birds fed the experimental diets for 2 d at d 8, 14 and 22. This feeding duration was important because observations from previous studies report that the effects of feeding Ca or P deficient diets for more than 48 h on AID of P and Ca could be confounded by homeostatic changes or adaptations in the digestive and absorptive ability of birds (Proszkwoweic-Weglarz and Angel, 2013; Li et al., 2018). Thus, feeding for 48 h could potentially reveal the true dietary impact of the low P diet and the efficacy of phytase at the different ages. Phytase efficacy was determined by comparing the response of birds derived from the numerical difference between responses from birds fed the low P diet with phytase supplementation from birds fed the NC and this was similar to methods from previous studies (Li et al., 2015, 2018). Utilization of P is usually a good indication of performance of birds especially using P digestibility, and retention, tibia ash and plasma P. It was observed in the current study that the difference in total tract retention of P between birds fed PC diet and birds fed the NC diet was more evident at d 14. This could be due to the fact that birds at this age have fairly developed gastro-intestinal tracts and gut enzymes. An inability of birds to fully hydrolyze phytate when fed the low P diet is evident as compared with birds at d 8 with underdeveloped gut or at d 22 where birds had developed mechanisms for P utilization and thus could adapt their gut

enzymes to utilize P more adequately in the low P diets. With addition of phytase, this theory could hold true as phytase was more efficacious at d 14 among birds fed the diets for 2 d. Amerah et al. (2007) reported that increased residence time in the upper gut improved digestion and absorption of nutrients, thus there is a possibility that birds fed at d 8 had shorter residence time in the gut and could not make use of the enzymes as effectively as birds at d 14 which had a relatively longer residence period. However, the efficacy of phytase in birds at d 22 was lower as compared with birds at d 14 and this could be because these birds had been on a commercial starter diet until d 20 and thus the effect of the low P diet at this age for a relatively brief time was not as severe. Consequently, the improvement due to phytase was not drastic even though birds had longer residence period in the gut. A similar pattern was observed for Ca with improvements in AID and retention of Ca which was in accordance with findings from previous studies (Onyango et al., 2005; Iyayi et al., 2013; Olukosi et al., 2013). Although limited studies have reported the age effect of phytase on AID of Ca, our findings were in accordance with Li et al. (2018), with phytase having more impact on Ca in birds at d 8 and 14 as compared with d 22 particularly when birds where fed phytase at 2,000 FTU/kg.

Duration of feeding effect was determined by comparing responses of birds fed the experimental diets for 2 d and 5 d until d 14 and for 2 d and 16 d until d 22. Results revealed that birds fed for 2 d had greater phytase efficacy for P retention than birds fed for 5 d and 16 d at d 14 and d 22 respectively. This further supported the theory that responses from birds fed beyond 48 h could be subject to homeostatic adaptations in the digestive ability of the birds (Li et al., 2015, 2018) as it could be that the birds had adapted to the low P concentration of the diet over the longer duration of feeding. Our results were in accordance with Li et al. (2015), who reported that birds fed low P diet supplemented with phytase at 500 FTU/kg for 2 d showed a higher phytase efficacy

on CP and AA digestibility than birds fed for 14 d at the same age. However, they reported no differences in the phytase efficacy when a higher dose of phytase was fed to the birds.

Predictably, the low P diet reduced tibia ash and thus bone mineralization as compared with birds fed the PC diet. Supplementation of phytase improved tibia ash regardless of age or duration of feeding, this was similar to observations from previous studies (Broz et al., 1994; Denbow et al., 1995; Dilger et al., 2004; Olukosi et al., 2013; Adeola, 2018). This is because P in addition to Ca are a component of hydroxyapatite in the bone and 80% of P found in the body is located in the bone, thus, insufficient P from low P diets will have a negative impact on bone formation and mineralization except in the presence of endogenous enzymes like phytase (Olukosi et al., 2013). However, in this study, there was no age effect on phytase efficacy on tibia ash as birds at d 8, 14 and 22 had similar improvements with phytase supplementation. However, there was a duration of feeding effect as birds fed for 5 d and 16 d at d 14 and d 22 respectively had a greater efficacy of phytase on tibia ash than birds fed for 2 d at the same age. This could be due to continuous deposition of P and Ca on the bones during this developmental phase (Dilger et al., 2004) from the prolonged exposure to the ameliorative effects of phytase in the low P diet. Tibia ash may reflect a cumulative process and may not react as quickly to changes in dietary P as observed in the ileal digestibility of P.

Plasma P has been identified as a good indicator of phytase efficacy (Aureli et al., 2011). Similarly with AID of P, plasma P increased with supplementation of phytase as compared with birds fed the NC regardless of age or duration of feeding due to the hydrolysis of phytate-P by phytase to release P into the bloodstream (Sebastian et al., 1996). Plasma Ca however, decreased with increasing phytase levels, which is similar to reports from Sebastian et al. (1996) and Viveros et al. (2002) but in contrast to reports from Jiang et al. (2013), who reported increasing plasma Ca

levels with supplementation of phytase and Aureli et al. (2011) who observed no effect of phytase on plasma Ca levels. There was no age effect on plasma P but there was a duration effect at d 22 with birds fed for 2 d having a higher average plasma P concentration than birds fed for 16 d, which could be due to effects already discussed with the duration of feeding effect on AID P. Plasma myo-inositol increased with increasing dietary phytase across all age and duration of feeding groups. This could be due to the partial dephosphorylation of dietary phytate by phytase in the gastric phase of the upper intestinal tract, thus, the absorption of the resulting esters in the small intestine are found in the blood where they can be further dephosphorylated into myo-inositol and free P (Cowieson et al., 2011). However, there was no effect of age or duration of feeding on plasma myo-inositol. Cowieson et al. (2017) observed that the reactivity of myo-inositol to dietary changes occur within hr when phytase supplemented diets were fed to pigs. This could possibly explain why there was no effect of duration of feeding on plasma myo-inositol as our experimental periods were in d. It is therefore possible that we may observe an effect of age or duration of feeding on plasma myo-inositol concentrations if time is changed from days to hours (i.e., 2 or 5 d vs. 16 h). Phytase did not affect plasma glucose, alkaline phosphatase, and uric acid and this was consistent with results from Aureli et al. (2011) and Safamehr and Attarhoseini (2011).

No negative effect of dosing birds with phytase at 2,000 FTU/kg on growth performance, nutrient utilization, bone mineralization and plasma indices were observed in the current study. There were improvements in responses greater or similar to birds on the PC diet and this was consistent with reports from previous studies (Cowieson et al., 2011; Taheri and Taherkhani, 2015; Adeola, 2018). The age and duration of feeding effect of super dose of phytase on mineral utilization followed similar trends with earlier discussion, however, the highest impact of the super

dosing on phytase efficacy on mineral utilization was observed in birds fed for 2 d at d 14. Thus, feeding for 2 d at d 14 would be valuable in testing the efficacy of phytase as the short duration of feeding avoids the confounding effects of the homeostasis and metabolism of the broiler chicken especially during a phase of prime growth and development.

2.6. References

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Table 2-1: Ingredients and nutrient composition of experimental diets for broiler chicken

Item	Positive Control	Negative Control	Phytase 1,000 FTU/kg	Phytase 2,000 FTU/kg
Ingredients, g/kg				
Corn	525.6	531.1	511.1	491.1
Soybean meal, 480g/kg CP	356.0	356.0	356.0	356.0
Soybean oil	50.0	50.0	50.0	50.0
Monocalcium phosphate	13.3	3.8	3.8	3.8
Limestone	15.3	19.3	19.3	19.3
Salt	4.0	4.0	4.0	4.0
Vitamin-mineral premix[1]	3.0	3.0	3.0	3.0
DL-Methionine	3.8	3.8	3.8	3.8
L-Lysine·HCL	2.9	2.9	2.9	2.9
L-Threonine	1.1	1.1	1.1	1.1
Chromic oxide premix[2]	25.0	25.0	25.0	25.0
Phytase premix[3]	0	0	20	40
Total	1,000.0	1,000.0	1,000.0	1,000.0
Calculated nutrients and energy, g/kg				
CP	224.8	225.3	225.3	225.3
ME, kcal/kg	3209.7	3228.8	3228.8	3228.8
Ca	9.0	9.0	9.0	9.0
P	6.5	4.5	4.5	4.5
Non-phytate P,	4.0	2.0	2.0	2.0
Analyzed nutrients, g/kg				
DM	890	884	887	893
Ca	13.1	13.9	13.5	13.4
Total P	7.5	5.4	5.3	5.4
Phytate P	3.0	2.8	2.8	2.9
Phytase activity, FTU/kg	<100	<100	1266	2693

[1]Supplied the following quantities per kg of diet: vitamin A, 5,484 IU; vitamin D_3, 2,643 ICU; vitamin E, 11 IU; menadione sodium bisulfite,4.38 mg; riboflavin, 5.49 mg; D-pantothenic acid, 11 mg; niacin, 44.1 mg; choline chloride, 771 mg; vitamin B_{12}, 13.2 µg; biotin, 55.2 µg; thiamine mononitrate, 2.2 mg; folic acid, 990 µg; pyridoxine hydrochloride, 3.3mg; I, 1.11 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; Se, 300 µg

[2]Prepared as 1 g chromic oxide added to 4 g corn.

[3]Prepared with ground corn to contain 50 FTU per g corn.

Table 2-2: Effect of phytase and age with different duration of feeding on growth performance and tibia ash contents of broiler chickens[1]

Item	Diet[2]	Final BW (g)	BW gain, g/bird	Feed intake, g/bird	G:F, g/kg	Tibia Ash, %
d 6-8	PC	202	58	70	836	46.2
	NC	191	47	64	737	42.1
	1,000	200	56	69	812	44.5
	2,000	203	59	71	825	45.0
d 12-14	PC	487	116	148	788	47.6
	NC	463	92	131	704	44.9
	1,000	473	103	136	754	47.3
	2,000	480	110	139	792	47.8
d 9-14	PC	478	240	295	814	47.3
	NC	444	206	261	790	39.1
	1,000	464	226	273	829	45.0
	2,000	470	232	277	836	46.0
d 20-22	PC	1006	158	236	672	49.4
	NC	1,000	152	232	656	47.4
	1,000	1002	154	236	661	48.8
	2,000	1005	156	237	666	49.3
d 6-22	PC	935	798	984	813	48.4
	NC	715	574	821	698	33.7
	1,000	881	743	972	765	45.0
	2,000	925	781	976	800	46.8
SEM		12.4	12.7	11.7	23.1	0.42
***P*-values**						
Diet		< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Duration of feeding		< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Diet x duration of feeding		< 0.01	< 0.01	< 0.01	0.65	< 0.01
PC vs. NC		< 0.01	< 0.01	< 0.01	0.861	< 0.01
Linear		< 0.01	< 0.01	< 0.01	1.00	< 0.01
Quadratic		0.03	0.03	< 0.01	0.29	< 0.01
d 6-8 vs. 12-14 vs. 20-22		< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
d 12-14 vs. 9-14		< 0.01	0.19	< 0.01	< 0.01	< 0.01
d 20-22 vs. 6-22		< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

[1]Data are means of 8 replicate cages

[2]Diets PC = Positive Control, NC = Negative Control, 1,000 = NC + 1,000 phytase units/kg, 2,000 = NC + 2,000 phytase units/kg

Table 2-3: Effect of phytase and age with different duration of feeding on nutrient digestibility and retention responses of broiler chickens[1]

Item[2]	Diet[3]	AID DM, %	AID P, %	AID Ca, %	TTR DM, %	TTR P, %	TTR Ca, %
d 6-8	PC	67	47.4	44.3	73.4	49.5	40.8
	NC	36	35.3	40.2	73.2	35.4	22.1
	1,000	66	45.5	42.0	71.1	56.1	38.1
	2,000	68	54.8	49.5	72.9	62.6	40.6
d 12-14	PC	71	53.3	49.9	71.7	48.1	43.5
	NC	68	35.0	42.9	72.2	32.4	24.3
	1,000	67	49.8	50.5	72.1	52.7	38.2
	2,000	72	64.1	55.7	71.7	63.0	40.8
d 9-14	PC	70	52.0	50.9	71.7	48.7	41.9
	NC	68	41.4	47.1	72.0	41.5	20.9
	1,000	68	55.5	52.5	71.2	59.6	36.0
	2,000	72	65.6	59.1	71.1	67.7	40.6
d 20-22	PC	72	50.4	43.9	71.5	43.3	39.0
	NC	69	42.6	40.9	70.8	32.0	30.5
	1,000	72	55.2	47.1	73.0	57.5	42.7
	2,000	75	65.3	51.0	71.9	56.8	45.9
d 6-22	PC	73	53.4	52.8	71.9	51.2	42.2
	NC	70	48.9	49.6	72.4	51.5	39.1
	1,000	73	66.9	55.2	72.7	70.5	43.9
	2,000	73	69.6	61.4	73.0	74.0	45.4
SEM		0.96	0.03	0.02	0.80	0.01	0.01
***P* values**							
Diet		< 0.01	< 0.01	< 0.01	1.00	< 0.01	< 0.01
Duration of feeding		< 0.01	< 0.01	< 0.01	0.24	< 0.01	< 0.01
Diet x duration of feeding		0.43	0.15	0.99	0.54	< 0.01	< 0.01
PC vs. NC		< 0.01	< 0.01	< 0.01	0.86	< 0.01	< 0.01
Linear		< 0.01	< 0.01	< 0.01	1.00	< 0.01	< 0.01
Quadratic		0.09	0.08	0.83	0.80	< 0.01	< 0.01
d 6-8 vs. 12-14 vs. 20-22		< 0.01	< 0.01	0.46	0.16	< 0.01	< 0.01
d 12-14 vs. 9-14		0.78	0.10	0.13	0.45	< 0.01	0.09
d 20-22 vs. 6-22		0.35	< 0.01	< 0.01	0.24	< 0.01	< 0.01

[1]Data are means of 8 replicate cages

[2]AID = apparent ileal digestibility, TTR = total tract retention

[3]Diets PC = Positive Control, NC = Negative Control, 1,000 = NC + 1,000 phytase units/kg, 2,000 = NC + 2,000 phytase units/kg

Table 2-4: Effect of age with different duration of feeding on phytase efficacy in mineral utilization and tibia ash of broiler chickens[1]

Item[2]	Phytase Inclusion[3]	AID P, %	AID Ca, %	TTR P, %	TTR Ca, %	Tibia Ash, %
d 6-8	1,000	11.2	1.8	19.6	13.6	2.4
	2,000	19.5	9.2	26.0	16.1	2.9
d 12-14	1,000	14.8	7.6	20.6	13.3	2.4
	2,000	29.1	12.8	30.7	16.5	2.9
d 9-14	1,000	14.1	5.4	14.5	7.2	5.9
	2,000	24.1	12.0	22.6	11.8	6.8
d 20-22	1,000	14.5	4.4	25.5	12.2	1.4
	2,000	21.0	6.7	23.0	15.6	1.9
d 6-22	1,000	18.1	4.3	18.9	4.8	11.3
	2,000	19.6	10.7	22.5	6.3	13.1
SEM		3.30	3.22	2.28	3.04	0.51
***P* values**						
Diet		< 0.01	< 0.01	< 0.01	0.12	0.01
Duration of feeding		0.40	0.54	0.02	< 0.01	< 0.01
Diet x Duration of feeding		0.41	0.94	0.07	0.99	0.64

[1]Data were obtained by subtracting responses of birds on NC diet from responses of birds on NC + 1000 or NC + 2000 FTU/kg phytase supplemented diets in each of 8 blocks of cages
[2]AID = apparent ileal digestibility, TTR = total tract retention
[3]Phytase 1,000= NC + 1,000 phytase units/kg, 2,000= NC + 2,000 phytase units/kg

Table 2-5: Effect of phytase and age with different duration of feeding on plasma metabolite concentrations of broiler chickens[1]

Item	Diet[2]	Myo-inositol, µmol/L	P, mg/dL	Ca, mg/dL	Alkaline Phosphatase, U/L	Glucose, mg/dL	Uric Acid, mg/dL
d 6-8	PC	182	7.8	13.5	30209	476	14.1
	NC	193	5.8	16.5	31056	457	16.1
	1,000	221	6.5	15.9	25447	452	15.9
	2,000	288	7.8	14.6	25812	514	18.4
d 12-14	PC	190	8.0	11.2	5572	444	11.9
	NC	211	6.0	13.2	5621	434	11.4
	1,000	254	8.4	12.3	6191	530	12.4
	2,000	267	7.5	11.8	4980	440	8.9
d 9-14	PC	158	7.5	13.2	16643	555	14.9
	NC	205	4.7	15.5	19125	499	13.3
	1,000	249	7.3	14.1	16634	494	14.5
	2,000	278	8.0	13.9	21352	532	16.4
d 20-22	PC	199	8.4	12.5	25893	588	15.6
	NC	170	6.0	15.9	17827	544	15.7
	1,000	276	6.8	12.6	16259	503	13.9
	2,000	283	8.0	13.0	19978	603	16.5
d 6-22	PC	149	7.1	11.0	11085	413	10.2
	NC	213	4.1	16.0	10107	488	10.6
	1,000	228	6.7	13.2	6696	418	10.7
	2,000	301	7.3	11.6	5759	411	11.5
SEM		27.4	0.56	0.48	2533.12	41.51	1.17
***P* values**							
Diet		< 0.01	< 0.01	< 0.01	0.13	0.85	0.49
Duration of feeding		0.98	0.04	< 0.01	< 0.01	< 0.01	<0.01
Diet x duration of feeding		0.86	0.54	0.03	0.43	0.48	0.19
PC vs. NC		0.19	< 0.01	< 0.01	0.48	0.67	0.89
Linear		< 0.01	< 0.01	< 0.01	0.47	0.55	0.22
Quadratic		0.76	0.04	< 0.01	0.17	0.58	0.51
d 6-8 vs.12-14 vs. 20-22		0.58	0.22	< 0.01	< 0.01	0.46	<0.01
d 12-14 vs. 9-14		0.68	0.29	0.04	0.39	0.18	0.43
d 20-22 vs. 6-22		0.63	< 0.01	0.02	0.12	0.31	0.63

[1]Data are means of 8 replicate cages

[2]Diets PC = Positive Control, NC = Negative Control, 1,000 = NC + 1,000 phytase units/kg, 2,000 = NC + 2,000 phytase units/kg

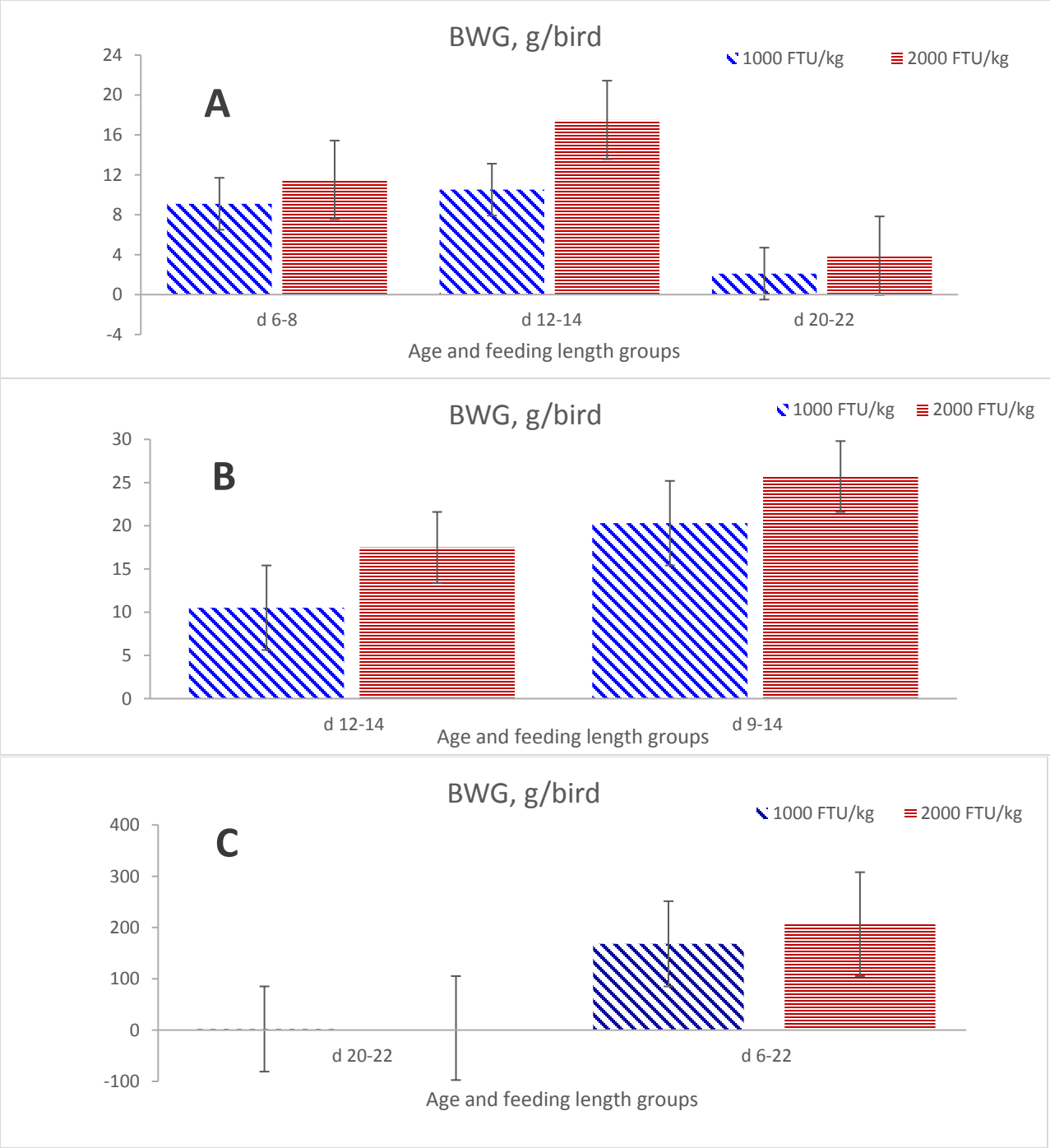
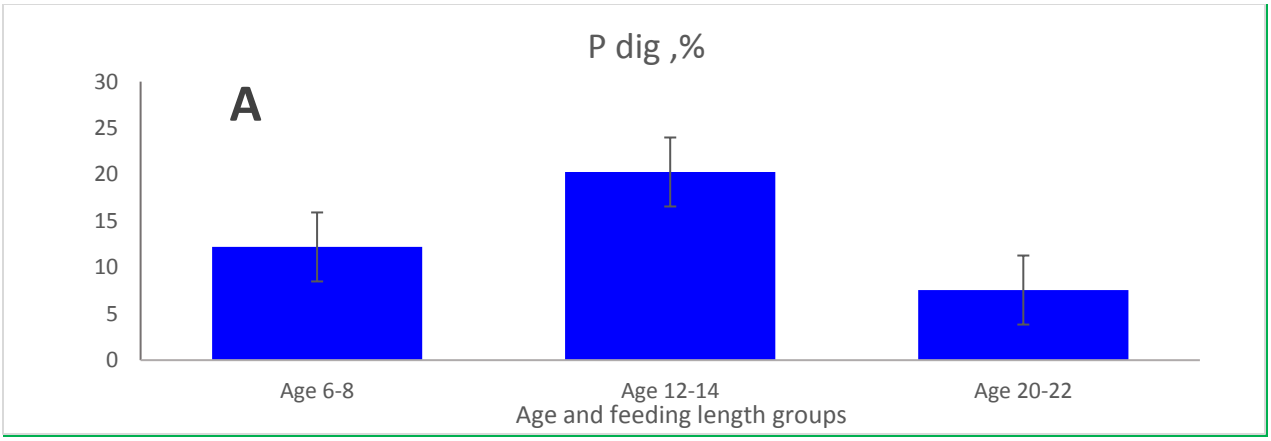
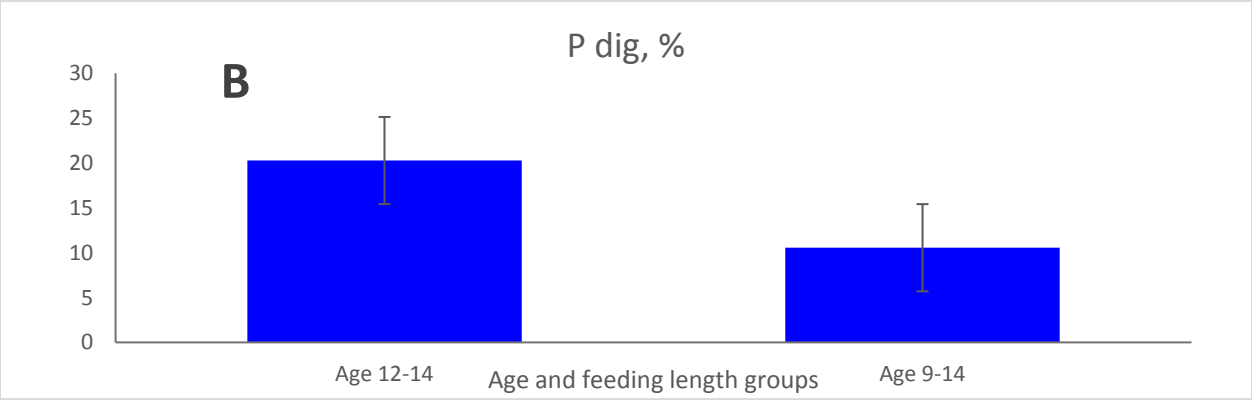


Figure 2-1: Phytase relative to NC diet derived by subtracting body weight gain (BWG, g/bird) in NC from 1000 or 2000 FTU/kg diet in each block. Panel A represents age effect for d 8, 14, and 22 when fed for 2 d from d 6 to 8, d 12 to 14, or 20 to 22 of age. Panel B represents age d 14 when fed for 2 or 5 d from d 12 to 14 or d 9 to 14 of age. Panel C represents age d 22 when fed 2 or 16 d from d 20 to 22 or d 6 to 22. Each bar represents a mean of 8 observations





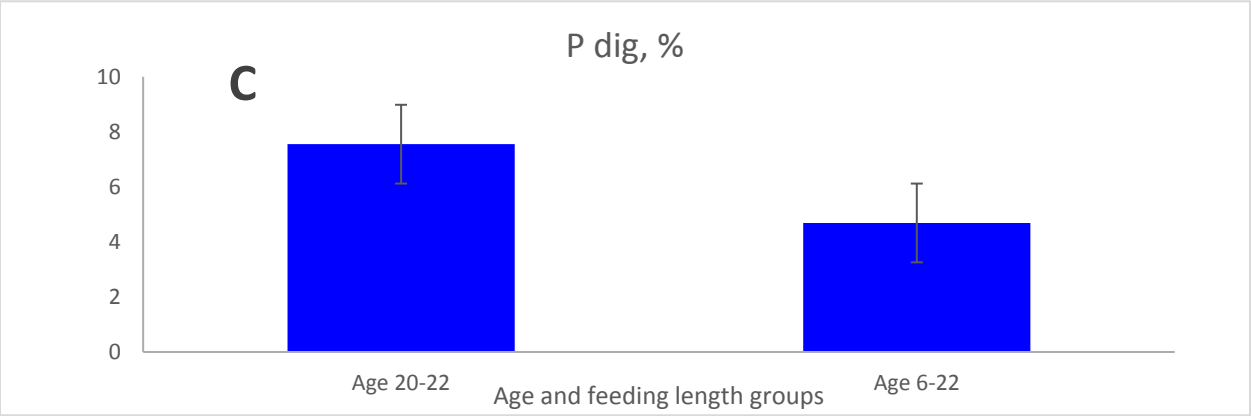


Figure 2-2: Difference in P digestibility (P Dig, %) of birds that received the NC relative to PC diet derived by subtracting P digestibility in NC from PC diet in each block. Panel A represents age effect for d 8, 14, and 22 when fed for 2 d from d 6 to 8, d 12 to 14, or 20 to 22 of age. Panel B represents age d 14 when fed for 2 or 5 d from d 12 to 14 or d 9 to 14 of age. Panel C represents age d 22 when fed 2 or 16 d from d 20 to 22 or d 6 to 22. Each bar represents a mean of 8 observations.

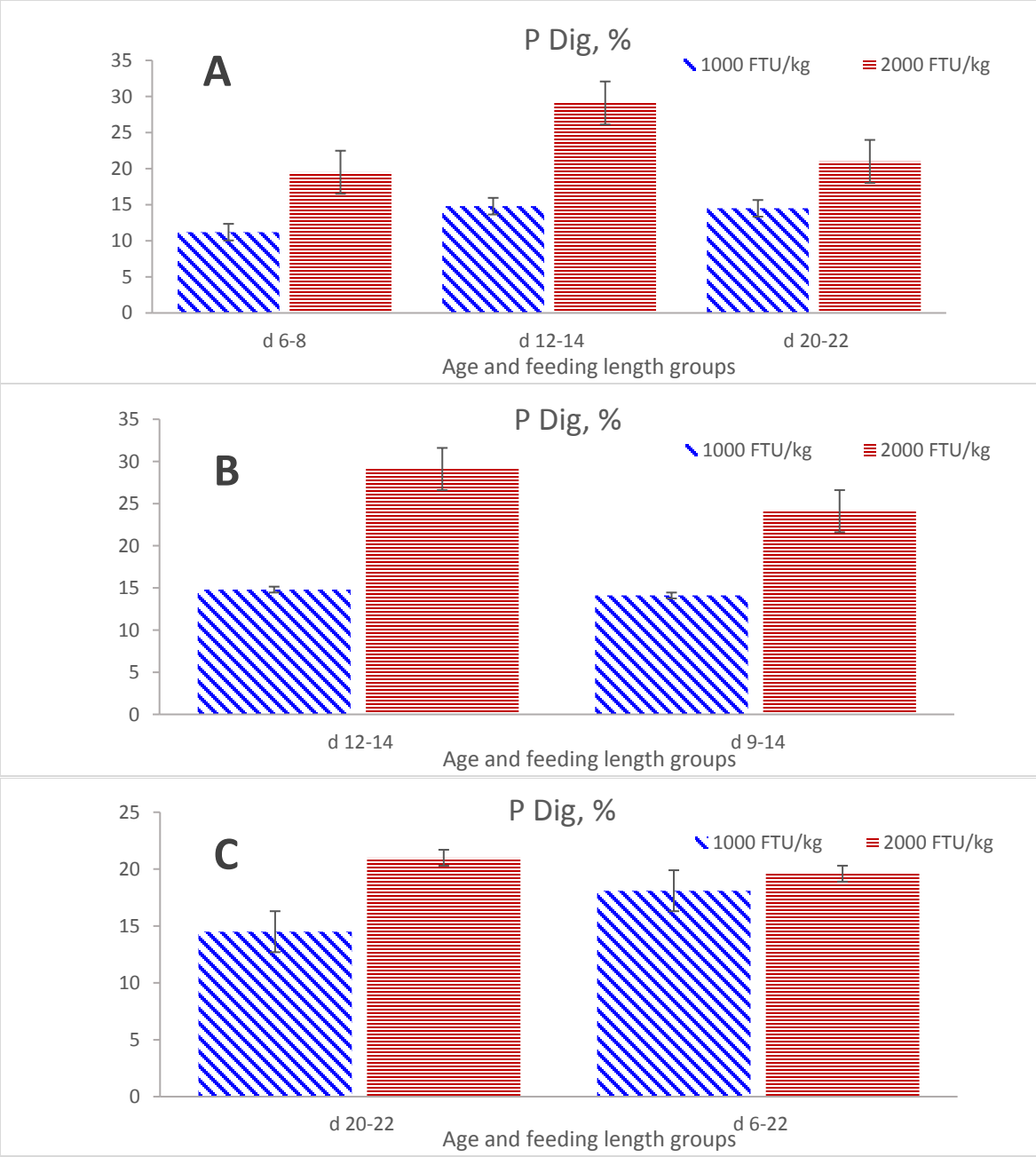


Figure 2-3: Phytase relative to NC diet derived by subtracting apparent P digestibility in NC from 1000 or 2000 FTU/kg diet in each block. Panel A represents age effect for d 8, 14, and 22 when fed for 2 d from d 6 to 8, d 12 to 14, or 20 to 22 of age. Panel B represents age d 14 when fed for 2 or 5 d from d 12 to 14 or d 9 to 14 of age. Panel C represents age d 22 when fed 2 or 16 d from d 20 to 22 or d 6 to 22. Each bar represents a mean of 8 observations.

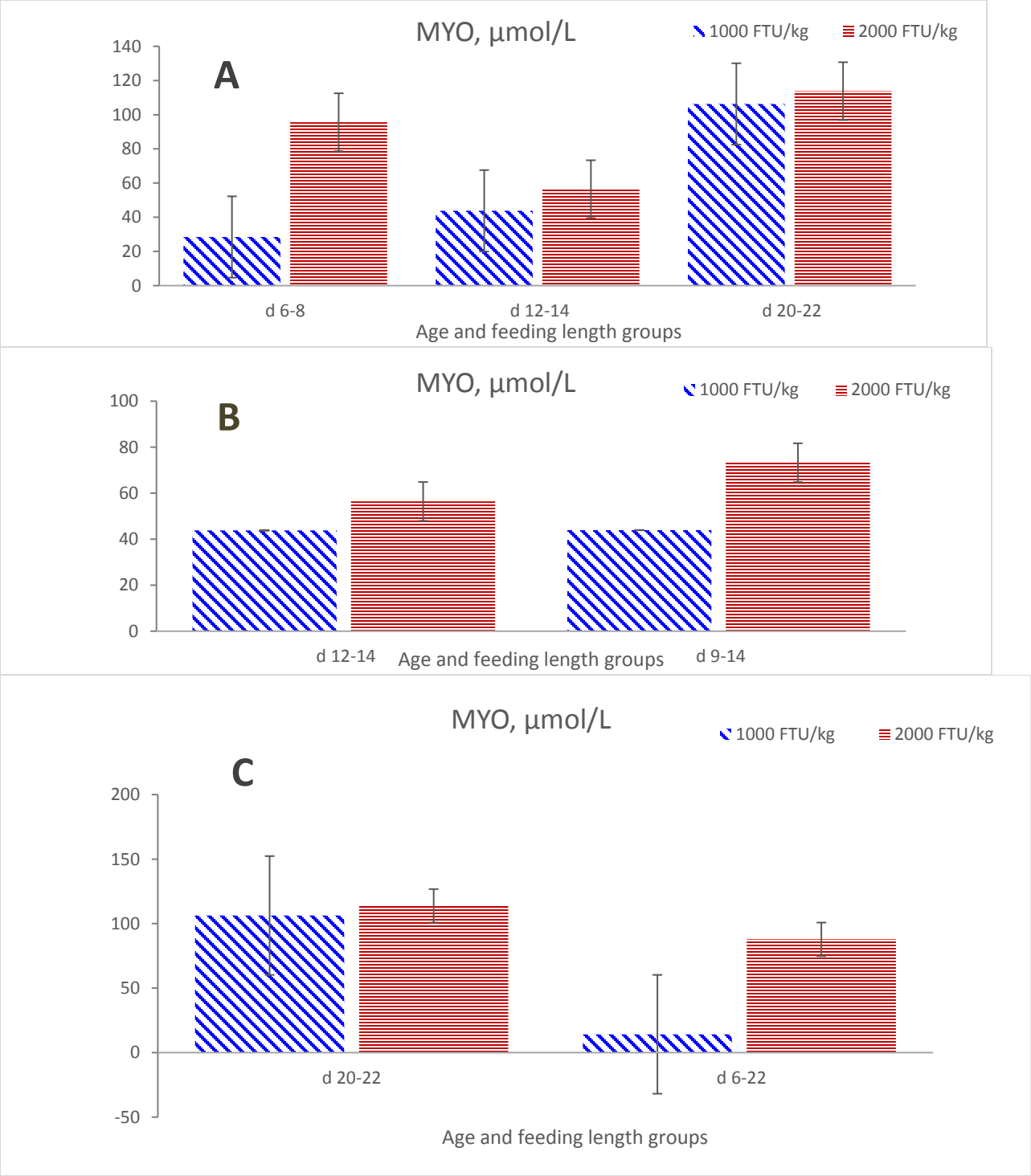


Figure 2-4: Phytase relative to NC diet derived by subtracting plasma concentrations of myoinositol (MYO umol/L) in NC from 1000 or 2000 FTU/kg diet in each block. Panel A represents age effect for d 8, 14, and 22 when fed for 2 d from d 6 to 8, d 12 to 14, or 20 to 22 of age. Panel B represents age d 14 when fed for 2 or 5 d from d 12 to 14 or d 9 to 14 of age. Panel C represents age d 22 when fed 2 or 16 d from d 20 to 22 or d 6 to 22. Each bar represents a mean of 8 observations.

CHAPTER 3. INFLUENCE OF AGE AND FEEDING LENGTH ON PHYTASE EFFICACY AND SUPER DOSING DURING THE STARTER PHASE OF BROILER CHICKENS

3.1. Abstract

Phytase is of importance to the poultry industry because of its ability to hydrolyze phytate and release phosphorus (P) for use by poultry, however, the effect of age on phytase efficacy and super dosing is not fully understood. A total of 864 broiler chicks were used to investigate the effect of age and feeding length on phytase efficacy using growth performance, mineral utilization and tibia ash as response criteria of evaluation. They were fed 3 diets including; a positive control (PC) (0.4% non-phytate P (nPP)), a negative control (NC) (0.2% nPP) and a NC diet supplemented with phytase at 2,000 FTU/kg. Birds were categorized into 4 age and feeding length groups with 8 replicates each including; a 2 and 5 d feeding length terminating on d 14 and 22 respectively. The experiment was arranged as a 3 x 2 x 2 factorial in a randomized complete block design. Birds fed the NC had decreased ($P < 0.01$) body weight, gain and feed efficiency across all the age groups as compared with birds fed the PC. Similarly, birds fed the phytase-supplemented diet had improved ($P < 0.01$) performance as compared to birds fed the NC regardless of age. Birds fed the phytase-supplemented diet for 2 d had improved ($P < 0.01$) P retention than birds fed for 5 d to d 14 or 22. There were no significant differences in P utilization between birds fed for 2 d to d 14 or 22 and birds fed for 5 d to both ages. However, phytase was more efficacious at d 14 than d 22 when mineral utilization was considered because the super dose of phytase elicited greater ($P < 0.01$) response in birds fed for 2 d to d 14. In contrast, percentage tibia ash improved ($P < 0.01$) in birds fed phytase supplemented diet for 5 d at both ages as compared with birds fed for 2 d. In conclusion, testing phytase products, even at high doses, for 2 d during the second week in the life cycle of broiler chicks, can be recommended from the results from this study.

Key words: age, broilers, feeding length, phosphorus, phytase efficacy

3.2. Introduction

Broiler chickens are a major source of animal protein to the human population (National Chicken Council, 2018) hence, research is continually carried out in diverse ways to improve their productivity while being cost effective and maintaining a safe environment. Waste from the broiler industry has been identified as one of the major sources of environmental pollution due to the high concentration of minerals including phosphorus (P) (Panda et al., 2007). When this waste is used as manure, P accumulates in the soil and if P is leached or runoff into water bodies may harm aquatic life and the environment in a process called eutrophication (Waldroup, 1999; Panda et al., 2007). Phytate is commonly found in several cereal and oilseed grains used in animal diets. Phytate binds to minerals such as P, calcium (Ca), magnesium, amino acids, and other essential nutrients forming insoluble complexes (Barrientos and Murphy, 1996; Cowieson and Bedford, 2009). These complexes are not easily hydrolyzed by broilers due to the hindrance in the activity of their endogenous phytase, resulting in the unavailability of nutrients to the birds and its loss to the environment (Selle et al., 2009). The use of exogenous phytase in broiler diets have been encouraged because of its ability to hydrolyze the phytate complex, and release P and other nutrients for use by the birds (Ravindran et al., 1995). Super dosing birds with phytase has resulted in further improvement in growth performance and nutrient utilization of birds compared with the industry recommended dose of 500 FTU/kg diet (Han et al., 2009; Rutherfurd et al., 2012). Studies have reported that high doses of phytase may lead to a complete breakdown of phytate in diets thus reducing its anti-nutritional effect and eliciting a better response in birds (Shirley and Edwards, 2003; Han et al., 2009). Age of birds have been known to influence their capacity to utilize nutrients especially during the early phase of rapid development (Batal and Parsons, 2002). Previous work from our lab reported that age of birds and feeding length has an impact on phytase

efficacy (Chapter 2). A reduction in the efficacy of phytase was observed with responses of birds fed a low P diet as they grew older than 2 wk. Birds fed phytase supplemented low P diets at d 14 post-hatching had increased growth performance, P and Ca utilization as compared with birds fed the same diets at d 8 and 22 post-hatching. Similarly, birds fed for 2 and 5 d had higher responses than birds fed for 16 d. However, it was not clear if there was any difference in the impact on phytase efficacy when birds were fed phytase supplemented low P diets for short periods (i.e. 2 or 5 d) at d 14 post-hatching, characterized by rapid development of organs and tissues, or at d 22 post-hatching, which is the beginning of the grower phase and the rapid accumulation of muscle in broilers. Thus, the objective of this study was to investigate the impact of these feeding lengths and age periods on phytase efficacy using a super dose of phytase (i.e. 2,000 FTU/kg phytase units).

3.3. Materials and Methods

3.3.1. Birds and Management

All protocols of animal experiments were reviewed and approved by the Purdue University Animal Care and Use Committee. A total of 864 day-old male broiler chicks (Cobb 500, Siloam Springs, AR.) were obtained from a commercial hatchery. Each bird was weighed individually, tagged, and allotted to cages. Birds were raised in heated brooder battery cages (model SB 4 T; Alternative Design Manufacturing, Siloam Springs, AR) in an environmentally controlled room. They had unrestricted access to water and were fed a commercial starter diet that met or exceeded the requirements of broiler chicks (NRC, 1994) until the beginning of the experimental periods.

3.3.2. Experimental Design and Procedure

This study was a randomized complete block design with a 3 x 2 x 2 factorial arrangement of treatments comprising; 3 experimental diets; a positive control (PC) diet (0.40% non-phytate P

(nPP)), negative control (NC) diet (0.20% nPP) and a NC diet supplemented with phytase at 2,000 FTU/kg; 2 feeding lengths (2 or 5 d), and 2 ages (d 14 or 22). Feeding length groups included a 2 and 5 d feeding length terminating at d 14 (i.e. d 12 to 14 and d 9 to 14) and at d 22 post-hatch (i.e. d 20 to 22 and 17 to 22). Each treatment had 8 replicates with 10 birds or 8 birds per cage for groups that were fed the experimental diets until d 14 or 22 respectively. The initial BW served as the blocking factor for the 12 treatments to ensure average weights across the diets. Two groups were fed the commercial starter diet until d 9 and 12 respectively and then fed the experimental diets until d 14 while the other two groups were fed the commercial diet until d 17 and 20 respectively and then fed the experimental diets until d 22.

3.3.3. Experimental Diets

Ingredient composition and nutrient and energy concentration of the experimental diets are shown in Table 3-1. The PC and NC diets were similar in ingredient composition but the levels of monocalcium phosphate and limestone were adjusted to give varying levels of nPP. Phytase (RONOZYME® HiPhos, DSM Nutritional Products, Switzerland) was combined with ground corn to form a premix containing 50 FTU/g. One FTU is defined as the quantity of enzyme required to liberate 1 µmol of inorganic phosphate/min from 5.0m*M* sodium phytate at pH 5.5 and 37˚C (Engelen at al., 1994). The phytate premix was then supplied at 40 g/kg to the NC diet to contain 2,000 FTU/kg. Ileal digestibility and total tract retention of nutrients in diets were determined by index method as described by Adeola and Walk (2013) and using chromic oxide, incorporated into the diets, as an indigestible marker.

3.3.4. Sample Collection and Chemical Analyses

Initial BW of birds were recorded at the start of each feeding length, while feed consumption and final BW were collected at the end of each period. Excreta was collected twice daily from each

cage towards the end of the experimental periods (i.e d 14 and 22). Excreta was oven dried at 55˚C for 5 d, ground and stored for further analyses. On d 14 and 22 post hatching, birds in 2 groups respectively were euthanized by CO_2 asphyxiation and blood samples were collected by cardiac puncture from the median weight bird in each cage into heparinized tubes. Plasma was obtained by centrifuging blood samples at 3,000 x g for 15 min at 4˚C (Jiang et al., 2013) and stored at −80˚C until further analyses. Ileal digesta was collected from the distal two-thirds of the ileum of all birds, flushed into plastic containers, pooled per cage, and stored at −20˚C until freeze dried. Dried samples were ground with a ZM 100 grinder (Retsch ZM 100, GmbH, Haan, Germany) and passed through a 0.5mm screen. The left tibia bone was collected from 4 birds with weights closest to the median weight per cage. Bone ash was determined from collected bones in a process previously described by Ogunwole et al. (2017). Dry matter (DM) was determined by placing samples in a drying oven at 105˚C for 24 h (The Precision Scientific Co., Chicago, IL; method 934.01; AOAC International, 2000). Chromium (Cr) concentration was determined in the diet, ileal digesta and excreta samples following a wet-ash digestion as previously described by Fenton and Fenton (1979). P concentration was determined from digested samples by spectrophotometry, with absorbance read at 450 nm (Spectronic 21D; Milton Roy Co., Rochester, NY). Ca concentrations in samples were determined by flame atomic absorption spectroscopy using a Varian Spectr.AA 220FS (Varian Australia Pty Ltd., Victoria, Australia; Iyayi et al., 2013). Phytase activity was estimated using methods described by Engelen et al. (1994). Blood plasma was analyzed for myo-inositol concentration by spectrophotometry using an ADVIA 1650 chemistry system (Bayer diagnostic, Puteaux, France).

3.3.5. Calculation and Statistical Analysis

Apparent ileal digestibility (AID) and total tract retention (TTR) of P and Ca in the diets was determined by the index method using the following equation (Dilger and Adeola, 2006):

$$\text{AID or TTR (\%)} = 100 - [(CR_I/CR_O) \times (N_O/N_I) \times 100]$$

where CR_I is the concentration of Cr in the diets, CR_O is the concentration of Cr in the excreta or ileal digesta, N_O is the nutrient concentration in the ileal digesta or excreta, and N_I is the nutrient concentration in the diet. All values were expressed as grams per kilogram of DM.

Data were analyzed using the GLM procedure of SAS as a 3 × 2 × 2 factorial arrangement of treatments with 3 diets, for 2 feeding periods (2 or 5 d) at 2 ages (d 14 or 22 post-hatching), with cage as the experimental unit. Statistical significance was set at $P \leq 0.05$. Contrast of PC vs. NC and NC vs. NC + 2000 phytase units/kg diets were used to examine the effect of responses of different levels of nPP and phytase respectively. The effect of age regardless of feeding length was examined using contrast within birds fed for the same length at different ages i.e. birds fed for 2 d at d 14 and 22 (i.e. d 12 to 14 vs. 20 to 22 post-hatching) and birds fed for 5 d at d 14 and 22 (i.e. d 9 to 14 vs. 17 to 22 post-hatching). The effect of feeding length regardless of diets was examined by comparing responses of birds fed for 2 and 5 d at d 14 (i.e. d 12 to 14 vs. 9 to 14 post hatching) and 22 (i.e. d 20 to 22 vs. 17 to 22 post hatching) respectively. Phytase efficacy was calculated by subtracting the nutrient digestibility of birds fed the negative control diet from the digestibility of birds fed the 2,000 FTU/kg phytase supplemented diet. Data was analyzed using the GLM procedure of SAS, orthogonal contrast were used to analyze the effect of age and feeding length on the phytase efficacy.

3.4. Results

Nutrient analyses and phytase activity in diets (Table 3-1) were within acceptable ranges when sampling and analysis variations were considered. Although, analyzed Ca concentration in the diets were higher than the formulated concentration which may be a result of limestone being used as a filler in mineral and vitamin premixes or as a flow agent in soybean meal. Phytase activity

in diets were analyzed at below 100 units/kg for the PC and NC diets and 2,210 units/kg for the NC diet supplemented with phytase at 2000 FTU/kg.

Growth performance data are presented in Table 3-2. Growth performance responses of birds fed the NC diet were lower ($P < 0.01$) than birds fed the PC diet. The effect of the NC on BWG as compared with the PC was more evident in birds fed for a shorter period at d 14 (2 d) and for a longer period at d 22 (5 d) thus, resulting in a diet*feeding length interaction ($P < 0.01$). Body weight gain (BWG) of birds fed the NC diet for 2 d were lower ($P < 0.01$) than birds fed the PC diet for the same length at d 14 and 22 (by 18 and 11% respectively). Similarly, BWG of birds fed the NC diet for 5 d until d 14 and 22 were lower ($P < 0.01$) than birds fed the PC for the same period at both ages (by 14 and 17% respectively). BWG of birds fed the NC supplemented with phytase at 2000 FTU/kg was linearly improved ($P < 0.01$) across all the ages and feeding lengths when compared with birds fed the NC diet. Age effect was evaluated by comparing responses of birds fed for the same length at different ages (i.e. 2 d feeding length at d 14 and 22 or 5 d feeding length at d 14 and 22), while feeding length effect was evaluated by comparing responses of birds fed for different lengths at the same age (i.e. 2 and 5 d feeding length at d 14 or at d 22). Results of age effect on BWG when comparing birds fed the phytase supplemented NC diets and the NC diets revealed that birds fed for 2 or 5 d at d 14 had lowered improvements in gain as compared with birds fed for the same period at d 22 respectively (Figure 3-1 A and B). Birds fed for a longer period at both ages had a greater improvement on weight gain with phytase supplementation as compared with birds fed for a shorter period when feeding length effect was considered (Figure 3-1 C and D). Similarly, birds fed the NC diet across all age and feeding length had reduced (Table 3-2; $P < 0.01$) feed intake and feed efficiency when compared to birds fed the PC diet. Similarly, birds fed the phytase supplemented diets had improved feed intake and efficiency when compared with birds fed the NC diet.

Birds fed the P deficient NC diet had reduced ($P < 0.01$) tibia ash when compared to birds fed the PC diet across all the feeding length and age groups (Table 3-2). However, the decline in the tibia ash was more apparent in birds fed for 5 d at d 14 and 22 (4.9 and 4.7 % respectively) than in birds fed for 2 d at the same ages (2.5 and 1.5% respectively). With phytase supplementation, birds had improved ($P < 0.01$) tibia ash when compared with birds fed the NC diet. Birds fed the phytase diet for 2 d at d 14 had a 1.5% improvement in tibia ash as compared with birds fed the NC diet for the same period. Similarly, birds fed the phytase diet for 5 d until d 14 had 4.7% improvement in tibia ash when compared to birds fed the NC diet. Birds fed the phytase supplemented diet for 2 and 5 d until d 22 had a 1.3 and 3.3% improvement in tibia ash as compared to birds fed the NC diet for the same period. The phytase induced improvements in tibia ash were more apparent at d 14 than at d 22 when both feeding lengths were considered.

Nutrient digestibility and retention responses of birds to diets are presented in Table 3-3. The AID of P in birds fed the NC diet were lower ($P < 0.01$) when compared with birds fed the PC diet across all age and feeding length groups. In birds fed the NC for 2 d until d 14, a 10% decrease in AID of P was observed when compared with birds fed the PC, while in birds fed the NC for 5 d until d 14, a 7.7% decrease in P digestibility was observed. Similarly, birds fed the NC diet for 2 d until d 22 had a 6% decrease in P digestibility as compared with birds fed the PC diet while birds fed the NC diet for 5 d until d 22 had a 9.5% decrease in P digestibility. The effect of age on the impact of the low P diet on birds were observed when comparing the P digestibility of birds fed the PC and the NC at d 14 and 22. Birds fed for 2 d until d 14 had an increased effect of the NC diet as compared with birds fed for 2 d until d 22 (Figure 3-2 A). However, birds fed for 5 d until d 22 had an increased effect of the NC than birds fed for 5 d until d 14 (Figure 3-2 B). The feeding length effect on the impact of the NC on birds were evaluated by comparing the P digestibility of birds fed for the same length until d 14 and 22. Thus, comparing birds fed the NC

for 2 and 5 d until d 14 or 22 revealed that birds fed for 2 d at d 14 had an increased impact on P digestibility than birds fed for 5 d while on d 22, the opposite was observed (Figure 3-2 C and D). Birds fed the phytase supplemented diets had improved P digestibility above birds fed the PC or NC diets. Birds fed the phytase diet for 2 d at d 14 and 22 had a 24 and 19% improvement in P digestibility over birds fed the NC diets for that same period. Meanwhile, birds fed the phytase diet for 5 d at d 14 and 22 had approximately a 19% improvement in P digestibility over birds fed the NC at the same period. Birds fed the NC diets had decreased ($P < 0.01$) P retention, Ca digestibility and retention when compared with birds fed the PC diets across all the periods. Phytase supplementation improved Ca digestibility and TTR of P and Ca in birds across all age and feeding length groups when compared with birds fed the NC diets. There was no age or feeding length effect on the AID or TTR of Ca. There was no age or feeding length effect on P digestibility however, there was an age effect on TTR of P with birds fed for 2 d at both ages having a lower average P retention than birds fed for 5 d at both ages. Phytase efficacy was determined by subtracting responses of birds fed the NC diet from birds fed the phytase supplemented NC diet and results are presented in Table 3-4. There was no significant effect of age or feeding length on phytase efficacy when P and Ca digestibility and retention were considered. However, in birds fed diet supplemented with phytase for 2 d, the magnitude of improvement in P digestibility was higher in birds fed until d 14 than in birds fed until d 22 (Figure 3-3 A). A similar trend was observed in birds fed for 5 d until d 14 and 22 (Figure 3-3 B). Birds fed for 2 d at both ages had an increased effect of phytase on P digestibility than birds fed for 5 d at both ages (Figure 3-3 C and D). There was no impact of age and feeding length on phytase efficacy in tibia ash (Table 3-4). However, phytase was more efficacious on tibia ash in birds fed for 5d until d 14 considering an increased ($P < 0.05$) improvement in tibia ash when compared with birds fed for 2 d until d 14. Plasma myo-inositol levels in birds fed the phytase supplemented diets until d 14 were higher than birds fed the

NC diet for the same period. However, birds fed the phytase supplemented diets for 5 d had a higher concentration of inositol as compared with birds fed for 2 d (Figure 3-4 A). Similarly, birds fed the phytase diets for 2 or 5 d until d 22 had a higher inositol concentration as compared with birds fed the NC diet for the same period until d 22 (Figure 3-4 B).

3.5. Discussion

Phytase supplementation in broiler nutrition has been known to improve growth performance, nutrient and mineral utilization, bone mineralization and the general well-being of birds, and this has been established by various studies over time (Broz et al., 1994; Sebastian et al., 1996; Ravindran et al., 1999; Leeson et al., 2000; Dilger et al., 2004; Olukosi et al., 2013; Ribeiro et al., 2016). The current study was not an exception to this trend as improvements on growth performance, mineral utilization and bone mineralization were observed with phytase supplementation. However, the study aimed to evaluate the impact of age and feeding length on phytase efficacy and super dosing. This was important because it has been established that age has an influence on the utilization of nutrients by broilers (Tarvid, 1995). Some studies have reported that birds in the first 2 wk of life, have increased energy and protein digestibility and utilization as compared with older birds due to the rapid growth of organs and tissues associated with this period (Batal and Parsons, 2002; Huang et al., 2005). Other studies have reported an increase in digestibility of nutrients with increasing age (Noy and Sklan, 1995; Sell, 1996). Feeding length has been observed to have an impact on P digestibility and retention. Li et al., (2018) reported that the effect of feeding low P diets to birds for greater than 48 h could potentially confound results of P digestibility as physiological adaptations could occur in birds. Following up on a previous study in our lab, feeding P deficient diets to birds for 2 or 5 d had the greatest impact on P digestibility and phytase efficacy as compared with feeding for a longer period of 16 d. However,

the study design did not allow for the determining if feeding for a short period would have the same effect on phytase efficacy at different time point in the starter phase of chickens. Therefore, this current study evaluated the age effect on phytase efficacy by comparing responses of birds fed for 2 d at d 14 and 22 and for 5 d at d 14 and 22. It also evaluated the feeding length effect on phytase efficacy by comparing responses for birds fed for 2 and 5 d at d 14 and at 22. Phytase efficacy was determined by subtracting responses of birds fed the NC from response of birds fed the phytase supplemented NC diet at each feeding length and age.

Birds fed the P deficient diet had lower weight gain, feed intake and feed efficiency as compared with birds fed the PC diet across all the ages and feeding lengths. Thus, proving that P is a limiting nutrient in the diet and supporting observations by previous studies where broilers fed low P diets had decreased growth performance (Dilger et al., 2004; Adeola and Walk, 2013). However, the effect of the low P diet on BWG was more evident in birds fed for 2 d at d 14 than in birds fed for 5 d at this same age. This could be due to the fact that P is one of the most important elements in biological life, P is needed for growth and development of cells and tissues, and also important in various biochemical reactions and in bone development (Berndt and Kumar, 2009; Jiang et al., 2013). Thus, an exposure to P deficiency for a short period and at a critical point in the lifecycle of the birds, characterized by the rapid growth of organs and tissues, could prove detrimental. However, birds may adapt to low P conditions over time (Proszkowiec-weglarz and Angel, 2013), this could explain why the impact on BWG after feeding for 5 d at d 14 was not as severe as feeding for 2 d. On the contrary, birds fed for 5 d at d 22 had a more severe effect of P deficiency on BWG than birds fed for 2 d. This could be due to birds being well developed at this phase. Thus, a short exposure to P deficiency may not be as severe as a longer exposure. This observation could be feed intake driven as the effect of the low P diet on feed intake was higher in birds fed for 5 d than in birds fed for 2 d at d 22. Phytase supplementation improved BWG, feed

intake, and efficiency across all groups. However, when age effect was considered at both feeding lengths, birds at d 22 had an increased improvement with phytase supplementation than birds at d 14. This may be feed intake driven as birds at this age will consume more diet than birds at d 14, resulting in more muscle development and weight gain. When feeding length effect was considered, birds fed the phytase supplemented diet for 5 d at both ages had increased weight gain than birds fed for 2 d at both ages, this was probably due to increased feed intake over a longer period.

Tibia ash was reduced in birds fed low P diets and increased in birds fed phytase supplemented low P diets, this was predictable as previous studies have observed this trend (Denbow et al., 1995; Onyango et al., 2004; Jiang et al., 2013; Pieniazek et al., 2017). Although no significant age or feeding length effect was observed on tibia ash, birds fed phytase supplementation for 5 d at both ages had increased improvement in tibia ash than birds fed for 2 d, this could be due to the fact that P and Ca constitute approximately 99% of bones in the body, thus, the longer the exposure of birds to P deficient diets, the greater the negative impact on tibia ash. Consequently, feeding phytase supplemented diets to birds for a longer period will ameliorate the impact of the low P diet on tibia ash. Birds at 14 d-of age had a slightly higher phytase induced improvement on tibia ash than birds at 22 d-of age regardless of the feeding length, this may be because birds at this phase are rapidly depositing hydroxyapatite on bones to increase its strength and to support their weight as they grow older (Dilger et al., 2004). Birds fed the P deficient diet had decreased P digestibility in accordance with observations from previous studies (Ravindran et al., 2000, 2006; Plumstead et al., 2008). Evaluating the age effect of P deficiency on P digestibility revealed that birds fed the NC for 2 d at d 14 had an increased reduction in AID of P when compared with birds fed for 2 d at d 22. This may be because birds at d 14 may be more sensitive to changes in dietary P than birds at d 22. Birds at d 22 had also been exposed to a commercial diet

until d 20 and so a change in dietary P for 2 d may not cause a severe decrease in P digestibility. Similarly, phytase improved P digestibility more so in birds fed for 2 d at d 14 than birds at d 22, thus showing that phytase was more efficacious at d 14. Considering feeding length effect, birds fed P deficient diets for 2 d at d 14 and 22 had lower P digestibility than birds fed for 5 d at both ages. This observation agreed with the previous study from our lab (Babatunde et al., under review) and other studies which revealed that feeding low P diet for more than 48 h could potentially confound results obtained from P digestibility. This is because, adaptative changes may be stimulated in the gastrointestinal tracts of birds to maintain P homeostasis (Yan et al., 2005; Li et al., 2014, 2015; Perryman et al., 2016). Phytase was more efficacious in birds fed for 2 d than in birds fed for 5 d at both ages, as increased P digestibility and retention was observed in birds fed phytase supplemented diet for 2 d at both ages possibly due to reasons previously discussed. Birds fed phytase supplemented diet at d 14 had greater improvements in AID of P than birds at d 22 further confirming that the second wk in the lifecycle of birds may be a better period to observe results of P digestibility when phytase efficacy trials are conducted in broiler chickens. Ca has been known to interact with phytate present in cereal and oilseed grains by forming complexes with phytate and preventing its hydrolysis and release to the animal (Tamin and Angel, 2003; Tamin et al., 2004; Plumstead et al., 2008). Invariably, addition of phytase to the diet breaks this complex bonds and releases Ca for use by the animal. This explains why Ca digestibility and retention were improved in birds fed diet with phytase supplementation and in agreement with previous studies (Silversides et al., 2004; Adeola and Walk, 2013; Paiva et al., 2014). A similar pattern with P utilization was observed when age and feeding length effect were evaluated. Plasma myo-inositol levels were increased in birds fed phytase supplemented diets and this was similar with observations reported by Cowieson et al., (2014). This is because phytase hydrolyzes phytic acid into inositol and orthophosphate in the digestive tract (Perryman et al., 2016). Inositol which

is absorbed into the blood, may be responsible for some of the benefits of phytase on growth performance, as it has recently been discovered to have insulin mimetic properties that stimulates the translocation of glucose transporters (GLUT4) to the plasma membranes of the small intestine (Cowieson et al., 2017).

In the current study, it was possible to evaluate the impact of age and feeding length on a super dose of phytase (2,000 FTU/kg) in broiler chickens. Various studies have examined the effect of a super dose level of phytase even up to 15,000 FTU/kg in poultry using growth performance, mineral utilization and tibia ash as parameters of evaluation without reporting any negative impact (Shirley and Edwards, 2003; Cowieson et al., 2006; Adeola, 2018). Similarly, there was no negative impact of using a high dose of phytase (2,000 FTU/kg) on the performance of broilers in this study. Instead, improvements in growth performance, P and Ca utilization and tibia ash were observed. Considering the previous discussion on the age and feeding length effect on P digestibility, it could be inferred that the greatest benefit of super dosing phytase on P utilization was observed when birds were fed phytase supplemented diet for 2 d until d 14. In conclusion, we can recommend based on results from this study, that phytase trials in broilers could be carried out for 2 d during the end of the second wk in the lifecycle of broilers for maximum efficiency.

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Table 3-1: Ingredients and nutrient composition of experimental diets of broiler chickens

Item	Positive Control	Negative Control	Phytase 2,000 FTU/kg
Ingredients, g/kg			
Corn	520.6	531.1	491.1
Soybean meal, 480g/kg CP	358.0	356.0	356.0
Soybean oil	53.0	50.0	50.0
Monocalcium phosphate	13.3	3.8	3.8
Limestone	15.3	19.3	19.3
Salt	4.0	4.0	4.0
Vitamin-mineral premix[1]	3.0	3.0	3.0
DL-Methionine	3.8	3.8	3.8
L-Lysine·HCL	2.9	2.9	2.9
L-Threonine	1.1	1.1	1.1
Chromic oxide premix[2]	25.0	25.0	25.0
Phytase premix[3]	0	0	40
Total	1,000.0	1,000.0	1,000.0
Calculated nutrients and energy, g/kg			
CP	225.3	225.3	225.3
ME, kcal/kg	3,222.5	3,228.8	3,228.8
Ca	9.0	9.0	9.0
P	6.5	4.5	4.5
Non-phytate P	4.0	2.0	2.0
Analyzed nutrients, g/kg			
DM	890	884	887
Ca	13	12.5	12.6
Total P	6.4	4.8	4.8
Phytate P	2.7	2.8	2.6
Phytase activity, FTU/kg	<100	<100	2,210

[1]Supplied the following quantities per kg of diet: vitamin A, 5,484 IU; vitamin D_3, 2,643 ICU; vitamin E, 11 IU; menadione sodium bisulfite,4.38 mg; riboflavin, 5.49 mg; D-pantothenic acid, 11 mg; niacin, 44.1 mg; choline chloride, 771 mg; vitamin B_{12}, 13.2 µg; biotin, 55.2 µg; thiamine mononitrate, 2.2 mg; folic acid, 990 µg; pyridoxine hydrochloride, 3.3mg; I, 1.11 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; Se, 300 µg.

[2]Prepared as 1 g chromic oxide added to 4 g corn.

[3]Prepared with ground corn to contain 50 FTU per g corn.

Table 3-2: Effect of phytase and age with different feeding length on growth performance and tibia ash contents of broiler chicken

Age, d	Feeding Length, d	Diet[1]	Final BW (g)	BW gain, g/bird	Feed intake, g/bird	G:F, g/kg	Tibia Ash, %	No. of replicates
14	2 (d 12-14)	PC	442	103	133	770	47.7	8
14	2 (d 12-14)	NC	424	84	122	689	45.2	8
14	2 (d 12-14)	2,000	437	97	128	762	46.7	8
14	5 (d 9-14)	PC	456	233	285	816	46.2	8
14	5 (d 9-14)	NC	423	200	268	741	41.3	8
14	5 (d 9-14)	2,000	446	223	276	808	46.0	8
22	2 (d 20-22)	PC	993	143	217	659	50.9	8
22	2 (d 20-22)	NC	976	127	211	599	48.7	8
22	2 (d 20-22)	2,000	991	142	210	676	50.0	8
22	5 (d 17-22)	PC	1013	376	529	711	50.9	8
22	5 (d 17-22)	NC	957	313	491	639	46.2	8
22	5 (d 17-22)	2,000	996	358	501	715	49.5	8
		PC	726	214	291	739	49	32
		NC	695	180	273	667	45	32
		2,000	718	205	279	740	48	32
14			438	157	202	764	46	48
22			988	243	360	666	49	48
	2		710	116	170	692	48	48
	5		715	284	392	738	47	48
	2	PC	718	123	175	714	49	16
	2	NC	700	105	167	644	47	16
	2	2,000	714	119	169	719	48	16
	5	PC	734	304	407	764	49	16
	5	NC	690	256	380	690	44	16
	5	2,000	721	291	389	761	48	16
14	2		434	95	128	740	47	24
14	5		442	219	276	789	44	24
22	2		986	137	213	644	50	24
22	5		989	349	507	688	49	24
***P* values**								
Diet			<0.01	<0.01	<0.01	<0.01	<0.01	
Age			<0.01	<0.01	<0.01	<0.01	<0.01	
Feeding Length			0.29	<0.01	<0.01	<0.01	<0.01	
Diet x Age			0.57	0.25	0.33	0.77	0.72	
Diet x Feeding Length			0.06	<0.01	0.05	0.96	0.02	
Age x Feeding Length			0.56	<0.01	<0.01	0.83	0.22	
Diet x Age x Feeding Length			0.56	0.22	0.21	0.94	0.80	
PC VS NC			<0.01	<0.01	<0.01	<0.01	<0.01	
NC VS 2,000			<0.01	<0.01	0.14	<0.01	<0.01	
d 12-14 vs. 9-14			0.25	<0.01	<0.01	<0.01	<0.01	
d 20-22 vs. 17-22			0.73	<0.01	<0.01	<0.01	0.09	
d 12-14 vs 20-22			<0.01	<0.01	<0.01	<0.01	<0.01	
d 9-14 vs 17-22			<0.01	<0.01	<0.01	<0.01	<0.01	

[1]Diets PC= Positie Control, NC= Negative Control, 2000= NC + 2000 phytase units/kg

Table 3-3: Effect of phytase and age with different feeding length on nutrient digestibility and retention responses of broiler chickens

Age, d	Feeding Length, d	Diet[2]	AID[1] DM, %	AID P, %	AID Ca, %	TTR[1] DM, %	TTR P, %	TTR Ca, %	No. of replicates
14	2 (d 12-14)	PC	72.0	56.0	58.2	66.5	50.1	44.7	8
14	2 (d 12-14)	NC	70.0	45.7	46.0	65.8	35.1	27.0	8
14	2 (d 12-14)	2,000	71.3	69.6	62.9	69.3	58.2	42.6	8
14	5 (d 9-14)	PC	70.2	55.0	59.6	70.0	54.9	45.0	8
14	5 (d 9-14)	NC	69.2	47.3	52.3	69.5	46.4	24.4	8
14	5 (d 9-14)	2,000	71.2	65.2	62.7	70.3	63.0	36.9	8
22	2 (d 20-22)	PC	70.7	54.4	50.4	69.6	48.9	46.2	8
22	2 (d 20-22)	NC	69.7	48.5	44.7	69.6	37.5	28.6	8
22	2 (d 20-22)	2,000	72.0	67.3	54.5	71.3	58.3	45.0	8
22	5 (d 17-22)	PC	71.1	58.3	57.5	71.4	51.8	45.5	8
22	5 (d 17-22)	NC	70.2	48.8	49.0	71.3	43.7	29.0	8
22	5 (d 17-22)	2,000	72.8	66.4	56.1	73.2	63.5	40.2	8
		PC	71.0	55.9	56.4	69.40	51.4	45.4	32
		NC	69.8	47.5	48.0	69.1	40.6	27.2	32
		2,000	71.8	67.1	59.0	71.0	60.8	41.1	32
14			70.6	56.4	57.0	68.6	51.3	36.7	48
22			71.1	57.3	52.0	71.1	50.6	39.1	48
	2		71.0	57.0	52.8	68.7	48.0	39.0	48
	5		70.8	56.8	56.2	71.0	53.9	36.8	48
14	2		71.1	57.1	55.7	67.2	47.8	38.1	24
14	5		70.1	55.8	58.2	70.0	54.8	35.4	24
22	2		70.8	56.7	49.9	70.2	48.2	40.0	24
22	5		71.3	57.8	54.2	72.0	53.0	38.2	24
***P* values**									
Diet			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	
Age			0.25	0.51	<0.01	<0.01	0.47	0.17	
Feeding Length			0.66	0.95	0.04	<0.01	<0.01	0.20	
Diet x Age			0.39	0.68	0.43	0.90	0.51	0.86	
Diet x Feeding Length			0.60	0.34	0.47	0.54	0.07	0.45	
Age x Feeding Length			0.06	0.34	0.57	0.37	0.23	0.77	
Diet x Age x Feeding Length			0.81	0.66	0.62	0.46	0.47	0.89	
PC VS NC			0.01	<0.01	<0.01	0.63	<0.01	<0.01	
NC VS 2,000			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	
d 12-14 vs. 9-14			0.11	0.47	0.27	<0.01	<0.01	0.27	
d 20-22 vs. 17-22			0.31	0.52	0.06	0.02	<0.01	0.48	
d 12-14 vs 20-22			0.61	0.83	0.01	<0.01	0.73	0.44	
d 9-14 vs 17-22			0.04	0.26	0.08	<0.01	0.18	0.24	

[1]AID = apparent ileal digestibility, TTR = total tract retention

[2]Diets PC = Positive Control, NC = Negative Control, 2,000 = NC + 2,000 phytase units/kg

Table 3-4: Effect of age and feeding length on phytase efficacy in mineral utilization and tibia ash of broiler chickens[1]

Age, d	Feeding Length, d	AID[2] P, %	AID Ca, %	TTR[2] P, %	TTR Ca, %	Tibia Ash, %	No. of replicates
14	2 (d 12-14)	23.94	16.87	23.15	15.57	1.5	8
14	5 (d 9-14)	17.95	10.32	16.68	12.49	4.8	8
22	2 (d 20-22)	18.85	9.84	20.89	16.34	1.3	8
22	5 (d 17-22)	17.67	7.09	19.85	11.25	3.4	8
14		20.95	13.60	19.92	14.03	3.1	16
22		18.26	8.46	20.37	13.79	2.3	16
	2	21.40	13.35	22.02	15.95	1.4	16
	5	17.81	8.71	18.27	11.87	4.1	16
***P* values**							
Age		0.41	0.10	0.82	0.96	0.45	
Feeding Length		0.27	0.14	0.06	0.37	0.02	
Age x Feeding Length		0.46	0.54	0.17	0.83	0.60	
d 12-14 vs. 9-14		0.19	0.14	0.02	0.63	0.04	
d 20-22 vs. 17-22		0.79	0.53	0.70	0.43	0.17	
d 12-14 vs 20-22		0.27	0.11	0.41	0.91	0.87	
d 9-14 vs 17-22		0.95	0.46	0.25	0.85	0.37	

[1]Data were obtained by subtracting responses of birds on NC diet from responses of birds on NC + 2000 FTU/kg phytase supplemented diets in each of 8 blocks of cages

[2]AID = apparent ileal digestibility, TTR = total tract retention

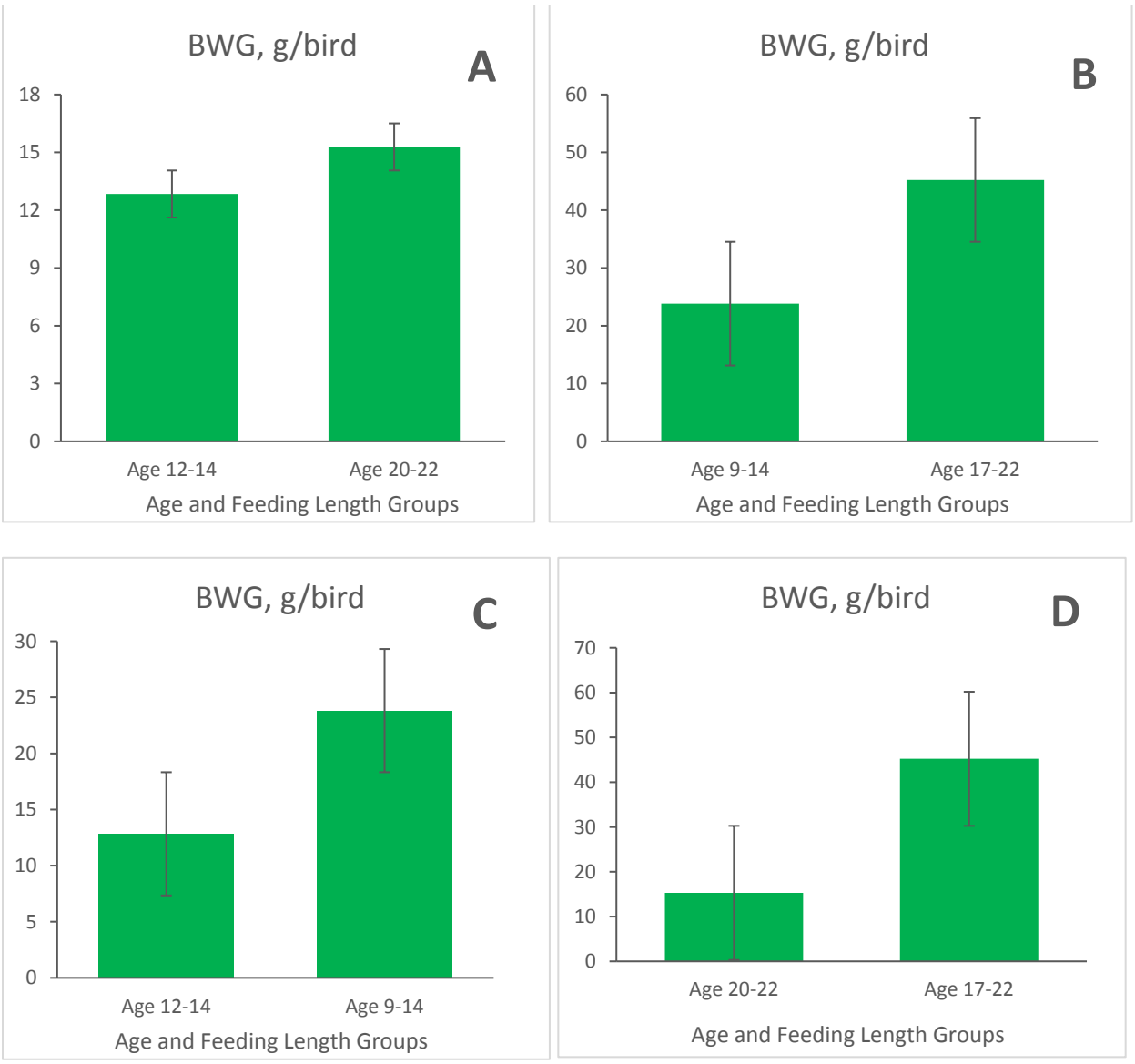


Figure 3-1: Phytase relative to NC diet derived by subtracting body weight gain (BWG, g/bird) in NC from 2000 FTU/kg diet in each block. Panel A represents age effect for d 14, and 22 when fed for 2 d from d 12 to 14, or 20 to 22 of age. Panel B represents age effect for d 14, and 22 when fed for 5 d from d 9 to 14, or 17 to 22 of age. Panel C represents age d 14 when fed for 2 or 5 d from d 12 to 14 or d 9 to 14 of age. Panel D represents age d 22 when fed 2 or 5 d from d 20 to 22 or d 17 to 22. Each bar represents a mean of 8 observations.

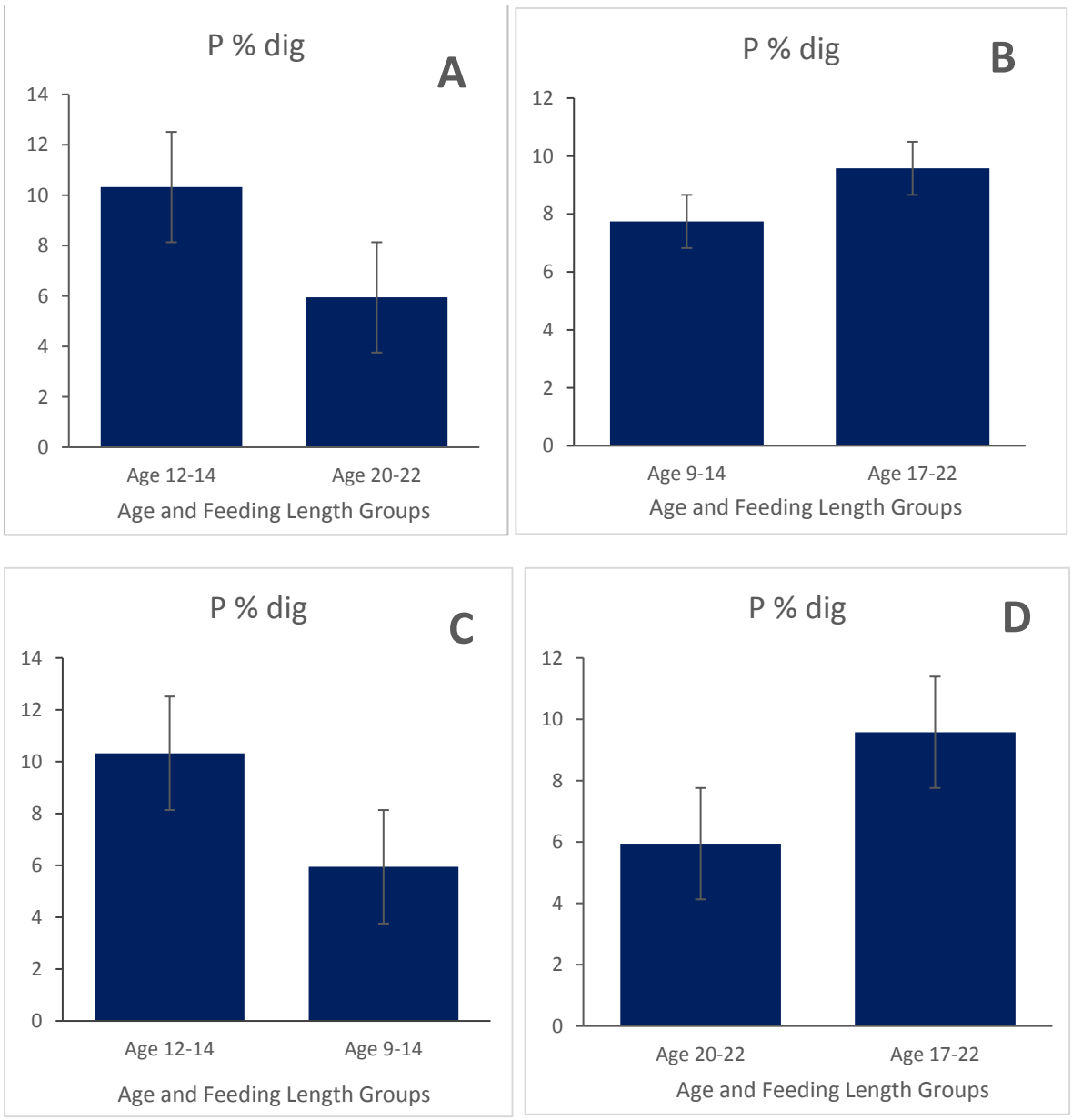


Figure 3-2: Difference in P digestibility (P Dig, %) of birds that received the NC relative to PC diet derived by subtracting P digestibility in NC from PC diet in each block. Panel A represents age effect for d 14, and 22 when fed for 2 d from d 12 to 14, or 20 to 22 of age. Panel B represents age effect for d 14, and 22 when fed for 5 d from d 9 to 14, or 17 to 22 of age. Panel C represents age d 14 when fed for 2 or 5 d from d 12 to 14 or d 9 to 14 of age. Panel D represents age d 22 when fed 2 or 5 d from d 20 to 22 or d 17 to 22. Each bar represents a mean of 8 observations.

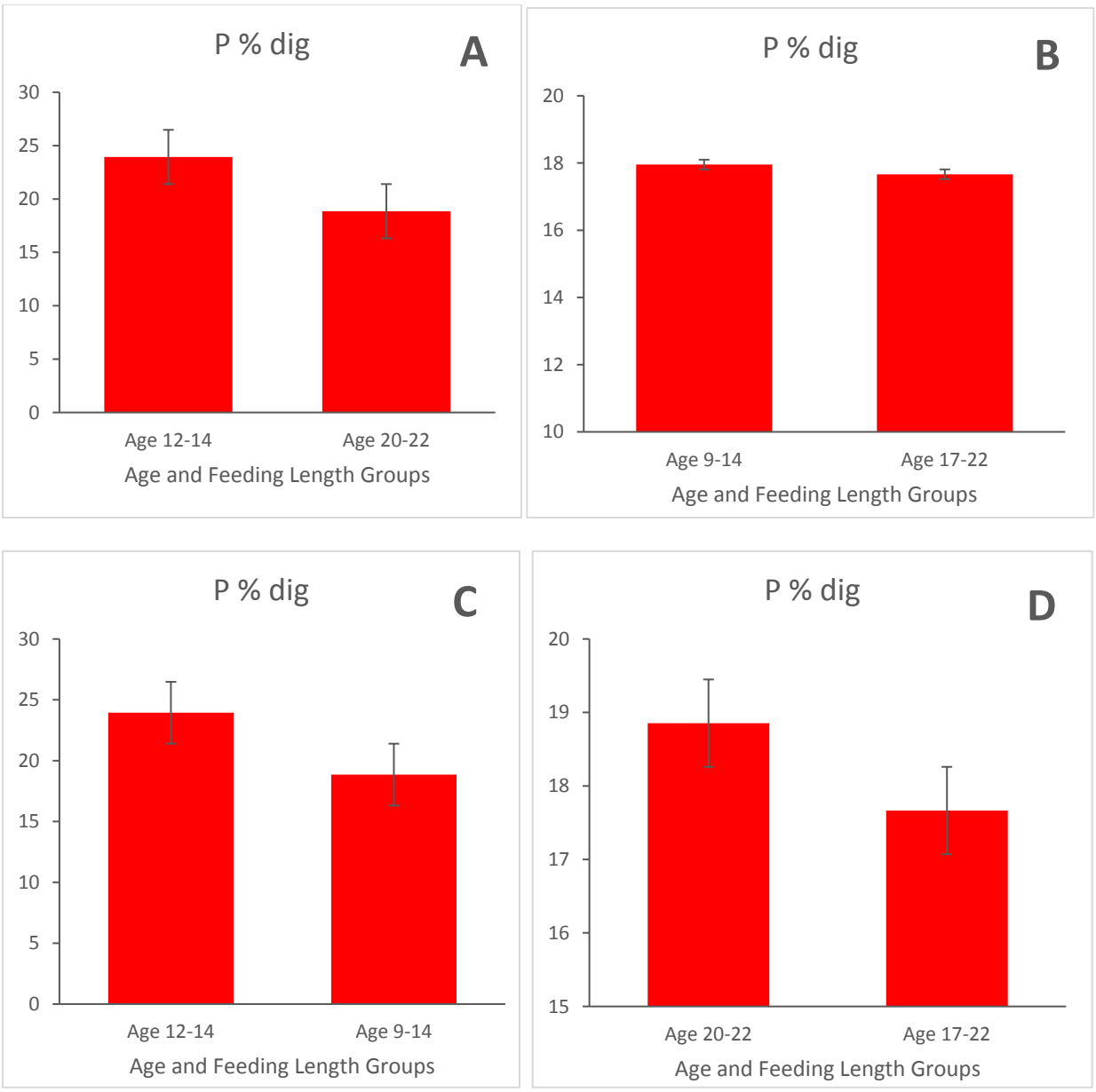
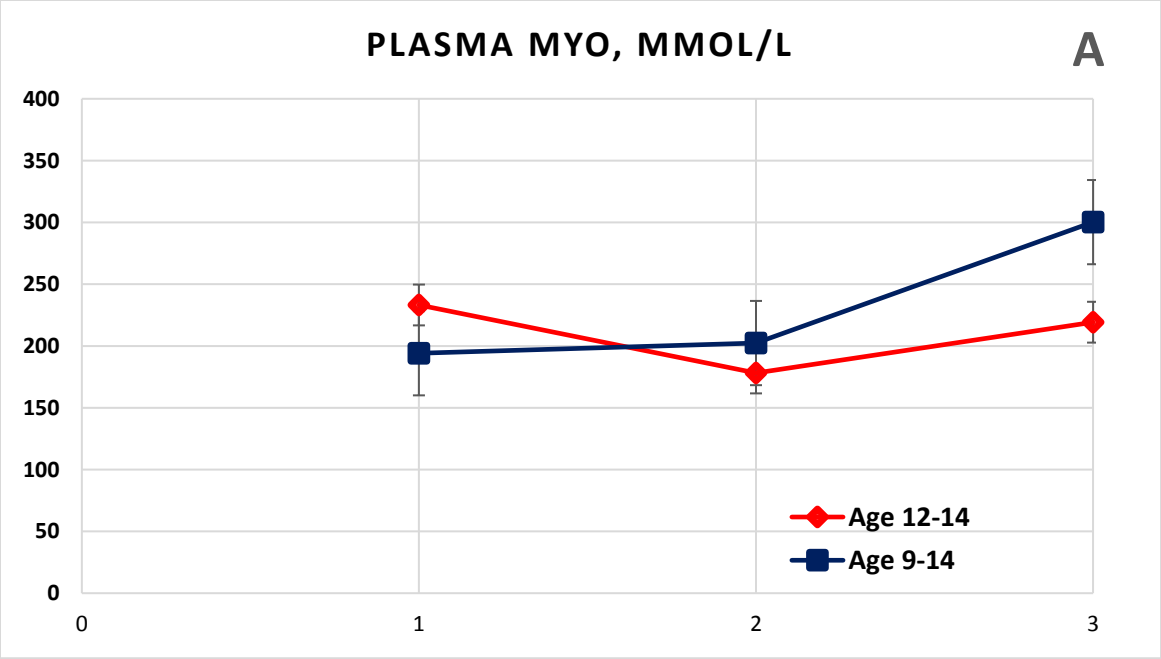


Figure 3-3: Phytase relative to NC diet derived by subtracting apparent P digestibility in NC from 2000 FTU/kg diet in each block. Panel A represents age effect for d 14, and 22 when fed for 2 d from d 12 to 14, or 20 to 22 of age. Panel B represents age effect for d 14, and 22 when fed for 5 d from d 9 to 14, or 17 to 22 of age. Panel C represents age d 14 when fed for 2 or 5 d from d 12 to 14 or d 9 to 14 of age. Panel D represents age d 22 when fed 2 or 5 d from d 20 to 22 or d 17 to 22. Each bar represents a mean of 8 observations.



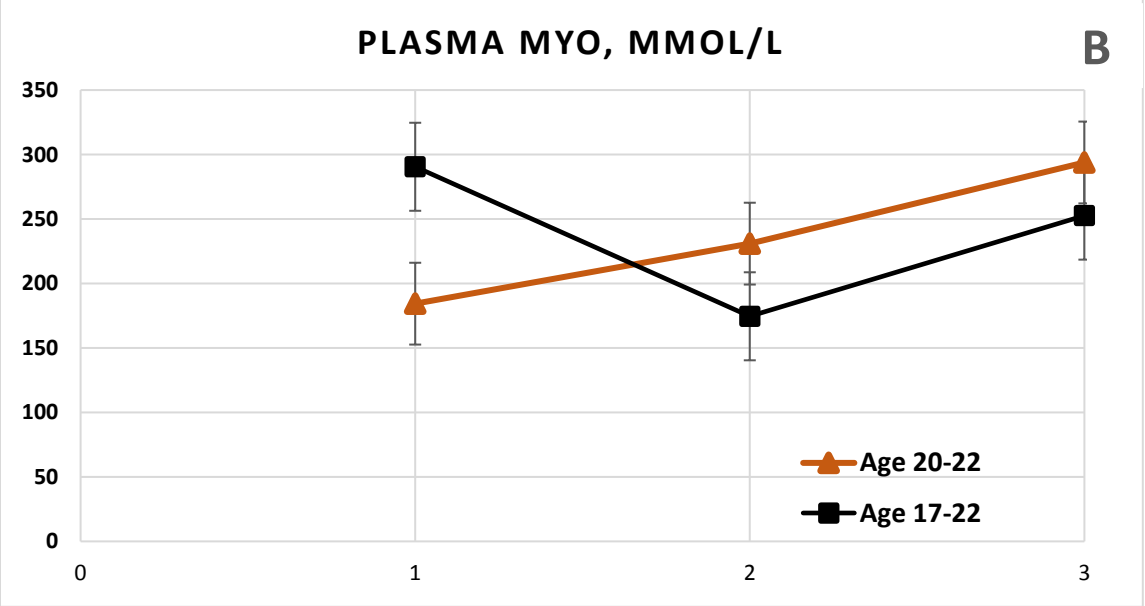


Figure 3-4: Plasma Myo-inositol (MYO) concentrations of broiler chickens fed PC diet (Point 1), NC diet (Point 2) and NC diet supplemented with phytase at 2,000 FTU/kg (Point 3). Panel A represents age d 14 when fed for 2 or 5 d from d 12 to 14 or d 9 to 14 of age. Panel B represents age d 22 when fed 2 or 5 d from d 20 to 22 or d 17 to 22. Each point represents a mean of 8 observations.

CHAPTER 4. SUMMARY

4.1. Summary

Phosphorus (P) is one of the most important minerals necessary for the sustenance of biological life. It plays its role in building up the skeletal system of animals, serving as a building block in the synthesis of RNA and DNA and activation and functioning in several biochemical reactions in living organisms. The loss of P from animal waste into the environment is considered one of the major causes of pollution and this is because animals such as broiler chickens cannot adequately hydrolyze phytate present in cereals and oilseeds used as feed ingredients (Panda et al., 2007; Cowieson and Bedford, 2009). Thus, the use of exogeneous enzymes such as phytase capable of hydrolyzing phytate and releasing P for use by broiler chickens has been encouraged. The age of broiler chickens has been known to influence the utilization of nutrients (Batal and Parsons, 2002). Feeding length has been known to influence P digestibility results obtained from broiler chickens. However, it is not clear if age or feeding length has an influence on the efficacy of phytase.

In chapter 1, the factors affecting nutrient utilization in broiler chickens, and the importance of P in broiler nutrition including sources, bioavailability, utilization and interaction with calcium (Ca) were reviewed. Subsequently, the influence of phytase on P utilization and broiler performance were examined. Most literature revealed that phytase improved growth performance, mineral and nutrient utilization and bone mineralization in broiler chickens fed P deficient diets. Furthermore, we examined factors that could influence the efficiency of phytase in broiler chickens including, sources of inorganic P, Ca levels, anti-nutritional factors, age of birds, and feeding length.

Chapter 2 was a study with the objective of investigating the influence of age and feeding length on phytase efficacy in broiler chickens during the starter phase. We hypothesized that there was no influence of these factors on phytase efficacy. The study investigated the efficacy of phytase in birds fed for 5 age and feeding length combinations including three 2-d feeding lengths to d 8, 14 and 22 respectively, a 5-d feeding length to d 14 and a 16-d feeding length to d 22 post hatching. Birds were fed diets including a positive control (P adequate diet), a negative control (P deficient diet) and a negative control with two levels of phytase inclusion (1,000 and 2,000 phytase units/kg). Results obtained from this study revealed that feeding the P deficient diet to birds for a short period (i.e. 2 or 5 d) had a higher negative effect on the growth performance and mineral utilization than feeding for a longer period (i.e. 16 d). We postulated that birds seemed to adapt to the P deficiency over a longer period through homeostatic changes in their digestive system, thus alleviating the negative impact of P deficiency as compared with feeding for a short period (Li et al., 2015, 2018). We also observed that birds fed the P deficient diets for 2 d to d 14 had a severe impact on mineral utilization than birds fed for the same period at d 8 or 22. Similarly, phytase was more effective in improving mineral utilization in birds fed for a short period than in birds fed for a longer period, and in birds at 14 d of age as compared with birds at 8 or 22 d of age. However, bone mineralization was severely reduced in bird fed for 16 d as compared with birds fed for 2 or 5 d. Phytase similarly increased bone mineralization in birds fed for a longer period as compared with birds fed for a shorter period. From our results, we could conclude that age and feeding length had an impact on phytase efficacy. However, we couldn't fully determine if feeding for a short period (i.e. 2 or 5 d) will have the same effect at all age points.

Chapter 3 included a second study which narrowed the feeding lengths to 2 or 5-d and fed birds for both periods until d 14 or 22 post hatching. Diets were similar with the previous study however, only 1 level of phytase inclusion was included (i.e. 2,000 phytase units/kg) as this elicited

a better response in the previous study. We observed a similar trend with the previous study has birds fed for 2 d at both ages had the greater negative impact of P deficiency on P and calcium digestibility and total tract retention as compared with birds fed for 5 d at both ages. While the impact on tibia ash was greater in birds fed for 5 d than in birds fed for 2 d. Similarly, phytase was more efficient in improving mineral utilization in birds fed phytase supplemented diets for 2 d than in birds fed for 5 d at both ages, and in birds fed at d 14 as compared with birds fed at d 22. Although this age effect was not statistically significant for all parameters observed, results revealed that the super dose of phytase numerically improved the mineral utilization in birds fed for 2 d until d 14 as compared with birds fed at other ages or feeding lengths.

From this research, it could be concluded that birds fed phytase supplemented diets for shorter periods had a greater efficiency on growth performance and nutrient utilization as compared with birds fed for a longer period particularly at the second week of their lifecycle. These results could potentially be important in studies testing new phytase products or comparing the efficiency of various sources of phytase in broiler chickens. Feeding these products to birds for a short period until d 14 could potentially reveal their maximum efficiency when compared to feeding at various time points or through the entire starter phase. Results from this research could also help farmers with determining the best age to supplement phytase into the diets of broiler chickens. Although current industry practices include phytase in broiler diets throughout the entire starter phase, it is possible that including a super dose of phytase at a critical phase and for a short period during the starter period could further boost the performance and nutrient utilization of birds thus increasing yield in the long run. Further research may be needed to investigate the effect of feeding low P diet for even shorter periods (24 or 36hr) to broiler chickens. This could test the sensitivity of birds to changes in dietary P or supplementation of phytase. The effect of age or

feeding length on P transporters in the gut may be equally important in explaining some of the results observed in this work.

4.2 References

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