EVALUATION OF PHYTASE IN BROILER AND LAYING HEN DIETS VARYING IN NUTRIENT DENSITIES AND MINERAL LEVEL

A Dissertation

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ABSTRACT

The use of phytase in broiler production has increased since its adoption as a feed additive. Published information supports the use of phytase to improve the digestibility of Ca and P; however, sources are conflicted about the effectiveness of phytase to improve amino acid and energy digestibility. Therefore, the objective of this research program was to explore the opportunity for use of high levels of phytase to improve poultry production parameters through increased nutrient utilization. It was hypothesized that through dietary manipulation of Ca and P levels, phytase would have a positive impact on growth performance and egg production.

In an experiment, broilers were fed diets with reduced digestible amino acid density and increasing levels of phytase. It was observed that as amino acid density was reduced, growth parameters suffered. However, increasing inclusion of phytase improved growth parameters and was able to recover growth performance lost with the reduction of amino acid density. The results of the experiment provide implications for industry nutritionists where elevating phytase inclusion can lead to improved breast yield and increased profits. Additionally, broiler production performance losses resulting from reducing feed costs through reduced amino acid density can be offset with a high level of phytase.

In a series of 2 trials, broilers were fed diets with reduced nutrient density (amino acid density and metabolizable energy), Ca, P and 2 levels of phytase. It was observed that increasing the inclusion of phytase led to improved growth, while reducing amino

acid density had a negative impact. Results identified the influence of Ca and P levels on the impact of phytase. In reduced nutrient diets, reducing Ca and P had a positive influence on broiler growth and allowed a high level of phytase to recover lost growth performance associated with reduced nutrient density. The implications of this data are that utilizing reduced nutrient densities to lower dietary cost, phytase can be positively influenced with a reduction of Ca and P through increased nutrient digestibility.

The effect of a high level of phytase on egg production of laying hens was evaluated. Laying hens were fed an industry-type positive control, a negative control with reductions in Ca, P and Na, while a high level of phytase was supplemented in the negative control. It was observed that the reduction of minerals had a negative impact on egg production and size; however, phytase was able to improve egg production and egg size. Additionally, the inclusion of phytase was able to recover apparent metabolizable energy (AME) that was lost with the reduction of minerals in the negative control. These data imply that a high inclusion of phytase fed to laying hens can improve nutrient digestibility of AME, Ca, P and Na leading to improved production of laying hens.

In conclusion, the use of high levels of phytase can improve poultry growth performance and production characteristics through improved nutrient digestibility. In broilers this is accomplished through increased breast yield, while in layers it is through increased egg production and size. This research program identifies the importance of adequate understanding of mineral and nutrient levels in poultry formulation with high levels of phytase.

DEDICATION

I would like to dedicate my dissertation work to my family and friends. I am extremely grateful for my wife, Halei, who has been my rock when things have gotten difficult. I appreciate all the laughter that we have shared which always reenergizes my soul when I am tired. Thank you for walking this path with me and encouraging me to study even when I wanted to stop.

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NOMENCLATURE

AMEn	Apparent metabolizable energy (corrected for nitrogen)
BW	Body weight
BXU	Birchwood xylanase unit
CP	Crude protein
dAA	Digestible amino acids
DDGS	Distillers dried grains with solubles
dig.Lys	Digestible lysine
FC	Feed consumption (gram/bird/d)
FCR	Feed conversion ratio
FTU	Phytase unit
GIT	Gastro-intestinal tract
HD	Hen day
IACUC	Institutional Animal Car and Use Committee
IP	Inositol phosphate
ME	Metabolizable energy
NC	Negative control
NPP	Non-phytate phosphorus
PC	Positive control
WOG	Without giblets

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CHAPTER I

INTRODUCTION

Poultry nutrition is an evolving field which is largely driven by least-cost formulation to meet broiler and laying hen dietary needs for optimum performance. Broiler rations in the USA are typically comprised of corn and soybean meal. Corn provides the bulk of the dietary energy through readily digestible carbohydrates, and soybean meal is used to meet the protein and amino acid requirements of the bird. Other ingredients in feed formulations typically consist of synthetic amino acids, sources of macro-minerals (phosphorus (P) and calcium (Ca)), and micro-mineral and vitamin supplements. However, cereal grains also contain phytate which exhibit anti-nutritive properties in monogastric animals such as birds. (Cowieson, et al., 2006a). Nearly two-thirds of the P stored in plants is in the form of phytate, which chelates P, Ca and cations (Rutherfurd, et al., 2012) to an inositol ring and is largely indigestible to monogastric animals. The inositol ring is 6-sided with polar phosphate esters located at each carbon bond in the ring; additionally, these inositol-phosphate (IP) esters are commonly referred to by their position on the inositol ring. Monogastric animals lack the endogenous enzyme (Cowieson, et al., 2011) capable of hydrolyzing the phytate molecule, thereby rendering the chelated minerals unavailable for absorption. Phytase is an enzyme that hydrolyzes the inositol molecule, thus making the chelated P, Ca and other minerals available for absorption. Since the adoption of phytase as a feed additive in poultry diets by Nelson, et al. (1968b), the advancement of phytase efficacy has improved phytate

hydrolyzation approximately from 35 to 40% in the early 1990s to between 60 and 70% with modern phytase products (Cowieson, et al., 2012).

Since phosphate sources are typically the third most expensive nutrient in poultry diets, it is important to have a tool that is cost feasible and reliable to not only successfully release P from chelation, but to also reduce the depletion of phosphate reserves and to minimize the environmental footprint of poultry production (Kiarie, et al., 2013). The use of phytase allows for the reduction of phosphate addition to the poultry diet as enzymatic breakdown of phytate will provide P from the inositol stored in the plant material, thereby creating an overall reduction of manure P content (Onyango, et al., 2005).

Standard phytase inclusion in broiler diets does offer benefits beyond a P and Ca reduction, as it has been shown to increase body weight (BW) and nutrient utilization in broilers fed diets that have reduced Ca and P levels (Cowieson, et al., 2006b). When included in laying hen diets, phytase releases P and Ca that are both necessary for eggshell formation and can improve egg production (Boling, et al., 2000). A study by Meyer and Parsons (2011) found that adding phytase to laying hen feed at a minimum inclusion of 150 FTU/kg with 0.105% non-phytate phosphorus (NPP) to laying hens was able to offer the same performance and egg production as a feed with 0.45% NPP and no phytase from 32 to 62 wk of age. Studies evaluating the efficacy of phytase have evolved into identifying benefits of elevated (super) doses (more than 3X) as compared to a standard inclusion. Super dosing phytase originally was investigated to identify additional hydrolyzation of the inositol ring. Shirley and Edwards (2003) observed that

inclusion of phytase at 12,000 FTU/kg resulted in almost complete hydrolyzation and release of phytate bound P. However, improvements in BW and feed conversion ratio (FCR) have repeatedly been shown when feeding super dose levels of phytase as numerous studies show additional benefits in broiler performance (Shirley and Edwards, 2003; Walk, et al., 2014; Pieniazek, et al., 2016a). Improvements were also identified in breast weight by Campasino, et al. (2014) who noted that birds fed a diet with 1,600 FTU/kg of phytase had increased breast weight of 49 g per bird compared to a control diet adequate in P and Ca. The benefits associated with elevated phytase and P release are well understood, however the growth promoting side effects have been examined less.

Walk (2016) hypothesized that higher levels of phytase are able to completely hydrolyze the inositol ring leading to a release of not only P, but Ca as well. The higher esters (IP6 and IP5) on the inositol ring are known to have a higher affinity for Ca with as many as 5 Ca ions (Selle, et al., 2009) for every P on the IP ester, and decreasing affinity moving down the esters, eventually returning to a 1:1 Ca to P release ratio at the lower esters. The use of phytase at elevated levels is sufficient to overcome the negative effects of a high Ca:P as the phytase not only breaks down the IP6 and IP5 esters, but can continue to the lower IP esters, helping to reduce the Ca:P. Additionally, extra dietary Ca can act as a buffer when ingested at lower pH, forming chelations with phytate (Taylor, 1965) and interfering with phytase's ability to hydrolyze the lower IP esters (Bedford and Rousseau, 2017). The work by Driver, et al. (2005), Walk (2016) and others demonstrate the importance of not over-feeding Ca in poultry diets and

understanding the usefulness of phytase as a tool to overcome the antagonistic effects of Ca on P.

The negative attributes credited to phytate are numerous and complex, however previous research has not been able to conclusively define its impact on protein digestibility in broilers. To assure that broilers are able to meet their growth potential, synthetic amino acids are often added to diets; however, these feed ingredients are among the costliest when formulating on a least-cost basis. Since phytate is a major anti-nutrient found in the majority of poultry feeds, it should be thoroughly investigated as it has been shown to impede protein utilization (Cowieson, et al., 2006a; Ravindran, et al., 2006). Kiarie, et al. (2013) hypothesized that phytate impacts protein digestibility at low pH where it binds to protein by attaching to the α-NH_2 groups of arginine, histidine and lysine, thereby rendering those amino acids unavailable for digestion.

Modern broiler diets are formulated with digestible lysine (dig.Lys) in ratio to the other essential amino acids, even though it is often the second limiting amino acid behind methionine (NRC, 1994). It is well established that protein plays an integral role in broiler growth, as reducing amino acid density in feed leads to reductions in growth performance, ultimately reducing breast meat weight (Dozier, et al., 2008). Early work by Munks, et al. (1945) demonstrated that breast tissue has the largest deposition of lysine, containing approximately 7% lysine, making the case that reducing amino acid density in today's broiler rations has an immediate detrimental effect on breast development. Kidd, et al. (2004) found that utilizing a diet with a high amino acid density increased BW and breast yield and improved FCR. However, in the same study,

it was identified that reducing amino acid density reduced BW and breast yield while increasing FCR. Due to the interactions with phytate, it is logical to assume that the use of phytase could offer improvements in protein digestion, however when this hypothesis has been applied, results have been inconsistent (Augspurger and Baker, 2004; Ravindran, et al., 2006; Pieniazek, et al., 2016b).

Therefore, the research program described herein was undertaken to determine if improvements in broiler performance could be achieved with high inclusion rates of phytase in diets that vary in amino acid density. Additionally, the effect of Ca and P levels on phytase efficacy was explored. The effect of a high inclusion of phytase in laying hen feeds was also explored in an attempt to determine the potential for improvements in growth at phytase inclusion rates above 500 FTU/kg.

CHAPTER II

LITERATURE REVIEW

Phytate

Anti-nutrient

Inositol hexaphosphate, more commonly known as phytic acid, or simply as phytate was first identified in 1872 by Pfeffer, however it took nearly 100 years for the chemical structure to be identified (Oberleas, 1973). Phytate is present in many plant-based feedstuffs and comprises around 70% of the total P in cereal grains (Erdman, 1979; Cowieson, et al., 2006a). Plants store P in the seed, which is also the primary plant material used in animal agriculture (Nelson, 1967). However, phytate concentration varies depending on the part of the plant and the maturity of the plant at time of harvest (Oberleas, 1973). Starting about 3 wk after pollination and increasing during maturity, P in plants is transferred to the seeds via roots and leaves where it is stored primarily as phytate for later use during germination (Erdman, 1979).

Animals require inorganic P for use in their skeletal systems as it is one of two major nutrients needed for the formation of bone. However, P is also needed on the cellular level as it is a key-component in reactions including those of the TCA cycle where energy is produced. Due to the chemical structure of the phytate molecule, hydrolysis of the ester bonds between the phosphate group and inositol ring is necessary prior to the bound P being utilized by the animal (Cowieson, et al., 2004). Nelson (1967) observed that phytate is largely indigestible, with only up to 10% being absorbed in poultry as they lack the endogenous enzyme capability of hydrolyzing the ester-

phosphate linkages. For example, approximately 30% of total P present in plant stuffs is considered to be available for use by poultry; and thus it is essential to provide additional ingredients in poultry diets with higher availability of P (NRC, 1994).

According to Kornegay (1996), phytate has a high affinity to chelate cations such as Ca, Fe, Zn, Mg and Cu; however these chelations are dependent upon pH (Erdman, 1979). An experiment by Davies and Nightingale (1975) illustrated the ability of phytate to chelate minerals in rats, as a ration including 10 g/kg (1%) of phytate significantly increased excretion of Fe, Cu, Mg and Zn. Maenz, et al. (1999) describes how cations can effectively inhibit the successful hydrolysis of phytate and ranked them from highest inhibition to least: Zn^{2+}>Fe^{2+}>Mn^{2+}>Fe^{3+}>Ca^{2+}>Mg^{2+}. This ranking is representative of a neutral pH of 7 where phytate hydrolysis was inhibited by 50% from chelations with 0.053 mM Zn^{2+} up to 4.87 mM Mg^{2+}. However, it was observed that the inhibitory effect on phytate hydrolysis decreased as the pH was reduced to 4.0. Phytates' ability to chelate these cations is predicated upon its chemical structure as the higher weighted inositol phosphate (IP) esters such as IP6 have increased ability to donate electrons and prefer to bind cations that have available protons compared to the lower weighted IP esters. According to Cheryan and Rackis (1980) cations are able to be complexed within a phosphate ester, between two esters, or even between phosphate esters of separate phytate molecules. The ability to share electrons from the phosphate esters creates the opportunity for phytate to attract minerals that desire electrons to correct their polarity. However, as minerals are chelated to phytate-phosphate esters, H^{+}

ions are made free, thereby reducing pH and creating the term “phytic acid” (Cheryan and Rackis, 1980).

Myo-inositol

Myo-inositol is a cyclical sugar alcohol that is widely distributed in plant and animal cells while being essential for cellular function (Cowieson, et al., 2013). In plants, free myo-inositol is generated during germination as phytate is hydrolyzed and then metabolically recycled for use as the plants primary source of sugar. Conversely, free myo-inositol is also created from D-Glucose 6-P with a series of enzymatic conversions to Ins(3)-P_1 and a series of phosphorylations resulting in inositol hexakisphosphate (IP6) which is then phosphorylated back to the inositol ring as phytate (Yoshida, et al., 1999; Loewus and Murthy, 2000). In preparation of phytate containing feedstuffs, chemical or enzymatic dephosphorylation may occur, resulting in myo-inositol esters containing anywhere from 1 to 5 phosphates attached (Knuckles, et al., 1989). In mammals, myo-inositol is created after phytate hydrolysis where the phosphate ester is cleaved from the inositol ring (Loewus and Murthy, 2000).

Myo-inositol is necessary for growth in plants and bacteria where enzymes naturally exist to dephosphorylate the myo-inositol of its phosphate esters (Loewus and Murthy, 2000). However, myo-inositol, when fed to animals, has attached phosphate esters which renders it an anti-nutrient that can inhibit protein digestion. In an experiment by Knuckles, et al. (1989), myo-inositol phosphate esters inhibited the digestion of casein by pepsin. It was observed that the extent of digestion was dependent upon the degree of phosphorylation as IP6 had reduced digestion and rotating

around the phytate molecule from IP5 to IP2 increased digestion as the molecular weight and polarity of the phosphate esters were reduced. Furthermore, Cowieson, et al. (2006a) demonstrated a similar effect where broilers fed IP6 had a significant reduction in the digestibility coeffecients of amino acids and N, while also leading to an increased excretion of endogenous minerals. This was supported by work from Ravindran, et al. (2000) where it was found that amino acid and energy availability were both reduced in the presence of IP6. However, a study by Żyła, et al. (2012) found that fully dephosphorylating the phytate esters led to the availability of free myo-inositol, which improved broiler growth. Furthermore, work by Cowieson, et al. (2013) demonstrated that free myo-inositol (0.15%) supplemented into diets deficient in Ca (0.71%) and NPP (0.30%) led to improved BW and FCR compared to a normal ration with +0.14% Ca and +0.12% NPP.

These experiments support the hypothesis that fully dephosphorylating phytate leads to improvements in bird performance via reductions of phytate esters leading to increased availability of free myo-inositol, P, Ca, other cations as well as protein and energy. It is thought that the mechanism behind improvements observed with free myo-inositol stems from its structure as it is a cyclical sugar alcohol similar to glucose (Cowieson, et al., 2013) which can increase the production of insulin (Cowieson, et al., 2015) to improve the absorbability of nutrients and energy leading to improved performance. However, as discussed by Cowieson, et al. (2013) free myo-inositol stems from a cascade of hydrolyzations ridding the phytate structure of phosphate esters.

Calcium

Anti-nutrient

Ca is abundant in the earth and as such it is a relatively low-cost ingredient for animal feeds. The low-cost and wide availability of Ca sources used in animal feeds generally lends to feeding Ca at higher than necessary levels to provide a safety margin for the bird (Bedford and Rousseau, 2017). Nutritionists historically have added Ca in excess of the bird's requirements as it is essential for bone formation, growth and excretion as shell in layers. The use of Ca is especially significant in rapidly developing strains of broilers that are commonly used in the poultry industry. Ca, while widely used in the body, is not necessarily readily available for digestion as it is chelated to phytate. It was shown by Nelson, et al. (1968a) that with inclusion of 1% phytate into the diet, as much as 36% of the total dietary Ca could be bound. It was identified by Erdman (1979) that per 1 mole of phytate, 3 to 6 moles of Ca are chelated and made insoluble at a neutral pH along with P. Luttrell (1993) reported phytate chelations are primarily centered around the higher molar weight IP6 esters where Ca is bound in a 5 to 1 ratio with P (Selle, et al., 2009) and that ratio is reduced as the IP esters decrease in molar weight from the 6 to the 1 position eventually falling to a 1 to 1 ratio. However, Luttrell (1993) also described how the affinity for phytate to bind cations largely depends on the number of phosphate substituents located on the inositol molecule as an IP3 would only have a mild affinity and would likely only attract Ca in a 1 to 1 ratio with P. It has also been noted (Erdman, 1979; Luttrell, 1993) that these chelations are pH dependent,

however Ca in and of itself does have an effect on the gastro-intestinal tract pH (Shafey, et al., 1991; Zeller, et al.).

According to a study by Shafey, et al. (1991), it was found that increasing the concentration of dietary Ca from 10.7 g/kg to 25.3 g/kg led to increased pH of the crop and ileum, but did not alter the pH of the rest of the GIT. Furthermore, increasing dietary Ca from 10.7 g/kg to 25.3 g/kg led to 70 to 92% of Ca, Fe, Mg and Zn becoming insoluble. An experiment conducted by Mohammed, et al. (1991) demonstrated that reducing dietary Ca content from 10 g/kg to 5 g/kg in low P diets (5 g/kg total P, 2.6 g/kg NPP) led to a 15% increase in phytate P digestibility in broiler chicks. Walk (2016) asserted that high dietary Ca can negatively influence broiler growth while reducing the availability of protein and amino acids in monogastrics through the formation of crystals, thus chelating cations and reducing nutrient availability. Furthermore, high levels of Ca will chelate with phytate in the small intestine, thereby reducing the availability of Ca and other cations. This was supported in a study by Li, et al. (2016) who demonstrated that increasing dietary Ca concentration from 0.7 to 1.0% led to significantly increased IP6 concentration in the crop, and decreased ileal disappearance of IP6 was observed with 62.3% disappearance at 0.7% Ca and 57.5% disappearance at 1.0% Ca. The study highlighted the effects of increasing Ca concentration on phytate degradation as the higher inclusion of Ca was able to form chelations more readily with phytate and prevent degradation.

Calcium Requirements

Ca is required by poultry for skeletal growth, metabolic needs and eggshell formation; however, the two breeds of poultry primarily used in animal agriculture vary completely in their requirements. According to the National Research Council (NRC (1994), pullets reared for egg production require Ca:NPP at approximately 2:1, which is similar to broilers as they mature. When pullets reach 18 wk of age they are placed on a pre-lay ration that increases the concentration of Ca to 2%, elevating the Ca:NPP to 6:1. The hen metabolizes Ca into the medullary bone and then uses those stores to secrete Ca as shell over the developing egg. Too little Ca or P can lead to deficiency symptoms including the presence of rickets (Taylor, 1965). Research has shown that once requirements are met, Ca:NPP becomes critical to growth and development as excess Ca can result in anti-nutritive effects, as discussed previously. Selle, et al. (2009) observed that phytate limits the availability of P and Ca as a result of insoluble Ca-phytate complexes which depend on the relative ratios of Ca to phytate. Amerah, et al. (2014) demonstrated in broilers fed a base of 0.28% NPP and increasing amounts of Ca that negative effects were observed in weight gain, feed consumption (FC), FCR and amino acid digestibility with higher Ca:NPP of 2.86:1 and 3.57:1. The reductions in performance were attributed to reduced phytate hydrolysis caused by the increased Ca forming chelations with phytate and the increased GIT pH observed as Ca can reduce acidity. As discussed by Walk (2016) and Bedford and Rousseau (2017), utilizing high concentrations of Ca with deficient levels of NPP is worse than having a low Ca:NPP;

as a NPP deficiency is enhanced in the presence of high Ca concentrations due to the chelating effect of excess Ca rendering both minerals insoluble.

Although not as exhaustively studied as in broilers, Ca research in laying hens is sufficient to be conclusive. The Ca mechanisms are similar in broilers until the hens reach sexual maturity around 18 wk, requiring a sharp increase in dietary Ca. Using a commercial strain of laying hens, Gordon and Roland (1998) demonstrated that increasing Ca from 2.5% to 3.1% significantly improved shell quality within 1 wk of supplementation. Including Ca at 2.5% in laying hen diets represents a deficiency, so increasing to 3.1% makes sense as Ca is the majority of the secretion by the uterus that forms the eggshell. Liu, et al. (2007) found in hens fed a PC with 3.30% Ca and 0.28% NPP and that reducing Ca by 0.12% and NPP by 0.13% led to overall reductions in FC, egg mass, shell strength, and digestibility of Ca, P, amino acids and N. This experiment highlighted the impact of reducing Ca and NPP to deficient levels resulting in a loss in performance.

Nutrient Density

Amino acid requirements have been well understood for decades in poultry for laying hens and broiler type chickens. Munks, et al. (1945) described how chickens are unable to synthesize the essential amino acids from CP. Then 1945 hen required 352 mg arginine, 111 mg histidine, 283 mg lysine, 78 mg tryptophan, 216 mg threonine, 315 mg phenylalanine, 286 mg methionine, 260 mg tyrosine and 132 mg cystine to produce 1 egg. It was also found that tyrosine and cystine could be supplemented by additional phenylalanine and methionine, respectively. Additionally, muscle tissue had differing

amino acid requirements compared to egg formation as muscle requires increased concentrations of arginine, histidine, lysine and threonine, while cystine and glycine are useful for feather formation. Over time these requirements have changed as the strains of birds have been genetically selected for their desired traits. Broilers are now able to reach their market weights in about 6 wk, compared to the 16 wk it required broilers of the 1950s (Schmidt, et al., 2009). An experiment by Schmidt, et al. (2009) compared the body conformations of a modern strain of broilers (Ross 708) compared to an unselected heritage line from the 1950's. It was found that at 5 wk of growth the modern broiler had reduced heart mass, earlier maturation of the liver, and 20% additional length of the jejunum and ileum. It was thought that these differences in organs provided for the breast weight to account for 18% of the total mass of the bird compared to the 9% of the heritage line. The increased length of the jejunum and ileum was concluded to provide increased nutrient absorbability leading to an improved FCR. Performance characteristics have continually changed since the vertical integration of the poultry industry and the ongoing efforts to improve poultry performance through genetics. However, these improvements in performance are correlated with better understanding of feed ingredients.

The use of synthetic amino acids has allowed nutritionists to exercise greater precision inclusions of limiting amino acids in formulation programs. The majority of feed mills in the poultry industry routinely add synthetic forms of lysine, methionine and threonine, while additional sources of isoleucine, valine and arginine are now available and becoming more economically feasible. In corn-soybean meal diets, the first limiting

amino acids, in order, are methionine, lysine and threonine. The fourth limiting amino acid can be valine, isoleucine, tryptophan or arginine depending on if the ration is an all-vegetable diet, includes animal by-products or more cereal grains (Berres, et al., 2010). Poultry feed mills generally mix rations with synthetic forms of lysine, methionine and threonine; however the CP content of dietary formulations is generally high enough that no deficiency symptoms of the fourth limiting amino acid is typically observed (Berres, et al., 2010). Crude protein in poultry diets is used as a safety margin in formulation for essential amino acids as the requirements for lysine, methionine and threonine are largely met using synthetic amino acids. However additional benefits of utilizing synthetic amino acids were identified by Jacob, et al. (1994) as a 2.5% reduction in CP with the supplementation of synthetic amino acids led to a 21% reduction of nitrogenous excretions (Ferguson, et al., 1998). Further reductions of CP may yet still be feasible given the utilization of synthetic forms of valine and isoleucine that allow for the precision formulation of the fourth limiting amino acid to be met, or perhaps through the use of an exogenous enzyme that can give credit for protein utilization.

Currently, nutritionists mostly choose to ratio essential amino acids to lysine on a digestible basis rather than setting requirements for each individual amino acid. This allows for simplicity in formulation as the ratio between amino acids will not change, however all inclusions will change based upon the identified inclusion of dig.Lys (Tillman and Dozier III, 2013). Utilizing amino acid ratios based on dig.Lys allows for nutritionists to supply a proper balance of protein (Berres, et al., 2010) and optimize the animal's use of the available protein. The inclusion of dig.Lys is regarded as highly

important for breast meat yield (Vieira and Angel, 2012), which is typically the driving force in broiler production. Kidd, et al. (1998) demonstrated that when broilers are fed diets with an increased amount of dig.Lys as identified by the NRC (1994) improvements can be observed in performance, breast meat weight and breast yield. The improvements in breast yield and weight are related to the role of lysine to increase protein accretion via satellite cells in breast tissue in the early stages of broiler growth.

An experiment by Kidd, et al. (2004) evaluated amino acid density on broiler growth performance through 49 d with the goal of identifying a feeding strategy that could be implemented by production nutritionists to increase breast yield. Diets with the high level of amino acid density were fed with CP and lysine at 22.50 and 1.37%, 21.50 and 1.20%, 18.50 and 1.08%, 17.50 and 1.03% in the starter, grower, finisher and withdraw phases, respectively. It was observed that utilizing the higher level of amino acid density led to improved performance throughout the study and increased carcass and breast yield at d 50. Dozier III, et al. (2006) determined that in broilers fed isonitrogenous rations with apparent metabolizable energy (AME) of 3,175 and 3,310 kcal/kg from d 30 to termination at d 59 performance was improved as AME was increased, however breast yield was reduced at the higher level of energy. Similar results to these studies were identified in a series of experiments conducted by Dozier, et al. (2007) where different approaches were used to target optimized breast meat yield in broilers from d 42 to 56. They utilized a high amino acid density at 18% CP, 0.98% lysine, 0.83% sulphur amino acids, and a moderate density at 16.2% CP, 0.88% lysine and 0.75% sulphur amino acids. Those amino acid densities were then crossed with 2

levels of AME consisting of a moderate (3,240 kcal/kg) and a low (3,140 kcal/kg) in the first experiment and a high (3,310 kcal/kg) and a moderate (3,220 kcal/kg) in the second experiment. In both experiments, it was observed that reducing dietary AME and amino acid density increased FC and FCR; however, the low level of AME (3,140 kcal/kg) actually led to increased breast meat yield (compared to 3,240 kcal/kg), which is likely due to the broilers increased FC of the birds leading to increased amino acid consumption and protein accretion in the breast. As expected, increases in breast yield were observed with increased amino acid density in both experiments. It was also observed that utilizing AME at 3,310 kcal/kg did lead to improved growth performance (BW, FC and FCR) compared to 3,220 kcal/kg, however the improvements in BW were attributed to fat deposition as carcass yield was reduced at 3,310 compared to 3,220 kcal/kg. The work by Dozier, et al. (2007) demonstrated the importance of amino acid density and ME in broiler diets, however it also supports the idea that nutritionists can improve breast yield by reducing AME and maintaining amino acid density.

Phytase

Overview

Phytase is perhaps the most researched enzyme in animal nutrition with thousands of experiments conducted since the late 1960s aimed at evaluating its efficacy and potential opportunities to increase inclusion rate. Phytase is an enzyme that is capable of hydrolyzing the phytate-inositol bonds rendering the P in phytate readily available for absorption by monogastric animals (Cowieson, et al., 2011). The utilization of phytase in poultry feeds can be traced back to the pioneering work

conducted by Nelson, et al. (1968b) who identified the potential for phytase to break down phytate and liberate phytate P. At the time of the work by Nelson, phytate was already identified as a major anti-nutrient and researchers were seeking ways to improve P release from phytate (Nelson, 1967). Nelson, et al. (1971) reported the use of an *Aspergillus spp.* phytase ranging from 950 to 7,600 FTU/kg combined with dietary phytate ranging from 0.18 to 0.24% phytate P in a corn-soybean meal diet. The results from that experiment indicated that increasing phytase inclusion did improve BW gain in a linear fashion, however bone ash saw very little improvement as the inclusion of phytase increased from 3,800 to 7,600 FTU/kg. It was identified that the disappearance of phytate P did increase in correlation with phytase inclusion and was maximized at 94.4% with the inclusion of 7,600 FTU/kg.

Since the work of Nelson in the late 1960s, it took until the adoption of phytase by the poultry and swine industries in 1991 for phytase to be relative in the market (Cowieson, et al., 2011). Phytases can generally be categorized into 2 types: 3-phytases, which begin hydrolyzing the phytate molecule at the IP3 ester and are bacterial or fungal in origin, and 6-phytases, which begin hydrolyzing the phytate molecule at the IP6 ester and originate from plants. Typically 6-phytases are derived from the fungi *Peniophora lycii,* or bacteria *Escherichia coli* (Lei and Porres, 2003; Selle and Ravindran, 2007). In the early 2010s, it was suggested by Kiarie, et al. (2013) that an estimated $3 to 5 billion (US) was saved per year in the global feed market through the use of exogenous enzymes in animal feeds. Of the exogenous enzymes being used worldwide, phytase

dominates at approximately 60% of the market due to its impacts on P liberation and ability to enhance animal performance.

The efficacy of phytase was evaluated in laying hens by Francesch, et al. (2005) over 24 wk with phytase inclusions at 0, 150, 300 and 450 FTU/kg added to corn or barley diets with CP at 17.0%, lysine at 0.85%, Ca at 3.6% and NPP at either 0.32% (11.2:1 Ca:NPP) for the positive control (PC) or 0.13% for the negatice control (NC). It was observed that without the supplementation of phytase to reduce the deficiency of NPP in the NC, the hens were not able to maintain the same production, weight gain, FC and bone ash as the birds supplemented with NPP in the PC. Furthermore, it was observed that P absorption increased linearly as phytase inclusion was elevated signifying that utilization of higher levels of phytase could further improve P retention.

An experiment conducted by Dilger, et al. (2004) utilized broiler chicks fed a corn-soybean meal ration through 42 d including a PC and a NC with reductions of -0.34% Ca, -0.26% NPP, and an increased energy of 60 kcal/kg in the starter while the grower had reductions of -0.28% Ca, -0.20% NPP and an increased energy of 48 kcal/kg. The NC diet was then supplemented with 6-phytase at 500, 750 and 1,000 FTU/kg and a 3-phytase included at 500 FTU/kg. It was observed that the 500 FTU/kg of both the 3-and 6-phytases had identical performance throughout the experiment, while increasing inclusion of the 6-phytase led to further growth improvements compared to 500 FTU/kg. However, it was observed that the 6-phytase did have increased tibia ash content compared to the 3-phytase, signifying that through d 22 it was more efficacious at releasing phytate P. This was also observed in a study conducted by Augspurger, et

al. (2003) where it was found that broiler chicks through d 14 fed 500 FTU/kg of 2 fungal 3-phytases or an *E. coli* 6-phytase had improved weight gain and tibia ash with the 6-phytase compared to the 3-phytases. Furthermore, it was identified that utilizing 500 FTU/kg of the 6-phytase led to improved P release compared to the 3-phytases with values at 0.108% vs 0.081% and 0.043%.

As discussed by Hatten, et al. (2001), supplementation of phytase can free chelated cations (Ca, Mg, Zn, Cu, N) and gastro-intestinal tract (Zeller, et al.) proteases bound to phytate complexes, leading to improvements in production parameters. Phytase has been proven to ameliorate the effect of phytate and also to improve performance in P deficient birds with an inclusion of 500 FTU/kg, resulting in the release of P at levels sufficient to meet nutritional needs (Cowieson, et al., 2011). Additionally, Onyango, et al. (2005) reported that use of phytase at 500 and 1,000 FTU/kg was sufficient to increase nutrient digestibility including amino acids, P, Ca and N which led to improvements in growth performance (BW and FC). Um and Paik (1999) found that laying hens fed diets containing phytase at 500 FTU/kg had improved retention of Ca, P, Mg, Fe and Zn leading to improvements in egg production. These experiments illustrate the effectiveness of phytase to improve production performance through increased nutrient digestibility. Many poultry companies currently utilize a higher level (super dose) of phytase as it was shown as long ago as Nelson, et al. (1971) that higher levels of phytase yield improvements over a lower inclusion of phytase.

Extra-phytic Effects

Super dosing phytase is generally understood as being equal to 3 times or more the normal inclusion of phytase (500 FTU/kg) (Lee, et al., 2016). A review by Selle and Ravindran (2007) suggested that increasing dietary P impedes the response of increased phytase inclusion. They hypothesized that the inhibition could stem from increased release of P prompting a Ca:NPP imbalance in the GIT, or possibly from the released inorganic P inhibiting the catalytic activity of phytase (Greiner, et al., 1993; Lei and Stahl, 2000). An experiment was conducted by Shirley and Edwards (2003) on broiler chicks through d 16 utilizing a corn-soybean meal diet with 22.2% CP, 0.88% Ca and a reduced NPP at 0.272% combined with increasing levels of a fungal phytase from 0 to 12,000 FTU/kg. They found that BW and gain:feed of those chicks was optimized at 12,000 FTU/kg while leading to a 95% reduction in phytate P compared to 40% with no phytase. However, while performance was reported to be optimized at 12,000 FTU/kg, it was recognized that few statistical differences were observed with inclusions between 1,500 and 12,000 FTU/kg. The lack of statistical differences between two inclusion rates raises the question of is an ultra-high inclusion of phytase is more beneficial than a reduced inclusion. Evaluating this idea further, Cowieson, et al. (2006b) fed broiler chicks through d 16 a PC diet with analyzed nutrients of 1.23% Ca and 0.51% NPP (2.40:1 Ca:NPP) and a NC with 1.23% Ca and 0.33% NPP (2.92:1 Ca:NPP). The NC was supplemented with an *E. coli* 6-phytase with inclusions of 0, 150, 300, 600, 1,200, 2,400 and 24,000 FTU/kg. It was observed that the reduction of minerals in the NC led to reduced performance (BW, FI and FCR) compared to the PC. Inclusions above 150

FTU/kg were sufficient to improve weight gain, toe ash and nutrient utilization compared to the NC diet. Phytase included above 1,000 FTU/kg led to improved nutrient utilization beyond those birds fed less than 1,000 FTU/kg. Furthermore, it was identified that the addition of phytase improved the digestibility of all amino acids. However, there was little improvement beyond 600 FTU/kg as the inclusion of 150 and 300 FTU/kg was sufficient to increase amino acid digestibility, which could help explain the performance improving ability of phytase as BW and FCR were both improved. Recovery of P from phytate hydrolysis was improved by 21% with the addition of phytase which improved the Ca:NPP to 2.69:1. Additionally Cowieson, et al. (2006b) observed that irrespective of treatment, the ratio of metabolizable Ca:P was roughly constant, suggesting that Ca influences P retention.

While reports on the effects of super dosing phytase on broiler performance are varied, it is clear from research that an effect does exist; however, its impact is likely related to dietary Ca and NPP levels. Walk (2016) asserts that in poultry diets with higher Ca content, phytase activity can be reduced as Ca binds to attachment sites on the phytate molecule at pH found in the GIT, thus rendering a reduced efficacy of phytase (Tamim and Angel, 2003). Given that a wide Ca:NPP has a negative impact on broiler performance (Shafey and McDonald, 1990), inclusion of low levels of a 6-phytase may hinder bird performance further as the preferred attachment site of 6-phytase to begin hydrolyzing phytate is the IP6 ester.

When discussing Ca:NPP, it should be understood that Ca is formulated on a total basis, however as identified by Nelson, et al. (1968a), 1% of dietary phytate can

chelate up to 35% of total dietary Ca so total does not imply digestibility. Historically, Ca:NPP have been higher to meet the dietary needs of birds while accounting for the nutrients that were unavailable due to phytate. Phytase has long been known to liberate both Ca and P; however, it doesn't create these nutrients, rather they are made available from hydrolyzations of the phytate molecule where the IP6 ester alone can release up to 5 Ca molecules for every P (Selle, et al., 2009). This interaction would thereby increase the digestible Ca:NPP and further hinder performance through reduced protein digestion from the increased GIT pH corresponding with the elevated presence of Ca as well as increased incidence of Ca crystals. Walk (2016) discusses how in high Ca diets, a standard dose of phytase (500 FTU/kg) should be sufficient to hydrolyze the IP6 ester, however an increased phytase inclusion should be sufficient to overcome the negative effects of high Ca levels and return the release ratio back to 1 Ca for every P with further hydrolyzation of the lower IP esters. So, unless the phytase inclusion is exceptionally high when formulating diets with higher than optimal Ca:NPP, reduced efficacy can be expected.

A report by Walk, et al. (2012) describe how in broilers reared to d 21, increasing the Ca:NPP from 1.4:1 to 2.8:1 led to reductions in BW and FC, while the utilization of phytase increased BW, FC and reduced FCR. Based upon the report by Walk, et al. (2012), nutritionists formulating diets containing phytase based upon the NRC (1994) recommended 2:1 Ca:NPP may consider revising to optimize potential growth. It was also observed by Walk, et al. (2012) that utilizing a 1.4:1 Ca:NPP ratio provided optimized BW with 500 FTU/kg and reduced the efficaciousness of the 2,500 FTU/kg

inclusion. Increasing Ca:NPP to 1.88:1 led to phytase at 500 and 2,500 FTU/kg providing similar BW, while further increasing Ca:NPP to 2.8:1 led to only slight improvements in BW as phytase was elevated. Utilizing phytase at 500 FTU/kg with a 2.8:1 Ca:NPP did provide improved BW compared to no inclusion, however a greater improvement was observed with 2,500 FTU/kg. Thus, it can be concluded that super dosing phytase can provide increased benefits, however Ca and NPP levels do play a role in the efficacy of phytase.

Gordon and Roland (1997) used laying hens from 21 to 38 wk of age to evaluate the efficacy of phytase added at 300 FTU/kg in diets with Ca at 4.00% and NPP ranging from 0.1% to 0.5%. It was observed that 0.1% NPP in the diet reduced egg production and FC, but the supplementation of phytase was able to improve performance to be equal to the other NPP levels without phytase. As noted in the study, no further improvements were observed with the inclusion of phytase to the range of NPP from 0.2% to 0.5%. This could perhaps be due to the deleterious effect of high Ca levels combined with the relatively low amount of NPP, however it is more likely the efficacy of phytase was lost as the birds NPP requirement was fulfilled with inclusion from 0.2% to 0.5%. Additionally, it is possible that had this study been drawn out, the lower inclusions (0.2% and 0.3%) of NPP may also have had differences observed with inclusion of phytase as depletion of P and Ca from the birds' stores could have taken longer to achieve. Selle and Ravindran (2007) hypothesized that the enhanced efficacy of lower inclusions of phytase could be due to layers holding feed in the crop for a longer period compared to broilers allowing increased time for phytate degradation. However it was

also observed that while phytase influences Ca availability, Ca also influences the efficacy of phytase (Scott, et al., 1999; Sohail and Roland Sr, 2000). It has since been theorized that increasing the inclusion of phytase in laying hen diets could improve performance, however this has not been conclusively identified.

Meyer and Parsons (2011) evaluated the addition of phytase at 150, 250 and 15,000 FTU/kg in a diet containing 3.8% Ca and 0.105% NPP. The experiment concluded that while the supplementation of phytase at 250 FTU/kg increased egg production at 61 to 62 wk of age compared to 150 FTU/kg, the addition of 15,000 FTU/kg conferred no improvements beyond that of the 250 FTU/kg inclusion. It can be concluded from the experiment that utilizing the low level of phytase at 150 FTU/kg was not able to liberate enough P to compensate for the deficient level of NPP fed, while the higher inclusions were more efficacious in meeting requirements. One of the reasons supporting the improvements related to super dosing phytase is its ability to hydrolyze not only IP6 but also the lower IP esters to rid the diet of anti-nutritive phytate (Bedford and Rousseau, 2017; Beeson, et al., 2017). As discussed by Bedford and Rousseau (2017), higher inclusions of Ca with respect to NPP not only reduce the hydrolyzability of IP6, but actually reduces the hydrolyzability of the lower IP esters to a greater degree. Additionally, Bedford and Rousseau (2017) go on to state that while Ca does have a negative effect on phytate hydrolyzability, Zn and Ca can form a mixed-mineral complex with phytate that keeps the IP esters completely unavailable for hydrolysis (Maenz, et al., 1999; Bedford and Rousseau, 2017).

The effects of mineral complexes on phytate hydrolysis via phytase are important to keep in mind when formulating rations, with a special consideration given for Ca as its impacts are multifactorial. However, in laying hens where higher Ca concentrations are required to maintain egg production and shell quality, the interactions may be different as the hen is able to absorb an increased amount of Ca into the blood stream that will see deposition in the medullary bone. To provide Ca for constant uptake by the hen, integrators often feed a mix of coarse and fine Ca sources. Where coarser materials typically take longer to solubilize due to its increased particle size allowing for Ca absorption when not consuming feed.

When added at super dose levels (1,500 FTU/kg) Skřivan, et al. (2018) found reduced efficacy of phytase compared to lower inclusions (300 FTU/kg). The decreased efficacy could be explained by the increased Ca:NPP of 19.5:1 and 23.5:1 that were observed in the formulation. To get a better response with higher levels of phytase, it might therefore be desirable to reduce the Ca:NPP as broilers have consistent improvements where the ratio is reduced below a 2:1 Ca:NPP that has been historically used. However, the ideal level has not been identified with the lack of literature pertaining to layers and high levels of phytase. Similar work was conducted by Kim, et al. (2017) where laying hens were fed a PC diet consisting of 3.91% Ca and 0.38% NPP and a NC diet with a -0.12% reduction in NPP to give a Ca:NPP of 15:1 which was then supplemented with the inclusion of phytase at 10,000, 20,000 and 30,000 FTU/kg. It was observed that the inclusion of phytase at 20,000 and 30,000 FTU/kg had increased production compared to the PC diet while all other parameters were similar. It was

hypothesized that utilizing the recommended level of Ca and a reduced NPP which increased the Ca:NPP, allowed phytase at 20,000 FTU/kg to release P and restore balance which led to the improvements in observed production. Futhering the idea proposed by Kim, et al. (2017), the increased production observed in the experiment could indicate that the higher levels of phytase combined with the reduced Ca:NPP compared to Skřivan, et al. (2018) led to a higher degree of phytate hydrolyzation which reduced the anti-nutritive effects of phytate. Improvements in performance related to phytase inclusion with a reduced Ca:NPP could lead to feeding strategies with super dose levels of phytase that confer benefits in layers more similar to those observed in broilers; however more work is needed in this area to be conclusive as research with laying hens fed high levels of phytase is very limited. Additionally, while adjusting Ca and NPP content of diets to manipulate the Ca:NPP is shown to be an important factor when deciding the efficacy of phytase, other factors require to be taken into account such as nutrient requirements and nutrient density.

Improvements in amino acid digestibility have been variable with multiple reports of phytase having no effects on protein utilization (Biehl and Baker, 1997; Boling-Frankenbach, et al., 2001; Augspurger and Baker, 2004) while others have accredited the enzyme with improvements (Ravindran, et al., 2001; Cowieson, et al., 2004; Ravindran, et al., 2006; Pieniazek, et al., 2016b). An experiment was conducted by Augspurger and Baker (2004) utilizing corn-soybean meal rations containing 23% CP, 0.89% Ca and 0.10% NPP (0.38% total P) fed to chicks through d 18 supplemented with phytase at 0, 500 and 10,000 FTU/kg. It was observed that the high inclusion of the

phytase conferred no benefits beyond that of the 500 FTU/kg inclusion and did not improve the protein efficiency ratio of the chicks. Evaluating the idea of phytase enhancing protein digestibility further, Cowieson, et al. (2004) fed d 42 broilers glucose, glucose with phytase at 1,000 FTU/kg, glucose with 1g of IP6, and glucose with 1g of IP6 and 1,000 FTU/kg of phytase. It was observed that IP6 significantly reduced absorption of amino acids and minerals while the addition of phytase to the IP6 increased the absorption of amino acids, Ca, Na and phytate P. Cowieson, et al. (2006a) found that broilers fed casein and IP6 had reduced digestibility coefficients of amino acids compared to broilers fed casein alone, however supplementing phytase into the mixture reversed this effect and increased the digestibility coefficients of amino acids signifying that protein digestibility was improved.

In an experiment conducted by Ravindran, et al. (2006), it was identified that phytase improved ileal protein and amino acid digestibility, however the magnitude of the effect was dependent upon the phytate concentration. Supplementation of phytase was successful at increasing AME. The diets used in the experiment increased Ca concentration from 0.75% to 0.82% to 0.89% as phytic acid was increased and all diets were approximately at a Ca:NPP of 3:1. It was reported that at the low phytate concentrations (0.104%) ileal digestibility coefficients increased in correlation to phytase inclusion. However, increasing phytate concentration to 0.118% and 0.136% reduced the efficacy of phytase at 1,000 FTU/kg as little improvement was observed compared to 500 FTU/kg. While increasing the phytate concentration reduced the efficacy of the higher dose of phytase, it is possible the effect of the Ca:NPP could have

reduced the efficacy of the higher inclusions of phytase. 1,000 FTU/kg should be more successful at hydrolyzing IP6 compared to a 500 FTU/kg inclusion translating to increased Ca release and reduction of pH in the GIT reducing the digestibility of amino acids. Phytase inclusion of 1,000 FTU/kg should not be considered sufficiently high enough to completely de-phytinize the diet and so in the diets containing the medium and high levels of phytic acid it may be possible that the phytase was optimized at 500 FTU/kg as levels beyond that were only partially successful at hydrolyzing the phytate. As stated by Selle, et al. (2006), phytase fed at standard levels (~500 FTU/kg) is not capable of hydrolyzing the entirety of dietary phytate thereby allowing the negative influence of phytate to still exist and disrupt digestion of protein and other nutrients. Higher levels of phytase could potentially de-phytinize diets by removing the negative effects of phytate while also improving nutrient digestibility.

The effects of increasing phytase inclusions on breast meat yields was investigated in an experiment by Campasino, et al. (2014) where broilers were fed rations consisting of 22.03% CP, 0.78% Ca and 0.31% NPP in the starter; 19.70% CP, 0.66% Ca and 0.25% NPP in the grower; 17.41% CP, 0.62% Ca and 0.23% NPP in the finisher. These diets were supplemented with phytase included at 400, 800, 1,200 and 1,600 FTU/kg. It was observed that as phytase inclusion increased, FCR decreased. Additionally, they reported the inclusion of 1,600 FTU/kg of phytase produced a 49 g heavier breast compared to the PC which was similar but with the addition of 0.14% Ca and 0.12% NPP. While the addition of phytase beyond 400 FTU/kg led to only a few statistical differences, it does support the idea that improvements with a super dose are

the result of increased nutrient digestibility and the increased breast size compared to the PC was likely attributed to increased amino acid digestibility from the higher inclusion of phytase. Amerah, et al. (2014) conducted an experiment consisting of 4 Ca:NPP (1.43:1, 2.14:1, 2.86:1 and 3.57:1) and 2 levels of phytase (0 and 1000 FTU/kg) where it was identified at d 21 that phytase supplementation increased phytate degradation and improved energy, amino acid and P digestibility while the digestibility of amino acids were correlated with phytate degradation.

Seeking to identify a relationship between phytase inclusion and nutrient digestibility, a series of experiments was conducted by Pieniazek, et al. (2016b). The first experiment utilized a NC diet with 0.23% and 0.19% NPP for the starter and grower phases respectively and included phytase added at 0, 250, 500 and 2,000 FTU/kg fed to broilers through d 21. The authors concluded that the inclusion of 2,000 FTU/kg of phytase significantly improved BW and FC compared to the lower inclusions while all inclusions of phytase increased dietary AME. Interestingly, compared to the NPP reduced control diet, elevating phytase inclusion to 2,000 FTU/kg increased the digestibility coefficients of cysteine, glycine, lysine, phenylalanine, proline, serine, arginine, aspartic acid and glutamic acid. In the second experiment experiment, a 42 d grow-out was conducted that included a PC with 0.93 and 0.45%, 0.84 and 0.41% and 0.77 and 0.38% Ca and NPP in the starter, grower and finisher phases, respectively. The NC was formulated to 0.60 and 0.28%, 0.60 and 0.24%, 0.60 and 0.20% Ca and NPP in the starter, grower and finisher phases, respectively, and was supplemented with phytase at 500 and 2,000 FTU/kg. It was observed that while the addition of phytase at 500

FTU/kg improved BW compared to the NC, it took 2,000 FTU/kg to have similar BW to the PC at d 42. Amino acid digestibility was not improved beyond the NPP reduced control diet with the lower inclusion of phytase, however increasing inclusion to 2,000 FTU/kg significantly increased the digestibility coefficients of all amino acids to be similar to the PC. The results published by Pieniazek, et al. (2016b) illustrate how increasing phytase inclusions can improve amino acid digestibility with respect to NPP availability as the NC diets in both experiments were possibly not only impacted from reduced growth performance from low levels of NPP, but the higher Ca:NPP likely affected the GIT pH with free Ca and reduced the effectiveness of pepsin to break down proteins. However, the super dose of phytase was sufficient to overcome these negative effects and improve amino acid digestibility, leading to improvements in growth performance correspondent of "extra-phosphoric" effects (Beeson, et al., 2017). Additionally, the increased AME observed in the study was likely associated with phytase increasing the digestibility of fat, protein and starch, allowing for energy to be made available (Selle and Ravindran, 2007).

As reviewed by Selle and Ravindran (2007), there is evidence that phytate interacts with lipids in corn where a Ca/Mg-phytate complex is formed, reducing the availability of energy. Additionally, they hypothesize that it could be possible that Ca-phytate and lipids may for metallic soaps in the GIT lumen, thereby reducing the absorption of energy. However, the inclusion of phytase should hydrolyze phytate before entering the lower GIT, thereby preventing soap formation and increasing AME. In agreement with with results observed by Pieniazek, et al. (2016b), it was proposed by

Selle and Ravindran (2007) that when utilizing phytase to increase amino acid digestibility, an increase in energy derived from protein would also be observed.

While super doses of phytase can improve nutrient digestibility, it remains to be determined if broilers fed a high level of phytase and reduced amino acid density can achieve similar performance to broilers fed a standard ration. The extra-phosphoric effects attributed to super doses of phytase could potentially improve broiler performance in amino acid reduced diets through increased destruction of the phytate molecule, reduction of Ca crystals and improvements in nutrient digestibility. This idea is supported by a review published by Cowieson, et al. (2017) in which it is argued that nutritionists should implement matrix values for lysine, methionine, cysteine and threonine between 2% and 6%, much like the established matrix values for P and CA. If this were proven through research to be effective, it could potentially lead to cost-savings for the poultry industry as the information could be used to better understand the relationships between protein digestion, Ca and phytase.

CHAPTER III

EVALUATION OF INCREASING LEVELS OF PHYTASE IN DIETS CONTAINING VARIABLE LEVELS OF AMINO ACIDS ON BROILER PERFORMANCE AND PROCESSING YIELDS[*]

Introduction

Synthetic amino acids, inorganic P and energy are commonly added in poultry diets to meet the nutritional needs of broilers to reach their maximum growth potential. However, nutritionists have tools such as exogenous enzymes that can reduce the cost of these nutrients in broiler rations by improving nutrient digestibility. One of the most commonly used exogenous enzymes is phytase. Phytase has a long history of use in the poultry industry, and was found to be useful in broiler diets by Nelson, et al. (1968b). Phytase allows for the reduction of inorganic P while also improving growth performance. Inositol hexaphosphate, more commonly known as phytate, is present in many plant-based feed stuffs, including mature cereal grains and oilseeds, and comprises about two-thirds of the P stored in plants (Adeola and Sands, 2003; Pieniazek, et al., 2016a). The main anti-nutritive property of phytate is related to its ability to chelate essential minerals and P rendering them unavailable for digestion by monogastric animals (Loewus and Murthy, 2000; Ravindran, et al., 2001). The anti-nutritive properties of phytate extend beyond chelation of essential minerals and P to include a

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reduction in digestible amino acids (dAA). The higher molecular weight IP esters (IP6 and IP5) are able to inhibit protein digestion by binding to pepsin and impair its ability to hydrolyze dietary proteins, leading to approximately a 12% reduction in true amino acid digestibility coefficients (Cowieson, et al., 2006a).

Phytase is an exogenous enzyme that is added to feed to de-phosphoralate the inositol hexaphosphate ring starting at the 3 and 6 positions, thus releasing P and other chelated minerals such as Zn, K, Mg and Ca for use by the animal (Rutherfurd, et al., 2012). Increasing phytase levels have been shown to improve the utilization of dietary NPP (Karimi, et al., 2013), while a higher than normal, or super dose of phytase, can improve meat yield, weight gain and reduce FCR in broilers (Cowieson, et al., 2011). It is widely thought that phytase can be supplemented into poultry diets to increase the utilization of amino acids as super dosing phytase can effectively "de-phytinize" the diet, allowing about 95% of the phytate P in corn-soybean meal diets to be available for digestion (Shirley and Edwards, 2003). This de-phytinization of the diet greatly reduces the content of the IP6 esters, which could allow for pepsin to be uninhibited while digesting protein.

Ca is widely regarded as a major micronutrient in feed formulation as it is necessary for proper growth performance and skeletal development, but may be fed in excess when including dietary phytase. Research by Walk (2016) detailed how high levels of dietary Ca can increase gastro-intestinal pH, acting as a buffer and lead to competition for attachment sites on the phytate molecule and potentially reduce the impact of phytase. Furthemore, Ca is bound to the higher phytate IP6 esters up to 5:1

with P (Selle, et al., 2009). Standard doses (1X) of phytase are only sufficient to hydrolyze the IP6 ester, but elevating the inclusion of phytase to super dose (3X) levels allows phytase to not only hydrolyze IP6, but also IP5, IP4, etc. thus returning the release ratio of Ca/P back to 1:1 (Walk, 2016). As such, it is important to utilize matrix values to account for the release of Ca as well as NPP when utilizing phytase.

Reducing dAA density can lower producers input costs. However, amino acid inclusion is highly correlated to achieving maximum bird performance as it is critical to optimizing breast meat yield and BW (Kidd, et al., 2004). Additionally, increasing dAA density has been shown by Dozier, et al. (2007) to reduce FCR and to increase breast meat yield. However, it is not clear in published literature if super dosing phytase is effective at increasing protein utilization. Augspurger and Baker (2004) reported no correlation between protein utilization and phytase dose when super dosing phytase in dAA reduced diets, while Ravindran, et al. (2001) reported ileal digestibility of amino acids to have linear increases that were minimal at 250 FTU/kg, but increased with further elevation of phytase inclusion. Rutherfurd, et al. (2004) also reported improvements in ileal amino acid digestibility with an average increase of 3.4% in low P diets with phytase included at both 500 and 750 FTU/kg, while Pieniazek, et al. (2016b) observed that total amino acid digestibility was increased by 7.3% with the addition of phytase at 2,000 FTU/kg in a 0.22% reduced available P (aP) diet. The differences in effects of phytase on variable dAA diets in reports led to the current objective which was to determine if elevated levels of phytase at 1,500 and 3,000 FTU/kg could have an

additional impact on bird performance and processing parameters beyond the inclusion of the standard 500 FTU/kg (1X) on diets that had variable levels of dAA.

Materials and Methods

Experimental Design

The impact of increasing levels of phytase on broiler performance and yield in diets containing varying levels of digestible amino acids was evaluated in a complete randomized block design arranged in a 3 x 3 factorial of treatments during a 44 d grow-out period. The experimental design consisted of 3 levels of phytase at 500, 1,500 and 3,000 FTU/kg that were added into 3 levels of dAA densities at 100% (control, Cobb-Vantress (2015) breeder recommendations), 95% of control and 90% of control for a total of 9 dietary treatments. Each treatment was fed to broilers in 7 replicate pens with 45 male Cobb 500 broilers placed in each pen. The industry reference diet, identified in this study as the control level of dAA with the addition of 500 FTU/kg, was used for individual contrast comparison between all other experimental treatments.

Experimental Diets

Diets were corn and soybean meal-based and were manufactured from 2 basal batches (Table III-1). The first basal batch was formulated to meet Cobb breeder recommendations for dAA density from which the control diet (100% dAA) was manufactured. The second basal batch was formulated to be iso-caloric to the first, but with a 10% reduction in dAA (90% of control). To complete the 3 levels of dAA, the control and 90% dAA density diets were blended in equal proportions resulting in a 5%

reduction in dAA density (95% of control). Xylanase[1] was added into all diets at 8,000 BXU/kg. Phytase[2] was included at a base level of 500 FTU/kg into both basal diets and with either corn starch or additional phytase added to achieve the 1,500 and 3,000 FTU/kg target levels. The matrix values used in the current experiment for the reductions of Ca and P were 0.12% aP and 0.15% Ca and are consistent with values used in the broiler industry when adding phytase at a recommended inclusion (500 FTU/kg). The same matrix values were also used for the 1,500 and 3,000 FTU/kg inclusions to observe the added benefits of elevating inclusion. The starter diet was fed from d 1 to 18, the grower diet from d 19 to 30, and the finisher diet from d 31 to 44 (termination of the trial). All diets were pelleted prior to allocation, with the starter diet being crumbled following pelleting. The conditioning time was 10 s, and temperature ranged between 75 and 80°C. Composite samples were collected during feed manufacturing for enzyme recovery (Table III-1) and nutrient analysis (Table III-2).

[1] Econase XT- AB Vista Feed Ingredients, Chesterfield, MO; using matrix values to account for 55 Kcal/kg release.
[2] Quantum Blue 5G- AB Vista Feed Ingredients, Chesterfield, MO

Table III-1. Diet formulations (100 and 90% dAA) and calculated nutrient content of diets and phytase[1] recovery fed to market broilers during the starter, grower and finisher phases. Xylanase[2] was added at 8,000 BXU/kg and phytase was added at 500 FTU/kg as a base level in all diets.

	Starter (%)		Grower (%)		Finisher (%)	
	100%	90%	100%	90%	100%	90%
Corn	55.63	61.73	64.50	69.68	66.98	71.14
Soybean Meal (48%)	32.51	27.28	26.50	22.03	23.54	19.94
DL-Methionine	0.29	0.25	0.25	0.20	0.21	0.17
Lysine HCL	0.24	0.25	0.23	0.24	0.20	0.19
L-Threonine	0.08	0.07	0.06	0.05	0.07	0.05
Soy Oil	1.92	1.37	0.82	0.08	1.63	1.05
Limestone	1.35	1.37	1.28	1.29	1.16	1.17
Monocalcium phosphate	0.78	0.82	0.67	0.70	0.50	0.52
Salt	0.34	0.33	0.29	0.18	0.23	0.16
Sodium Bicarbonate	--	0.02	0.03	0.18	0.12	0.24
TAMU Trace Minerals[1]	0.05	0.05	0.05	0.05	0.05	0.05
TAMU Vitamins[2]	0.25	0.25	0.25	0.25	0.25	0.25
Corn Dried Distillers' Grain with solubles	5.00	5.00	5.00	5.00	5.00	5.00
Xylanase[3]	0.01	0.01	0.01	0.01	0.01	0.01
Phytase[4]	0.01	0.01	0.01	0.01	0.01	0.01
Salinomycin[5]	0.05	0.05	0.05	0.05	0.05	0.05

[1]Trace mineral premix added at this rate yields 150 mg manganese, 125 mg zinc, 16.5 mg iron, 5 mg copper, 1 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.
[2] Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.
[3] Econase XT®- AB Vista Feed Ingredients, Chesterfield, MO.
[4]Quantum Blue 5G®- AB Vista Feed Ingredients, Chesterfield, MO.—Enzyme recovery for the starter phase was 412, 603, and 679 for the 100, 95, and 90% for 500 FTU/kg; 1520, 1570, and 1360 FTU/ kg for the 100, 95, and 90% for 1500 FTU/kg; and 2630, 2100 and 2210 FTU/kg for the 100, 95, and 90% for 3000 FTU/kg. Phytase recovery for the grower was 503, 407, and 573 FTU/kg for the 100, 95, and 90% for 500 FTU/kg; 1230, 2250, and 1560 FTU/kg for the 100, 95, and 90% for 1500 FTU/kg; 2710, 2950 and 2640 FTU/kg for the 100, 95, and 90% for 3000 FTU/kg; Phytase recovery for the finisher diet was 466, 563, and 603 FTU/kg for the 100, 95, and 90% for 500 FTU/kg, 1570, 1450, and 1600 FTU/kg for the 100, 95, and 90% for 1500 FTU/kg; 2520, 2930 and 2290 FTU/kg for the 100, 95, and 90% for 3000 FTU/kg. 1 unit of phytase activity is defined as the amount of enzyme which liberates 1.0 µmole of inorganic phosphorus from $4.2X10^{-2}$ M phytate per minute at pH 2.5 and temperature of 37°C.
[5]Sacox 60®, Huvepharma Inc., Peachtree City, GA.

Table III-1. Continued

	Starter (%)		Grower (%)		Finisher (%)	
	100%	90%	100%	90%	100%	90%
Calculated nutrient level (%)						
Crude protein	22.27	20.23	20.07	18.31	18.80	17.36
Crude fat	4.75	4.05	3.93	3.35	4.80	4.35
Fiber	2.92	2.85	2.88	2.82	2.82	2.77
M.E.- kcal/kg	2992	2992	3047	3047	3124	3124
Calcium	0.90	0.90	0.84	0.84	0.76	0.76
Tot. Phosphorus	0.57	0.56	0.53	0.52	0.48	0.48
Av. Phosphorus	0.45	0.45	0.42	0.42	0.38	0.38
Sodium	0.19	0.19	0.18	0.18	0.18	0.19
Dig Arginine	1.28	1.13	1.12	0.99	1.03	0.93
Dig Methionine	0.60	0.53	0.53	0.47	0.49	0.43
Dig TSAA	0.89	0.80	0.80	0.72	0.74	0.67
Dig Lysine	1.20	1.08	1.05	0.95	0.95	0.86
Dig Threonine	0.78	0.70	0.69	0.62	0.65	0.59
Dig Tryptophan	0.22	0.20	0.19	0.17	0.18	0.16

Table III-2. Analyzed nutrient content of diets fed to market broilers during the starter, grower and withdraw phases.

	100% dAA			95% dAA			90% dAA		
	Starter	Grower	Finisher	Starter	Grower	Finisher	Starter	Grower	Finisher
Moisture (%)	12.38	12.14	11.94	12.09	11.73	12.30	12.00	11.90	12.58
Dry Matter (%)	87.62	87.86	88.06	87.91	86.27	87.70	88.00	88.10	87.42
Crude protein (%)	20.7	19.1	17.6	21.2	18.6	19.1	20.3	18.1	17.1
Crude fat (%)	5.34	3.72	4.61	4.12	2.88	4.30	3.04	3.12	4.38
Fiber (acid detergent) (%)	5.2	4.4	3.4	4.7	3.6	3.9	5.1	3.8	3.2
Ash (%)	5.57	4.06	3.70	5.86	4.46	3.73	5.55	4.15	3.64
Total Dig. Nutrients (%)	74.6	74.8	76.9	73.2	74.2	75.8	72.4	74.7	76.2
Metabolizable Energy (Kcal/kg)	2,992	3,014	3,124	2,948	2,992	3,036	2,904	3,014	3,102

Animals and Management Practices

On d of hatch, 2,520 Cobb 500 male broiler chicks were randomly allotted to floor pens and dietary treatments based on initial BW. Broilers were placed in 1.83 m x 1.83 m pens equipped with tube feeders and nipple drinkers with used litter from 2 previous flocks that was top dressed with fresh pine shavings. Chicks were provided age

appropriate supplement heat and given access to feed and water *ad libitum*. All broilers and feed were weighed on the d of dietary changes (d 18, 30, and 44) for calculation of average BW and determination of FC for the calculation of mortality corrected FCR. Upon completion of the trial (d 45), following an 8-hour feed withdrawal, 5 birds per replicate pen were randomly selected, individually weighed and processed for determination of carcass part weights and yields. Animal care was provided in accordance with an approved protocol by the Texas A&M University Institutional Animal Care and Use Committee (IACUC).

Statistical Analysis

All data were subjected to a 3 X 3 factorial analysis with main effect means deemed significantly different at $P \leq 0.05$ and were separated using Duncan's Multiple Range Test. A one-way ANOVA using the General Linear Model was conducted to identify differences in individual treatment means. Additionally, individual contrasts were run to compare individual treatment means to the reference diet (control + 500 FTU/kg phytase).

Results

Performance

Throughout the trial, no significant interactions were observed between level of phytase and amino acid density. Reducing amino acid densities at both levels decreased ($P = 0.001$) average broiler BW at d 18 and 30 as compared to the control level of amino acids (Table III-3). At the conclusion of the trial on d 44, the reduction of 10% in amino acid density decreased ($P = 0.012$) average male BW compared to the control fed

broilers with the 5% reduction being intermediated. Dietary phytase level impacted BW. On d 18, increasing the amount of supplemental phytase to either 1,500 or 3,000 FTU/kg increased ($P \leq 0.05$) male BW compared to the 500 FTU/kg. On d 44, the highest level of phytase evaluated (3,000 FTU/kg) increased ($P \leq 0.05$) BW compared to the standard 500 FTU/kg, while the 1,500 FTU/kg inclusion was intermediate. Individual contrast comparisons regarding BW indicate supplementation of phytase at 1,500 and 3,000 FTU/kg significantly increased ($P \leq 0.05$) early male BW in the control diet as compared to the industry reference diet (control + 500 FTU/kg phytase). Reducing the amino acid density by 10% decreased ($P \leq 0.05$) BW on d 18 and 30 compared to the reference diet, however, increasing the level of phytase from 500 to 1,500 FTU/kg eliminated this negative impact of amino acid density.

Table III-3. Body weight (BW) and percent mortality of broilers fed variable levels of amino acids and phytase[1] through 44 d.

% Amino Acids	Phytase FTU/kg	BW (kg)				Mortality (%)
		d 0 (g)	d 18	d 30	d 44	Total
100	500	39.0	0.747	1.837	3.308	3.49
100	1,500	39.0	0.776*	1.886	3.371	3.81
100	3,000	39.0	0.782*	1.884	3.391	5.08
95	500	39.0	0.737	1.800	3.313	4.04
95	1,500	39.0	0.760	1.805	3.263	1.90
95	3,000	39.0	0.758	1.841	3.371	4.47
90	500	39.0	0.727**	1.762**	3.246	3.81
90	1,500	39.0	0.750	1.821	3.294	4.42
90	3,000	39.0	0.749	1.796	3.293	4.15
Amino Acid Density		Main Effect Means				
90		0.039	0.742[b]	1.793[b]	3.278[b]	4.126
95		0.039	0.753[b]	1.815[b]	3.316[ab]	3.472
100		0.039	0.767[a]	1.869[a]	3.356[a]	4.126
Phytase FTU/kg						
	500	0.039	0.738[b]	1.800	3.289[b]	3.779
	1500	0.039	0.762[a]	1.837	3.309[ab]	3.379
	3000	0.039	0.762[a]	1.840	3.352[a]	4.566
p-value						
Amino Acid Density		0.459	<0.001	0.001	0.012	0.656
Phytase		0.801	<0.001	0.075	0.049	0.343
Amino Acid Density x Phytase		0.041	0.902	0.716	0.274	0.532
Pooled-SEM		<0.001	0.003	0.009	0.013	0.345
CV			3.1	3.9	3.1	70.1

[a-b] Means within a column with different superscripts differ at P≤0.05

[1]Quantum Blue 5G, AB Vista, Stilwell, KS

*Denotes an increased (P≤0.05) value on an individual contrast comparison to the industry reference (100% dAA & phytase at 500 FTU/kg)

**Denotes a reduced (P≤0.05) value on an individual contrast comparison to the industry reference (100% dAA & phytase at 500 FTU/kg)

The main effects means for FC (Table III-4) was not impacted by either increasing inclusions of phytase or density of dAA throughout the trial. However, mortality corrected FCR (Table III-4) was effected by levels of phytase and dAA densities. During the starter phase of production, increases (P≤0.05) in FCR were observed incrementally with each reduction in amino acid density. During the grower phase, reductions of 5 and 10% amino acid density increase FCR compared to the

control diet. While no differences were observed in finisher FCR, cumulative FCR between 0 and 30 and 0 and 44 d of age was impacted by the reduction of amino acid density as the reduction at 5 and 10% negatively ($P \leq 0.05$) impacted observed FCR. Phytase level also impacted observed FCR. During the starter phase, increasing the level of phytase above 500 FTU/kg decreased ($P \leq 0.05$) FCR. Cumulatively through 30 d of age, elevating the amount of phytase to 1,500 FTU/kg reduced ($P \leq 0.05$) FCR compared to the standard 500 FTU/kg inclusion. However, phytase level needed to significantly reduce FCR through d 44 was 3,000 FTU/kg as the 1,500 FTU/kg was similar to the 500 FTU/kg. Mortality corrected FCR was adjusted for BW at the conclusion of the trial using a conversion factor of 27 g of BW equal to 1 point of FCR. Main effects means for levels of dAA for adjusted FCR (Table III-3) from d 0 to 44 revealed an incremental increase ($P \leq 0.05$) in weight adjusted FCR with each reduction in amino acid density. Increasing phytase inclusion to 3,000 FTU/kg improved ($P \leq 0.05$) weight adjusted FCR as compared to the 500 FTU/kg inclusion with the 1,500 FTU/kg level being intermediated. Individual contrast comparisons regarding FCR indicate that phytase inclusion at 3,000 FTU/kg in the control diet reduced FCR through d 18 and cumulatively through d 44, while the inclusion of 1,500 and 3,000 FTU/kg in the 10% dAA reduced diet was able to recover FCR to be similar to the reference diet. Although the inclusion of 3,000 FTU/kg to the 10% dAA reduced diet had increased FCR through d 30, it was able to improve FCR in the finisher phase compared to the reference diet. These individual contrasts confirm the ability of phytase at super dose levels to improve performance in diets with reduced dAA.

Table III-4. Feed conversion ratio and feed consumption of broilers fed variable levels of amino acids and phytase[1] through 44 d.

% Amino Acids	Phytase FTU/kg	Feed Conversion Ratio (FCR)						Feed Consumption (g/bird/d)				
		d 0 to 18	d 18 to 30	d 30 to 44	d 0 to 30	d 0 to 44	[2]Adj d 0 to 44	d 0 to 18	d 18 to 30	d 30 to 44	d 0 to 30	d 0 to 44
100	500	1.322	1.629	1.958	1.496	1.693	1.697	57.8	174.1	207.1	88.9	116.3
100	1,500	1.300	1.601	1.952	1.470	1.674	1.659	58.7	175.1	207.6	89.0	114.7
100	3,000	1.287**	1.620	1.942	1.474	1.672	1.650**	58.0	174.8	212.4	89.1	115.9
95	500	1.347	1.688	1.917	1.540*	1.706	1.708	58.1	173.8	209.4	89.3	115.7
95	1,500	1.304	1.691	1.933	1.515	1.696	1.714	57.9	178.5	202.4	88.9	116.3
95	3,000	1.318	1.671	1.911	1.517	1.688	1.672	58.3	178.5	209.2	89.6	116.1
90	500	1.355*	1.678	1.955	1.537*	1.723*	1.747*	56.9	170.3	207.7	86.2	112.7
90	1,500	1.344	1.638	2.000	1.509	1.717	1.725	58.2	174.8	212.4	87.7	113.2
90	3,000	1.343	1.715*	1.882**	1.550*	1.694	1.703	58.5	174.6	202.5	89.3	115.2
Amino Acid Density		Main Effect Means										
90		1.348[a]	1.677[a]	1.946	1.532[a]	1.711[a]	1.725[a]	57.9	173.2	207.5	87.7	113.7
95		1.323[b]	1.683[a]	1.920	1.524[a]	1.697[a]	1.698[b]	58.1	174.0	207.0	89.3	115.6
100		1.303[c]	1.616[b]	1.951	1.480[b]	1.680[b]	1.669[c]	58.2	174.6	209.0	89.0	115.6
Phytase FTU/kg												
	500	1.341[a]	1.665	1.944	1.524[a]	1.708[a]	1.718[a]	57.6	172.7	208.1	88.2	114.9
	1,500	1.316[b]	1.643	1.961	1.498[b]	1.696[ab]	1.699[ab]	58.3	173.2	207.5	88.5	114.7
	3,000	1.316[b]	1.669	1.911	1.514[ab]	1.685[b]	1.675[b]	58.3	176.0	208.0	89.3	115.7
p-value												
Amino Acid Density		<0.001	0.001	0.340	<0.001	0.001	<0.001	0.747	0.754	0.721	0.099	0.239
Phytase		0.009	0.360	0.083	0.009	0.015	0.008	0.220	0.183	0.968	0.284	0.764
Amino Acid Density x Phytase		0.531	0.342	0.308	0.214	0.821	0.708	0.434	0.184	0.058	0.510	0.892
Pooled-SEM		0.004	0.009	0.010	0.005	0.004	0.007	0.200	0.800	1.278	0.300	0.760
CV		2.6	4.2	4.1	2.5	1.6	3.1	2.4	3.9	4.9	3.0	5.2

[1]Quantum Blue 5G, AB Vista, Stilwell, KS

[2]Adjusted FCR was done by 1 point of FCR equal to 32 grams of BW.

[a-c] Means within a column with different superscripts differ at P≤0.05

*Denotes an increased (P≤0.05) value on an individual contrast comparison to the industry reference (100% dAA & phytase at 500 FTU/kg)

**Denotes a reduced (P≤0.05) value on an individual contrast comparison to the industry reference (100% dAA & phytase at 500 FTU/kg)

Processing Parameters

Live weight of processed birds and carcass weight without giblets (WOG) was not impacted by amino acid density or phytase level. However, reducing amino acid density by 10% reduced breast weight ($P \leq 0.05$) and breast meat yield ($P < 0.05$) compared to the control fed broilers while the 5% reduction was intermediated (Table III-5). Phytase level impacted breast meat yield as increasing the level of phytase to 1,500 FTU/kg increased ($P \leq 0.05$) breast meat yield compared to the 500 FTU/kg fed broilers. Reducing the amino acid density increased ($P < 0.05$) fat pad yield incrementally. An individual contrast comparison highlighted the ability of elevated levels of phytase (3,000 FTU/kg) to increase breast weight in the control diet (100% dAA) by 39.5 g compared to the industry reference. Additionally, contrast comparisons on breast yield identified increased ($P \leq 0.05$) yield with elevated levels of phytase at 1,500 and 3,000 FTU/kg in the control diet (100% dAA) compared to the industry reference diet.

Table III-5. Processing weights and percent yield of broilers fed variable levels of amino acids and phytase[1] through 44 d.

% Amino Acids	Phytase FTU/kg	Weight					Yield (%)			
		Live (kg)	Carcass (kg)	Breast (g)	Tender (g)	Fatpad (g)	Carcass	Breast	Tender	Fatpad
100	500	3.330	2.567	644.0	135.4	34.2	77.10	25.09	5.28	1.33
100	1,500	3.349	2.582	613.8	132.1	34.7	77.10	25.85*	5.12**	1.34
100	3,000	3.433	2.646	683.5*	139.4	37.9	77.09	25.83*	5.27	1.43
95	500	3.314	2.600	653.4	133.5	39.3*	78.75	25.09	5.14	1.52*
95	1,500	3.268	2.553	650.0	131.9	36.1	78.34	25.45	5.17	1.41
95	3,000	3.367	2.587	650.3	134.9	35.5	76.84	25.12	5.21	1.38
90	500	3.262	2.501	623.5	132.6	37.7	76.68	24.91	5.30	1.51*
90	1,500	3.377	2.597	658.5	134.9	36.9	76.91	25.35	5.20	1.42
90	3,000	3.317	2.570	631.9	132.7	39.5*	77.54	24.58	5.16	1.54*
Amino Acid Density		Main Effect Means								
90		3.319	2.556	638.0[b]	133.4	38.0	77.04	24.95[b]	5.22	1.49[a]
95		3.316	2.580	651.2[ab]	133.5	36.9	77.98	25.22[ab]	5.17	1.43[ab]
100		3.370	2.598	665.1[a]	135.6	35.6	77.10	25.59[a]	5.22	1.37[b]
Phytase FTU/kg										
	500	3.302	2.556	640.3	133.8	37.1	77.51	25.03[b]	5.24	1.45
	1,500	3.331	2.578	658.7	133.0	35.9	77.45	25.55[a]	5.16	1.39
	3,000	3.372	2.601	655.2	135.7	37.6	77.15	25.18[ab]	5.21	1.45
p-value										
Amino Acid Density		0.417	0.324	0.032	0.288	0.136	0.24	0.015	0.517	0.04
Phytase		0.313	0.272	0.156	0.242	0.333	0.824	0.052	0.219	0.325
Amino Acid Density x Phytase		0.535	0.242	0.217	0.207	0.147	0.426	0.333	0.194	0.240
Pooled-SEM		0.021	0.013	4.9	0.8	0.5	0.27	0.10	0.02	0.02
CV		5	3.9	6	4.4	10.7	2.8	3.2	3	10.7

[a-c] Means within a column with different superscripts differ at (P≤0.05)

[1]Quantum Blue 5G, AB Vista, Stilwell, KS

* Denotes an increased (P≤0.05) value on an individual contrast comparison to the industry reference (100% dAA & phytase at 500 FTU/kg)

**Denotes a reduced (P≤0.05) value on an individual contrast comparison to the industry reference (100% dAA & phytase at 500 FTU/kg)

Discussion

While no interactions were observed between dAA and phytase, individually, reducing dAA density in this study did reduce broiler performance and increasing phytase inclusion to 1,500 and 3,000 FTU/kg was shown to improve performance. Through contrasts, this experiment demonstrated that utilization of these higher inclusions of phytase was able to improve performance (BW and FCR) in reduced dAA density diets to similar levels as the industry reference (100% dAA + 500 FTU/kg) supporting the hypothesis that utilizing elevated levels of phytase in dAA reduced diets could confer additional benefits beyond 500 FTU/kg.

The 10% reduction in dAA density in this study would be considered a dramatic and unrealistic scenario under commercial conditions. This extreme reduction in amino acid density was incorporated into the experimental design to illustrate the added benefits of super dosing phytase in an effort to recover performance and yield losses associated with feeding lower density diets. It was decided to formulate the diets to be iso-caloric so as to isolate the effects of phytase and dAA on bird performance as metabolizable energy has been well documented by Dozier, et al. (2007) to effect bird performance, however a future study to evaluate diets containing similar protein to energy ratios may be beneficial. Research by Kidd, et al. (2004) demonstrated that reducing dAA density from 1.25% to 1.15% lysine, reduced broiler weights by 600 g at d 49 and observed a cumulative FCR increase of 0.42 through d 49. Their findings, while more drastic in BW, still support that reducing dAA density in the current study by 10% led to reductions in cumulative performance at d 44 with decreased BW by 62 g,

breast weight by 20.5 g and adjusted FCR increased by 5 points. The authors surmise that while the differences were significant in the current trial, the more drastic differences found by Kidd, et al. (2004) could be due to the combination of higher dAA levels and increased grow-out time compared to the current study.

The benefits of super dosing phytase is well documented to enhance growth performance such as improving BW and FCR (Shirley and Edwards, 2003; Pieniazek, et al., 2016b). This was demonstrated in the current trial with the inclusion of phytase at 3,000 FTU/kg improving cumulative FCR and final BW to the standard dose, while improvements were identified with 1,500 FTU/kg, at the conclusion of the trial it was intermediate. Using individual contrasts, it was observed that the benefits in growth performance with phytase at 1,500 and 3,000 FTU/kg in the 95 and 90% dAA density diets were improved ($P<0.05$) to levels comparable to that of the reference diet. A possible explanation could be that the elevated levels of phytase were able to de-phytinize the diet and released increased levels of Ca, P, starch and amino acids. This effect was subdued with the standard phytase inclusion as it was released far more Ca (Walk, 2016) than P and the release of amino acids was not registered in bird performance or carcass yield due to forming complexes with the excess Ca, as the matrix value used was the same for all levels of phytase. Driver, et al. (2005) hypothesizes that broilers are susceptible to having extra dietary Ca forming complexes with P as the birds are not able to mineralize higher levels of Ca completely as it forms complexes with P to reduce mineral uptake, but it was noted that the inclusion of phytase can overcome the negative effects of an increased Ca:P. The P and Ca release matrix used in this trial only

accounted for release up to 500 FTU/kg inclusion of phytase with no additional credits being given to the 1,500 and 3,000 FTU/kg inclusions. Results by Li, et al. (2015) indicate that in birds from d 7 to 21, increasing the level of dietary Ca from 0.65 to 0.95% decreases the apparent ileal digestibility of all amino acids and that there is an interaction between Ca and phytase on the AID of leucine, methionine, threonine, valine and cysteine. Li, et al. (2015) also reported that from d 19 to 21, as Ca concentration increased, the AID of these amino acids was negatively affected due to the complexes formed from the Ca-amino acid interaction. Therefore, it is not unreasonable to surmise that an excess of Ca produced by the recommended dose of phytase led to reductions in amino acid digestibility and explains why the recommended dose had the lowest performance standards throughout the current trial. However, Walk (2016) asserts that elevating phytase inclusion can overcome the increased Ca chelation by hydrolyzing the higher and lower IP esters to return the release ratio of Ca back to 1:1 with P and improve amino acid retention. Although not measured in the current experiment, it can be taken from the data that this effect was likely responsible for the improved performance responses observed with the elevated dosages of phytase across all dAA diets.

Dozier, et al. (2007) conducted a broiler study from d 42 to 56, with 2 levels of amino acid density: moderate AA (16.2% CP, 0.88% Lys, and 0.75% TSAA) and high AA (18.0% CP, 0.98% Lys, and 0.83% TSAA) crossed with 2 levels of dietary AME: low (3,140 kcal/kg) and moderate (3,240 kcal/kg). It was noted at the conclusion of the trial that the broilers fed the low AA diets increased FC by 101 g/bird, increased FCR by

6 points and reduced breast meat yield by 0.5%. In the current study, the 10% reduction in dAA density led to a 27 g and 0.64% decrease ($P \leq 0.05$) in breast weight and yield compared to the control. Breast yield was also improved ($P \leq 0.05$) by 0.52% with the increased dosage of phytase at 1,500 FTU/kg which was also observed by Campasino, et al. (2014) that had broilers receiving 1,600 FTU/kg in a diet with Ca and P reduced by 0.14 and 0.13% increasing breast meat by 49 g compared to a PC diet with adequate levels of Ca and P. In the current experiment, other than the main effects differences in dAA density, the addition of 500 FTU/kg of phytase to the 90% dAA diets had reduced BW at d 18 and d 30 as well as increased cumulative FCR compared to the industry reference highlight the significance of the dAA density reductions. However, elevating the inclusion of phytase to 1,500 and 3,000 FTU/kg was able to recover the lost performance associated with the 90% dAA density. These improvements may be due to mechanisms proposed by Cowieson, et al. (2011) in which high dosages of phytase: 1) liberates more phosphate or restored Ca:P proportionate release, 2) effectively destroyed phytate to eliminate the anti-nutritive effect, and 3) generation of myo-inositol with lipotrophic effects. The lack of interactions in the current experiment show the consistency of phytase' impact regardless of dAA level which improved nutrient digestibility and may be related to reduced nutrient chelation. Cowieson, et al. (2011) reports that because the release values of Ca are asymptotic and are the most dramatic at an inclusion of 500 FTU/kg while P is relatively linear, then a super dose has a greater capability of additional performance improvements. Super dosing phytase has consistently been shown to further influence growth performance beyond a standard

dose in a beneficial manner (Onyango, et al., 2005; Campasino, et al., 2014; Walk, et al., 2014; Pieniazek, et al., 2016b) similar to the responses observed at 1,500 and 3,000 FTU/kg in the current study.

Overall, the results indicate that super dosing phytase has the potential to improve broiler performance and processing parameters and can recover performance loss associated with reductions in dAA density. The authors realize the -10% dAA reductions used in this study may not be realistic for practical application; however, the -5% dAA reduction could be considered practical and did not have the severe reductions in growth and processing yields identified with the -10% dAA reduction. This data can provide nutritionists information regarding the potential to reduce diet cost through reduced nutrient density, specifically dAA, without loss of performance by capitalizing on the benefits of elevated levels of phytase inclusion.

CHAPTER IV

EVALUATION OF HIGH DOSAGES OF PHYTASE WITH REDUCTIONS IN AMINO ACID, ENERGY, CALCIUM AND PHOSPHORUS DENSITY ON BROILER PERFORMANCE AND PROCESSING YIELDS

Introduction

Commercial broiler production companies aim to produce birds of different sizes and meat yields depending on their respective markets, and as such target different nutritional strategies to achieve the desired bird growth. However, in all broiler markets, feed represents over half of the cost of rearing and processing the broilers for sale. In an effort to control and lower feed costs, producers regularly evaluate new ways to reduce diet inputs through means such as sourcing less expensive materials or implementing nutritional strategies such as feed additives that improve growth performance. Over the past three decades, a considerable amount of research has been conducted to identify new technologies to achieve these goals. The continued development, understanding and improvement of the enzyme market and products has resulted in a current cost savings of approximately $3 to 5 billion US per year (Adeola and Cowieson, 2011). Enzyme use in feed manufacturing has been dominated by phytase at approximately 60% of the market place, followed by non-phytins (carbohydrases, proteases and lipases) in the remaining 40% (Kiarie, et al., 2013).

Phytate (inositol hexaphosphate) is the major form of P stored in cereal grains (Erdman, 1979) and is largely unavailable to monogastric animals for absorption due to a lack of endogenous phytase in the GIT (Zeller, et al.) (Cowieson, et al., 2006a).

Phytate also exhibits many antagonistic effects such as the ability to form complexes with inorganic P and cations (Mg, Ca, Zn, Cu, Fe (Oberleas, 1973)) and to even inhibit pepsin, thereby reducing the digestion of proteins (Knuckles, et al., 1989). Phytate can effectively chelate Ca at the IP6 ester in a 5 to 1 ratio with P (Selle, et al., 2009; Cowieson, et al., 2011). Since the 1970's, phytase has been evaluated based on its ability to hydrolyze phytate bound P and increase the bioavailability of P in the diet (Nelson, et al., 1968b). Phytase research evolved to identify the relationship between Ca and phytate as it was identified by Nelson, et al. (1968a) that 1% inclusion of phytate effectively bound up to 35% of total Ca.

High inclusions of phytase (12,000 FTU/kg) have been proven to effectively "de-phytinize" feed, recovering around 95% of the phytate P for digestion (Shirley and Edwards, 2003). Additionally, elevated dosages of phytase have shown to improved performance in broilers including BW, FCR, and even processing yields (Campasino, et al., 2014; Pieniazek, et al., 2016b; Smith, et al., 2018). Phytase has shown the ability to improve growth performance (BW, FCR) with higher levels of phytase (<1,500 FTU/kg) in diets with reduced amino acid density to have similar performance to a non-reduced diet supplemented with a normal level of phytase (500 FTU/kg) (Smith, et al., 2018). As previously mentioned, phytate can bind to pepsin and inhibit the digestion of proteins, and this was confirmed by an experiment by Cowieson, et al. (2006a) where birds were fed a diet with 21% protein during the grower phase, and then precision fed a ration containing 5 g of casein in 50 mL of water with and without IP6. It was concluded that the presence of IP6 led to an overall reduction of amino acid digestibility of

approximately 12%. Research has also shown that adding a high level of dietary phytase can improve the retention of N, P, metabolizable energy and amino acids (Dilger, et al., 2004; Amerah, et al., 2014; Pieniazek, et al., 2016b), which could lead to additional cost-savings by reducing the inclusion of soybean meal and crystalline amino acids in addition to P and Ca.

Ca is commonly fed at higher levels than needed in broiler diets as a safety margin due to its wide availability and relative in-expensive cost (Bedford and Rousseau, 2017), but can have negative consequences particularly when utilized at levels beyond requirement. High levels of Ca can reduce protein and amino acid digestibility in non-ruminants by elevating GIT pH. Ca can also reduce the ability of phytase to hydrolyze phytate by chelating to the inositol ring (Walk, 2016). Walk (2016) summarizes that phytase at recommended levels (~500 FTU/kg) is only sufficient to effectively hydrolyze the IP6 esters; however, super dosing phytase (3x the recommended dose) can not only break down the IP6, but also the IP5, IP4, IP3 and even IP2 to return the Ca release back to a 1:1 ratio with P. Returning the release back to 1:1 is critical as literature reviewing phytase inclusion questions Ca and P levels in broiler diets with the idea that when Ca is fed in excess of NPP, it hinders P digestibility (Bedford and Rousseau, 2017). Bedford and Rousseau (2017) also stated that excess Ca negatively influences the hydrolysis of the phytate esters by phytase, creating the need for producers to increase inclusion of inorganic P to maintain performance. Therefore, the objective of the current experiments was to evaluate multiple levels of phytase in diets varying in nutrient density (ME and dAA) and minerals (Ca and P).

Materials and Methods

Experimental Design

Trial 1 was conducted as a 3 X 2 X 2 randomized complete block design. To attain the factorial design, 3 feeding regimens were utilized. Feeding regimen A consisted of a control level of amino acid density and metabolizable energy. Feeding regimen B consisted of dAA fed at 98%, 97%, 96% and 96% of the control during the starter, grower, finisher and withdraw phases, respectively. Feeding regimen C consisted of dAA fed at 98%, 96%, 94% and 94% of the control during the starter, grower, finisher and withdraw phases, respectively. Feeding regimens B and C both received similar reductions in metabolizable energy (ME) to consist of 99%, 98%, 97% and 97% of the control ME during the starter, grower, finisher and withdraw phases, respectively. Each feeding regimen was split into 2 mineral densities including a positive control (PC; no mineral reduction) and a negative control (NC; PC -0.12% Ca, -0.15% aP). Additionally, each of those treatments were further split and fed 2 levels of phytase[3] inclusion (1,000 and 4,500 FTU/kg) for a total of 12 experimental treatments.

Trial 2 was designed similarly to Trial 1, however it only consisted of feeding regimens A and C in addition to the 2 levels of minerals (PC and NC (-0.10% Ca, starter and grower; -0.12% Ca, finisher and withdraw), and -0.15% aP)) and 2 levels of phytase inclusion (1,000 and 4,500 FTU/kg) for a total of 8 experimental treatments. Additional

[3] Grainzyme® Agrivida Inc., Woburn, MA 01801

NC reductions in the second trial were formulated to account for increased mineral intake due to increased FC observed in the latter phases of the first Trial.

Experimental Diets

Dietary formulations for the 3 feeding regimens differing in nutrient density in Trial 1 are presented in Tables IV-1 and IV-2. Regimen A was formulated to provide dAA and ME at levels exceeding those recommended by the National Research Council (NRC, 1994). Regimen B reduced dAA by 2% in the starter, 3% in the grower and 4% in the finisher and withdraw phases. Regimen C was reduced dAA 2% in the starter, 4% in the grower and 6% in the finisher and withdraw phases. Regimens B and C were paired with reductions in metabolizable energy at 1% in the starter, 2% in the grower and 3% in the finisher and withdraw phases. All reductions of essential amino acids were ratioed to dig.Lys and were maintained through both experiments. During both trials, sand was utilized as a non-digestible filler to account for the differences in weight of losing Ca and P density in the NC diets. The basal diets were mixed for each phase in 3 separate batches and were mixed with the lower inclusions for each micronutrient and added additional micro-ingredients for each treatment to obtain the targeted nutrient level for the PC and NC diets, respectively. Additionally, 1,000 FTU/kg was added in the basal diet for the baseline phytase level. Treatments with the high dose of phytase received an additional 3,500 FTU/kg at the time of mixing individual treatments to reach the 4,500 FTU/kg target. The enzyme utilized in this experiment is a naturally occurring phytase expressed in corn as described by Yoshida, et al. (1999).

Table IV-1. Diet formulations with variable AA, energy (regimens A, B and C) and mineral density, calculated nutrient content of diets and phytase[1] recovery fed to Ross 708 broilers during the starter and grower phases of Trial 1.

Dietary phase	Starter				Grower					
Feeding regime	A		B and C		A		B		C	
AA density	100% AA		98% AA		100% AA		97% AA		96% AA	
ME density	100% ME		99% ME		100% ME		98% ME			
Mineral level	PC	NC	PC	NC	PC	NC	PC	NC	PC	NC
Ingredient (%)										
Corn %	58.01	58.08	60.14	60.14	64.48	64.49	67.80	67.80	68.38	68.39
Soybean meal %	35.61	35.59	34.29	34.29	29.14	29.14	27.30	27.30	26.79	26.79
DL Methionine %	0.27	0.27	0.26	0.26	0.26	0.26	0.25	0.25	0.25	0.25
Lysine %	0.13	0.13	0.14	0.14	0.19	0.19	0.21	0.21	0.21	0.21
L Threonine %	0.04	0.03	0.03	0.03	0.06	0.06	0.06	0.06	0.06	0.06
Soy Oil %	2.02	2.00	1.20	1.20	2.22	2.22	0.71	0.71	0.62	0.62
Limestone %	1.53	1.51	1.53	1.52	1.39	1.37	1.40	1.38	1.40	1.38
Monocalcium P %	1.69	0.97	1.69	0.98	1.49	0.78	1.50	0.79	1.50	0.79
Salt %	0.41	0.41	0.41	0.41	0.31	0.31	0.27	0.27	0.26	0.25
Sodium Bicarb %	-	-	-	-	0.13	0.14	0.19	0.20	0.21	0.21
Vitamins[2] %	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Mineral Premix[3] %	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Salinomycin[4] %	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Sand %	0.00	0.70	0.00	0.72	0.00	0.72	0.00	0.73	0.00	0.73
Calculated Nutrients (%)										
Crude protein	22.51	22.50	22.05	22.05	20.00	20.00	19.39	19.40	19.20	19.20
Crude Fat	4.56	4.54	3.82	3.82	4.94	4.94	3.55	3.55	3.49	3.49
Ca	0.96	0.84	0.96	0.84	0.86	0.74	0.86	0.74	0.86	0.74
Av. P	0.48	0.33	0.48	0.33	0.43	0.28	0.43	0.28	0.43	0.28
AME (kcal/kg)	3,000	3,000	2,970	2,970	3,080	3,080	3,018	3,018	3,018	3,018
Dig. Methionine	0.57	0.57	0.56	0.56	0.54	0.54	0.52	0.52	0.52	0.52
Dig. TSAA	0.87	0.87	0.85	0.85	0.81	0.81	0.79	0.79	0.78	0.78
Dig. Lysine	1.18	1.18	1.16	1.16	1.07	1.07	1.04	1.04	1.03	1.03
Dig. Tryptophan	0.24	0.24	0.23	0.23	0.20	0.20	0.19	0.19	0.19	0.19
Dig. Threonine	0.77	0.77	0.75	0.75	0.71	0.71	0.68	0.68	0.68	0.68
Dig. Arginine	1.37	1.37	1.33	1.33	1.18	1.18	1.13	1.13	1.11	1.11
Sodium	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18

[1]Recovery (FTU/kg) of Grainzyme® Agrivida Inc., Woburn, MA 01801- Listed as 100% dAA PC 1,000, 100% dAA PC 4,500, 100% dAA NC 1,000, 100% dAA NC 4,500, 98% dAA PC 1,000, 98% dAA PC 4,500, 98% dAA NC 1,000, 98% dAA NC 4,500, 96% dAA PC 1,000, 96% dAA PC 4,500, 96% dAA NC 1,000, 96% dAA NC 4,500 respectively for each phase. Starter: 2,176, 4,353, 759, 5,204, 823, 4,513, 1,168, 3,869, 806, 3,085, 890, 4,630. Grower: 819, 5,160, 659, 5,533, 1,159, 4,108, 1,168, 3,841, 604, 3,668, 1,078, 5,520. Finisher: 404, 4,419, 1,105, 4,509, 1,310, 3,288, 1,183, 4,082, 983, 3,612, 1,066, 4,149. Withdraw: 598, 3,092, 823, 4,612, 1,112, 3,464, 862, 4,259, 659, 4,590, 1,282, 3,998.

[2]Vitamin premix added at this rate yields 22,045 IU vitamin A, 7,716 IU vitamin D_3, 91 IU vitamin E, 0.04 mg B_{12}, 11.9 mg riboflavin, 91.8 mg niacin, 40.4 mg d-pantothenic acid, 261.1 mg choline, 2.9 mg menadione, 3.50 mg folic acid, 14.3 mg pyroxidine, 5.87 mg thiamine, 1.10 mg biotin per kg diet. The carrier is ground rice hulls**.**

[3]Trace mineral premix added at this rate yields 120 mg of manganese, 72 mg of total zinc, 3.4 mg of copper, 2.33 mg of iodine, 0.2 mg of total selenium, 0.009 g of *bacillus subtilis.*

[4]Sacox 60, Huvepharma Inc., Peachtree City, GA.

Table IV-1. Continued

Dietary phase	Starter				Grower					
Feeding regime	A		B and C		A		B		C	
AA density	100% AA		98% AA		100% AA		97% AA		96% AA	
ME density	100% ME		99% ME		100% ME		98% ME			
Mineral level	PC	NC	PC	NC	PC	NC	PC	NC	PC	NC
Analyzed nutrients (%)										
Moisture	11.68	11.68	11.97	11.97	11.18	11.18	11.93	11.93	10.87	10.87
Crude protein	21.1	21.1	21.4	21.4	19.0	19.0	17.3	17.3	18.6	18.6
Crude fat	4.87	4.87	4.21	4.21	4.53	4.53	3.62	3.62	3.30	3.30
P	0.83	0.68	0.75	0.88	0.72	0.65	0.78	0.58	0.73	0.58
Ca	1.25	1.30	1.11	1.36	1.15	1.00	1.22	0.93	1.12	0.94

Table IV-2. Diet formulations with variable AA, energy (regimens A, B and C) and mineral density, calculated nutrient content of diets and phytase[1] recovery fed to Ross 708 broilers during the finisher and withdrawal phases of Trial 1.

Dietary phase	Finisher						Withdrawal					
Feeding regime	A		B		C		A		B		C	
AA density	100% AA		96% AA		94% AA		100% AA		96% AA		94% AA	
ME density	100% ME		97% ME				100% ME		97% ME			
Mineral level	PC	NC	PC	NC	PC	NC	PC	NC	PC	NC	PC	NC
Ingredient (%)												
Corn %	68.70	68.69	73.20	73.21	74.28	74.28	71.10	71.13	75.57	75.57	76.40	76.40
Soybean meal %	24.30	24.30	22.00	22.00	21.06	21.06	22.20	22.19	19.96	19.96	19.25	19.25
DL Methionine %	0.24	0.23	0.22	0.22	0.21	0.21	0.22	0.21	0.20	0.20	0.19	0.19
Lysine %	0.20	0.20	0.22	0.22	0.22	0.22	0.20	0.20	0.22	0.22	0.22	0.22
L Threonine %	0.08	0.07	0.08	0.08	0.08	0.08	0.07	0.07	0.07	0.07	0.07	0.07
Soy Oil %	2.99	3.00	0.75	0.75	0.60	0.60	2.92	2.91	0.65	0.65	0.53	0.53
Limestone %	1.31	1.29	1.33	1.31	1.33	1.31	1.22	1.20	1.24	1.22	1.23	1.22
Monocalcium P %	1.39	0.67	1.40	0.68	1.40	0.69	1.26	0.54	1.27	0.55	1.27	0.55
Salt %	0.19	0.19	0.14	0.14	0.11	0.11	0.14	0.13	0.09	0.08	0.07	0.07
Sodium Bicarb %	0.31	0.31	0.38	0.38	0.42	0.42	0.38	0.38	0.45	0.46	0.47	0.48
Vitamins[2] %	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Mineral Premix[3] %	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Salinomycin[4] %	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Sand %	0.00	0.74	0.00	0.72	0.00	0.73	0.00	0.72	0.00	0.73	0.00	0.73
Calculated nutrients (%)												
Crude protein	18.01	18.00	17.28	17.28	16.92	16.92	17.18	17.18	16.48	16.48	16.20	16.20
Crude Fat	5.82	5.82	3.75	3.74	3.63	3.63	5.81	5.81	3.71	3.71	3.62	3.62
Ca	0.80	0.68	0.80	0.68	0.80	0.68	0.74	0.62	0.74	0.62	0.74	0.62
Av. P	0.40	0.25	0.40	0.25	0.40	0.25	0.37	0.22	0.37	0.22	0.37	0.22
AME (kcal/kg)	3,168	3,168	3,073	3,073	3,073	3,073	3,190	3,190	3,093	3,093	3,093	3,093
Dig. Methionine	0.49	0.49	0.47	0.47	0.46	0.46	0.46	0.46	0.44	0.44	0.43	0.43
Dig. TSAA	0.74	0.74	0.71	0.71	0.70	0.70	0.70	0.70	0.67	0.67	0.66	0.66
Dig. Lysine	0.95	0.95	0.91	0.91	0.89	0.89	0.90	0.90	0.87	0.87	0.85	0.85
Dig. Tryptophan	0.18	0.18	0.16	0.16	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.15
Dig. Threonine	0.65	0.65	0.62	0.62	0.61	0.61	0.61	0.61	0.59	0.59	0.58	0.58
Dig. Arginine	1.03	1.03	0.97	0.97	0.95	0.95	0.97	0.97	0.92	0.92	0.90	0.90
Sodium	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18

[1]Recovery (FTU/kg) of Grainzyme® Agrivida Inc., Woburn, MA 01801- Listed as 100% dAA PC 1,000, 100% dAA PC 4,500, 100% dAA NC 1,000, 100% dAA NC 4,500, 98% dAA PC 1,000, 98% dAA PC 4,500, 98% dAA NC 1,000, 98% dAA NC 4,500, 96% dAA PC 1,000, 96% dAA PC 4,500, 96% dAA NC 1,000, 96% dAA NC 4,500 respectively for each phase. Starter: 2,176, 4,353, 759, 5,204, 823, 4,513, 1,168, 3,869, 806, 3,085, 890, 4,630. Grower: 819, 5,160, 659, 5,533, 1,159, 4,108, 1,168, 3,841, 604, 3,668, 1,078, 5,520. Finisher: 404, 4,419, 1,105, 4,509, 1,310, 3,288, 1,183, 4,082, 983, 3,612, 1,066, 4,149. Withdraw: 598, 3,092, 823, 4,612, 1,112, 3,464, 862, 4,259, 659, 4,590, 1,282, 3,998.

[2]Vitamin premix added at this rate yields 22,045 IU vitamin A, 7,716 IU vitamin D_3, 91 IU vitamin E, 0.04 mg B_{12}, 11.9 mg riboflavin, 91.8 mg niacin, 40.4 mg d-pantothenic acid, 261.1 mg choline, 2.9 mg menadione, 3.50 mg folic acid, 14.3 mg pyroxidine, 5.87 mg thiamine, 1.10 mg biotin per kg diet. The carrier is ground rice hulls**.**

[3]Trace mineral premix added at this rate yields 120 mg of manganese, 72 mg of total zinc, 3.4 mg of copper, 2.33 mg of iodine, 0.2 mg of total selenium, 0.009 g of *bacillus subtilis.*

[4]Sacox 60, Huvepharma Inc., Peachtree City, GA.

Table IV-2. Continued

Dietary phase	Finisher						Withdrawal					
Feeding regime	A		B		C		A		B		C	
AA density	100% AA		96% AA		94% AA		100% AA		96% AA		94% AA	
ME density	100% ME		97% ME				100% ME		97% ME			
Mineral level	PC	NC	PC	NC	PC	NC	PC	NC	PC	NC	PC	NC
Analyzed nutrients (%)												
Moisture	11.66	11.66	12.12	12.12	11.32	11.32	13.48	13.48	13.36	13.36	13.43	13.43
Crude protein	16.3	16.3	16.0	16.0	15.9	15.9	16.0	16.0	15.2	15.2	15.6	15.6
Crude fat	5.47	5.47	3.68	3.68	2.88	2.88	5.09	5.09	2.93	2.93	2.98	2.98
P	0.65	0.57	0.70	0.56	0.68	0.53	0.66	0.49	0.70	0.48	0.67	0.46
Ca	1.15	1.01	1.25	1.00	1.26	1.12	0.98	0.73	1.21	0.77	1.04	0.73

For the second trial (Tables IV-3 and IV-4), regimen A was again formulated to provide dAA and metabolizable energy sufficient for growth and development similar to the first Trial. Regimen C was again utilized and had reductions of 2%, 4%, 6%, 6% dAA and 1%, 2%, 3%, 3% ME through the starter, grower, finisher and withdrawal phases, respectively. The basal diets were mixed for each phase in 2 separate batches and were mixed with the lower inclusions for each micronutrient and added additional micro-ingredients for each treatment to obtain the targeted nutrient level for the PC and NC diets, respectively. Additionally, 1,000 FTU/kg was added in the basal diet for the baseline phytase level. Treatments with the high dose of phytase received an additional 3,500 FTU/kg at the time of mixing individual treatments to reach the 4,500 FTU/kg target. It is worth noting that based on the differences in FC observed in the first Trial, the calculated mineral differences of the basal diets were adjusted from -0.10 to -0.12% in the finisher and withdraw phases in an effort to equalize mineral intake. Additionally, further reductions were added at 0.06% Ca and 0.03% P between the non-nutrient reduced PC and nutrient reduced PC diets in the grower, finisher and withdraw phases.

Table IV-3. Diet formulations with variable AA, energy (regimens A and C) and mineral density, calculated nutrient content of diets and phytase[1] recovery fed to Ross 708 broilers during the starter and grower phases of Trial 2.

Dietary phase	Starter				Grower			
Feeding regime	A		C		A		C	
AA density	100% AA		98% AA		100% AA		96% AA	
ME density	100% ME		99% ME		100% ME		98% ME	
Mineral level	PC	NC	PC	NC	PC	NC	PC	NC
Ingredient (%)								
Corn %	58.54	58.54	60.64	60.64	64.65	64.65	68.73	68.73
Soybean meal %	35.49	35.50	34.20	34.20	29.10	29.10	26.70	26.70
DL-Methionine %	0.27	0.268	0.26	0.26	0.27	0.27	0.25	0.25
Lysine HCL %	0.13	0.13	0.14	0.14	0.20	0.20	0.21	0.21
L-Threonine %	0.35	0.35	0.35	0.35	0.07	0.07	0.07	0.07
Soy Oil %	1.84	1.84	1.03	1.03	2.17	2.17	0.50	0.50
Limestone %	1.43	1.47	1.44	1.47	1.36	1.40	1.27	1.31
Monocalcium Phosphate %	1.55	0.84	1.55	0.84	1.45	0.74	1.31	0.60
Salt %	0.41	0.41	0.41	0.41	0.31	0.31	0.25	0.25
Sodium Bicarbonate %	-	-	-	-	0.14	0.14	0.21	0.21
Vitamins[1] %	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Mineral Premix[2] %	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Salinomycin[3] %	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Sand %	-	0.67	-	0.67	-	0.67	-	0.67
Calculated nutrients (%)								
Crude protein	22.50	22.5	22.05	22.05	20.00	20.00	19.20	19.20
Calcium	0.90	0.80	0.90	0.80	0.84	0.74	0.78	0.68
Av. Phosphorus	0.45	0.30	0.45	0.30	0.42	0.27	0.39	0.24
AME Poultry (kcal/kg)	3,000	3,000	2,970	2,970	3,080	3,080	3,018	3,018
Dig. Methionine	0.57	0.57	0.56	0.56	0.54	0.54	0.52	0.52
Dig. Lysine	1.18	1.18	1.16	1.16	1.07	1.07	1.03	1.03
Dig. TSAA	0.87	0.87	0.86	0.86	0.81	0.81	0.78	0.78
Dig. Tryptophan	0.24	0.24	0.23	0.23	0.20	0.20	0.19	0.19
Dig. Threonine	0.77	0.77	0.75	0.75	0.71	0.71	0.68	0.68
Dig. Arginine	1.36	1.36	1.33	1.33	1.18	1.18	1.11	1.11
Sodium	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18

[1]Recovery (FTU/kg) of Grainzyme® Agrivida Inc., Woburn, MA 01801- Listed as 100% dAA PC 1,000, 100% dAA PC 4,500, 100% dAA NC 1,000, 100% dAA NC 4,500, reduced dAA PC 1,000, reduced dAA PC 4,500, reduced dAA NC 1,000, reduced dAA NC 4,500 respectively for each phase. Starter: 485, 2,264, 899, 2,441, 437, 1,746, 833, 2,364. Grower: 416, 2,386, 690, 2,502, 690, 2,502, 548, 2,489, 561, 2,521. Finisher: 718, 2,606, 841, 2,939, 520, 2,982, 944, 2,754. Withdraw: 420, 2,141, 679, 2,145, 339, 2,236, 728, 2,380.

[2]Vitamin premix added at this rate yields 22,045 IU vitamin A, 7,716 IU vitamin D_3, 91 IU vitamin E, 0.04 mg B_{12}, 11.9 mg riboflavin, 91.8 mg niacin, 40.4 mg d-pantothenic acid, 261.1 mg choline, 2.9 mg menadione, 3.50 mg folic acid, 14.3 mg pyroxidine, 5.87 mg thiamine, 1.10 mg biotin per kg diet. The carrier is ground rice hulls**.**

[3]Trace mineral premix added at this rate yields 120 mg of manganese, 72 mg of total zinc, 3.4 mg of copper, 2.33 mg of iodine, 0.2 mg of total selenium, 0.009 g of *bacillus subtilis.*

[4]Sacox 60, Huvepharma Inc., Peachtree City, GA

Table IV-3. Continued

Dietary phase	Starter				Grower			
Feeding regime	A		C		A		C	
AA density	100% AA		98% AA		100% AA		96% AA	
ME density	100% ME		99% ME		100% ME		98% ME	
Mineral level	PC	NC	PC	NC	PC	NC	PC	NC
Analyzed nutrients (%)								
Crude Protein	22.80	22.80	22.5	22.5	20.4	20.40	18.00	18.00
Crude fat	4.20	4.20	3.50	3.50	4.55	4.55	3.20	3.20
Crude fiber	2.50	2.50	3.70	3.70	3.50	3.50	2.80	2.80
Calcium	1.39	1.03	1.05	0.91	0.92	0.77	0.95	0.80
Phosphorus	0.74	0.67	0.71	0.65	0.72	0.56	0.75	0.55

Table IV-4. Diet formulations with variable AA, energy (regimens A, B and C) and mineral density, calculated nutrient content of diets and phytase[1] recovery fed to Ross 708 broilers during the finisher and withdraw phases of Trial 2.

Dietary phase	Finisher				Withdrawal			
Feeding regime	A		C		A		C	
AA density	100% AA		94% AA		100% AA		94% AA	
ME density	100% ME		97% ME		100% ME		97% ME	
Mineral level	PC	NC	PC	NC	PC	NC	PC	NC
Ingredient (%)								
Corn %	69.04	69.04	74.57	74.56	71.29	71.30	76.47	76.47
Soybean meal %	24.25	24.25	21.00	21.00	22.15	22.15	19.25	19.25
DL-Methionine %	0.23	0.23	0.21	0.21	0.21	0.21	0.19	0.19
Lysine HCL %	0.20	0.20	0.22	0.22	0.20	0.20	0.22	0.22
L-Threonine %	0.07	0.07	0.08	0.08	0.07	0.07	0.07	0.07
Soy Oil %	2.87	2.87	0.50	0.50	2.86	2.86	0.50	0.05
Limestone %	1.24	1.23	1.17	1.15	1.19	1.17	1.11	1.09
Monocalcium Phosphate %	1.29	0.58	1.16	0.45	1.21	1.49	1.08	0.37
Salt %	0.19	0.19	0.11	0.11	0.14	0.13	0.07	0.07
Sodium Bicarbonate %	0.31	0.31	0.42	0.42	0.38	0.38	0.48	0.48
Vitamins[2] %	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Mineral Premix[3] %	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Salinomycin[4] %	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Sand %	-	0.73	0.26	0.99	-	0.73	0.28	1.00
Calculated nutrients (%)								
Protein	18.01	18.01	16.92	16.92	17.17	17.17	16.21	16.21
Calcium	0.76	0.64	0.70	0.58	0.72	0.60	0.66	0.54
Av. Phosphorus	0.38	0.23	0.35	0.20	0.36	0.21	0.33	0.18
AME Poultry (kcal/kg)	3,168	3,168	3,074	3,073	3,190	3,190	3,093	3,093
Dig. Methionine	0.49	0.49	0.46	0.46	0.46	0.46	0.43	0.43
Dig. Lysine	0.95	0.95	0.89	0.89	0.90	0.90	0.85	0.85
Dig. TSAA	0.74	0.74	0.70	0.70	0.70	0.70	0.66	0.66
Dig. Tryptophan	0.18	0.18	0.16	0.16	0.16	0.16	0.15	0.15
Dig. Threonine	0.65	0.65	0.61	0.61	0.61	0.61	0.58	0.58
Dig. Arginine	1.03	1.03	0.95	0.95	0.97	0.97	0.90	0.90
Sodium	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18

[1]Recovery (FTU/kg) of Grainzyme® Agrivida Inc., Woburn, MA 01801- Listed as 100% dAA PC 1,000, 100% dAA PC 4,500, 100% dAA NC 1,000, 100% dAA NC 4,500, reduced dAA PC 1,000, reduced dAA PC 4,500, reduced dAA NC 1,000, reduced dAA NC 4,500 respectively for each phase. Starter: 485, 2,264, 899, 2,441, 437, 1,746, 833, 2,364. Grower: 416, 2,386, 690, 2,502, 690, 2,502, 548, 2,489, 561, 2,521. Finisher: 718, 2,606, 841, 2,939, 520, 2,982, 944, 2,754. Withdraw: 420, 2,141, 679, 2,145, 339, 2,236, 728, 2,380.

[2]Vitamin premix added at this rate yields 22,045 IU vitamin A, 7,716 IU vitamin D_3, 91 IU vitamin E, 0.04 mg B_{12}, 11.9 mg riboflavin, 91.8 mg niacin, 40.4 mg d-pantothenic acid, 261.1 mg choline, 2.9 mg menadione, 3.50 mg folic acid, 14.3 mg pyroxidine, 5.87 mg thiamine, 1.10 mg biotin per kg diet. The carrier is ground rice hulls**.**

[3]Trace mineral premix added at this rate yields 120 mg of manganese, 72 mg of total zinc, 3.4 mg of copper, 2.33 mg of iodine, 0.2 mg of total selenium, 0.009 g of *bacillus subtilis.*

[4]Sacox 60, Huvepharma Inc., Peachtree City, GA.

Table IV-4. Continued

Dietary phase	Finisher				Withdrawal			
Feeding regime	A		C		A		C	
AA density	100% AA		94% AA		100% AA		94% AA	
ME density	100% ME		97% ME		100% ME		97% ME	
Mineral level	PC	NC	PC	NC	PC	NC	PC	NC
Analyzed nutrients (%)								
Crude protein	17.90	17.90	17.50	17.50	16.90	16.90	15.90	15.90
Crude fat	5.44	5.44	3.50	3.50	5.18	5.18	3.69	3.69
Crude fiber	2.60	2.60	4.00	4.00	2.80	2.80	2.90	2.90
Calcium	1.11	0.84	0.85	0.64	1.00	0.79	0.79	0.68
Phosphorus	0.68	0.53	0.69	0.48	0.59	0.47	0.53	0.42

For both trials, pelleting temperature was maintained at 75 C with a conditioning time of 12 s. The starter phase was fed as a crumble from d 1 to 14, while the grower was fed as a pellet from d 15 to 28, the finisher as a pellet from d 29 to 42, and the withdrawal as a pellet from d 43 to termination.

Animals and Management Practices

For Trial 1, 18 Ross 708 male broilers were placed into 1.83 m X 0.91 m floor pens with an overall stocking density of 0.88 m^2 per broiler and a total of 1,944 birds for a 49 d assay period. For Trial 2, 36 Ross 708 male broilers were placed into 1.83 m X 1.83 m floor pens with an overall stocking density of 0.88 m^2 per broiler and a total of 2,304 birds for a 48 d assay period. Birds in both trials were placed into floor pens with fresh pine shavings. Birds were provided with tube feeders (13.5 kg), nipple drinkers and age-appropriate supplemental heat. In both trials, BW and FC were measured, and FCR was calculated on d 14, 28, 42 and at the termination of the trial (d 49 for Trial 1 and d 47 for Trial 2). Following an 8-hour feed withdrawal (on d 50 for Trial 1 and d 48 for Trial 2), 5 birds per replicate pen were randomly selected and processed at Texas A&M University's pilot processing facility for determination of processing weights and yields. All animal care and husbandry were provided in accordance with a Texas A&M University IACUC approved protocol.

Statistical Analysis

The data for both trials were subjected to a 3 X 2 X 2 and a 2 X 2 X 2 Factorial Analysis of Variance (Skřivan, et al.) using the General Linear Model for all data with main effect means deemed significantly different at $P \leq 0.05$. In instances of significant

interactions between factors, a one-way ANOVA was performed where means were deemed significantly different at ($P \leq 0.05$). Main effect means and treatment means were further separated by Duncan's Multiple Range Test.

Results

Trial 1

Performance

Through d 14, BW (Table IV-5) was increased ($P \leq 0.05$) both by increasing phytase inclusion from 1,000 to 4,500 FTU/kg as well as increasing mineral density. By d 28 and 42, those main effects differences transferred into interactions between nutrient density X minerals and phytase X minerals. Phytase had an increased ($P \leq 0.05$) response with the reduction of Ca and P, while the reduction of minerals in the reduced nutrient density diets served to increase ($P \leq 0.001$) BW. By d 49, a 3-way interaction implied that reducing the nutrient density gave the combination of the high inclusion of phytase and reduced minerals improved responses to achieve similar BW as the PC control diet.

Table IV-5. Body weights (BW) of Ross 708 broilers fed diets with reduced amino acid, metabolizable energy and mineral densities supplemented with phytase[1] through 49 d in Trial 1.

				Body weight (kg)			
TRT	Regimen*	Phytase FTU/kg	Mineral	d 14	d 28	d 42	d 49
1	A	1,000	PC	0.439	1.454^{cd}	2.748^{bc}	3.458^{abc}
2	A	4,500	PC	0.450	1.535^{a}	2.913^{a}	3.587^{a}
3	A	1,000	NC	0.422	1.445^{de}	2.769^{bc}	3.415^{bc}
4	A	4,500	NC	0.444	1.491^{bc}	2.853^{ab}	3.556^{ab}
5	B	1,000	PC	0.438	1.380^{f}	2.573^{d}	3.148^{d}
6	B	4,500	PC	0.447	1.475^{bcd}	2.778^{bc}	3.464^{abc}
7	B	1,000	NC	0.437	1.455^{cd}	2.763^{bc}	3.486^{abc}
8	B	4,500	NC	0.446	1.459^{cd}	2.723^{c}	3.428^{bc}
9	C	1,000	PC		1.405^{ef}	2.605^{d}	3.208^{d}
10	C	4,500	PC		1.457^{cd}	2.709^{c}	3.346^{c}
11	C	1,000	NC		1.477^{bcd}	2.774^{bc}	3.437^{abc}
12	C	4,500	NC		1.517^{ab}	2.809^{bc}	3.504^{ab}
Main Effect Means							
Nutrients	Regimen A			0.439	1.482	2.825	3.504
	Regimen B			0.442	1.445	2.717	3.381
	Regimen C				1.464	2.724	3.374
Phytase FTU/kg		1,000		0.435^{b}	1.438	2.711	3.359
		4,500		0.447^{a}	1.490	2.798	3.481
Mineral			NC	0.438^{b}	1.475	2.782	3.471
			PC	0.443^{a}	1.454	2.729	3.368
P-values	Nutrients			0.302	0.002	<0.001	<0.001
	Phytase			<0.001	<0.001	<0.001	<0.001
	Mineral			0.021	0.003	0.004	<0.001
	Nutrients X Phytase			0.106	0.716	0.579	0.878
	Nutrients X Mineral			0.078	<0.001	0.005	0.002
	Phytase X Mineral			0.195	0.010	0.002	0.010
	Nutrients X Phytase X Mineral			0.250	0.230	0.128	0.013

[a-f]Means within a column with different superscripts differ at (P≤0.05)

[1]Grainzyme, Agrivida, Woburn, MA 018301

*Regimens B and C contained similar AA and energy density during the starter phase with -2% AA density and -1% ME compared to Regimen A.

FCR (Table IV-6) revealed that reducing nutrient density through d 14 as well as in the withdraw phase (d 43 to 49) led to increased ($P \leq 0.05$) FCR. However, through d 42 and 49, interactions between phytase X mineral density affected performance, where the higher inclusion of phytase had improved FCR only within the PC diets. Cumulatively through d 28 and through d 49 (adjusted), a 3-way interaction revealed that the high inclusion of phytase improved FCR with both mineral densities within the 94% dAA diets. However, within the control and 96% dAA diets, the higher inclusion of phytase was only able to reduce FCR in the PC diets while the mineral reductions of the NC resulted in increased FCR.

Table IV-6. Feed conversion ratio of Ross 708 broilers fed diets with reduced amino acid, metabolizable energy and mineral densities supplemented with phytase[1] through 49 d in Trial 1.

TRT	Regimen*	Phytase FTU/kg	Mineral	Feed conversion ratio (FCR)							
				d 0 to 14	d 15 to 28	d 0 to 28	d 29 to 42	d 1 to 42	d 43 to 49	d 1 to 49	**Adj. 1 to 49
1	A	1,000	PC	1.227	1.496^{fg}	1.420^{fg}	1.808^{c}	1.608^{d}	1.989	1.694^{c}	1.682^{cd}
2	A	4,500	PC	1.227	1.456^{h}	1.393^{h}	1.797^{c}	1.585^{d}	2.065	1.674^{c}	1.622^{d}
3	A	1,000	NC	1.21	1.452^{h}	1.389^{h}	1.800^{c}	1.587^{d}	2.073	1.679^{c}	1.681^{cd}
4	A	4,500	NC	1.221	1.482^{g}	1.410^{g}	1.779^{c}	1.587^{d}	2.047	1.677^{c}	1.635^{d}
5	B	1,000	PC	1.254	1.631^{a}	1.522^{a}	1.926^{ab}	1.709^{a}	2.119	1.790^{a}	1.875^{a}
6	B	4,500	PC	1.246	1.550^{cd}	1.462^{c}	1.878^{b}	1.662^{c}	2.076	1.737^{b}	1.724^{bc}
7	B	1,000	NC	1.242	1.530^{de}	1.445^{de}	1.878^{b}	1.658^{c}	2.031	1.734^{b}	1.713^{c}
8	B	4,500	NC	1.234	1.539^{cd}	1.457^{cd}	1.929^{ab}	1.670^{c}	2.038	1.745^{b}	1.743^{bc}
9	C	1,000	PC		1.581^{b}	1.481^{b}	1.946^{a}	1.696^{ab}	2.192	1.790^{a}	1.857^{a}
10	C	4,500	PC		1.557^{c}	1.469^{bc}	1.913^{ab}	1.675^{bc}	2.159	1.763^{ab}	1.786^{b}
11	C	1,000	NC		1.552^{cd}	1.469^{bc}	1.875^{b}	1.660^{c}	2.154	1.753^{b}	1.747^{bc}
12	C	4,500	NC		1.511^{ef}	1.436^{ef}	1.925^{ab}	1.664^{c}	2.101	1.748^{b}	1.721^{bc}
Main Effect Means											
Nutrients	Regimen A			1.224^{b}	1.471	1.403	1.798	1.593	2.05^{b}	1.681	1.655
	Regimen B			1.244^{a}	1.565	1.473	1.900	1.674	2.047^{b}	1.752	1.764
	Regimen C				1.550	1.464	1.915	1.674	2.151^{a}	1.763	1.778
Phytase FTU/kg		1,000		1.240	1.542	1.455	1.873	1.653	2.096	1.740	1.759
		4,500		1.235	1.515	1.437	1.868	1.639	2.071	1.724	1.705
Mineral			NC	1.232	1.510	1.433	1.862	1.636	2.074	1.723	1.707
			PC	1.242	1.546	1.459	1.878	1.655	2.093	1.741	1.757
P-values	Nutrients			0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001	<0.001
	Phytase			0.525	<0.001	<0.001	0.747	0.010	0.390	0.014	<0.001
	Mineral			0.146	<0.001	<0.001	0.161	<0.001	0.501	0.003	<0.001
	Nutrients X Phytase			0.496	0.016	0.021	0.493	0.782	0.484	0.695	0.905
	Nutrients X Mineral			0.653	<0.001	<0.001	0.481	0.564	0.607	0.286	0.007
	Phytase X Mineral			0.980	<0.001	<0.001	0.006	<0.001	0.994	0.008	0.003
	Nutrients X Phytase X Mineral			0.998	<0.001	<0.001	0.124	0.331	0.280	0.192	0.017

[a-f]Means within a column with different superscripts differ at (P≤0.05)

[1]Grainzyme, Agrivida, Woburn, MA 018301

* Regimens B and C contained similar AA and energy density during the starter phase with -2% AA density and -1% ME compared to Regimen A.

**Adjusted FCR was done by 1 point of FCR equal to 32 g of BW.

During the starter phase, FC (Table IV-7) followed as expected with increased (P=0.004) FC when reducing nutrient density, increased (P≤0.001) FC when including a higher level of phytase and was reduced (P=0.003) FC when Ca and P was removed. During the grower from d 15 to 28 and cumulatively through d 28 and d 42, it was observed that a nutrient density X mineral interaction had an increased (P≤0.05) effect on FC when reducing both nutrient density and mineral density; however in the control diets, the PC had increased FC compared to the NC. Cumulatively through d 49, a 3-way interaction was observed that as nutrients and minerals were removed and phytase was increased, FC was generally increased (P=0.050) with the exception of the control diets where the mineral reduced diets were similar to the PC 1,000 FTU/kg phytase regardless of phytase inclusion.

Table IV-7. Feed consumption and litter P content of Ross 708 broilers fed diets with reduced amino acid, metabolizable energy and mineral densities supplemented with phytase[1] through 49 d in Trial 1.

				Feed consumption (FC) (g/bird/d)							(ppm)
TRT	Regimen*	Phytase FTU/kg	Mineral	d 0 to 14	d 15 to 28	d 0 to 28	d 29 to 42	d 1 to 42	d 43 to 49	d 1 to 49	Litter P
1	A	1,000	PC	34.4	108.8^{e}	71.3^{de}	170.0^{abc}	102.3^{bc}	201.8	117.2^{bcd}	14,211
2	A	4,500	PC	35.5	112.8^{abcd}	73.9^{abc}	178.4^{a}	106.5^{ab}	199.1	120.1^{abc}	15,316
3	A	1,000	NC	32.9	104.9^{f}	68.8^{f}	170.0^{abc}	100.7^{cd}	202.5	114.6^{de}	10,543
4	A	4,500	NC	34.7	111.2^{cde}	72.5^{bcde}	173.6^{ab}	104.6^{abc}	204.5	119.3^{abcd}	12,485
5	B	1,000	PC	34.7	108.1^{ef}	70.6^{ef}	162.7^{c}	97.5^{d}	188.5	111.5^{e}	13,563
6	B	4,500	PC	35.6	113.8^{abc}	74.3^{ab}	176.5^{ab}	104.6^{abc}	204.9	119.0^{abcd}	14,712
7	B	1,000	NC	34.6	111.2^{cde}	72.3^{bcde}	176.6^{ab}	105.4^{abc}	210.2	120.4^{ab}	11,112
8	B	4,500	NC	35.2	111.7^{bcde}	73.0^{abcde}	175.5^{ab}	104.9^{abc}	203.1	119.5^{abcd}	11,061
9	C	1,000	PC		109.4^{de}	71.8^{cde}	167.5^{bc}	102.3^{bc}	188.3	115.0^{cde}	15,070
10	C	4,500	PC		112.8^{abcd}	73.7^{abcd}	172.4^{ab}	103.9^{abc}	196.0	117.4^{bcd}	15,050
11	C	1,000	NC		115.5^{ab}	74.3^{ab}	174.2^{ab}	105.3^{abc}	202.0	120.0^{abc}	10,244
12	C	4,500	NC		115.8^{a}	75.4^{a}	177.6^{a}	108.5^{a}	206.2	122.8^{a}	11,264
Main Effect Means											
Nutrients	Regimen A			34.4^{b}	109.4	71.6	173.0	103.5	201.8	117.8	12,983
	Regimen B			35.0^{a}	111.3	72.6	172.8	103.1	201.7	117.6	12,488
	Regimen C				113.4	73.8	172.9	105.0	198.1	118.8	12,907
Phytase FTU/kg		1,000		34.3^{b}	109.6	71.5	170.2	102.3	198.6	116.4	12,493^{b}
		4,500		35.3^{a}	113.0	73.8	175.7	105.5	202.5	119.7	13,106^{a}
Mineral			NC	34.5^{b}	111.7	72.7	174.6	104.9	204.8^{a}	119.4	10,977^{b}
			PC	35.1^{a}	111.0	72.6	171.2	102.9	196.3^{b}	116.7	14,651^{a}
P-values	Nutrients			0.004	<0.001	0.001	0.997	0.169	0.369	0.495	0.563
	Phytase			<0.001	<0.001	<0.001	0.002	<0.001	0.104	<0.001	0.006
	Mineral			0.003	0.258	0.742	0.059	0.02	0.001	0.003	<0.001
	Nutrients X Phytase			0.104	0.161	0.330	0.857	0.735	0.688	0.828	0.637
	Nutrients X Mineral			0.055	<0.001	0.001	0.068	0.010	0.316	0.004	0.149
	Phytase X Mineral			0.597	0.150	0.291	0.045	0.231	0.082	0.227	0.821
	Nutrients X Phytase X Mineral			0.179	0.082	0.147	0.267	0.076	0.079	0.050	0.183

[a-f]Means within a column with different superscripts differ at (P≤0.05)

[1]Grainzyme, Agrivida, Woburn, MA 018301

* Regimens B and C contained similar AA and energy density during the starter phase with -2% AA density and -1% ME compared to Regimen A.

Litter and Processing

Litter P concentration (Table IV-7) confirmed the impact that varying dietary mineral level has on litter mineral content as the litter from pens of broilers fed the PC diet had elevated levels of P in the litter. Elevating phytase inclusion was effective at hydrolyzing additional phytate; however, this additional hydrolysis resulted in higher P litter content. The elevation of litter P with the higher inclusion of phytase is counter-intuitive as research has repeatedly found increasing phytase to increase P retention. Therefore, the results may have been skewed from practices involving litter collection as only a few samples were analyzed per replicate and litter quality varied across each pen. Concerning processing results (Table IV-8), a nutrient X mineral density interaction was observed for WOG, breast and tender weight. Aside from the expected results of reducing mineral density in the control diets leading to a reduction ($P \leq 0.05$) in processing weights, the reduced minerals combined with the reduced nutrients for an increasing effect where the NC diets had improved weights compared to the PC. Breast and total processing yield (Table IV-8) were affected by decreasing nutrient density as broilers consuming the 96% dAA diet maximized breast meat yield compared to broilers fed the control diet, while 94% dAA produced intermediate results. The increased yield associated with the reduction in dAA can be explained by the increased FC that was observed allowing for an increased amount of dig.Lys to be absorbed and accreted into the tissue. The differences observed in breast yield correlated to the differences observed in total white meat yield.

Table IV-8. Processing weights and yields of Ross 708 broilers fed diets with reduced amino acid, metabolizable energy and mineral densities supplemented with phytase[1] through 49 d in Trial 1.

TRT	Regimen*	Phytase FTU/kg	Mineral	Weight			Yield (%)			
				WOG (kg)	Breast (g)	Tender (g)	WOG	Breast	Tender	Total
1	A	1,000	PC	2.809^{bcd}	845.2^{abc}	147.7^{bc}	79.90^{ab}	30.10	5.30	35.40
2	A	4,500	PC	2.986^{a}	892.2^{a}	159.2^{a}	80.40^{ab}	29.90	5.30	35.20
3	A	1,000	NC	2.749^{bcd}	811.6^{bc}	148.5^{bc}	79.80^{ab}	29.50	5.40	34.90
4	A	4,500	NC	2.858^{ab}	847.9^{abc}	152.8^{abc}	80.20^{ab}	29.70	5.40	35.00
5	B	1,000	PC	2.670^{d}	806.3^{c}	145.5^{bc}	79.80^{ab}	30.20	5.50	35.60
6	B	4,500	PC	2.838^{bc}	867.9^{ab}	148.6^{bc}	80.00^{ab}	30.60	5.20	35.80
7	B	1,000	NC	2.855^{ab}	867.0^{ab}	151.8^{abc}	79.90^{ab}	30.40	5.30	35.70
8	B	4,500	NC	2.815^{bcd}	858.2^{abc}	150.8^{bc}	79.80^{ab}	30.50	5.40	35.90
9	C	1,000	PC	2.697^{cd}	812.9^{bc}	146.8^{bc}	79.30^{b}	30.10	5.50	35.60
10	C	4,500	PC	2.733^{bcd}	814.2^{bc}	144.5^{c}	80.40^{a}	29.80	5.30	35.10
11	C	1,000	NC	2.808^{bcd}	857.4^{abc}	151.1^{bc}	80.60^{a}	30.50	5.40	35.90
12	C	4,500	NC	2.801^{bcd}	846.9^{abc}	153.2^{ab}	79.70^{ab}	30.20	5.50	35.70
Main Effects										
Nutrients	Regimen A			2.850	849.2	152.0	80.10	29.80^{b}	5.30	35.10^{b}
	Regimen B			2.794	849.9	149.2	79.90	30.40^{a}	5.30	35.80^{a}
	Regimen C			2.761	833.4	148.9	80.00	30.20^{ab}	5.40	35.60^{ab}
Phytase FTU/kg		1,000		2.765	833.8	148.6	79.90	30.10	5.40	35.50
		4,500		2.838	854.6	151.5	80.10	30.10	5.40	35.50
Mineral			NC	2.814	848.2	151.4	80.00	30.10	5.40	35.50
			PC	2.790	840.3	148.7	80.00	30.10	5.30	35.40
P-values	Nutrients			0.026	0.308	0.17	0.685	0.025	0.507	0.028
	Phytase			0.007	0.04	0.055	0.259	0.924	0.457	0.782
	Mineral			0.359	0.408	0.079	0.822	0.826	0.411	0.677
	Nutrients X Phytase			0.142	0.181	0.052	0.611	0.511	0.757	0.596
	Nutrients X Mineral			0.009	0.005	0.034	0.617	0.209	0.760	0.269
	Phytase X Mineral			0.054	0.131	0.47	0.045	0.903	0.231	0.676
	Nutrients X Phytase X Mineral			0.394	0.397	0.228	0.078	0.767	0.171	0.957

[a-d]Means within a column with different superscripts differ at (P≤0.05)

[1]Grainzyme, Agrivida, Woburn, MA 018301

* Regimens B and C contained similar AA and energy density during the starter phase with -2% AA density and -1% ME compared to Regimen A.

Trial 2

Performance

At the conclusion of the starter phase, d 14, a 3-way interaction provided an increased ($P \leq 0.05$) effect on BW (Table IV-9). Elevating phytase inclusion had a greater effect on the PC diets while the NC diets BW's were similar; however, the greatest effect was observed in the control PC diet. At d 28 and d 41, a nutrient X phytase interaction was observed where increasing phytase inclusion in the nutrient reduced diets had less of an improvement ($P \leq 0.05$) in BW between inclusion levels compared to phytase inclusions in the control diet. Day 41 also had an interaction between phytase X minerals where it was observed that increasing the inclusion of phytase increased ($P \leq 0.05$) BW with the PC diets as opposed to the NC diets.

Table IV-9. Body weight (kg) and body weight gain (kg) of Ross 708 broilers fed diets with reduced amino acid, metabolizable energy and mineral densities supplemented with phytase[1] through 48 d in Trial 2.

				Body weight (kg)			
TRT	Regimen*	Phytase FTU/kg	Mineral	d 14	d 28	d 41	d 47
1	A	1,000	PC	0.414^{c}	1.491^{cd}	2.968^{b}	3.553
2	A	4,500	PC	0.455^{a}	1.594^{a}	3.096^{a}	3.680
3	A	1,000	NC	0.428^{b}	1.493^{cd}	2.997^{b}	3.588
4	A	4,500	NC	0.433^{b}	1.564^{ab}	3.067^{a}	3.661
5	C	1,000	PC	0.435^{b}	1.477^{d}	2.878^{d}	3.464
6	C	4,500	PC	0.449^{a}	1.525^{bc}	2.955^{bc}	3.530
7	C	1,000	NC	0.429^{b}	1.460^{d}	2.903^{cd}	3.478
8	C	4,500	NC	0.432^{b}	1.470^{d}	2.907^{cd}	3.488
Main Effect Means							
Nutrients	Regimen A			0.433	1.535	3.032	3.620^{a}
	Regimen C			0.436	1.483	2.911	3.490^{b}
Phytase FTU/kg		1,000		0.427	1.480	2.937	3.521^{b}
		4,500		0.442	1.538	3.006	3.590^{a}
Mineral			NC	0.431	1.497	2.968	3.554
			PC	0.438	1.522	2.974	3.557
P-values	Nutrients			0.147	<0.001	<0.001	<0.001
	Phytase			<0.001	<0.001	<0.001	<0.001
	Mineral			0.002	0.013	0.658	0.858
	Nutrients X Phytase			0.003	0.004	0.032	0.071
	Nutrients X Mineral			0.131	0.278	0.677	0.528
	Phytase X Mineral			<0.001	0.075	0.016	0.111
	Nutrients X Phytase X Mineral			0.014	0.883	0.772	0.982

[a-d]Means within a column with different superscripts differ at (P≤0.05)

[1]Grainzyme, Agrivida, Woburn, MA 018301

* Regimens B and C contained similar AA and energy density during the starter phase with -2% AA density and -1% ME compared to Regimen A.

During the starter and grower phases (d 1 to 14, 14 to 28) a 3-way interaction had an increased ($P \leq 0.05$) effect on FCR (Table IV-10) in the nutrient reduced diets as well as the control PC diet with the low level of phytase in the starter. For the remainder of the experiment, FCR was impacted by a phytase X mineral interaction and a nutrient X mineral interaction. The phytase X mineral interaction had an increasing ($P \leq 0.05$) effect on FCR with increasing phytase inclusion in mineral reduced NC diets. The nutrient X mineral interaction had an increased effect on mineral density improving ($P \leq 0.05$) FCR within the control diets as Ca and P was removed. However, mineral density did not have a significant affect in the nutrient reduced diets.

Table IV-10. Feed conversion ratio of Ross 708 broilers fed diets with reduced amino acid, metabolizable energy and mineral densities supplemented with phytase[1] through 48 d in Trial 2.

				Feed conversion ratio (FCR)								
TRT	Regimen*	Phytase FTU/kg	Mineral	d 1 to 14	d 14 to 28	d 1 to 28	d 28 to 41	d 1 to 41	ADJ 1 to 41	d 41 to 47	d 1 to 47	**ADJ 1 to 47
1	A	1,000	PC	1.328^{a}	1.525^{b}	1.474^{c}	1.787^{bc}	1.630^{d}	1.642^{d}	1.966^{ab}	1.684^{b}	1.684^{c}
2	A	4,500	PC	1.236^{c}	1.496^{bc}	1.427^{de}	1.776^{bc}	1.598^{e}	1.562^{e}	1.980^{a}	1.657^{c}	1.634^{d}
3	A	1,000	NC	1.233^{c}	1.514^{b}	1.439^{d}	1.716^{d}	1.579^{f}	1.580^{e}	1.907^{b}	1.633^{d}	1.621^{d}
4	A	4,500	NC	1.237^{c}	1.470^{c}	1.409^{e}	1.764^{c}	1.583^{ef}	1.558^{e}	1.936^{ab}	1.640^{cd}	1.606^{d}
5	C	1,000	PC	1.284^{b}	1.595^{a}	1.510^{a}	1.856^{a}	1.680^{a}	1.725^{a}	1.936^{ab}	1.722^{a}	1.748^{a}
6	C	4,500	PC	1.260^{bc}	1.563^{a}	1.480^{bc}	1.834^{a}	1.653^{bc}	1.670^{cd}	1.990^{a}	1.707^{a}	1.713^{bc}
7	C	1,000	NC	1.271^{b}	1.563^{a}	1.483^{bc}	1.814^{ab}	1.650^{c}	1.685^{bc}	1.978^{a}	1.704^{a}	1.726^{ab}
8	C	4,500	NC	1.273^{b}	1.590^{a}	1.503^{ab}	1.838^{a}	1.669^{ab}	1.704^{ab}	1.985^{a}	1.720^{a}	1.739^{ab}
Main Effect Means												
Nutrients	Regimen A			1.259	1.501	1.437	1.761	1.597	1.586	1.947	1.653	1.632
	Regimen C			1.272	1.578	1.494	1.836	1.663	1.696	1.972	1.713	1.731
Phytase FTU/kg		1,000		1.279	1.549	1.476	1.793	1.635	1.658	1.947	1.686	1.695
		4,500		1.252	1.530	1.455	1.803	1.626	1.623	1.973	1.681	1.669
Mineral			NC	1.254	1.534	1.459	1.783	1.620	1.632	1.951	1.674	1.673
			PC	1.277	1.545	1.472	1.813	1.666	1.650	1.968	1.693	1.691
P-values	Nutrients			0.069	<0.001	<0.001	<0.001	<0.001	<0.001	0.108	<0.001	<0.001
	Phytase			0.001	0.020	0.001	0.302	0.059	<0.001	0.099	0.287	0.002
	Mineral			0.003	0.204	0.029	0.003	<0.001	0.030	0.289	<0.001	0.030
	Nutrients X Phytase			0.033	0.036	0.008	0.359	0.249	0.460	0.789	0.251	0.071
	Nutrients X Mineral			0.003	0.315	0.048	0.243	0.006	0.630	0.029	0.001	0.019
	Phytase X Mineral			<0.001	0.176	0.009	0.010	<0.001	<0.001	0.605	0.001	0.003
	Nutrients X Phytase X Mineral			0.022	0.024	0.176	0.741	0.590	0.628	0.317	0.874	0.941

[a-e]Means within a column with different superscripts differ at (P≤0.05)

[1]Grainzyme, Agrivida, Woburn, MA 018301

* Regimens B and C contained similar AA and energy density during the starter phase with -2% AA density and -1% ME compared to Regimen A.

**Adjusted FCR was done by 1 point of FCR equal to 32 g of BW.

FC (Table IV-11) during the starter phase (d 1 to 14) was increased by reducing nutrient density ($P=0.002$), increasing phytase inclusion ($P=0.008$), and reducing mineral density ($P<0.001$). With the exclusion of the withdraw (d 41 to 47) of the trial, FC was increased ($P \leq 0.05$) by increasing phytase inclusion, while reducing mineral density from the PC to the NC increased FC with the exception of the finisher (d 28 to 41) where only phytase had significant impacts.

Table IV-11. Feed consumption of Ross 708 broilers fed diets with reduced amino acid, metabolizable energy and mineral densities supplemented with phytase[1] through 48 d in Trial 2.

				Feed consumption (FC) (g/bird/d)						
TRT	Regimen*	Phytase FTU/kg	Mineral	d 1 to 14	d 14 to 28	d 1 to 28	d 28 to 41	d 1 to 41	d 41 to 47	d 1 to 47
1	A	1,000	PC	34.2	116.9	74.8	186.7	111.5	226.9	123.4
2	A	4,500	PC	35.8	121.5	78.3	190.0	115.2	226.9	127.0
3	A	1,000	NC	33.4	115.0	74.0	183.6	110.3	224.1	122.2
4	A	4,500	NC	33.9	117.4	75.0	188.7	112.0	227.3	124.0
5	C	1,000	PC	35.3	118.5	76.6	183.8	112.1	224.7	123.8
6	C	4,500	PC	36.0	120.1	77.7	186.9	113.8	222.1	125.2
7	C	1,000	NC	34.5	115.1	74.5	186.0	111.4	224.9	123.3
8	C	4,500	NC	34.7	117.7	75.8	185.8	112.1	224.2	123.6
Main Effect Means										
Nutrients	Regimen A			34.3[b]	117.7	75.5	187.3	112.3	226.3	124.1
	Regimen C			35.1[a]	117.9	76.2	185.6	112.4	224.0	124.0
Phytase FTU/kg		1,000		34.4[b]	116.4[b]	75.0[b]	185.0[b]	111.3[b]	225.1	123.2[b]
		4,500		35.1[a]	119.2[a]	76.7[a]	187.8[a]	113.3[a]	225.1	124.9[a]
Mineral			NC	35.3[a]	119.3[a]	76.9[a]	186.9	113.2[a]	225.1	124.9[a]
			PC	34.1[b]	116.3[b]	74.8[b]	186.0	111.4[b]	225.1	123.3[b]
P-values	Nutrients			0.002	0.833	0.172	0.062	0.825	0.244	0.818
	Phytase			0.008	<0.001	<0.001	0.002	<0.001	0.997	0.006
	Mineral			<0.001	<0.001	<0.001	0.347	0.001	0.998	0.011
	Nutrients X Phytase			0.300	0.310	0.277	0.120	0.121	0.416	0.130
	Nutrients X Mineral			0.530	0.944	0.910	0.128	0.339	0.554	0.382
	Phytase X Mineral			0.134	0.649	0.200	0.661	0.138	0.518	0.242
	Nutrients X Phytase X Mineral			0.578	0.242	0.126	0.161	0.644	0.856	0.804

[a-e]Means within a column with different superscripts differ at (P≤0.05)

[1]Grainzyme, Agrivida, Woburn, MA 018301

* Regimens B and C contained similar AA and energy density during the starter phase with -2% AA density and -1% ME compared to Regimen A.

Processing

Reviewing processing weights and yields (Table IV-12), both WOG and breast weight were affected by nutrient density as reducing dAA/energy led to reductions ($P \leq 0.05$) in weight. A 3-way interaction was observed on tender weight where reducing mineral density in the control diet reduced the impact of the high level of phytase on tender weight, while reducing nutrient density and minerals increased the efficacy of the high level of phytase. When evaluating yields, only tender yield had significant differences which were due to a 3-way interaction where no differences were observed between mineral density or phytase inclusion in the control diets. Reducing nutrient density allowed for both mineral density and phytase inclusion to provide differences with the mineral reduced low phytase inclusion diet having the lowest yield, and the PC diet with the low phytase inclusion having the highest yield.

Table IV-12. Processing weights and yields of Ross 708 broilers fed diets with reduced amino acid, metabolizable energy and mineral densities supplemented with phytase[1] through 48 d in Trial 2.

TRT	Regimen*	Phytase FTU/kg	Mineral	Weight (g)			Yield (%)		
				WOG	Tender	Breast	WOG	Tender	Breast
1	A	1,000	PC	2,761.8	148.1^{abc}	840.7	78.36	5.37^{abc}	30.42
2	A	4,500	PC	2,810.6	150.6^{ab}	864.1	78.83	5.36^{abc}	30.69
3	A	1,000	NC	2,806.6	153.0^{a}	866.6	78.40	5.46^{ab}	30.85
4	A	4,500	NC	2,803.3	149.5^{ab}	854.2	78.46	5.34^{abc}	30.42
5	C	1,000	PC	2,682.7	146.8^{bc}	819.2	78.71	5.48^{a}	30.58
6	C	4,500	PC	2,745.3	145.0^{bc}	838.8	78.36	5.29^{bc}	30.53
7	C	1,000	NC	2,732.9	143.5^{c}	842.5	78.78	5.26^{c}	30.82
8	C	4,500	NC	2,713.4	146.3^{bc}	840.8	78.70	5.39^{abc}	30.94
Main Effect Means									
Nutrients	Regimen A			2,795.6^{a}	150.3	856.4^{a}	78.52	5.34	30.59
	Regimen C			2,718.6^{b}	145.4	835.3^{b}	78.64	5.36	30.73
Phytase FTU/kg		1,000		2,746.0	147.9	842.2	78.57	5.39	30.67
		4,500		2,768.2	147.9	849.4	78.59	5.34	30.65
Mineral			NC	2,750.1	147.6	840.7	78.57	5.37	30.55
			PC	2,764.0	148.1	851.0	78.59	5.36	30.76
P-values	Nutrients			<0.001	<0.001	0.012	0.325	0.546	0.493
	Phytase			0.229	0.999	0.378	0.844	0.231	0.957
	Mineral			0.447	0.734	0.209	0.868	0.796	0.204
	Nutrients X Phytase			0.974	0.704	0.830	0.062	0.678	0.692
	Nutrients X Mineral			0.793	0.270	0.775	0.149	0.256	0.427
	Phytase X Mineral			0.071	0.786	0.085	0.786	0.176	0.396
	Nutrients X Phytase X Mineral			0.683	0.040	0.657	0.179	0.008	0.211

[a-c]Means within a column with different superscripts differ at (P≤0.05)

[1]Grainzyme, Agrivida, Woburn, MA 018301

* Regimens B and C contained similar AA and energy density during the starter phase with -2% AA density and -1% ME compared to Regimen A.

Discussion

The ability of phytase to improve BW is well documented (Olukosi, et al., 2006; Cowieson, et al., 2011; Pieniazek, et al., 2016b) along a range of inclusions, however it was impacted through its interactions with both nutrient and mineral densities in the current experiment. Reducing nutrient density in both studies resulted in reduced BW. BW reductions correlating with reductions in dAA have been reported by Kidd, et al. (2004) where broilers fed an industry level (21.5% CP, 1.25% Lys) of dAA had increased ($P<0.05$) BW compared to broilers fed reduced (20.5% CP, 1.15% Lys) dAA diets throughout the study, which agrees with the current trials. Similarly, Smith, et al. (2018) found that reducing dAA from 1.20% dig.Lys to 1.08% dig.Lys led to reductions in performance including BW and FCR, but those losses in performance were able to be recovered by the addition of a high inclusion of phytase (1,500 & 3,000 FTU/kg). In Trial 1, performance was reduced in correlation with nutrient density. However, the reduction of Ca and P influenced the observed impact of high phytase where performance (BW and adj. FCR) was able to be recovered. This could be due to a reduction of Ca chelation formed from phytate binding with extra Ca (as in the PC) inhibiting the efficacy of the lower inclusion of phytase as previously studied by Walk (2016) and Bedford and Rousseau (2017). Walk (2016) and Luttrell (1993) describe how the breakdown of the higher esters of inositol hexaphosphate (IP6) have an increased release of Ca at 5 molecules (Selle, et al., 2009) for every IP6 P cleaved. This ratio is reduced to 1:1 by the time the lower IP3 and IP2 esters are broken down which means that utilizing a high level of phytase can be restorative of balance to the Ca:P and

furthers the potential to improve performance (Cowieson, et al., 2011). Utilizing phytase in Trial 1 presumably removed the negative attributes of phytate, while the disproportionate release of Ca and P with the lower-level of phytase created an anti-nutritive component further binding Ca. However, the effect of the Ca bonds was reduced with the higher inclusion of phytase at 4,500 FTU/kg. The positive influence of the reduced mineral levels and elevated phytase inclusion on the nutrient reduced diets could be explained by Bedford and Rousseau (2017) where it was observed that adding Ca, P or a combination thereof with the presence of phytase can increase the concentration of IP6 as well as the lower esters (Li, et al., 2016; Beeson, et al., 2017). A lack of hydrolysis of IP esters would indicate Ca and P to be anti-nutritive and can lead to performance reductions through the chelation of Ca with phytate which is where a high inclusion of phytase is important to overcome the anti-nutritive effects and restore balance. The positive influence of the reduced Ca and P diets was lost in the reduced nutrient dense diets of the Trial 2. It is worth noting that the nutrient reductions in Trial 1 were locked going into the grower phase, while nutrient reductions increased in each successive phase of Trial 2 as it was -2%, -4%, -6% and -6% dAA in the starter, grower, finisher and withdraw phases, respectively. Those increases in nutrient reduction led to an overall observed reduction ($P<0.001$) in BW at the conclusion of Trial 2, while elevating phytase inclusion improved ($P<0.001$) BW compared to the lower level.

Ca to P ratios are important to broiler physiology and development as Ca is widely used in the body for skeletal strength and growth as well as in basic biochemical reactions for muscles to contract (Bedford and Rousseau, 2017). A series of experiments

by Driver, et al. (2005) demonstrated in broiler chicks from d 0 to 16 that phytase (657 FTU/kg) is most efficacious in diets that were low in NPP and high in Ca and that the efficacy of phytase was reduced as Ca was reduced or NPP was increased. Furthermore, they went on to identify that phytase had the greatest response at 0.86% Ca and 0.20% NPP. This could explain the lack of interactions with mineral density at the conclusion of Trial 2 as the further reduction of Ca to P levels were possibly too low for these broilers to maintain performance compared to the PC even with the addition of phytase. The differences in calculated nutrients between the current Trials includes -0.06% Ca and -0.03% NPP between the first and second Trial starter phases. Some of the observed differences between the Trials could stem from additional reductions in Trial 2 of Ca and NPP that were added into the formulation in an attempt to normalize consumption between treatments as mineral consumption was increased in correlation with FC during Trial 1. Those further reductions took the level of Ca down by -0.06% and NPP by -0.03% in the nutrient reduced PC diet compared to the control PC in the grower, finisher and withdraw diets while the -0.12% Ca and -0.15% NPP reductions were applied based on the PC formulations for each nutrient density. These additional reductions could explain the lack of sensitivity and interactions observed in Trial 2 as the Ca level was reduced beyond that of what was identified by Driver, et al. (2005).

Zhai, et al. (2013) reports that with a higher level of dAA, FC was reduced through d 14 while no differences were observed after the starter period, which correlates to the current Trials where decreasing nutrient density led to reductions in FC through d 14 in both Trials. In the current Trials, Trial 1 had a 3-way interaction

between phytase X nutrients X mineral density, while no interactions occurred in Trial 2, presumably from the further reduction of Ca and P. The 3-way interaction signified that the reduction of Ca and P in the control diets had an opposite effect on FC as it was reduced which could be explained by the birds eating to compensate for energy. These results parallel an experiment by Dozier, et al. (2007) from d 42 to 56 in which broilers fed a diet reduced in ME (3,140 kcal/kg) had increased FC compared to birds fed a normal ME (3,240 kcal/kg). However, in the current Trial, the reduction of nutrients and removal of Ca and P increased FC while the efficacy of the higher level of phytase was reduced as nutrients and minerals were removed. Observing the FC data from Trial 2, it is clear that elevating the phytase inclusion was able to increase FC which is in agreement with literature (Dilger, et al., 2004; Olukosi, et al., 2013). The increase in FC with the elevated dose of phytase can be attributed to the phytase eliminating the majority (greater than 73.5% at 3,000 FTU/kg (Shirley and Edwards, 2003)), if not all, of the anti-nutritive properties of phytate enabling the broiler to fully utilize dietary nutrients. However, reducing the Ca and P density was also found to increase consumption as the birds attempted to consume more feed to compensate for the reduction of available Ca and P. A similar trend was observed in an experiment by Li, et al. (2015) in broilers through d 19 where reducing Ca from 0.80% to 0.65% with 0.20% NPP and no phytase led to increased FC. Conversely, the increases in FC in the current Trial along with the increased BW of the NC birds in both studies led to improved ($P<0.05$) FCR. FCR in the first trial was greatly affected by an interaction between phytase X minerals, as reducing the Ca and P density enabled phytase to have an

increased effect, which could point to the reduced mineral density in the first trial being closer to the ideal matrix values for phytase in these experiments. Phytase has been shown to improve FCR (Pieniazek, et al., 2016b) and to even offer increased improvements with elevated doses beyond normal inclusions (Ravindran, et al., 2001; Cowieson, et al., 2011; Smith, et al., 2018). Elevating phytase inclusion did seem to improve FCR compared to the lower level, however it wasn't clear at all times due to the interactions phytase had with both mineral and nutrient density.

The reductions in nutrient density of both Trials led to reductions in WOG, breast and tender weights which is in agreement with Dozier, et al. (2007) and Kidd, et al. (2004). In Trial 1, elevating phytase inclusion did tend to increase WOG, breast and tender weights in the control and 96% dAA PC diets; however, that improvement was lost with the 94% dAA diet which was due to the phytase X mineral interaction. The interaction between nutrient X mineral density implied the nutrient reduced NC diets to be statistically similar to the non-nutrient reduced PC diet for WOG, breast and tender weights, which was not observed in Trial 2. In Trial 2, it was found that reducing nutrient density reduced breast weight which is in agreement with an experiment by Smith, et al. (2018) where breast weight was reduced by 27g with a reduction in dig.Lys of 0.12%. Tender weight was reduced by the reduction of nutrient density as well as the further reduced levels of Ca and P in Trial 2. The increased breast weight and yield stemming from the reduction of nutrients in the Trial 1 was also observed by Dozier, et al. (2007) where it was demonstrated that reducing ME from 3,240 to 3,140 kcal/kg would increase breast yield due to increased consumption as the broilers attempted to

consume more energy to meet dietary needs but were also increasing their intake of dAA which coincidentally led to increased FCR as well. This correlation was not observed of breast weight and yield in Trial 2, which is likely due to the reduction of nutrients being too much for the broilers to compensate for by increasing FC. Additionally, published research identified similar trends where reducing nutrient density led to reduced breast weight/yield (Kidd, et al., 2004; Smith, et al., 2018).

The results from these Trials indicate the effectiveness in which phytase can improve performance in nutrient reduced diets. Further improvements were identified with the reduction in P and Ca in the NC diets of Trial 1. Additionally, the reductions of Ca and P levels in combination with the nutrient reductions in Trial 2 were likely too extensive for phytase to improve beyond the 1,000 FTU/kg inclusion. The interactions described in this paper highlight the multiple considerations (mineral density, nutrient density) that must be given when including phytase in broiler diets and this information can be utilized as a tool for nutritionists to help identify the correct levels of Ca and P to further improve the efficacy of phytase in diets consisting of different nutrient levels.

CHAPTER V

EVALUATION OF A SUPER DOSE OF PHYTASE IN PRODUCTION LAYERS FROM 18 TO 60 WEEKS

Introduction

Phytase as a nutritional tool was first found to be useful in poultry by Nelson, et al. (1968b) in the 1960s, and has since been shown to improve bird performance. Cleaving phytate bonds allows for increased nutrient digestion of P, Ca, mineral cations and even proteins which, in broilers, leads to improvements in BW and FCR (Cowieson, et al., 2011; Walk, 2016; Pieniazek, et al., 2017; Smith, et al., 2018). While the effects of phytase on broiler growth are well documented, the amount of research pertaining to laying hens is significantly less and more dated. Multiple nutrient requirement differences exist between layer and broiler diets, with laying hens requiring significantly greater amounts of Ca for egg production. As such, the ability for phytase addition to reduce dietary inclusions of inorganic P and Ca in laying hen diets can be beneficial towards reducing input costs as well as reducing the excretion of P (Lim, et al., 2003).

Research by Van der Klis, et al. (1997) and Gordon and Roland (1997) showed that phytase supplementation in layer diets is adequate to improve phosphate utilization from phytate sources. Walk (2016) asserted that in broilers the efficacy of phytase is hampered by high levels of dietary Ca which can bond with phytate and block the action of phytase. Furthermore, it is suggested that using high levels of phytase can overcome the negative effects of the extra Ca by enabling more enzyme to break down the substrate before moving further into the digestive tract where it is inactivated from

changes in pH. In instances where excess Ca descends further into the digestive tract, it is thought to disrupt growth performance by precipitating with phytate and forming insoluble complexes with proteins, thereby reducing protein digestion (Amerah, et al., 2014). While in egg production, laying hens are fed Ca at approximately 4 to 6 times the levels of broilers; however, the majority of that is dedicated to the formation of the eggshell. If Ca is fed at levels below the hen's requirement, the hen will pull Ca from reservoirs in the medullary bone, eventually leading to a decline in egg production. If Ca is supplemented at adequate levels, no negative impact is observed upon medullary stores. Gordon and Roland (1998) found that supplementing phytase at 300 FTU/kg in laying hen diets improved eggshell quality through factors related to increased NPP and Ca utilization stemming from phytate degradation. Additionally, the authors reported an interaction between phytase and P with improvements in eggshell quality as well as FC, rate of lay and egg weights being the greatest with diets supplemented 0.1% NPP compared to 0.3% NPP. It is possible the increased efficacy of phytase at the lower NPP inclusion demonstrated the inhibitory effect of NPP on the catalytic actions of phytase (Selle and Ravindran, 2007), thereby muting the response. Phytase has also been documented to improve egg production, egg size, specific gravity and eggshell quality by improving P utilization with phytase inclusions of 100 to 1,300 FTU/kg (Boling, et al., 2000; Francesch, et al., 2005; Wu, et al., 2006; Żyła, et al., 2012). However, when it comes to high levels of phytase, the literature is not as clear on the benefits of phytase. Casartelli, et al. (2005) described how at peak production hens fed 1,000 FTU/kg of phytase with 0.12% NPP (a decrease of 0.24% compared to the control) egg weights

were reduced compared to the control fed hens. Additionally, it was identified that eggshell percentage and specific gravity were both increased with phytase supplementation, while no significant effects were observed on FC, FCR or hen day (HD) egg production. In an experiment leading up to and through peak production, Skřivan, et al. (2018) reported that in hen diets containing 3.5% Ca and 0.18% NPP, egg production was reduced between 4.5% and 7.4% with phytase inclusion at 1,500 FTU/kg compared to 0 or 300 FTU/kg. Furthermore, the authors noted that the high level of phytase negatively influenced egg weights as well as FCR. However, an increase in eggshell breaking strength was also observed. Skřivan, et al. (2018) concluded that the reduction in performance with 1,500 FTU/kg was due to increased levels of Ca fed in layers relative to broilers where further improvements are typically observed with higher inclusions. Finally, a study by Kim, et al. (2017) demonstrated that in laying hens fed high dosages (10,000, 20,000 or 30,000 FTU/kg) of phytase added to negative control (NC) diets consisting of 3.91% Ca and 0.26% NPP between 42 and 47 wk of age, no negative effects were found in egg weight, FCR, or egg quality. Additionally, the authors reported that the inclusion of 20,000 and 30,000 FTU/kg resulted in increased egg production compared to the positive control (PC (NC + 0.12% NPP). The objective of this experiment, therefore, was to determine the effect of super dosing phytase on the performance, production efficiency, and egg quality in laying hens from pre-peak through 60 wk of age.

Materials and Methods

Experimental Design

The experiment was designed as a randomized complete block design consisting of 3 treatments: a positive control (PC), a negative control (NC) which was formulated to have reductions of 0.10% P, 0.10% Ca and 0.02% Na compared to the PC, and the NC with the addition of phytase[4] at a 6x rate (1,800 FTU/kg) recommended by the enzyme manufacturer. These 3 treatments were randomly assigned into a complete block design from 18 to 60 wk of age.

Experimental Diets

The PC diet (Table V-1) was majority corn and soybean meal and formulated to primary breeder recommendations based upon daily FC (International, 2016). The NC diet was mixed separately from the PC and was split into 2 rations with one ration to receive the inclusion of a super dose of phytase (1,800 FTU/kg). All rations included vitamin D_3 at 0.05% in addition to the vitamin premix and were fed as a mash throughout the study. During the course of the study, 3 feed phases were fed. Phase 1 was fed for 18 to 35 wk, phase 2 for 36 to 55 wk, and phase 3 for 56 to 60 wk. Feed phases were changed based upon egg production level as outlined by the Hy-Line W-36 management guide, with egg production during phase 1 extending to wk 35 for the desired 2% drop below peak. Phase 2 was fed longer than expected according to the Hy-Line W-36 management guide (wk 48 to 49) as it took until wk 55 for the hens to drop to

[4] Ronozyme HiPhos- DSM Nutritional Products, Parsipanny, NJ 07054

89% egg production. Phase 3 was then fed from wk 56 until the termination of the study at wk 60. During the final wk of the study, titanium dioxide was added as an indigestible marker to the rations at 0.04% inclusion.

Table V-1. Diet formulations, calculated nutrient, analyzed nutrient content and phytase recovery of diets fed to Hy-Line W-36 laying hens during Phase 1, 2 and 3.

	Phase 1		Phase 2		Phase 3	
	18 to 35 wk		36 to 55 wk		56 to 60 wk	
% Inclusion	PC	NC	PC	NC	PC	NC
Corn	57.79	58.94	58.52	58.83	63.54	64.60
Soybean Meal	25.49	25.34	25.62	26.21	22.06	21.92
DL Methionine (98.5%)	0.32	0.32	0.17	0.16	0.17	0.17
L-Lysine HCl	0.20	0.20	-	-	0.03	0.04
L-Threonine (98.5%)	0.11	0.11	-	-	-	-
Soybean Oil	3.54	3.13	3.30	3.01	2.19	1.81
Fine Calcium Carbonate	7.01	6.95	7.09	7.03	6.79	6.73
Course Calcium Carbonate	3.00	3.00	3.00	3.00	3.00	3.00
Mono-calcium Phosphate	1.66	1.18	1.46	0.98	1.29	0.81
Sodium Chloride	0.24	0.19	0.28	0.25	0.24	0.24
Sodium Bicarbonate	0.30	0.30	0.20	0.18	0.34	0.34
Trace Minerals[1]	0.05	0.05	0.05	0.05	0.05	0.05
Vitamins[2]	0.25	0.25	0.25	0.25	0.25	0.25
Hydrated D3[3]	0.05	0.05	0.05	0.05	0.05	0.05
Calculated Nutrients (%)						
Crude Protein	17.72	17.75	17.50	17.81	16.23	16.25
Crude Fat	5.96	5.59	5.75	5.48	4.81	4.47
Calcium	4.15	4.05	4.15	4.05	4.00	3.90
Total Phosphorus	0.67	0.57	0.63	0.53	0.59	0.49
Non-Phytate Phosphorus	0.45	0.35	0.41	0.31	0.37	0.27
Sodium	0.20	0.18	0.19	0.17	0.21	0.21
ME kcal/kg	2882	2882	2871	2871	2860	2860
Dig. Methionine	0.56	0.56	0.42	0.42	0.40	0.40
Dig. Lysine	0.96	0.96	0.81	0.83	0.75	0.75
Dig. Threonine	0.67	0.67	0.57	0.58	0.52	0.52
Dig. TSAA	0.80	0.80	0.66	0.66	0.63	0.63
Analyzed Nutrients (%)						
Crude Protein	17.20	16.40	16.70	17.40	15.00	16.0
Crude Fat	5.87	5.35	5.75	5.03	3.86	3.61
ME kcal/kg	3,058	3,036	2,948	2,948	2,860	2,816
Phosphorus	0.72	0.59	0.68	0.56	0.58	0.47
Calcium	4.56	4.37	4.70	4.24	4.17	4.02
Sodium	0.18	0.17	0.18	0.16	0.20	0.22
Phytase Recovery (FTU/kg)						
	PC	NC	NC + Phytase	PC	NC	NC + Phytase	PC	NC	NC + Phytase
Mix 1	<50	<50	2,675	<50	<50	1,860	<50	<50	1,886
Mix 2	<50	<50	2,323	<50	<50	1,973	<50	<50	1,527
Mix 3	<50	<50	2,159	<50	<50	2,200			

[1]Trace mineral premix added at this rate yields 60.0 mg manganese, 60 mg zinc, 60 mg iron, 7 mg copper, 0.4 mg iodine, a minimum of 6.27 mg calcium and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[2]Vitamin premix added at this rate yields 22,045 IU vitamin A, 7,716 IU vitamin D_3, 91 IU vitamin E, 0.04 mg B_{12}, 11.9 mg riboflavin, 91.8 mg niacin, 40.4 mg d-pantothenic acid, 261.1 mg choline, 2.9 mg menadione, 3.50 mg folic acid, 14.3 mg pyroxidine, 5.87 mg thiamine, 1.10 mg biotin per kg of diet. The carrier is ground rice hulls.

[3]Hy-D®- DSM Nutritional Products, Inc., Parsipanny, NJ.

Animal Practices and Management

Hy-Line W-36 pullets (16 wk of age) were housed in an A-frame cage design and fed a common pre-lay ration until the start of the study at wk 18. At wk 18, the birds were randomized and placed into cages on an equal BW basis, with 3 hens placed per cage for a total area of 82 in^2 per bird. Three continuous cages for a total of 9 hens comprised each experimental unit, and there were 12 replicates per treatment for a total of 324 hens placed for a 42-wk assay period. Hens were provided feed and water *ad libitum*. At 20, 40, and 60 wk of age, hens were individually weighed to determine BW. Beginning at wk 20, feed disappearance was calculated every 4 wk in addition to egg quality measurements including specific gravity, albumen height, Haugh units, eggshell thickness and eggshell breaking strength. Specific gravity was measured according to Hamilton (1982) using a range of saline solutions with density increasing in increments of 0.005. Eggshell thickness was measured using an Orca Egg Sonar[5] which measured thickness to 0.001 mm. Breaking strength was calculated using a strength-force reader[6] and recorded as kg/cm^2. Haugh units were calculated by correlating egg weights (W) and albumen height (H) into the equation provided by Eisen, et al. (1962):

$HU=100 \log (H-1.7W^{0.37}+7.6)$.

Egg production was monitored daily while egg weights were recorded every 2 wk according to USDA size guidelines of peewee (< 37 g), small (38 to 43 g), medium (44

[5] Egg Force Reader™, Orka Food Technologies Ltd., Ramat HaSharon, Israel
[6] Egg Shell Thickness Gauge, Orka Food Technologies Ltd., Ramat HaSharon, Israel

to 49 g), large (50 to 55 g), extra-large (56 to 62 g) and jumbo (greater than 63 g). At wk 60, following a 24-hour collection period, fecal material was collected from trays positioned underneath each replicate for determination of apparent metabolizable energy with N (nitrogen) correction (AMEn) (kcal/kg). The calculation for AMEn was determined following the formula from Leeson and Summers (2001):

$$\mathbf{AMEn = GH_{Feed} - ((GH_{Fecal}*(TiO_{2Feed}/TiO_{2Fecal}) - 8.22*(Feed_N - Fecal_N*(TiO_{2Feed}/TiO_{2Fecal})))}.$$

Where GH represents gross heat utilizing a bomb calorimeter[7], TiO_2 represents titanium determination according to the method by Short, et al. (1996) utilizing a spectrophotometer[8], and N values were calculated utilizing a N analyzer[9]. At the conclusion of the study, 3 hens per replicate were randomly selected (1 per cage) and euthanized and the left tibia removed to determine bone ash weight and percent. All animal care and husbandry was provided in accordance with a Texas A&M University IACUC approved protocol.

Statistical Analysis

The data for this trial was analyzed using a Fit model in JMP 12 with treatment, wk, and the interaction of treatment X wk as fixed effects. Means were separated using Student's T-test with $P \leq 0.05$ considered significant. For bone ash weight, bone ash

[7] Parr 6400 Bomb Calorimeter, Parr Instrument Company, Moline, IL 61265
[8] Thermo Fisher Scientific Genesys 10S UV-Vis, Thermo Fisher Scientific, Waltham, MA 02451
[9] Rapid N Cube, Elementar Americas Inc., Ronkonkoma, NY 11779

percent and AMEn, data was analyzed using a one-way ANOVA in SPSS software where means ($P \leq 0.05$) were further separated by Duncan's Multiple Range Test.

Results

Performance

Hen BW and FC were not significantly affected throughout the trial (Table V-2). HD egg production (Table V-2) was reduced ($P<0.001$) with the reduction in dietary minerals (NC diet), however the addition of phytase to the NC resulted in greater HD egg production compared to both control treatments ($P \leq 0.05$). Average egg weight (Table V-2) was also decreased ($P<0.001$) with the reduction of dietary minerals in the NC, while the inclusion of phytase increased ($P \leq 0.05$) egg weight compared to the NC, however it did not reach the level of the PC. The percent of eggs in the peewee size was greater ($P=0.012$) with the reduction in dietary minerals in the NC, while the inclusion of phytase reduced the incidence of peewee eggs to be equal to the PC. No differences were noted for percent small egg production; however, percent medium and large egg production was greater ($P<0.001$) with the reduction in dietary minerals of both NC diets. Percent production of extra-large and jumbo eggs was reduced ($P<0.001$, $P=0.002$) in correlation with the reduction of minerals in the NC diet, however the inclusion of phytase to the NC diet increased percent production of jumbo egg production to be equal to the PC. Cumulative grams of feed consumed per grams of egg produced (Table V-2) was increased ($P=0.012$) with the reduction in minerals in the NC, while the inclusion of phytase was able to reduce conversion to be similar to the PC.

Table V-2. Performance analysis between treatments of Hy-Line W-36 hens fed control diets with the addition of phytase[1] from 18 to 60 wk of age.

TRT	% Hen Day	Average Egg Wt (g)	Peewee (%)	Small (%)	Medium (%)	Large (%)	Extra Large (%)	Jumbo (%)	Average Bird Wt (g)	Bird Wt Gain (g)	g Feed per g Egg	g of Feed per Egg	Feed Consumption (g/d/bird)
PC	93.0[b]	63.01[a]	0.2[b]	3.3	6.7[b]	40.0[b]	46.0[a]	4.0[a]	1530.9	178.0	1.96[b]	121.9	113.9
NC	92.1[c]	61.83[c]	1.2[a]	3.0	9.2[a]	46.0[a]	37.0[b]	3.0[b]	1538.1	181.7	2.01[a]	122.5	114.2
NC + Phytase	94.8[a]	62.37[b]	0.4[b]	2.5	8.9[a]	46.0[a]	37.0[b]	5.0[a]	1537.2	185.0	1.96[b]	120.7	114.7
SEM	0.1423	0.0449	0.0015	0.0020	0.0026	0.0049	0.0048	0.0021	4.8441	5.3683	0.0071	0.4356	0.3474
P-values	<0.001	<0.001	0.012	0.302	<0.001	<0.001	<0.001	0.002	0.805	0.873	0.012	0.225	0.642

Means within a column with no common superscripts are significantly different ($P \leq 0.05$) using Student's T test

Analysis using Fit Model of JMP 12: TRT, Week, TRTxWeek=Fixed Effects

[1]Ronozyme HiPhos- DSM Nutritional Products, Parsipanny, NJ 07054

Grams of feed per gram of egg produced throughout the trial (Table V-3) was influenced by laying hen age, as expected, with the lowest conversion recorded during wk 41 to 44. FC (Table V-3) increased ($P \leq 0.05$) as the hens aged with the lowest FC observed at the beginning of the trial from wk 18 to 20 and increased until peak FC was observed from wk 41 to 44. After wk 44, FC was sharply reduced between wk 45 to 48 in correlation with conversion during the same period and increased again through the end of the study.

Table V-3. Feed conversion (g of feed to produce 1 g of egg; g of feed to produce 1 egg), and feed consumption (g/bird/d) of Hy-Line W-36 hens fed control diets with the addition of phytase[1] from 18 to 60 wk.

Time Period (wk)	g Feed per g Egg	g of Feed/Egg		Time Period (wk)	Feed Consumption (g/bird/d)
21 to 24	2.31^{a}	120^{cd}		18 to 20	93.0^{f}
25 to 28	1.99^{b}	119^{d}		21 to 24	109.8^{e}
29 to 32	2.00^{b}	124^{b}		25 to 28	115.4^{d}
33 to 35	1.98^{b}	124^{b}		29 to 32	121.1^{b}
36 to 40	1.92^{c}	122^{bcd}		33 to 35	120.6^{b}
41 to 44	2.02^{b}	129^{a}		36 to 40	119.0^{bc}
45 to 48	1.74^{d}	111^{e}		41 to 44	124.2^{a}
49 to 53	1.90^{c}	122^{bcd}		45 to 48	107.3^{e}
54 to 60	1.92^{c}	124^{bc}		49 to 53	116.3^{cd}
				54 to 60	115.9^{d}

Means within a column with no common superscripts are significantly different ($P<0.05$) using Student's T test
Analysis using Fit Model of JMP 12: TRT, Week, TRTxWeek=Fixed Effects
[1]Ronozyme HiPhos- DSM Nutritional Products, Parsipanny, NJ 07054

Egg Quality

Specific gravity (Table V-4) was increased ($P<0.001$) with the reduction in minerals of the NC, while the inclusion of phytase reduced specific gravity to levels similar to that of the PC. It should be noted that the differences observed in specific gravity were very minute and likely not of consequence to industry nutritionists. Albumen height and Haugh units (Table V-4) were not impacted by treatment, however albumen height was reduced ($P\leq0.05$) as hen age increased. The reduction in minerals of the NC led to increased ($P=0.019$) eggshell thickness (Table V-4) while the inclusion of phytase was equal to the PC. The increased specific gravity and eggshell thickness of the NC was likely associated with reduced egg weight that was observed when minerals were removed in the NC. Shell thickness was reduced ($P\leq0.05$) as the study progressed which may potentially be associated with egg size increasing as the birds aged. While no differences between treatments were observed in breaking strength (Table V-4), it increased from the start of the trial until its peak at wk 28 before decreasing throughout the remainder of the study. For all egg quality measurements, treatment had no effect on phase averages. However, all measurements decreased with each successive phase.

Table V-4. Egg quality from Hy-Line W-36 hens fed control diets with the addition of phytase[1] from 18 to 60 wk.

TRT	Specific Gravity	Albumen Height (mm)	Haugh Units	Shell Thickness (mm)	Breaking Strength (kg/cm^2)
PC	1.090^{a}	8.45	90.34	0.447^{b}	4.80
NC	1.090^{a}	8.40	90.37	0.451^{a}	4.85
NC + Phytase	1.089^{b}	8.33	89.80	0.448^{b}	4.83
SEM	0.0001	0.032	0.175	0.001	0.011
P-value	<0.001	0.333	0.320	0.019	0.095
wk of age					
20	1.100^{a}	9.34^{a}	99.10^{a}	0.454^{b}	4.97^{cd}
24	1.098^{b}	9.09^{ab}	95.39^{b}	0.471^{a}	5.13^{b}
28	1.093^{c}	8.73^{cd}	92.51^{cd}	0.457^{b}	5.23^{a}
32	1.090^{e}	8.97^{bc}	93.40^{c}	0.452^{b}	5.01^{c}
36	1.090^{e}	8.58^{d}	90.92^{de}	0.448^{c}	4.92^{cd}
40	1.091^{d}	8.59^{d}	91.08^{de}	0.439^{d}	4.89^{d}
44	1.086^{g}	8.69^{cd}	91.62^{de}	0.433^{e}	4.48^{h}
48	1.088^{f}	6.96^{e}	80.79^{f}	0.450^{c}	4.78^{e}
52	1.086^{g}	6.23^{f}	75.87^{g}	0.441^{d}	4.62^{f}
56	1.085^{h}	8.58^{d}	90.75^{e}	0.442^{d}	4.58^{fg}
60	1.080^{i}	8.54^{d}	90.43^{e}	0.441^{d}	4.50^{gh}
Data by phase					
PC	1.088	8.30	88.80	0.45	4.77
NC	1.089	8.08	87.93	0.45	4.80
NC + Phytase	1.088	8.16	88.17	0.45	4.79
SEM	0.000	0.043	0.255	0.001	0.013
P-value	0.131	0.140	0.381	0.145	0.740
Feed phase					
20 to 32	1.095^{a}			0.460^{a}	5.10^{a}
36 to 52	1.088^{b}	7.79^{b}	86.03^{b}	0.442^{b}	4.74^{b}
56 to 60	1.082^{c}	8.57^{a}	90.57^{a}	0.441^{b}	4.53^{c}

Means within a column with no common superscripts are significantly different ($P \leq 0.05$) using Student's T test

Analysis using Fit Model of JMP 12: TRT, Week, TRTxWeek=Fixed Effects

[1]Ronozyme HiPhos- DSM Nutritional Products, Parsipanny, NJ 07054

Lab Analysis

Percent bone ash (Table V-5) was unaffected by treatment. The reduction of minerals in the NC did not significantly impact bone ash weight (Table V-5) compared to the PC. However, an increase ($P \leq 0.05$) in bone ash weight was observed with the inclusion of phytase compared to the PC. It was expected that bone ash weight would be reduced with the reduction of minerals in the NC compared to the PC. It is possible that Ca in the reduced diet was above levels indicative of a deficiency and so the hens were not nutritionally stressed to metabolize from their medullary stores. Bone ash results reflect that the hens utilized minerals to maintain skeletal integrity and sacrificed HD production and egg weight due to the Ca and P reductions of the NC. The inclusion of phytase was able to liberate minerals from the NC diet to maintain skeletal integrity while also not sacrificing HD production and weight. AMEn (Table V-5) was reduced ($P<0.001$) by 56 kcal/kg in relation to the reduction in minerals of the NC, while the addition of phytase was able to recover 38 kcal/kg to become intermediate.

Table V-5. Bone ash weight, percent ash and apparent metabolizable energy nitrogen corrected (AMEn) of Hy-Line W-36 hens at 60 wk of age that were fed control diets with the addition of phytase[1] from 18 to 60 wk.

TRT	Ash Wt (g)	Ash (%)	AMEn (kcal/kg)
PC	8.005[b]	48.2	2,931[a]
NC	8.464[ab]	47.2	2,874[b]
NC + Phytase	8.630[a]	46.9	2,912[ab]
P-value	0.050	0.459	<0.001
SEM	0.1	0.5	13.1

[a-b]Means within a column with different superscripts differ at <0.05.

[1] Ronozyme HiPhos- DSM Nutritional Products, Parsipanny, NJ 07054

Discussion

As no differences were identified in BW or FC, it is reasonable to assume that the differences in nutrient availability between the PC and NC manifested themselves with negative impacts on production performance as differences in egg size and lay rate that were observed throughout the study between the NC and PC fed hens. The use of phytase can be concluded to have been largely successful at liberating P and Ca as HD production and egg size were increased compared to the NC throughout the experiment which was in contrast to the reductions in egg size and HD production observed by Casartelli, et al. (2005) and Skřivan, et al. (2018) at high levels of phytase. As shell egg producers target the production of large and extra-large eggs, the use of a high level of phytase can be useful as it was able to elevate % large egg production compared to the PC. Conversely, liquid egg markets also may benefit from a high phytase inclusion as % HD production was increased overall with the inclusion of phytase, meaning that more liquid egg mass was produced giving an edge to producers.

The exact reason for the observed increase in HD production compared to the PC fed laying hens is unknown, but contridicts the published results of Skřivan, et al. (2018) and Casartelli, et al. (2005). Perhaps the positive effects in the current study could be based on the reduced Ca:NPP fed in this study (11.57:1) compared to the others (Casartelli, et al. (2005) 30.33:1, Skřivan, et al. (2018) 19.5:1). It has been noted that Ca:NPP play a significant role in performance, as increasing the ratio negatively influences perfomance (Walk, 2016). In the high dosing study by Kim, et al. (2017), an intermediate Ca:NPP (15.04:1) between the current study and the one conducted by

Skřivan, et al. (2018) was utilized. Kim, et al. noted that 20,000 and 30,000 FTU/kg was sufficient to increase HD production compared to the PC, while no detrimental effects were noted in the other parameters. Logically it follows that as the Ca:NPP decreases, hen performance increases due to the reduction of Ca in ratio with NPP. It is important to keep these ratios low as phytase breaks apart the phytate molecule starting at the heavier IP6 ester and can release 5 Ca for every P (Selle, et al., 2009), however that ratio decreases as more phytase is available to help liberate the lower IP esters and lower the Ca:NPP release ratio back towards a 1:1 (Walk, 2016; Smith, et al., 2018). In the instance of a wide Ca:NPP, a lower level of phytase could have an optimal effect as it would limit the amount of Ca released, while a high inclusion would release an increased amount of Ca from subsequent destructions of the degradeable IP esters culminating in the widening of the Ca:NPP. In the current study, the Ca:NPP provided enough P to negate the negative effects of the higher release values associated with a high dosage of phytase as observed in egg production, and the other performance metrics.

Additionally, it was noted by Carlos and Edwards (1998) in a series of experiments that fed 600 FTU/kg phytase to 56 wk old hens for 9 wk, and then followed up with phytase at 600 FTU/kg fed to 24 wk old hens for 8 wk. In both experiments there were no effects on egg weight or specific gravity but phytase did increase BW and tibia bone ash signifying that the phytase was able to improve the birds digestibility of the Ca and P minerals. In the current study, the use of phytase was able to improve Ca and P digestibility; however, it resulted in improved egg weight compared to the NC, increased tibia ash % compared to the PC while no differences in BW or tibia ash weight

were observed with the inclusion of phytase. Increasing tibia ash % can be illustrative of improved hen welfare as the phytase is liberating all of the Ca and P necessary to maintain their medullary bone stores. The effect of NPP on bird performance and egg metrics has been demonstrated by Wu, et al. (2006) where it was observed that increasing the NPP content of laying hen diets from 0.11 to 0.26% affected FC (2.8 g/hen/d increase), HD production (3.1% increase), egg weight (0.52 g increase) and specific gravity (0.001 reduction). Phytase has long been recognized to release P from the phytate molecule, thereby increasing the amount of NPP available for absorption, and this release lead to the improvements observed by Wu, et al. (2006) where hens fed 0.11% NPP supplemented with 300 FTU/kg of phytase to have similar performance as the 0.26% NPP fed birds with no phytase. Ceylan, et al. (2003) also demonstrated a positive correlation with egg weight as NPP inclusion increases. Those correlations translated into the current study, where P liberated from phytate in hens fed phytase allowed for the improvements in egg weight, HD production, and FCR. Additionally, while the inclusion of phytase did not recover all of the lost egg weight compared to the PC, the eggs on average were in the same USDA grade as the PC but increased HD production by 1.7%. This translates into increased profits for egg producers as the hens are able to increase their overall output while shaving dietary costs with reduced P.

While a high Ca:NPP can have negative impacts on egg production, the importance of Ca has been noted in experiments (Sohail and Roland, 2000; Lim, et al., 2003; Skřivan, et al., 2018) where increasing Ca leads to improvements in shell thickness, breaking strength and specific gravity. However, in the current study this

impact did not follow as there were no differences between the PC and NC on specific gravity or breaking strength. The 0.10% reduction in Ca in the NC diet led to an increase in shell thickness, but this was attributed to smaller egg size and lower HD production of NC fed hens throughout the trial as a smaller egg means a reduced surface area for deposition thereby leading to an overall increase in shell thickness. Aside from implications of shell quality, interior quality was also not affected in this experiment by either the reductions in Ca and NPP or phytase inclusion. The study by Kim, et al. (2017) found that super dosing phytase had no impact on interior egg quality, while a study by Lim, et al. (2003) with phytase at 300 FTU/kg demonstrated that low NPP decreased Haugh units and low Ca increased it. Additionally, Skřivan, et al. (2018) found that the supplementation of phytase at both 300 and 1,500 FTU/kg reduced Haugh units while also increasing shell breaking strength.

In broiler studies, phytase has been shown to improve nutrient digestibility (Cowieson, et al., 2006b; Selle, et al., 2009; Pieniazek, et al., 2017) which is accomplished by phytase not only hydrolyzing the phytate molecule, but also helping to degrade the plant cell wall. These degradations release additional nutrients which can be hydrolyzed by endogenous enzymes, thus leading to improvements in metabolizable energy retention. While the mode of action should be the same as in broilers, this has been studied little in production hens. Phytase has been shown by Liebert, et al. (2005) to not have an effect on AMEn, although a positive benefit of phytase inclusion was observed in the current study where the hens fed phytase were able to recover 38

kcal/kg, which represented approximately two-thirds of the difference observed between the NC and PC fed hens.

Overall, the results of this study indicate that super dosing phytase has the potential to improve layer performance through increased nutrient digestibility leading to improved egg production and energy digestibility. However, these improvements do not apply to interior egg quality, and the observed improvements are likely related to a lower Ca:NPP.

CHAPTER VI

CONCLUSION

The inclusion of phytase in broiler and layer hen feeding programs can be a useful tool that improves bird performance and production through increased nutrient digestion. The opportunities to improve performance and production have been well explored, however seem to point towards phytase efficacy being effected by Ca and NPP levels. Published literature has identified mineral balance to be critical in determining the efficacy of phytase as too little NPP or too much Ca can hinder phytate degradation thereby trapping nutrients. Furthermore, while utilizing phytase is commonplace in the poultry industry, there exists opportunities for producers to improve bird performance with increased inclusion. Understanding the mechanisms to maximize enzyme potential is beneficial to producers who are seeking to improve performance while reducing cost through mineral reduction or even amino acid density.

When evaluating increasing levels of phytase in diets containing variable levels of amino acids on broiler performance and processing yields in Chapter III, phytase was successful at improving performance at higher inclusions. Including phytase at high levels (1,500 and 3,000 FTU/kg) allowed for improved nutrient digestibility leading to increased BW, reductions in FCR and increased breast yield compared to a standard 500 FTU/kg inclusion. Additionally, it was identified that reducing dAA density by 5% and 10% negatively affects broiler performance and yield with increases in FCR through d 44 by 3.1 points and by 5.6 points on a weight adjusted basis. This also led to reduced BW by 78 g, breast weight by 27 g and breast yield by 0.64%. Finally, super dosing

phytase at 1,500 and 3,000 FTU/kg can improve performance in broilers negatively impacted by reductions in dietary dAA to levels comparable to a typical industry diet containing 500 FTU/kg of phytase. The results of Chapter III indicate that increasing levels of phytase is sufficient to improve nutrient digestibility and recover performance, thus allowing for the reduction in formulated dAA to spare dietary costs.

During the evaluation of high dosages of phytase with reductions in amino acid density, and Ca and P on broiler performance and processing yields in Chapter IV, it was observed that there were multiple interactions between all variables. It has been theorized that higher Ca and NPP levels influence the efficacy of high dosages of phytase more dramatically than a low dosage and this was observed. Including a high dosage (4,500 FTU/kg) of phytase in Ca and NPP reduced diets improved broiler growth performance and processing weights to include BW, FC, FCR, breast weight and tender weight compared to a low dosage (1,000 FTU/kg). However, reducing Ca and NPP too far can lead to reduced efficacy of phytase in broilers as observed in Trial 2. As reported in published literature and observed in Chapter III, reducing nutrient density negatively impacts growth performance and processing weights including the increase of adjusted FCR at d 49 by 12.3 points, BW reduction by 130 g, and a reduction in breast weight of 21 g. Additionally, it was observed that the high inclusion of phytase could be utilized to recover losses in performance associated with nutrient reductions. The results of Chapter IV indicate that utilizing a high dosage of phytase can be advantageous towards minimizing costs through reduced energy, dAA, Ca and NPP. Additionally, when predicting growth response, it may be beneficial for producers to understand the effects

of different Ca and NPP levels on the efficacy of different inclusions of phytase as demonstrated here.

When evaluating a super dose of phytase in production layers from 18 to 60 wk in Chapter V, positive impacts from a high inclusion of phytase were observed. Published literature on the effects of high inclusions of phytase indicated laying hens can have a negative response, likely from the low levels of NPP being fed. However, the current research presented here disagrees and supports the idea that high levels of phytase can be beneficial to egg production. It was observed that feeding a NC with 0.10% reduction of Ca and NPP from a PC with 4.15% Ca and 0.45% NPP was sufficient to reduce egg production by 0.9%, egg weight by 1.2 g, and increased feed-to-egg conversion by 5 points. However, including a super dose of phytase at 1,800 FTU/kg increased egg production by 1.75% beyond the PC, increased egg weight by 0.5 g, improved feed-to-egg conversion by 5 points to be similar to the PC. Additionally, phytase increased recovery of AMEn by 38 kcal/kg from the NC diet signifying that it was successful at liberating nutrients additional nutrients from phytate in the NC diet. However, at the conclusion of the experiment, it was observed that super dosing phytase did not impact interior egg quality. The results of Chapter V indicate that phytase is capable of improving layer hen performance throughout a production cycle. These improvements are likely due to increased hydrolyzability of the phytate molecule allowed by low levels of Ca and NPP that correspond to reduced incidences of Ca chelations enabling improvements in egg production, egg weights and feed-to-egg conversion. It is important for producers to understand the viability of a current

generation phytase to liberate nutrients and improve performance in laying hens where increased inclusion levels have historically been looked over for negative attributes.

The ability of phytase to impact bird performance is derived from its ability to liberate bound nutrients such as Ca, P, amino acids and energy for digestion, thereby providing major nutrients needed for growth. The results of these experiments indicate a strong ability for high inclusions of phytase to confer benefits beyond a low-level inclusion while also allowing for increased nutrient digestibility to overcome the negative effects of reducing nutrient density. Additionally, it should be noted that utilizing reduced Ca levels may attribute to the improved efficacy of high dosages of phytase by reducing incidence of Ca chelations to phytate and inhibiting hydrolysis. The results of these experiments should serve as a resource to nutritionists seeking to identify the performance improving benefits of phytase and the mechanisms influencing the incorporation of high inclusions.

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