ABSTRACT

NUSAIRAT, BASHEER MAHMOUD. Effect of Soybean Meal Varieties, Phytase Enzyme, and Particle Size of Corn on the Performance of Broilers. (Under the direction of Dr. John Brake).

Phosphorus (P) has been considered to be one of the most expensive feed ingredients in poultry production. Excess fecal excretion of P has also created environmental concerns since it has been shown to contribute to eutrophication of water bodies. Several strategies have been proposed and can be adopted to reduce the excretion of P and reduce incorporation into diets. The use of feed ingredients such as low phytate P (LP) grains (e.g. corn, canola) and legumes (e.g. LP SBM) that have been genetically selected to contain more available P and less phytate P have been proposed. The use of exogenous feed enzymes such as phytase that liberate P from phytate molecule (an anti-nutritional factor in feed) has become successful in poultry production. Last, modification of gastrointestinal tract functions through feed particle size manipulation provided a promising means to improve digestibility of available nutrients in feed. Therefore, in this series of experiments, these three strategies were investigated with broilers. In the first experiment, chicks were raised to 21 d in battery cages to study the effect of feeding coarse corn (CC) and SBM varieties (LP and normal phytate (NP)) from 9-21 d. Results showed that 50%CC produced a larger gizzard and smaller proventriculus while phytate P digestibility was improved by either feeding 50%CC or NP SBM. In the second experiment, 300 FTU/kg of phytase was investigated in addition to CC and SBM varieties. Chicks were again raised to 21 d of age in battery cages. Results showed that feeding either 50%CC or LP SBM improved P digestibility, and increased gizzard weight at 21 d. Adding 300 FTU/kg improved total P digestibility in the presence of

0%CC but 50%CC. Furthermore, adding 300 FTU/kg to the LP SBM diet improved total P digestibility compared to the NP SBM diet. In the third experiment, the same experimental dietary combinations were used with male chicks raised in floor pens to 35 d. The AdjFCR was improved by phytase addition to the NP diet but was poorer with the LP diet from 23 to 35 d. The improvement of total P digestibility was better when phytase was used in combination with LP SBM than with NP SBM. On the other hand, adding phytase to either 0%CC or 50%CC improved total P digestibility. In the fourth experiment, a combination of SBM varieties with phytase enzyme either added “on top” of diet or incorporated with a matrix value, or without phytase were investigated. Female chicks were raised in battery cages to 35 d and treatments started after 21 d. Feeding LP-LS SBM produced a smaller gizzard and increased BW gain from 22-35 d as compared to NP diets. Feeding 300 FTU/kg of phytase added “on top” of diet or with matrix value improved total P digestibility compared with the non phytase control treatment.

Effect of Soybean Meal Varieties, Phytase Enzyme, and Particle Size of Corn on the Performance of Broilers

by
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DEDICATION

I dedicate this dissertation to my beloved wife Rasha, who supported and helped me in each step of the way. A special feeling of gratitude is extended to my loving family, especially to my late father Mahmoud and my late mother Fatima who left fingerprints of grace and courage in my life that will not be forgotten. To my brothers Mutasem, Belal, Nedal, Ahmad, and Bashar, and to my sisters, Seham and Khetam.

BIOGRAPHY

Basheer Nusairat is the sixth of the eight siblings of Mahmoud Nusairat and Fatima Nusairat. He was born in Jordan where he received his education. He obtained his Bachelor of Science degree in Animal Science from Jordan University of Science and Technology in 2002. After earning his BSc, he worked as a breeder farm supervisor in a commercial poultry company in Jordan. Then he moved to another company where he worked as a grandparent farm supervisor. At that time, he decided to pursue a Masters of Science degree in 2005 at Jordan University of Science and Technology while working part time in a commercial feed mill. He received a Masters degree in Animal Science with a Poultry Nutrition concentration in 2008 where his research mainly focused on broiler performance and histology of the small intestine as affected by feeding different levels of garlic powder. After graduation, he worked full time as a feed mill manager at the same feed mill. In 2010, he and his wife decided to move to the USA to pursue their PhD degrees at North Carolina State University where he joined Dr. Brake's lab and worked on the effect of soybean meal varieties, corn particle size, and addition of phytase on broiler live performance and nutrient digestibility. He hopes to use his knowledge and experience in serving and improving the poultry industry.

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INTRODUCTION

1.1. Environmental Concerns

Essentiality of P was first identified in cattle and sheep in 1912 by Sir Arnold Theiler (Theiler, 1912). However, P supplementation of poultry and swine diets was begun only in the 1950's. A global concern regarding the accumulation of P in soils and threat to surface water quality, due to water surface runoff or leaching of P (Pautler and Sims, 2000; CAST, 2002), has developed during the subsequent period of time. This P has often been generated from land application of poultry litter. These concerns have arisen because P has been shown to contribute to the eutrophication of water bodies because excess P used by aquatic plants for their growth reduced oxygen availability for aquatic animals. Eutrophication has reduced the biological, economic, and aesthetic value of the affected water bodies (Bennet et al., 1999; Sharpley and Moyer, 2000; CAST, 2002). This enrichment of surface waters by plant nutrients, according to Withers et al. (1995), was a form of pollution that restricted the potential use of affected water. This problem has led to vigorous research to reduce the quantity of P released into the environment as a product of the poultry industry as well as other animal industries.

The environmental implications resulting from the soil P level have led to federal regulations that limit the amount of P from manure that can be applied to land, since over 20% of the nation's streams and rivers have been negatively impacted by nonpoint source pollution with animal agriculture identified as one of the contributors (Environmental

Protection Agency, 2003). Crops require a N:P ratio of 6-11:1, while the N:P ratio in manure was reported to be 2-3:1 (Daniels et al., 1998). Therefore, the application of poultry manure as fertilizer to meet the crop N requirement has generally resulted in excessive P application to land relative to crop P removal. One of the factors that has contributed to the large amount of P in manure and litter was that poultry were not inherently able to fully utilize the phytate bound P fraction contained in the diet.

1.2. Phytate P in Cereal Grains and Feed

Phytate, or *myo*-inositol 1,2,3,4,5,6-hexa*kis* dihydrogen phosphate has been shown to contain 28.2% P and to be the predominant form of P in grains accounting for 65% to 80% of P in corn and ~ 70% of P in soybean meal (SBM). The aleurone particles have been reported to be the major site for phytate accumulation in monocotyledonous seeds, while the globoid crystals of protein bodies were the site of phytate storage in dicotyledonous seeds (Cosgrove and Irving, 1980). Phytate has been shown to exist in its ionic form in most portions of the plant but has occurred predominantly as salts of Ca, Mg, and K in mature seeds. This salt-chelated form of phytate has been referred to as phytin and was often found complexed with proteins and amino acids in the seed (Angel et al., 2002). This chelation has led to phytin having antinutritional effects and being insoluble at pH 5 or above.

Only 21% of the total P in corn and 35% in SBM (NRC, 1994) was reported to be readily available for intestinal absorption, while the remainder was excreted in the feces due to the lack of sufficient endogenous intestinal phytase. As a result, animal nutritionists have been compelled to include significant amounts of expensive, but highly bioavailable, inorganic sources such as dicalcium phosphate and defluorinated phosphate in diets. This has been a successful means to maintain performance of birds, but has been identified as having potentially detrimental effects on the environment.

Therefore, to improve the utilization of phytate P in the diet and reduce total P in the manure, exogenous phytase enzymes of microbial or fungal origin were developed and have become commonly included in diets of monogastric animals. These actions have resulted in decreased inclusion of inorganic P without affecting poultry performance (Angel et al., 2002; Applegate et al., 2003).

Other strategies considered to economically reduce the impact of P on the environment have included genetic selection of animals for lower P requirements (Punna and Ronald, 1999), reduced P safety margins during feed formulation, use of novel feed ingredients such as low phytate phosphorus (LP) grains (corn, canola) and legumes (LP SBM) (Wilcox et al., 2000), and inclusion of feed additives such as phytase, citric acid, and 25-hydroxycholecalciferol (25OHD3). The general consensus of researchers that have investigated the effects of diet amendments such as reduced total P, phytase addition, or the inclusion of LP grains in diets of broilers and turkeys was that these strategies were able to reduce total P in broiler litter and manure by 29 to 45% (Applegate et al., 2003).

Phytate P availability can be improved by phytase enzyme inclusion in the feed, the amount and location of phytate in seeds was reported to differ considerably between grains. In corn, 80% of phytate was found in the germ portion of the kernel with the remainder in the auleurone layer (Raboy et al., 2000). In contrast, wheat and rice contained phytate predominantly in the outer bran and auleurone layers of the kernel (Angel et al., 2002). Phytate in most oilseeds and legumes has been reported to be chelated with protein and distributed throughout the kernel inside subcellular inclusions called globoids. An exception to this was the soybean in which phytate was distributed throughout the kernel with no specific location (Angel et al., 2002). The function of phytate in corn was initially viewed as a P and mineral storage compound but phytate and its lower esters have more recently been shown to play important roles in the regulation of the free inorganic P pool, control of cellular processes during germination by immobilization of divalent cations, as a competitor for ATP during the rapid biosynthesis of phytate as the seed approached maturity and dormancy was induced, and intra-cellular signaling (Vogelmaier et al., 1996; Angel et al., 2002). Research has shown that LP diets dramatically decreased P excretion into the environment without affecting growth (Sugiura et al., 1999; Waldroup et al., 2000; Veum et al., 2001).

The LP mutation in corn and barley was achieved by suppression of the lpa-1-1 allele that resulted in decreased expression of the first enzyme in the phytate pathway, *myo*-inositol-3-phosphate synthase (Pilu et al., 2005). This mutation, while being non-lethal, has variable effects on grain yield and seed functionality.

The LP variants of soybean lines were in some instances shown to have reduced seedling emergence and yield due to the location of the lpa-1 mutation that greatly affected its impact on crop yield. Selection within LP lines for increased emergence has resulted in a decrease in total P, inorganic P, and other forms of P (Oltmans et al., 2005). On the other hand, while reductions in seed phytate had negative effects in some studies, other researchers have succeeded in isolating two independent LP mutants of soybean lines with an 80% reduction in phytate P without significant changes to seed functionality (Wilcox et al., 2000).

Appropriate inclusion of degermed dehulled corn, as a reduced phytate P feed ingredient, has previously been shown to not negatively affect the performance of broilers or swine (Moeser et al., 2002; Applegate, 2005).

1.3. Feed Milling and Particle Size Reduction

The first step in manufacturing commercial poultry feed has involved mixing ground grains with a protein source such as SBM. Amerah et al. (2007) reported that reduction of particle size of the grains was a two-step process involving the disruption of outer seed coat and the exposure of endosperm.

In the USA, the single major cereal grain has been corn. In other regions of the world, such as Europe, a number of different cereals grains may be present in the diet, including corn, wheat, barley, and oats. In the USA corn is ground prior to mixing, while in Europe cereals and other ingredients have been mixed together before grinding in order to address the complexities of mill flow when using multiple cereal ingredients. Grinding has most often been accomplished with a hammermill. The resultant particle size can be measured in terms of both geometric mean and standard deviation (ASAE, 2009).

Particle size reduction has increased the number of particles and increased the surface area allowing for greater exposure of feed ingredients to digestive enzymes. However, the extent of particle size reduction was critical as the birds may encounter difficulties in consuming very coarse or very fine particles. In recent years, concerns about particle size has increased as the industry continued to search for ways to optimize feed utilization and improve production efficiency. Moreover, the milling of the seeds has been shown to influence bird performance through influence digestive tract development.

Two processing machines have been commonly used to reduce the particle size of grains. These have been hammermill and roller mill (Koch, 1996; Waldroup, 1997).

A hammermill has been typically comprised of a set of hammers moving at high speed in a grinding chamber, which reduced the size of grains until the particles were able to pass through a screen of designated size (Figure 1). Thus, the size and spectrum of particles produced depended on the screen size and the hammer tip speed (Koch, 1996). The efficiency of the hammermill was influenced by a number of factors, including grain type, grain moisture content, screen size, screen area, peripheral speed, hammer width and design, number of hammers, hammer tip to screen clearance, feed rate, power of the motor, and speed of air flow through the mill (Martin, 1985).

The roller mill was typically comprised of one or more pairs of horizontal rollers in a supporting frame, the distance between which may be varied according to the particle size required (Figure 2). The grain size was reduced by a constant compression force as it passed between the rotating rollers. Roller mills were more efficient and required less energy for grinding than the hammermill. The roller mill also produced a more uniform particle size distribution with a lower proportion of fines (GMD, < 500 μm) than the hammermill (Nir and Ptichi, 2001), although the particle size and spectrum may have varied with the type of corrugation on the rollers and the type of grain (Martin, 1985).

The main purpose of grinding feed ingredients was to increase particle homogeneity, improve the batching and mixing efficiency, decrease ingredient segregation during further handling, enhance pelleting quality and efficiency, and improve nutrient digestibility by increasing substrate surface area. Several investigations have been conducted to determine the optimal grain particle size in poultry feed, specifically for corn particle size (Hastings and Higgs, 1980).

Particle size has a paradoxical role in poultry digestion, especially concerning the interaction between gizzard activity and gastrointestinal tract motility and function. It was previously thought that finely ground grain would enhance nutrient utilization and growth efficiency due to increased surface area (Goodlad, 2002). However, larger particles have enhanced gizzard activity and increased retention time for nutrients by slowing the passage rate, while promoted better digestibility through prolonged enzymatic processing.

The response of intestinal absorptive capability to structural material has been observed with conflicting results concerning villi number and height, and villi crypt depth (Drozdowski et al., 2009). Reduced relative duodenal weights were found in birds fed coarse particle diets (Nir et al., 1995), and a similar pattern was also reported in birds fed diets containing whole wheat (Gabriel et al., 2003).

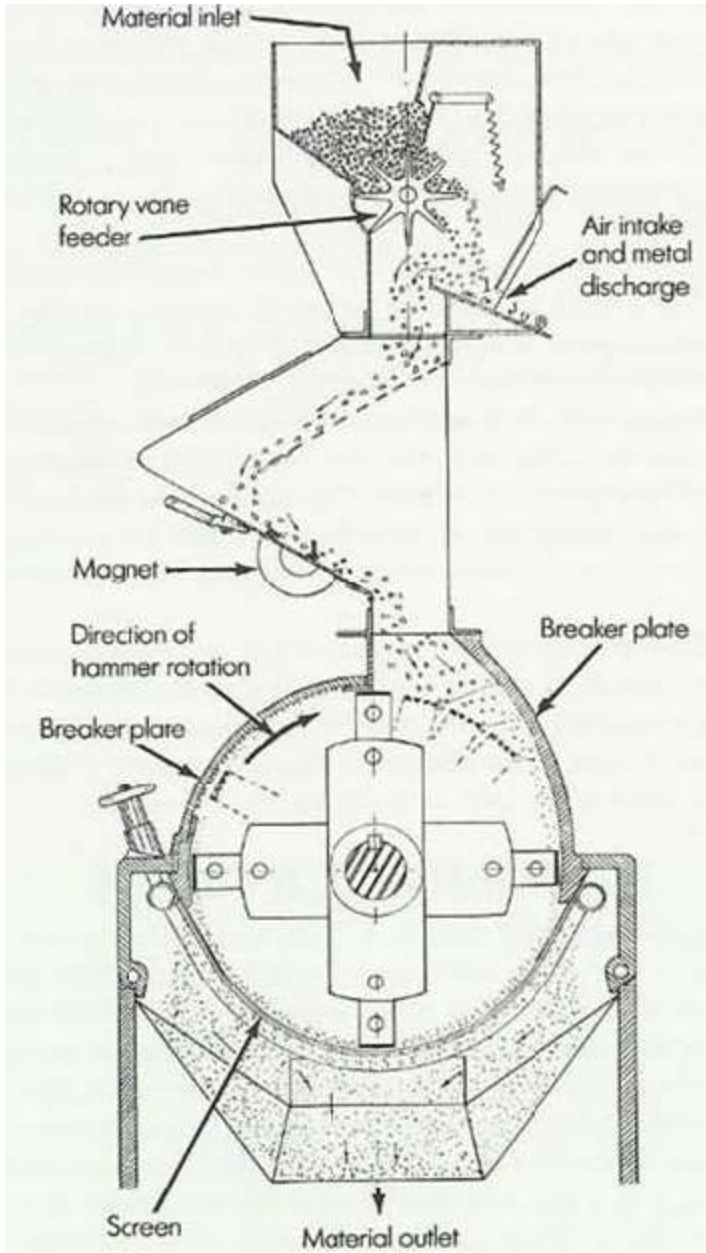

Figure I-1. Hammermill design.

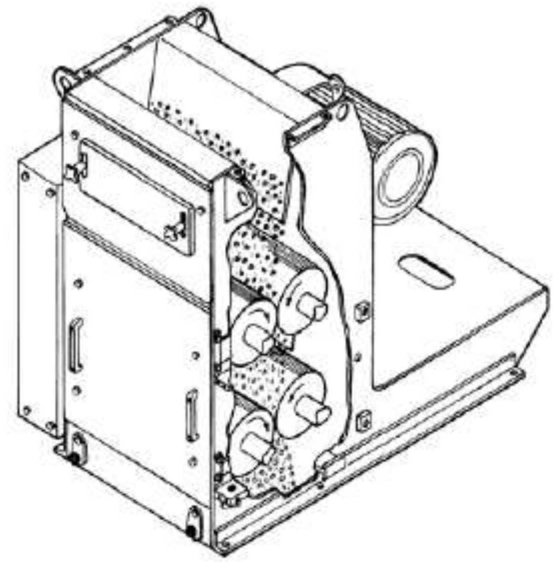

Figure I-2. Roller mill design.

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CHAPTER II

LITERATURE REVIEW

Phosphorus (P) has been recognized as an essential nutrient for plants and animals and was critically important for optimal production of poultry. Phosphorus was the second most important mineral in the body after Calcium (Ca). About 80% of the body's P was found in the skeleton, and the remaining 20% was contained in nucleotides, such as ATP, nucleic acid, phospholipids, and a host of other phosphorylated compounds essential for metabolism (Suttle, 2010).

Compounds containing P were important constituents of all living cells, and the salts formed from P have been found to be essential for maintaining acid-base balance in cells (Leeson and Summers, 2001). Inorganic P was also essential for muscle coordination, metabolism of carbohydrates, amino acids, and fat as well as nervous tissue function, normal blood chemistry, and transport of fatty acids and other lipids (Suttle, 2010).

Because corn and soybean meal (SBM) have constituted a substantial portion of diets for poultry, the major source of P in poultry feed has been SBM. The amount of total P in SBM was approximately 0.62% P (NRC, 1994), but it was not all available since it was bound to phytate (Figure 1). Corn has been considered to be a poor source and contain approximately 0.28% P (NRC, 1994). Inorganic sources of P, such as dicalcium phosphate, have been used to meet the remaining P requirements of poultry although it was very expensive compared to corn and SBM.

The poultry industry has always investigated means to reduce production costs. Feed has represented 60-80% of the expenses in broiler production (Murakami et al., 2007), and P has become one of the most expensive ingredients in broiler production as it has become a limited global resource (Summers, 1997) required for both plant and animal production.

Researchers and industry worldwide have made P a global focus not only for its effects on the cost of production, but also on broiler performance, environmental concerns, leg health, and animal welfare (Overturf et al., 2003).

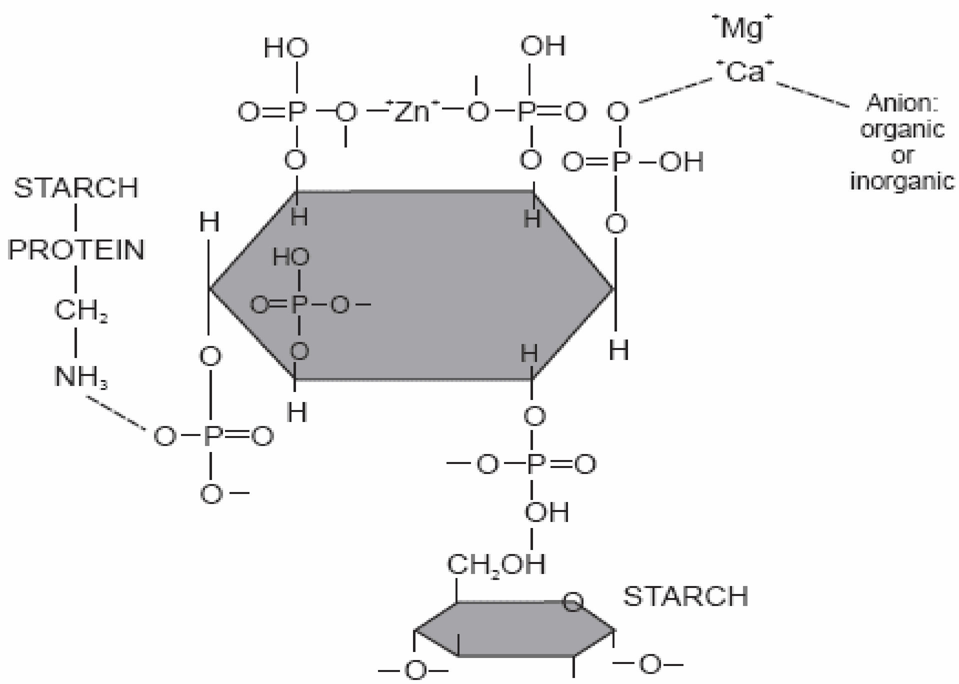

Figure II-1. Phytate molecule

2.1. Effect of soybean meal varieties on broiler performance

Broiler diets have been generally made from plant based feed ingredients. Among these ingredients was SBM where more than 50% of SBM produced in the United States was utilized by poultry. SBM has been commonly added to poultry diets as a source of amino acids, and when blended with corn, the complimentary combination supplied nearly adequate nutrients to support normal growth. Dilger and Adeola (2005) reported that feeding two varieties of SBM, conventional and low phytate SBM to chicks over 7 d of age increased BW gain and feed intake of all chicks, but with the feed efficiency increased >4-fold for conventional SBM and >5-fold for low phytate SBM. In a study by Baker et al. (2011), the inclusion of either SBM-high protein (SBM-HP) or SBM-low oligosaccharide (SBM-LO) resulted in a greater nutritional value in diets of broiler chicks because of the increased concentration of digestible amino acids compared to a conventional SBM, but there was no significant differences in BW, feed intake, BW gain, and gain:feed ratio. However, the cost of diets containing SBM-HP and SBM-LO was lower since they required less SBM in the diets compared to conventional SBM diets. On the other hand, Perryman et al. (2013) reported that broilers fed diets formulated with SBM had greater BW gain and lower feed conversion ratio (FCR) than birds fed diets formulated with conventional SBM from 1-14 d of age. Feeding low phytate SBM that spent 45 min in the extractor has been shown to increase feed intake and BW gain compared with LP SBM that spent 90 min in the extractor due to a higher concentration of amino acids as well as total essential amino acids and higher concentration of total P in the former (Karr-Lilienthal et al. 2005). This suggested that the essential amino acid constituents of SBM were heat labile.

2.2. Effect of soybean meal varieties on nutrient digestibility

Several low phytate varieties of corn, wheat, oats, barley, rice, and soybeans have been developed with reduced phytate P concentrations of between 50 and 95% (Raboy et al., 2000; Raboy, 2002; Guttieri et al., 2004; Israel et al., 2005; Oltmans et al., 2005). A study conducted by Sands et al. (2003) showed that the relative bioavailability of P from low-phytate SBM was 15-25% greater compared to conventional SBM when fed to broiler chickens. Moreover, the improved P digestibility and retention of P in diets containing low phytate SBM was consistent with studies that compared low phytate versus conventional sources of corn (Li et al., 2000), and barley (Thacker et al., 2003).

Dilger and Adeola (2006) found no differences in pre-cecal digestibility of P which ranged from 79-89% in low phytate or conventional SBM. However, in spite of no change in pre-cecal P digestibility when birds received a low phytate SBM, P retention of broilers was greater when diets contained low phytate SBM (77% vs 60%) and was attributed to a 57% reduction in phytate P. However, this was much greater than previous digestibility estimates of ~ 50% (Rutherford et al., 2002) and was attributed to the low P concentrations of the diets used in this study. The influence of the amount of Ca and P together and their effect on P digestibility using different varieties of SBM, high phytate (HP) SBM, and low phytate (LP) SBM was investigated by Plumstead et al. (2008). They found that the apparent prececal digestibility of P decreased in a curvilinear manner with increasing dietary Ca and was greater for diets containing LP SBM compared with either commercial or HP SBM. The prececal Ca digestibility increased 2-fold when dietary Ca level was increased from 0.47 to 1.16% which also increased the output of phytate P per kg of DMI at the distal ileum. The apparent P digestibility was improved from 51.69 and 52.24% to 65.44% when LP SBM was included in diets in place of either commercial SBM or HP SBM, respectively. These results were consistent with Karr-Lilienthal et al. (2005) who reported that P bioavailability from SBM prepared from low phytate SBM was 1.5 times greater than from SBM extracted for 45 min. Moreover, Karr-Lilienthal et al. (2005) showed that roosters fed conventional SBM extracted for 45 min (traditional extraction time) had higher total essential, total nonessential, and total amino acid digestibility compared with SBM extracted 60 min.

2.3. Effect of soybean meal varieties on organ function

The information regarding the influence of SBM variety on organs was scarce. Mojarrad et al. (2012) did not find any difference in full gizzard and proventriculus weights among different SBM sources processed at different temperature and time. However, raw soybeans have caused pancreatic gland enlargement because the trypsin inhibitors (anti-nutritional factors) stimulated pancreatic enlargement via a humoral mechanism, possibly pancreozymin (Khayambashi and Lyman,1969). In a similar manner, Moeser et al. (2002) studied the effect of degermed, dehulled corn on gastrointestinal tract and liver weights of pigs and did not find any significant difference as compared with feeding whole grain corn.

2.4. Particle Size

Particle size reduction of grains has involved disruption of the outer seed coat and fracture of the endosperm (Amerah et al., 2007). The purpose of particle size reduction was to increase the surface area of the digesta available for interaction with digestive enzymes (Engberg et al., 2004).

The reduction of feed particle size has been described as the second largest energy cost after that of pelleting in the broiler industry (Reece et al., 1985). So, reducing feed particles to finer size required greater energy use and reduced feed production rate. Thus, any reduction in energy consumption from grinding could significantly lower feed cost. Many of the diets that have been used in EU countries contained whole grains of wheat, sorghum, barley, or oats in recent years. Although, poultry diets in the US has been primarily based on ground corn, feeding some large particle size corn may produce benefits similar to the effects observed from whole grain feeding (Parsons et al., 2006).

The official method for particle size determination measured particle geometric mean diameter (Dgw) and the standard deviation (Sgw) by using a Ro-tap device that contained sieves that allowed different particle size materials to pass according to the method of determining and expressing fineness of feed materials by sieving as defined by ASAE S319.3 (Figure 2).

Figure II-2. Ro-tap device with sieves. ASAE, 2009.

2.5. Effect of corn particle size on broiler live performance

Coarse particle size cereal grains have been shown to affect poultry performance, but the results of these studies have not been consistent. Nir et al. (1995) reported that broilers fed wheat and sorghum mash diets with coarser particles exhibited greater BW and improved feed efficiency as compared to those fed finely ground grains. Reece et al. (1985) reported greater BW and better feed efficiency when birds consumed diets that contained corn at 1,343 µm as compared to corn at 814 µm. Moreover, Noy and Skylan (1990) and Batal and

Parsons (2002) reported that increasing feed particle size improved nutrient digestion during the first few weeks of a bird's life, which may be an important step to improve BW gain potential. Moreover, Nusairat et al. (2012) reported in a cage study conducted to 21 d of age that 50% coarse corn inclusion had no effect on BW gain after 9 d of age, which suggested that feed intake and digestive functions may have adapted quickly in broiler chicks. In contrast, Charbeneau and Roberson (2004) reported significantly decreased BW gain at 7 and 15 d when the particle size of corn in diets fed to poults increased from 600 to 1,100 µm. In addition, Douglas et al. (1990) reported that BW and feed efficiency were adversely affected when chicks were fed diets that contained 1,470 µm coarse corn as compared with medium size 947 µm corn. Moreover, the addition of whole grains has shown diverse results as well. Ravindran et al. (2006) reported that chicks fed whole wheat had lower BW due to poorer feed consumption as compared to those receiving ground wheat. These authers suggested that young chicks may have difficulties swallowing the whole wheat. In contrast, Elwinger et al. (1992) reported that when whole wheat was used to replace ground wheat, the dry matter content of the litter increased. This finding could help to keep the broiler house floor drier and reduce ammonia emissions and wet litter problems.

2.6. Effect of corn particle size on nutrient digestibility

The effect of particle size on the digestibility of cereal grains has been studied extensively in poultry and swine diets (Goodband et al., 1995). According to Kilburn and Edwards (2004) increasing the particle size of commercial SBM from 891 µm to 1,239 µm improved mineral utilization and FCR in semi-purified diets. Nir et al. (1995) suggested that larger particles were better suited to the intestinal tract because they increased gizzard size and stimulated peristalsis more than smaller particles. Larger particles slowed digestion within the gizzard and small intestine. Thus, feed passage rate was slowed, which resulted in greater reflux of intestinal contents and increased exposure of nutrients to digestive enzymes (Figure 3). Although particle size reduction has been stated to improve digestion of nutrients by increasing the surface area available to digestive enzymes, studies that relate particle size to digestibility of nutrients were limited and, in the case of grains, equivocal. Kilburn and Edwards (2001) reported that fine grinding of corn increased the true metabolizable energy values of mash diets, but the opposite effect was observed with pelleted diets.

Peron et al. (2005) found that fine grinding of wheat improved starch digestibility and apparent metabolizable energy (AME) compared to coarse grinding. On the other hand, coarse grinding of corn has been reported to increase the efficiency of nitrogen and lysine retention in broilers fed mash diets (Parsons et al., 2006). Amerah et al. (2007b) reported that coarse grinding tended to improve AME in wheat-based diets but not in corn-based diets. In contrast, Svihus et al. (2004) found no effect of wheat particle size on dietary AME.

In terms of mineral availability, coarse grinding appeared preferable to fine grinding. Large corn particle size corn has been shown to significantly improve Ca, total P, and phytate P utilization in broilers (Kasim and Edwards, 2000; Kilburn and Edwards, 2001). Lu et al. (2011) reprted that the retention ratio of P, Ca, and crude protein was increased when geese were fed whole corn compared with ground corn.

Bone ash and plasma P levels of broilers were improved after the feeding of coarse (1239 µm) rather than fine (891µm) SBM (Kilburn and Edwards, 2004).

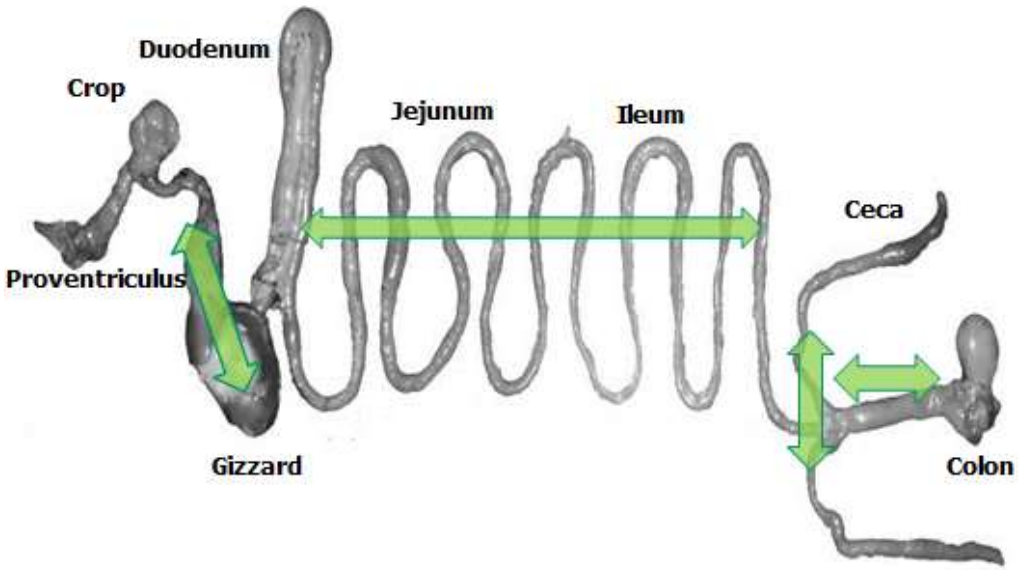

Figure II-3. Broiler digestive tract depicting reverse peristalsis (locations of peristalsis according to Denbow, 2000).

2.7. Effect of corn particle size on organ function

Previous studies with cereal grains have demonstrated that particle size can influence gizzard development. A well-developed gizzard has been associated with improved gut motility (Ferket, 2000), which increased the retention time of the feed in the upper part of the intestine. This promoted better digestion and reduced the risk of coccidiosis and other enteric diseases (Bjerrum et al., 2005). The gizzard has been described as the principal physical food-processing organ in avian species (Moore, 1993), which reduced the size of ingested items principally by shear (Moore, 1998). Therefore, increased feed particle size slowed the passage rate of nutrients through the gizzard with potentially improved nutrient digestibility (Nir et al., 1994a). In addition, feeding whole grains has been associated with enhanced gut development and less occurrence of proventricular swelling (Jones and Taylor, 2001). However, this may have increased mortality due to ascites through occlusion of the thoracic cavity, thereby impairing heart and lung function (Jones and Cumming, 1993). Jacobs et al. (2010) reported a 19% increase in gizzard weight as corn particle size increased from 557 to 1,387 μm. These results were similar to those of Nir et al. (1994a) who found that when 1-d-old broiler chicks were fed coarse 2,010-2,100 μm and medium 1,130-1,230 μm corn particles, gizzard weight increased by 26 to 41% as compared with chicks fed fine 570-670 μm particles. The relative weight of gizzard and small intestine have been shown to decrease when birds were fed pelleted rather than mash diets (Nir et al., 1995) as pelleted diets have generally contained finer particles.

Feed with larger particle size has been shown to influence the development of other segments of the digestive tract in birds fed mash diets. Nir et al. (1994b) reported hypertrophy of the small intestine and reduced intestinal pH when fine mash diets were fed to broilers.

2.8. Feed Additives

2.8.1. Phytase Enzymes

Phytase enzymes (*myo*-inositol hexaphosphate hydrolases) catalyze the hydrolysis of phytic acid (*myo*-inositol hexaphosphate, IP6), phosphate ester bonds and yield free inorganic P (P_i) and one or more lower phosphoric esters of *myo*-inositol penta*kis*-, terta*kis*-, tris-, bis-, and monophosphates (Wyss et al., 1999). Phytases are widely distributed in plants, animals, and microorganisms, and two classes of phytase enzymes have been characterized that consist of a 3-phytase that initiates de-phosphorylation of IP6 on the phytate molecule at the 3 position to yield 1,2,4,5,6-penta*kis*phosphate and P_i, whereas 6-phytases initiate de-phosphorylation at the 6 position of the inositol ring and yield 1,2,3,4,5 penta*kis*phosphate and P (Angel et al., 2002). The pH optima at which these enzymes hydrolyze inositol phosphates have been shown to be an important distinction. Four possible sources of phytase can be found in the digestive tract of the animal and include 1) phytase from feed ingredients, 2) exogenous microbial or fungal phytase that were added to the diet, 3) endogenous phytase secreted by the intestinal mucosa, and 4) phytase produced by microflora in the lower gastrointestinal tract of the animal (Angel et al., 2002).

The low bioavailability of minerals in common feedstuffs, especially in a corn-soy based diet, has been shown to be a result of two factors. First, most of the P present in ingredients of plant origin was in the form of phytate, or inositol hexa*kis*phosphate, where six phosphate groups were attached by phosphoester bonds to an inositol ring. The strong chelating capacity of these phosphoester bonds for minerals such as Mg, Zn, Fe, and Ca and other nutrients such as carbohydrate and protein renders a portion less available. Second, poultry species and swine have been shown to lack the capacity to produce adequate phytase to optimally utilize the P from these organic sources (Maenz and Classen, 1998).

The availability of P in feedstuffs of plant origin is generally very low (Herland and Oberleas, 1999; Ravindran et al., 1999). Bioavailability estimates of P in corn and SBM for pigs and poultry range from 10-30% (Jongbloed and Kemme, 1990). So, this low availability of phytate P has posed two problems for poultry producers. First, inorganic P supplements lead to the excretion of a large amount of P in manure. Second, phytate limits availability of P and several other essential nutrients as mentioned above. Phytase supplementation has become an efficient tool to improve bioavailability of P present in animal feedstuffs and to reduce the amount of phytate-derived P excreted to the environment. Phytase activity has been measured as FTU. One FTU has been generally defined as the amount of enzyme required to liberate 1 μmol of inorganic P min^{-1} from 5.5 m*M* of Na phytate at pH 5.5 and 37º C (Qian et al., 1997).

2.8.2. Definition of phosphorus terms

Total phosphorus (total P) has generally referred to the total amount of analyzed elemental P that would be analyzed following digestion of the sample after the inorganic P content (P_i) had been determined by atomic absorption spectrophotometry, inductively coupled plasma spectroscopy, or a colorimetric method.

Non Phytate Phosphorus (NPP) has represented a chemically defined entity calculated by subtracting the analyzed phytate P content of ingredients from their analyzed total P content (Angel and Applegate, 2001). The term NPP has been predominantly used in poultry nutrition as an expression of the phosphorus requirement of birds (NRC, 1994).

Available phosphorus (AvP) has been commonly used as a second method in feed formulation to express the amount of P from feed ingredients. The term AvP has often been misused and frequently erroneously interchanged with NPP. The classical definition of AvP, which has also been known as relative bioavailable P, was the amount of P from a feed ingredient or diet that was available at tissue level to the bird. AvP was further used to express the nutritional requirement for P. The most common method that has been used to assess the amount of AvP in feed ingredients has been a slope-ratio procedure using a low-P semi-synthetic diet that was supplemented with graded levels of P from a reference source of

P such as monocalcium phosphate, or monosodium phosphate and the response (tibia ash, toe ash, or BW gain) determined (Soares, 1995). The AvP content of the test ingredient was then determined by comparing the relative response obtained from the test ingredient to that of the reference standard.

Apparent digestible P, or absorbed P has been defined as the difference in the amount of P consumed from the diet and that arriving at the terminal ileum. When values were corrected for the endogenous P contribution from intestinal secretions and desquamation of epithelium lining the digestive tract, *true digestible P* values resulted.

2.9. Effect of phytase enzyme on broiler performance

Many studies have produced inconsistent results with regards to the effect of phytase in poultry diets and its relation to the adequacy of nutrients such as Ca and available P. Hassanabadi et al. (2008) added 0, 250, 500, 750, 1,000, and 1,250 FTU/kg of phytase to broilers raised to 28 d of age and showed that phytase had no effect on live BW, daily BW gain, feed intake, and FCR. The experimental diets were nutritionally adequate in all nutrients as well as Ca and non phytate P (nPP). On the other hand, Cowiesn et al. (2013) reported that when 500 FTU/kg of microbial phytase was added to diets insufficient in Ca and digestible P and fed to broilers raised to 42 d of age, phytase improved BW gain and FCR with the effect most pronounced in the finisher phase. Also, when 1,000 FTU/kg of phytase was added to different sources of SBM, a significant increase in BW gain and improvement in feed to gain ratio was observed, but feed intake was not affected (Manangi and Coon, 2006). Powell et al. (2008) reported that adding 1,200 FTU/kg phytase to broilers during the grower period (14-32 d) decreased average daily gain (ADG), average daily feed intake (ADFI), FCR, and BW. During the finisher period phytase further decreased ADFI and BW. Nusairat et al. (2013) reported that when phytase was added at 300 FTU/kg of feed to LP SBM for broilers raised to 35 d of age on floor pens, the FCR was not improved compared to NP SBM from 23-35 d of age, and that was probably related to lack of substrate available to the enzyme.

In breeder trials, research has shown that the use of phytase at recommended levels could be a cost-effective, safe option to reduce the inclusion of inorganic P in broiler breeder diets without affecting the number of chicks per hen housed (Plumstead et al., 2007) or egg production (Berry et al., 2003).

2.10. Effect of phytase enzyme on nutrient digestibility

Optimal growth and efficiency in poultry was determined by nutrient digestibility and bioavailability, which has meant that improved digestion and bioavailability would be expected to provide nutrient requirements of poultry for optimum live growth performance. Exogenous phytase has improved the availability of P, released trace minerals from hydrolysis of phyate, possibly increased starch digestibility, and increased the availability of amino acids that should improve the live performance of broilers. Many studies have been conducted to determine the effect of phytase enzyme on digestibility of P, Ca, and amino acids in broiler and breeder diets. Adding phytase enzyme at two levels (500 and 750 FTU/kg of phytase) improved P digestibility of low P diets compared to non-supplemented low P diets due to the reduced P uptake in low P diets and the same effect of improving amino acid digestibility (Rutherfurd et al., 2004). Furthermore, when phytase was added to different sources of SBM (Manangi and Coon, 2006), the total P and phytate P were reduced in excreta. This produced an increased digestibility and availability of P that was reflected in improved BW of broiler chicks at 21 d of age compared to diets without phytase.

In a study conducted by Applegate et al. (2003), a combination of LP corn plus phytase (600 FTU/kg) had no effect on broiler live performance to 49 d of age but significantly reduced P intake of birds in this treatment combination and reduced total P concentration of the litter to a greater extent than diets with either phytase or LP corn alone. This was explained by increased P digestibility and availability for the broilers.

2.11. Effect of phytase enzyme on organ function

The digestive tract has several sections involved in P digestion and absorption. The crop, proventriculus, and gizzard regions were where partial solubilization of the ingested compounds, and eventually the hydrolysis of phytates by exogenous phytase occur. Solubilization occurs in the gastric area; absorption in the small intestines and according to Breves and Scröder (1991), the large intestine was of minor importance for total P absorption. The optimal brush border phytase activity of the small intestine was reported to be between pH 5.5 and 6.5 (Maenz and Classen, 1998). Phytase activity in diets and gastrointestinal tracts of 3 to 5-wk-old chickens fed diets with added P and microbial phytase were studied by Leibert et al. (1993). Phytase activity in the crop increased as phytase level increased and P level was increased, while the activity of phytase decreased in small intestine.

According to Nusairat et al. (2013), adding phytase to diets of broiler chicks raised up to 21 d of age produced broiler with a smaller proventriculus and larger gizzard compared to birds fed diets without phytase.

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CHAPTER III

Effect of low phytate and normal phytate soybean meal and dietary corn particle size on live performance, organ weight, and nutrient digestibility of young male broilers

3.1. ABSTRACT

A 2 X 2 factorial experiment was conducted to compare the effect of normal (NP) and low phytate (LP) varieties of soybean meal (SBM) in the presence of either 0% (0%CC) or 50% coarse corn (50%CC) on male broiler performance raised to 21 d of age in battery cages. From 1 to 9 d, all birds received two pre-starter diets (0%CC or 50%CC) with the same source of SBM. From 9 to 21 d birds received diets with either NP-50%CC, NP-0%CC, LP-50%CC, or LP-0%CC combinations with 8 pens of 8 broilers each for each combination. Cage BW, BW gain, and feed intake were measured and adjusted feed conversion ratio (AdjFCR) was calculated. Ileal and diet total phosphorus (P) were measured to estimate P digestibility, and gizzard and proventriculus weights were also measured. Although BW (208 versus 203 g) and AdjFCR (1.10 versus 1.13 g:g) were improved ($P \leq 0.05$) with 0%CC diets at 9 d of age this difference did not persist to 21 d of age. There were no main effects of LP versus NP that persisted to 21 d of age as well. However, upon necropsy at 21 d of age, the LP diets produced lower ($P \leq 0.05$) proventriculus weight while the 50%CC diets produced larger ($P \leq 0.0001$) gizzards and smaller ($P \leq 0.0001$) proventriculus weights. Examination of the interaction means revealed that the greatest ($P \leq 0.05$) proventriculus weight was with the NP-0%CC combination, which suggested a difference in ingesta pH or digestive function that could affect phytate hydrolysis. This conjecture was supported by the finding that ileal

phytate P was numerically less (P = 0.79) in the NP-0%CC than NP-50%CC diets and was greater (P = 0.79) than in the presence of LP diets. Results of the current study indicated that feeding 50%CC or using NP SBM improved ($P \leq 0.05$) phytate P digestibility.

Key words: Broilers, low phytate, soybean meal, phosphorus, feed particle size

3.2. INTRODUCTION

More than 50% of all soybean meal (SBM) produced in the United States has been utilized by poultry, primarily because of its high-quality protein content (Baker, 2000). However, there are several antinutritional factors present in soybeans that prevent complete utilization of some of the nutrients. One approach has been to reduce the amount of phytic acid and increase available P in the seeds via genetic selection. Low-phytic acid genotypes of soybeans, barley, and corn have been developed that produce seeds with a reduced phytic acid P of 50 to >90% (Raboy et al., 2000). On the other hand, interest in the effects of feed or grain particle size has increased in recent years as the industry has been searching for techniques to optimize utilization of feed and improve live production efficiency. The grinding of whole grain for broiler feeds constitutes the second greatest energy expenditure after pelleting (Reece et al., 1985). Therefore, feeding coarse corn (CC) has considered beneficial from the viewpoint of energy expenditure. Promotion of the development of gizzard was another nutritional strategy that can be achieved by manipulating feed particle size (Nir et al., 1995; Engberg et al., 2002). A well-developed gizzard was associated with improvement in gut motility (Ferket, 2000), may prevent pathogenic bacteria from entering the small intestine (Bjerrum et al., 2005), and reduced the risk of coccidiosis and other enteric diseases (Cumming, 1994; Engberg et al., 2002; Bjerrum et al., 2005). Also, according to Lentle (2005), greater proportions of coarse particles of grain in the feed resulted in greater numbers of coarse particles transiting the gizzard, which may increase the permeability of digesta to enzymes and improve digestive efficiency. The objective of this study was to determine the effect of phytate modified SBM varieties and corn particle size on broiler digestive tract development, broiler live performance, and nutrient digestibility to 21 d of age.

3.3. MATERIALS AND METHODS

Birds and Experimental Diets

All animal work was conformed to the Guide for Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010). Broiler chicks were hatched from eggs obtained from Ross 344 x 708 (Aviagen, Huntsville, AL) broiler breeders housed at the institution. Chicks were feather-sexed at hatching, and 576 male chicks were permanently identified with neck tags and 12 chicks were randomly allocated to 48 electrically heated battery brooders located within one environmentally controlled room. To reduce vertical temperature and lighting differences among the cages in the battery brooders, only the middle 4 tiers of cages in each 6-tier battery brooder were utilized (344 chicks). Brooding temperatures within cages were initially set at 33^{0}C and reduced gradually to 29^{0}C by 21 d of age. All diets were formulated to meet or exceed the National Research Council (1994) suggested requirements. From 1-8 d, all birds received a standard corn-soy broiler starter diet containing 2.81 kcal ME/g and 22.45% CP, with either fine or coarse corn particle size (Table 1). For diets with fine particle size (0%CC), all of the corn in the diet was ground using a hammermill (Model 1522, Roskamp Champion, Waterloo, IA) equipped with two 2.4 mm screens to obtain a particle size of 229 µm. For diets with coarse particle size (50%CC), half of the corn in the diet was ground with a two-pair roller mill with a gap setting of 0% opening (0.18 mm) on the top pair of rollers and 100% opening (7.16 mm) on the bottom pair of rollers (Model C128829, RMS, Tea, SD) to obtain a coarse particle size of 1095 μm, and 50% through a hammermill to obtain a fine particle size of 229 μm. Particle size distribution was determined by ASAE S319.3 (ASAE, 2003). Feeder was covered with screen wires that had openings of 2.54 x 2.54 cm. Screen wires were used to reduce feed wastage, and to make the birds meal eaters by consuming feed as wires were shaken three times daily to keep the feed available. To reduce variation in chick BW between cages during the ileal digestibility evaluation, all chicks were individually weighed at 9 d and chicks with extreme BW were eliminated so that 8 chicks per cage with a mean chick BW of 206 ± 2 g remained. Group body weight (BW), and feed intake were measured at 0, 8, 15, and 21 d, and BW gain was calculated by time interval. Mortality was weighed and recorded twice daily for adjusted feed conversion ratio (AdjFCR) calculations.

At 9 d of age the birds were then allocated to their pre-assigned experimental diet in a 2 x 2 design of 4 diets with 8 replicate cages of 8 birds per diet and fed the experimental diets from 9-21 d of age. From 9-21 d, 4 experimental diets were formulated using two basal diets that differed in their inclusion of coarse particle size corn (fine and coarse) and either NP or LP SBM (Table 2). The LP and NP SBM were analyzed for their composition. The LP SBM contained 51.23% CP, 0.81 total P. The NP SBM contained 50.48% CP and 0.69% total P. Titanium dioxide (TiO_2) and Celite were included in diets as indigestible markers to allow the calculation of apparent nutrient digestibility. All diets were mixed in a horizontal twin shaft ribbon mixer (TRDB126-0604, Hayes and Stolz Ind. Mfg. Co., Fort Worth, TX) to produce the mash diets, mash feed was then (conditioned at 85°C for 45 seconds) and pelleted with a ring die (4.4 mm by 35 mm) pellet mill (Model PM1112-2, California Pellet

Mill Co., Crawfordsville, IN). Pellets were then cooled with ambient air in a counter-flow cooler (Model VK09 × 09KL, Geelen Counterflow USA Inc., Orlando, FL) and crumbled. The chemical composition of NP and LP SBM was slightly different, but for the purpose of this short study, these differences were ignored.

Sample Collection and Analyses

At 21 d of age, 8 chicks from each cage were weighed, killed by cervical dislocation, and the terminal 13 cm of ileum was removed 3 cm anterior to the ileo-cecal junction. Ileal contents were gently expressed, pooled per cage, and frozen.

Frozen samples of feed were dried in oven for 24 hour at 95 to 100°C for dry matter (Method 934.01, AOAC, 2006) and ileal digesta were freeze-dried (Virtis Freezemobile - Model 12XL, Warminster, PA) and ground prior to analysis for total P using acid digestion of a 0.5 g dried sample with 12 mL of 6N HCl and total P quantified using inductively-coupled plasma optical-emission spectrometry (ICP–OES; 4300DV, Perkin-Elmer, Wellesley, MA 02481) detection. The TiO_2 of diets and ileal samples were determined using the method of Myers et al. (2004).

Calculations

The apparent percentage prececal nutrient digestibility (PcND%), expressed as a percentage of dry matter (DM) nutrient concentration, was calculated using the index method based on the following equation: (Dilger and Adeola, 2006).

$$PcND\% \text{ or } TNR\% = 100-[(TiO_{diet}/\ TiO_{out}) \times (Nut_{out}/\ Nut_{diet}) \times 100]$$

Where TiO_{diet} was the initial TiO_2 concentration in the diet, Nut_{diet} was the initial dietary concentration of the nutrient being assessed, and TiO_{out} and Nut_{out} were the respective concentrations of either TiO_2 or nutrient in the ileal digesta or excreta, respectively.

Statistical Analysis

Two-way ANOVA was used to determine the main effects (SBM and corn particle size) and their interaction using the GLM procedure of SAS (2009; SAS version 9.2, SAS Institute, Cary, NC, USA) in a randomized complete block design with a factorial structure of 4 factorial treatments in 4 blocks and 2 replicate cages per treatment per block. Cage served as the experimental unit. Differences were considered significant at $P \leq 0.05$, although effects with *P*-values between 0.06 and 0.10 are mentioned in the text when the data suggested a numerical trend.

3.4. RESULTS AND DISCUSSION

Effect of SBM Varieties on Performance

The effect of SBM variety on broiler BW, BW gain, feed intake, and AdjFCR is shown in Table 3. Broiler BW was determined at 1, 9, 16, and 21 d of age. The LP SBM was included in the diets from 9 d. At 16 d, birds consuming LP SBM had lower BW ($P \leq 0.05$), however, at 21 d BW was comparable between birds consuming both varieties of SBM, suggesting that birds were still adjusting to the LP SBM at 16 d but by 21 d birds were fully adjusted and gaining BW faster than birds consuming NP SBM. This could represent the time required for birds to adapt to such a new feed ingredient. It was clear from the BW gain results that birds consuming LP SBM gained less ($P \leq 0.05$) BW from 10 to 16 d, which suggested a period of adaptation. However, from 17-21 d BW gain was greater ($P \leq 0.05$) for birds consuming LP SBM. The overall 21 d BW gain was comparable among the SBM varieties as was feed intake and AdjFCR (Table 3). Limestone inclusion rate increased from 0.76 to 0.90% when changing from pre-starter to starter diets at 9 d, but feed intake was not negatively influenced as the increase in limestone was marginal. Dilger and Adeola (2006) reported that feeding graded levels of conventional and LP SBM to chicks increased BW gain and feed intake as SBM inclusion increased, with birds fed LP SBM exhibiting lower BW gain and feed intake. Similar findings were also reported by Karr-Lili et al. (2005). Differences in reported results could be due to the fact that response observed by the investigators was due to the graded levels of each SBM variety rather than the variety itself. In the current study we only used one level of either NP or LP SBM.

Effect of SBM Varieties on Organ Weight

The effect of SBM variety on absolute and relative gizzard and proventriculus weights is shown in Table 4. The gizzard and proventriculus were harvested at 21 d and were not affected by SBM, however, the proventriculus was smaller ($P \leq 0.05$) in birds consuming LP SBM. Since organs were harvested at 21 d, the proventriculus weight suggested that it was part of the adaptation process and may have indicated that birds reduced the amount of secreted acid or pepsin in the presence of LP SBM.

Effect of SBM Varieties on Total Phosphorus and Phytate Phosphorus Digestibility

The effect of SBM variety on total P and phytate P digestibility is shown in Table 5. Total P digestibility was comparable among the different SBM varieties but phytate P digestibility was reduced ($P \leq 0.05$) in LP SBM diets (76.09 vs 72.54%), probably due to lower initial phytate P content. Plumstead et al. (2008) reported that the apparent prececal P absorption per kg of DM intake was comparable between commercial and LP SBM diets, and no differences among SBM cultivars for the percentage apparent prececal disappearance of phytate P were observed. Karr-Lili et al. (2005) demonstrated that by decreasing the amount of phytate present in SBM, a larger proportion of P and potentially other minerals were rendered more available to the animal.

Effect of Corn Particle Size on Performance

The effect of %CC on broiler BW, BW gain, feed intake, and AdjFCR is shown Table 3. Diets had either 0% coarse corn (0%CC) or 50% coarse corn (50%CC). Inclusion of CC in the diets had no effect on BW from 1 to 9 d. However, BW gain was greater ($P \leq 0.05$) for birds consuming 0%CC but this difference were not evident at 16 d and thereafter (Table 3). Nir et al. (1994) reported similar results in which birds that received a medium particle size diet (769 μm) from hatching to 7 d of age exhibited improved BW and feed efficiency as compared to those fed a more coarse diet (1,200 μm). The BW gain exhibited a linear decrease as corn particle size increased (commercial broiler chick) during the first week, with BW for the two largest corn particle sizes (1,210 and 1,387 μm) being significantly less than the two smaller corn particle sizes (557 and 858 μm) (Jacobs et al., 2010). Feed intake was comparable among the different corn particle size diets in the present study. Birds consuming 0%CC exhibited improved ($P \leq 0.05$) FCR from 1-9 d (1.10 versus 1.13 g:g) and from 10-16 d (1.39 versus 1.41 g:g), which implied that birds required a period of time to adapt to the larger particle size of corn (~14 days) compared to a shorter adaptation period for the LP SBM (~7 days). For the 17-21 d period, AdjFCR was comparable among the different corn particle size diets. Nir et al. (1994) reported that birds consuming different particle size grains, which were ground using a hammermill, exhibited no significant differences in performance at 7 d of age, but performance improved at 21 d of age in birds that consumed medium and coarse mash diets. There was no interaction effect on live performance (Table 6).

Effect of Corn Particle Size on Organ Weight

The effect of %CC on gizzard and proventriculus weights is shown in Table 4. Feeding 50%CC increased ($P \leq 0.01$) gizzard weight but decreased ($P \leq 0.01$) proventriculus weight when compared to 0%CC. This indicated that larger particles stimulated gizzard muscle activity to provide proper mixing of the large particles with other secretions. However, greater mixing time probably required less acid and pepsin secretion from the proventriculus, which could explain the reduced proventriculus weight. There was an interaction between SBM and CC for proventriculus weight (Table 7) where birds consuming 50%CC with either LP or NP SBM had the smallest proventriculus that indicated that particle size had a greater effect on proventriculus weight than did SBM. Our findings were in agreement with the previous findings of Nir et al. (1994) who reported greater gizzard development and lower gizzard pH in 7-d-old chicks fed medium or coarse particle size diets as compared with those consuming fine particle diets. Taylor and Jones (2004) reported that feeding whole grains reduced the size of the proventriculus and increased gizzard musculature. Jacobs et al. (2010) reported that gizzard weight increased as corn particle size in the diet increased with the greatest gizzard weight observed when chicks were fed the largest corn particle size of 1,387 μm.

Effect of Corn Particle Size on Total Phosphorus and Phytate Phosphorus Digestibility

The effect of %CC on total P and phytate P digestibility is shown in Table 5. As expected, birds consuming 50%CC had better overall nutrient digestibility probably due to altered peristalsis and increased retention time of digesta in the GIT, which improved digestion and nutrient absorption. This was evidenced by phytate P being improved ($P \leq 0.01$) as compared to birds consuming 0%CC. There was no interaction effect on total P digestibility (Table 8). Coarse grinding of corn has been reported to increase the efficiency of nitrogen and lysine retention in broilers fed mash diets (Parsons et al., 2006). Large particle size corn has also been shown to significantly improve calcium, total P, and phytate P utilization in broilers (Kasim and Edwards, 2000).

3.5. CONCLUSION

Results of the current study demonstrated that 50%CC produced larger gizzard and smaller proventriculus weights and LP diets produced a smaller proventriculus compared to NP, which suggested that digestive functions could both respond to and affect phytate hydrolysis. Numerically, LP diets had greater total P digestibility possibly due to more available P compared to NP diets, and 50%CC improved phytate P digestibility compared to 0%CC, which may have been due to increased retention time for digesta in the GI tract. This provided more time for endogenous enzymes to work. The LP diets possessed low phyate so digestibility was lower as compared to NP diets.

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Table III-1. Pre-starter diets used to 8 d of age

	Dietary Treatments[1]	
	0 %CC	50% CC
Ingredients	**Commercial NP SBM**	**Commercial NP SBM**
	(%)	
Fine corn	58.90	29.45
Coarse corn (CC)	---	29.45
Soybean meal (48%CP)	36.33	36.33
Poultry fat	0.50	0.50
Limestone	0.76	0.76
Dicalcium phosphate (18.5%P)	1.99	1.99
Salt	0.50	0.50
DL-Methionine	0.24	0.24
L-Lysine	0.03	0.03
L-Threonine	0.07	0.07
Choline chloride (60%)	0.20	0.20
Vitamin premix[2]	0.05	0.05
Mineral premix[3]	0.20	0.20
Coccidiostat[4]	0.05	0.05
Selenium premix[5]	0.10	0.10
Vermiculite (inert filler)	0.07	0.07
Calculated nutrient content		
Metabolizable energy (kcal/g)	2.81	2.81
Crude protein (%)	22.45	22.45
Calcium (%)	0.90	0.90
Available phosphorus (%)	0.45	0.45
Lysine (%)	1.24	1.24
Methionine (%)	0.59	0.59
Threonine (%)	0.82	0.82
Methionine + cysteine (%)	0.93	0.93
Sodium (%)	0.21	0.21

[1]Either 0% coarse corn (0%CC) or 50% coarse corn (50%CC) were fed in combination with normal phytate (NP) soybean meal (SBM).
[2]Vitamin premix supplied the following per kg of diet: 6,614 IU vitamin A, 1,984 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 1.98 mg menadione (K_3), 1.98 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 3.97 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.
[3]Mineral premix supplied the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; cobalt, 1 mg.
[4]Coccidiostat supplied monensin sodium at 99 mg/kg of feed.
[5]Selenium premix provided 0.2 mg Se (as Na_2SeO_3) per kg of diet.

Table III-2. Starter diets used from 9 to 21 d of age

Ingredients	Dietary Treatments[1]			
	0 %CC		50% CC	
	NP SBM	LP SBM	NP SBM	LP SBM
	(%)			
Fine corn	58.24	58.24	29.12	29.12
Coarse corn (CC)	---	---	29.12	29.12
Soybean meal	31.95	31.95	31.95	31.95
Poultry fat	0.95	0.95	0.95	0.95
Limestone	0.90	0.90	0.90	0.90
Dicalcium phosphate (18.5%P)	1.75	1.75	1.75	1.75
Salt	0.48	0.48	0.48	0.48
DL-Methionine	0.23	0.23	0.23	0.23
L-Threonine	0.09	0.09	0.09	0.09
Choline chloride (60%)	0.19	0.19	0.19	0.19
Vitamin premix[2]	0.05	0.05	0.05	0.05
Mineral premix[3]	0.20	0.20	0.20	0.20
L-Lysine	0.07	0.07	0.07	0.07
Coccidiostat[4]	0.05	0.05	0.05	0.05
Selenium premix[5]	0.09	0.09	0.09	0.09
Titanium Dioxide	1.00	1.00	1.00	1.00
Vermiculite (inert filler)	3.76	3.76	3.76	3.76
Calculated nutrient content				
Metabolizable energy (kcal/g)	2.73	2.73	2.73	2.73
Crude protein (%)	20.13	20.13	20.13	20.13
Calcium (%)	0.89	0.89	0.89	0.89
Available phosphorus(%)	0.40	0.40	0.40	0.40
Lysine (%)	1.14	1.14	1.14	1.14
Methionine (%)	0.54	0.54	0.54	0.54
Threonine (%)	0.77	0.77	0.77	0.77
Methionine + cysteine (%)	0.86	0.86	0.86	0.86
Sodium (%)	0.20	0.20	0.20	0.20

[1]Either 0% coarse corn (0%CC) or 50% coarse corn (50%CC) were fed in combination with normal phytate (NP) soybean meal (SBM) and low phytate (LP) SBM.
[2]Vitamin premix supplied the following per kg of diet: 6,614 IU vitamin A, 1,984 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 1.98 mg menadione (K_3), 1.98 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 3.97 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.
[3]Mineral premix supplied the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; cobalt, 1 mg.
[4]Coccidiostat supplied monensin sodium at 99 mg/kg of feed.
[5]Selenium premix provided 0.2 mg Se (as Na_2SeO_3) per kg of diet.

Table III-3. Soybean meal (SBM) variety and percentage coarse corn (%CC) effects on broiler body weight (BW), body weight gain (BWG), feed intake (FI), and adjusted feed conversion ratio (AdjFCR)

Variable	Age	Dietary treatments						Source of variability	
		SBM variety[2]		SE[1]	%CC		SE[1]	SBM	%CC
		NP	LP		0	50			
		(g)			(g)			(*P*-value)	
BW	1	-	-	-	45.6	45.4	0.2	-	0.63
	9	-	-	-	208.4	203.0	2.1	-	0.09
	16	542.7[a]	527.8[b]	4.2	539.6	530.9	4.2	0.02	0.16
	21	862.7	861.9	6.1	863.5	861.1	6.1	0.92	0.78
BWG	1-9	-	-	-	161.4[a]	155.3[b]	1.8	-	0.03
	1-16	-	-	-	494.1	485.4	4.1	-	0.16
	1-21	-	-	-	817.9	815.7	6.0	-	0.80
	10-16	334.6[a]	324.5[b]	2.4	331.3	327.8	2.4	0.01	0.33
	17-21	320.0[b]	334.1[a]	3.3	323.9	330.3	3.3	0.01	0.17
	10-21	645.6	658.6	4.9	655.1	658.1	4.9	0.56	0.66
FI	1-9	-	-	-	179.1	175.3	1.9	0.40	0.19
	10-16	464.6	456.2	3.5	458.8	462.0	3.6	0.10	0.53
	17-21	477.3	483.5	5.7	473.8	486.9	5.5	0.45	0.11
		(g:g)			(g:g)				
AdjFCR	1-9	-	-	-	1.10[B]	1.13[A]	0.01	0.51	0.01
	10-16	1.39	1.41	0.01	1.39[b]	1.41[a]	0.01	0.23	0.04
	10-21	1.44	1.43	0.01	1.42	1.44	0.01	0.37	0.19
	17-21	1.52	1.45	0.04	1.47	1.50	0.04	0.23	0.65
	1-16	-	-	-	1.29[b]	1.31[a]	0.01	-	0.03
	1-21	-	-	-	1.36	1.38	0.01	-	0.07

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Standard error (SE) for n=16 cages of 12 birds initially and 8 birds/cage after selection at 9 d.
[2]Soybean meal treatments started at day 10.

Table III-4. Soybean meal (SBM) variety and percentage coarse corn (%CC) effects on absolute and relative gizzard and proventriculus weights at 21 d of age

Variable	SBM variety NP	SBM variety LP	SE[1]	%CC 0	%CC 50	SE[1]	Source of variability SBM	Source of variability %CC
	(g)			(g)			(P-value)	
Gizzard weight	14.54	14.59	0.2	13.19[B]	15.95[A]	0.2	0.81	0.0001
Proventriculus weight	5.42[a]	5.08[b]	0.1	6.00[A]	4.51[B]	0.1	0.05	0.0001
	(g/100g BW)			(g/100g BW)				
Gizzard	1.55	1.55	0.02	1.40[B]	1.70[A]	0.02	0.90	0.0001
Proventriculus	0.58	0.54	0.01	0.64[A]	0.48[B]	0.01	0.08	0.0001

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Standard error (SE) for n=16 cages of 8 birds each.

Table III-5. Effect of soybean meal (SBM) variety and percentage coarse corn (%CC) on total phosphorus (P) and phytate phosphorus (Phytate P)

Variable	Dietary treatments						Source of variability	
	SBM variety		**SE[1]**	**%CC**		**SE[1]**	**SBM**	**%CC**
Digestibility	**NP**	**LP**		**0**	**50**			
	(% DM[2])			(% DM[2])			(*P*-value)	
Total P	65.73	68.95	2.7	64.81	69.87	2.7	0.40	0.20
Phytate P	76.09^{A}	72.54^{B}	0.8	72.67^{B}	75.96^{A}	0.8	0.01	0.01

^{A,B}Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Standard error (SE) for n=16 cages of 8 birds each.
[2]Percent DM of feed and ileal samples were used to calculate nutrient digestibility on dry matter basis.

Table III-6. Effect of soybean meal (SBM) variety by percentage coarse corn (%CC) interaction on broiler body weight (BW), body weight gain (BWG), feed intake (FI), and adjusted feed conversion ratio (AdjFCR)

Variable	**Age**	**Dietary treatments**					**Source of variability**
		NP SBM		**LP SBM**		**SE[2]**	**SBM×%CC**
		0	**50**	**0**	**50**		
		(g)					(P-value)
BW	16	537.6	547.8	531.4	524.2	6.0	0.81
	21	868.2	857.3	858.9	846.9	8.6	0.34
BWG	10-16	337.1	323.0	325.4	323.6	3.4	0.63
	17-21	320.3	319.8	327.4	340.8	4.5	0.14
	10-21	657.5	651.8	652.8	664.4	6.8	0.21
FI	10-16	463.1	466.1	454.6	457.9	5.0	0.97
	17-21	470.7	483.9	476.9	490.0	8.0	0.99
		(g:g)					
AdjFCR	10-16	1.38	1.41	1.41	1.42	0.01	0.56
	10-21	1.42	1.46	1.43	1.43	0.01	0.19
	17-21	1.49	1.55	1.46	1.44	0.06	0.47

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[2]Standard error (SE) for n=8 cages of 12 birds initially and 8 birds/cage after selection at 9 d.

Table III-7. Effect of soybean meal (SBM) variety by percentage coarse corn (%CC) interaction on absolute and relative gizzard and proventriculus weights

Variable	Dietary treatments					Source of variability
	NP SBM		LP SBM		SE[2]	SBM×%CC
	0	50	0	50		
	(g)					(*P*-value)
Gizzard weight	13.23	15.85	13.15	16.04	0.2	0.56
Proventriculus weight	6.34[a]	4.49[c]	5.64[b]	4.54[c]	0.2	0.04
	(g/100g BW)					
Gizzard	1.39	1.70	1.40	1.70	0.02	0.76
Proventriculus	0.67	0.48	0.61	0.48	0.02	0.13

[a-c]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[2]Standard error (SE) for n=8 cages of 8 birds each.

Table III-8. Effect of soybean meal (SBM) variety by percentage coarse corn (%CC) interaction on total phosphorus (P) and phytate phosphorus (Phytate P)

Variable	Dietary treatments					Source of variability
	NP SBM		LP SBM		SE[2]	SBM×%CC
Digestibility	**0**	**50**	**0**	**50**		
	(% DM[3])					(*P*-value)
Total P	63.49	67.97	66.14	71.77	0.7	0.88
Phytate P	74.61	77.58	70.74	74.34	3.8	0.79

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[2]Standard error (SE) for n=8 cages of 12 birds initially and 8 birds/cage after selection at 9 d.
[3]Percent DM is feed and ileal samples were used to calculate nutrient digestibility on dry matter basis.

CHAPTER IV

Effects of phytase enzyme in combination with 50% coarse corn in broiler diets containing low phytate versus normal phytate soybean meal on male broiler live performance, development of the gizzard and proventriculus, and total phosphorus digestibility

4.1. ABSTRACT

This study investigated the effect of phytase enzyme addition in combination with either 0 or 50% coarse corn (CC) to crumbled diets containing low phytate (LP) versus normal phytate (NP) soybean meal (SBM) on performance of broiler raised to 21 d of age. From 1 to 8 d, all birds received four diets (0%CC or 50%CC), either with (300 FTU/kg) or without (0 FTU/kg) phytase with a single commercial NP-type SBM. From 9 to 21 d, birds were fed either NP-0%CC, NP-50%CC, or LP-0%CC, LP-50%CC, with and without phytase, in a 2 × 2 × 2 arranged. Diets were adjusted for phytase contribution to available calcium and phosphorus (P) but no adjustments were made for differences in total P among the SBM sources. There were 8 pens of 8 birds each for each interaction cell from 9 to 21 d. Broiler BW gain, feed intake, adjusted feed conversion ratio (AdjFCR), and weights of gizzard and proventriculus were measured. Ileal contents were collected for total P digestibility calculation. Results of necropsy at 21 d showed that gizzard weight was increased ($P \leq 0.05$) when birds consumed either 50%CC (13.71 versus 16.82 g) or LP SBM (15.01 versus 15.51 g). On the other hand, proventriculus weight was reduced ($P \leq 0.05$) by feeding either 50%CC (4.98 versus 5.99 g) or 300 FTU/kg (5.32 versus 5.64 g), but was not

affected by SBM variety. Total P digestibility was improved ($P \leq 0.001$) when feeding either 0%CC (44.81 versus 47.89%), LP SBM (39.72 versus 52.98%), or 300 FTU/kg phytase (42.46 versus 50.06%). Digestibility of total P was better in LP than NP SBM, with further improvement in digestibility as enzyme was added to NP SBM and further increase as enzyme was added to LP SBM.

Adding phytase improved total P digestibility compared to 0 FTU/kg where digestibility was greater ($P \leq 0.001$) in the presence of 0%CC (42.55 versus 53.24%) versus 50%CC (42.74 versus 46.88%). These data suggested that the digestive environment created by both SBM phytate level and CC affected phytase function and these effects may involve changes in gizzard and proventriculus weight and function.

Key words: Broilers, low phytate, soybean meal, corn particle size, phytase

4.2. INTRODUCTION

Nutrients, mainly phosphorus (P) in plant feed ingredients has been reported to be a component of phytate (Harland and Morris, 1995; Harland and Oberleas, 1999) that has the ability to chelate di- and trivalent cations and interact with proteins, amino acids, and carbohydrates such that their bioavailability for poultry was reduced (Angel et al., 2002). Such nutrients were not available to poultry since they lacked the endogenous phytase required for phytate hydrolysis and the release of nutrients (Cosgrove and Irving, 1980; Bedford, 2000). Exogenous feed enzymes such as phytase have become widely used in poultry nutrition to release significant amounts of bound P and improve P bioavailability in diets (Kornegay, 1996; Kies, 1999) with variation in phytase efficacy depending on its source (i.e. yeasts, fungi, and bacteria). It has been shown that phytase was effective when calcium (Ca) and non-phytate P (nPP) concentrations in the diets were reduced (Yan et al., 2001) or when added to diets adequate in Ca and nPP (Watson et al., 2005).

Soybean meal (SBM) has been used as a major protein source in corn-SBM based broiler diets for many years. Approximately 50-60% of the total P in SBM was found in the form of phytate P (Nelson et al., 1968; Leske and Coon, 1999) which was not available. Therefore, poultry diets have been supplemented with inorganic sources of P to meet nutritional requirements. However, excess P supplementation has led to excess P excretion, which has caused eutrophication of fresh water (Honeyman, 1993). Genetically selected LP SBM has been developed as an alternative to conventional to SBM to reduce the environmental impact of P (Raboy, 2002; Sands et al., 2003). In addition to this alternative, improving nutrient digestion can also minimize nutrient excretion into the environment.

Larger particles of feed have stimulated reverse peristalsis in the gastrointestinal tract (Nir et al., 1995) to slow feed passage, which increased the exposure time of nutrients to digestive enzymes and improved nutrient digestibility. Therefore, the objectives of this experiment were to determine the effects of phytase, genetically selected LP SBM, and corn particle size in a corn-SBM based diet on live performance, gizzard and proventriculus weights, and total P digestibility of broiler raised to 21 d of age.

4.3. MATERIALS AND METHODS

Dietary Treatments

Practical diets with 2 soybean meal (SBM) varieties, 2 different particle sizes of corn, and 2 levels of phytase were formulated. A total of 8 corn-SBM based diets treatments were tested with 2 SBM varieties (low phytate (LP) and normal phytate (NP)), and 2 coarse corn (CC) inclusion levels (0% and 50% CC) in the presence or absence of 300 FTU/kg *Escherichia coli*-derived research-grade phytase enzyme (Danisco Animal Nutrition, Malborough, UK), phytase recovery in feed was confirmed (Danisco Animal Nutrition, Denmark). One FTU has been generally defined as the amount of enzyme required to liberate 1 μmol of inorganic P min^{-1} from 5.5 m*M* of Na phytate at pH 5.5 and 37º C (Qian et al., 1997). Table 1 illustrates the chemical composition of the SBM varieties. Fine particles were obtained by grinding corn in a hammermill (Model 1522, Roskamp Champion, Waterloo, IA) equipped with two 2.4 mm screens to obtain an average particle size of 314 μm, while coarse particles with an average size of 1190 μm were prepared with a two-pair roller mill with a gap setting of 0% opening (0.18 mm) on the top pair of rollers and 100% opening (7.16 mm) on the bottom pair of rollers (Model C128829, RMS, Tea, SD). Particle size distribution was determined by ASAE S319.3 (ASAE, 2003). Tables 2 and 3 detail the experimental diets. From 1-8 d, all birds were fed pre-starter diets with commercial NP SBM with either 0 or 50% inclusion of CC with or without phytase (Table 1). All diets were formulated to be iso-caloric and iso-nitrogenous and calculated to contain 2.85 kcal/g metabolizable energy (ME), and 22.0% crude protein (CP). From 9-21 d, birds were fed a starter diet with either NP SBM or LP SBM and a CC inclusion of either 0 or 50% with or without phytase, while maintaining the same dietary specification (Table 2). Titanium dioxide (TiO2) was included in starter diets as an indigestible marker to allow for the calculation of apparent nutrient digestibility. Feed and water were provided for ad libitum intake. Pre-starter and starter diets were both fed as crumbles. All diets were mixed in a horizontal twin shaft ribbon mixer (TRDB126-0604, Hayes and Stolz Ind. Mfg. Co., Fort Worth, TX) to produce the mash diets. Mash feed was then (conditioned at 85°C for 45 seconds) and pelleted with a ring die (4.4 mm by 35 mm) pellet mill (Model PM1112-2, California Pellet Mill Co., Crawfordsville, IN) and pellets were cooled with ambient air in a counter-flow cooler (Model VK09 × 09KL, Geelen Counterflow USA Inc., Orlando, FL) and crumbled. Diets that contained phytase were formulated to contain 0.15% less Ca and available phosphorus (AvP) as suggested by the enzyme manufacturer.

Birds Management and Data Collection

The care of the birds in these studies conformed to the Guide for Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010). Broiler chicks were hatched from eggs collected from a 45-wk-old Ross 344 × 708 (Aviagen, Huntsville, AL) breeder flock. Eggs were incubated under standard conditions. Chicks were feather-sexed at hatching, and a total of 1,152 male chicks were permanently identified with neck tags and randomly allocated to each of 64 heated battery brooders located within two environmentally controlled brooding rooms. To reduce vertical temperature and lighting differences among the cages in the battery brooders, only the middle 4 tiers of cages in each 6-tier battery brooder were utilized. Twelve extra birds were placed in each of the upper and lower cages and utilized for necropsy of gizzard and proventriculus at 8 and 15 d. The temperature within cages was initially set at 33°C and reduced gradually to 29°C. Feeders were weighed at the start and end of each feeding period. Feeders were covered with screen wires that had openings of 2.54 × 2.54 cm to reduce feed wastage. Screens were moved three times daily to keep the feed available.

Body weight (BW) and feed intake by cage were measured at 1, 8, 15, and 21 d, and BW gain was calculated by time interval. Mortality was weighed and recorded twice daily for adjusted feed conversion ratio (AdjFCR) calculations. Three birds per cage were randomly chosen from upper and lower cages and necropsied for gizzard and proventriculus at 8 and 15 d of age. At 21 d of age, 32 birds per treatment from the center tiers of cages were killed by cervical dislocation, necropsied for weights of gizzard and proventriculus, and the terminal 13 cm of ileum was removed 3 cm anterior to the ileo-cecal junction. Ileal contents were gently expressed, pooled per cage, and frozen for nutrient digestibility determination.

Chemical Analyses

The TiO_2 in diets and ileal samples was determined using the method of Myers et al. (2004). Frozen samples of feed were dried in an oven for 24 h at 95 to 100°C to determine dry matter (Method 934.01, AOAC, 2006). The percentage dry matter of ileal digesta was determined by freeze drying (Virtis Freezemobile – Model 12XL, Warminster, PA). Samples of feed and dried ileal digesta were ground (< 2 mm) for analysis. Diets and ileal samples were analyzed for total P.

Statistical Methods

A randomized complete block design with a 2 × 2 × 2 factorial arrangement of treatments was used (SBM × CC × Phytase). The general linear model of SAS (2009; SAS version 9.2, SAS Institute, Cary, NC, USA) was used to analyze each measured variable. Treatment means were partitioned by LSMEANS and were considered statistically different when $P \leq 0.05$, although effects with *P*-values between 0.06 and 0.10 are mentioned in the text when the data suggested a numerical trend. Group averages for all birds in a single cage were used for live performance data analysis, whereas averages of 3 randomly selected birds per cage were used for the other measurements. The experimental unit for statistical analysis of measured parameters was the cage.

4.4. RESULTS AND DISCUSSION

Effect of Dietary Treatments on Live Performance

Table 4 shows the effect of SBM variety, %CC, and phytase on BW, BW gain, feed intake, and AdjFCR at 8, 15, and 21 d of age. Diets were formulated with a commercial NP SBM for the first 8 d. Therefore, there was no effect of SBM variety on measured variables before 9 d. The LP and NP SBM was included in the diets starting at 9 d but BW, feed intake, BW gain, and AdjFCR were not affected by SBM variety at 15 and 21 d of age. Baker et al. (2011) reported that feeding different varieties of SBM (conventional, high protein, and low oligosaccharide) had no effect on broiler live performance as long as diets were formulated with the same true metabolizable energy. In this study feed intake was not negatively affected by increasing the inclusion rate of limestone from a minimum of 0.92 to 1.34% from 9-21 d of age. Fraga (1994) reported that feeds with more than 4% Ca to layers may reduce feed palatability and interfere with feed intake. Similar effect may be evident at lower inclusion in broilers.

Diets had either 0% coarse corn (0%CC) or 50% coarse corn (50%CC) for the entire study (Table 4) but had no effect on either BW or feed intake (Table 4). However, BW gain was greater ($P \leq 0.05$) in birds consuming 0%CC from 9-15 d. Conversely, from 16-21 d, BW gain was greater ($P \leq 0.05$) for the birds consuming 50%CC. From 9-15 d birds had similar feed intake, but BW gain was greater in birds consuming 0%CC possibly due to less energy expenditure for mechanical grinding of larger particles that preserved more available energy for growth. However, from 16-21 d, feed intake tended ($P = 0.09$) to increase, which indicated an adaptation to CC in birds consuming 50%CC that resulted in increased BW gain. The AdjFCR was improved ($P \leq 0.05$) from 1-8 d in birds that consumed 0%CC, however, this improvement disappeared thereafter. Results to 16 d of age were similar to those of Douglas et al. (1990), who reported that feeding of coarse particle mash diets of either sorghum or corn that were ground in a roller mill depressed BW gain and feed efficiency in broilers compared with those fed diets with finely hammermill ground grains. However, according to Amerah et al. (2007) feeding wheat-based mash diets with coarser grinding resulted in greater BW gain, feed intake, and improved FCR compared to medium particle size wheat.

Adding phytase to the diets from 1 d of age did not affect BW of the birds (Table 4). However, BW gain from 9-15 d was greater ($P \leq 0.05$) in birds consuming diets containing 300 FTU/kg phytase. For the period from 16-21 d, feed intake was also increased ($P \leq 0.01$) by phytase inclusion. Adding phytase tended ($P = 0.09$) to improve AdjFCR from 9-15 d, but the opposite was evident from 16-21 d. These results differed from those of Yan et al. (2001) who reported that using 800 FTU/kg of phytase improved BW gain and FCR of broilers raised to 3 wk of age, probably because of the presence of LP SBM and CC.

Table 5 illustrates the SBM variety × %CC interaction for broiler BW, BW gain, feed intake, and AdjFCR. There were no interactions with respect to live performance.

Results of the SBM variety × phytase interaction effect on broiler BW, BW gain, feed intake, and AdjFCR are presented in Table 6. The two SBM varieties were added to the diets after 9 d. Feed intake for the 16-21 d period was greater ($P \leq 0.05$) in birds consuming NP diets with phytase, while other dietary combinations were comparable. Table 7 shows the phytase × %CC interaction for broiler BW, BW gain, feed intake, and AdjFCR. An interaction was only observed for BW gain from 9-15 d, where BW gain was decreased ($P \leq 0.05$) in birds consuming diets formulated with 50%CC without phytase. This response could be related to an assumed longer gut retention time and slower digesta passage rate in the presence of 50%CC, which phytase modified by enhancing digestion.

Table 8 shows the 3-way interaction effect of SBM variety by %CC and phytase on broiler live performance. The AdjFCR for the period from 9-15 d was the only variable affected ($P \leq 0.05$) by the 3-way interaction. Adding phytase to LP SBM in the presence of 50%CC improved AdjFCR but this improvement was not observed when formulating with NP SBM. In the absence of phytase at 0%CC, LP SBM produced better AdjFCR than NP SBM. In the absence of phytase, using 0%CC with LP SBM improved AdjFCR compared to 50%CC.

Effect of Dietary Treatments on Organ Weights

Table 9 shows the effect of SBM variety, %CC, and phytase on absolute and relative gizzard and proventriculus weights at 15 and 21 d of age. Absolute gizzard and proventriculus weights and relative gizzard and proventriculus weights were comparable at 15 d of age, while absolute and relative gizzard weights were greater ($P \leq 0.05$) at 21 d in birds consuming diets containing LP SBM. At 8, 15, and 21 d, absolute and relative gizzard weights were greater ($P \leq 0.001$) in birds that consumed 50%CC (Table 9), which was probably due to increased demand for mechanical digestion and mixing with digestive secretions required to deal with coarse material. These results were comparable to those of

Peron et al. (2005) who reported that coarse particles that remained after pelleting significantly increased gizzard weights compared to fine particles. On the other hand, absolute proventriculus weight was reduced in birds that consumed 50%CC at 8 ($P \leq 0.01$), 15 ($P \leq 0.05$), and 21 ($P \leq 0.001$) d of age. This suggested reduced secretion of HCl and pepsin in the presence of large particles of corn, probably due to increased digesta retention time that provided more time for acid and enzyme activity.

The phytase effect on absolute and relative gizzard and proventriculus weights measured at 8, 15, and 21 d of age are presented in Table 9. Adding phytase resulted in a smaller absolute proventriculus ($P \leq 0.05$) at 21 d of age, which suggested less secretory activity by the proventriculus in the presence of phytase while the gizzard was not affected by phytase.

Table 10 illustrates the SBM variety × %CC interaction effects for absolute and relative gizzard and proventriculus weights at 15 and 21 d of age. At 15 d of age, LP SBM reslted in a larger ($P \leq 0.05$) absolute and relative proventriculus weight in the presence of 0%CC, while in the presence of NP SBM, CC had no effect on absolute and relative proventriculus weight. At 21 d of age absolute ($P \leq 0.001$) and relative ($P \leq 0.01$) gizzard weights were larger when 50%CC was used in combination with either NP or LP SBM. However, using 0%CC with NP produced smaller absolute ($P \leq 0.001$) and relative gizzard ($P \leq 0.01$) weights compared to 0%CC with LP SBM.

The effect of SBM variety × phytase interaction is shown in Table 11. In the presence of phytase, LP diets produced a greater ($P \leq 0.05$) absolute gizzard weight at 15 d of age compared to LP SBM without phytase, while diets formulated with NP SBM were intermediate. At 21 d of age, adding phytase to LP SBM produced a larger ($P \leq 0.05$) relative gizzard weight compared to NP SBM without phyatse with others being.

Table 12 shows the phytase × %CC interaction effects for absolute and relative gizzard and proventriculus weights at 8, 15, and 21 d of age. At 8 d of age, in the absence of phytase, using 50%CC produced a larger ($P \leq 0.01$) relative gizzard weight compared to 0%CC. However, when phytase was added, the effect of CC on relative gizzard weight disappeared. A similar effect was observed ($P \leq 0.05$) at 21 d for relative gizzard weight.

Table 13 shows the 3-way interaction of %CC × SBM × phytase effect on absolute and relative gizzard and proventriculus weights. In general, birds consuming 50%CC had larger gizzard weights ($P \leq 0.01$) compared to birds consuming 0%CC. In the presence of 0%CC, 300 FTU/kg increased gizzard weight in the presence of LP SBM, while with 50%CC present, NP SBM increased gizzard weight relative to LP SBM at 300 FTU/kg with LP versus NP SBM having no effect at 0 FTU/kg.

Effect of Dietary Treatments on Total Phosphorus Digestibility

The effect of SBM variety, CC, and phytase addition on total P digestibility on a DM basis are shown in Table 14. Diets formulated with LP SBM improved total P digestibility as compared to NP diets ($P \leq 0.001$) probably due to higher available P in LP SBM. These results were comparable with findings reported for numerically higher P digestibility of the LP barley compared with NP barley variants in broilers (Leytem et al., 2007) and turkey poults (Li et al., 2001). Diets that contained 0%CC improved total P digestibility ($P \leq 0.001$) compared to 50%CC probably due to increased surface area of digesta available for endogenous and exogenous enzymes action. These results were similar to Carre et al. (2002)

who found a negative relationship between the hardness of wheat and the digestibility of starch contained therein in pelleted diets. Adding 300 FTU/kg phytase improved total P digestibility ($P \leq 0.001$), as expected.

There was no interaction of SBM variety and CC (Table 15) on total P digestibility. On the other hand, the interaction between SBM variety and phytase (Table 16) affected ($P \leq 0.001$) total P digestibility. These data show digestibility of LP to be better than NP SBM and a stepwise increase in digestibility of total P as enzyme was added to NP SBM and further increase as enzyme was added to LP SBM. Adding phytase improved ($P \leq 0.001$) total P digestibility (Table 17) compared to 0 FTU/kg, but the effect was reduced by 50%CC probably due to the phytate content in the large yellow pieces (O'Dell and Boland, 1976). There was no 3-way interaction effect of SBM variety, CC, and phytase on total P digestibility (Table 18).

4.5. CONCLUSION

Inclusion of 50%CC resulted in larger of gizzard weights and smaller proventriculus weights. Furthermore, broilers fed LP SBM diets exhibited a larger gizzard and improved digestibility of total P probably due to more available P in LP SBM and decreased digesta passage rate. Adding phytase at 300 FTU/kg also improved total P digestibility as expected. Therefore, under these experimental conditions, the inclusion of 50%CC in broiler diets could provide a potential advancement to broiler nutrition through its influence on gizzard and proventriculus thus altering the entire GI tract function, digesta passage rate, and retention time. Further improvement could be achieved by using feed ingredients with more available nutrients such as genetically selected LP SBM, which had more available P. Adding phytase further liberated available nutrients in feed ingredients.

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Table IV-1. Chemical analysis[1] of SBM varieties

Composition	Commercial NP SBM	NP SBM	LP SBM
	(%)		
Moisture	11.43	11.43	9.19
Protein	48.73	49.55	51.38
Fiber	2.60	6.40	6.40
Ash	6.12	5.78	5.78
Total Phosphorus	0.66	0.68	0.74
Calcium	0.29	0.19	0.13

[1]Results obtained from Carolina Analytical Services laboratories.

Table IV-2. Pre-starter diets used to 8 d of age

	Dietary Treatments[1]			
	0 %CC		50% CC	
	Commercial NP SBM		Commercial NP SBM	
Ingredients	**0 FTU/kg**	**300 FTU/kg**	**0 FTU/kg**	**300 FTU/kg**
	(%)			
Fine corn	57.64	59.16	28.82	29.58
Coarse corn (CC)	---	---	28.82	29.58
Soybean meal (48% CP)	36.28	36.04	36.28	36.04
Poultry fat	1.74	1.20	1.74	1.20
Limestone	0.92	1.07	0.92	1.07
Dicalcium phosphate (18.5% P)	2.00	1.06	2.00	1.06
Salt	0.50	0.50	0.50	0.50
DL-Methionine	0.25	0.24	0.25	0.24
L-Lysine	0.07	0.07	0.07	0.07
L-Threonine	0.10	0.10	0.10	0.10
Choline chloride (60%)	0.10	0.10	0.10	0.10
Vitamin premix[2]	0.05	0.05	0.05	0.05
Mineral premix[3]	0.20	0.20	0.20	0.20
Coccidiostat[4]	0.05	0.05	0.05	0.05
Selenium premix[5]	0.10	0.10	0.10	0.10
Phytase[6]	---	0.03	---	0.03
Vermiculite (inert filler)	0.00	0.03	0.00	0.03
Calculated nutrient content				
Metabolizable energy (kcal/g)	2.85	2.85	2.85	2.85
Crude protein (%)	22.00	22.00	22.00	22.00
Calcium (%)	0.90	0.75	0.90	0.75
Available phosphorus (%)	0.45	0.30	0.45	0.30
Lysine (%)	1.26	1.26	1.26	1.26
Methionine (%)	0.58	0.58	0.58	0.58
Threonine (%)	0.84	0.84	0.84	0.84
Methionine + cysteine (%)	0.92	0.92	0.92	0.92
Sodium (%)	0.21	0.21	0.21	0.21

[1]Either 0% coarse corn (0%CC) or 50% coarse corn (50%CC) were fed in combination with normal phytate (NP) soybean meal (SBM) with either no (0 FTU/kg) or 300 FTU/kg phytase enzyme.
[2]Vitamin premix supplied the following per kg of diet: 6,614 IU vitamin A, 1,984 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 1.98 mg menadione (K_3), 1.98 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 3.97 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.
[3]Mineral premix supplied the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; cobalt, 1 mg.
[4]Coccidiostat supplied monensin sodium at 99 mg/kg of feed.
[5]Selenium premix provided 0.2 mg Se (as Na_2SeO_3) per kg of diet.
[6]Phytase enzyme added at 300 FTU/kg.

Table IV-3. Starter diets used from 9 to 21 d of age

	Dietary Treatments[1]							
	0 %CC				50% CC			
	NP SBM		LP SBM		NP SBM		LP SBM	
Ingredients	**0 FTU/kg**	**300 FTU/kg**	**0 FTU/kg**	**300 FTU/kg**	**0 FTU/kg**	**300 FTU/kg**	**0 FTU/kg**	**300 FTU/kg**
	(%)							
Fine corn	56.24	57.76	56.24	57.76	28.12	28.88	28.12	28.88
Coarse corn (CC)	---	---	---	---	28.12	28.88	28.12	28.88
Soybean meal	34.91	34.69	34.91	34.69	34.91	34.69	34.91	34.69
Poultry fat	3.68	3.15	3.68	3.15	3.68	3.15	3.68	3.15
Limestone	1.34	1.48	1.34	1.48	1.34	1.48	1.34	1.48
Dicalcium phosphate (18.5% P)	1.45	0.51	1.45	0.51	1.45	0.51	1.45	0.51
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
DL-Methionine	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
L-Threonine	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Choline chloride (60%)	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Mineral premix[3]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
L-Lysine	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Coccidiostat[4]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Selenium premix[5]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Phytase[6]	---	0.03	---	0.03	---	0.03	---	0.03
Titanium Dioxide	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vermiculite (inert filler)	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Calculated nutrient content								
Metabolizable energy (kcal/g)	2.85	2.85	2.85	2.85	2.85	2.85	2.85	2.85
Crude protein (%)	22.00	22.00	22.00	22.00	22.00	22.00	22.00	22.00
Calcium (%)	0.90	0.75	0.90	0.75	0.90	0.75	0.90	0.75
Available phosphorus(%)	0.45	0.30	0.45	0.30	0.45	0.30	0.45	0.30
Lysine (%)	1.26	1.26	1.26	1.26	1.26	1.26	1.26	1.26
Methionine (%)	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54
Threonine (%)	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84
Methionine + cysteine (%)	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92
Sodium (%)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20

[1]Either 0% coarse corn (0%CC) or 50% coarse corn (50%CC) were fed in combination with normal phytate (NP) soybean meal (SBM) and low phytate (LP) SBM with either no (0 FTU/kg) or 300 FTU/kg phytase enzyme.

[2]Vitamin premix supplied the following per kg of diet: 6,614 IU vitamin A, 1,984 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 1.98 mg menadione (K_3), 1.98 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 3.97 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.

[3]Mineral premix supplied the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; cobalt, 1 mg.

[4]Coccidiostat supplied monensin sodium at 99 mg/kg of feed.

[5]Selenium premix provided 0.2 mg Se (as Na_2SeO_3) per kg of diet.

[6]Phytase enzyme added at 300 FTU/kg.

Table IV-4. Effect of soybean meal (SBM) variety, percentage coarse corn (%CC), and phytase on broiler body weight (BW), body weight gain (BWG), feed intake (FI), and adjusted feed conversion ratio (AdjFCR)

Variable	Age		Dietary Treatments[1]									Source of Variability		
			SBM variety[3]		SE[2]	%CC		SE[2]	Phytase (FTU/kg)		SE[2]	SBM	%CC	Phytase
		n	NP	LP		0	50		0	300				
	(d)		(g)									(P-value)		
BW	1	32	-	-	-	45.0	45.4	0.2	45.4	45.0	0.2	-	0.09	0.08
	8	32	-	-	-	228.4	227.8	1.6	229.1	227.0	1.6	-	0.99	0.35
	15	32	589.0	590.0	2.5	591.8	587.2	2.5	588.1	590.9	2.5	0.77	0.20	0.44
	21	32	997.1	1004.2	6.6	996.4	1004.8	6.6	997.9	1003.4	6.6	0.44	0.37	0.56
BWG	1-8	32	-	-	-	183.4	182.3	1.6	183.7	182.0	1.6	-	0.65	0.48
	9-15	32	355.3	359.3	1.7	360.0[a]	354.5[b]	1.7	354.4[b]	360.2[a]	1.7	0.11	0.03	0.02
	16-21	32	405.5	414.2	4.4	402.0[b]	417.6[a]	4.4	409.8	409.9	4.4	0.17	0.02	0.99
	9-21	32	763.4	774.5	6.1	764.7	773.2	6.1	765.2	772.7	6.1	0.20	0.33	0.39
FI	1-8	32	-	-	-	197.3	199.6	1.5	199.7	197.	1.5	-	0.26	0.22
	9-15	32	459.0	459.9	2.3	459.1	459.8	2.3	459.2	459.7	2.3	0.76	0.84	0.88
	16-21	32	522.7	518.6	4.2	515.6	525.7	4.2	512.5[B]	528.7[A]	4.2	0.49	0.09	0.01
	9-21	32	981.6	978.5	5.9	974.7	985.4	5.9	971.7	988.4	5.9	0.71	0.21	0.06
			(g:g)											
AdjFCR	1-8	32	-	-	-	1.08[b]	1.10[a]	0.01	1.09	1.08	0.01	-	0.02	0.59
	9-15	32	1.29	1.28	0.01	1.28	1.30	0.01	1.30	1.28	0.01	0.39	0.08	0.09
	16-21	32	1.30	1.25	0.01	1.29	1.26	0.02	1.25	1.30	0.02	0.09	0.21	0.07
	9-21	32	1.29	1.27	0.01	1.28	1.28	0.01	1.27	1.29	0.01	0.09	0.70	0.35

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Either 0% coarse corn (0%CC) or 50% coarse corn (50%CC) were fed in combination with normal phytate (NP) soybean meal (SBM) and low phytate (LP) SBM with either no (0 FTU/kg) or 300 FTU/kg phytase enzyme.
[2]Standard error (SE) for number of cages (n) shown containing 12 birds/cage prior to 8 d of age, and 8 birds/cage after 8 d.
[3]Soybean meal treatments started at day 9.

Table IV-5. Effect of soybean meal (SBM) variety by percentage coarse corn (%CC) interaction on broiler body weight (BW), body weight gain (BWG), feed intake (FI), and adjusted feed conversion ratio (AdjFCR)

Variable	Age	n	Dietary Treatments[1] NP SBM 0%CC	NP SBM 50%CC	LP SBM 0%CC	LP SBM 50%CC	SE[2]	Source of Variability SBM×%CC
	(d)		(g)					(*P*-value)
BW	15	16	589.6	588.4	593.9	586.1	3.5	0.36
	21	16	990.6	1003.5	1002.2	1006.2	9.3	0.63
BWG	9-15	16	357.1	353.6	363.0	355.5	2.4	0.41
	16-21	16	395.8	415.2	408.3	420.1	6.3	0.55
	9-21	16	758.1	788.9	771.3	777.6	8.6	0.79
FI	9-15	16	458.8	459.1	459.4	460.4	3.2	0.91
	16-21	16	518.9	526.4	512.2	525.0	6.0	0.66
	9-21	16	977.7	985.5	971.6	985.4	8.4	0.72
			(g:g)					
AdjFCR	9-15	16	1.29	1.30	1.27	1.30	0.01	0.51
	16-21	16	1.33	1.27	1.26	1.25	0.03	0.34
	9-21	16	1.30	1.28	1.26	1.27	0.02	0.34

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[1]Either 0% coarse corn (0%CC) or 50% coarse corn (50%CC) were fed in combination with normal phytate (NP) soybean meal (SBM) and low phytate (LP) SBM.
[2]Standard error (SE) for number of cages (n) shown containing 12 birds/cage prior to 8 d of age, and 8 birds/cage after 8 d.

Table IV-6. Effect of soybean meal (SBM) variety by phytase interaction on broiler body weight (BW), body weight gain (BWG), feed intake (FI), and adjusted feed conversion ratio (AdjFCR)

Variable	Age	n	Dietary Treatments[1] NP SBM 0 FTU/kg	NP SBM 300 FTU/kg	LP SBM 0 FTU/kg	LP SBM 300 FTU/kg	SE[2]	Source of Variability SBM×Phytase
	(d)		(g)					(P-value)
BW	15	16	586.6	591.3	589.6	590.4	3.5	0.59
	21	16	992.2	1001.9	1003.7	1004.7	9.3	0.64
BWG	9-15	16	352.1	358.6	356.7	361.9	2.4	0.79
	16-21	16	405.5	405.4	414.1	414.3	6.3	0.98
	9-21	16	757.6	769.2	772.8	776.2	8.3	0.63
FI	9-15	16	458.1	459.8	460.2	459.6	3.2	0.72
	16-21	16	507.8[b]	537.5[a]	517.2[b]	520.1[b]	6.0	0.03
	9-21	16	965.9	997.3	977.4	979.6	8.4	0.09
			(g:g)					
AdjFCR	9-15	16	1.30	1.28	1.29	1.27	0.01	0.91
	16-21	16	1.25	1.34	1.25	1.26	0.03	0.11
	9-21	16	1.27	1.31	1.27	1.26	0.02	0.19

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).

[1]Either normal phtate (NP) soybean meal (SBM) and low phytate (LP) SBM were fed in combination of either no (0 FTU/kg) or 300 FTU/kg phytase enzyme.

[2]Standard error (SE) for number of cages (n) shown containing 12 birds/cage prior to 8 d of age, and 8 birds/cage after 8 d.

Table IV-7. Effect of phytase by percentage coarse corn (%CC) interaction on broiler body weight (BW), body weight gain (BWG), feed intake (FI), and adjusted feed conversion ratio (AdjFCR)

Variable	Age	n	Dietary Treatments[1]				SE[2]	Source of Variability
			0 FTU/kg		300 FTU/kg			Phytase×%CC
			0%CC	50%CC	0%CC	50%CC		
	(d)		(g)					(P-value)
BW	1	16	45.1	45.7	44.9	45.1	0.2	0.40
	8	16	230.2	228.1	226.6	227.5	2.3	0.99
	15	16	592.5	583.7	591.0	590.8	3.5	0.22
	21	16	995.4	999.2	996.5	1010.2	9.3	0.57
BWG	1-8	16	185.1	182.3	182.7	182.4	2.3	0.46
	9-15	16	359.8[a]	349.0[b]	360.3[a]	360.2[a]	2.4	0.03
	16-21	16	403.8	415.8	400.3	419.5	6.3	0.57
	9-21	16	763.6	766.8	765.8	779.6	8.6	0.54
FI	1-8	16	199.4	200.0	195.1	199.2	2.0	0.38
	9-15	16	459.0	459.4	459.2	460.2	3.2	0.93
	16-21	16	505.5	519.5	525.6	531.9	6.0	0.52
	9-21	16	964.5	978.8	984.8	992.0	8.4	0.67
			(g:g)					
AdjFCR	1-8	16	1.08	1.10	1.07	1.09	0.01	0.99
	9-15	16	1.28	1.32	1.28	1.28	0.01	0.11
	16-21	16	1.25	1.25	1.33	1.27	0.03	0.29
	9-21	16	1.26	1.28	1.30	1.27	0.02	0.18

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[1]Either 0% coarse corn (0%CC) or 50% coarse corn (50%CC) were fed in combination of either no (0 FTU/kg) or 300 FTU/kg phytase enzyme.
[2]Standard error (SE) for number of cages (n) shown containing 12 birds/cage prior to 8 d of age, and 8 birds/cage after 8 d.

Table IV-8. Effect of soybean meal (SBM) variety by percentage coarse corn (%CC) by phytase interaction on broiler body weight (BW), body weight gain (BWG), feed intake (FI), and adjusted feed conversion ratio (AdjFCR)

Variable	Age		Dietary Treatments[1]									Source of Variability
			0 %CC				50% CC					
			NP SBM		LP SBM		NP SBM		LP SBM		SE[2]	SBM×%CC ×Phytase
		n	0 FTU/kg	300 FTU/kg	0 FTU/kg	300 FTU/kg	0 FTU/kg	300 FTU/kg	0 FTU/kg	300 FTU/kg		
	(d)						(g)					(P-value)
BW	15	8	588.4	590.9	596.7	591.1	584.9	591.8	582.5	589.8	5.0	0.55
	21	8	987.3	993.9	1005.4	999.1	997.0	1010.1	1002.1	1010.4	13.1	0.83
BWG	9-15	8	354.6	359.6	365.0	361.0	349.6	357.6	348.3	362.7	3.4	0.11
	16-21	8	399.0	392.5	406.7	408.0	412.0	418.3	419.6	420.6	8.8	0.66
	9-21	8	753.6	762.5	773.7	769.0	761.6	775.9	771.9	783.4	12.1	0.75
FI	9-15	8	460.3	457.2	457.6	461.3	455.9	462.3	462.9	458.0	4.5	0.16
	16-21	8	499.6	538.3	511.4	513.0	516.0	536.7	522.9	527.0	8.4	0.39
	9-21	8	960.0	995.4	969.0	974.2	971.9	999.1	985.8	985.0	11.9	0.95
							(g:g)					
AdjFCR	9-15	8	1.30[abc]	1.27[bc]	1.25[c]	1.28[bc]	1.30[ab]	1.29[abc]	1.33[a]	1.26[bc]	0.02	0.03
	16-21	8	1.25	1.40	1.26	1.26	1.25	1.28	1.25	1.25	0.04	0.25
	9-21	8	1.27	1.33	1.25	1.27	1.28	1.29	1.28	1.26	0.02	0.93

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[1]Either 0% coarse corn (0%CC) or 50% coarse corn (50%CC) were fed in combination with normal phytate (NP) soybean meal (SBM) and low phytate (LP) SBM with either no (0 FTU/kg) or 300 FTU/kg phytase enzyme.
[2]Standard error (SE) for number of cages (n) shown containing 12 birds/cage prior to 8 d of age, and 8 birds/cage after 8 d.

Table IV-9. Effect of soybean meal (SBM) variety, percentage coarse corn (%CC), and phytase on absolute and relative gizzard and proventriculus weights at 8, 15, and 21 d of age

Variable	Age	n	SBM variety[3] NP	SBM variety[3] LP	SE[2]	%CC 0	%CC 50	SE[2]	Phytase (FTU/kg) 0	Phytase (FTU/kg) 300	SE[2]	SBM	%CC	Phytase
	(d)					(g)						(*P*-value)		
Gizzard	8	16	-	-	-	7.12[B]	7.72[A]	0.1	7.40	7.44	0.1	-	0.001	0.78
Proventriculus	8	16	-	-	-	2.33[A]	2.19[B]	0.04	2.28	2.24	0.04	-	0.01	0.50
						(g/100g BW)								
Gizzard	8	16	-	-	-	2.98[B]	3.29[A]	0.04	3.17	3.14	0.04	-	0.001	0.88
Proventriculus	8	16	-	-	-	0.98[a]	0.93[b]	0.02	0.96	0.94	0.02	-	0.03	0.39
						(g)								
Gizzard	15	16	11.12	11.17	0.3	10.51[B]	11.78[A]	0.2	11.03	11.26	0.2	0.89	0.001	0.45
Proventriculus	15	16	3.50	3.85	0.2	3.88[a]	3.47[b]	0.1	3.78	3.57	0.2	0.17	0.02	0.25
						(g/100g BW)								
Gizzard	15	16	1.86	1.88	0.04	1.73[B]	2.00[A]	0.03	1.85	1.89	0.03	0.81	0.001	0.37
Proventriculus	15	16	0.59	0.64	0.02	0.64	0.59	0.02	0.63	0.60	0.02	0.18	0.06	0.16
						(g)								
Gizzard	21	16	15.01[b]	15.51[a]	0.2	13.71[B]	16.82[A]	0.2	15.12	15.39	0.2	0.04	0.001	0.25
Proventriculus	21	16	5.41	5.55	0.1	5.99[A]	4.98[B]	0.1	5.64[a]	5.32[b]	0.1	0.37	0.001	0.05
						(g/100g BW)								
Gizzard	21	16	1.41[b]	1.46[a]	0.01	1.30[B]	1.57[A]	0.01	1.42	1.45	0.01	0.05	0.001	0.07
Proventriculus	21	16	0.51	0.52	0.01	0.57[A]	0.47[B]	0.01	0.53	0.50	0.01	0.52	0.001	0.13

Dietary Treatments[1]: SBM variety, %CC, Phytase (FTU/kg); Source of Variability: SBM, %CC, Phytase.

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).

[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).

[1]Either 0% coarse corn (0%CC) or 50% coarse corn (50%CC) were fed in combination with normal phytate (NP) soybean meal (SBM) and low phytate (LP) SBM with either no (0 FTU/kg) or 300 FTU/kg phytase enzyme.

[2]Standard error (SE) for n=16 cages of 8 birds each.

[3]Soybean meal treatments started at day 9.

Table IV-10. Effect of soybean meal (SBM) variety by percentage coarse corn (%CC) interaction on absolute and relative gizzard and proventriculus weights at 15 and 21 d of age

Variable	Age	n	Dietary Treatments[1]				SE[2]	Source of Variability
			NP SBM		LP SBM			SBM×%CC
			0%CC	50%CC	0%CC	50%CC		
	(d)		(g)					(P-value)
Gizzard	15	8	10.15	12.08	10.86	11.48	0.4	0.12
Proventriculus	15	8	3.46[b]	3.54[b]	4.31[a]	3.39[b]	0.2	0.05
			(g/100g BW)					
Gizzard	15	8	1.68	2.07	1.79	1.93	0.06	0.07
Proventriculus	15	8	0.57[b]	0.61[b]	0.70[a]	0.57[b]	0.03	0.02
			(g)					
Gizzard	21	8	13.04[C]	16.98[A]	14.37[B]	16.65[A]	0.2	0.001
Proventriculus	21	8	5.81	5.01	6.16	4.95	0.2	0.20
			(g/100g BW)					
Gizzard	21	8	1.25[C]	1.58[A]	1.34[B]	1.57[A]	0.02	0.01
Proventriculus	21	8	0.56	0.47	0.58	0.47	0.02	0.55

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Either 0% coarse corn (0%CC) or 50% coarse corn (50%CC) were fed in combination with normal phtate (NP) soybean meal (SBM) and low phytate (LP) SBM.
[2]Standard error (SE) for n=8 cages of 8 birds each.

Table IV-11. Effect of soybean meal (SBM) variety by phytase interaction on absolute and relative gizzard and proventriculus weights at 15 and 21 d of age

Variable	Age	n	Dietary Treatments[1] NP SBM 0 FTU/kg	NP SBM 300 FTU/kg	LP SBM 0 FTU/kg	LP SBM 300 FTU/kg	SE[2]	Source of Variability SBM×Phytase
	(d)		(g)					(P-value)
Gizzard	15	8	11.36[ab]	10.87[ab]	10.70[b]	11.64[a]	0.3	0.02
Proventriculus	15	8	3.67	3.33	3.88	3.82	0.2	0.43
			(g/100g BW)					
Gizzard	15	8	1.88	1.87	1.81	1.91	0.05	0.26
Proventriculus	15	8	0.61	0.57	0.66	0.62	0.03	0.97
			(g)					
Gizzard	21	8	14.85	15.17	15.40	15.62	0.2	0.83
Proventriculus	21	8	5.52	5.30	5.76	3.35	0.2	0.57
			(g/100g BW)					
Gizzard	21	8	1.40[b]	1.43[ab]	1.44[ab]	1.47[a]	0.02	0.05
Proventriculus	21	8	0.52	0.50	0.54	0.50	0.02	0.52

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[1]Either normal phytate (NP) soybean meal (SBM) and low phytate (LP) SBM were fed in combination of either no (0 FTU/kg) or 300 FTU/kg phytase enzyme.
[2]Standard error (SE) for number of cages n=8 cages of 8 birds each.

Table IV-12. Effect of phytase by percentage coarse corn (%CC) interaction on absolute and relative gizzard and proventriculus weights at 8, 15, and 21 d of age

Variable	**Age**	**n**	**Dietary Treatments[1]**				**SE[2]**	**Source of Variability**
			0 FTU/kg		**300 FTU/kg**			**Phytase×%CC**
			0%CC	**50%CC**	**0%CC**	**50%CC**		
	(d)		(g)					(*P*-value)
Gizzard	8	8	6.96	7.83	7.27	7.62	0.2	0.19
Proventriculus	8	8	2.34	2.22	2.27	2.15	0.2	0.64
			(g/100g BW)					
Gizzard	8	8	2.89[C]	3.37[A]	3.07[B]	3.20[AB]	0.1	0.01
Proventriculus	8	8	0.97	0.95	0.98	0.90	0.02	0.18
			(g)					
Gizzard	15	8	10.43	11.63	10.58	11.93	0.3	0.80
Proventriculus	15	8	3.99	3.56	3.78	3.37	0.2	0.95
			(g/100g BW)					
Gizzard	15	8	1.73	1.96	1.73	2.08	0.05	0.41
Proventriculus	15	8	0.66	0.60	0.61	0.58	0.02	0.63
			(g)					
Gizzard	21	8	13.5	16.75	13.91	16.88	0.2	0.55
Proventriculus	21	8	6.28	5.00	5.69	4.95	0.2	0.10
			(g/100g BW)					
Gizzard	21	8	1.26[c]	1.58[a]	1.34[b]	1.57[ab]	0.02	0.03
Proventriculus	21	8	0.58	0.47	0.55	0.46	0.02	0.49

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Either 0% coarse corn (0%CC) or 50% coarse corn (50%CC) were fed in combination of either no (0 FTU/kg) or 300 FTU/kg phytase enzyme.
[2]Standard error (SE) for n=8 cages of 8 birds each.

Table IV-13. Effect of soybean meal (SBM) variety by percentage coarse corn (%CC) by phytase interaction on absolute and relative gizzard and proventriculus weights at 15 and 21 d of age

Variable	Age	n	Dietary Treatments[1]								SE[2]	Source of Variability
			0 %CC				50% CC					SBM×%CC ×Phytase
			NP SBM		LP SBM		NP SBM		LP SBM			
			0 FTU/kg	300 FTU/kg	0 FTU/kg	300 FTU/kg	0 FTU/kg	300 FTU/kg	0 FTU/kg	300 FTU/kg		
	(d)		(g)									(*P*-value)
Gizzard	15	4	10.62	9.69	10.25	11.47	12.11	12.05	11.15	11.81	0.5	0.23
Proventriculus	15	4	3.70	3.23	4.28	4.33	3.65	3.43	3.48	3.30	0.3	0.50
			(g/100g BW)									
Gizzard	15	4	1.71	1.65	1.75	1.82	2.05	2.09	1.87	2.00	0.07	0.79
Proventriculus	15	4	0.59	0.55	0.72	0.68	0.62	0.60	0.58	0.56	0.04	0.93
			(g)									
Gizzard	21	4	13.15[D]	12.94[D]	13.85[D]	14.89[C]	16.55[AB]	17.41[A]	16.95[AB]	16.36[B]	0.3	0.01
Proventriculus	21	4	6.08	5.54	6.48	5.84	4.97	5.05	5.04	4.85	0.2	0.80
			(g/100g BW)									
Gizzard	21	4	1.23	1.27	1.28	1.40	1.57	1.59	1.59	1.54	0.03	0.08
Proventriculus	21	4	0.57	0.55	0.60	0.55	0.47	0.46	0.48	0.46	0.02	0.77

[a-d]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A-D]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Either 0% coarse corn (0%CC) or 50% coarse corn (50%CC) were fed in combination with normal phytate (NP) soybean meal (SBM) and low phytate (LP) SBM with either no (0 FTU/kg) or 300 FTU/kg phytase enzyme.
[2]Standard error of mean (SE) n=4 cages of 8 birds each.

Table IV-14. Effect of soybean meal (SBM) variety, percentage coarse corn (%CC), and phytase on total phosphorus (P) digestibility

Variable	Dietary Treatments[1]									Source of Variability		
	SBM variety		SE[2]	%CC		SE[2]	Phytase (FTU/kg)		SE[2]	SBM	%CC	Phytase
Digestibility	NP	LP		0	50		0	300				
	(%DM[3])									(P-value)		
Total P	39.72[B]	52.98[A]	0.58	47.89[A]	44.81[B]	0.58	42.46[B]	50.06[A]	0.6	0.001	0.001	0.001

[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Either 0% coarse corn (0%CC) or 50% coarse corn (50%CC) were fed in combination with normal phytate (NP) soybean meal (SBM) and low phytate (LP) SBM with either no (0 FTU/kg) or 300 FTU/kg phytase enzyme.
[2]Standard error (SE) for n=16 cages of 8 birds each.
[3]Percent DM of feed and ileal samples were used to calculate nutrient digestibility on dry matter basis.

Table IV-15. Effect of soybean meal (SBM) variety by percentage coarse corn (%CC) interaction on total phosphorus (P) digestibility

Variable	**Dietary Treatments**[1]					**Source of Variability**
	NP SBM		**LP SBM**		**SE**[2]	**SBM×%CC**
Digestibility	**0%CC**	**50%CC**	**0%CC**	**50%CC**		
	(%DM[3])					(P-value)
Total P	40.60	38.84	55.18	50.78	0.8	0.12

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[1]Either 0% coarse corn (0%CC) or 50% coarse corn (50%CC) were fed in combination with normal phytate (NP) soybean meal (SBM) and low phytate (LP) SBM.
[2]Standard error (SE) for n=8 cages of 8 birds each.
[3]Percent DM of feed and ileal samples were used to calculate nutrient digestibility on dry matter basis.

Table IV-16. Effect of soybean meal (SBM) variety by phytase interaction on total phosphorus (P) digestibility

Variable	Dietary Treatments[1]					Source of Variability
	NP SBM		LP SBM		SE[2]	SBM×Phytase
Digestibility	0 FTU/kg	300 FTU/kg	0 FTU/kg	300 FTU/kg		
	(%DM[3])					(*P*-value)
Total P	38.43^{D}	41.01^{C}	46.86^{B}	59.11^{A}	0.8	0.001

[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).

[1]Either normal phtate (NP) soybean meal (SBM) and low phytate (LP) SBM were fed in combination of either no (0 FTU/kg) or 300 FTU/kg phytase enzyme.

[2]Standard error (SE) for n=8 cages of 8 birds each.

[3]Percent DM of feed and ileal samples were used to calculate nutrient digestibility on dry matter basis.

Table IV-17. Effect of phytase by percentage coarse corn (%CC) interaction on total phosphorus (P) digestibility

Variable	**Dietary Treatments[1]**				**SE[2]**	**Source of Variability**
	0 FTU/kg		**300 FTU/kg**			**Phytase×%CC**
Digestibility	**0%CC**	**50%CC**	**0%CC**	**50%CC**		
	(%DM[3])					(*P*-value)
Total P	42.55^{C}	42.74^{C}	53.24^{A}	46.88^{B}	0.8	0.001

^{A,B}Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).

[1]Either 0% coarse corn (0%CC) or 50% coarse corn (50%CC) were fed in combination of either no (0 FTU/kg) or 300 FTU/kg phytase enzyme.

[2]Standard error (SE) for n=8 cages of 8 birds each.

[3]Percent DM of feed and ileal samples were used to calculate nutrient digestibility on dry matter basis.

Table IV-18. Effect of soybean meal (SBM) variety by percentage coarse corn (%CC) by phytase interaction on total phosphorus (P) digestibility

Variable	Dietary Treatments[1]									Source of Variability
	0 %CC				50% CC					
	NP SBM		LP SBM		NP SBM		LP SBM			
Digestibility	0 FTU/kg	300 FTU/kg	0 FTU/kg	300 FTU/kg	0 FTU/kg	300 FTU/kg	0 FTU/kg	300 FTU/kg	SE[2]	SBM×%CC ×Phytase
	(%DM[3])									(*P*-value)
Total P	37.38	43.82	47.72	62.65	39.48	38.20	46.00	55.57	1.2	0.48

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[1]Either 0% coarse corn (0%CC) or 50% coarse corn (50%CC) were fed in combination with normal phytate (NP) soybean meal (SBM) and low phytate (LP) SBM with either no (0 FTU/kg) or 300 FTU/kg phytase enzyme.
[2]Standard error (SE) for n=4 cages of 8 birds each.
[3]Percent DM of feed and ileal samples were used to calculate nutrient digestibility on dry matter basis.

CHAPTER V

Effect of adding phytase to low phytate and normal phytate SBM diets in combination with coarse corn on male broiler live performance, gizzard and proventriculus weights, total phosphorus digestibility, bone ash, and bone strength

5.1. ABSTRACT

This study evaluated the effects of adding phytase in combination with either 0% or 50% coarse corn (CC) to crumbled broiler diets containing either normal phytate (NP) or low phytate (LP) soybean meal (SBM) on broiler live performance, total phosphorus (P) digestibility, bone ash, and bone breaking strength of broilers grown to 35 d of age. A total of 512 Ross 708 male broiler chicks were assigned to 32 floor pens in 4 blocks with 16 birds per pen. Common corn-SBM diets either with (300 FTU/kg) or without (0 FTU/kg) phytase in combination with either 0%CC or 20%CC were fed to 8 pens per diet to 9 d of age. From 10 to 35 d, a 2 × 2 × 2 factorial arrangement of SBM type (LP and NP), phytase addition (with and without), and CC (0 and 50%) with 4 pens per diet. Birds and feed were weighed at 9, 22, and 35 d of age, and 3 birds per pen were necropsied at 35 d to determine total P digestibility. Tibia bones were excised to determine bone strength and percentage ash. Data from 10 to 35 d were analyzed as a randomized complete block design. From 23 to 35 d, feed intake decreased ($P \leq 0.01$) when phytase was added to the LP (2,131 versus 1,996 g) but not NP diet. BW at 35 d was decreased ($P \leq 0.001$) by phytase in the LP diet (2,183 versus 2,034

g) but increased when phytase was added to the NP diet (2,249 versus 2,094 g). The AdjFCR was improved ($P \leq 0.001$) by phytase addition to the NP diet (1.64 versus 1.78 g:g) but was poorer with the LP diet (1.84 versus 1.71 g:g) from 23 to 35 d. When birds consumed 300 FTU/kg phytase with LP SBM a decreased ($P \leq 0.01$) gizzard weight compared to other combinations at 22 d was observed. Phytase improved ($P \leq 0.05$) total P digestibility in both LP (93.4 versus 86.6%) and NP (88.8 versus 76.3%) diets. Adding phytase to either 0%CC or 50%CC improved ($P \leq 0.05$) total P digestibility. Digestibility was 84.5% versus 78.5% for 0%CC, and 91.4% versus 90.7% for 50%CC with and without phytase, respectively. Phytase decreased ($P \leq 0.001$) bone strength in both LP and NP diets. On the other hand, adding phytase to LP SBM decreased ($P \leq 0.001$) bone ash percentage (55.8 versus 51.4 %). Results demonstrated that phytase improved live performance and total P digestibility of broilers fed diets formulated with NP SBM. Adding phytase to LP SBM diets resulted in poorer live performance, reduced bone breaking strength, and decreased percentage bone ash, which could be explained by a dietary imbalance associated with increased total P digestibility.

Key words: Broilers, low phytate SBM, phytase, total P digestibility, bone ash

5.2. INTRODUCTION

Soybean has served as a major human food and animal feed component based on its nutritional and health values. It has become an important dietary source of protein, fat, fiber, minerals, and vitamins. However, components present in soybeans like trypsin inhibitors and phytate have acted as anti-nutritional factors that interfered with protein digestion or chelated nutritionally essential elements including P, Ca, Zn, and Fe (Liener, 1994; Hurrell, 2003). Soybean meal (SBM) has become a common ingredient that was mixed with corn and other cereals to develop balanced diets for swine and poultry. The amount of total P in SBM has been listed as approximately 0.62% (NRC, 1994), but it was not all available since a portion was bound to phytate. The bioavailability of P from conventional SBM was significantly less than the bioavailability of P in LP SBM. In fact, the SBM produced from LP soybeans was almost 50% higher in P bioavailability compared with the highest bioavailability achieved for NP SBM irrespective of time in the extractor. The increased P bioavailability was largely due to decreased phytate concentrations in the LP SBM (Karr et al., 2005).

In order to improve efficiency, the broiler industry has typically fed crumbled pellets for the first 1-2 wk of a broiler's life because birds fed diets in a mash (non-pelleted) form have generally been reported to spend more time feeding than those provided pellets. Further, chicks were found to utilize pellets more efficiently than mash because they spent less energy consuming the feed (Jensen et al., 1962). Nevertheless, performance of broilers was prepared to be equal when diets were fed in both mash and crumble form from roller mill ground yellow corn with a geometric mean diameter of 1,343 μm as compared with diets from hammer mill (4.76 mm screen) ground yellow corn with a geometric mean diameter of 814 µm (Reece et al., 1985).

Increased particle size has been reported to have other benefits from increased retention time of digesta such as when P was limiting, more P was retained in the body for physiological functions, which resulted in less P being excreted in the feces (Li et al., 2000).

Dietary phytase has been shown to increase phytate P hydrolysis up to 72.4% with total P retention of 58% for broilers fed diets with SBM as the only source of phytate P (Leske and Coon, 1999). The effectiveness of microbial phytase in improving bioavailability of P and Ca in monogastric animal diets has been well documented (Coelho et al., 1999), since phytase from *E. coli* has also been shown to have high proteolytic stability compared with other phytases prepared from *Aspergillus and Bacillus* (Igbasan et al., 2000). Therefore, this research aimed to investigate the influence of SBM variety, corn particle size, and phytase addition on live performance, total P digestibility, bone breaking strength, and bone ash of broilers grown to 35 d in floor pens.

5.3. MATERIALS AND METHODS

Dietary Treatments

This study was conducted at North Carolina State University, Prestage Department of Poultry Science. A total of 8 corn-soybean based dietary treatments were tested with 2 SBM varieties (low phytate (LP) and normal phyate (NP)), and 2 corn particle size (0% and 50% coarse corn (CC)), in the presence or absence of 300 FTU/kg *Escherichia coli*-derived research-grade phytase enzyme (Danisco Animal Nutrition, Marlborough, UK). One FTU has been generally defined as the amount of enzyme required to liberate 1 μmol of inorganic P min^{-1} from 5.5 m*M* of Na phytate at pH 5.5 and 37º C (Qian et al., 1997). Table 1 illustrates the chemical composition of the different SBM varieties. Fine corn particles were obtained by grinding corn in a hammermill (Model 1522, Roskamp Champion, Waterloo, IA) equipped with two 2.4 mm screens to obtain an average particle size of 314 μm, while CC particles with an average particle size of 1,190 μm were obtained by grinding corn with a two-pair roller mill with a gap setting of 0% opening (0.18 mm) on the top pair of rollers and 100% opening (7.16 mm) on the bottom pair of rollers (Model C128829, RMS, Tea, SD). Particle size distribution was determined by ASAE S319.3 (ASAE, 2003). Tables 2 and 3 illustrate the dietary treatments for starter and grower, respectively. From 1-9 d, all birds were fed starter diets with commercial NP SBM and with 20% inclusion of CC in the coarse diets. From 10-35 d, CC birds were fed a grower diet with a CC inclusion rate of 50% and the same particle size of corn. Titanium dioxide (TiO_2) was included in grower diets as an indigestible marker to allow for the calculation of apparent nutrient digestibility. Feed and water were provided for *ad libitum* consumption. Starter and grower diets were both fed as crumbles. All diets were mixed in a horizontal double twin shaft mixer (TRDB126-0604, Hayes and Stolz Ind. Mfg. Co., Fort Worth, TX). The mash was conditioned at 85°C for 45 seconds, and then pelleted with a ring die (4.4 mm by 35 mm) pellet mill (Model PM1112-2, California Pellet Mill Co., Crawfordsville, IN). Pellets were immediately cooled with ambient air in a counter-flow cooler (Model VK09 × 09KL, Geelen Counterflow USA Inc., Orlando, FL) and crumbled. Corn obtained from hammermill and roller mill were analyzed for their total P content according to particle size. Particles below 297 µm were collected between sieve number 50 and the pan, particles between 298-1190 µm were collected from sieve numbers 16-50, and particles above 1190 µm were collected from sieve number above 16 according to ASAE S319.3 procedure (ASAE, 2003). Diets that contained phytase were formulated to contain 0.15% less calcium (Ca) and available phosphorus (AvP).

Birds Management and Data Collection

The care of the birds in these studies conformed to the Guide for Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010). Eggs from a 37-wk-old Ross 344 × 708SF (Aviagen, Huntsville, AL) broiler breeder flock were collected and identified by breeding pen (16 pens) and then incubated under standard conditions. Chicks were hatched, sexed, and maintained separate by their breeder pen at all times until placement. A total of 512 male chicks were permanently identified with neck tags and placed in 32 floor pens with 16 birds per pen (1 chick per breeder pen source). Pen floors were covered with new softwood shavings. Each pen had 2 tube feeders and 1 bell drinker, plus a plastic font drinker during the first 7 d. Three feeder trays were initially placed in every pen, then reduced to 2 feeders at 5 d, and finally to 1 at 8 d. The final supplemental feeder was removed at 9 d prior to the change to grower feed. Contents of the trays were screened and dumped back into the tube feeders at 9 d. Total starter feed per bird was equalized to 0.48 kg per live chick at 9 d. Feeders were shaken twice daily to maintain flow of feed from the tubes into the pans until 22 d of age. Shavings were cleaned from feeders as needed. From 23-35 d, feeders were shaken three times daily. The lighting program during the first 7 d was 23 h of light that was reduced to 21 h of light up to 21 d. After 21 d of age, only 13 h of natural light was used. The initial house temperature was set at 32°C and gradually reduced to 25°C. Feeders were weighed at the start and the end of each feeding period.

Pen body weight (BW), and feed intake were measured at 1, 9, 22, and 35 d, and BW gain was calculated by time interval. Mortality was weighed and recorded twice daily for adjusted feed conversion ratio (AdjFCR) calculations by time interval. Three birds per pen were selected randomly and necropsied for gizzard and proventriculus weights at 9 and 22 d. At 35 d of age average BW per pen was determined and 3 birds near the average BW of each pen were selected, killed by cervical dislocation, and necropsied for weights of gizzard and proventriculus. The terminal 13 cm of ileum was removed 3 cm anterior to the ileo-cecal junction, ileal contents were gently expressed, pooled per pen, and frozen for nutrient digestibility determination. Tibia bones were excised for bone strength measurement. Bones were broken using the 3-point bending test and sheared midshaft using a crosshead speed of 30 mm/min to minimize splintering (Crenshaw et al., 1981). Bones were then ashed at 600°C overnight in a muffle furnace (Hall et al., 2003).

Chemical Analyses

The TiO_2 of diets and ileal samples were determined using the method of Myers et al. (2004). Frozen samples of feed were dried in oven for 24 h at 95 to 100°C for dry matter (Method 934.01, AOAC, 2006). The dry matter percentage of ileal digesta was determined by freeze drying (Virtis Freezemobile, Model 12XL, Warminster, PA). Samples of feed and dried ileal digest were ground (< 2 mm) for analysis. Diets and ileal samples were analyzed for total P.

Statistical Methods

A randomized complete block design with a 2 × 2 × 2 factorial arrangement of treatments was used (SBM × CC × Phytase). The general linear model of SAS (2009; SAS version 9.2, SAS institute, Cary, NC, USA) was used to analyze live performance. Variable means were partitioned by LSMEANS and were considered statistically different when $P \leq 0.05$, although effects with P-values between 0.06 and 0.10 were mentioned in the text when the data suggested a numerical trend. Group averages for all birds in a single pen were used for live performance data analysis, whereas averages of 2 or 3 randomly selected birds per pen were used for the other measurements. The experimental unit for statistical analysis of measured parameters was pen.

5.4. RESULTS AND DISCUSSION

Effect of Dietary Treatments on Live Performance

The effect of SBM variety, %CC, and phytase on broiler BW, BW gain, feed intake, and AdjFCR are show in Table 4. LP SBM was included in the diets from 9 d. The BW gain for the period from 23-35 d was greater ($P \leq 0.05$) in birds consuming NP SBM, which was also reflected in an improved ($P \leq 0.05$) AdjFCR for the same period. In contrast, Li et al. (2000) found that broilers had comparable BW when fed either LP corn or NP corn diet. Feed intake was not negatively affected when changing from starter to grower diets at 10 d probably because limestone level decreased in the grower.

For the first 9 d, diets contained either 0% coarse corn (0%CC) or 20% coarse corn (20%CC), and from 10-35 d diets were formulated with either 0%CC or 50%CC. Inclusion of CC in the diets had no effect on BW and feed intake measured throughout the experiment (Table 4). However, BW gain from 23-35 and 10-35 d was greater ($P \leq 0.05$) in birds consuming 0%CC, which was probably due to availability of new wood shavings that provided some coarse material to stimulate gizzard function when consumed. The AdjFCR was improved ($P \leq 0.01$) from 23-35 d in birds that consumed 0%CC, and the same pattern was observed from 1-35 d ($P \leq 0.05$). These results disagreed with findings of Lentle et al. (2006) who reported that diets with a higher relative proportion of coarser particles resulted in better feed efficiency in broilers. Also, in a study conducted on particle size of SBM, Kilburn and Edwards (2004) reported that increasing the particle size of commercial SBM from 891 μm to 1,239 μm improved mineral utilization and FRC in semi-purified diets.

Results of phytase effect on live performance are shown in Table 4. Adding 300 FTU/kg improved ($P \leq 0.05$) AdjFCR from 1-22 d of age. According to Yan et al. (2000), an increase in BW at 21 d for broilers was observed with the addition of phytase. The effect of SBM variety by %CC on BW, BW gain, feed intake, and AdjFCR is shown in Table 5. There was no interaction effect of SBM variety by %CC on broiler BW, BW gain, feed intake, or AdjFCR. Table 6 shows the SBM variety × phytase interaction for broiler BW, BW gain, feed intake, and AdjFCR. Adding phytase to NP SBM improved BW at 35 d but decreased ($P \leq 0.001$) BW in the presence of LP SBM. The same effect was observed for BW gain ($P \leq 0.001$) and feed intake ($P \leq 0.01$) from 23-35 d. This effect was also observed for AdjFCR where adding phytase to NP SBM improved ($P \leq 0.001$) AdjFCR from 23-35 d and resulted in poorer AdjFCR when added to LP SBM. In contrast to our findings, Manangi and Coon (2006) reported that adding phytase to different commercial sources of SBM had no effect on feed intake, BW, BW gain, and FCR of broiler chicks.

The phytase × %CC interaction effect on broiler BW, BW gain, feed intake, and AdjFCR are presented in Table 7. In the absence of phytase, 50%CC decreased ($P \leq 0.05$) BW at 9, 22, and 35 d. However, when phytase was added to 50%CC diets BW was improved ($P \leq 0.001$) with the same effect observed for BW gain and feed intake. However, AdjFCR was not affected. Results may indicate that birds required a period of time to adapt to large particle size corn probably because birds distinguished the differences in feed particle size by mechanoreceptors located in the beak (Gentle, 1979) or the gizzard required time to adapt.

The three-way interaction of SBM variety, %CC, and phytase on BW, BW gain, feed intake, and AdjFCR is shown in Table 8. Broiler BW at 35 d was affected by the 3-way interaction ($P \leq 0.05$). Adding phytase to NP SBM improved BW when using 50%CC, but adding phytase to LP SBM reduced BW when fed with either 0 or 50%CC. The same effect was observed for BW gain from 10-35 d ($P \leq 0.05$). In the presence of 0%CC, adding phytase to NP SBM did not further improve BW gain from 23-35 d. However, when phytase was added to NP SBM and 50%CC the BW gain was improved ($P \leq 0.01$). On the other hand, adding phytase to LP SBM at either 0 or 50%CC decreased BW gain. The AdjFCR for the 23-35 d period was improved ($P \leq 0.05$) when 50%CC diets were fed in combination with NP SBM and 300 FTU/kg phytase. AdjFCR was also improved by 0%CC in combination with NP SBM and 300 FTU/kg phytase. Improved AdjFCR was probably due to more phytate substrate in NP SBM diets upon which the phytase could act to release nutrients which was further improved by the presence of 0%CC that had greater surface area.

Effect of Dietary Treatments on Organ Weights

The effect of SBM variety, %CC, and phytase on absolute and relative gizzard and proventriculus weights at 22 and 35 d of age is shown in Table 9. Feeding NP SBM produced a larger ($P \leq 0.01$) gizzard, a larger proventriculus ($P \leq 0.05$), and greater ($P \leq 0.05$) relative gizzard weight at 22 d, however, these differences were no longer evident at 35 d. The changes in proventriculus weight indicated that there was an adaptation process where birds may have initially reduced the amount of secreted acid in the presence of LP SBM. Feeding 50%CC produced a smaller proventriculus ($P \leq 0.01$) at 22 d but the gizzard was not affected by CC inclusion (Table 9). The smaller proventriculus suggested less secretion of HCl and pepsin in the presence of large particles of corn. Our findings were consistent with other studies where feeding whole grains had been associated with enhanced gut development and reduced proventricular swelling (Jones and Taylor, 2001). Also, Jacobs et al. (2010) reported a 19% increase in gizzard weight as corn particle size increased. Adding phytase had no effect on absolute and relative gizzard and proventriculus weights (Table 9). The effect of SBM variety by %CC interaction on absolute and relative gizzard and proventriculus weights at 22 and 35 d of age is shown in Table 10. No significant interaction was observed between SBM variety and CC. The effect of SBM variety by phytase interaction on absolute and relative gizzard and proventriculus weights at 22 and 35 d of age is shown in Table 11. Adding phytase to the NP diet did not affect gizzard weight at 22 d, but when phytase was added to the LP diet, gizzard weight was reduced ($P \leq 0.01$). On the other hand, relative proventriculus weight measured at 22 d was reduced ($P \leq 0.05$) when phytase was added to the NP diet but was not affected in the LP diets. These differences were no longer evident at 35 d of age, which might have been due to an adaptation by the gizzard.

The phytase × %CC interaction effect on absolute and relative gizzard and proventriculus weights at 9, 22, and 35 d of age is depicted in Table 12 . At 9 d of age, adding phytase to 50%CC produced increased weights of gizzard ($P \leq 0.01$) and proventriculus ($P \leq 0.001$). Large feed particles have been frequently reported to stimulate gizzard weight and activity, which slowed passage rate of digesta through the gizzard (Nir et al., 1994) and thus increased the exposure time of nutrients to digestive enzymes. This, in turn, may have improved energy utilization and nutrient digestibility (Carre, 2000). In the presence of phytase and 50%CC, pepsin and acid secretion may have been stimulated as evidenced by a larger proventriculus ($P \leq 0.001$). Relative proventriculus weight was larger ($P \leq 0.01$) at 22 d when phytase was added to 0%CC, but when phytase was added to 50%CC relative proventriculus weight was reduced. The 3-way interaction of SBM × %CC × phytase on absolute and relative gizzard and proventriculus weight is shown in Table 13. No significant effects were found.

Effect of Dietary Treatments on Phosphorus Digestibility, Bone Strength and Ash

The effects of SBM variety, %CC, and phytase on total P digestibility, bone breaking strength, and percentage bone ash are presented in Table 14. Total P digestibility was greater ($P \leq 0.0001$) in birds receiving LP SBM probably due to an initially higher available P in LP

SBM. These results were similar to findings reported by Leytem et al. (2007), where feeding low phytate barley to broilers produced greater P digestibility compared to normal phytate barley. Diets formulated with 0%CC had higher ($P \leq 0.05$) total P digestibility that could have been due to increased surface area of the fine particles. Similar findings were reported by Peron et al. (2005) who found that feeding pelleted diets made from finely ground wheat improved apparent metabolizable energy for broilers compared to pelleted diets made from coarsely ground wheat. Adding phytase increased P digestibility by approximately 10% compared to a non phytase diet ($P \leq 0.0001$). These results were in agreement with findings reported regarding the efficacy of microbial phytase in hydrolyzing phytate P, improving its utilization by swine and poultry, and consequently leading to the reduction in P excretion (Simons et al., 1990; Jongbloed et al., 1992; Baxter et al., 2003). The interaction of SBM and %CC on total P digestibility is shown in Table 15. Total P digestibility was not affected by the SBM × %CC interaction. The interaction of SBM variety and phytase on total P digestibility, breaking strength of tibia, and bone ash percentage is shown in Table 16. On the other hand, total P digestibility was improved ($P \leq 0.05$) when phytase was used in combination with either LP or NP SBM. The interaction of phytase and %CC on total P digestibility, breaking strength of tibia, and bone ash percentage is shown in Table 17. In the absence of phytase, 0%CC exhibited improved total P digestibility ($P \leq 0.05$), but when 300 FTU/kg of phytase was added, differences in digestibility attributed to particle size disappeared with higher improvement of total P digestibility in 50% CC. Further improvent due to phytase addition could be explained by the total P content present in 50%CC particles larger than 1190 μm when ground using roller mill (Table 19). The total P content in fine corn obtained from hammermill was consistent among the different particles of corn while grinding corn using roller mill resulted in higher amount of total P in the larger particles (>1190 µm) which were the predominant particles (72.3%) obtained from roller mill. The three way-interaction of SBM variety, %CC, and phytase on total P digestibility, breaking strength of tibia, and bone ash percentage is shown in Table 18. No significant effect were found.

Bone breaking strength was associated with percentage bone ash, where bones with greater breaking strength possessed an increased percentage ash that indicated greater bone mineralization (Driver et al., 2003). Adding 300 FTU/kg phytase to feed decreased ($P \leq 0.001$) bone breaking strength and decreased percentage ash ($P \leq 0.01$), which indicated reduced mineralization in bones of birds on this treatment probably due to a mineral imbalance in this diet (Table 14). Using LP SBM decreased percentage bone ash ($P \leq 0.05$) but had no effect on bone breaking strength. Furthermore, corn particle size did not affect percentage ash or bone breaking strength. Percentage ash and breaking strength were not affected by the SBM × %CC interaction (Table 15). Adding 300 FTU/kg phytase to NP SBM did not affect percentage bone ash or breaking strength. However, when phytase was added to LP SBM, percentage ash and bone breaking strength were decreased ($P \leq 0.001$) as shown in Table 16. These results were in agreement with Yan et al. (2001) who found a significant interaction between nPP and phytase. They reported that phytase supplementation improved tibia ash at lower levels of nPP. However, at higher levels of nPP that approached or surpassed the amount needed to maximize tibia ash, the addition of phytase was of little or no benefit.

5.5. CONCLUSION

Results demonstrated that phytase improved live performance and total P digestibility of broilers fed diets formulated with NP SBM. Adding phytase to LP SBM diets resulted in poorer live performance, bone breaking strength, and percentage bone ash, which could be explained by a dietary imbalance that had a negative effect on intestinal Ca and P balance in the LP SBM environment. When adding phytase to LP diet, the dosage should be adjusted, so as to not exceed the total phytate P available and have to make a balance for both total P and available P when using LP SBM or LP diets. The negative effect of 50%CC on total P digestibility could be ameliorated by adding phytase.

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Table V-1. Chemical analysis[1] of soybean meal (SBM) varieties

Composition	Commercial NP SBM	NP SBM	LP SBM
	(%)		
Moisture	11.43	11.43	9.19
Protein	48.73	49.55	51.38
Fiber	2.60	6.40	6.40
Ash	6.12	5.78	5.78
Total Phosphorus	0.66	0.68	0.74
Calcium	0.29	0.19	0.13

[1]Results obtained from Carolina Analytical Services laboratories.

Table V-2. Starter diets used to 9 d of age

	Dietary Treatments			
	0 %CC		20% CC	
	Commercial NP SBM		Commercial NP SBM	
Ingredients	**0 FTU/kg**	**300 FTU/kg**	**0 FTU/kg**	**300 FTU/kg**
	(%)			
Fine corn	59.73	59.73	47.78	47.78
Coarse corn (CC)	---	---	11.95	11.95
Soybean meal (48% CP)	34.74	34.74	34.74	34.74
Poultry fat	1.00	1.00	1.00	1.00
Limestone	0.93	1.07	0.93	1.07
Dicalcium phosphate (18.5% P)	2.01	1.08	2.01	1.08
Salt	0.50	0.50	0.50	0.50
DL-Methionine	0.24	0.24	0.24	0.24
L-Lysine	0.10	0.10	0.10	0.10
L-Threonine	0.10	0.10	0.10	0.10
Choline chloride (60%)	0.20	0.20	0.20	0.20
Vitamin premix[1]	0.05	0.05	0.05	0.05
Mineral premix[2]	0.20	0.20	0.20	0.20
Coccidiostat[3]	0.05	0.05	0.05	0.05
Selenium premix[4]	0.03	0.03	0.03	0.03
Phytase[5]	---	0.03	---	0.03
Vermiculite (inert filler)[6]	0.13	0.89	0.13	0.89
Calculated nutrient content				
Metabolizable energy (kcal/g)	2.87	2.87	2.87	2.87
Crude protein (%)	22.00	22.00	22.00	22.00
Calcium (%)	0.90	0.75	0.90	0.75
Available phosphorus (%)	0.45	0.30	0.45	0.30
Lysine (%)	1.26	1.26	1.26	1.26
Methionine (%)	0.58	0.58	0.58	0.58
Threonine (%)	0.83	0.83	0.83	0.83
Methionine + cysteine (%)	0.92	0.92	0.92	0.92
Sodium (%)	0.21	0.21	0.21	0.21

[1]Vitamin premix supplied the following per kg of diet: 6,614 IU vitamin A, 1,984 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 1.98 mg menadione (K_3), 1.98 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 3.97 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.
[2]Mineral premix supplied the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; cobalt, 1 mg.
[3]Coccidiostat supplied monensin sodium at 99 mg/kg of feed.
[4]Selenium premix provided 0.2 mg Se (as Na_2SeO_3) per kg of diet.
[5]Phytase enzyme added at 300 FTU/kg.
[6]Vermiculite was used as an inert filler.

Table V-3. Grower diets used from 10 to 35 d of age

	Dietary Treatments							
	0 %CC				50% CC			
	NP SBM		LP SBM		NP SBM		LP SBM	
Ingredients	**0 FTU/kg**	**300 FTU/kg**	**0 FTU/kg**	**300 FTU/kg**	**0 FTU/kg**	**300 FTU/kg**	**0 FTU/kg**	**300 FTU/kg**
					(%)			
Fine corn	63.17	63.17	63.54	63.54	31.59	31.59	31.77	31.77
Coarse corn	---	---	---	---	31.58	31.58	31.77	31.77
Soybean meal	27.17	27.17	26.65	26.65	27.17	27.17	26.65	26.65
Poultry fat	1.05	1.04	1.00	1.00	1.05	1.04	1.00	1.00
Limestone	0.92	1.07	1.32	1.46	0.92	1.07	1.32	1.46
Dicalcium phosphate (18.5% P)	1.55	0.62	0.96	0.03	1.55	0.62	0.96	0.03
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
DL-Methionine	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
L-Lysine	0.10	0.10	0.14	0.14	0.10	0.10	0.14	0.14
L-Threonine	0.08	0.08	0.09	0.09	0.08	0.08	0.09	0.09
Choline chloride (60%)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin premix[1]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Mineral premix[2]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Coccidiostat[3]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Selenium premix[4]	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Phytase[5]	---	0.03	---	0.03	---	0.03	---	0.03
Titanium Dioxide	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vermiculite (inert filler)[6]	3.78	4.54	4.12	4.88	3.78	4.54	4.12	4.88
Calculated nutrient content								
Metabolizable energy (kcal/g)	2.84	2.84	2.84	2.84	2.84	2.84	2.84	2.84
Crude protein (%)	19.00	19.00	19.00	19.00	19.00	19.00	19.00	19.00
Calcium (%)	0.76	0.61	0.76	0.61	0.76	0.61	0.76	0.61
Available phosphorus(%)	0.38	0.23	0.38	0.23	0.38	0.23	0.38	0.23
Lysine (%)	1.08	1.08	1.08	1.08	1.08	1.08	1.08	1.08
Methionine (%)	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46
Threonine (%)	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73
Methionine + cysteine (%)	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79
Sodium (%)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20

[1]Vitamin premix supplied the following per kg of diet: 6,614 IU vitamin A, 1,984 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 1.98 mg menadione (K_3), 1.98 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 3.97 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.

[2]Mineral premix supplied the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; cobalt, 1 mg.

[3]Coccidiostat supplied monensin sodium at 99 mg/kg of feed.

[4]Selenium premix provided 0.2 mg Se (as Na_2SeO_3) per kg of diet.

[5]Phytase enzyme added at 300 FTU/kg.

[6]Vermiculite was used as an inert filler.

Table V-4. Effect of soybean meal (SBM) variety, percentage coarse corn (%CC), and phytase on broiler body weight (BW), body weight gain (BWG), feed intake (FI), and adjusted feed conversion ratio (AdjFCR)

Variable	Age	Dietary Treatments									Source of Variability		
		SBM variety[2]		SE[1]	%CC		SE[1]	Phytase (FTU/kg)		SE[1]	SBM	%CC	Phytase
		NP	LP		0	50		0	300				
	(d)	(g)			(g)			(g)			(P-value)		
BW	1	-	-	-	40	40	0.16	40	40	0.2	-	0.88	0.64
	9	-	-	-	216	220	2.49	217	219	2.5	-	0.29	0.70
	22	940	939	11.76	943	935	11.76	932	947	11.8	0.94	0.63	0.40
	35	2171	2108	26	2176	2103	26.08	2138	2141	26.1	0.10	0.07	0.93
BWG	1-9	-	-	-	176	180	2.53	177	178	2.5	-	0.30	0.72
	10-22	720	723	10.67	727	715	10.67	715	728	10.7	0.87	0.44	0.41
	23-35	1231^{a}	1170^{b}	17.54	1233^{a}	1168^{b}	17.54	1203	1195	17.5	0.02	0.02	0.66
	10-35	1952	1892	25.47	1960^{a}	1883^{b}	25.47	1921	1923	25.5	0.11	0.05	0.96
	1-22	-	-	-	903	895	11.78	892	906	11.8	-	0.64	0.41
	1-35	-	-	-	2136	2063	26.09	2098	2101	26.1	-	0.07	0.94
FI	1-9	-	-	-	227	229	4.07	230	226	4.1	-	0.73	0.47
	10-22	1095	1078	11.25	1093	1081	11.25	1093	1081	11.3	0.30	0.46	0.47
	23-35	2094	2064	25.06	2100	2059	25.06	2094	2064	25.1	0.40	0.26	0.40
	10-35	3189	3412	33.45	3192	3139	33.45	3187	3145	33.5	0.33	0.27	0.38
	1-22	-	-	-	1319	1309	13.33	1322	1306	13.3	-	0.60	0.40
	1-35	-	-	-	3419	3368	33.99	3417	3370	34.0	-	0.30	0.34
		(g:g)			(g:g)			(g:g)					
AdjFCR	1-9	-	-	-	1.05	1.04	0.01	1.06	1.04	0.01	-	0.75	0.24
	10-22	1.52	1.49	0.02	1.50	1.51	0.02	1.53	1.49	0.02	0.24	0.68	0.07
	23-35	1.71^{b}	1.77^{a}	0.02	1.71^{B}	1.78^{A}	0.02	1.74	1.73	0.02	0.02	0.01	0.88
	10-35	1.64	1.66	0.02	1.63	1.67	0.02	1.66	1.63	0.02	0.23	0.06	0.30
	1-22	-	-	-	1.37	1.39	0.01	1.39^{a}	1.36^{b}	0.01	-	0.10	0.02
	1-35	-	-	-	1.54^{b}	1.56^{a}	0.01	1.56	1.55	0.01	-	0.04	0.12

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Standard error (SE) for 16 pens containing 16 birds/pen prior to 9 d of age, 13 birds/pen after 9 d, and 10 birds/pen after 22 d.
[2]Soybean meal treatments started at 10 d of age.

Table V-5. Effect of soybean meal (SBM) variety by percentage coarse corn (%CC) interaction on broiler body weight (BW), body weight gain (BWG), feed intake (FI), and adjusted feed conversion ratio (AdjFCR)

Variable	Age	Dietary Treatments					Source of Variability
		NP SBM		LP SBM		SE[2]	SBM×%CC
		0	50	0	50		
	(d)	(g)					(*P*-value)
BW	22	931	949	956	922	16.6	0.14
	35	2191	2152	2162	2055	36.9	0.37
BWG	10-22	716	725	739	706	15.1	0.19
	23-35	1260	1203	1206	1133	24.8	0.74
	10-35	1975	1928	1945	1839	36.0	0.43
FI	10-22	1090	1100	1095	1061	15.9	0.18
	23-35	2111	2078	2088	2040	35.4	0.83
	10-35	3201	3178	3184	3101	47.3	0.54
		(g:g)					
AdjFCR	10-22	1.52	1.52	1.48	1.51	0.02	0.60
	23-35	1.68	1.74	1.73	1.81	0.02	0.75
	10-35	1.62	1.66	1.64	1.69	0.02	0.70

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[2]Standard error (SE) for n=8 pens of 10 birds/pen.

Table V-6. Effect of soybean meal (SBM) variety by phytase interaction on broiler body weight (BW), body weight gain (BWG), feed intake (FI), and adjusted feed conversion ratio (AdjFCR)

Variable	Age	Dietary Treatments					Source of Variability
		NP SBM		LP SBM		SE[2]	SBM×Phytase
		0 FTU/kg	300 FTU/kg	0 FTU/kg	300 FTU/kg		
	(d)			(g)			(P-value)
BW	22	933	947	932	946	16.6	0.98
	35	2094^{B}	2249^{A}	2183^{A}	2034^{B}	36.9	0.001
BWG	10-22	711	729	718	726	15.1	0.72
	23-35	1161^{B}	1302^{A}	1251^{A}	1088^{C}	24.8	0.001
	10-35	1872^{BC}	2031^{A}	1970^{AB}	1815^{C}	36.0	0.001
FI	10-22	1082^{ab}	1108^{a}	1103^{a}	1054^{b}	21.9	0.03
	23-35	2058^{AB}	2131^{A}	2131^{A}	1996^{B}	28.8	0.01
	10-35	3140^{AB}	3239^{A}	3234^{A}	3050^{B}	48.1	0.01
				(g:g)			
AdjFCR	10-22	1.53	1.52	1.54	1.45	0.02	0.10
	23-35	1.78^{AB}	1.64^{C}	1.71^{BC}	1.84^{A}	0.02	0.001
	10-35	1.68^{A}	1.59^{B}	1.64^{AB}	1.68^{A}	0.02	0.01

^{a,b}Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
^{A,B}Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[2]Standard error (SE) for n=8 pens of 10 birds/pen.

Table V-7. Effect of phytase by percentage coarse corn (%CC) interaction on broiler body weight (BW), body weight gain (BWG), feed intake (FI), and adjusted feed conversion ratio (AdjFCR)

Variable	Age	Dietary Treatments					Source of Variability
		0 FTU/kg		300 FTU/kg		SE^2	Phytase×%CC
		0	50	0	50		
	(d)	(g)					(*P*-value)
BW	1	40	40	41	40	0.2	0.64
	9	229^A	206^B	203^B	235^A	3.5	0.001
	22	969^A	896^B	918^B	975^A	16.6	0.001
	35	2219^a	2057^b	2133^{ab}	2149^{ab}	36.9	0.03
BWG	1-9	189^A	165^B	163^B	194^A	3.6	0.001
	10-22	740^a	690^b	715^{ab}	741^a	15.1	0.03
	23-35	1250	1162	1216	1174	24.8	0.35
	10-35	1990	1852	1930	1915	36.0	0.10
	1-22	929^A	855^B	877^B	935^A	16.2	0.001
	1-35	2179^a	2017^b	2093^{ab}	2109^{ab}	36.9	0.03
FI	1-9	242^A	218^B	212^B	239^A	5.8	0.001
	10-22	1132^A	1053^B	1054^B	1108^A	15.2	0.001
	23-35	2134	2055	2065	2062	35.4	0.29
	10-35	3266^a	3108^b	3119^b	3170^{ab}	47.3	0.04
	1-22	1373^A	1271^B	1265^B	1347^A	18.9	0.001
	1-35	3508^a	3326^b	3330^b	3410^{ab}	48.0	0.02
		(g:g)					
AdjFCR	1-9	1.05	1.07	1.05	1.02	0.01	0.35
	10-22	1.53	1.53	1.48	1.49	0.04	0.67
	23-35	1.71	1.78	1.70	1.77	0.03	0.99
	10-35	1.64	1.68	1.62	1.66	0.02	0.96
	1-22	1.38	1.40	1.36	1.37	0.02	0.75
	1-35	1.56	1.57	1.53	1.56	0.01	0.33

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[2]Standard error (SE) for n=8 pens containing 16 birds/pen prior to 9 d of age, 13 birds/pen after 9 d, and 10 birds/pen after 22 d.

Table V-8. Effect of soybean meal (SBM) variety by percentage coarse corn (%CC) by phytase interaction on broiler body weight (BW), body weight gain (BWG), feed intake (FI), and adjusted feed conversion ratio (AdjFCR)

Variable	Age	Dietary Treatments								SE[3]	Source of Variability SBM×%CC× Phytase
		0 %CC				50% CC					
		NP SBM		LP SBM		NP SBM		LP SBM			
		0 FTU/kg	300 FTU/kg	0 FTU/kg	300 FTU/kg	0 FTU/kg	300 FTU/kg	0 FTU/kg	300 FTU/kg		
	(d)	(g)									(*P*-value)
BW	22	958	904	980	931	907	991	884	960	23.5	0.83
	35	2198^{abc}	2184^{abc}	2240^{ab}	2083^{cd}	1990^{d}	2314^{a}	2125^{bd}	1985^{bd}	52.2	0.05
BWG	10-22	729	702	750	727	717	756	687	725	21.3	0.64
	23-35	1240^{A}	1280^{A}	1261^{A}	1152^{B}	1058^{BC}	1323^{A}	1241^{A}	1025^{C}	35.1	0.01
	10-35	1969^{abc}	1982^{abc}	2011^{abc}	1879^{cd}	1775^{d}	2080^{a}	1928^{bc}	1750^{d}	66.9	0.03
FI	10-22	1112	1068	1152	1039	1053	1147	1054	1069	22.5	0.87
	23-35	2130	2093	2139	2038	1986	2170	2124	1955	50.1	0.06
	10-35	1969	1982	2011	1879	1775	2080	1928	1750	51.0	0.13
		(g:g)									
AdjFCR	10-22	1.53	1.52	1.54	1.43	1.52	1.52	1.54	1.47	0.03	0.65
	23-35	1.72^{bc}	1.64^{c}	1.70^{bc}	1.77^{b}	1.84^{a}	1.64^{c}	1.72^{bc}	1.91^{a}	0.04	0.03
	10-35	1.65	1.59	1.64	1.64	1.72	1.60	1.65	1.73	0.03	0.11

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[3]Standard error (SE) for n=4 pens of 10 birds/pen.

Table V-9. Effect of soybean meal (SBM) variety, percentage coarse corn (%CC), and phytase on absolute and relative gizzard and proventriculus weights at 9, 21, and 35 d of age

Variable	Age	SBM variety[2] NP	SBM variety[2] LP	SE[1]	%CC 0	%CC 50	SE[1]	Phytase (FTU/kg) 0	Phytase (FTU/kg) 300	SE[1]	SBM	%CC	Phytase
	(d)	(g)			(g)			(g)			(P-value)		
Gizzard	9	-	-	-	6.13	6.24	0.14	6.07	6.30	0.1	-	0.58	0.25
Proventriculus		-	-	-	1.76	1.70	0.04	1.73	1.73	0.04	-	0.30	0.99
		(g/100g BW)			(g/100g BW)			(g/100g BW)					
Gizzard		-	-	-	2.90	2.90	0.07	2.85	2.95	0.07	-	0.96	0.33
Proventriculus		-	-	-	0.82	0.79	0.02	0.81	0.80	0.02	-	0.20	0.77
		(g)			(g)			(g)					
Gizzard	22	15.42[A]	13.81[B]	0.34	14.67	14.55	0.34	14.99	14.24	0.3	0.01	0.79	0.12
Proventriculus		3.92[a]	3.68[b]	0.02	3.95[A]	3.65[B]	0.08	3.85	3.75	0.1	0.03	0.01	0.38
		(g/100g BW)			(g/100g BW)			(g/100g BW)					
Gizzard		1.59[a]	1.47[b]	0.04	1.50	1.57	0.04	1.57	1.50	0.04	0.02	0.12	0.14
Proventriculus		0.41	0.39	0.01	0.40	0.40	0.01	0.40	0.40	0.01	0.20	0.52	0.47
		(g)			(g)			(g)					
Gizzard	35	27.85	27.14	0.64	27.56	27.42	0.64	27.85	27.14	0.6	0.44	0.88	0.44
Proventriculus		6.60	6.46	0.1	6.43	6.63	0.1	6.58	6.48	0.1	0.35	0.17	0.47
		(g/100g BW)			(g/100g BW)			(g/100g BW)					
Gizzard		1.31	1.30	0.03	1.31	1.30	0.03	1.32	1.29	0.03	0.76	0.98	0.60
Proventriculus		0.31	0.31	0.01	0.30	0.32	0.01	0.31	0.31	0.01	0.81	0.10	0.74

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Standard error (SE) for n=16 pens using 3 birds/pen.
[2]Soybean meal treatments started at 9 d of age.

Table V-10. Effect of soybean meal (SBM) variety by percentage coarse corn (%CC) interaction on absolute and relative gizzard and proventriculus weights at 22 and 35 d of age

Variable	Age	Dietary Treatments					Source of Variability
		NP SBM		LP SBM		SE[2]	SBM×%CC
		0	50	0	50		
	(d)	(g)					(P-value)
Gizzard	22	15.44	15.40	13.91	13.70	0.5	0.85
Proventriculus		4.03	3.81	3.87	3.50	0.1	0.50
		(g/100g BW)					
Gizzard		1.54	1.64	1.45	1.50	0.05	0.66
Proventriculus		0.40	0.41	0.40	0.38	0.01	0.21
		(g)					
Gizzard	35	28.12	27.58	27.00	27.27	0.9	0.66
Proventriculus		6.36	6.84	6.50	6.42	0.1	0.06
		(g/100g BW)					
Gizzard		1.33	1.29	1.28	1.32	0.05	0.35
Proventriculus		0.30	0.32	0.31	0.31	0.01	0.25

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).

[2]Standard error (SE) for n=8 pens using 3 birds/pen.

Table V-11. Effect of soybean meal (SBM) variety by phytase interaction on absolute and relative gizzard and proventriculus weights at 22 and 35 d of age

Variable	Age	Dietary Treatments					Source of Variability
		NP SBM		LP SBM		SE[2]	SBM×Phytase
		0 FTU/kg	300 FTU/kg	0 FTU/kg	300 FTU/kg		
	(d)	(g)					(*P*-value)
Gizzard	22	15.18^{A}	15.66^{A}	14.79^{A}	12.82^{B}	0.5	0.01
Proventriculus		3.98	3.87	3.72	3.64	0.1	0.90
		(g/100g BW)					
Gizzard		1.61	1.58	1.53	1.42	0.05	0.38
Proventriculus		0.42^{a}	0.39^{b}	0.38^{b}	0.40^{b}	0.01	0.03
		(g)					
Gizzard	35	27.36	28.34	28.34	25.94	0.9	0.07
Proventriculus		6.73	6.46	6.44	6.49	0.1	0.26
		(g/100g BW)					
Gizzard		1.30	1.33	1.34	1.26	0.05	0.23
Proventriculus		0.32	0.30	0.30	0.32	0.01	0.07

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[2]Standard error (SE) for n=8 pens using 3 birds/pen.

Table V-12. Effect of phytase by percentage coarse corn (%CC) interaction on absolute and relative gizzard and proventriculus weights at 9, 22, and 35 d of age

Variable	Age	Dietary Treatments					Source of Variability
		0 FTU/kg		300 FTU/kg		SE[2]	Phytase×%CC
		0	50	0	50		
	(d)	(g)					(*P*-value)
Gizzard	9	6.31^{AB}	5.82^{B}	5.94^{B}	6.65^{A}	0.2	0.01
Proventriculus		1.92^{A}	1.54^{B}	1.60^{B}	1.86^{A}	0.06	0.001
		(g/100g BW)					
Gizzard		2.74^{b}	2.95^{ab}	3.05^{b}	2.85^{ab}	0.10	0.05
Proventriculus		0.84	0.78	0.81	0.80	0.02	0.40
		(g)					
Gizzard	22	15.20	14.77	14.15	14.32	0.5	0.53
Proventriculus		4.00	3.70	3.90	3.60	0.1	0.98
		(g/100g BW)					
Gizzard		1.50	1.64	1.49	1.50	0.05	0.19
Proventriculus		0.39^{B}	0.41^{A}	0.41^{A}	0.38^{B}	0.01	0.01
		(g)					
Gizzard	35	27.04	28.66	28.08	26.19	0.9	0.06
Proventriculus		6.56	6.61	6.31	6.65	0.1	0.32
		(g/100g BW)					
Gizzard		1.27	1.36	1.34	1.25	0.05	0.06
Proventriculus		0.31	0.31	0.30	0.32	0.01	0.47

^{a,b}Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
^{A,B}Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[2]Standard error (SE) for n=8 pens using 3 birds/pen.

Table V-13. Effect of soybean meal (SBM) variety by percentage coarse corn (%CC) by phytase interaction on absolute and relative gizzard and proventriculus weights at 22 and 35 d of age

Variable	Age	0 %CC				50% CC				SE[3]	Source of Variability
		NP SBM		LP SBM		NP SBM		LP SBM			SBM×%CC× Phytase
		0 FTU/kg	300 FTU/kg	0 FTU/kg	300 FTU/kg	0 FTU/kg	300 FTU/kg	0 FTU/kg	300 FTU/kg		
	(d)	(g)									(*P*-value)
Gizzard	22	15.01	15.87	15.39	12.43	15.35	15.45	14.20	13.20	0.7	0.16
Proventriculus		4.21	3.86	3.79	3.95	3.75	3.87	3.66	3.34	0.4	0.08
		(g/100g BW)									
Gizzard		1.47	1.62	1.53	1.36	1.75	1.54	1.54	1.47	0.90	0.07
Proventriculus		0.41	0.39	0.37	0.43	0.43	0.39	0.39	0.37	0.01	0.21
		(g)									
Gizzard	35	26.58	29.66	27.15	26.50	28.14	27.02	29.17	25.37	1.3	0.70
Proventriculus		6.55	6.17	6.57	6.44	6.92	6.76	6.31	6.54	0.2	0.81
		(g/100g BW)									
Gizzard		1.27	1.40	1.28	1.28	1.33	1.25	1.40	1.24	0.06	0.74
Proventriculus		0.31	0.29	0.30	0.31	0.33	0.31	0.30	0.32	0.01	0.84

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).

[3]Standard error (SE) for n=4 pens using 3 birds/pen.

Table V-14 . Effect of soybean meal (SBM) variety, percentage coarse corn (%CC), and phytase on total P digestibility, breaking strength of tibia, and percentage bone ash

Variable	Dietary Treatments									Source of Variability		
	SBM variety		SE[1]	%CC		SE[1]	Phytase (FTU/kg)		SE[1]	SBM	%CC	Phytase
	NP	LP		0	50		0	300				
	(% DM[4])									(*P*-value)		
Total P digestibility	82.55[B]	90.00[A]	0.85	87.94[a]	84.61[b]	0.85	81.48[B]	91.07[A]	0.9	0.0001	0.02	0.0001
Bone ash	54.97[a]	53.55[b]	0.49	54.02	54.50	0.49	55.25[A]	53.28[B]	0.5	0.05	0.49	0.01
	(N[5])											
Breaking strength	323.70	314.66	6.24	318.70	319.66	6.24	338.74[A]	299.62[B]	6.2	0.31	0.91	0.0001

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Standard error (SE) for n= 16 pens using 3 birds/pen.
[4]Percent DM of feed and ileal samples were used to calculate nutrient digestibility on dry matter basis. Ash was calculated from dried bones.
[5]Newton (N) represented maximum force required to break the bones.

Table V-15. Effect of soybean meal (SBM) variety by perecntage coarse corn (%CC) interaction on total P digestibility, breaking strength of tibia, and percentage bone ash

Variable	Dietary Treatments					Source of Variability
	NP SBM		LP SBM		SE[2]	SBM×%CC
	0%CC	50%CC	0%CC	50%CC		
	(% DM[4])					(*P*-value)
Total P digestibility	84.21	80.90	91.67	88.33	1.2	0.99
Bone ash	54.33	55.61	53.72	53.39	0.7	0.25
	(N[5])					
Breaking strength	318.24	329.17	319.16	310.16	8.8	0.26

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).

[2]Standard error (SE) for n= 8 using 3 birds/pen.

[4]Percent DM of feed and ileal samples were used to calculate nutrient digestibility on dry matter basis. Ash was calculated from dried bones.

[5]Newton (N) represented maximum force required to break the bones.

Table V-16. Effect of soybean meal (SBM) variety by phytase interaction on total P digestibility, breaking strength of tibia, and percentage bone ash

Variable	Dietary Treatments					Source of Variability
	NP SBM		LP SBM			SBM×Phytase
	0 FTU/kg	300 FTU/kg	0 FTU/kg	300 FTU/kg	SE[2]	
	(% DM[4])					(*P*-value)
Total P digestibility	76.33[c]	88.78[b]	86.63[b]	93.37[a]	1.2	0.03
Bone ash	54.75[A]	55.19[A]	55.75[A]	51.36[B]	0.7	0.001
	(N[5])					
Breaking strength	328.21[AB]	319.19[B]	349.27[A]	280.04[C]	8.8	0.001

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[2]Standard error (SE) for n= 8 pens using 3 birds/pen.
[4]Percent DM of feed and ileal samples were used to calculate nutrient digestibility on dry matter basis. Ash was calculated from dried bones.
[5]Newton (N) represented maximum force required to break the bones.

Table V-17. Effect of phytase by percentage coarse corn (%CC) interaction on total P digestibility, breaking strength of tibia, and percentage bone ash

Variable	Dietary Treatments				SE[2]	Source of Variability
	0 FTU/kg		300 FTU/kg			Phytase×%CC
	0%CC	50%CC	0%CC	50%CC		
	(% DM[4])					(*P*-value)
Total P digestibility	84.46[b]	78.50[c]	91.42[a]	90.73[a]	1.2	0.04
Bone ash	54.53	55.97	53.52	53.04	0.7	0.17
	(N[5])					
Breaking strength	344.19	333.30	293.21	306.03	8.8	0.18

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[2]Standard error (SE) for n= 8 pens using 3 birds/pen.
[4]Percent DM of feed and ileal samples were used to calculate nutrient digestibility on dry matter basis. Ash was calculated from dried bones.
[5]Newton (N) represented maximum force required to break the bones.

Table V-18. Effect of soybean meal (SBM) variety by percentage coarse corn (%CC) by phytase interaction on total P digestibility, breaking strength of tibia, and percentage bone ash

Variable	Dietary Treatments									Source of Variability
	0 %CC				50% CC					
	NP SBM		LP SBM		NP SBM		LP SBM			
	0 FTU/kg	300 FTU/kg	0 FTU/kg	300 FTU/kg	0 FTU/kg	300 FTU/kg	0 FTU/kg	300 FTU/kg	SE[3]	SBM×%CC ×Phytase
	(% DM[4])									(*P*-value)
Total P digestibility	80.15	88.28	88.77	94.57	72.52	89.28	84.49	92.17	1.7	0.17
Bone ash	53.48	55.17	55.57	51.87	56.10	55.21	55.93	50.86	1.0	0.69
	(N[5])									
Breaking Strength	334.53	301.95	353.85	284.47	321.90	336.44	344.70	275.61	12.5	0.19

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[3]Standard error (SE) for n= 4 pens using 3 birds/pen.
[4]Percent DM of feed and ileal samples were used to calculate nutrient digestibility on dry matter basis. Ash was calculated from dried bones.
[5]Newton (N) represented maximum force required to break the bones.

Table V-19. Total phosphorus (P) content relative to sieve fraction in ground corn

Corn particle size (μm)	P content	Sieve fraction[1]
Hammermill (Fine corn)	——— (%) ———	
< 297	0.32	30.6
298-1190	0.34	63.0
> 1190	0.39	6.4
Roller mill (Coarse corn)		
< 297	0.14	8.0
298-1190	0.29	19.7
> 1190	0.34	72.3

[1]Particles below 297 μm were collected between sieve number 50 and the pan, particles between 298-1190 μm were collected from sieve numbers 16-50, and particles above 1190 μm were collected from sieve number above 16. ASAE S319.3 (ASAE, 2003).

CHAPTER VI

Effect of adding phytase to low phytate low stachyose and normal phytate SBM diets on broiler live performance, total phosphorus digestibility, and bone ash

6.1. ABSTRACT

This study evaluated the effects of phytase in diets containing normal phytate (NP) versus low phytate (LP) low stachyose (LS) soybean meal (LP-LS SBM) on BW, feed intake, FCR, total phosphorus (P) digestibility, and bone ash of broilers grown to 35 d of age. A total of 216 Ross 708 female broiler chicks were assigned to 36 cages in 2 batteries with 6 birds per cage. From 1 to 21 d, a common corn-SBM starter diet was fed. From 22 to 35 d, a 2 × 3 factorial arrangement of SBM type (LP-LS and NP SBM) and phytase additions were used with 6 cages per diet. Phytase treatments were no phytase (CON), matrix phytase in which phytase was given a matrix value (MX), and on-top (OT) phytase in which phytase was not given a matrix value. Birds and feed were weighed at 1, 21, 28, and 35 d of age, and all birds were necropsied at 35 d of age to determine gizzard and proventriculus weights, total P digestibility, and percentage bone ash. Data from 22 to 35 d were analyzed as a randomized complete block design. The BW gain was greater ($P \leq 0.05$) in birds that consumed LP-LS SBM from 22 to 35 d (763 versus 733 g), which was reflected in an improved ($P \leq 0.05$) FCR by LP SBM for the same period (1.93 versus 2.00 g:g). Feeding LP-LS SBM produced smaller ($P \leq 0.0001$) gizzard weight (18.71 versus 20.32) compared to NP SBM. Phytase had no effect on BW, BW gain, feed intake, FCR, and gizzard or proventriculus weights. Total P

digestibility was increased ($P \leq 0.0001$) when birds were fed NP SBM (68.52 versus 58.39 %), which was associated with improved ($P \leq 0.001$) bone ash. Adding phytase either as MX or OT produced better ($P \leq 0.0001$) total P digestibility compared to non phytase diet (65.55 and 68.55 versus 56.26 %). Based on these results, it was concluded that feeding LP-LS SBM improved BW gain and FCR. Furthermore, gizzard weight was reduced in birds consuming LP-LS SBM, which could be related to reduced digestive demand and led to improved live performance. Giving a matrix value for phytase improved total P digestibility, although the improvement in live performance did not reach significant levels.

Key words: Broilers, normal phytate SBM, low phytate SBM , phytase

6.2. INTRODUCTION

Dietary energy in poultry diets has a significant cost. A large portion of corn production has been diverted for ethanol production and oil from oilseeds have also been moved away from animal agriculture to satisfy biodiesel production demands (Donohue and Cunningham, 2009). These trends have intensified efforts to identify strategies to enhance energy utilization of cereal grains and oilseed meals have been investigated since dietary energy affects the cost of feed production. Several strategies have been implemented to reduce the concentration of raffinose and stachyose of SBM to enhance dietary energy utilization with poultry (Coon et al., 1990; Parsons et al., 2000; Ghazi et al., 2003) because stachyose and raffinose were two types of oligosaccharides that cannot be cleaved due to an absence of α-1,6-galactosidase in the intestinal tract of poultry (Zuo et al., 1996). Novel soybean varieties have been genetically selected for reduced raffinose and stachyose content, which may lead to increased apparent metabolizable energy and higher concentrations of digestible amino acids for broilers fed such meals (Parsons et al., 2000; Baker et al., 2011; Perryman and Dozier, 2012) thus allowing nutritionists to formulate diets with lower inclusions of supplemental fat. The presence of phytate in the diet has been reported to limit the availability of P and other minerals due to chelation (Biehl et al., 1995; Traylor et al., 2001). Phytate has decreased nutrient and mineral availability due to the lack of phytase in the gastrointestinal tract of monogastric animals (Cosgrove and Irving, 1980).

Over-processing of SBM will lead to reduced lysine and cysteine digestibility and subsequently to a reduced growth performance of broilers (Parsons et al., 1992). Adding microbial phytase to broiler diets often increased apparent digestibility of protein and amino acids as well as enhanced the utilization of phytate phosphorus (Sebastian et al., 1997). Therefore, the objective of this study was to evaluate the effects of different varieties of SBM in the presence or absence of phytase enzyme on live performance, total P digestibility, and percentage bone ash of broiler females raised to 35 d.

6.3. MATERIALS AND METHODS

Dietary Treatments

This study was conducted at North Carolina State University, Prestage Department of Poultry Science. A total of 6 corn-SBM based dietary treatments were tested with 2 SBM varieties (low phytate low stachyose (LP-LS) and normal phyate (NP)), and 3 phytase enzyme treatments that utilized 300 FTU/kg *Escherichia coli*-derived commercial-grade phytase enzyme (Danisco Animal Nutrition, Malborough, UK). One FTU has been generally defined as the amount of enzyme required to liberate 1 μmol of inorganic P min^{-1} from 5.5 m*M* of Na phytate at pH 5.5 and 37º C (Qian et al., 1997). Table 1 illustrates the chemical composition of the different SBM varieties. These identity preserved SBM were slightly under processed to preserve their nutrients as indicated by the urease activity values. From 1-21 d, all birds were fed a common starter diet with commercial NP SBM (Table 2). From 22-35 d, birds were fed one of six grower diets (Table 3). Titanium dioxide (TiO_2) was included in grower diets as an indigestible marker to allow for the determination of apparent nutrient digestibility. The starter diets were mixed in a horizontal twin shaft ribbon mixer (TRDB126-0604, Hayes and Stolz Ind. Mfg. Co., Fort Worth, TX) to produce the mash diets that were conditioned at 85°C for 45 seconds, and then pelleted with a ring die (4.4 mm by 35 mm) pellet mill (Model PM1112-2, California Pellet Mill Co., Crawfordsville, IN). Pellets were cooled with ambient air in a counter-flow cooler (Model VK09 × 09KL, Geelen Counterflow USA Inc., Orlando, FL) and crumbled. The grower diets were fed in mash form diet with the corn ground in a hammermill (Model 1522, Roskamp Champion, Waterloo, IA) with an average particle size of 400 μm. Particle size distribution was determined by ASAE S319.3 (ASAE, 2003). All diets were mixed in a horizontal twin shaft ribbon mixer (Davis and Sons, Bonner Spring, Kansas) and each batch was sampled and analyzed individually. The three phytase treatments were either no phytase (CON), matrix phytase in which phytase was given a matrix value (MX), and on-top (OT) phytase in which phytase was not given a matrix value. When employed, phytase was given a matrix value of 0.15% for both calcium (Ca) and available phosphorus (AvP). These phytase treatments were in 2 × 3 factorial treatment design with either NP or LP SBM.

Birds Management and Data Collection

Care of the birds in this study conformed to the Guide for Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010). Eggs from a 40-wk-old Ross 344 × 708SF (Aviagen, Huntsville, AL) breeder flock maintained at the site were collected and incubated under standard conditions. A total of 216 female chicks were hatched, sexed, permanently identified with neck tags, and randomly distributed among 2 battery cages in 36 cages with 6 birds per cage (56×68×36 cm length×width×height, respectively). The lighting program during the first 7 d was 23 h of light that was reduced to 21 h of light up to 21 d.

After 21 d of age, only 14 h of light was used. The initial room temperature was set at 32°C and gradually reduced to 25°C. Feed and water were provided for *ad libitum* consumption. Feed was covered with plastic screen wires that had openings of 2.54 x 2.54 cm to reduce feed wastage, and screens were moved three times daily to stimulate feed consumption. Feeders and feed were weighed at the start and end of each feeding period.

Birds were weighed individually at 15 d and redistributed by BW among cages to create uniform BW distribution between the cages. Group BW and feed intake were measured at 1, 21, 28, and 35 d, and BW gain and feed conversion ratio (FCR) were calculated by time interval. There was no mortality during the experimental period. At 35 d of age, birds were killed by cervical dislocation, gizzard and proventriculus were necropsied and weighed and tibia bones were excised for ashing, bones were ashed at 600°C overnight in a muffle furnace (Hall et al., 2003). The terminal 13 cm of ileum was removed 3 cm anterior to the ileo-cecal junction, ileal contents were gently expressed, pooled per cage, and frozen for further nutrient digestibility determination.

Chemical Analyses

The TiO_2 of diets and ileal samples were determined using the method of Myers et al. (2004). Frozen samples of feed were dried in an oven for 24 h at 95 to 100°C for dry matter determination (Method 934.01, AOAC, 2006). The dry matter percentage of ileal digesta was determined by freeze drying (Virtis Freezemobile - Model 12XL, Warminster, PA). Samples of feed and dried ileal digesta were ground (<2 mm) prior to analysis. Diets and ileal samples were analyzed for total P.

Statistical Methods

A randomized complete block design with a 2 × 3 factorial arrangement of treatments was used (SBM × Phytase). The general linear model of SAS (2009; SAS version 9.4, SAS institute, Cary, NC, USA) was used to analyze measured variables. Variable means were partitioned by LSMEANS and were considered statistically different when $P \leq 0.05$, although effects with P-values between 0.06 and 0.10 were mentioned in the text when the data suggested a numerical trend. Cage averages for all birds in a single cage (6 birds/cage) were experimental unit. The experimental unit for statistical analysis of measured parameters was cage and each battery was block.

6.4. RESULTS AND DISCUSSION

Effect of Dietary Treatments on Live Performance

The effect of SBM variety and phytase on BW, BW gain, feed intake, and FCR at 1, 22, 28, and 35 d of age is shown in Table 4. The LP-LS SBM was included in the diets from 21 d. The BW gain was greater in birds that consumed LP-LS SBM from 22-35 and from 29-35 d of age ($P \leq 0.05$) which was reflected in an improved FCR ($P \leq 0.05$) for the same time periods. This was associated with raffinose and stachyose content in LP-LS SBM which seemed to be more effective than supplying dietary exogenous galactosidase enzyme (Angel et al., 1988; Irish et al., 1995). These results were similar to Perryman et al. (2013) who reported that feeding low oligosaccharide SBM to conventional roosters from 1-14 d improved BW gain and FCR compared to conventional SBM. The same research group illustrated that feeding ultra-low oligosaccharide SBM resulted in better live performance compared to either low oligosaccharide SBM or conventional SBM when fed to broilers from 1-28 and 1-42 d of age. The feed intake and BW of broilers were not affected by SBM varieties at 28 and 35 d of age. Phytase had no effect on BW, BW gain, feed intake, and FCR. However, according to Hall et al. (2003), adding phytase to corn-SBM based diet improved live performance when phytase was added form 0 to 6,000 FTU/kg, showing the expected increase in efficiency. Commercial-grade phytase was used in this experiment compared to research-grade phytase in previuos experiments which probably contributed to differents and results obtained with regard to FCR. The same grades of phytase were used with broiler breeder where egg production differed based on phytase grade (un published data). The interaction effect of SBM variety and phytase on broiler BW, BW gain, feed intake, and AdjFCR is shown in Table 5. There was no SBM variety × phytase interaction effect on broiler BW, BW gain, feed intake, and FCR.

Effect of Dietary Treatments on Organ Weights

Table 6 shows the effect of SBM variety and phytase on absolute and relative gizzard and proventriculus weights measured at 35 d of age. Absolute gizzard weight was smaller ($P \leq 0.0001$) in LP-LS SBM diet, probably due to less digestive demand elicited by the LP-LS SBM. A similar trend was observed for the relative gizzard weight (g/100g), which was smaller ($P \leq 0.0001$) in birds that consumed LP-LS SBM. On the other hand, absolute and relative proventriculus weights were comparable among SBM varieties. According to

Aderemi (2003), the enlargement of liver and pancreas when fed cassava root diets to pullets was possibly due to compensatory metabolic activities aimed to reduce intrinsic anti-nutritional factors. Absolute and relative gizzard and proventriculus weights were not affected by phytase treatments (Table 6). The interaction effect of SBM variety and phytase on absolute and relative gizzard and proventriculus weights is shown in Table 7. There was no effect also of the combination of SBM and phytase on measured organs at 35 d of age.

Effect of Dietary Treatments on Total Phosphorus Digestibility and Bone Ash

The effect of SBM variety and phytase on total P digestibility and bone ash percentage at 35 d of age is shown in Table 8. Birds that consumed NP SBM diet had improved P digestibility ($P \leq 0.0001$) and increased ($P \leq 0.001$) percentage bone ash compared to the LP-LS SBM diet. Moreover, Parson et al. (2000) showed that feeding low oligosacharride SBM to broilers improved raffinose and stachyose digestibility compared to conventional SBM, however this improvement did not reach statistical difference. Phytase improved total P digestibility. Feeding diets with the phytase OT treatment resulted in improved P digestibility ($P \leq 0.0001$) followed by the phytase matrix (MX) treatment. Our results were in agreement with Namkung and Leeson (1999) who reported that feeding broiler diets with phytase exhibited greater apparent metabolizable energy digestibility broilers compared to control diets. However, Koher et al. (2000) reported that feeding broilers a high dosage of ß-galactanase added to SBM resulted in improved apparent metabolizable energy and reduced ileal protein digestibility. The effect of SBM variety and phytase interaction on total P digestibility and bone ash percentage at 35 d of age is shown in

Table 9. There was no interaction effect of SBM varieties and phytase treatments on total P digestibility and percentage bone ash.

6.5. CONCLUSION

Based on these results, it was concluded that feeding LP-LS SBM improved BW gain and FCR. Gizzard weight was reduced in birds consuming LP-LS SBM, which could be related to reduced digestive demand. Thiis was probably due to the presence of more digestible nutrients and less stachyose in LP-LS SBM, which required less grinding. The gizzard may no have been working as a passage rate regulator under these conditions since particle size of corn was small. The LP-LS SBM diets had less total P than expected that was reflected in a reduced total P digestibility for these diets. Therefore, it was considered important to balance both total P and available P when using LP-LS SBM or other LP grains. Using a matrix value for phytase improved total P digestibility but the improvement in broiler live performance did not reach significance.

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Table VI-1. Chemical analysis[1] of soybean meal (SBM) varieties

Composition	Commercial NP SBM	NP SBM	LP-LS SBM
Urease value	0.01	0.41	0.21
		(%)	
Moisture	11.43	11.49	10.20
Protein	48.73	50.81	50.27
Fiber	2.60	5.10	5.10
Ash	6.12	5.58	5.75
Total Phosphorus	0.66	0.71	0.77
Calcium	0.29	0.25	0.17

[1]Results obtained from Carolina Analytical Services laboratories.

Table VI-2. Starter diet used to 21 d of age

Ingredients	Common starter
	(%)
Corn[1]	59.60
Soybean meal (48%CP)	35.11
Poultry fat	1.00
Limestone	0.78
Dicalcium phosphate (18.5%P)	2.02
Salt	0.50
DL-Methionine	0.24
L-Lysine	0.09
L-Threonine	0.11
Choline chloride (60%)	0.20
Vitamin premix[2]	0.05
Mineral premix[3]	0.20
Coccidiostat[4]	0.05
Selenium premix[5]	0.05
Calculated nutrient content	
Metabolizable energy (kcal/g)	2.85
Crude protein (%)	22.00
Calcium (%)	0.90
Available phosphorus (%)	0.45
Lysine (%)	1.26
Methionine (%)	0.58
Threonine (%)	0.84
Methionine + cysteine (%)	0.92
Sodium (%)	0.20

[1]Corn was ground with a hammermill to an average particle size of 400μm.
[2]Vitamin premix supplied the following per kg of diet: 6,614 IU vitamin A, 1,984 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 1.98 mg menadione (K_3), 1.98 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 3.97 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.
[3]Mineral premix supplied the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; cobalt, 1 mg.
[4]Coccidiostat supplied monensin sodium at 99 mg/kg of feed.
[5]Selenium premix provided 0.2 mg Se (as Na_2SeO_3) per kg of diet.

Table VI-3. Grower diets used from 22 to 35 d of age

Ingredients	Dietary Treatments[1]					
	NP SBM			LP-LS SBM		
	0 FTU/kg (CON)	300 FTU/kg (MX)	300 FTU/kg (OT)	0 FTU/kg (CON)	300 FTU/kg (MX)	300 FTU/kg (OT)
	(%)					
Corn[2]	66.00	66.00	66.00	66.24	66.24	66.24
Soybean meal	27.10	27.10	27.10	26.60	26.60	26.60
Poultry fat	0.50	0.50	0.50	0.50	0.50	0.50
Limestone	0.81	0.95	0.81	1.20	1.35	1.20
Dicalcium phosphate (18.5%P)	1.52	0.59	1.52	0.93	0.00	0.93
Salt	0.50	0.50	0.50	0.50	0.50	0.50
DL-Methionine	0.15	0.15	0.15	0.15	0.15	0.15
L-Lysine	0.10	0.10	0.10	0.14	0.14	0.14
L-Threonine	0.08	0.09	0.08	0.09	0.09	0.09
Choline chloride (60%)	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin premix[3]	0.05	0.05	0.05	0.05	0.05	0.05
Mineral premix[4]	0.20	0.20	0.20	0.20	0.20	0.20
Coccidiostat[5]	0.05	0.05	0.05	0.05	0.05	0.05
Selenium premix[6]	0.05	0.05	0.05	0.05	0.05	0.05
Phytase[7]	0.00	0.02	0.02	0.00	0.02	0.02
Titanium Dioxide	0.50	0.50	0.50	0.50	0.50	0.50
Vermiculite (inert filler)[8]	2.19	2.95	2.17	2.60	3.36	2.58
Calculated nutrient content						
Metabolizable energy (kcal/g)	2.87	2.87	2.87	2.87	2.87	2.87
Crude protein (%)	19.00	19.00	19.00	19.00	19.00	19.00
Calcium (%)	0.76	0.61	0.76	0.76	0.61	0.76
Available phosphorus (%)	0.38	0.23	0.38	0.38	0.23	0.38
Lysine (%)	1.08	1.08	1.08	1.08	1.08	1.08
Methionine (%)	0.46	0.46	0.46	0.46	0.46	0.46
Threonine (%)	0.73	0.73	0.73	0.73	0.73	0.73
Methionine + cysteine (%)	0.79	0.79	0.79	0.79	0.79	0.79
Sodium (%)	0.20	0.20	0.20	0.20	0.20	0.20

[1]Either NP or LP-LS SBM in combination of three phytase treatments were either no phytase (CON), matrix phytase in which phytase was given a matrix value (MX), and on-top (OT) phytase in which phytase was not given a matrix value.
[2]Corn was ground with a hammermill to an average particle size of 400µm.
[3]Vitamin premix supplied the following per kg of diet: 6,614 IU vitamin A, 1,984 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 1.98 mg menadione (K_3), 1.98 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 3.97 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.
[4]Mineral premix supplied the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; cobalt, 1 mg. [5]Coccidiostat supplied monensin sodium at 99 mg/kg of feed. [6]Selenium premix provided 0.2 mg Se (as Na_2SeO_3) per kg of diet. [7]Phytase enzyme added at 300 FTU/kg. [8]Vermiculite was used as an inert filler.

Table VI-4. Effect of soybean meal (SBM) variety, and phytase on broiler body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR)

Variable	Age	Dietary Treatments							Source of Variability	
		SBM variety			Phytase FTU/kg[1]				SBM	Phytase
		NP	LP-LS	SE[2]	0 FTU/kg (CON)	300 FTU/kg (MX)	300FTU/kg (OT)	SE[3]	*P*-value	
	(d)	(g)								
BW	28	1234	1220	8.6	1221	1230	1231	10.6	0.29	0.79
	35	1601	1625	11.4	1597	1630	1612	14.0	0.15	0.26
BWG	22-28	366	359	8.6	365	359	363	10.6	0.57	0.92
	29-35	368[b]	404[a]	12.3	376	400	382	15.1	0.05	0.49
	22-35	733[b]	763[a]	10.7	740	759	745	13.2	0.05	0.57
FI	22-28	730	734	4.4	730	736	730	5.4	0.54	0.69
	29-35	729	736	4.7	729	739	728	5.8	0.32	0.36
	22-35	1459	1469	6.4	1459	1475	1458	7.8	0.25	0.26
		(g:g)								
FCR	22-28	2.00	2.09	0.06	2.00	2.11	2.02	0.08	0.35	0.58
	29-35	2.02[a]	1.84[b]	0.06	1.99	1.88	1.91	0.07	0.05	0.53
	22-35	2.00[a]	1.93[b]	0.03	1.99	1.94	1.96	0.03	0.05	0.67

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).

[1]The three phytase treatments were either no phytase (CON), matrix phytase in which phytase was given a matrix value (MX), and on-top (OT) phytase in which phytase was not given a matrix value.

[2]Standard error (SE) for n= 18 cages with 6 birds per cage.

[3]Standard error (SE) for n= 12 cages with 6 birds per cage.

Table VI-5. Effect of soybean meal (SBM) variety by phytase interaction on broiler body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR)

Variable	Age	Dietary Treatments[1]						SE[2]	Source of Variability
		NP SBM			LP-LS SBM				SBMxPhytase
		0 FTU/kg (CON)	300 FTU/kg (MX)	300 FTU/kg (OT)	0 FTU/kg (CON)	300 FTU/kg (MX)	300 FTU/kg (OT)		*P*-value
	(d)	(g)							
BW	28	1230	1241	1230	1213	1219	1231	15.0	0.72
	35	1578	1623	1604	1616	1638	1621	19.9	0.83
BWG	22-28	371	368	358	358	350	368	15.1	0.61
	29-35	348	381	374	404	419	390	21.5	0.67
	22-35	719	750	732	762	769	758	18.7	0.81
FI	22-28	731	732	727	729	739	733	7.7	0.82
	29-35	716	741	730	743	737	727	8.3	0.12
	22-35	1447	1473	1457	1472	1477	1460	11.1	0.54
		(g:g)							
FCR	22-28	1.97	1.99	2.03	2.03	2.23	2.00	0.11	0.45
	29-35	2.14	1.95	1.96	1.85	1.80	1.87	0.10	0.61
	22-35	2.04	1.97	1.99	1.93	1.92	1.93	0.05	0.83

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[1]Either NP or LP-LS SBM in combination of three phytase treatments were either no phytase (CON), matrix phytase in which phytase was given a matrix value (MX), and on-top (OT) phytase in which phytase was not given a matrix value.
[2]Standard error (SE) for n=6 cages with 6 birds per cage.

Table VI-6. Effect of soybean meal (SBM) variety and phytase on absolute and relative gizzard and proventriculus weights at 35 d of age

Variable	Age	Dietary Treatments							Source of Variability	
		SBM variety			Phytase FTU/kg[1]				SBM	Phytase
		NP	LP-LS	SE[2]	0 FTU/kg (CON)	300 FTU/kg (MX)	300 FTU/kg (OT)	SE[3]	*P*-value	
	(d)	(g)								
Gizzard	35	20.32[A]	18.71[B]	0.2	19.17	19.52	19.85	0.3	0.0001	0.28
Proventriculus		4.57	4.57	0.1	4.59	4.48	4.63	0.1	0.99	0.62
		(g/100g BW)								
Gizzard		1.20[A]	1.10[B]	0.01	1.30	1.15	1.17	0.03	0.0001	0.34
Proventriculus		0.27	0.27	0.01	0.27	0.26	0.27	0.01	0.99	0.56

[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]The three phytase treatments were either no phytase (CON), matrix phytase in which phytase was given a matrix value (MX), and on-top (OT) phytase in which phytase was not given a matrix value.
[2]Standard error (SE) for n=109 birds form a total of 18 cages.
[3]Standard error (SE) for n=72 birds form a total of 12 cages.

Table VI-7. Effect of soybean meal (SBM) variety by phytase interaction on absolute and relative gizzard and proventriculus weights at 35 d of age

Variable	Age	Dietary Treatments[1]						SE[2]	Source of Variability
		NP SBM			LP-LS SBM				SBMxPhytase
		0 FTU/kg (CON)	300 FTU/kg (MX)	300 FTU/kg (OT)	0 FTU/kg (CON)	300 FTU/kg (MX)	300 FTU/kg (OT)		*P*-value
	(d)				(g)				
Gizzard	35	19.86	20.78	20.31	18.49	18.25	19.40	0.4	0.14
Proventriculus		4.59	4.51	4.59	4.58	4.45	4.68	0.2	0.89
					(g:g)				
Gizzard		1.17	1.23	1.19	1.09	1.07	1.14	0.03	0.07
Proventriculus		0.27	0.27	0.27	0.27	0.26	0.27	0.01	0.79

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[1]Either NP or LP-LS SBM in combination of three phytase treatments were either no phytase (CON), matrix phytase in which phytase was given a matrix value (MX), and on-top (OT) phytase in which phytase was not given a matrix value.
[2]Standard error (SE) for n=36 birds form a total of 6 cages.

Table VI-8. Effect of soybean meal (SBM) variety and phytase on total phosphorus (P) digestibility and percentage bone ash at 35 d of age

Variable	Dietary Treatments							Source of Variability	
	SBM variety			Phytase FTU/kg[1]				SBM	Phytase
	NP	LP-LS	SE[2]	0 FTU/kg (CON)	300 FTU/kg (MX)	300 FTU/kg (OT)	SE[3]	*P*-value	
	(%DM[4])								
Total P digestibility	68.52^{A}	58.39^{B}	1.1	56.26^{B}	65.55^{A}	68.55^{A}	1.4	0.0001	0.0001
Bone ash	57.12^{A}	55.90^{B}	0.2	56.51	56.64	56.39	0.3	0.001	0.84

[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).

[1]The three phytase treatments were either no phytase (CON), matrix phytase in which phytase was given a matrix value (MX), and on-top (OT) phytase in which phytase was not given a matrix value.

[2]Standard error (SE) for n= 18 cages with 6 birds per cage.

[3]Standard error (SE) for n= 12 cages with 6 birds per cage.

[4]Percent DM of feed and ileal samples were used to calculate nutrient digestibility on dry matter basis. Ash was calculated from dried bones.

Table VI-9. Effect of soybean meal (SBM) variety by phytase interaction on total phosphorus (P) digestibility and percentage bone ash at 35 d of age

Variable	Dietary Treatments[1]							Source of Variability
	NP SBM			LP-LS SBM			SE[2]	SBMxPhytase
	0 FTU/kg (CON)	300 FTU/kg (MX)	300 FTU/kg (OT)	0 FTU/kg (CON)	300 FTU/kg (MX)	300 FTU/kg (OT)		*P*-value
	(%DM[3])							
Total P digestibility	61.91	68.82	74.83	50.61	62.29	62.27	1.9	0.28
Bone ash	57.13	57.03	57.19	55.88	56.24	55.59	0.4	0.62

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[1]Either NP or LP-LS SBM in combination of three phytase treatments were either no phytase (CON), matrix phytase in which phytase was given a matrix value (MX), and on-top (OT) phytase in which phytase was not given a matrix value.
[2]Standard error (SE) for n=6 birds form a total of 6 cages.
[3]Percent DM of feed and ileal samples were used to calculate nutrient digestibility on dry matter basis. Ash was calculated from dried bones.

SUMMARY AND CONCLUSION

Possible strategies intended to improve endogenous P availability and digestibility in broilers were investigated. These approaches included using a blend of small and large particles, exogenous phytase, and incorporation of a genetically selected ingredient that contained an increased amount of available P, i.e. low phytate soybean meal (LP SBM). A series of four experiments were conducted. In the first experiment to 21 d of age, diets with 50%CC decreased BW gain and had the poorest FCR as compared to 0%CC during the first 9 d of age although feed intake was not affected. These effects probably reflected the negative effect of the energy required for increased muscular activity in the young broiler before 50%CC could produce a digestive benefit. Furthermore, this effect disappeared after 9 d probably due to the adaptation process whereby the GIT benefited from decreased rate of digesta passage relative to the energy requirement thereof. The digestive system responded to 50%CC as evidenced by an increased gizzard weight, undoubtedly as a result of muscular activity, and a smaller proventriculus probably due to decreased secretory activity of HCl and pepsin. The inclusion of 50%CC increased digesta retention time, which probably explained an improved phytate P digestibility. An adaptation period of approximately one week appeared to be required for the GIT to fully utilize LP SBM as evidenced by BW gain being improved with age. The decreased phytate P digestibility of LP SBM compared to NP SBM to 21 d of age was probably due to simply less phytate substrate in LP SBM and relatively less phytase produced in birds younger than 21 d of age.

In the second experiment, the same factors and conditions were used as in the first experiment with 300 FTU/kg phytase enzyme as an additional factor to examine any further improvement in live performance and P digestibility to 21 d of age. The same responses due to CC and LP SBM for live performance and organ weights were observed as in the first experiment. However, using 50%CC decreased P digestibility probably due to less surface area available for digestive enzymes. Total P content relative to sieve fraction in ground corn was obtained after grinding with either hammermill or roller mill. In corn obtained from both hammermill and roller mill, the P content increased as particle size increased but P content in fine corn obtained from hammermill was relatively more consistent among the different sieve fractions of corn while grinding corn using the roller mill resulted in a greater amount of total P in the larger particles (>1190 μm), which were the predominant particles (72.3%). The P digestibility was increased when using LP SBM probably due to initially higher available P in the LP SBM. Adding 300 FTU/kg phytase improved broiler live performance and P digestibility compared to 0 FTU/kg through hydrolyzing phytate that obviously liberated more P and possibly other nutrients as well.

The first two experiments were conducted in battery cages where there was no access to wood shavings used as litter. The third experiment was conducted in floor pens with new wood shavings. The same experimental factors were utilized as in the second experiment but the birds were raised to 35 d. Using 50%CC produced no beneficial effect on BW gain or FCR probably because birds had access to two sources of coarse material (new wood shavings and CC). This was supported by the fact that there was no effect of CC on gizzard weight, which clearly demonstrated that these broilers were consuming wood shavings such that they derived no further digestive benefit from CC across all treatments. Results of a previous study conducted in cages at our laboratory showed that gizzard weights of birds that consumed 0%CC were smaller than gizzard weights in this floor pen experiment. There was no caking of this litter because stocking density was not high, which provided access to the wood shavings throughout the experimental period. Additionally, P digestibility was decreased by 50%CC but increased by LP SBM and phytase, which was consistent with previous experiments. The inclusion of LP SBM decreased BW gain and FCR with no recovery as the birds aged. This finding differed from previous experiment probably because of a different formulation approach. In the first two experiments, LP and NP SBM were not given a matrix value, but were directly substituted for commercial SBM. In the third experiment, LP and NP SBM were given a matrix value that gave credit for the greater available P in LP SBM. Therefore, less inorganic P was added to diets formulated with LP compared to NP SBM diets which could have probably resulted in the negative effect observed on BW gain and FCR. Adding phytase to 50%CC diets improved BW, BW gain, feed intake, and P digestibility where the larger particles of corn that contained high P were further hydrolyzed by phytase, which released P and other nutrients bound by phytate and improved live performance. However, adding phytase to LP SBM decreased BW, BW gain, and feed intake. It was possible that giving a high P matrix value to LP SBM resulted in the formulation of an imbalanced diet where phytase addition exacerbated the imbalance further and birds consumed less feed, which was reflected in a lower BW and BW gain.

In the fourth experiment the effects of a low phytate-low saccharide SBM variety and phytase were again investigated in battery cages. The LP-LS SBM was included in diets from

22-35 d when dietary energy requirements would have thought to been increased. The inclusion of LP-LS SBM improved BW gain and FCR as expected. Gizzard weight was decreased by the inclusion of LP-LS SBM, which probably reflected a decreased digestive demand as the LP-LS SBM was fed for a shorter period of time at an older age compared to previous experiments and was more digestible. The LP-LS SBM diets had less total P than expected, which was reflected in a reduced total P digestibility compared to NP SBM. This was further implicated by the reduced gizzard weight, which probably indicated the loss of gizzard function in controlling retention time as less muscular activity occurred in LP-LS SBM diets. Adding phytase either "on top" of the diet or formulated with a matrix value both improved P digestibility.

Based upon these results, it was concluded that when using CC in poultry nutrition, the inclusion percentage should be lower than 50% in the starter period to avoid negative effects. Furthermore, the beneficial effect of 50%CC was improved by adding phytase to diets, which could be related to the variable P content in CC when using larger particles of corn (>1190 µm) that contained more P compared to smaller particles of corn. Furthermore, it was determined to be very important to correctly balance both total P and available P when using LP-LS SBM or other LP grains in the presence of phytase. Adding phytase improved total P digestibility consistently. The investigated approaches were proven beneficial in releasing more P from ingredients would have consequently reduced environmental impact and feed cost.