## ABSTRACT

PLUMSTEAD, PETER WILLIAM. Strategies To Reduce Fecal Phosphorus Excretion In The Broiler Industry Without Affecting Performance. (Under the direction of John Thomas Brake.)

Total phosphorus (P) in broiler manure has been reduced by decreasing dietary non phytate P (NPP) in combination with added phytase or low phytate (LP) grains. However, few studies have investigated the effects of diet amendment on performance and P excretion from broiler breeders, while the reported effects on water soluble P (WSP) from broilers have been inconsistent. To address this, broiler breeders were fed reduced NPP diets and phytase during the rearing (10-21 wk) and laying (22-64 wk) phase (Chapter 1). Decreasing dietary NPP below 0.37% reduced fertility, which was hypothesized to result from insufficient P intake during rearing. Decreasing NPP in breeder laying diets to 0.19% (no phytase) or 0.09% (with phytase) had no effect on eggs or chicks produced, but reduced manure total P and WSP by 39 to 42%, respectively. Chapters 2, 3, and 4 investigated reduced available P (AvP), phytase, phytate level, and the calcium (Ca):AvP or Ca:NPP ratios in broiler diets on ileal P and amino acid (AA) absorption, P retention, and total P and WSP in manure. Reduced AvP and phytase decreased manure total P. However, manure WSP and the ratio of WSP:total P were controlled by the dietary Ca:AvP ratio. Effects of Ca:AvP on WSP were dependent on the initial dietary phyate level and were mediated by altered phytate hydrolysis, precipitation of CaP complexes and urinary P output. Including LP soybean meal (SBM) in diets decreased total P and WSP by 49% and 56%, respectively, while phytase increased the WSP:total P ratio in manure. Maximum P retention and minimum total P output occurred when the dietary Ca:NPP ratio was 2.34:1 and 2.53:1 in LP and high phytate (HP) SBM diets, respectively. Dietary Ca had no effect on amino acid (AA) absorption in HP SBM but decreased AA absorption in LP SBM diets when Ca was $\geq$0.93%. Therefore, while a wider

Ca:NPP ratio could decrease manure WSP, this may negatively affect AA absorption when diets contain LP SBM.

# STRATEGIES TO REDUCE FECAL PHOSPHORUS EXCRETION IN THE BROILER INDUSTRY WITHOUT AFFECTING PERFORMANCE

by

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A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

**NUTRITION**

and

**POULTRY SCIENCE**

Raleigh, NC

2007

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## DEDICATION

This dissertation is dedicated to the loving memory of my father John William Alan Plumstead and to my mother Gisela Gertrud Draeger Plumstead who through an abundance of love, guidance, and support have made me all that I am and whose friendship has added immeasurable riches to my life.

## BIOGRAPHY

Plumstead, Peter William, the son of John William Alan Plumstead and Gisela Gertrud Draeger Plumstead, was born on May 27, 1971 in Nairobi, Kenya. Peter completed his high school education at Woodridge College in South Africa and then enrolled in the Department of Animal Science at the University of Pretoria and was awarded a B.S. (Agric.) degree in Animal Science in 1994, followed by a B.S. (Hons.) degree in Animal Science in 1995. After completion of his undergraduate degrees Peter accepted a position as a research scientist at the Animal Nutrition and Animal Products Institute, a branch of the South African Agricultural Research Council. From 1998 to 2002 Peter held the position of Senior Nutritionist at Meadow Feeds, Natal. After gaining practical experience in animal nutrition he chose to pursue his graduate education under the guidance of Dr. John Brake at North Carolina State University. Peter was awarded an M.S. degree with a double major in Nutrition and Poultry Science in 2005. The title of his thesis was "Response of young broilers to graded levels of dietary protein and amino acids." While at North Carolina State University Peter has been recognized for his graduate research by receiving the MB Dutch Garner Award for his M.S. thesis in the Department of Poultry Science; the Andrews Ph.D. fellowship from North Carolina State University, and five graduate student research awards for presentations at meetings of both the Southern Poultry Science Society and the Poultry Science Association. Peter was also an active member of the Poultry Science Graduate Student Association where he held positions of vice president and president. He is also a member of the Poultry Science Association and was inducted into Phi Kappa Phi and Gamma Sigma Delta academic honor societies while at North Carolina State University.

## ACKNOWLEDGEMENTS

The author would like to express his deep appreciation and thanks to Dr. John T. Brake who through his mentorship, constructive criticism, and friendship has contributed substantially to his professional and personal development in a manner that has extended far beyond the scope of the graduate degree program. Appreciation is also expressed to Drs. Rory Maguire, Jerry Spears, Jason Osborne, and Jon Allen for serving on the graduate committee and providing helpful direction, criticism, and insight during the course of my graduate program.

The technical assistance and friendship of fellow graduate students Hugo Romero-Sanchez, Kelly Brannan, and Nirada Leksrisompong are greatly valued while a very special thanks goes to Susan Creech who has not only helped considerably in all technical aspects of my work but whose support and advice has also contributed substantially to my personal development. Gratitude is also extended to the staff at the Lake Wheeler Road Field Laboratory and, in particular, to Terry Reynolds, Bill Stuart and Paul Otto for their hard work and attention to detail throughout the execution of my research. Special gratitude is also expressed to Corina Rosuita for her help in the preparation and submission of the manuscripts presented in this work. I am also greatly indebted to my mentors Professors Gerrie Smith and Kas Holtzhausen and my good friends Ben Holtzhausen, Mary Fosnaught, and Merida Roets for their lasting friendship and support.

The United Soybean Board and North Carolina Agricultural Foundation are acknowledged for financial support of the studies reported in this dissertation.

Finally, the author is deeply indebted to Dr. April Leytem from the USDA-ARS who completed most of the analytical work and without whose hard work, insight, and friendship this dissertation would not have been possible.

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# INTRODUCTION

The broiler industry has encountered increasing pressure to develop strategies to reduce the environmental impact of the large amounts of manure (feces and urine) and litter (manure mixed with bedding) that are generated from both broiler and broiler breeder operations. A single broiler chicken weighing 2.8 kg at 42 d of age has been estimated to produce between 1.0 and 1.3 kg of manure that contained approximately 4% total nitrogen (N) and 2% total phosphorus (P) (Saylor et al., 2001; Haggard et al., 2003). Further investigations have shown that as 41% of the P consumed by broiler breeders and 45% of the P consumed by broilers was excreted, the cumulative amount of P excreted by broiler breeders and broilers, respectively, would be 165 g or 11.6 g elemental P over a 64-wk or 42-d period, respectively. Therefore, as the annual production of broiler breeders and broilers in the United States was approximately 60 million and 9 billion, respectively, the total elemental P contained in the manure and litter produced would approximate 115,000 tons (Plumstead et al., 2006).

Land application of the manure and litter from poultry as fertilizer for crops has been the most cost-effective method of disposing of the large amounts of manure and litter that have been produced annually. However, crops require a N:P ratio of 6-11:1, while the N:P ratio in manure was on average 2-3:1 (Daniels et al., 1998). Therefore, the application of poultry manure as fertilizer to meet the crop N requirement has resulted in excessive P application to land relative to crop P removal. The continued application of manure and litter to soils has led to an increase in soil test P concentrations in areas of intensive poultry production (Pautler and Sims, 2000). High P levels in soils have substantial environmental implications as this was shown to increase P contained in surface water runoff (Pautler and

Sims, 2000; CAST, 2002). The associated increase in P concentrations in receiving water has in-turn been shown to increase aquatic plant growth, deplete oxygen, and cause eutrification that results in reduced biological, economic, and aesthetic value of these water bodies (Bennet et al., 1999; Shapley and Moyer, 2000; CAST, 2002).

These environmental implications of a high soil test P level have led to federal regulations that limit the amount of P from manure that can be applied to land (Environmental Protection Agency, 2003). Furthermore, in addition to total P, a strong positive correlation was also shown between the water soluble P (WSP) in broiler litter and the amount of P that leached from a soil (Sharpley and Moyer, 2000) and P contained in surface water runoff (Kleinman et al., 2000). Therefore, to further control P losses from soils regulatory agencies in large poultry producing regions such as Arkansas, Maryland, and North Carolina have included a measure of the WSP in litter and manure into their P loss assessment tools. Therefore, the rate at which manure and litter can be applied to land in large poultry producing regions has become largely regulated by the amount of total P and WSP contained therein.

One of the factors that has contributed to the large amounts of P in manure and litter was that poultry are not inherently able to fully utilize the phytate bound P fraction contained in the diet. Phytate, or *myo*-inositol 1,2,3,4,5,6-hexa*kis* dihydrogen phosphate has been shown to be the predominant form of P in grains with 65% to 80% of P in corn and ~ 70% of P in soybean meal (SBM) occurring in the phytate form. 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 commonly included in diets of monogastric animals (Angel et al., 2002; Applegate et al., 2003). In addition to the dietary inclusion of

phytase the total P in manure and litter from broilers and turkeys was also reduced by decreasing the dietary non-phytate P (NPP) closer to the minimum nutritional requirement of the bird (Maguire et al., 2004; Vadas et al., 2004; Angel et al., 2005). Finally, research has also investigated the use of low-phytate (LP) variants of corn and soybeans that contained a higher proportion of NPP:phytate and that were shown to reduce both total and WSP in litter from broilers (Applegate et al., 2003; Wilcox et al., 2005).

The general consensus of researchers that have investigated the effects of diet amendments such as reduced NPP, 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 litter and manure from broilers by 29 to 45% (Applegate et al., 2003; Maguire et al., 2004; Smith et al., 2004; Vadas et al., 2004; McGrath et al., 2005; Angel et al., 2006). However, in spite of extensive research that investigated the combined effects of NPP and phytase in broilers, there have been comparably few reports that have described similar effects on P fractions in manure from broiler breeders.

Furthermore, while effects of diet amendment on P excretion from broilers were shown to consistently decrease the total P in litter, the reported effects of phytase on WSP have been inconclusive and highly controversial (Applegate and Angel, 2003). Phytase addition to poultry diets was shown to decrease the litter WSP concentration by 35.6% (Applegate et al., 2003) and by 29% (McGrath et al., 2005). However, in other studies, phytase supplementation to diets had no effect on litter WSP concentration (Saylor et al., 2001; Maguire et al., 2004; McGrath et al., 2005), while in two other studies, amendment of diets with phytase increased the concentration of WSP in the litter (Delaune et al., 2001; Vadas et al., 2004).

Recently, Leytem and Maguire (2006) showed that the proportion of WSP in litter decreased when phytate P of the manures and litters increased. Therefore, dietary factors that influence the amount of phytate excreted by the birds could potentially alter the WSP fraction of the resultant manures and litters. Wise (1983) concluded that the molar ratio of Ca:phytate in the diet was the primary factor that determined the extent of phytate hydrolysis in the intestines due to increased formation of insoluble Ca-phytate complexes. Therefore, as the molar ratio of Ca:phytate in the intestines was altered by the inclusion of dietary phytase enzymes or LP grains, it could be expected that the proportion of phytate in the manure and litter and its effect on WSP could also vary accordingly.

In addition to inhibiting phytate hydrolysis in the intestines of poultry, the addition of excess Ca to diets was also shown to also affect P utilization by increased precipitation of insoluble CaP complexes in the digesta and excreta (Driver et al., 2005; Toor et al., 2005) that would furthermore alter the proportion of P that was soluble in litter or manure. A review of the existing literature revealed that many previous studies that investigated effects of reduced dietary NPP and phytase did not also consider the potential impact of varying dietary Ca concentration on total and WSP concentrations in litter (Applegate et al., 2003; Maguire et al., 2004; Vadas et al., 2004).

Therefore, as comparably few studies had investigated effects of diet amendment on excretion of P from broiler breeders the first objective of this research was to further evaluate effects of reduced dietary NPP and phytase on the total P or WSP concentration of the broiler breeder pullet rearing litter and broiler breeder laying manure, while also quantifying the potential benefits of these strategies on the performance of broiler breeders to 64 wk of age. Secondly, as it was evident from the literature that the dietary phytate concentration and ratio

of Ca:phytate may affect the proportion of WSP in broiler litter, the effects of AvP, phytase, phytate, and Ca levels on total P, WSP, and litter P composition in broilers was investigated. Finally, as other workers had suggested that as the amino acid availability in broiler diets was dependent on the dietary phytate level, the effect of including LP grains on the apparent digestibility of amino acids at the terminal ileum was also investigated.

# LITERATURE REVIEW

## Phosphorus Accumulation in Soils and the Environment

While being an essential nutrient for both plants and animals, excessive P accumulation in soils and water has important environmental implications. An accumulation of P in soils increases the risk of P losses to sensitive bodies of water (Sims et al., 2000) and can lead to increased P concentrations of surface waters (Pautler and Sims, 2000; CAST, 2002). The large amount of P in surface water runoff from agricultural lands and increased P concentration in receiving bodies of water have in-turn been shown to increase aquatic plant growth, deplete oxygen, and cause eutrophication that results in reduced biological, economic, and aesthetic value of these bodies of water (Bennet et al., 1999; Sharpley and Moyer, 2000; CAST, 2002). As a result of these negative effects of elevated P concentrations in soils and water on down-stream aquatic systems, research has been conducted to understand the underlying reasons for excessive P levels in soils, as well as factors controlling the subsequent transport of P to aquatic systems .

Generally, the accumumlation of P in soils results from an excess of P inputs onto the land versus P exports off site. The difference between P inputs and exports has also been termed the ‘P mass balance’ and can be calculated for a geographic region of any scale or size. In order to have a sustainable system over the long term and prevent regional P accumulation, the P mass balance must be neither positive or negative, i.e. total P inputs (imports) into an area must be less than or, at the very least, equal to total P outputs (exports) (Beede, 2003). Predominant P inputs have been shown to be generally in the form of animal manure and inorganic fertilizer, as well as inputs from other sources, such as livestock

mineral and feed supplements, nutrients contained within precipitation, crop residues, and nitrogen accretion by legumes (NRC, 1993). Predominant pathways of P export occur via harvested farm products (either crops or animal products) and to a lesser extent via hydrologic exports in the form of runoff and leaching losses although, for simplicity, the latter have frequently been not included in the calculation of mass nutrient balances (Bennet et al., 1999; NRC, 1993).

A positive mass balance of P in many states has resulted from the consolidation of animal agriculture into very small isolated areas that were geographically separate from the region of feed grain production. Thus, intensive animal production practices can generate regional and farm-scale nutrient surpluses where nutrient imports in feed and mineral fertilizer exceed nutrient exports in crops and animal products (Sharpley *et al.*, 1994; Sims *et al.*, 1998). On a more localized scale, accumulation of P on lands was frequently the result of continued application of manure as a fertilizer. The reason for this lies in the fact that application of animal manures to land has remained the most efficient method of disposing of the large quantities of manure generated by confined animal feeding operations. However, due to an unfavorable nitrogen:total P ratio relative to crop requirements, the continual application of manures to soils to meet crop nitrogen needs, which has been common practice, often leads to excess application of P and an accumulation of soil test P concentrations (Sims et al., 2000).

The environmental implications of high P levels in soils has led to federal regulations that restrict or limit the amount of P from manure that can be applied to soils (Environmental Protection Agency, 2003). Further, in addition to total P in poultry litter, the water soluble P fraction (WSP) has been identified as an important environmental risk factor, as P losses in

runoff following land application of litter have been related to the WSP in the litter applied (Maguire et al., 2005). Therefore, considerable research resources have been invested to address means whereby P concentrations in animal manure can be reduced without impacting animal performance, while other research has focused on studying the fate of manure P following land application.

**Phosphorus Forms and Definitions**

When evaluating phosphorus (P) in feed, manures, litters, soils, and in runoff water, it has become important to distinguish between different forms of P since the complex chemistry of P affects its availability to the animal in the feed, as well as its reactivity in soil and aquatic environments.

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

The term *Phytate Phosphorus (Phytate P)* was developed to describe organic forms of P contained in a phosphorylated cyclic sugar alcohol form called *myo*-inositol 1,2,3,4,5,6-hexa*kis* dihydrogen phosphate (commonly known as phytate) (Angel et al., 2002). Phytate has been shown to be synthesized by plants and exist in its ionic form in most portions of the plant, but occurs predominantly as a salt of Ca, Mg, and K in mature seeds. This chelated form of phytate has been referred to as phytin and can also complex with proteins and amino acids in the seed (Angel et al., 2002). The phytate molecule contained 28.2% P and was the predominant form of P in grains with 65-80% of P in corn and ~70% of P in soy occurring in

the phytate form (Raboy et al., 2000; Oltmans et al., 2005). The phytate molecule has been reported to be poorly digested by monogastric animals as these animals do not produce sufficient amounts of phytase enzyme required to hydrolyze the carbon-phosphate bonds on the myo-inositol ring. Phytate hydrolysis and the availability of phytate-bound P can be increased by addition of exogenous phytase sources of microbial or fungal origin (Angel et al., 2002).

*Non Phytate Phosphorus (NPP)* represents 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 was also 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-phosphorus 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 body weight 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.

However, estimates of the AvP content of ingredients vary considerably and were shown to be primarily dependent on the biological response variable selected, the source and purity of the reference standard, and experimental conditions of the study (Soares, 1995). For example, in a review of the AvP of dicalcium phosphate added to broiler feed, Soares (1995) showed that published values from ten different studies ranged from 81% to 123%. Since "available P" was expressed relative to the standard reference material used and was also dependent on the biological response variable (tibia ash, toe ash, or body weight gain), it was not only possible to obtain values greater than 100%, but the determined values of AvP could also be expected to vary considerably with differences in the reference material used, the preferred response variable selected, as well as being affected by the experimental conditions of the specific study.

*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.

*Retained P* has defined the total P from the diet that was retained by the bird. This was measured by determining dietary P intake and then subtracting the amount of P that was excreted in the urine and feces. The retained phosphorus system has been extensively utilized in Holland where it has become known as the "opneembare phosphor system."

*Soluble Phosphorus (soluble P)* has been used to define the amount of P in soil, manure, or water, that was soluble. By definition, soluble P was defined as the P fraction that will pass through a 0.45-um-pore filter (NRC, 1993). Soluble P has also been synonymous with *WSP*, and *water extractable P (WEP)* and was typically determined in manures by

shaking a sample of manure in distilled water and analyzing the P in suspension following filtration of the sample through a 0.45-um-pore filter. Following extraction, the P in suspension was most frequently analyzed using the Murphy and Riley (1962) colorimetric procedure, or analyzed using Inductively Coupled Plasma Spectrophotometry (ICP).

*Particulate Phosphorus (Particulate P)* comprised particles in soil or water that do not pass through a 0.45um filter and represented the P fraction tightly bound to sediment and organic matter by anion adsorption (NRC, 1993; Sturgul and Bundy, 2004).

*Soil Test Phosphorus (STP)* represented P in soil that was available for uptake by plants and represented an index of the nutrient supplying capacity of the soil, relative to crop demand rather than being a measure of the total P in the soil (Daniels et al., 1998; Sturgul and Bundy, 2004). Numerous variations exist in analytical methods used to assess STP. Examples of these include Mehlich I P, Mehlich III P, Olsen P and the Bray P1 methods. Since large differences between these methods in the proportion of total P represented by STP have been reported, method-specific threshold values of STP have been established that serve as guide to determine optimum, and maximum rates of P application to soils.

**Functions of Phytate in Plants and Development of Low-phytate Grains**

Phytate bound P was shown to be the predominant form of P in grains with 65-80% of total P in corn and ~70% of total P in soy occurring in the phytate form (Raboy et al., 2000; Oltmans et al., 2005). However, 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 $P_i$ 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 approaches maturity and dormancy was induced; and intra-cellular signaling (Vogelmaier et al., 1996, Angel et al., 2002). In spite of the apparent importance of phytate in physiological and biochemical processes of the plant, low phytate, non-lethal mutants of corn, wheat, oats, barley, rice, and soybeans have been developed (Raboy et al., 2000; Raboy, 2002; Guttieri et al., 2004; Oltmans et al., 2005; Israel et al., 2006) with reductions in the phytate P concentrations of 50 to 95%.

In most instances, total seed P concentration remained unchanged as a result of corresponding increases in $P_i$ concentrations, and in some cases, increased lower order *myo*-inositol phosphates containing five or fewer P esters. (Raboy, 2002; Israel et al., 2006). The low phytate 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) which, while being non-lethal, has variable effects on grain yield and seed functionality. Although the phytate concentration was shown to not be essential to seed function of low phytate mutants of corn, the decrease in phytate was accompanied by reduced seed weights (Raboy et al., 2000).

Bregitzer and Raboy, (2006) also showed low phytate variants of barley to have reduced seed test weights and percentage plump kernels. However, the location of the lpa-1 mutation in this study greatly affected its impact on crop yield and had the smallest effect on yield when concentrated in the auleurone layer. Low phytate variants of soybean lines were in some instances shown to have reduced seedling emergence and yield, while selection within low phytate lines for increased emergence resulted in a decrease in total P, inorganic P, and other forms of P (Oltmans et al., 2005). However, while reductions in seed phytate had negative effects in some studies, other researchers have succeeded in isolating two independent low phytate mutants of soybean lines with an 80% reduction in phytate P, without significant changes to seed functionality (Wilcox et al., 2000).

## Strategies to Decrease Diet P without Impacting Animal Performance

### *1. Feeding P Closer To Dietary P Requirements*

Two approaches have been used to more accurately match dietary P to the requirements of the bird. In the first instance, research has focused on more accurately describing the dietary P requirement of modern strains of poultry, while in the second instance the P intake of birds was reduced by increasing the number of phases in the dietary regimen.

#### *1.1. Re-Defining P Requirements for New Genetics*

***P Requirements of Broilers.*** Estimates by the NRC (1994) of the NPP requirements of broilers from 0-3 wk of 0.45% NPP and from 4 – 6 wk of and 0.35% NPP were based on studies that, for the most part, used non-commercial strains of broilers (NRC, 1994). More

recent research has suggested that NPP requirements of modern strains of broilers were considerably lower than previous recommendations by the NRC (1994). Yan et al. (2001) reported the NPP requirement of Cobb 500 male broilers from 3 – 6 wk of age to be 0.33%. In a similar manner, Angel at al. (2000 a,b), using Ross 308 broilers and a more industry typical four-phase feeding program, reported an NPP requirement for maximum tibia ash and body weight gain, respectively, of 0.32% and 0.28% from 18-32 d. The NPP requirements decreased from 0.24% to 0.19% and from 0.16% to 0.11% during the finisher (32-42 d) and withdrawal phases (42-49 d), respectively, such that no added dicalcium phosphate was needed in diets at the lowest NPP level of 0.11%.

Estimates of the reduced NPP requirement of modern strains of broilers were further supported by Dhandu et al. (2000) who suggested that the NPP requirement of Ross 308 broilers from 33 to 42 d was not higher than 0.23%; while in a similar series of experiments, Ling et al. (2000) confirmed the NPP requirements of modern broiler strains to range between 0.32 and 0.26% in the grower phase, 0.26 and 0.18% in the finisher phase, and from 0.19 to 0.14% during the withdrawal phase. Although the estimates of Angel et al. (2000 a,b), Dhandu et al. (2000), and Ling et al. (2000) were not directly comparable to those of the NRC (1994) because these authors had used different broiler strains and a four phase feeding program (Applegate et al., 2003), on a whole, these data suggested that the dietary NPP requirement after 3 weeks of age was somewhat lower than NRC (1994) recommendations.

In spite of to the reported lower NPP requirement of modern broiler strains, NPP levels in conventional broiler diets were often increased by nutritionists to create a “safety margin” in dietary formulations (Knowlton et al., 2004). This increase in dietary NPP levels above minimum requirements was justified because of the associated negative effects of a P

deficiency on animal performance and to account for a large variation in the available P content of ingredients (Soares, 1995).

***P Requirements of Broiler Breeders.*** Comparatively little research has been conducted that has directly investigated the P requirements of broiler breeders and frequently, levels of NPP in diets for broiler breeders have been extrapolated from work conducted in laying hens. The NRC (1994) stated that, with the possible exception of trace minerals and vitamins, the major nutrient requirements for egg-type breeders to produce a hatching egg were the same as that required to produce an egg for human consumption. Accordingly, the daily NPP requirement was 250 mg per 100 g of feed intake, which would be equivalent to 0.40% NPP in the diet assuming a feed intake of 160 g per day (NRC, 1994).

In a recent study, Berry et al. (2003) showed that hen-day egg production of broiler breeders housed in cages responded to phytase added to diets with 0.3% NPP. As phytase was able to contribute approximately 0.1% AvP to the diet, these data suggested that the NPP requirements of caged broiler breeders were at least equivalent to the 0.40% NPP recommended by the NRC (1994). However, in commercial production systems broiler breeders have not been housed in cages, estimates of the NPP requirements of cage layers may not be directly applicable to broiler breeders housed in conventional slat-litter production facilities.

Singsen et al. (1962) showed that P levels in litter accumulated to approximately three times that of feed and suggested that coprophagy reduced the dietary requirements of phosphorus for laying hens housed on litter. This was confirmed Bootwalla (1982) who estimated that broiler breeders could supplement their P intake by 140 mg per day by the consumption of old litter. Litter intake of broiler breeders was further shown to increase

when diets deficient in Ca were fed (Harms et al., 1984), which suggested that broiler breeders increased nutrient intake from litter when the supply of Ca in the diet was limiting. In a review of commercial P levels in broiler breeder diets used in Holland, Tuijl (1998) reported that a 30% reduction in total dietary P was correlated with increased mortality, leg problems, and reduced egg-shell quality and hatchability towards the end of lay. In response to the reduced performance and production problems observed in the field, the revised retained P requirements of broiler breeders of the CVB (1997) were reported by these authors to be 0.29% and 0.24% from onset of lay to 40 wk of age and from 41 wk of age to the end of lay, respectively.

To further investigate the P requirements of broiler breeders, Brake et al. (2003) conducted two experiments in which dietary NPP levels in broiler breeder diets fed from 21 wk of age were reduced from 0.40% to as low as 0.10% with or without added phytase. Results showed that performance of broiler breeders in the first experiment was not affected by the lowest level of dietary P when diets contained small quantities of wheat bran, which had some phytase activity, and summer ambient temperatures were moderate. However, during the second experiment the wheat bran was removed from the diet and ambient temperatures were high, the mortality rate of broiler breeder females on diets that contained 0.10% NPP with no added phytase was 9.38% compared to 3.13% or 4.17% when breeders were fed diets with either 0.10% NPP with phytase, or 0.40% NPP with no added phytase, respectively.

These data suggested that the NPP requirement of broiler breeders was increased by high ambient temperatures but that good performance and low mortality rates under high ambient temperatures could be supported by feeding diets with 0.10% NPP and added

phytase during the breeder laying period. This finding was supported by a further experiment in which no differences in broiler breeder mortality, eggs per hen-housed, or chicks per hen-housed were found when broiler breeder diets contained 0.11% NPP with added phytase (Plumstead et al., 2007). However, while reduced dietary NPP levels did not reduce the number of eggs or chicks produced per hen-housed, a decrease in fertility was observed when dietary NPP levels were reduced below 0.40%. The reduction in fertility observed by Plumstead et al. (2007) may be attributed to reduced dietary P levels being fed during the rearing diets from as early as 11 wk of age, as Brake et al. (2003) showed no effects on fertility when diets with 0.10% NPP with phytase were fed from 21 wk of age.

In support of these data, Wilson et al. (1980) showed that maximum egg production and hatchability from broiler breeders in litter floor pens were supported when birds received diets with 0.41% total P. As the broiler breeder laying diet used in that study was predominantly corn-soy based, the estimated requirement of 0.41% total P would be equivalent to approximately 0.15 to 0.20% NPP. Therefore, based on the reports by Wilson et al. (1980), Brake et al. (2003), and Plumstead et al. (2007) there was a substantial amount of evidence that suggested that the NPP requirements of broiler breeders housed in slat-litter production facilities were considerably lower than previous estimates of 0.40% NPP (NRC, 1994; Berry et al., 2003) for breeder hens housed in cages.

### *1.2. Phase Feeding*

Phosphorus requirements of poultry, expressed as a percentage of the diet decrease as animals age (NRC, 1994). Conventional feeding programs utilized only three different diets over an 8-wk growth period and resulted in considerable periods of nutrient deficiencies or

excesses (CAST, 2002). An increase in the number of diets fed during the growth period should be expected to result in a smaller disparity between the nutrient requirement of the animal and nutrient intake and reduced excretion of excess nutrients (Ferket et al., 2002). For example, the revised NPP requirements for broilers that were developed by the University of Maryland utilized a four-phase feeding program to 49 d of age that, compared to industry standard diets, was able to significantly reduce total P intake of broilers by 2.42 g per bird (Applegate et al., 2003).

## *2. Feed Additives and Diet Modification*

### *2.1. Phytase Enzymes*

Phytase enzymes (*myo*-inositol hexaphosphate hydrolases) catalyze the hydrolyses of phosphate ester bonds and yield free $P_i$ and one or more lower phosphoric esters of *myo*-inositol penta*kis*-, terta*kis*-, tris-, bis-, and monophosphates (Wyss et al. 1999). Two classes of phytase enzymes have been characterized that consist of a 3-phytase that initiates hydrolyses on the phytate molecule at the 3 position to yield 1,2,4,5,6-penta*kis*phosphate and inorganic P while 6-phytases initiate de-phosphorylation at the 6 position of the inositol ring and yield 1,2,3,4,5 penta*kis*phosphate and inorganic P (Angel et al., 2002). The pH optima at which they hydrolyze inositol phosphates has been shown to be an important distinction between phytase enzymes.

Fungal phytase (*A. fumigatus*) was shown to have a broad pH optima with 80% of the maximal activity occurring in a pH range of between 4.0 and 7.3. The commercial variant of aspergillus phytase (A. niger) was shown to have two pH optima of 2.0-2.5 and 5.5. In contrast, *E. coli* derived phytases (*E. nidulans*) had a narrow pH optimum near pH 6.5 (Wyss

et al., 1999; Angel et al., 2002). The pH optimum of phytase activity has been reportated to be important in that it determined the site of maximum phytase activity within the gastrointestinal tract of the animal. 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 phytases that were added to the diet; 3) endogenous phytases secreted at the intestinal mucosa; and 4) phytases produced by microflora in the lower gastrointestinal tract of the animal (Angel et al., 2002).

While intestinal brush border phytase activity was shown to exist in both 4-wk-old broiler chickens and mature laying hens, as a result of a larger intestinal surface area the total intestinal phytase activity was 35% greater in the older hens (Maenz and Claasen, 1998). The phytase activity of the intestinal mucosa, combined with endogenous phytases associated with cereal grains such as wheat and wheat by-products result in a portion of phytate bound P being hydrolyzed in poultry feed without supplementation of exogenous phytase enzymes. Van der Klis and Versteegh (1996) estimated that the percentage phytate P hydrolyzed by 3-wk-old broilers ranged from 2% to 80% in a variety of feedstuffs. In these experiments hydrolyses of phytate P from corn and soybean meal was 16% and 61%, respectively. However, the extent of phytate P hydrolyses of soybean meal was shown to be dependent on level of both calcium and phosphorus in the diet and decreased from 69% to as low as 36% when the calcium and available phosphorus levels in the diet were increased from 0.5% and 0.18%, respectively, to 0.83% and 0.30% (Van der Klis and Versteegh, 1996).

This inhibitory effect of calcium on phytate P hydrolyses was also investigated by Tamin et al. (2004) who showed that in the absence of calcium or exogenous phytase enzyme, 69.2% of dietary phytate P was hydrolyzed by the terminal ileum. Calcium additions

in the form of $CaCO_3$ to levels normally found in broiler diets (0.90%) decreased phytate P hydrolyses to 25.4% and reduced apparent P absorption in the bird from 67.9% to as low as 29.4%. Applegate et al. (2003) also showed differences in the intestinal phytase activity and phytate hydrolyses between commercial broiler strains that was independent of the effect of calcium on phytate hydrolyses. The effect of calcium on decreasing phytate P hydrolyses was also not limited to intestinal phytases and varied between sources of exogenous phytase enzymes.

In a study that compared the efficacy of *P. lycii* and *A. niger* phytases to hydrolyze phytate P, a 2.1 fold difference in efficacy was observed between phytases, while adverse effects of calcium on phytate P hydrolyses were dependent on the source of phytase enzyme (Tamin et al., 2004). The mechanism whereby calcium reduced phytate hydrolyses was thought to occur primarily by the formation of stable calcium-phytate complexes (Maenz et al., 1999; Angel et al., 2002). These larger molecules may no longer be available for hydrolyses by the enzyme either as a result of changes to the phytate P structure that precluded it from binding to the substrate binding site of the enzyme or due to the reduced solubility of the calcium-phytate complex that caused it to precipitate out of solution. In addition to calcium, other di- and trivalent cations can also form stable complexes with phytate and result in reduced hydrolyses of the phytate P.

The binding of cations to phytate was pH dependent with the order of potency of minerals to inhibit phytate P hydrolyses at neutral pH reported in the order of $Zn^{2+} >> Fe^{2+} > Mn^{2+} > Fe^{3+} > Ca^{2+} > Mg^{2+}$ (Maenz et al., 1999; Angel et al., 2002). Driver et al. (2005) further showed that the response of broiler tibia ash to phytase was greatest at high Ca levels and low levels of NPP in the diet and that these decreased as dietary Ca was reduced. Based

on these results these authors concluded that “it was virtually impossible to determine a single equivalency value for phytase supplements”. In spite of an overwhelming amount of data that showed hydrolyses of phytate P by phytase enzyme to be dependent on a multitude of dietary factors, these have frequently not been considered when phytase was included in poultry feed.

However, due to differences in the pH optima and efficacy of the type of phytase enzyme, differences in standard inclusion rates of commercially produced phytases have become common. Standard recommendations have been that 500 FTU $kg^{-1}$ inclusion of an *A. Niger* phytase enzyme (BASF, Natuphos 5000) was equivalent to 0.10% P from monocalcium phosphate. Due to a lower efficacy and narrow pH optimum, inclusion of 750 FTU $kg^{-1}$ feed from *P. Lycii* phytase enzyme was considered equivalent to 0.10% P from monocalcium phosphate, while the new-generation *E. coli*-derived phytases were marketed as 500 FTU $kg^{-1}$ being able to replace 0.13% NPP in the feed (BASF Corp., Mount Olive, NJ; DSM Nutritional Products, Basel Switzerland; Syngenta Animal Nutrition; Research Triangle Park, NC). 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).

### *2.2. Incorporation of low phytate grains in diets*

Feeding grains that contain a high amount of digestible P as a result of a reduced phytate concentration in the diet has the potential to greatly increase P retention and decrease manure P concentrations (CAST, 2002; Penn et al., 2004). 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). Dilger and Adeola (2006) found no differences in pre-cecal digestibility of P from low phytate or conventional soybean meals that ranged from 79-89%. However, this was much higher 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. However, in spite of no change in pre-cecal P digestibility when birds received a low phytate soybean meal, P retention of broilers was greater when diets contained low phytate soybean meal (77% vs. 60%) and was attributed to a 57% reduction in phytate P (Dilger and Adeola, 2006).

While not directly comparable to P digestibility or P retention studies, Karr-Lilienthal et al. (2005) determined P bioavailablity, as measured using a slope ratio assay with percentage tibia ash as the response variable, and found that this was at least 50% greater for low phytate soybean meal compared to conventional soybean meal. It was also interesting to note that increased extraction time during the processing of conventional soybean meal increased the relative P bioavailability by 23% and was attributed to a decrease in phytate P due to the increased degree of soybean processing. Comparable studies have also been conducted using low phytate (LP) corn in broilers. Replacement of yellow dent corn with LP corn in diets significantly increased tiba ash in turkeys (Yan et al., 2003). However, improved P retention from diets that contained LP corn was dependent on the dietary P concentration.

Li et al. (2000) showed that at low dietary available P concentrations of 0.28%, chicks fed lP corn retained 15.3% more P compared to chicks fed conventional corn. However, increased P retention from LP corn was reduced to only 6.7% when the available P concentration in the diet was increased to 0.45%. The reduced P retention with increased

available P was attributed to chicks retaining more P for physiologic functions when this was limiting in the diet. An increase in the dietary available P concentration decreased the relative deficiency of chicks and reduced the benefit of incorporating LP corn. In addition to dietary inclusion of LP corn alone, combined effects of LP corn and phytase have also been investigated. Applegate et al. (2003) showed that a combination of LP corn and phytase had no effect on broiler performance to 49 d of age but significantly reduced P intake of birds on this treatment and reduced total P concentration of the litter to a greater extent than diets with phytase or LP corn alone. To our knowledge, no studies have been published that evaluated the potentially additive effect of including both LP corn and low phytate soybean meals on broiler performance and P excretion.

### *2.3. Other feed additives*

Vitamin D3 and its metabolites, 25-hydroxycholecalciferol ($25[OH]_2D_3$) and 25-hydroxy-cholecalciferol ($1,25[OH]_2D_3$), have shown potential for use as a dietary strategy to improve retention and reduce P excretion from broilers (Ferket et al., 2002). Biehl and Baker (1997) showed $1,25[OH]_2D_3$ to increase phytate P utilization in chicks without affecting the activity of intestinal phytase. Further, the beneficial effect of $1,25[OH]_2D_3$ appeared to be additive to that of phytase. This was suggested by Biehl et al. (1995) who showed that while feed intake, BW gain, and tibia ash in chicks were increased by both phytase and $1,25[OH]_2D_3$ independently, the response was greater when phytase and $1,25[OH]_2D_3$ were combined in the diet. While the mode of action of Vitamin $D_3$ on phytate P utilization was unclear, it has long been known that vitamin $D_3$ increases calcium absorption by stimulating synthesis of calcium binding proteins in enterocytes (Cromwell, 1996).

Based on this, Biehl and Baker (1997) postulated that increased phytate P hydrolyses by vitamin $D_3$ may be caused by increased calcium absorption that decreased calcium concentrations in the intestinal lumen. The associated reduction in the formation of calcium-phytate complexes could in turn, increase phytate P hydrolyses by phytases present at the mucosal surface. In addition to vitamin $D_3$, citric acid additions to diets were also shown to increase phytate P hydrolyses. Bolingen-Frankenbach et al. (2001) found that 4% citric acid added to corn-soy based broiler diets was capable of replacing the equivalent of 0.10% supplemental P from inorganic sources. These authors speculated that, as citric acid was a strong chelator of calcium, added citric acid could increase hydrolyses of phytate P by chelating free calcium ions in the gut, thereby reducing the formation of Ca-phytate complexes. Based on these results it would appear that feed additives such as vitamin $D_3$ and citric acid may act in a similar manner by reducing inhibitory effects of free ionic calcium on hydrolyses of phytate P by intestinal or exogenous phytase enzymes.

**Phosphorus Composition of Poultry Manures and Litters**

A thorough understanding of the content and form of P in poultry manure and litter is required to develop guidelines that will minimize environmental impact following land application (Sharpley and Moyer, 2000; Maguire et al., 2004). The total P concentration of manure and litter varied greatly from 19,500 to 36,100 mg $kg^{-1}$ in manure and 11,300 to 22,400 mg $kg^{-1}$ in litter and were dependent on differences in the physiological maturity of the birds, diet composition, manure and litter collection method, manure treatment, and storage (Sharpley and Moyer, 2000; Ferket et al., 2002; Toor et al., 2005). Since total P concentrations in manures provide no information on the forms of P in manures and their

impacts on plant availability, mobility, and fate following land application, fractionation of the total P in manures has been performed to enhance our knowledge of the forms of P present and their potential reactivity and mobility in the environment (Toor et al., 2005).

Sharpley and Moyer (2000) characterized the total P contained in manures and litter into organic and inorganic P fractions via sequential extraction with water, dilute bases (NaOH, $NaHCO_3$) and acid (HCl) using a modification of the soil P fractionation previously described by Hedley et al. (1982). In this study, 84% of the total P in manure from laying hens was inorganic P and increased to as much as 90% in broiler litter. Sequential extraction of the inorganic P fractions showed that 25% and 26% of inorganic P was water soluble P, while further sequential extractions were bicarbonate (25% and 31%); hydroxide (1% and 5%), and acid (32% and 29%) extractable P in manure and litter, respectively. On comparing their results with a sequential extraction of poultry manure by Leinweber (1996) the authors noted that, while water extractable and bicarbonate extractable fractions were similar, differences existed in the hydroxide and acid extractable P forms that may be explained by differences in type and P formulation of the diet (Sharpley and Moyer, 2000).

Measuring proportions of the water and bicarbonate extractable P fractions in manures and litter was important because these P forms were readily soluble and therefore susceptible to runoff and leaching following land application. This was examined in the same study by Sharpley and Moyer (2000) who found that while both bicarbonate and water extractable fractions were correlated to the amount of P leached from the soil following 5 simulated rainfall events, this was significantly more so for the water extractable fraction.

More recently, Phosphorus-31 nuclear magnetic resonance spectroscopy (P-NMR) has been used to characterize P fractions in manures (Turner, 2004) and in sequential Hedley

extracts of manures (Turner and Leytem, 2004; Maguire et al., 2004)). Results have shown that P in broiler litter was comprised mainly of orthophosphate and orthophosphate mono-esters, with few traces of phospholipids, and no detectable polyphosphates or DNA. Turner (2004) reported that 59% of total manure P in broiler litter was present as phytate. Similar values were obtained by Turner and Leytem (2004) who reported that of the total P in broiler litter, 41% occurred as phosphate P and 58% as phosphate mono-esters that consisted almost entirely of phytate. Phytate P fractions amounting to 63% of total P were also reported by Maguire et al. (2004).

This could have important environmental implications as Leytem et al. (2006) reported a positive linear relationship ($r^2 = 0.98$) between the proportion of manure phytate P and manure WSP. Speciation of the sequential extracts of broiler litter by P-NMR revealed that 89% and 82% of P in water and $NaHCO_3$ extracts occurred as phosphate P while only 6% and 18% of the P in water and $NaHCO_3$ extracts occurred as phosphate mono-esters. These results again confirmed that because of the relative degree of insolubility of phytate P, this was contained predominantly in NaOH and HCl extracted fractions. Similar conclusions were drawn by Maguire et al. (2004), who also found a 73% correlation between NaOH-P and phytate P, as measured by P-NMR, while the soluble P fraction was most strongly correlated with P as orthophosphate ($r^2 = 0.91$).

**Relative Importance of Total P and Soluble P in Manures**

Total P in manures was important as this determined the amount of P that was added to soils following land application of manure. Manure total P concentrations have been shown to be, at the same rate of manure application, correlated with STP levels. Therefore,

the increase in STP following manure application was primarily determined by the manure total P when manures were applied on a nitrogen basis. Alternatively, if manure was applied on a phosphorus basis (i.e., limited to the amount of P that will be removed from the soil after crops have been removed) the manure total P determined the total amount of manure that can be applied per hectare (ha) of land.

In the first instance, when manures were applied on a nitrogen basis, diet amendment that resulted in a reduction in total manure P concentration without changes in manure N concentration led to an increased manure N:P ratio. On average, manures have contained a ratio of N:P of 2-3:1 (Daniels et al., 1998; Sistani et al., 2001), however, plants require a ratio of N:P of between 6-11:1, or stated in a different manner, the requirement of crops for N is 3 – 5 times greater than the requirement for P (Daniels et al., 1998). Because of the difference in the required N:P ratio of plants and the N:P ratio in manure, application of poultry litter based on crop N requirements was estimated to add 135 kg P $ha^{-1}$, relative to crop removal of only 25 kg P $ha^{-1}$ (Mozaffari and Sims, 1994). Over time, the practice of N based manure application has led to the widespread increase in STP concentrations to well above that required for local crop production (Pautler and Sims, 2000).

The legislative response to high STP and water quality problems associated with non-point losses of P have led to the implementation of guidelines that regulate P application based on either existing STP levels, or on a calculated P-index for a specific field (Penn et al., 2004). Soils that have a STP or P index above a maximum threshold values have become subject to reduced P application rates from manure such that some farms would no longer have sufficient land for application of the manure produced, while alternate methods of manure disposal could be expected to increase production costs (Johnson et al., 2005).

Therefore, a reduction in manure total P concentration that resulted from dietary amendment would potentially increase the amount of manure that could be applied per ha when P application rates were followed, or would reduce the relative excess of P applied when manures were applied on a nitrogen basis. This was shown by Penn et al. (2004) who calculated that phytase amendment of turkey diets, in conjunction with reduced dietary NPP levels, reduced manure total P by 41% and increased the amount of wet manure needed to provide 60 kg total P $ha^{-1}$ by 87%. In a similar manner, Maguire et al. (2004) calculated that when applied on an N basis, after accounting for crop P removal, a reduction in litter total P of 38% would reduce the relative degree of P excess by as much as 46%. However, in addition to determining manure total P, measuring soluble P in manure was important as the soluble P concentration greatly affected the environmental impact of manures under certain conditions. A positive correlation was shown between soil soluble P and dissolved reactive phosphorus (DRP) in runoff water (Pote et al., 1999). This was confirmed by Sharpley and Moyer (2000) who found a 98% correlation between soluble P and the amount of P leached from the soil following 5 simulated rainfall events that suggested that soluble P was a good indicator to estimate the potential of manure to contribute to P runoff after surface application (Sharpley and Moyer, 2000). Similar increases in DRP in runoff from fields following application of manures with a high soluble P concentration were also observed by McGrath et al. (2005).

Therefore, the importance of determining soluble P in manure and litters was based on data that suggested an increase in the concentration of soluble P in litter or manure also increased P losses from surface applied manures that had not had sufficient time to react with soil and convert to more stable forms (Penn et al., 2004; Sturgul and Bundy, 2004). Further, a

high concentration of soluble P or a high ratio of soluble P:total P would also likely increase P runoff from irrigated pastures where DRP was the dominant fraction in runoff (Sharpley et al., 1992). The ratio of soluble P:total P would become particularly important when converting to a P-based nutrient management plan as an increased proportion of soluble P would increase application rates of soluble P at the same rate of total P application (Maguire et al., 2005).

However, the relevant importance of the soluble P fraction in manures as a determinant of P runoff decreased both over time and when manures were incorporated in soils. These time-dependent effects of the relevant importance of soluble P were shown by Maguire et al. (2004) who noted that application of litter with reduced soluble P:total P decreased soil soluble P 5 days following litter application. However, effects of dietary treatment on soil soluble P were no longer present after 29 days. Therefore, while soluble P and the ratio of soluble P:total P were important factors driving DRP losses within a short time period following manure application, dietary strategies that reduced litter total P were more important over the longer term, as this reduced P surpluses and the positive mass balance of P.

**Effects of Diet Amendment on Phosphorus Fractions in Manure and Litter**

***Effects on Total Phosphorus.*** A single broiler chicken was estimated to produce 1 kg of poultry litter that contained approximately 4% total N and 2% total phosphorus (Haggard et al., 2003). Comparable estimates were also made by Saylor et al. (2001) who measured total litter production levels per bird of 0.465 kg per kg broiler or 1.3 kg per 2.8 kg broiler at 42 d of age. Importantly, in the study by Saylor et al. (2001) a 0.2% reduction in diet NPP

with added phytase reduced the litter produced by 13% to 0.406 kg $kg^{-1}$ bird, while this was associated with a 32% decrease in total litter P concentration from 14,320 mg/kg to 9,698 mg/kg. Numerous other examples exist that showed substantial reductions in total litter P when dietary NPP was reduced either alone, or in combination with phytase.

For example, Maguire et al. (2004) evaluated litter P concentrations from broilers and turkeys. Feeding closer to NPP requirements decreased total P in litter from turkeys from 19% to 33%; and from 10% to 17% in broiler litter. In the same study, phytase addition decreased total P in broiler litter by 17 to 24% and by 7 to 24% in turkey litter. In this study, the combination of reduced NPP, closer to the requirement of the birds, and added phytase had the biggest impact on litter total P with reductions of 38% and 31% in turkey and broiler litter, respectively (Maguire et al., 2004). Similar observations were made by Applegate et al. (2003), Vadas et al. (2004), Smith et al. (2004), Angel et al. (2005), McGrath et al. (2005), and Angel et al. (2006) with the general consensus that reduced NPP regimens, combined with phytase, could reduce litter total P fractions by at least 29%, while this could be as high as 45% (Vadas et al., 2004).

Sequential fractionation of P in broiler and turkey litters showed that the water and $NaHCO_3$ extractable P fractions increased when birds were fed high NPP diets. The NaOH-P extractable fractions that represent primarily organic phytate P were not altered by diet NPP level but were reduced between 27 to 38% in litters from phytase amended diets fed to broilers and by 25 to 32% in turkey litter (Maguire et al., 2004). Similar results were obtained by McGrath et al. (2005) who suggested that excess P added to diets as calcium phosphate was converted to highly labile water and $NaHCO_3$ extractable P forms in the litter and that the relative amounts of these soluble P fractions could be reduced simply by feeding

broilers closer to the NPP requirement. In these studies, the reduction in total P and phytate P in the litter, as a result of phytase addition to diets, provided evidence of improved P digestion and absorption by the bird and were most likely caused by increased hydrolysis of phytate by phytase enzyme (Rutherford et al., 2002; McGrath et al., 2005).

Diet amendment using grains with a low phytate P and high available P (HAP) content was also shown to have similar effects as phytase on litter total P concentration. In a study by Penn et al. (2004) the inclusion of HAP corn, phytase, or a combination of HAP corn with phytase in diets reduced total P by 40, 41, and 42%, respectively, but there was no additive effect of both phytase and HAP corn. Saylor et al. (2001) found that HAP corn alone, without reductions in diet NPP or added phytase only, reduced litter total P by 8.3%, while the combination of HAP corn and phytase reduced litter total P in an additive manner by 30.8%. These findings were supported in a review by Maguire et al. (2005) who concluded that reductions in total P were greatest when dietary strategies to reduce P excretion were combined. The potential for reductions in manure and litter total P by combined technologies such as phytase, HAP corn, and 25OH-$D_3$ was estimated to be at least 40% (CAST, 2002).

***Effects On Soluble Phosphorus.*** While effects of reduced diet NPP combined with phytase and HAP corn have led to consistent reductions in total P concentrations of litter and manure, effects on soluble P have been more variable (Applegate et al., 2003). Phytase addition to diets was shown to decrease soluble P concentration by 35.6% (Applegate et al., 2003) and by 29% (McGrath et al., 2005). However, in other studies, phytase supplementation to diets had no effect on soluble P concentration (Saylor et al., 2001; Maguire et al., 2004; Angel et al., 2005; McGrath et al., 2005), while in two other studies,

amendment of diets with phytase increased the concentration of soluble P in the litter (Delaune et al., 2001; Vadas et al., 2004).

A possible cause of variations in reported effects of phytase on soluble P was reported by Angel et al. (2005) who investigated changes in manure soluble P after excretion by the bird. Results showed there were no effects of phytase on manure soluble P when manure was frozen immediately following excretion, or when an antibiotic was added to the manure. In contrast, a rapid increase in soluble P was observed when manure was incubated following excretion. This suggested that soluble P of manures could be altered by microbial degradation of organic P fractions, while phytase addition per se did not affect soluble P in the excreta. Effects of other dietary amendments such as HAP grains and 25OH-$D_3$ either alone, or in combination with phytase, on litter soluble P have also been reported.

Saylor et al. (2001) showed that use of HAP corn, or phytase alone had no effect on soluble P in broiler litter but that a combination of HAP corn and phytase reduced soluble P concentrations by 44%. In contrast, Applegate et al. (2003) showed no differences in litter soluble P when broilers where fed diets containing HAP corn with phytase compared to litter from diets that contained a commercial corn variant and phytase. Other dietary amendments such as reduced diet NPP in combination with 25OH-$D_3$ were also shown to not affect soluble P concentration in litter (Maguire et al., 2004).

**Effect of Storage and Handling on P Forms in Manure**

Angel et al. (2005) showed that variable effects of diet amendment on soluble P concentrations may, in part, be caused by microbial influence on P fractions of manures following excretion. In addition, factors such as the moisture content of the diet, handling of

the manure, and duration of storage, may mediate the extent of the microbial influence reported by Angel et al. (2005). McGrath et al. (2005) reported an interaction of litter moisture level and storage time on P fractions in broiler litter. In this study, storage of dry litter resulted in a gradual increase in soluble P with time but soluble P concentrations in wet litter increased rapidly after the onset of storage and tended to reach a plateau after 4 months. Increases in soluble P during storage of wet litter were also related to diet as the greatest increase in soluble P of 229% occurred in litter from birds that had been fed the diet with the highest NPP level and no phytase.

McGrath et al. (2005) showed that phytase added to broiler diets did not contribute to increased soluble P in stored litter, while reductions in dietary NPP level with or without phytase additions produced soluble P concentrations that were lower than that of the control after 15 months of storage. Storage of wet litter was also shown to impact total P concentration that was attributed to loss of carbon due to microbial decomposition of the litter (McGrath et al., 2005). Further, effects of storage and moisture on litter total P and soluble P fractions could be explained by changes in the P-forms in the litter.

Storage of wet litter increased orthophosphate concentrations and decreased phytate concentrations to a much greater extent than did storage of dry litter that had higher concentrations of less soluble NaOH and HCl-P fractions. These changes in P-forms were attributed to increased microbial phytate degradation under high moisture conditions that were also shown to result in significant increases in soil soluble P concentrations and TRP and DRP in runoff following a rainfall simulation study (McGrath et al., 2005). This study showed that benefits of diet modification on litter soluble P could be completely ameliorated following prolonged storage of litter under moist conditions. Similar adverse effects of

moisture on litter soluble P fractions in manure from broiler breeders were also shown by Maguire et al. (2006). These data highlight the importance of correct manure moisture management both before collection as well as during subsequent storage.

## REFERENCES


Angel, R. C., W. J. Powers, T. J. Applegate, N. M. Tamin, and M. C. Christman. 2005. Influence of phytase on water soluble phosphorus in poultry and swine manure. J. Environ. Qual. 34:563-571.

Angel, R., and T. Applegate. 2001. Phytase use -- what do we know? Pages 250-263 in Proc. of the 62nd Minnesota Nutrition Conf. and Minnesota Corn Growers Assn. Tech. Symp., Bloomington, MN.

Angel, R., N. M. Tamim, T. J. Applegate, A. S. Dhandu, and L. E. Ellestad. 2002. Phytic acid chemistry: influence on phytin-phosphorus availability and phytase efficacy. J. Appl. Poult. Res. 11:471-480.

Angel, R., T. J. Applegate, and M. Christman. 2000a. Effects of dietary non-phytate phosphorus (nPP) on performance and bone measurements in broilers fed on a four-phase feeding system. Poultry Sci. 79 (Suppl. 1):22.

Angel, R., T.J. Applegate, M. Christman, and A.D. Mitchel. 2000b. Effect of dietary non-phytate phosphorus (nPP) level on broiler performance and bone measurements in the starter and grower phase. Poultry Sci. 79(Suppl. 1):21-22.

Angel, R., W. W. Saylor, A. D. Mitchell, W. Powers, and T. J. Applegate. 2006. Effect of dietary phosphorus, phytase, and 25-hydroxycholecalciferol on broiler chicken bone mineralization, litter phosphorus, and processing yields. Poult. Sci. 85:1200-1211.

Applegate, T. J., B. C. Joern, D .L. Nussbaum-Wagler, and R. Angel. 2003. Water-soluble phosphorus in fresh broiler litter is dependent upon phosphorus concentration fed but not on fungal phytase supplementation. Poult. Sci. 82:1024-1029.

Beede, D. 2003. Ration phosphorus management: Requirements and excretion. Pages 145-151 in Proc. Four-State Applied Nutrition and Mmanagement Conf., LaCrosse, WI.

Bennet, E. M., T. Reed-Andersen, J. N. Houser, J. R. Gabriel, and S. R. Carpenter. 1999. A phosphorus budget for the lake Mendota watershed. Ecosystems 2:69-75.

Berry, W. D., J. B. Hess, R. J. Lien, and D. A. Roland. 2003. Egg production, fertility, and hatchability of breeder hens receiving dietary phytase. J. Appl. Poult. Res. 12: 264-270.

Biehl, R. R., and D. H. Baker. 1997. 1α-hydroxycholecalciferol does not increase the specific activity of intestinal phytase but does improve phosphorus utilization in both cecectomized and sham-operated chicks fed cholecalciferol-adequate diets. J. Nutr. 127:2054-2059.

Biehl, R. R., D. H. Baker, and H. F. Deluca. 1995. 1α-hydroxylated cholecalciferol compounds act additively with microbial phytase to improve phosporus, zinc, and manganese utilization in chicks fed soy-based diets. J. Nutr. 125:2407-2416.

Bolingen-Frankenback, S. D., J. L. Snow, C. M. Parsons, and D. H. Baker. 2001. The effect of citric acid on the calcium and phosphorus requirements of chicks fed corn-soybean meal diets. Poult. Sci. 80:783-788.

Bootwalla, S. M. 1982. Performance of broiler breeders on different feeding schedules and different flooring systems. M.S. Thesis, Univ. Florida.

Brake, J., C. V. Williams, and B. A. Lenfestey. 2003. Optimization of dietary phosphorus for broiler breeders and their progeny. Pages 77-83 in Proc. of Alltech's 19th Annual Symposium, Lexington, KY. Nottingham Univ. Press, Nottingham.

Bregitzer, P., and V. Raboy. 2006. Effects of four independent low-phytate mutations on barley agronomic performance. Crop Sci. 46: 1318-1322.

CAST (Council for Agricultural Science and Technology). 2002. Animal diet modification to decrease the potential for nitrogen and phosphorus pollution. Issue paper no. 21.

Centraal Veevoeder Bureau (CVB). 1997. Definitief Systeeem Opneembaar Fosfor voor pluimvee, Nr 20. Centraal Veevoederbureau, Postbus 2176 8203 AD, Leylystad, The Netherlands.

Cromwell, G. L. 1996. Vitamin D3 in swine nutrition. P.101-109. In M.B. Coelho, and E.T. Kornegay (eds.). Phytase in animal nutrition and waste management. A BASF reference manual, Mount Olive, New Jersey.

Daniels, M., T. Daniel, D. Carman, R. Morgan, J. Langston, and K. van Deventer. 1998. Soil phosphorus levels: Concerns and recommendations. University of Arkansas Cooperative Extension Service Publication. FSA1029. http://www.sera17.ext.vt.edu/Documents/ Soil_P_Levels_Concerns_and_Recommendations.pdf (Accessed 05.19.2006).

DeLaune, P. B., P. A. Moore, Jr., D. C. Carman, T. C. Daniel, and A. N. Sharpley. 2001. Development and validation of a phosphorus index for pastures fertilized with animal manure. Proc. International Symposium Addressing Animal Production and Environmental Issues, Research Triangle Park, Raleigh, NC.

Dhandu, A. S., R. Angel, T. J. Applegate, and B. Ling. 2000. Non-phytate phosphorus requirement of broilers in the finisher phase of a four-phase feeding program. Poultry Sci. 79 (Suppl. 1):11.

Dilger, R. N., and O. Adeola. 2006. Estimation of true phosphorus digestibility and endogenous phosphorus loss in growing chicks fed conventional and low phytate soybean meals. Poult. Sci. 85:661-668.

Driver, J. P., G. M. Pesti, R. I. Bakalli, and H.M. Edwards. 2005. Effects of calcium and non phytate phosphorus concentrations on phytase efficacy in broiler chicks. Poult. Sci. 84:1406-1417.

Environmental Protection Agency. 2003. National pollutant discharge elimination system permit regulation and effluent guidelines and standards for concentrated animal feeding operations: Final rule. Fed. Regist. 68: 7175-7274.

Ferket, P.R., E. van Heughten, T. A. T .G. van Kempen, and R. Angel. 2002. Nutritional strategies to reduce environmental emissions from nonruminants. J. Anim. Sci. 80 (E. Suppl. 2):E168-E182).

Guttieri M, D. Bowen, J. A. Dorsch, V. Raboy, and E. Souza. 2004. Identification and characterization of a low phytic acid wheat. Crop Sci. 44:418-424.

Haggard, B. E., P. A. Moore, Jr., I. Chaubey, and E. H. Stanley. 2003. Nitrogen and phosphorus concentrations and export from an Ozark plateau catchment in the United States. Biosystems Eng. 86(1):75-85.

Harms, R. H., S. M. Bootwalla, and H. R. Wilson. 1984. Performance of broiler breeder hens on wire and litter floors. Poult. Sci. 63:1003-1007.

Hedley, M .J., J. W. B. Stewart, and B. S. Chauhan. 1982. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. Soil Sci. Soc. Am. J. 46:970-976.

Israel, D. W., P. Kwanyuen, and J. W. Burton. 2006. Genetic variability for phytic acid phosphorus and inorganic phosphorus in seeds of soybeans in maturity groups V, VI, and VII. Crop Sci. 46:67-71.

Johnson, A. M., D. L. Osmond, and S. C. Hodges. 2005. Predicted impact of North Carolina's phosphorus indexing tool. J. Environ. Qual. 34:1801-1810.

Karr-Lilienthal, L. K., P. L. Utterback, C. Martinez Amezcua, C. M. Parsons, N. R. Merchen, and G. C. Fahey, Jr. 2005. Relative bioavailability of phosphorus and true amino acid digestibility by poultry as affected by soybean extraction time and use of low phytate soybeans. Poult. Sci. 84:1555-1561.

Knowlton, K. F., J. S. Radcliffe, C. L. Novak, and D. A. Emmerson. 2004. Animal management to reduce phosphorus losses to the environment. J. Anim. Sci. 82 (E. Suppl.):E173-195.

Leinweber, P. 1996. Phosphorus fractions in soils from and area with high density livestock population. Z. Pflanzeneraehr. Bodenkd. 159:251-256.

Leytem, A. B., D. R. Smith, P. A. Thacker, and T. J. Applegate. 2006. The influence of manure phytic acid on phosphorus solubility in calcareous soils. Soil Sci. Soc. Am. J. (Submitted).

Li, Y.C., D. R. Ledoux, T. L. Veum, V. Raboy, and D. S. Ertl. 2000. Effects of low phytic acid corn on phosphorus utilization, performance, and bone mineralization in broiler chicks. Poult. Sci. 79:1444-1450.

Ling, B., R. Angel, T. J. Applegate, N.G. Zimmerman, and A. S. Dhandu. 2000. The non-phytate phosphorus requirements of broilers in a four-phase feeding program. Poult. Sci. 79(Suppl. 1):11.

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

Maenz, D. D., C. M. Engele-Schaan, R.W. Newkirk, and H. L. Claasen. 1999. The effect of minerals and mineral chelators on the formation of phytase-resistant and phytase susceptible forms of phytic acid in solution and in a slurry of canola meal. Anim. Feed Sci. Technol. 81:177-192.

Maguire, R. O., J. T. Sims, W. W. Saylor, B. L. Turner, R. Angel, and T. J. Applegate. 2004. Influence of phytase addition to poultry diets on phosphorus forms and solubility in litters and amended soils. J. Environ. Qual. 33:2306-2316.

Maguire, R. O., Plumstead, P. W., and J. Brake. 2006. Impact of diet, moisture, location and storage on soluble phosphorus in broiler breeder manure. J. Environ. Qual. 35:858-865.

Maguire, R. O., Z. Dou, J. T. Sims, J. Brake, and B. C. Joern. 2005. Dietary strategies for reduced phosphorus excretion and improved water quality. J. Environ. Qual. 34:2093-2103.

McGrath, J. M., J. T. Sims, R. O. Maguire, W. W. Saylor, R. Angel, and B. L. Turner. 2005. Broiler diet modification and litter storage: Impacts on phosphorus in litters, soils, and runoff. J. Environ. Qual. 34:1896-1909.

Mozaffari, P. M. and J. T. Sims. 1994. Phosphorus availability and sorption in and Atlantic Coastal Plain watershed dominated by intensive, animal-based agriculture. Soil Sci. 157:97-107.

Murphy, J., and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta. 27:31-36.

National Research Council (NRC). 1993. Soil and water quality: an agenda for agriculture. National Academy Press, Washington, DC.

National Research Council (NRC). 1994. Nutrient requirements for poultry. 9th revised ed. National Academy Press, Washington, D.C.

Oltmans, S. E., W. R. Fehr, G. A. Welke, V. Raboy, and K. L. Peterson. 2005. Agronomic and seed traits of soybean lines with low-phytate phosphorus. Crop Sci. 45:593–598.

Pautler, M. and T. J. Sims. 2000. Relationships between soil test phosphorus, soluble phosphorus, and phosphorus saturation in Delaware soils. Soil. Sci. Soc. Am. J. 64:765-773.

Penn, C. J., G. L. Mullins, L. W. Zelazny, J. G. Warren, and J. M. McGrath. 2004. Surface runoff losses of phosphorus from Virginia soils amended with turkey manure using phytase and high available phosphorus corn diets. J. Environ. Qual. 33:1431-1439.

Pilu R. M. Landoni E. Cassani E. Doria, and E. Nielsen. 2005. The maize lpa241 mutation causes a remarkable variability of expression and some pleiotropic effects Crop Sci.45:2096-2105.

Plumstead, P. W., A. B. Leytem, R. O. Maguire, E. Oviedo, and J. T. Brake. 2006. Manipulation of dietary calcium, phosphorus and phytase in broilers: 1. Effects on performance. Poult. Sci. 85 (Suppl. 1):89.

Plumstead, P. W., H. Romero-Sanchez, R. O. Maguire, and J. Brake. 2006. Effects of dietary phosphorus level and phytase in broiler breeder rearing and laying diets on performance and phosphorus excretion. Poult. Sci. 86: (in press).

Plumstead, P. W., H. Romero-Sanchez, R. O. Maguire, N. Leksrisompong and J. Brake. 2005a. Reducing fecal phosphorus excretion 1: Effects of dietary phosphorus and phytase in broiler breeder rearing and laying diets on water soluble fecal phosphorus during rearing and subsequent reproductive performance. Page 36 in: The development of alternate technologies for the processing and use of animal waste. Proc. Animal Waste Management Symposium, Research Triangle Park, NC.

Plumstead, P. W., H. Romero-Sanchez, R. O. Maguire, N. Leksrisompong and J. Brake. 2005b. Reducing fecal phosphorus excretion 2: Vertical effects of dietary phosphorus and phytase in broiler breeder diets on performance and water soluble phosphorus excretion of broiler progeny fed reduced phosphorus diets. Page 37 in: The development of alternate technologies for the processing and use of animal waste. Proc. Animal Waste Management Symposium, Research Triangle Park, NC.

Pote, D. H., T. C. Daniel, D. J. Nichols, A. N. Sharpley, P. A. Moore Jr., D. M. Miller, and D. R. Edwards. 1999. Relationship between phosphorus levels in three ultisols and phosphorus concentrations in runoff. J. Environ. Qual. 28:170-175.

Qian, H., E. T. Kornegay, and D. M. Denbow. 1997. Utilization of phytate phosphorus and calcium as influenced by microbial phytase, cholecalciferol, and the calcium:total phosphorus ratio in broiler diets. Poult. Sci. 76:37-46.

Raboy, 2002. Progress in breeding low phytate crops. J. Nutr. 132:502s-505s.

Raboy, V., P. F. Gerbasi, K. A. Young, S. D. Stoneberg, S. G. Pickett, A. T. Bauman, P. N. Murthy, W. F. Sheridan, and D. S. Ertl. 2000. Origin and seed phenotype of maize with low phytic acid 1-1 and low phytic acid 2-1. Plant. Phys. 124:355-368.

Rutherfurd, S. M., T. K. Chung, and P. J. Moughan. 2002. The effect of microbial phytase on ileal phosphorus and amino acid digestibility in the broiler chicken. Br. Poult. Sci. 44:598-606.

Saylor, W. W., J. T. Sims, G. W. Malone, and M. F. Lavahun. 2001. Use of phytase and high available phosphorus corn in broiler diets: Impact on litter phosphorus levels and solubility. P. 43-57. In Proc. Maryland Nutr. Conf., College Park, MD.

Self-Davis, M. L., and P. A .Moore, Jr. 2000. Determining water soluble phosphorus in animal manure. P. 74–76. In G.M. Pierzynski (ed.). Methods of phosphorus analysis for soils, sediments, residuals and waters. Southern Cooperative Series Bulletin #396, North Carolina.

Sharpley, A. N., Chapra, S. C., Wedephol, R., Sims, J. T., Daniel, T. C. and Reddy, K. R. 1994. Managing agricultural phosphorus for protection of surface waters: Issues and options. J. Environ. Qual. 23:437-451.

Sharpley, A. N., S .J. Smith, and O. R. Jones. 1992. The transport of bioavailable phosphorus in agricultural runoff. J. Environ. Qual. 21:30-35.

Sharpley, A., and B. Moyer. 2000. Phosphorus forms in manure and compost and their release during simulated rainfall. J. Environ. Qual. 29:1462-1469.

Sims, J. T., A. C. Edwards, O. F. Schoumans, and R. R. Simard. 2000. Integrating soil phosphorus testing into environmentally based agricultural practices. J. Environ. Qual. 29:60-71.

Sims, J. T., Simard, R. R. and Joern, B. C. (1998) Phosphorus loss in agricultural drainage: Historical perspective and current research. J. Environ. Qual. 27:277-293.

Singsen, E. P., A. H. Spandorf, L. D. Matterson, J. A. Serafin, and J. J. Tlustohowciz. 1962. Phosphorus in the nutrition of the adult hen. 1. Minimum phosphorus requirements. Poult. Sci. 41: 1401-1414.

Sistani, K. R., D. M. Miles, D. E. Rowe, G. E. Brink, and S. L. McGowen. 2001. Impact of drying method, dietary phosphorus levels, and methodology on phosphorus chemistry of broiler manure. Commun. Soil Sci. Plant Anal. 32 :2783-2793.

Smith, D. R., P. A. Moore, Jr., C. V. Maxwell, B. E. Haggard, and T. C. Daniel. 2004. Reducing phosphorus runoff from swine manure with dietary phytase and aluminum chloride. J. Environ. Qual. 33:1048-1054.

Soares, J. H., Jr. 1995. Phosphorus Bioavailability. Pages 257-294 in Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins. C. B. Ammerman, D. H. Baker, A. J. Lewis. Eds.Academic Press, London, UK.

Sturgul, S. J., and L. G. Bundy. 2004. Understanding soil phosphorus. An overview of phosphorus water quality and agricultural management practices. http://ipcm.wisc.edu /pubs/pdf/ UnderstandingSoilP04.pdf (Accessed 06.11.2006).

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

Toor, G. S., J. D. Peak, and J. T. Sims. 2005. Phosphorus speciation in broiler litter and turkey manure produced from modified diets. J. Environ. Qual. 34: 687-697.

Turner, B. L. 2004. Optimizing phosphorus characterization in animal manures by solution phosphorus-31 nuclear magnetic resonance spectroscopy. J. Environ. Qual. 22:757-766.

Turner, B. L. and A. B. Leytem. 2004. Phosphorus compounds in sequential extracts of animal manures: chemical speciation and a novel fractionation procedure. Environ. Sci. Tech. 38:6101-6108.

Vadas, P. A., J. J. Meisinger, L. J. Sikora, J. P. McMurtry, and A. E. Sefton. 2004. Effect of poultry diet on phosphorus in runoff from soils amended with poultry manure and compost. J. Environ. Qual. 33:1845-1854.

Van der Klis, J. D. and H. A. J. Versteegh. 1996. Phosphorus nutrition of poultry. Pages 71-83. In Recent Developments in Poultry Nutrition. Ed. P. C. Garnsworthy, J. Wiseman, and W. Haresign, Nottingham University Press, Nottingham, UK.

Van Tuijl, O. A. 1998. Field observations and practical implications resulting from reductions in the phosphorus content of breeder and broiler diets. World's Poult. Sci. J. 54:359-363.

Vogelmaier, S. M., M. E. Bembenek, A. I. Kaplin, G. Dorman, J. D. Olzeweski, G. D. Prestwich, and S.H. Snyder. 1996. Purified inositol hexakisphosphate kinase is an ATP synthase:diphospoinositol pentakisphosphate as a high energy phosphate donor. Proc. Natl. Acad. Sci. USA 93:4305-4310.

Wilcox, J. R., G. S. Premachandra, K. A. Young, and V. Raboy. 2000. Isolation of high seed inorganic p, low-phytate soybean mutants. Crop Sci. 40: 1601-1605.

Wilson, H. R., E. R. Miller, H. Harms, and B. L. Damron. 1980. Hatchability of chicken eggs as affected by dietary phosphorus and calcium. Poult. Sci. 59:1284-1289.

Wyss, M., R. Brugger, A. Kronenberger, R. Remy, R. Fimbel, G. Oesterhelt, M. Lehmann, and A. P. G. M. van Loon. 1999. Biochemical characterization of fungal phytases (myo-inositol hexakisphosphate phosphohydrolases): catalytic Properties. Appl. Environ. Microbiol. 2:367-373.

Yan, F., C. A. Fritts, P. W. Waldroup, H. L. Stilborn, D. Rice, C. R. Crum, Jr., and V. Raboy. 2003. Comparison of normal and high available phosphorus corn with and without phytase supplementation in diets for male large white turkeys grown to market weights. Int. J. Poult. Sci. 2 :83-90.

Yan, F., J. H. Kersey, and P. W. Waldroup. 2001. Phosphorus requirements of broiler chicks three to six weeks of age as influenced by phytase supplementation. Poultry Sci. 80:455-459.

# CHAPTER 1

## Effects of Phosphorus Level and Phytase in Broiler Breeder Rearing and Laying Diets on Live Performance and Phosphorus Excretion

**ABSTRACT** Effects of reduced dietary non-phytate phosphorus (NPP) level and phytase on broiler breeder performance and phosphorus (P) concentrations in the litter and manure were investigated. Day-old Ross 308 breeder pullets and Ross 344 cockerels were reared sex-separate in a conventional blackout growing house and fed standard starter and grower diets to 9 wk of age. At 10 wk four treatments (A, B, C, D) were each assigned to four floor pens of 68 pullets and one pen of 50 cockerels. From 10 – 21 wk dietary treatments A – D contained 0.37%, 0.27%, 0.27%, and 0.17% NPP, respectively, with 300 FTU/kg phytase added to treatments B and D. At 21 wk of age birds were photostimulated and transferred to a two-thirds slat-litter breeder house with 16 pens of 60 pullets and 6 cockerels each. A breeder layer diet was fed from 22 – 64 wk and NPP levels of treatments A – D were adjusted to 0.37%, 0.27%, 0.19% and 0.09%, respectively, and phytase addition to treatments B and D increased to 500 FTU/kg. Analysis of the litter from growing pens showed no effect on litter total P when phytase replaced 0.1% NPP. However, decreasing diet NPP by 0.1% without adding phytase reduced litter total P by 18%. Water soluble P (WSP) and the WSP:total P ratio decreased when grower diet NPP level was reduced to 0.17% with added phytase and was positively correlated with litter moisture levels in growing pens. During the breeder laying period a reduction in NPP from 0.37% to 0.09% with added phytase reduced both manure total P and WSP by 42%. Hen-day egg production and feed efficiency were improved on the lowest NPP diet with phytase but fertility decreased when dietary NPP was reduced below 0.37%. Results showed that phytase inclusion in a broiler breeder diet at the expense of all added P from dicalcium phosphate reduced manure total P and WSP concentrations by 42% without affecting the number of chicks produced per hen housed.

## INTRODUCTION

The application of animal manures to land remains the most efficient method of utilizing the large quantities of manure generated by confined animal feeding operations (Olson and Paterson, 2005). However, due to an unfavorable nitrogen:total phosphorus (P) ratio relative to crop requirements, the continual application of manures to soils often leads to an accumulation of excessive Soil Test P concentrations (Sims et al., 2000) that in turn has led to federal regulations to limit the amount of P from manure that can be applied to soils (Environmental Protection Agency, 2003). Further, in addition to total P in poultry litter, the Water Soluble P fraction (WSP) has been identified as an important environmental risk factor as P losses in runoff following land application of litter have been related to the WSP in the litter applied (Maguire et al., 2005). To date, strategies to decrease P accumulation in soils have focused primarily on reducing both total P and WSP fractions in the litter by reducing levels of inorganic P supplementation to diets in combination with added phytase enzymes (Applegate et al., 2003; Angel et al., 2005). Angel et al. (2000a,b) demonstrated that the dietary non-phytate phosphorus (NPP) levels of broiler diets could be reduced substantially from National Research Council (1994) recommendations without affecting broiler performance. Applegate et al. (2003) further showed that when combined with phytase, the revised NPP regimens reduced litter total P and WSP fractions by as much as 30% and 50%, respectively. However, in spite of extensive research that investigated the combined effects of NPP and phytase in broilers, there have been comparably few reports that have described similar effects on total P and WSP fractions in manure from broiler breeders. Studies have shown that combinations of reduced dietary NPP and added phytase had either no effect or, in some instances, increased egg production from broiler breeder hens (Berry et al., 2000, 2003; Li et al., 2002; Brake et al., 2003). As these previous

authors did not describe treatment effects on total P and WSP in the manure or litter, the objectives of the present study were to further evaluate effects of reduced dietary NPP and phytase on the performance of broiler breeders, while also quantifying the potential benefits of these strategies to reduce the total P or WSP concentration of the pullet rearing litter and broiler breeder manure.

## MATERIALS AND METHODS

### *Definitions*

Since the dietary NPP and available P (AvP) content of diets fed to poultry have often been erroneously interchanged, the term AvP utilized in this experiment referred to relative bioavailable P as determined using a slope ratio assay with monocalcium P as the reference standard (Apke at al., 1987; Soares, 1995), and NPP represented a chemically defined entity calculated by subtracting the analyzed P content of ingredients from their analyzed phytate P content (Angel and Applegate, 2001).

### *Pullet and Breeder Management*

There were 1,088 day-old Ross 308 breeder pullets and 200 day-old Ross 344 cockerels placed and reared sex-separate in a blackout growing house with either 68 female or 50 male chicks randomly assigned to each of 16 female or four male pens of 3.96 x 3.96 m. The lighting program consisted of 23 h light per day for one week followed by 8L:16D to 21 wk of age. Other aspects of management and house infrastructure were as generally described by Brake and Baughman (1989). At 21 wk, 60 pullets from each of the 16 female rearing pens as well as 6 cockerels from the pen of males on the same treatment were moved to each of 16 breeder pens located within a curtain-sided breeder laying house. Laying pen dimensions were similar to the

pens of the growing house while two-thirds of each breeder pen was covered with raised plastic slats while a scratch area with fresh pine shavings constituted the remaining one-third of each pen. Each pen contained one automatic waterer, one 12-hole galvanized nest box, five tube feeders with male exclusion grills for females that were located above the slats, and one tube feeder for males located over the scratch area. At 20 and 22 wk the photoperiod was increased to 14 and 15 h, respectively, after which it was further increased to 15.5 h at 5% lay and again to 16 h at 50% lay. All eggs laid were collected twice daily and stored at 18.6 C and 70% relative humidity until incubated in Jamesway 252B[1] incubators. Fertility of eggs was determined from sets of 60 eggs per pen that were incubated biweekly from 28 to 64 wk of age. Treatment identity of hatching eggs was maintained by breeder pen throughout the incubation and hatching process. After 21.5 d of incubation all eggs that did not hatch were broken out and examined macroscopically and the percentage fertility and stage of embryonic mortality calculated. All eggs that were accidentally cracked were deleted from the analyses.

***Pullet and Breeder Diets***

From placement to 3 wk of age, all chicks received a common pullet starter diet (2,925 kcal/kg ME and 17.66% CP) followed by a common grower diet (2,925 kcal/kg ME and 15.96% CP) to 9 wk of age (Table 1.1). From 10 wk of age, four female pens and one male pen were randomly assigned to one of four dietary treatment groups (A, B, C, D) that received diets with different combinations of AvP or NPP in the presence or absence of a fungal derived phytase (Allzyme SSF[2]) during the growing (10-21 wk) and laying period (22-64 wk) (Table 1.2). For the grower period, diets fed to Treatment A contained 0.37% NPP with no added phytase.

[1] Jamesway Incubator Company, Ft. Atkinson, WI 53538
[2] Alltech, Inc., Nicholasville, KY 40356

Treatment B diets contained 0.27% NPP with 300 FTU phytase. Treatment C diets contained 0.27% NPP but with no added phytase. The reductions in NPP between Treatments A and B were based on recommendations of the phytase manufacturer that the addition of 300 FTU phytase in pullet rearing diets was able to replace the equivalent of 0.10% NPP from dicalcium phosphate. In a similar manner, the NPP level of the grower diet fed to Treatment D was reduced by 0.10% from that in Treatment C to 0.17%, while simultaneously adding 300 FTU phytase. Further, to reduce the phytase contribution from endogenous sources, the wheat bran and a portion of corn, poultry fat, and vermiculite was removed from the diet formulation and pelleted separately as a premix that consisted of 37% corn, 58% wheat bran, 1.6% poultry fat, and 3.4% vermiculite. The reduction in basal phytase activity of the pelleted bran premix was confirmed prior to amending diets with quantities that reflected the original ingredient profiles in each formulation. Treatment identity of pens was maintained during transfer from the rearing facility to the production facility. During the 42-wk breeder phase the NPP level of Treatment A was 0.37% with no added phytase; while Treatment B contained 0.27% NPP and 500 FTU added phytase. The NPP level of Treatment C was further reduced to 0.19% with no added phytase. Breeder Treatment D with 0.09% NPP was created by removing all remaining dicalcium phosphate from breeder diets fed to Treatment C while adding 500 FTU of phytase. Phytase inclusion in breeder diets for Treatments B and D was increased to 500 FTU / kg during the breeder layer phase to increase the likelihood of detecting potentially deleterious effects of phytase on the performance of the broiler breeder progeny, as had been reported previously (Brake et al., 2003).

***Manure Collection***

Litter samples were collected at 21 wk from five locations within each of the 20 growing pens and from four locations from each of the 16 breeder pens at 65 wk after the birds had been removed. Sampling areas in the growing pens consisted of four areas 1m from each corner along the diagonal tangent of the pen and one area in the center of the pen. Samples from the breeder house consisted of three samples from beneath the slatted floor area and one sample from the scratch area. The sampling location under the slated floor area was the same in each pen and consisted of one sample drawn from under the drinker, one sample drawn from under a feeder, and one sample drawn from an area not directly under a feeder or drinker. The five samples from each growing pen were mixed and a composite sample prepared while the four samples from each breeder pen were analyzed individually. The moisture content of all litter and manure samples was determined by drying samples overnight at 105 C. The analytical procedure for total P and WSP analyses of the litter was as described by Maguire et al. (2006).

***Statistical Methods***

A pen of birds was the experimental unit for all live production measurements. Egg production, fertility, and hatchability data were analyzed on a cumulative basis from 29-64 wk. Litter data from the rearing phase were subjected to one-way ANOVA using a completely randomized design with five replicate pens per treatment. Upon initial analysis of the data a significant correlation was found between litter moisture and litter WSP ($r = 0.74$, $P \leq 0.001$). To account for this correlation the percentage litter moisture was log transformed and then included as a covariate in the subsequent statistical analyses to assess treatment effects on WSP and the ratio of WSP:total P in the rearing litter. Manure samples from breeder pens were analyzed using a split-plot design with treatment as the main plot and sample area within each pen as the sub-plot. Because pen was the experimental unit, within pen variation in the manure analyses was not

considered in the estimation of treatment effects during the breeder phase and spatial effects within individual pens were reported previously by Maguire et al. (2006). The general linear model (GLM) procedure of SAS Institute (1996) was used to analyze the continuous variables of the broiler breeder production data. Fertility data was analyzed as categorical data, where each individual egg was taken as a binomial event, either fertile or infertile, using the generalized model (GENMOD) procedure of SAS Institute (1996). The results of the rearing litter and breeder manure analyses were interpreted using the mixed procedure of SAS Institute (1996). Means were partitioned using protected LSMEANS and statements of statistical significance were based upon $P \leq 0.05$ unless otherwise stated.

## RESULTS

### *Effect of Treatment on Phosphorus Excretion*

Analyses of the rearing litter showed no reduction in the total P concentration of the litter when 300 FTU of phytase was added to pullet grower diets at the expense of 0.1% NPP at either the 0.37% or 0.27% NPP level (Treatments A versus B and C versus D (Table 1.3)). However, a 0.1% reduction in NPP without the simultaneous addition of phytase decreased the litter total P by 18% with no effects on the WSP (Treatments A versus C). Treatment D exhibited the lowest WSP and also had the lowest WSP:total P ratio as there was a strong correlation between the percentage litter moisture and WSP concentration ($r = 0.74$, $P \leq 0.001$) while the moisture level in the litter showed no correlation with the litter total P concentration (Table 1.3).

In the breeder manure a reduction of 0.10% NPP from 0.37% to 0.27% with 500 FTU of phytase had no effect on total P or the ratio of WSP:total P, but decreased WSP by 28.6%. (Treatments A versus B (Table 1.4)). The further reduction in NPP from 0.27% to 0.19% without

added phytase decreased the manure total P concentration by an additional 29%, but had no effect on WSP or the ratio of WSP:total P (Treatments B versus C). A somewhat surprising result was that the further reduction in diet NPP from 0.19% to 0.09% in combination with 500 FTU phytase had no additional effect on either the litter total P or percentage WSP (Treatments C versus D (Table 1.4)). The overall reduction in dietary NPP from 0.37% to 0.09% with 500 FTU added phytase decreased both total and WSP by 42% without altering the relative proportion of WSP as judged by the WSP:total P ratio (Treatments A versus D (Table 1.4)).

***Treatment Effects on Broiler Breeder Performance***

The removal of all added dicalcium phosphate with the simultaneous addition of 500 FTU phytase (Treatment D) increased hen-day egg production relative to all other treatments (Table 1.5) while there was no effect of dietary treatment on the percentage female or male breeder mortality (data not shown). The percentage fertility decreased when dietary NPP was reduced below 0.37% (Treatments B, C, and D) but there was no treatment effect on the number of eggs or chicks produced per hen housed. The increased hen-day egg production of hens that received the 0.09% NPP + phytase treatment (Treatment D) produced a numerical reduction of feed consumed per dozen eggs laid ($P = 0.06$).

## DISCUSSION

While the authors were not aware of previous research that reported effects of NPP level and phytase on broiler breeder pullets housed in floor pens, comparable research in broilers has shown that phytase addition, in combination with a reduction in dietary NPP closer to broiler nutritional requirements, decreased both total P and WSP in the broiler litter by as much as 30%

and 50%, respectively (Applegate et al., 2003). In one of the few research papers that investigated these effects in broiler breeder pullets, Lilburn et al. (2004) reported that the replacement of 0.10% NPP with phytase in 6-wk-old broiler breeder pullets housed in battery cages decreased fecal P by 17% during a 48 h collection study. However, these results can not be extrapolated to breeder pullets reared on litter due to the considerable re-cycling of fecal P by birds housed on litter (Harms et al., 1984). In contrast to the results of Applegate et al. (2003), our investigation using broiler breeder pullets reared on litter showed no effect on litter total P when phytase replaced 0.10% NPP in diets with either 0.37% or 0.27% NPP (Table 1.3). Importantly, the same reduction of 0.1% NPP from 0.37% to 0.27% NPP without adding phytase decreased the litter total P concentration by 18.0% (Table 1.3). This lack of an effect of phytase on reducing litter total P can most likely be explained on the basis of the recycling of P from the litter. Tamin et al. (2004) showed that, in the absence of dietary calcium, broilers had a high inherent capacity to hydrolyze phyate P, as evidenced by its disappearance from the small intestine. Since these breeder pullets were feed restricted and housed in floor pens over a 22-wk period, it could also be expected that they would be able to hydrolyze a considerable amount of the residual phytate P in the litter they consumed. While we did not verify this mechanism by means of a mass balance study it could be expected that the digestion of residual bound phytate in the litter would effectively ameliorate the potential benefits of phytase over time, and would also explain why a reduction in litter total P was only observed when dietary NPP was reduced without the simultaneous addition of phytase. However, while the reductions in litter total P may potentially be explained on this basis, previous reports concerning WSP showed the effects of reduced dietary NPP with or without phytase to be, at best, variable (Angel and Applegate, 2001). Results from some broiler studies showed phytase application in combination with

reduced NPP to not alter the proportion of WSP in the litter (Applegate et al., 2003), while other studies reported a decrease in the proportion of WSP (Saylor et al., 2001), and in one case phytase supplementation even increased the proportion of WSP in broiler litter (Vadas et al., 2004). To help explain these conflicting results, Leytem et al. (2006) showed that there was a 94% correlation between the proportion of manure total P that was WSP and the amount of manure P that was present as phytate P. Microbial fermentation of the phytate P fraction has also increased WSP in the feces (Angel et al., 2005), while the proportion of WSP was further influenced by the manure moisture level (Maguire et al., 2006) in other studies. The effects of microbial fermentation of the phytate P fraction over time were also previously demonstrated by the work of McGrath et al. (2005) who showed that the proportion of WSP increased during long term storage of wet but not dry broiler litter. Since the litter from the broiler breeder pullets was kept in the rearing house for 22 wk, effects of moisture and the extent of microbial degradation of the phytate P fraction may be greater than in conventional broiler studies conducted over a much shorter time period. These effects of moisture on litter WSP were suggested by our data that showed a highly significant correlation (74%) between percentage litter moisture and WSP concentration. Further, when moisture was included in a model to explain treatment differences in the WSP fraction it was seen that both moisture level and dietary treatment had significant effects on the percentage WSP, but that only the litter moisture level was influential in altering the WSP as a proportion of litter total P (Table 1.3). After correcting for the effects of litter moisture, our data supported the previous findings of Applegate et al. (2003), specifically, that reduced NPP diets in combination with phytase reduced the percentage WSP in the litter, but had no effect on the relative proportion of WSP:total P.

In a similar manner, reductions in the dietary NPP with or without phytase did not affect the proportion of WSP in manure produced from 22-64 wk of age. The concentration of total P and WSP in the breeder manure again reflected the positive benefits that could be obtained by decreasing dietary NPP levels either alone or in combination with dietary phytase enzymes and showed a potential reduction in total P of 39% or 42% when NPP was reduced from 0.37% to either 0.19% or 0.09% with or without phytase, respectively (Table 1.4). Although the reduction in NPP from 0.19% to 0.09%, while adding phytase provided no additional benefits in further reducing manure total P, this did allow the complete removal of all added dicalcium phosphate from the diet while also having a positive effect on the percentage hen-day egg production. Li et al. (2002) previously reported an increased egg production of 0.55% from 26-40 wk of age and a 2% improvement in hatchability when NPP was reduced from 0.30% to 0.10% and phytase was added to diets of broiler breeders housed in slat/litter pens. However, since there were only two dietary treatments in their study, it was not possible to differentiate between the effects of NPP and phytase. In contrast to the positive effect on egg production that was shown in our study and the study by Li et al. (2002), Brake et al. (2003) were not able to find any change in broiler breeder performance when all added dicalcium phosphate was replaced by phytase in two previous studies. The reduction in fertility that occurred in our study when dietary NPP was reduced below 0.37% had not been previously reported in broiler breeders. The effect of NPP level on fertility in our study might have been related to the early start (10 wk of age) of the reduced NPP treatments and warrants further study. Nevertheless, in spite of the potentially detrimental effect on fertility, the numerically higher egg production of treatments that had received less than 0.37% NPP contributed to there being no treatment differences in the number of chicks produced per hen-housed.

Our calculations have shown that a broiler breeder hen housed to 64 wk of age consumed 397 g of total dietary P of which about 41% or 165 g per bird was excreted in the litter and manure produced (Plumstead et al., 2005). Data from the present study suggested that the replacement of 0.10% dietary NPP with phytase had little effect on reducing total litter P in diets fed to pullets that had been reared on litter. However, once transferred to a two-thirds slat litter breeder house, the replacement of all added P from dicalcium phosphate from diets fed to broiler breeders with 500 FTU of phytase could provide substantial benefits to producers by reducing the concentrations of P in manure from broiler breeders by as much as 42% without affecting the number of chicks produced per breeder hen housed.

## REFERENCES


Angel, R., and T. Applegate. 2001. Phytase use -- what do we know? Pg. 250-263 in Proc. 62nd Minnesota Nutrition Conf. and Minnesota Corn Growers Assn. Tech. Symp., Bloomington, MN.

Angel, R., T. J. Applegate, and M. Christman. 2000a. Effects of dietary non-phytate phosphorus (nPP) on performance and bone measurements in broilers fed on a four phase feeding system. Poult. Sci. 79 (Suppl. 1): 21-22 (Abstr.).

Angel, R., T. J. Applegate, M. Christman, and A. D. Mitchell. 2000b. Effect of dietary non-phytate phosphorus (nPP) level on broiler performance and bone measurements in the starter and grower phase. Poult. Sci. 79 (Suppl. 1): 22 (Abstr.).

Angel, R., W. J. Powers, T. J. Applegate, N. M. Tamin, and M. C. Christman. 2005. Influence of phytase on WSP in poultry and swine manure. J. Environ. Qual. 34: 563-571.

Apke, M. P., P. E. Waibel, K. Larntz, L. Metz, S. L. Noll, and M. Walser. 1987. Phosphorus availability bioassay using bone ash and bone densitometry as response criteria. Poult. Sci. 66:713-720.

Applegate, T. J., B. C. Joern, D. L. Nussbaum-Wagler, and R. Angel. 2003. Water soluble phosphorus in fresh broiler litter is dependent upon phosphorus concentration fed but not on fungal phytase supplementation. Poult. Sci. 82: 1024-1029.

Berry, W. D., J. X. Zhang, P. Liu, D. A. Roland, and G. R. McDaniel. 2000. Effect of supplemental dietary phytase on egg production, fertility and hatchability of broiler breeder hens. Poult. Sci. 79 (Suppl. 1): 62 (Abstr.)

Berry, W. D., J. B. Hess, R. J. Lien, and D. A. Roland. 2003. Egg production, fertility, and hatchability of breeder hens receiving dietary phytase. J. Appl. Poult. Res. 12: 264-270.

Brake, J. and G. R. Baughman. 1989. Comparison of lighting regimens during growth on subsequent seasonal reproductive performance of broiler breeders. Poult. Sci. 68:79-85.

Brake, J., C. V. Williams, and B. A. Lenfestey. 2003. Optimization of dietary phosphorus for broiler breeders and their progeny. Pages 77-83 in Proc. of Alltech's 19th Annual Symposium, Lexington, KY. Nottingham Univ. Press, Nottingham.

Environmental Protection Agency. 2003. National pollutant discharge elimination system permit regulation and effluent guidelines and standards for concentrated animal feeding operations: Final rule. Fed. Regist. 68: 7175-7274.

Harms, R. H., S. M. Bootwalla, and H. R. Wilson. 1984. Performance of broiler breeder hens on wire and litter floors. Poult. Sci. 63:1003-1007.

Li, H., W. D. Berry, J. B. Hess, R. J. Lien, D. A. Roland, and S. Oates. 2002. Phytase supplementation for rearing and production of heavy strain broiler breeders. Poult. Sci. 80 (Suppl. 1): 4 (Abstr.).

Lilburn, M. S., A. Mitchell, and E. E. M. Pierson. 2004. The effects of supplemental enzyme (Avizyme 1502) and phytase (Phyzyme) on phosphorus nutrition in broiler breeders. Poult. Sci. 83 (Suppl. 1): 145 (Abstr.).

Leytem, A. B., D. R. Smith, P. A. Thacker, and T. J. Applegate. 2007. The influence of manure phytic acid on phosphorus solubility in calcareous soils. Soil Sci. Soc. Am. J. 70:1629-1638.

Maguire, R. O., J. T. Sims, and T. J. Applegate. 2005. Phytase supplementation and reduced phosphorus turkey diets reduce phosphorus loss in runoff following litter application. J. Environ. Qual. 34:359-369.

Maguire, R. O., P. W. Plumstead, and J. Brake. 2006. Impact of diet, moisture and storage on soluble phosphorus in broiler breeder manure. J. Environ. Qual. 35:858-865.

McGrath, J. M., J. T. Sims, R. O. Maguire, W. W. Saylor, R. Angel, and B. L. Turner. 2005. Broiler diet modification and litter storage: impacts on broiler litter phosphorus. J. Environ. Qual. 34:1896-1909.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th Rev. Ed., Natl. Acad. Press, Washington, DC.

Olson, B. M., and B. A. Paterson. 2005. Implications of moving to a phosphorus based system for manure application. Pages 1-7 in Manure Management Planning: The Essentials. Cooperative Extension Series. Alberta Agriculture, Food and Rural Development, Edmonton, AB, Canada.

Plumstead, P. W., H. Romero-Sanchez, R. O. Maguire, N. Leksrisompong and J. Brake. 2005. Reducing fecal phosphorus excretion 1: Effects of dietary phosphorus and phytase in broiler breeder rearing and laying diets on water soluble fecal phosphorus during rearing and subsequent reproductive performance. Pg. 36 in The development of alternate technologies for the processing and use of animal waste. Proc. Animal Waste Management Symposium, Research Triangle Park, NC.

SAS Institute, 1996. SAS® Users Guide: Statistics. Version 8.0. SAS Institute, Cary, NC.

Saylor, W. W., J. T. Sims, G. W. Malone, and M. F. Lavahun. 2001. Use of phytase and high available phosphorus corn in broiler diets: Impact on litter phosphorus levels and solubility. Pages 43-57 in Proc. of the Maryland Nutrition Conf., College Park, MD.

Soares, J. H., Jr. 1995. Phosphorus Bioavailability. Pg. 257-294 in Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins. C. B. Ammerman, D. H. Baker, A. J. Lewis ed. Academic Press, London, UK.

Sims, J. T., A. C. Edwards, O. F. Schoumans, and R. R. Simard. 2000. Integrating soil phosphorus testing into environmentally based agricultural practices. J. Environ. Qual. 29:60-71.

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

Vadas, P. A., J. J. Meisinger, L. J. Sikora, J. P. McMurty, and A. E. Sefton. 2004. Effect of poultry diet on phosphorus in runoff from soils amended with poultry manure and compost. J. Environ. Qual. 33: 1845-1854.

**Table 1.1.** Formulation and calculated analyses of the common broiler breeder starter and grower diets fed to 9 weeks of age.

| Ingredients | Starter Diet[1] | Grower Diet[1] |
|---|---|---|
| | (%) | |
| Corn | 60.37 | 63.72 |
| Soybean meal (48% CP) | 21.43 | 16.54 |
| Wheat bran | 13.00 | 15.00 |
| Poultry fat | 1.00 | 0.50 |
| Limestone | 1.33 | 1.39 |
| Dicalcium phosphate | 1.56 | 1.57 |
| Sodium chloride | 0.50 | 0.55 |
| Premixes[2] | 0.68 | 0.68 |
| L-Threonine | - | 0.02 |
| DL-Methionine | 0.13 | 0.03 |
| | 100.00 | 100.00 |
| Calculated nutrients[3] | | |
| Moisture, % | 11.34 | 10.45 |
| ME, kcal/kg | 2,925 | 2,925 |
| Crude protein, % | 17.66 | 15.96 |
| Lysine, % | 0.85 | 0.77 |
| Methionine + Cysteine, % | 0.71 | 0.63 |
| Threonine, % | 0.64 | 0.56 |
| Calcium, % | 0.95 | 0.95 |
| Available phosphorus, %[4] | 0.45 | 0.45 |
| Non-phytate phosphorus, %[5] | 0.38 | 0.37 |
| Sodium, % | 0.20 | 0.22 |

[1] Starter and grower diets fed from 0 – 3 wk and 4 - 9 wk, respectively.

[2] Premixes provided the following per kg diet: vitamin A, 13,200 IU; vitamin $D_3$, 4,000 IU; vitamin E, 66 IU; vitamin $B_{12}$, 39.6 µg; riboflavin, 13.2 mg; niacin, 110 mg; d-pantothenate, 22 mg; menadione ($K_3$), 4 mg; folic acid, 2.2 mg; thiamine, 4 mg; pyridoxine, 8 mg; d-biotin, 252 µg; selenium (as $Na_2SeO_3$), 0.30 mg; manganese, 120 mg; zinc, 120 mg, iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; cobalt, 1.0 mg; choline chloride 1,200 mg; coccidiostat, 500 mg.

[3] Nutrient compositions calculated from proximate analyses of all ingredients. Final diet composition confirmed by proximate analyses.

[4] Calculated available phosphorus using the slope ratio method (Soares, 1995).

[5] Calculated percentage non-phytate bound phosphorus (Angel and Applegate, 2001).

**Table 1.2.** Formulation and calculated analyses of dietary treatments fed during the rearing and production phase.

| Ingredients | Grower Treatments[1] | | | | Breeder Treatments[1] | | | |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | A | B | C | D |
| | (%) | | | | | | | |
| Corn | 63.68 | 63.68 | 63.68 | 63.68 | 70.53 | 70.53 | 70.53 | 70.53 |
| Soybean meal (48% CP) | 15.86 | 15.86 | 15.86 | 15.86 | 18.61 | 18.61 | 18.61 | 18.61 |
| Wheat bran[2] | 15.03 | 15.03 | 15.03 | 15.03 | - | - | - | - |
| Poultry fat | 0.72 | 0.72 | 0.72 | 0.72 | 2.00 | 2.00 | 2.00 | 2.00 |
| Limestone | 1.13 | 1.43 | 1.43 | 1.73 | 5.99 | 6.29 | 6.53 | 6.83 |
| Dicalcium phosphate | 1.58 | 1.04 | 1.04 | 0.50 | 1.52 | 0.99 | 0.54 | - |
| Filler (vermiculite) | 0.67 | 0.81 | 0.91 | 1.05 | - | 0.13 | 0.44 | 0.58 |
| Sodium chloride | 0.56 | 0.56 | 0.56 | 0.56 | 0.50 | 0.50 | 0.50 | 0.50 |
| Premixes[3] | 0.68 | 0.68 | 0.68 | 0.68 | 0.68 | 0.68 | 0.68 | 0.68 |
| Phytase premix[4] | - | 0.10 | - | 0.10 | - | 0.10 | - | 0.10 |
| L-Threonine | 0.04 | 0.04 | 0.04 | 0.04 | 0.11 | 0.11 | 0.11 | 0.11 |
| DL-Methionine | 0.05 | 0.05 | 0.05 | 0.05 | 0.06 | 0.06 | 0.06 | 0.06 |
| Calculated nutrients[5] | | | | | | | | |
| Moisture, % | 10.37 | 10.37 | 10.37 | 10.37 | 12.90 | 12.90 | 12.90 | 12.90 |
| ME, kcal/kg | 2,925 | 2,925 | 2,925 | 2,925 | 2,925 | 2,925 | 2,925 | 2,925 |
| Crude protein, % | 15.60 | 15.60 | 15.60 | 15.60 | 15.00 | 15.00 | 15.00 | 15.00 |
| Lysine, % | 0.75 | 0.75 | 0.75 | 0.75 | 0.76 | 0.76 | 0.76 | 0.76 |
| Methionine + Cysteine, % | 0.63 | 0.63 | 0.63 | 0.63 | 0.63 | 0.63 | 0.63 | 0.63 |
| Threonine, % | 0.64 | 0.64 | 0.64 | 0.64 | 0.64 | 0.64 | 0.64 | 0.64 |
| Calcium, % | 0.85 | 0.85 | 0.85 | 0.85 | 2.70 | 2.70 | 2.70 | 2.70 |
| Available phosphorus, %[6] | 0.45 | 0.45 | 0.35 | 0.35 | 0.40 | 0.40 | 0.22 | 0.22 |
| Non-phytate phosphorus, %[7] | 0.37 | 0.27 | 0.27 | 0.17 | 0.37 | 0.27 | 0.19 | 0.09 |
| Sodium, % | 0.22 | 0.22 | 0.22 | 0.22 | 0.20 | 0.20 | 0.20 | 0.20 |

[1] Grower and breeder treatments applied from 10 – 21 wk and from 22 – 64 wk, respectively.

[2] During mixing of the diets, the wheat bran and a portion of the corn, poultry fat, and vermiculite were included in the form of a pelleted premix that contained 58% wheat bran, 37% corn, 1.6% poultry fat, and 3.4% vermiculite.

[3] Premixes provided the following per kg diet: vitamin A, 13,200 IU; cholecalciferol, 4,000 IU; vitamin E, 66 IU; vitamin $B_{12}$, 34.6 µg; riboflavin, 13.2 mg; niacin, 110 mg; pantothenic acid, 22 mg; menadione ($K_3$), 4 mg; folic acid, 2.2 mg; thiamine, 4 mg; pyridoxine, 8 mg; d-biotin, 252 µg; selenium (as $Na_2SeO_3$), 0.30 mg; manganese, 120 mg; zinc, 120 mg, iron, 180 mg; copper, 10 mg; iodine, 2.5 mg; cobalt, 1.0 mg; choline chloride 1,200 mg; coccidiostat, 500 mg.

[4] Phytase premix analyzed to contain 1,098 FTU/g and supplied 300 FTU and 500 FTU phytase in the grower and breeder diets, respectively. Allzyme SSF, Alltech Inc., Nicholasville, KY 40356.

[5] Nutrient compositions calculated from proximate analyses of all ingredients. Final diet composition confirmed by proximate analyses.

[6] Calculated available phosphorus using the slope ratio method (Soares, 1995).

[7] Calculated percentage non-phytate bound phosphorus (Angel and Applegate, 2001).

**Table 1.3.** Total phosphorus (P) and water soluble phosphorus (WSP) in broiler breeder pullet rearing litter as affected by changes in dietary non-phytate phosphorus (NPP) level in pullet grower diets with or without phytase enzyme[1] and fed from 10 - 21 wk of age.

| Treatment | NPP | Added Phytase[1] | Moisture | Total P | WSP | WSP : Total P Ratio |
|---|---|---|---|---|---|---|
| | (%) | (FTU/kg) | (%) | (mg/kg DM) | | (g:g) |
| A | 0.37 | - | 25.6 | 10,411.5 $^{A}$ | 1,573.4 $^{A}$ | 15.1 $^{a}$ |
| B | 0.27 | 300 | 23.0 | 10,114.3 $^{A}$ | 1,570.5 $^{A}$ | 15.6 $^{a}$ |
| C | 0.27 | - | 19.8 | 8,535.1 $^{B}$ | 1,218.7 $^{A}$ | 14.2 $^{a}$ |
| D | 0.17 | 300 | 15.1 | 8,469.1 $^{B}$ | 713.5 $^{B}$ | 8.7 $^{b}$ |
| | SEM | | 3.70 | 381.1 | 153.5 | 1.6 |
| Probability of treatment effect | | | 0.254 | 0.003 | 0.004 | 0.031 |
| Probability of moisture effect | | | | 0.743 | 0.003 | 0.006 |
| Probability of treatment effect after adjusting for moisture covariate | | | | - | 0.019 | 0.120 |

$^{a,b}$ Means within the same column with no common superscript differ significantly ($P \leq 0.05$).

$^{A,B}$ Means within the same column with no common superscript differ significantly ($P \leq 0.01$).

[1] Allzyme SSF, Alltech Inc., Nicholasville, KY 40356.

**Table 1.4.** Total phosphorus (P) and water soluble phosphorus (WSP) in manure from breeders as affected by changes in non-phytate phosphorus (NPP) level of breeder layer diets with or without phytase enzyme[1] and fed from 22 – 64 wk of age.

| Treatment | NPP | Added Phytase[1] | Moisture | Total P | WSP | WSP : Total P Ratio |
|---|---|---|---|---|---|---|
| | (%) | (FTU/kg) | (%) | (mg/kg DM) | | (g:g) |
| A | 0.37 | - | 41.4 | 32,341 [A] | 1,316 [A] | 4.42 |
| B | 0.27 | 500 | 46.3 | 27,905 [A] | 940 [B] | 3.52 |
| C | 0.19 | - | 42.0 | 19,729 [B] | 743 [B] | 3.78 |
| D | 0.09 | 500 | 45.1 | 18,713 [B] | 759 [B] | 4.09 |
| SEM | | | 2.00 | 1,519 | 147 | 0.29 |
| Probability of treatment effect | | | 0.262 | <0.001 | 0.007 | 0.564 |

[A,B] Means within the same column with no common superscript differ significantly ($P \leq 0.01$).

[1] Allzyme SSF, Alltech Inc., Nicholasville, KY 40356.

**Table 1.5.** Effect of varying dietary non-phytate phosphorus (NPP) level with or without added phytase enzyme[1] on performance variables of broiler breeders from 29 to 64 wk of age.

| Treatment | NPP | Added Phytase[1] | Eggs Per Hen Housed | Feed Per Dozen Eggs | Hen-Day Egg Production | Fertility[2] | Chicks Per Hen Housed |
|---|---|---|---|---|---|---|---|
| | (%) | (FTU/kg) | (n) | (g) | (%) | | (n) |
| A | 0.37 | - | 158.0 | 2,999 $^{xy}$ | 62.09 $^{b}$ | 97.5 $^{A}$ | 143.6 |
| B | 0.27 | 500 | 164.5 | 3,038 $^{x}$ | 62.12 $^{b}$ | 95.0 $^{B}$ | 145.2 |
| C | 0.19 | - | 163.9 | 3,068 $^{x}$ | 61.24 $^{b}$ | 95.5 $^{B}$ | 145.3 |
| D | 0.09 | 500 | 170.8 | 2,875 $^{y}$ | 65.18 $^{a}$ | 95.3 $^{B}$ | 152.4 |
| SEM | | | 3.74 | 46.7 | 0.893 | - | 3.26 |
| Probability of treatment effect | | | 0.178 | 0.059 | 0.041 | <0.001 | 0.269 |

$^{x,y}$ Means within the same column with no common superscript differ significantly ($P \leq 0.10$).
$^{a,b}$ Means within the same column with no common superscript differ significantly ($P \leq 0.05$).
$^{A,B}$ Means within the same column with no common superscript differ significantly ($P \leq 0.01$).
[1] Allzyme SSF, Alltech Inc., Nicholasville, KY 40356.
[2] The standard error for fertility was not available due to the categorical nature of the data.

## CHAPTER 2

### Effects of Dietary Phosphorus, Phytase, and Calcium on Total and Water Soluble Phosphorus in Broiler Litter

**ABSTRACT** Previous research that evaluated the effects of reduced phosphorus (P) and phytase in broiler diets on water soluble P (WSP) in litter has produced conflicting results. To further investigate the effects of dietary available phosphorus (AvP), calcium (Ca), and phytase on P excretion in broilers, 18 dietary treatments were applied in a randomized complete block design to each of four replicate pens of 28 broilers from 18 to 42 d of age. Treatments consisted of three levels of AvP (0.35%, 0.30%, and 0.25%) combined with three levels of Ca (0.80%, 0.69%, and 0.57%) and two levels of phytase (0 and 600 FTU $kg^{-1}$). Phytase was added at the expense of 0.10% P from dicalcium phosphate. Broiler BW and feed intake was determined at 28 and 39 d of age and fresh litter was collected from pens when the broilers were 41 d of age. Results showed no effects of Ca, AvP, or phytase on broiler BW but mortality was increased when diets contained less than 0.35% AvP. The inclusion of phytase at the expense of inorganic P or reductions in AvP decreased litter total P from 28% to 43%. Litter WSP decreased by up to 73% with an increasing dietary Ca:AvP ratio, irrespective of phytase addition. The ratio of WSP:total P in litter decreased as the dietary Ca:AvP ratio increased and was greater in the phytase amended diets. This study showed that reduced AvP with phytase diets decreased litter total P, but that the ratio of Ca:AvP in the diets was primarily responsible for effects on WSP. These findings suggest that studies that evaluate effects of diet amendment on WSP in the litter should consider the ratio of Ca:AvP in the diet as an additional explanatory variable that can substantially affect the response obtained.

## INTRODUCTION

Phosphorus (P) has been shown to be an essential nutrient for both plants and animals, but excessive P in soil has also been shown to have important negative environmental implications. The accumulation of P in soils has increased the risk of P losses to bodies of water (Sims et al., 2000) that can subsequently increase aquatic plant growth, deplete oxygen, and cause eutrification that reduced the biological and asthetic value of these bodies of water (Sharpley and Moyer, 2000; CAST, 2002). The environmental implications of high P levels in soils has led to federal regulations that restrict or limit the amount of P from manure that can be applied to soils (Environmental Protection Agency, 2003). Although total P in poultry litter has been a concern, the water soluble P fraction (WSP) has also been identified as an important environmental risk factor, as P losses in runoff following land application of litter have been related to the WSP in the litter that was applied (Maguire et al., 2005).

To reduce P concentrations in animal manure and litter, primary strategies have included reducing excessive P in the diet by reducing dietary non-phytate P (NPP) concentrations closer to minimum animal nutrient requirements, and the inclusion of phytase enzymes that increase the availability to the animal of phytate bound P. The general consensus of studies that investigated reduced dietary NPP regimens in combination with phytase was that this could reduce litter total P from 29% to 45% (Applegate et al., 2003; Smith et al., 2004; Vadas et al., 2004; McGrath et al., 2005; Angel et al., 2006). However, while reduced dietary NPP regimens and phytase have both been shown to reduce total P in litter and manures, effects on WSP have been variable. Phytase addition to poultry diets was shown to decrease the litter WSP concentration by 35.6% (Applegate et al., 2003) or by 29% (McGrath et al., 2005). However, in other studies, phytase supplementation to diets had no

effect on litter WSP concentration (Saylor et al., 2001; Maguire et al., 2004; McGrath et al., 2005) but amendment of diets with phytase increased the concentration of WSP in the litter in two other studies (Delaune et al., 2001; Vadas et al., 2004).

In addition to diet modification, the P composition of litters themselves has been shown to influence the amount of WSP. An increased proportion of phytate P in poultry litters and manures was shown to have a substantial impact on P solubility, as the proportion of WSP was shown to decrease when phytate P of the manures and litters increased (Leytem and Maguire, 2006). Therefore, dietary factors that influenced the amount of phytate excreted by the birds could potentially alter the WSP fraction of the resultant manures and litters.

Minerals such as Ca and other di- and trivalent cations supplemented to diets have been reported to reduce the disappearance of phytate from the small intestine of broilers (Maenz et al., 1999; Angel et al., 2002), which would increase phytate concentrations in the excreta.

In addition to inhibiting phytate hydrolysis in poultry, the addition of Ca to diets can cause the precipitation of insoluble CaP complexes in litter and make the P less soluble. Toor et al. (2005) showed that as dietary Ca increased there was an increase in insoluble CaP precipitates in manures and litters. Furthermore, while dietary Ca has been shown to impact the extent of phytate hydrolysis, many studies that investigated effects of reduced dietary NPP and phytase did not also consider the potential impact of varying dietary Ca concentration on total and WSP concentrations in litter (Applegate et al., 2003; Maguire et al., 2004; Vadas et al., 2004).

The variable effect of diet modification on WSP in litters remains a concern from a litter management standpoint, as litter WSP has been shown to be highly correlated with P losses from land applied litter. It was evident from the disparity in the literature that there may be factors other than dietary P and phytase supplementation that influenced WSP in poultry litters. To address this, the present study investigated the effects of AvP, phytase, and Ca levels on total P, WSP, and litter P composition in broilers.

## MATERIALS AND METHODS

### *Definitions*

The term available P (AvP) utilized in this experiment referred to relative bioavailable P as determined using a slope ratio assay with dicalcium P as the reference standard (Apke at al., 1987; Soares, 1995), and NPP represented a chemically defined entity calculated by subtracting the analyzed phytate P from total P in the diet.

### *Broiler Feeding Trial*

Broiler chicks were hatched from eggs produced by a 33-wk-old Ross 344 male x Ross 508 female broiler breeder flock housed at the North Carolina State University Chicken Educational Unit. Chicks were feather-sexed after hatching and permanently identified with neck tags. Fourteen male and 14 female chicks were randomly allocated to each of 72 floor pens (3.5 $m^2$) that contained fresh pine shavings. The chicks were given *ad libitum* access to water and a standard broiler starter diet that met or exceeded National Research Council (1994) requirements (Table 2.1). The quantity of starter feed provided per pen was adjusted to provide 907 g per bird alive at 7 d of age.

To account for position effects within the broiler house the 72 pens were divided into four quarters (blocks) and treatments randomly assigned within each block. The treatment structure was a 3 x 3 x 2 factorial with three levels of AvP (0.35%, 0.30%, 0.25%), three levels of Ca (0.80%, 0.69%, 0.57%), and two levels of a bacterial phytase enzyme[1] (0, or 600 FTU $kg^{-1}$). Differences in AvP and Ca in diets were achieved by varying the concentration of dicalcium phosphate and limestone in a standard basal grower diet that was formulated to meet or exceed National Research Council (1994) recommendations, except for NPP and Ca (Tables 2.1 and 2.2). Phytase enzyme inclusion in diets mimicked industry practice of the replacement of 0.10% NPP from dicalcium phosphate with 600 FTU of added phytase (Angel et al., 2006). Fine river sand was included as inert filler during diet formulation to allow variable inclusion rates of dicalcium phosphate, limestone, and phytase premix. The composition of final diets was confirmed by analysis for crude protein, amino acids, Ca, and P. To ensure that birds had continuous access to feed and that all birds received the same amount of starter feed, grower diets were added to tube feeders on top of the remaining starter feed at 14 d. Male and female BW and mixed sex feed consumption per pen was determined at 14, 28, and 39 d of age and BW of mortality was recorded twice daily and used to calculate the adjusted feed conversion (AdjFCR) of mixed sex pens.

***Litter Collection and Analysis***

Litter samples were collected at 41 d of age from three areas within each pen, mixed thoroughly, and a sub-sample taken. Fresh litter samples were homogenized in a blender and then immediately analyzed for WSP by shaking the equivalent of 1g dry litter with 100 mL deionized water for 1 h, filtering through a 0.45 μm membrane, and analyzing total WSP by

[1] Syngenta Animal Nutrition, Research Triangle Park, NC 27709

inductively-coupled plasma atomic-emission spectrometry (ICP–AES). The remaining samples were immediately frozen (-80°C), lyophilized, and ground (2 mm) for analysis. Analysis of the litters were as follows: (i) total elements (Ca and P) were determined by microwave-assisted digestion of a 0.5 g dried sample with 8 mL of concentrated $HNO_3$ and 2 mL of 30% $H_2O_2$ (v/v) with P and Ca quantified using inductively-coupled plasma optical-emission spectrometry (ICP–OES) detection and (ii) phytate P was determined by acid extraction followed by high performance liquid chromatography (HPLC) analysis (Kwanyuen and Burton, 2005). All of the P values reported in the text were as elemental P.

***Statistical Analyses***

There were four replicate pens per treatment arranged in a randomized complete block design with four blocks. The ratio of litter WSP to total P (WSP:Total P) was calculated by taking the WSP and dividing by total P for each pen, the ratio of litter phytate P to total P (phytate P:Total P) was calculated in the same manner.

All variables were tested for normality using the Shapiro-Wilk test with the PROC CAPABILITY procedure (SAS Institute, 2004). Where results suggested non-normality, variables were log-transformed prior to statistical analyses, with untransformed numbers presented in the text. All data were analyzed using the general linear models (GLM) procedure (SAS Institute, 2004). A pen of birds served as the experimental unit with 18 pens randomized among all combinations of dietary AvP (3 levels), dietary Ca (3 levels), and phytase (2 levels) within each block. Data were analyzed using a full factorial effects model that included block effects and all possible main effects and interaction effects among treatment factors. Further inspection of the effects of Ca and AvP revealed that the ratio of Ca:AvP appeared to be a good predictor of the response in WSP and the WSP:total P ratio. A

best fit model was selected by using a forward stepwise regression that included the independent variables AvP, Ca, phytase, Ca:AvP, and Ca:AvP$^2$ (quadratic term). Model fit was evaluated by comparing the mean square error of the full factorial effects model with that of the reduced, more parsimonious model.

## RESULTS

The determined Ca and total P of grower diets were in general agreement with formulated values (Table 2.2). Reductions in the dietary Ca or AvP concentration from 0.80% to 0.57%, or 0.35% to 0.25%, respectively, had no effect on broiler BW gain, feed intake, or AdjFCR from 0-28 d or from 0–39 d of age (Table 2.3). Furthermore, addition of phytase at the expense of 0.10% P from dicalcium phosphate had no effect on any of the broiler performance variables measured. However, while reduced dietary Ca or phytase had no effect on mortality rate, early mortality (0-28 d) increased from 1.04% to 3.27% when dietary AvP was reduced below 0.35%. The higher mortality on low AvP diets (< 0.35% AvP) at 28 d also resulted in a higher cumulative mortality at 39 d of age (Table 2.3).

The litter P chemical characteristics are shown in Table 2.4 and statistical significance of the factorial analysis of treatment effects are shown in Table 2.5. Total P concentrations in litters from the 18 treatments ranged from 6,111 to 10,647 mg P kg$^{-1}$. Statistical analysis of the litter total P indicated significant differences in the response in total P due to both dietary AvP and phytase, while dietary Ca and all interactions were not significant (Table 2.5). As dietary AvP increased, the total P in the litter also increased, while total litter P from broilers fed the phytase amended diets were approximately 20% lower than broilers fed diets without phytase supplementation (Table 2.4, Figure 2.1).

The litter WSP concentrations ranged from 631 to 2,363 mg P $kg^{-1}$, while the ratio of litter WSP:total P ranged from 0.08 to 0.28. The ratio of WSP:total P in the litter decreased in a linear manner when dietary AvP was decreased from 0.35% to 0.25%, or when Ca was increased from 0.57% to 0.80% (Table 2.4, Figure 2.2 a,b). There was no interaction of Ca x AvP for either litter WSP concentration or the ratio of WSP:total P in the litter. Phytase addition at the expense of 0.10% P from dicalcium phosphate had no effect on litter WSP but the average litter WSP:total P ratio for the phytase amended diets were 18% higher than diets without phytase supplementation. Litter phytate P concentrations ranged from 3,148 to 6,664 mg P $kg^{-1}$ and were on average 43% lower when diets contained phytase. Litter total P concentrations were positively correlated with the litter phytate P concentrations ($r^2$ = 0.78; Figure 2.3). A strong negative correlation was also observed between litter WSP and the ratio of phytate P:total P for diets without phytase ($r^2$=0.72) and diets with phytase ($r^2$=0.80). In addition, the average phytate P:total P ratio in the litters from diets with phytase supplementation were 30% lower than that of the litters from phytase amended diets (Figure 2.4).

Further inspection of the effects of dietary factors revealed that the ratio of dietary Ca:AvP was a good predictor of the response obtained in litter WSP and WSP:total P ratio. This can be seen in Figure 2.2 a,b where a linear reduction in WSP and the WSP:total P ratio resulted when the dietary Ca:AvP ratio increased, either due to a decrease in AvP from 0.35% to 0.25% at a fixed level of Ca, or when Ca was increased from 0.57% to 0.80% with AvP constant. Stepwise regression analysis confirmed that the response in litter WSP and WSP:total P could be described by a model that included Ca:AvP, Ca:$AvP^2$, and phytase (Table 2.5). The more parsimonious model that described the response in litter WSP and

WSP:total P in terms of the ratio of dietary Ca:AvP (both linear and quadratic terms) and phytase resulted in an improved fit with less unexplained variance, as indicated by the smaller mean square error of this model, compared to the full factorial effects model that had more parameters (Table 2.5). As the Ca:AvP ratio increased, the litter WSP and WSP:total P ratio decreased in a curvilinear manner (Figures 2.5 and 2.6). After accounting for the effects of Ca:AvP, the litter WSP:total P ratio was greater when diets contained added phytase compared to diets without phytase supplementation.

## DISCUSSION

The 39 d broiler BW across all treatments of 2,460 g in the present experiment was considerably higher than the breed guideline of 2,102 g for the same age (Ross Breeders, 2005) and indicated excellent growth. Although no effects of reductions in dietary AvP to 0.25% in the grower diet were observed for BW gain to 28 d or 39 d, the increase in early mortality when diets contained less than 0.35% AvP suggested that AvP intake of broilers on diets with 0.30% and 0.25% AvP did not meet the nutritional requirements for P. This was consistent with previous research that showed the NPP requirement of broilers from 18 to 32 d of age to lie between 0.28% and 0.32% for maximum BW gain and tibia ash, respectively (Angel et al., 2000 a,b). Furthermore, while the higher 28 d mortality of broilers that received diets with <0.35% AvP suggested that these birds were deficient in P, the severity of P deficiency would have decreased towards the end of the study as the NPP requirement of broilers from 32 to 42 d of age was shown to be as low as 0.19% to 0.24% (Angel et al., 2000 a,b).

While reduced dietary AvP in the present study had only small effects on broiler performance, the positive correlation between dietary AvP level and litter total P shown in Figure 2.1 was consistent with similar studies in broilers that had evaluated effects of reduced dietary P on litter P concentration. For example, Maguire et al. (2004) demonstrated that reducing NPP concentrations in broiler feed (11-40% reduction in NPP over four feeding phases) decreased the total P in the resulting litter by 10 to 17%. McGrath et al. (2005) also found that reducing total dietary P levels (6-19% reduction in NPP with four feeding phases) reduced broiler litter total P concentration by 18%.

The addition of phytase to diets resulted in a 20% reduction in litter total P at all levels of dietary AvP and was consistent with results from other studies that reported reductions of 13% to 33% in total P excretion due to phytase addition (Applegate et al., 2003; Miles et al., 2003; Maguire et al., 2004; Vadas et al., 2004; Angel et al., 2005). As phytase was commonly added to diets at the expense of 0.10% P from an inorganic source of P such as dicalcium phosphate (Angel et al., 2006), and feed intake of broilers in the present study was not affected by phytase addition, the reduced P concentrations in the litter due to phytase could be attributed to a decrease in the P consumed by birds receiving diets with phytase. In addition to decreased litter total P, phytase addition to diets in the present study was also shown to decrease the amount of phytate in the litter (Table 2.4) that resulted in a lower ratio of total P:phytate P in diets with phytase compared to diets with no phytase (Figure 2.4). Similar reductions in litter phytate P from broilers fed supplemental phytase have previously been shown in several studies (Maguire et al., 2004; McGrath et al., 2005; Toor et al., 2005). In the present study, an increase in dietary Ca generally decreased phytate P hydrolysis leading to greater excretion of phytate P. This detrimental effect of dietary Ca on phytate

hydrolysis was previously reported by Tamin et al. (2004) and was thought to occur primarily due to the formation of stable Ca-phytate complexes (Maenz et al., 1999; Angel et al., 2002). The small negative effects of increased dietary AvP on phytate degradation in the present study has also been previously reported. Ravindran et al. (2000) demonstrated that increasing dietary AvP concentrations resulted in reduced P digestibility, presumably due to a decrease in phytate hydrolysis. Van der Klis and Versteegh (1996) demonstrated a decrease in phytate P hydrolysis of between 26 and 48% when AvP was increased in diets fed to broilers while the Ca:AvP ratio in the diet was held constant.

The results of the factorial analysis of factors that affected WSP concentrations in litter was in agreement with previous studies that had reported a reduction in litter WSP when dietary AvP or NPP was reduced (Applegate et al., 2003; Vadas et al., 2004). However, the present study also showed that an increase in dietary Ca concentration from 0.57% to 0.80% resulted in a decrease in the WSP concentration of similar magnitude as that obtained when AvP in diets was reduced from 0.35% to 0.25% (Figure 2.2a). The significant main effects of either AvP or Ca on WSP concentration that were evident in the factorial analysis of the data also corresponded to a simultaneous increase in the ratio of dietary Ca:AvP (Figure 2.2a). The stepwise model selection procedure confirmed that the decrease in litter WSP that was observed with reductions in diet AvP or increased diet Ca could be described as a quadratic function of an increasing ratio of Ca:AvP in the diet. These effects of the Ca:AvP ratio in the diet on WSP in the litter could arise from either of two distinct mechanisms. In the first instance, examination of available literature has shown that the WSP in litter or manure decreased in a linear manner with increasing phytate concentration (Maguire et al., 2004; Toor et al., 2005; Leytem et al., 2006). This observation was supported

by previous workers who showed that P compounds extracted in deionized water were predominantly inorganic P, while the majority of phytate P was only released with stronger extractions such as HCl or NaOH (Turner and Leytem, 2004). Therefore, litters that have a greater proportion of phytate P would also be expected to have lower WSP concentrations. It has been well established that the dietary Ca concentration had a considerable effect on the extent of intestinal phytate hydrolysis (Maenz et al., 1999; Angel et al., 2002; Tamin et al., 2004) and this was confirmed in the present study when diets with high Ca concentrations also generally had more phytate in the litter. The second mechanism whereby a high Ca:AvP ratio in the diet could reduce WSP in the litter would be by a reduced proportion of inorganic P in the litter that was soluble, presumably due to formation of stable CaP complexes (Figure 2.7). This was consistent with previous observations of Driver et al. (2005) who showed that a high ratio of Ca:AvP reduced intestinal absorption of P due to the increased precipitation of Ca:P, as well as due to reduced hydrolysis of phytate by phytase enzymes. Furthermore, the formation of stable CaP complexes in litter and manure with increasing dietary Ca have been reported previously (Cooperband and Ward Good, 2002; Toor et al., 2005). Van der Klis and Vesteegh (1996) showed that the optimal Ca:AvP ratio that maximized both absorption and retention of P in broilers was approximately 2.2:1, while the Ca:AvP ratio in the present study was varied from 1.62:1 to 3.2:1. Therefore, based on the observations by previous workers at a high dietary Ca:AvP, both phytate P hydrolysis and P absorption in the present study would have been reduced due to the formation of CaP precipitates in the gut. Conversely, at lower dietary Ca:AvP ratios, more phytate would have been hydrolyzed, which would be expected to increase the amount of P absorbed by the bird. However, as a

result of an imbalance of Ca relative to P, the excess P that was absorbed was presumably excreted in the urine, which would increase WSP in the litter.

The curvilinear reduction in the ratio of WSP:total P in the litter with increasing Ca:AvP ratio in the diet (Figure 2.6) could also be explained by a similar mechanism as that used for litter WSP. However, while phytase addition did not alter the WSP concentration in the litter, supplementation of diets with phytase at the expense of 0.10% P from dicalcium phosphate increased the proportion of litter P that was soluble by approximately 18% after the effect of the Ca:AvP ratio on litter WSP:total P ratio had been accounted for (Figure 2.6). This increase in the ratio of WSP:total P in the present study should be expected as additions of phytase have been shown to reduce the amount of phytate P in the litter, which would subsequently increase the proportion of P that was soluble. However, the increase in the WSP:total P ratio from phytase addition contradicted previous observations of Applegate et al. (2003) who concluded that phytase addition to broiler diets reduced the WSP concentration in litter but did not alter the proportion of WSP relative to litter total P. In contrast to the findings by Applegate et al. (2003) and in agreement with results of the present study, Vadas et al. (2004) showed that broiler manure from diets equivalent in Ca:AvP with and without phytase supplementation had a greater proportion of WSP in litters from phytase amended diets.

As the results of the present study showed that both WSP and the ratio of WSP:total P were largely controlled by the ratio of Ca:AvP, this should be considered when evaluating results of other studies that have reported effects of phytase on WSP in litter and manure. For example, in the study by Applegate et al. (2003) effects of reduced diet NPP or phytase on WSP and the WSP:total P ratio were evaluated without altering the percentage dietary Ca.

This resulted in an increase in the weighted average ratio of Ca:NPP from 2.2:1 in their standard industry diet to 3.0:1 in the phytase amended diets and 3.5:1 in the diets with both reduced NPP and phytase. Therefore, the effect of the ratio of Ca:AvP on both WSP and the WSP:total P ratio shown in the present study suggested that the effects of either reduced NPP or phytase shown in the study by Applegate et al. (2003) may have been severely confounded by the wider Ca:AvP ratio of these treatments. Similar conclusions may be drawn when evaluating findings of similar studies by Dhandu et al. (2002), Maguire et al. (2003), and Vadas et al. (2004), all of whom had investigated effects of reduced NPP diets with or without added phytase on the WSP in litter without accounting for the potentially confounding effects of a widening Ca:NPP ratio in the dietary treatments in which NPP was reduced alone or in combination with phytase.

In conclusion, the present study showed that both WSP and the WSP:total P ratio were predominantly controlled by the ratio of Ca:AvP in the diet. This finding suggested that the prediction of the response in litter WSP and WSP:total P ratio in future studies that examine effects of diet amendment on litter WSP would be more accurate using a combination of dietary treatments in which the dependence of the response in litter WSP and WSP:total P ratio would be captured through the dietary Ca:AvP ratio. Earlier research that had examined the influence of dietary modification on WSP excretion did not take into account the effects of dietary Ca and the resulting Ca:AvP or Ca:NPP ratio on WSP excretion, which may explain some of the variation in the reported results. Since poultry producing regions such as Arkansas, Maryland, and North Carolina have included WSP in litter and manure as a measure to assess the potential impacts of land application on P losses, a better

understanding of factors that affect WSP levels in poultry litters and manures must be developed in order to better manage these with respect to environmental protection.

## REFERENCES


Angel, R., T. J. Applegate, and M. Christman. 2000a. Effects of dietary non-phytate phosphorus (nPP) on performance and bone measurements in broilers fed on a four-phase feeding system. Poultry Sci. 79 (Suppl. 1):22.

Angel, R., T. J. Applegate, M. Christman, and A. D. Mitchel. 2000b. Effect of dietary non-phytate phosphorus (nPP) level on broiler performance and bone measurements in the starter and grower phase. Poultry Sci. 79 (Suppl. 1):21-22.

Angel, R., N. M. Tamim, T. J. Applegate, A. S. Dhandu, and L. E. Ellestad. 2002. Phytic acid chemistry: influence on phytin-phosphorus availability and phytase efficacy. J. Appl. Poult. Res. 11:471-480.

Angel, R. C., W. J. Powers, T. J. Applegate, N. M. Tamin, and M. C. Christman. 2005. Influence of phytase on water soluble phosphorus in poultry and swine manure. J. Environ. Qual. 34:563-571.

Angel, R., W. W. Saylor, A. D. Mitchell, W. Powers, and T. J. Applegate. 2006. Effect of dietary phosphorus, phytase, and 25-hydroxycholecalciferol on broiler chicken bone mineralization, litter phosphorus, and processing yields. Poult. Sci. 85:1200-1211.

Angel, R., N. M. Tamim, T. J. Applegate, A. S. Dhandu, and L. E. Ellestad. 2002. Phytic acid chemistry: influence on phytin-phosphorus availability and phytase efficacy. J. Appl. Poult. Res. 11:471-480.

Apke, M. P., P. E. Waibel, K. Larntz, L. Metz, S. L. Noll, and M. Walser. 1987. Phosphorus availability bioassay using bone ash and bone densitometry as response criteria. Poult. Sci. 66:713-720.

Applegate, T. J., B. C. Joern, D. L. Nussbaum-Wagler, and R. Angel. 2003. Water-soluble phosphorus in fresh broiler litter is dependent upon phosphorus concentration fed but not on fungal phytase supplementation. Poult. Sci. 82:1024-1029.

CAST (Council for Agricultural Science and Technology). 2002. Animal diet modification to decrease the potential for nitrogen and phosphorus pollution. Issue paper no. 21.

Cooperband, L. R., and L. Ward Good. 2002. Biogenic phosphate minerals in manure: Implications for phosphorus loss to surface waters. Environ. Sci. Tech. 36:5075-5082.

DeLaune, P. B., P. A. Moore, Jr., D. C. Carman, T. C. Daniel, and A. N. Sharpley. 2001. Development and validation of a phosphorus index for pastures fertilized with animal manure. Pg. 92-103 in Proc. Int. Symp. Addressing Animal Production and Environmental Issues, Research Triangle Park, Raleigh, NC.

Dhandu, A. S., R. Angel, and W. W. Saylor. 2002. Effect of reduced dietary non phytin phosphorus (nPP) levels with or without phytase and 25-hydroxycholecalciferol (25OHD3) on litter total phosphorus (TP) and soluble phosphorus (SP). Poult. Sci. 80(Suppl. 1):75.

Driver, J. P., G. M. Pesti, R. I. Bakalli, and H. M. Edwards. 2005. Effects of calcium and non phytate phosphorus concentrations on phytase efficacy in broiler chicks. Poult. Sci. 84:1406-1417.

Environmental Protection Agency. 2003. National pollutant discharge elimination system permit regulation and effluent guidelines and standards for concentrated animal feeding operations: Final rule. Fed. Regist. 68: 7175-7274.

Kwanyuen, P., and J. W. Burton. 2005. A simple and rapid procedure for phytate determination in soybean and soybean products. J. Am. Oil Chemists' Soc. 82:81-85.

Leytem, A. B., and R. O. Maguire. 2006. Environmental implications of inositol phosphates in animal manures. In Inositol Phosphates: Linking Agriculture and the Environment. B. L. Turner, A. E Richardson, and E. J. Mullaney ed. CAB International, Wallingford, UK.

Leytem, A. B., D. R. Smith, T. J. Applegate, and P. A. Thacker. 2006. The influence of manure phytic acid on phosphorus solubility in calcareous soils. Soil Sci. Soc. Am. J. 1629-1638.

Maenz, D. D., C. M. Engele-Schaan, R. W. Newkirk, and H. L. Claasen. 1999. The effect of minerals and mineral chelators on the formation of phytase-resistant and phytase susceptible forms of phytic acid in solution and in a slurry of canola meal. Anim. Feed Sci. Technol. 81:177-192.

Maguire, R. O., J. T. Sims, and T. J. Applegate. 2005. Phytase supplementation and reduced phosphorus turkey diets reduce phosphorus loss in runoff following litter application. J. Environ. Qual. 34:359-369.

Maguire, R. O., J. T. Sims, J. M. McGrath, and C. R. Angel. 2003. Effect of phytase and vitamin D metabolite (25-OH-$D_3$) in turkey diets on phosphorus solubility in manure-amended soils. Soil Sci. 168:421-433.

Maguire, R. O., J. T. Sims, W. W. Saylor, B. L. Turner, R. Angel, and T. J. Applegate. 2004. Influence of phytase addition to poultry diets on phosphorus forms and solubility in litters and amended soils. J. Environ. Qual. 33:2306-2316.

McGrath, J. M., J. T. Sims, R. O. Maguire, W. W. Saylor, R. Angel, and B. L. Turner. 2005. Broiler diet modification and litter storage: Impacts on phosphorus in litters, soils, and runoff. J. Environ. Qual. 34:1896-1909.

Miles, D. M., P. A. Moore, D. R. Smith, D. W. Rice, H. L. Stilborn, D. R. Rowe, B. D. Lott, S. L. Branton, and J. D. Simmons. 2003. Total and water-soluble phosphorus in broiler litter over three flocks with alum litter treatment and dietary inclusion of high available phosphorus corn and phytase supplementation. Poult. Sci. 82:1544-1549.

National Research Council. 1994. Nutrient requirements for poultry. 9th revised ed. National Academy Press, Washington, DC.

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

Ross Breeders. 2005. Ross x Ross 508 North American Broiler Performance Objectives. Aviagen, TN. http://www.aviagen.com/pdf/Ross_NA/508_Supplement.htm.

SAS Institute. 2004. SAS® Users Guide: Statistics. Version 9.3.1. SAS Institute, Cary, NC.

Saylor, W. W., J. T. Sims, G. W. Malone, and M. F. Lavahun. 2001. Use of phytase and high available phosphorus corn in broiler diets: Impact on litter phosphorus levels and solubility. Pg. 43-57 In Proc. Maryland Nutr. Conf., College Park, MD.

Sharpley, A., and B. Moyer. 2000. Phosphorus forms in manure and compost and their release during simulated rainfall. J. Environ. Qual. 29:1462-1469.

Sims, J. T., A. C. Edwards, O. F. Schoumans, and R. R. Simard. 2000. Integrating soil phosphorus testing into environmentally based agricultural practices. J. Environ. Qual. 29:60-71.

Smith, D. R., P. A. Moore, D. M. Miles, B. E. Haggard, and T. C. Daniel. 2004. Decreasing phosphorus runoff losses from land-applied poultry litter with dietary modifications and alum addition. J. Environ. Qual. 33:2210-2216.

Soares, J. H., Jr. 1995. Phosphorus Bioavailability. Pg. 257-294 In Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins. C. B. Ammerman, D. H. Baker, A. J. Lewis ed., Academic Press, London, UK.

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

Toor, G. S., J. D. Peak, and J. T. Sims. 2005. Phosphorus speciation in broiler litter and turkey manure produced from modified diets. J. Environ. Qual. 34: 687-697.

Turner, B. L. 2004. Optimizing phosphorus characterization in animal manures by phosphorus-31 nuclear magnetic resonance spectroscopy. J. Environ. Qual. 33:757-766.

Turner, B. L. and A. B. Leytem. 2004. Phosphorus compounds in sequential extracts of animal manures: chemical speciation and a novel fractionation procedure. Environ. Sci. Technol. 38:6101-6108.

Vadas, P. A., J. J. Meisinger, L. J. Sikora, J. P. McMurtry, and A. E. Sefton. 2004. Effect of poultry diet on phosphorus in runoff from soils amended with poultry manure and compost. J. Environ. Qual. 33:1845-1854.

van der Klis, J. D., and H. A. J. Versteegh. 1996. Phosphorus nutrition of poultry. Pg. 71-83 In Recent Developments in Poultry Nutrition. P.C. Garnsworthy, J. Wiseman, and W. Haresign ed. Nottingham University Press, Nottingham, UK.

**Table 2.1.** Formulation and calculated analyses of the starter diet and basal grower diets.

| Ingredients | Starter Diet[†] | Basal Grower Diet[†,‡] |
|---|---|---|
| | (g $kg^{-1}$) | |
| Corn | 556.0 | 604.5 |
| Soybean meal | 328.9 | 302.0 |
| Poultry meal | 35.0 | - |
| Poultry fat | 41.5 | 3.8 |
| Limestone[‡] | 10.8 | ... |
| Dicalcium phosphate[‡] | 12.7 | ... |
| Sodium chloride | 5.0 | 5.0 |
| Premixes[§] | 6.2 | 6.2 |
| Lysine HCl | 0.7 | 0.5 |
| L-Threonine | 0.8 | 0.4 |
| DL-Methionine | 2.5 | 1.6 |
| Phytase premix[‡,¶] | - | ... |
| | | |
| Calculated nutrients[#] | | |
| Moisture, % | 11.08 | 11.07 |
| ME, kcal/g | 3.11 | 3.20 |
| Crude protein, % | 23.00 | 20.00 |
| Lysine, % | 1.33 | 1.13 |
| Methionine + Cysteine, % | 1.01 | 0.84 |
| Threonine, % | 0.89 | 0.75 |
| Ca, % [‡] | 1.00 | ... |
| Non phytate phosphorus, % [¶] | 0.45 | ... |
| Sodium, % | 0.22 | 0.20 |

[†] Quantity of starter diet fed was adjusted to 907 g per bird alive at 7 d of age after which birds were fed the grower diet to 42 d of age.

[‡] The inclusion rate of limestone, dicalcium phosphate, and a bacterial phytase premix (Syngenta Animal Nutrition Inc., Research Triangle Park, NC 27709) was adjusted in the basal grower diet and varied from 7.1 to 16.2, 3.4 to 14.6, and 0.00 or 0.2 g $kg^{-1}$, respectively, to make 18 diets that had combinations of three levels of available phosphorus of 0.25, 0.30, or 0.35 %, three levels of calcium of 0.57, 0.69, or 0.80 %, and either 0 or 600 FTU/kg added phytase enzyme.

[§] Premixes provided the following per kg diet: vitamin A, 13,200 IU; vitamin $D_3$, 4,000 IU; vitamin E, 66 IU; vitamin $B_{12}$, 39.6 µg; riboflavin, 13.2 mg; niacin, 110 mg; d-pantothenate, 22 mg; menadione ($K_3$), 4 mg; folic acid, 2.2 mg; thiamine, 4 mg; pyridoxine, 8 mg; d-biotin, 252 µg; selenium (as $Na_2SeO_3$), 0.30 mg; manganese, 120 mg; zinc, 120 mg, iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; cobalt, 1.0 mg; choline chloride 1,200 mg; coccidiostat, 700 mg.

[¶] Phytase premix was included at the expense of 0.1% phosphorus from dicalcium phosphate and resulted in non phytate phosphorus levels of 0.35, 0.30, or 0.25 % in treatments with no added phytase enzyme and 0.25, 0.20, or 0.15 % in treatments with 600 FTU/kg added phytase enzyme.

[#] Nutrient compositions calculated from proximate analyses of all ingredients. Final diet composition confirmed by proximate analyses (Table 2.2).

**Table 2.2.** Calculated and determined nutrient analyses of dietary treatments.

| Calculated and determined nutrient analyses | Grower Diet | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Ca, calculated, % | 0.80 | 0.80 | 0.80 | 0.69 | 0.69 | 0.69 | 0.57 | 0.57 | 0.57 |
| Ca, determined, % | 0.70 | 0.80 | 0.66 | 0.61 | 0.68 | 0.75 | 0.60 | 0.55 | 0.50 |
| Total P calculated, % | 0.58 | 0.53 | 0.48 | 0.58 | 0.53 | 0.48 | 0.58 | 0.53 | 0.48 |
| Total P, determined, % | 0.61 | 0.60 | 0.52 | 0.59 | 0.57 | 0.52 | 0.62 | 0.56 | 0.52 |
| NPP, calculated, %[1] | 0.35 | 0.30 | 0.25 | 0.35 | 0.30 | 0.25 | 0.35 | 0.30 | 0.25 |
| Phytate P, calculated, %[2] | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 |
| Calculated AvP, %[3] | 0.35 | 0.30 | 0.25 | 0.35 | 0.30 | 0.25 | 0.35 | 0.30 | 0.25 |
| Calculated phytase, FTU/kg | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Calculated Ca:AvP ratio | 2.29 | 2.66 | 3.20 | 1.97 | 2.30 | 2.76 | 1.62 | 1.90 | 2.28 |
| | Grower Diet | | | | | | | | |
| | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| Ca, calculated, % | 0.80 | 0.80 | 0.80 | 0.69 | 0.69 | 0.69 | 0.57 | 0.57 | 0.57 |
| Ca, determined, % | 0.78 | 0.80 | 0.63 | 0.86 | 0.65 | 0.68 | 0.58 | 0.58 | 0.48 |
| Total P calculated, % | 0.48 | 0.43 | 0.38 | 0.48 | 0.43 | 0.38 | 0.48 | 0.43 | 0.38 |
| Total P, determined, % | 0.53 | 0.50 | 0.46 | 0.55 | 0.51 | 0.44 | 0.55 | 0.49 | 0.46 |
| NPP, calculated, %[1] | 0.25 | 0.20 | 0.15 | 0.25 | 0.20 | 0.15 | 0.25 | 0.20 | 0.15 |
| Phytate P, calculated, %[2] | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 |
| Calculated AvP, %[3] | 0.35 | 0.30 | 0.25 | 0.35 | 0.30 | 0.25 | 0.35 | 0.30 | 0.25 |
| Calculated phytase, FTU/kg[4] | 600 | 600 | 600 | 600 | 600 | 600 | 600 | 600 | 600 |
| Calculated Ca:AvP ratio | 2.29 | 2.67 | 3.20 | 1.97 | 2.30 | 2.76 | 1.63 | 1.90 | 2.28 |

[1] Non phytate P (NPP) was defined as the calculated difference between total phosphorus (P) and phytate P.

[2] Calculated from the analyzed phytate P concentration of all ingredients and the respective inclusion rate in diets.

[3] Calculated available P (AvP) using the slope ratio method (Soares, 1995).

[4] Calculated from analyzed activity of 1,098 FTU/g of added phytase premix (Syngenta Animal Nutrition, Inc., Research Triangle Park, NC 27709). One phytase U was defined as the amount of enzyme required to liberate 1 µmol of inorganic P from 1.5 m*M* of Na phytate at pH 5.5 and 37 C.

**Table 2.3.** Performance of broilers fed varying levels of calcium (Ca), and available phosphorus (AvP) with and without added phytase in the grower diet from 14 to 39 d of age.

| | Body weight gain | | Feed intake | | AdjFCR[1] | | Mortality | |
|---|---|---|---|---|---|---|---|---|
| Main Effect | 0-28 d | 0-39 d | 0-28 d | 0-39 d | 0-28 d | 0-39 d | 0-28 d | 0-39 d |
| Ca | (g) | | | | (g:g) | | (%) | |
| 0.80% | 1,323 | 2,420 | 2,040 | 3,897 | 1.54 | 1.61 | 2.83 | 3.13 |
| 0.69% | 1,312 | 2,418 | 2,033 | 3,926 | 1.55 | 1.62 | 2.08 | 2.68 |
| 0.57% | 1,333 | 2,426 | 2,035 | 3,911 | 1.53 | 1.61 | 2.23 | 2.23 |
| SEM | 10.9 | 14.3 | 18.4 | 25.9 | 0.01 | 0.01 | 0.01 | 0.01 |
| AvP | | | | | | | | |
| 0.35% | 1,328 | 2,427 | 2,039 | 3,917 | 1.54 | 1.61 | 1.04[b] | 1.34[b] |
| 0.30% | 1,321 | 2,419 | 2,042 | 3,908 | 1.55 | 1.62 | 3.27[a] | 3.57[a] |
| 0.25% | 1,319 | 2,416 | 2,027 | 3,909 | 1.54 | 1.62 | 2.83[a] | 3.13[a] |
| SEM | 10.9 | 14.3 | 18.4 | 25.9 | 0.01 | 0.01 | 0.01 | 0.01 |
| Added Phytase | | | | | | | | |
| 0 FTU/kg | 1,318 | 2,414 | 2,029 | 3,906 | 1.54 | 1.62 | 2.68 | 3.08 |
| 600 FTU/kg | 1,327 | 2,429 | 2,043 | 3,916 | 1.54 | 1.61 | 2.08 | 2.28 |
| SEM | 10.0 | 12.8 | 16.9 | 22.7 | 0.01 | 0.01 | 0.01 | 0.01 |
| | Probability | | | | | | | |
| Ca | 0.325 | 0.694 | 0.758 | 0.665 | 0.158 | 0.867 | 0.442 | 0.268 |
| AvP | 0.414 | 0.516 | 0.466 | 0.773 | 0.948 | 0.682 | 0.021 | 0.029 |
| Phytase | 0.288 | 0.242 | 0.312 | 0.679 | 0.992 | 0.382 | 0.335 | 0.228 |
| Ca*AvP | 0.353 | 0.602 | 0.672 | 0.974 | 0.128 | 0.463 | 0.617 | 0.799 |

[a-b] Means within the same column with no common superscript differ significantly ($P \leq 0.05$).
[1] Mortality corrected feed conversion rate (AdjFCR).

**Table 2.4.** Effect of diet available P (AvP) and calcium (Ca) on total P, water soluble P (WSP) and WSP:total P ratio in litter from broilers

| Diet calcium, % | No added phytase | | | + 600 FTU/kg added phytase[1] | | |
|---|---|---|---|---|---|---|
| | Diet AvP, % | | | | | |
| | 0.35 | 0.30 | 0.25 | 0.35 | 0.30 | 0.25 |
| | (Litter total P, mg kg$^{-1}$) | | | | | |
| 0.80 | 9,902$^{a}$ | 8,699$^{b}$ | 8,232$^{bc}$ | 8,141$^{bc}$ | 6,714$^{de}$ | 6,920$^{de}$ |
| 0.69 | 10,418$^{a}$ | 8,657$^{b}$ | 8,156$^{bc}$ | 7,553$^{bcd}$ | 7,274$^{cde}$ | 6,343$^{e}$ |
| 0.57 | 10,647$^{a}$ | 8,509$^{b}$ | 7,632$^{bcd}$ | 8,304$^{bcd}$ | 7,267$^{cde}$ | 6,111$^{e}$ |
| Pooled SEM | 428.7 | | | | | |
| | (Litter WSP, mg kg$^{-1}$) | | | | | |
| 0.80 | 1,165$^{cdef}$ | 984$^{defg}$ | 631$^{g}$ | 1,209$^{cdef}$ | 900$^{efg}$ | 807$^{fg}$ |
| 0.69 | 1,760$^{b}$ | 1,230$^{cdef}$ | 934$^{defg}$ | 1,353$^{bcde}$ | 1,225$^{cdef}$ | 923$^{efg}$ |
| 0.57 | 2,363$^{a}$ | 1,416$^{bcd}$ | 1,002$^{defg}$ | 2,290$^{a}$ | 1,598$^{bc}$ | 1,044$^{defg}$ |
| Pooled SEM | 286.2 | | | | | |
| | (Litter WSP:total P ratio) | | | | | |
| 0.80 | 0.12$^{def}$ | 0.11$^{ef}$ | 0.08$^{f}$ | 0.15$^{cde}$ | 0.13$^{cde}$ | 0.12$^{def}$ |
| 0.69 | 0.17$^{cde}$ | 0.14$^{cde}$ | 0.11$^{ef}$ | 0.18$^{bc}$ | 0.17$^{bc}$ | 0.15$^{cde}$ |
| 0.57 | 0.22$^{b}$ | 0.17$^{cd}$ | 0.13$^{cde}$ | 0.28$^{a}$ | 0.22$^{b}$ | 0.17$^{cd}$ |
| Pooled SEM | 0.03 | | | | | |
| | (Phytate P, mg kg$^{-1}$)[2] | | | | | |
| 0.80 | 6,664$^{a}$ | 5,722$^{ab}$ | 5,971$^{ab}$ | 3,889$^{c}$ | 3,393$^{d}$ | 3,436$^{cd}$ |
| 0.69 | 6,304$^{a}$ | 5,616$^{b}$ | 5,603$^{b}$ | 3,515$^{c}$ | 3,576$^{cd}$ | 3,148$^{de}$ |
| 0.57 | 6,129$^{a}$ | 5,824$^{ab}$ | 5,629$^{b}$ | 3,337$^{d}$ | 3,166$^{de}$ | 2,760$^{e}$ |
| Pooled SEM | 91.2 | | | | | |

[a-e] Means within columns within variables with different superscripts differ significantly ($P \leq 0.05$).

[1] Syngenta Animal Nutrition, Inc., Research Triangle Park, NC 27709. One phytase unit (FTU) was defined as the amount of enzyme required to liberate 1 µmol of inorganic P from 1.5 m*M* of Na phytate at pH 5.5 and 37 C.

[2] Phytate P analyzed by HPLC methodology (Kwanyuen and Burton, 2005).

**Table 2.5.** Effects of dietary variables on litter total phosphorus (P), water soluble phosphorus (WSP), and the ratio of water soluble to total phosphorus (WSP:total P) for both the full factorial model and a reduced model containing dietary calcium (Ca) to available phosphorus (AvP) ratios (Ca:AvP).

| | Litter P Characteristic | | |
|---|---|---|---|
| Diet Variable | Total P (mg kg $^{-1}$) | WSP (mg kg $^{-1}$) | WSP:total P (ratio) |
| | | Pr > F | |
| Full Factorial Effects Model | | | |
| Ca | 0.988 | <0.0001 | <0.0001 |
| AvP | <0.0001 | <0.0001 | <0.0001 |
| Phytase | <0.0001 | 0.757 | <0.0001 |
| Ca*AvP | 0.291 | 0.368 | 0.698 |
| Ca*phytase | 0.732 | 0.609 | 0.743 |
| AvP*phytase | 0.175 | 0.513 | 0.646 |
| Ca*AvP*phytase | 0.652 | 0.819 | 0.967 |
| Reduced model | | | |
| Ca:AvP | 0.0115 | <0.0001 | 0.009 |
| Ca:AvP$^2$ | 0.0349 | 0.012 | 0.095 |
| Phytase | <0.0001 | 0.745 | <0.0001 |
| | | Mean Square Error | |
| Full Factorial Effects Model | 8,081.99 | 0.0683 | 0.0574 |
| Reduced Model | 999,351.1 | 0.0621 | 0.0513 |

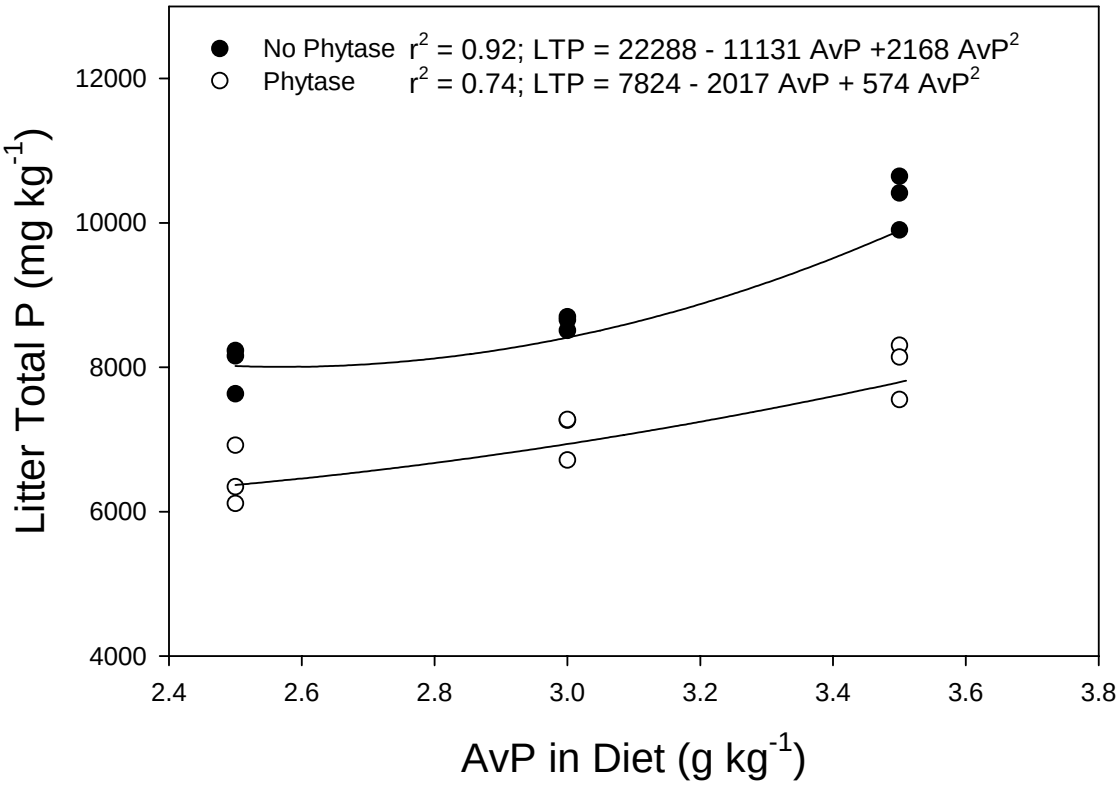


**Figure 2.1.** The effect of dietary available phosphorus (AvP) on litter total phosphorus (P) concentrations for both non-phytase and phytase amended diets.

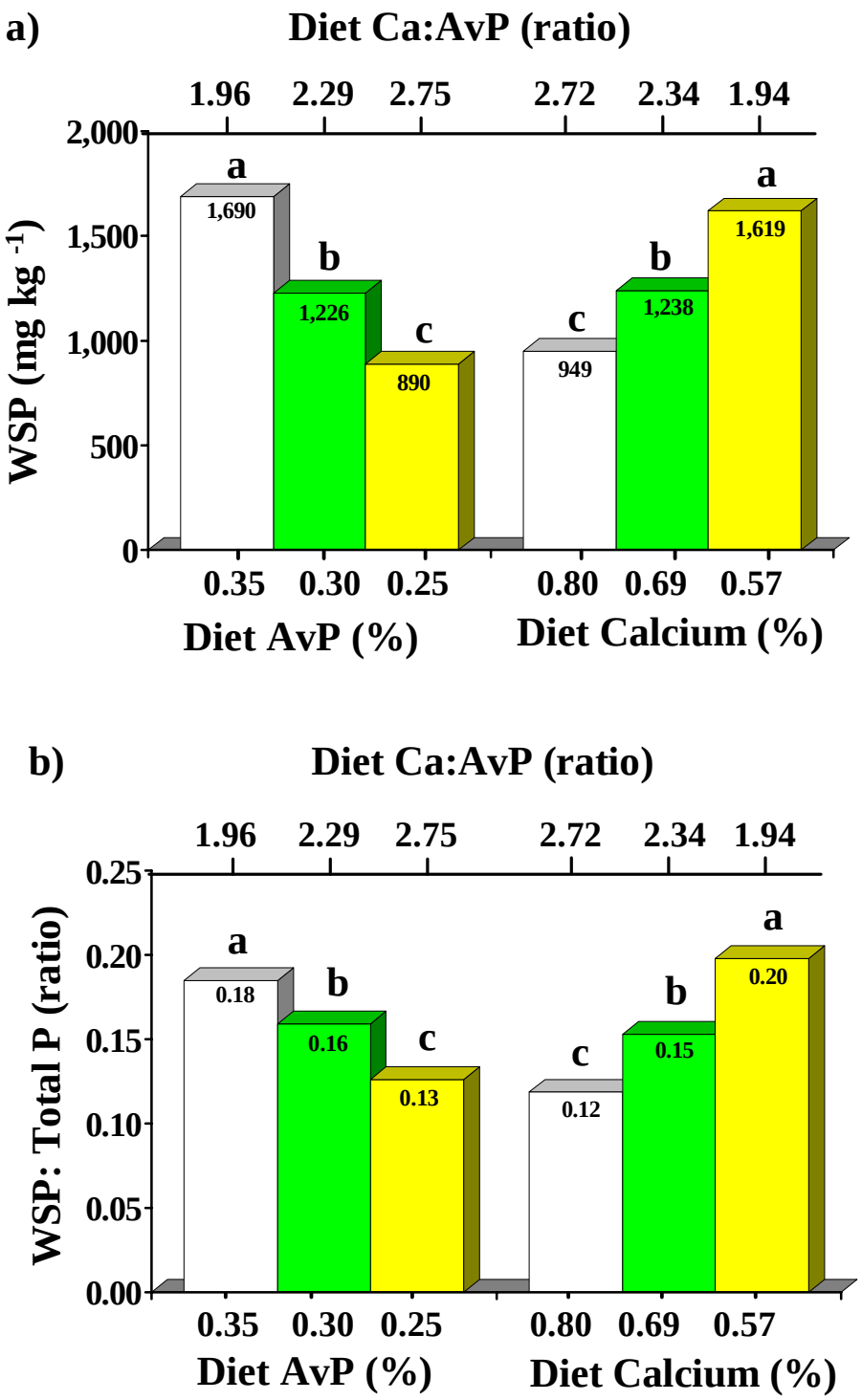


**Figure 2.2 (a-b).** Effects of calcium (Ca), available phosphorus (AvP), and the ratio of Ca:AvP in broiler grower diets on water soluble phosphorus (WSP) (a) and the ratio of WSP:Total P (b) in broiler litter.
[a-c] Bars with different superscripts differ significantly ($P \leq 0.05$).

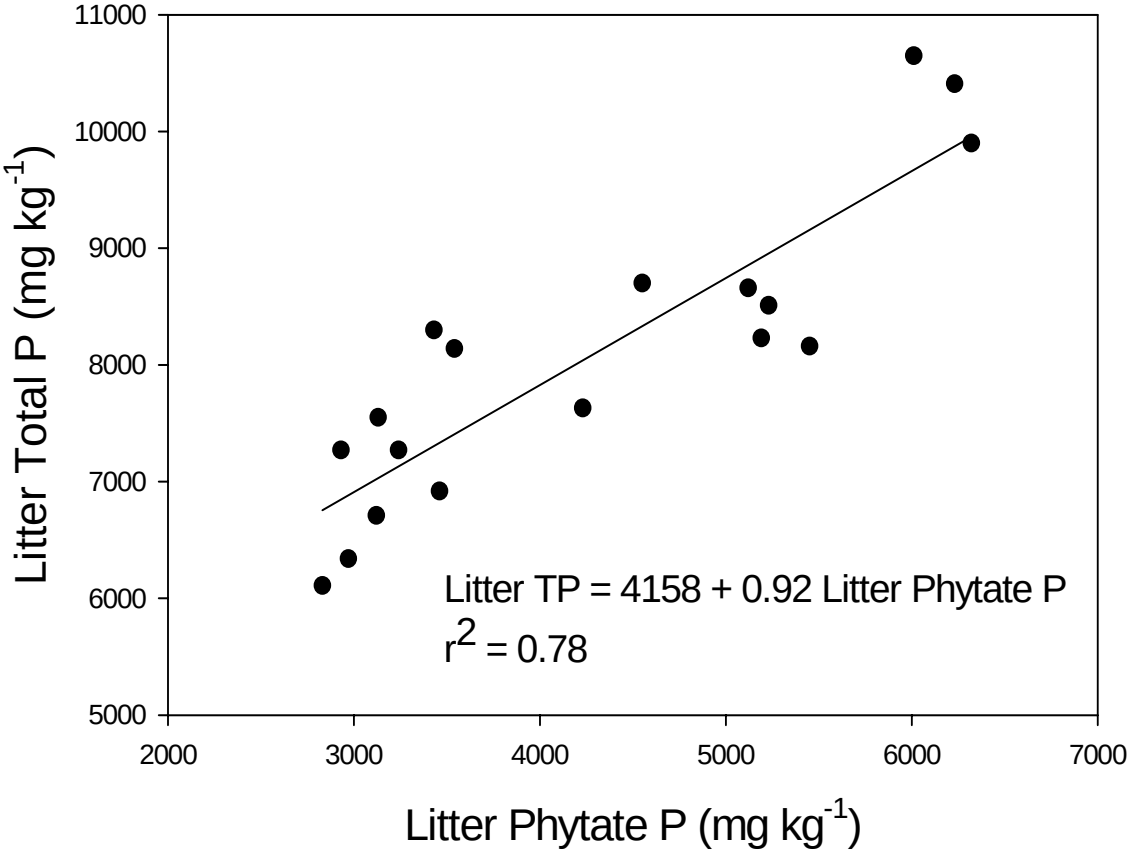


**Figure 2.3.** The relationship between litter phytate phosphorus (P) concentration and litter total P (TP) concentrations for all diets.

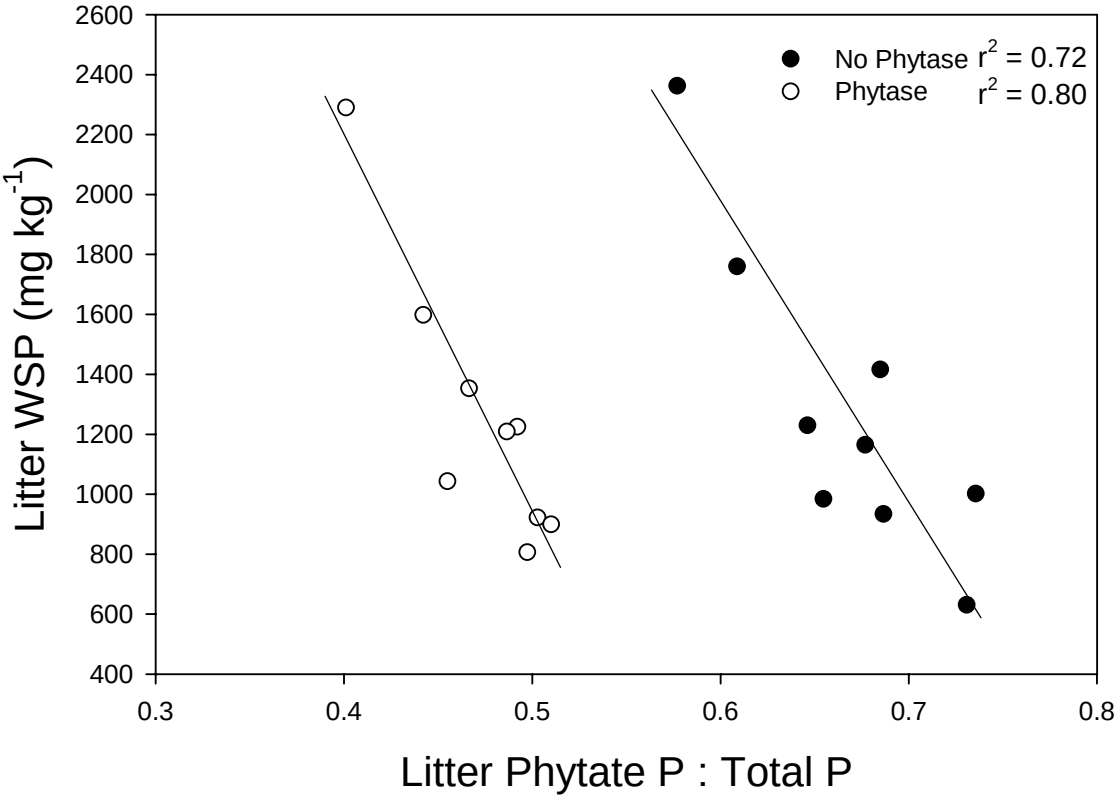


**Figure 2.4.** The relationship between the litter phytate phosphorus (P) to total P ratio and litter water soluble P (WSP) for both non-phytase and phytase amended diets.

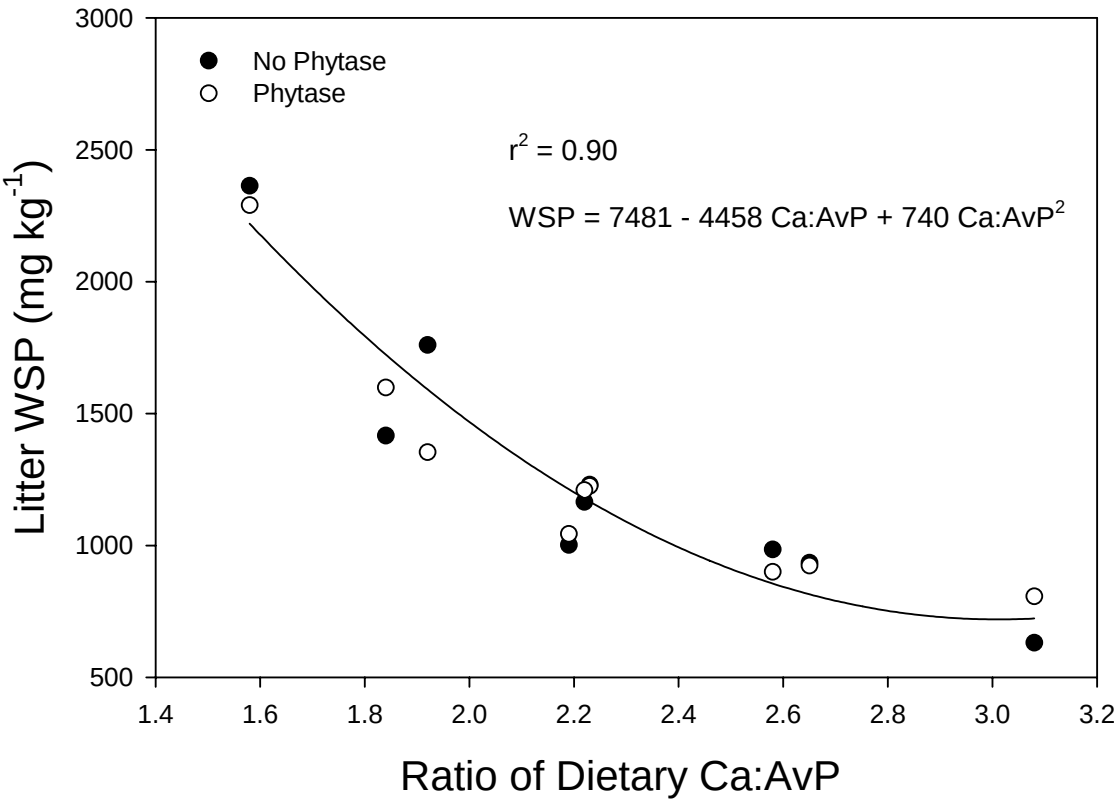


**Figure 2.5.** The influence of the dietary calcium (Ca) to available phosphorus (AvP) ratio on the litter water soluble phosphorus (WSP) concentration of both non-phytase and phytase amended diets.

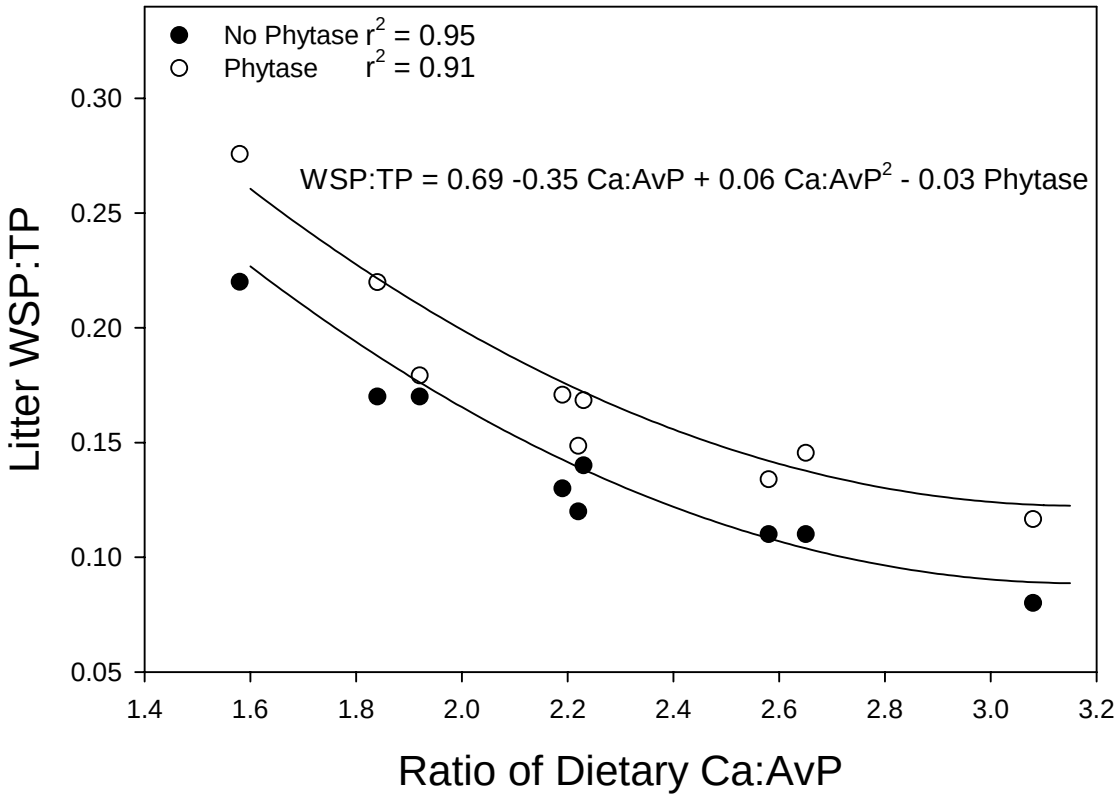


**Figure 2.6.** The influence of the dietary calcium (Ca) to available phosphorus (AvP) ratio on the litter water soluble phosphorus (WSP) to total phosphorus (TP) ratio of both non-phytase and phytase amended diets.

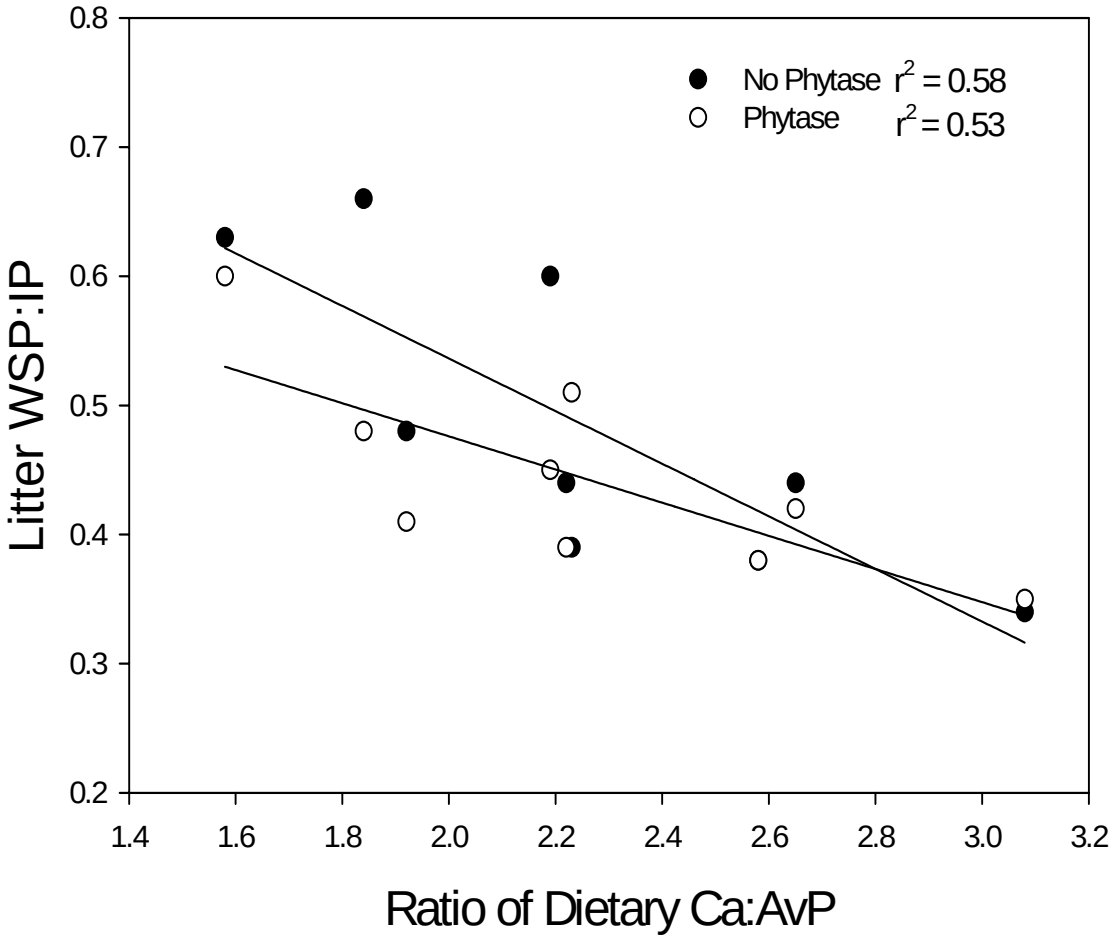


**Figure 2.7.** The relationship of dietary calcium (Ca) to available phosphorus (AvP) ratio and the fraction of inorganic P (IP) in litter that was water soluble (WSP).

## CHAPTER 3

### Interaction of Calcium and Phytate in Broiler Diets. 1. Effects on Apparent Prececal Phosphorus Utilization and Total and Water Soluble Phosphorus Excretion

**ABSTRACT** High concentrations of Ca in broiler diets have been shown to reduce phytate hydrolysis in the intestines and alter phytate phosphorus (P) excretion and the amount of water soluble P (WSP) in the litter. Although low phytate (LP) varieties of soybean meal (SBM) have been developed, it was not well known how dietary Ca in combination with reduced dietary phytate concentrations would affect the dietary ratio of Ca:non phytate P (NPP) required for optimal P retention and the total P and WSP in the manure. A factorial treatment structure was used with four levels of dietary Ca from 0.47% to 1.16% and three levels of phytate P (0.28%, 0.24%, and 0.10%). Varying dietary phytate P levels were obtained by utilizing SBM from three varieties of soybeans with different phytate P concentrations. Ross 508 broiler chicks were fed one of the 12 diets from 16-21 d of age. Excreta was collected from 16-17 d and from 19-20 d of age and ileal digesta was collected at 21 d of age. Apparent prececal P absorption decreased when dietary Ca concentration increased and was higher when diets contained low phytate (LP) SBM. The apparent absorption of Ca and percentage phytate P hydrolysis at the distal ileum was not reduced when dietary phytate P concentration increased. Including LP SBM in diets reduced total P output in the excreta by 49% compared to conventional SBM. The optimum ratio of Ca:NPP that resulted in the highest P retention and lowest P excretion was 2.53:1, 2.40:1, and 2.34:1 for diets with 0.28%, 0.24%, and 0.10% phytate P. The WSP was 48% lower with LP SBM diets, while the effect of increasing Ca on WSP and the WSP:total P ratio was greater when diets contained conventional or high phytate (HP) SBM. These data suggested that the inclusion of LP SBM was able to reduce total P output by 49% and WSP by 48% and that the response in WSP to increasing dietary Ca was dependent on the phytate concentration in the diet.

## INTRODUCTION

Legislation aimed at the control of pollution has limited the application of animal manure to land based on its phosphorus (P) content, which has greatly increased the need to develop strategies that will improve the utilization of dietary P and reduce P excretion (Beede, 2003; Maguire et al., 2005). The P in poultry manure and litter has been shown to be derived from undigested dietary P, endogenous P secreted into the gastrointestinal tract, and endogenous P excreted via the urine (Dilger and Adeola, 2006). Therefore, dietary strategies aimed at reducing P excretion should both increase the amount of P absorbed in the small intestine and minimize urinary P losses by increasing the retention of P in the body. However, in addition to the effects on total P excretion, the impact of diet modification on P forms in the manure must be considered as these have been shown to also alter the P in manure that was water soluble (WSP) (Leytem and Maguire, 2006). The WSP fraction can have a substantial impact on the potential of P in manure or litter to contribute to P runoff after application of manure or litter to land, as Sharpley and Moyer (2000) showed a 98% correlation between WSP in manure and the amount of P leached from soils following simulated rainfall.

Soybean meal (SBM) has been routinely added to poultry diets as a primary source of protein, but has also been found to contribute up to 30% of the total dietary P in a corn and SBM based broiler diet (Plumstead et al., 2004). However, over 60% of the total P contained in SBM has been reported to be present as salts of phytic acid (myo-inositol 1,2,3,4,5,6 hexa*kis* dihydrogen phosphate), also known as phytate, which has been reported to be poorly digested by monogastric animals (Nelson et al., 1968a; Raboy et al., 1984). The utilization of phytate P in poultry diets, measured as disappearance of phytate P from the distal ileum, was also highly variable, ranging from 25.4% to 69.2% (Rutherford et al., 2002; Tamin et al., 2004). A considerable part of the large variation in

the extent of phytate P hydrolysis in the small intestine was shown to be caused by differences in the dietary calcium (Ca) concentration (Tamin et al., 2004). A high dietary Ca concentration has been thought to reduce hydrolysis of phytate P by endogenous and exogenous phytase enzymes as a result of the formation of insoluble Ca-phytate complexes (Nelson, 1980; Sands et al., 2003; Tamin and Angel, 2003; Tamin et al., 2004). Furthermore, a high ratio of dietary Ca to non phytate phosphorus (NPP) in the diet was also shown to antagonize the absorption and availability of inorganic, soluble forms of P, due to increased precipitation of insoluble Ca:P complexes (Hurwitz and Bar, 1971). The concentration of phytate in the diet and extent of phytate hydrolysis in the digestive tract can also have substantial environmental implications as the proportion of WSP decreased when the proportion of phytate P in the excreta increased (Leytem and Maguire, 2006).

To improve the utilization of P from SBM, and reduce total P excretion in manure, low phytate varieties of soybeans have been selectively bred that have similar nutritive value and total P concentrations but contain considerably reduced levels of phytate P (Wilcox et al., 2000; Raboy, 2002). However, reducing the dietary phytate P concentration by including low phytate grains would also presumably increase the molar ratio of Ca:phytate P in the intestine, which would influence the fate of phytate P (Wise, 1983). Early findings by Nelson et al. (1968b) demonstrated a 50% reduction in the Ca requirement for maximum bone ash when the dietary phytate P content was reduced in broiler diets. This was hypothesized to be caused by the ability of 1% phytate in a diet to chelate 0.36% Ca thus decreasing the amount of Ca absorbed and increasing the Ca required for maximum bone ash. This would also alter the optimum dietary ratio of Ca:NPP. However, Nelson et al. (1968b) did not determine potential effects of Ca and phytate level on intestinal phytate hydrolysis and excretion of P in broilers. Our previous

research (Chapter 2) had suggested that the WSP in broiler litter was dependent on the ratio of Ca:available phosphorus (AvP) in the diet. However, as the effect of Ca:AvP on WSP was shown to be mediated by the degree of phytate hydrolysis and the formation of insoluble CaP complexes, it could be expected that the extent whereby dietary Ca affected WSP in the litter may also be dependent on the initial phytate concentration in the diet.

Therefore, the objective of this study was to determine if the dietary Ca concentration for optimal hydrolysis of phytate P, P absorption in the intestine, and overall P retention in broilers was altered when the diets contained different phytate P concentrations from inclusion of either high phytate (HP) Prolina, conventional, or low phytate (LP) SBM. In addition, we examined the impact of dietary Ca and phytate concentration on total P and WSP concentrations in manure to determine if the effects of Ca on WSP that were previously shown in Chapter 2 were dependent on the initial concentration of phytate in the diet.

## MATERIALS AND METHODS

### *Animals and Management*

Broiler chicks were hatched from eggs obtained from Ross 344 x 508 broiler breeders housed at the institution. Chicks were feather-sexed at hatching and 816 male chicks were permanently identified with neck tags and 17 chicks were randomly allocated to each of 48 electrically 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. Brooding temperatures within cages were initially set at 33º C and reduced gradually to 25º C by 21 d of age. From 1-15 d all birds had ad libitum access to feed and water and received a standard corn-soy broiler starter diet containing

3,150 kcal ME $kg^{-1}$, 23.0% CP, 0.90% Ca, and 0.45% NPP. To reduce variation in chick BW between pens all chicks were individually weighed at 12 d and chicks with extreme BW eliminated so that 13 chicks per pen with a mean chick BW of 342 $\pm$ 12 g remained.

***Dietary Treatments***

To obtain practical broiler diets with different phytate P concentrations, SBM from three different cultivars of soybeans that differed in their natural phytate content were utilized (Table 3.1). The HP Prolina SBM and the LP SBM were processed from soybeans selected for either improved protein or low phytate P content. A Commercial SBM with similar protein and amino acid concentration, while being intermediate in its phytate concentration, was selected as a control. Further, to increase the range of phytate P in the final diets degermed dehulled (DGDH) corn with low levels of phytate P was used in formulation.

Three basal diets that differed in their source of SBM were formulated to contain similar nutrient profiles with the exception of dietary Ca (Table 3.2). Finely ground limestone was added to each of the basal diets at the expense of washed sand to obtain graded levels of dietary Ca (0.47, 0.70, 0.93, and 1.16%). Titanium dioxide ($TiO_2$) and celite were included in diets as indigestible markers to allow the calculation of apparent nutrient absorption and overall retention. To further reduce differences between diets, the formulated moisture content was fixed at 11.5% by varying the inclusion of water while soy oil added to diets was held constant at 6.5%. The analyzed phytate P levels were 0.28, 0.24, and 0.10% for the HP Prolina, Commercial, and LP SBM diets, respectively (Table 3.2). All diets were mixed in two 25 kg batches in a bakery style vertical mixer and each batch was sampled and analyzed individually. At 15 d of age birds were fasted for 16 h after which the 12 experimental diets were each assigned to four pens of 13 birds and fed from 16-21 d of age.

***Sample Collection and Analyses***

Excreta were collected from metal pans lined with clean plastic beneath each cage during each of two 24 h periods. Collection 1 commenced once birds started the experimental diets at 16 d of age and had been fasted for 16 h, while Collection 2 commenced at 19 d after an adaptation period of 72 h. Following each collection the excreta from each pan was homogenized, sub-sampled, and immediately frozen. At 21 d of age 10 chicks from each pen were weighed, killed by cervical dislocation, and the terminal 13 cm of ileum removed 3 cm anterior to the ileo-cecal junction. Ileal contents were gently expressed, pooled per cage, and frozen. Feeders were weighed at the start and end of Collection 1 and again at the end of Collection 2 to calculate the mean feed intake per bird.

The percentage dry matter of ileal digesta and manure was determined by drying a fresh sub-sample of manure in an oven at 100°C d for 24 h. Fresh manure samples were immediately analyzed for WSP by shaking the equivalent of 1 g dry manure with 100 mL deionized water for 1 h, filtering through a 0.45 μm membrane, and analyzing total WSP by inductively-coupled plasma atomic-emission spectrometry (ICP–AES; Optima 2000, Perkin Elmer, Wellesley, MA 02481). The remaining manure samples were frozen, lyophilized, and ground (<2 mm) for analysis.

Frozen samples of feed, ileal digesta, and excreta were lyophilized and ground prior to analysis for total elements and phytate as follows: (i) total elements (Ca and P) were determined by microwave-assisted digestion of a 0.5 g dried sample with 8 mL of concentrated $HNO_3$ and 2 mL of 30% $H_2O_2$ (v/v) with all elements quantified using inductively-coupled plasma optical-emission spectrometry (ICP–OES; 4300DV, Perkin-Elmer, Wellesley, MA 02481) detection and (ii) phytate P was determined by acid extraction followed by high performance liquid chromatography analysis (HPLC; Agilent

HPLC 1100 series, Agilent Technologies, Wilmington, DE 19808; Kwanyuen and Burton, 2005). The $TiO_2$ of diets, ileal and excreta samples was determined using the method of Short et al. (1996).

***Calculations and Statistical Analyses***

The apparent percentage prececal nutrient digestibility ($PcND_{\%}$) and overall percentage nutrient retention ($TNR_{\%}$), expressed as a percentage of dry matter (DM) nutrient concentration were calculated using the index method based on the following equation: (Dilger and Adeola, 2006).

$$PcND_{\%} \text{ or } TNR_{\%} = 100-[(TiO_{diet}/ TiO_{out}) \times (Nut_{out}/ Nut_{diet}) \times 100] \quad [1]$$

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.

To account for differences in dietary nutrient concentrations, the apparent amount (g) of nutrients absorbed per kg of DM intake (DMI) at the terminal ileum ($PcNA_g$), as well as the total retention of dietary nutrients per kg of DMI, ($TNR_g$) was calculated using the equation:

$$PcNA_g \text{ or } TNR_g \text{ (g/kg DMI)} = PcND_{\%} \text{ or } TNR_{\%} \times \text{Nutrient Intake / kg DMI} \quad [2]$$

where $PcND_{\%}$ or $TNR_{\%}$ were the percentage nutrient digestibility or retention calculated in [1]. Further, the nutrient output per kg DMI at the terminal ileum ($PcNE_g$,)

or total nutrients excreted ($TNE_g$) per kg DMI were calculated using the ratio of $TiO_2$ intake to $TiO_2$ output (Dilger and Adeola, 2006):

$$PcNE_g \text{ or } TNE_g \text{ (g/kg DMI)} = NcE \times (TiO_{diet} / TiO_{out}) \quad [3]$$

where NcE was the concentration of the respective nutrient in the ileal digesta or excreta; $TiO_{diet}$ was the initial $TiO_2$ concentration in the diet and $TiO_{out}$ was the concentration of $TiO_2$ in the ileal digesta or excreta. Finally, the sum of

$$PcNA_g + PcNE_g \text{ or } TNR_g + TNE_g = \text{Dietary nutrient intake /kg DMI.} \quad [4]$$

All data were analyzed using the Mixed Models procedure (SAS Institute, 2004). There were four replicate cages per treatment arranged in a randomized complete block design. There were four blocks with two blocks of four cages located in each of two rooms. A cage of birds served as the experimental unit. Data were analyzed using a factorial effects model that included SBM source (3 levels), dietary Ca (4 levels), and all two-way interactions as fixed effects with block as a random effect. Orthogonal polynomial contrasts were used to assess the significance of linear or quadratic models to describe the response in the dependent variable to increasing Ca level. Where appropriate, means separation was carried out using Tukeys HSD with an alpha level of 0.05. Further, the breakpoint in the dietary Ca dose at which a plateau in total P retention and output of P in the excreta was reached was determined using segmented regression models in PROC NLIN (SAS Institute, 2004). Statements of statistical significance were based upon $P < 0.05$ unless otherwise stated.

## RESULTS

Analysis of the SBM for total inositol phosphate (IP) esters and phytate P are shown in Table 3.1. The majority of IP found in all of the SBM was in the form of IP6, with decreasing concentrations of the lower IP esters. The concentration of IP esters was lower in the LP SBM than the other SBM sources with the exception of IP3 which had a slightly greater concentration than the other sources. The HP Prolina SBM had a greater concentration of IP5, which accounted for 19% of the total IP content, while there was only ~11% IP5 for both the Commercial and LP SBM.

The calculated and analyzed nutrient content of diets are presented in Table 3. Analyzed dietary crude protein, amino acids, Ca, and P levels were in good agreement with formulated values and small deviations in the analyzed Ca and P values were attributed to sampling and analytical error. The analyzed phytate P concentration of samples of SBM shown in Table 3.1 was determined from samples of SBM drawn at the time of diet mixing and was lower compared to values used in diet formulation, which had been obtained from initial screening of this SBM several months prior to the onset of the study. Therefore, although all diets had been formulated to contain the same calculated NPP of 0.35% by variable addition of feed grade monobasic calcium phosphate ($CaH_4[PO_4]_2 \cdot H_2O$), differences between formulated and analyzed phytate P levels of the diets resulted in the analyzed diet NPP values of the HP Prolina diets being higher (0.40%), than the Commercial, and LP SBM diets (0.31% and 0.32%, respectively). Broilers appeared healthy throughout the 5-d experimental period with the exception of one bird on the HP Prolina SBM diet with 0.70% Ca that died on the second day after the onset of the feeding study. The feed intake of chicks over the 5-d experimental period was remarkably consistent between sources of SBM and was 422 $\pm$ 4.3 g for the HP Prolina diets, 424 $\pm$ 4.7 g for the Commercial SBM diets, and 424 $\pm$ 3.2 g

for birds consuming the LP SBM diets. The BW selection process at 12 d and similar feed intake of chicks on different treatments produced no differences in the 21 d BW of the 10 chicks that were randomly sampled per pen for collection of the ileal digesta.

***Apparent Prececal Absorption and Output of P, Ca, and Phytate P at the Distal Ileum***

The apparent prececal digestibility of P decreased in a curvilinear manner with increasing dietary Ca level and was significantly higher for diets containing the LP SBM (Table 3.4, Figure 3.1a). The apparent prececal P absorption per kg of DMI was not different between the Commercial and LP SBM diets, but as a result of the higher analyzed NPP levels, was greater in diets that contained HP Prolina SBM. Further, a significant interaction of dietary Ca level and SBM source suggested that increasing dietary Ca concentration resulted in a more rapid reduction in the amount of prececal P absorbed from the HP Prolina SBM diets per kg DMI. This also resulted in a more rapid increase in the quantity of P output per kg DMI at the distal ileum (Figure 3.1 c).

There were no differences between SBM for the apparent prececal disappearance of phytate P, which decreased linearly from 20.15% to 5.87% when dietary Ca was increased from 0.47% to 1.16% (Table 3.4). Differences in the initial diet phytate P intake per kg DMI resulted in more phytate P absorbed per kg of DMI in the HP Prolina and Commercial SBM diets (Figure 2 b), while the output of intact phytate P at the distal ileum was lowest for the LP SBM (Table 3.4). Increasing dietary Ca concentrations also increased the output of phytate P per kg DMI at the distal ileum and was independent of the dietary phytate P concentration.

Although there were differences in dietary phytate P concentrations, there was no effect of SBM and no interaction of SBM and dietary Ca level on apparent prececal Ca digestibility, or amount of Ca absorbed per kg DMI that increased two-fold when dietary

Ca level was raised from 0.47% to 1.16% (Table 3.4). However, in spite of more Ca being absorbed the increase in dietary Ca from 0.47% to 1.16% also resulted in a significant stepwise increase in the output of Ca from the distal ileum from 2.78 g $kg^{-1}$ to as high as 7.92 g $kg^{-1}$ DMI (Table 3.4).

***Total Retention and Excretion of P, Ca, and Phytate P in the Excreta During the first 24 hr (Collection 1) or Following a 3 d Adaptation Period (Collection 2)***

The percentage P and Ca retention was consistently higher for Collection 2, after birds had been adapted to diets for 72 h (Table 3.5). There was also a strong negative correlation between the percentage P retention and the analyzed dietary phytate P concentration during both Collection 1 (r = -0.916; $P<0.0001$), and during Collection 2 (r = -0.922; $P<0.0001$). During Collection 1, the percentage dietary P retained increased from 32.01% to 39.37% when Commercial SBM replaced HP Prolina SBM in diets and to 53.78% when LP SBM replaced HP Prolina SBM. Following the 3-d adaptation period, the percentage P retention during Collection 2 was 38.95%, 46.89%, and 63.37% for the HP Prolina, Commercial, and LP SBM, respectively.

Increasing the dietary Ca level resulted in a linear increase in P retention during Collection 1 (Table 3.5), while the response in P retention to increasing Ca levels during Collection 2 differed between sources of SBM, which could be described by a quadratic function that reached a definite plateau (Figure 3.3 a,b). Segmented regression analysis of the response in P utilization (i.e. retention and excretion) with increasing dietary Ca determined the breakpoint in the dietary Ca% at which the plateau in P utilization was reached, which was 0.996 $\pm$ 0.057% Ca for the HP Prolina SBM diets, 0.739 $\pm$ 0.038% Ca for the Commercial SBM, and 0.743 $\pm$ 0.053% Ca for the LP SBM diets (Figure 3.3 a-c). Total P excretion per kg of DMI during Collection 2 generally reflected differences in

P retention and the significant interaction of SBM and Ca level suggested that the initial reduction in excreta P output per kg of DMI with increasing dietary Ca concentration differed between sources of SBM, being lower for diets that contained LP SBM (Figure 3.3c). There was no interaction of dietary Ca level and source of SBM for phytate P output per kg of DMI in the excreta from Collection 2, but phytate P was four-fold lower when LP SBM rather than HP Prolina SBM was included in the diet.

***Characteristics of Phosphorus in Excreta During the first 24 hr (Collection 1) or Following a 3-d Adaptation Period (Collection 2)***

The excreta P characteristics of Collection 1 are shown in Table 3.6 and that of Collection 2 are shown in Table 3.7. The highest total P concentration in the excreta from Collection 1 of 14.87 g P $kg^{-1}$ was found in manure from birds fed the HP Prolina SBM with 0.47% Ca. Replacing the HP Prolina SBM with either Commercial or LP SBM at the same dietary Ca decreased total P in the excreta from 14.87 g P $kg^{-1}$ to 11.54 and 6.47 g P $kg^{-1}$, respectively. Although total P was generally lower in Collection 2, the effects of decreasing dietary phytate concentration by replacing HP Prolina SBM with either Commercial or LP SBM followed the same order as for Collection 1, namely HP Prolina > Commercial > LP SBM. There was no interaction of source of SBM with Ca level during both Collection 1 and 2. Increasing the dietary Ca from 0.47% to 1.16% during Collection 1 and 2 resulted in small reductions in excreta total P, irrespective of the source of SBM. In contrast to effects of Ca on total P, effects of Ca on the WSP in excreta were dependent on the source of SBM, as indicated by a significant SBM x Ca interaction. There was a significant reduction in WSP of excreta from both Collection 1 and Collection 2 in the order of HP Prolina > Commercial > LP SBM (Table 3.8). Increasing the dietary Ca concentration resulted in a linear decrease in the WSP during

both collection periods. However, the effect of increased dietary Ca on WSP was greatest in the HP Prolina and Commercial SBM as indicated by the steeper slope of regression equations that described the response in WSP to increasing Ca compared to that when diets contained LP SBM (Table 3.8, Figure 3.4 a-b). In contrast to the effects of SBM source on WSP, the WSP:total P ratio was not altered when Commercial or LP SBM replaced HP Prolina SBM in diets. However, after a 3-d adaptation period, the WSP:total P ratio of excreta from Collection 2 was significantly lower when broilers had received diets with LP SBM compared to diets with either Commercial or HP Prolina SBM. There was also a significant Ca x SBM source interaction for the WSP:total P ratio. Increasing dietary Ca percentage from 0.47% to 1.16% in Collection 2 resulted in a more rapid decrease in the WSP:total P ratio when diets contained either HP Prolina or Commercial SBM compared to diets with LP SBM.

The phytate P in excreta from both Collection 1 and 2 was lowest in the LP SBM treatment with no significant differences between the remaining diets. The ratios of phytate P:total P in excreta from Collection 1 ranged from 0.18 to 0.59, with the average ratio following the trend Commercial > HP Prolina > LP SBM. Effects of Ca on phytate in the excreta from Collection 1 were somewhat variable when diets contained HP Prolina SBM but phytate in excreta increased linearly when diets contained either Commercial or LP SBM and the Ca concentration was increased from 0.47% to 1.16%. Following a period of adaptation to the test diets, there was a significant effect of dietary Ca on manure phytate P:total P. The manure phytate P:total P was negatively correlated with WSP within SBM with correlation coefficients of -0.36 to -0.94 for Collection 1 and -0.70 to -0.90 for Collection 2.

## DISCUSSION

Previous workers who have investigated effects of phytate P concentration on P utilization obtained a range of phytate P in their diets either by adding synthetic sources of calcium or magnesium phytate to diets (Waldroup et al., 1963a), by including graded levels of SBM (Dilger and Adeola, 2006), by reducing dietary phytate intake by pre-treating ingredients with phytase enzymes (Nelson et al., 1968b), or by including low phytate variants of SBM in diets (Sands et al., 2003; Dilger and Adeola, 2006). In the present study graded levels of dietary phytate P from SBM that ranged from 0.10 to 0.28% were obtained by combining a single source of DGDH corn with three sources of SBM that differed in their natural concentration of phytate P. Inclusion of DGDH corn has previously been shown to reduce phytate P in diets without negatively affecting the performance of broilers or swine (Moeser et al., 2002; Applegate, 2005). Therefore, as diets in the present study contained similar levels of DGDH corn and SBM, they were similar in physical attributes that resulted in similar feed intake. Therefore, differences in P utilization observed in this study could be attributed to differences in the Ca, P, NPP, and phytate P content of diets rather than the physical attributes and consumption of the diets.

The higher determined NPP concentration of the HP Prolina SBM diets was unintentional and resulted from a lower than expected analyzed phytate concentration determined in a sample of SBM that was drawn when diets were mixed, compared to a previous sample drawn from the HP Prolina SBM 14 months earlier. It was not known if the differences in analyzed phytate in the HP Prolina SBM were due to sampling error, or if some hydrolysis of phytate to lower order IP esters had occurred during storage.

***Phytate P Disappearance and Apparent Absorption of P at the Distal Ileum***

The antagonism of dietary Ca on the apparent absorption of P from the small intestine has been well established and shown to be dependent on both the absolute Ca and P concentrations and the ratio of Ca to P in the diet (Hurwitz and Bar, 1971; van der Klis and Versteegh, 1996). Several other studies have also found reduced P digestibility (Al-Masri, 1995), broiler performance, and bone mineralization (Waldroup et al., 1963b; Qian et al., 1997; Driver et al., 2005) when the Ca:P ratio was widened in diets with low levels of P. The negative effect of Ca on P on the apparent absorption of P in the intestines has been thought to result from two potential mechanisms. In the first instance, elevated concentrations of dietary Ca were hypothesized to increase the formation of insoluble Ca-phytate complexes that reduced phytate P hydrolysis by endogenous or exogenous phytase enzymes (Nelson, 1980; Wise, 1983; Qian et al., 1997). Secondly, excess Ca relative to inorganic P increased the formation of inorganic CaP precipitates, which decreased the concentrations of soluble forms of P in the intestinal lumen and reduced P absorption (Hurwitz and Bar, 1971).

Given these two separate mechanisms whereby dietary Ca could potentially reduce the apparent absorption of P, the present authors hypothesized at the onset of this study that the response in apparent prececal P absorption and overall P utilization (retention and excretion) to increasing dietary Ca concentration may be different when diets contained different concentrations of phytate. Our results showed that increased dietary Ca concentrations, which also widened the Ca:NPP ratio, reduced the apparent prececal P digestibility and P absorption per kg DMI in a curvilinear manner (Figure 3.1 a,b) consistent with previous research (van der Klis and Versteegh, 1996). However, in spite of substantial differences in the analyzed dietary phytate P concentrations of diets with different sources of SBM, there was no difference in the rate at which prececal P

digestibility was reduced when dietary Ca was increased. This lack of any interaction of Ca and SBM source on apparent prececal P digestibility became particularly evident when the LP SBM and Commercial SBM diets, which had similar mean analyzed concentrations of NPP (0.32 and 0.31%, respectively), while having large differences in the concentration of phytate P (0.10 and 0.24%, respectively) and large differences in the levels of added monocalcium phosphate (0.59% or 1.09%, respectively) were compared. In spite of these differences, the decrease in the percentage apparent prececal P digestibility and P absorption per kg DMI was remarkably similar when Ca concentration in diets was increased from 0.47 to 1.16% (Figure 3.1 a,b).

While there was no interaction of dietary Ca and SBM source on prececal P digestibility, there was an improvement in apparent P digestibility from 51.69% and 52.54% to 65.44% when LP SBM was included in diets in place of either HP Prolina or Commercial SBM, respectively (Tables 3.4 and 3.5). Dilger and Adeola (2006) showed no differences between LP and Conventional SBM in the amounts of endogenous P secreted into the ileum. Therefore, the higher apparent prececal P digestibility found in the present study was most likely due to the higher percentage P absorbed and not due to reduced endogenous P losses. Importantly, the similar amounts of P absorbed per kg DMI between the LP and Commercial SBM diets suggested that as determined NPP levels in these diets were similar, the inclusion of the LP SBM in diets was able to replace the additional 0.5% monocalcium phosphate that had been added to the Commercial SBM diets to maintain a similar level of NPP.

The higher apparent P absorption per kg DMI in diets containing the HP Prolina SBM and the significant interaction observed between Ca and SBM source for apparent P absorption per kg DMI was most likely caused by the higher analyzed NPP concentration in the HP Prolina SBM that resulted in a more rapid decrease in apparent P absorption

when dietary Ca concentrations were increased. The improved P digestibility and retention of P in diets containing LP SBM was consistent with previous studies that compared low phytate versus conventional sources of SBM (Sands et al., 2003), corn (Li et al., 2000), and barley (Thacker et al., 2003). In contrast to these findings, Dilger and Adeola (2006) found no significant difference in the apparent prececal P digestibility of LP or conventional SBM, which was 85% and 82.6%, respectively. However, in the study by Dilger and Adeola (2006) diets contained no added Ca or P from inorganic mineral sources. Tamin et al. (2004) showed that in the absence of an added inorganic Ca to diets, broilers were able to hydrolyze 69.20% of the dietary phytate P, which was reduced to 25.40% when 0.5% Ca from $CaCO_3$ was added to a corn-soy diet. Therefore, in the study of Dilger and Adeola (2006) the absence of added Ca and inorganic P sources would presumably have facilitated a high rate of phytate P hydrolysis, which may have ameliorated any differences in P absorption between sources of SBM with low or high concentrations of phytate P.

In our study, the mean disappearance of phytate P at the distal ileum when diets contained 0.47% Ca was 20.15% (Table 3.4), which was lower than previous estimates by Leske and Coon (1999) of 34.9% in diets with 0.5% Ca. The lower phytate P hydrolysis in our study was most likely due to the younger age of birds and the inclusion of higher levels of Ca and NPP, both of which have been shown to reduce the extent of phytate P utilization by broilers (Nelson, 1980; van der Klis and Versteegh, 1996). Based on a lower percentage phytate hydrolysis, with increased dietary Ca, Wise et al. (1983) suggested that the molar ratio of Ca:phytate in diets was one of the main factors determining the fate of phytate P.

At a single level of dietary Ca, diets containing LP SBM in the present study would have had a molar ratio of Ca:phytate P in the intestine over two-fold higher than

diets containing the Commercial or HP Prolina SBM. However, this increase in the Ca:phytate ratio did not affect the mean percentage phytate P disappearance by the time digesta had reached the distal ileum. However, in agreement with previous findings, a high dietary Ca concentration linearly decreased the percentage disappearance of phytate P by the distal ileum which, in turn, would have contributed to the decrease from 64.32 to 50.50% in the apparent prececal absorption of P at the highest level of dietary Ca (Nelson and Walker, 1963; van der Klis and Versteegh, 1996; Tamin et al., 2004). Therefore, these data suggested that the percentage dietary phytate hydrolyzed in the small intestine may be affected to a greater extent by the concentration of Ca added to diets as $CaCO_3$ rather than by the ratio of Ca:phytate per se.

***Effects of Ca:NPP Ratio and Phytate P Concentration on Overall P Retention***

It has been well established that the kidney plays an important role in the regulation of Ca and P resorption (Al-Masri, 1995). Therefore, while low levels of dietary Ca consistently increased prececal phytate hydrolysis, apparent P digestibility, and P absorption/kg DMI, overall P retention responded positively to increasing dietary Ca concentrations and reached a plateau after which no further improvements occurred. This was consistent with previous observations by van der Klis and Versteegh (1996), who showed that while increased dietary Ca concentration reduced the percentage P absorption, the overall retention of P increased as dietary Ca increased. Segmented regression analyses estimated the optimum Ca concentration at which P retention was maximized to be 0.74% Ca when diets contained Commercial SBM (0.308% NPP) or LP SBM (0.317% NPP). Therefore, when diets contained similar levels of NPP (Commercial and LP SBM), the dietary Ca level at which P retention was maximized was not affected by the large differences in the dietary phytate content. Maximum P retention was reached

in the HP Prolina SBM diet at 0.996% dietary Ca but mean analyzed NPP levels of these diets were also higher (0.395% NPP). Therefore, the Ca:NPP ratio at which P retention was maximized was approximately 2.53:1 for the HP Prolina SBM diets, 2.40:1 for the Commercial SBM diets, and 2.34:1 for the LP SBM diets.

The Ca:NPP ratio for maximum P retention, and minimal P excretion estimated in the present study was similar to the optimal Ca:AvP ratio of 2.2:1 and 2.3:1 estimated by van der Klis and Versteegh (1996) for LP and HP diets, respectively. Also, a Ca:NPP ratio of 2.22:1 can be calculated from the NRC (1994) estimate of the Ca and NPP requirements for 0-3-wk-old broilers of 1.00% and 0.45%, respectively. While the present data only showed small effects of dietary phytate concentration Ca absorption and retention, Nelson et al. (1968b) suggested that the Ca requirement at which maximum bone ash was obtained in 3-wk-old chicks was increased by at least 50% when dietary phytate levels were increased from 0.0% to 1.25%. The increased Ca requirement demonstrated in the study by Nelson et al. (1968b) was hypothesized to be caused by increased binding of Ca in the intestine, as 1% phytate was found to be able to bind 0.36% Ca.

Diets in the present study contained analyzed phytate concentrations of 0.975, 0.849, and 0.357% for diets containing HP Prolina, Commercial, or LP SBM, respectively. However, the large range in phytate had no effect on apparent Ca absorption. Therefore, the ratio of absorbed Ca:P was similar between sources of SBM, which may explain why the dietary Ca:NPP at which maximum P retention occurred only varied from 2.34 to 2.57:1 for the LP and HP SBM diets, respectively, in spite of a 2.7 fold difference in the dietary phytate concentration.

***Effects of Ca and SBM source on Total P and Soluble P in Excreta***

In the present study the improved P retention and reduced P output per kg DMI was also reflected in a reduction in total P excreted of 42% and 49% when LP SBM replaced Commercial SBM at the same dietary NPP in diets in Collection 1 and 2, respectively. This was comparable to previous estimates by Jang et al. (2003) and Penn et al. (2004) that had examined the impact of reduced dietary phytate on P excretion by including LP corn in diets of broilers and turkeys and found reductions in manure total P of 33% and 40%, respectively. While the difference between HP Prolina and LP SBM in excreta total P of 56% and 63% in Collection 1 and 2 in the present study was even higher, this could not be attributed to differences in phytate content of the diets alone. The HP Prolina diets had a higher determined NPP compared to the Commercial and LP SBM diets that was previously shown to increase total P in litter from broilers (Chapter 2) and was also supported by previous work that showed consistent reductions in litter total P when broilers or turkeys were fed diets that contained lower levels of NPP (Applegate et al., 2003; Maguire et al., 2004; McGrath et al., 2005).

The inclusion of LP SBM in diets in favor of Commercial SBM also resulted in a 46% and 48% reduction in WSP in excreta from Collection 1 and 2, respectively. This supported previous findings of a reduction in litter WSP of 48% in turkeys (Penn et al., 2004) and 33% in broiler litter (Smith et al., 2005) when diets with reduced phytate content by including LP corn were fed. As dietary NPP was also shown to affect the WSP of litter (Chapter 2), the higher NPP level of the HP Prolina SBM diets most likely contributed to the increase in WSP in the excreta of HP Prolina SBM compared to either Commercial or LP SBM diets.

The linear reduction in litter WSP and the WSP:total P ratio that was found in the present study when dietary Ca was increased at a constant level of NPP during both

collection periods supported the findings in Chapter 2 that both WSP and the WSP:total P ratio in excreta from broilers was reduced as the dietary Ca:NPP ratio increased. However, the significant Ca x SBM source interaction in the present study suggested that the relationship between dietary Ca and either WSP or the WSP:total P ratio was dependent on the dietary phytate concentration. At higher dietary phytate concentrations (HP Prolina or Commercial SBM) the slope of the response in both WSP and WSP:total P to increasing dietary Ca (higher Ca:NPP ratio) was greater than when diets contained reduced phytate concentrations from inclusion of LP SBM (Table 3.8, Figures 3.4 and 3.5). The dietary adaptation period also affected the response in excreta WSP:total P to dietary Ca as there was no difference in the slope of the response to Ca between SBM in Collection 1. However, after a 3-d adaptation period there was a greater effect of increased Ca on WSP:total P when diets contained more phytate (Table 3.8, Figure 3.5).

The effects of Ca on WSP were attributed to a combination of several factors. At the lowest Ca level of 0.47% more P was retained when diets contained LP SBM versus Commercial SBM and the NPP level was similar (Figure 3.3 a,b). However, there was no difference in the amount of P absorbed per kg DMI between LP and Commercial SBM diets. Therefore, while similar amounts of P were absorbed at the lowest dietary Ca, less of the absorbed P was retained by birds fed the Commercial SBM diets. The difference between P absorbed and retained would have resulted in a higher urinary P excretion in Commercial SBM diets that contributed to the higher excreta P output and higher WSP in the excreta. As dietary Ca was increased from 0.47% to 1.16% there was a reduction in phytate P disappearance from the distal ileum (Fig 2 a,b) that contributed to the reduced prececal P absorption and also increased the proportion of phytate P in the excreta (Table 3.7). In the present study there was a strong negative correlation between the proportion of phytate in the excreta and WSP, particularly when birds were allowed a dietary

adaptation, as in Collection 2 (r=-0.70 to 0.90, $P < 0.002$). Previous workers have shown that P compounds extracted in water were predominantly inorganic P and that the majority of phytate P was only extracted when solutions contained HCl or NaOH (Turner and Leytem, 2004). Therefore, the progressive reduction in WSP as dietary Ca was increased could be explained by the corresponding increase in the ratio of phytate P:total P in the litter that was the result of an increase in the amount of absorbed P that was retained, as well as a linear reduction in phytate hydrolysis. This confirmed previous observations in Chapter 2, while the effect of Ca on WSP in the present study was further dependent on the phytate content of the diet. There was a 56% reduction in WSP when LP SBM replaced Commercial SBM and the dietary Ca was 0.47%. However, the benefit of LP SBM versus Commercial SBM on reduced WSP was only 26% when diets contained 1.16% Ca.

In conclusion, the present study demonstrated that the ratio of Ca:NPP at which total P excretion was minimized ranged from 2.3:1 to 2.5:1 when diets contained a wide range of phytate P from 0.10 to 0.28%. However, further research may be needed to ascertain the effects of such a wide Ca:P ratio on long-term broiler performance and economic returns. At the optimum Ca:NPP ratio, the inclusion of LP SBM in diets was able to reduce total P excretion to 1.70 g $kg^{-1}$ DMI, a reduction of 49% compared to broilers fed diets that contained Commercial SBM. Furthermore, the WSP of the excreta was dependent on both the phytate content of the diet and the dietary Ca concentration. At the Ca:NPP ratio of 2.3:1 where P output was minimized when diets contained LP SBM, the WSP in excreta was 53% lower compared to diets that had Commercial SBM. Therefore, as LP SBM was able to effectively replace 0.5% monocalcium phosphate without affecting the amount of P absorbed by the distal ileum, its inclusion in broiler diets in place of conventional SBM would result in a considerable cost-saving per ton of

feed, while LP SBM at the optimal Ca:NPP ratio was also able to reduce total P output by 49% and WSP by 53%.

## REFERENCES


Al-Masri, M. R. 1995. Absorption and excretion of phosphorus in growing broiler chicks, as influenced by calcium and phosphorus ratios in feed. Br. J. Nutr. 74:407-415.

Angel, R., N. M. Tamim, T. J. Applegate, A. S. Dhandu, and L. E. Ellestad. 2002. Phytic acid chemistry: influence on phytin-phosphorus availability and phytase efficacy. J. Appl. Poult. Res. 11:471-480.

Applegate, T. J. 2005. The nutritional value of degermed-dehulled corn for broiler chickens and its impact on nutrient excretion. Poult. Sci. 84:742-747.

Applegate, T. J., B. C. Joern, D. L. Nussbaum-Wagler, and R. Angel. 2003. Water-soluble phosphorus in fresh broiler litter is dependent upon phosphorus concentration fed but not on fungal phytase supplementation. Poult. Sci. 82:1024-1029.

Beede, D. 2003. Ration phosphorus management: Requirements and excretion. Pages 145-151 in Proc. Four-State Applied Nutrition and Management Conference, LaCrosse, WI.

Dilger R. N., and O. Adeola. 2006. Estimation of true phosphorus digestibility and endogenous phosphorus loss in growing chickens fed conventional and low-phytate soybean meals. Poult. Sci. 85:661-668.

Driver, J. P., G. M. Pesti, R. I. Bakalli, and H. M. Edwards. 2005. Effects of calcium and non phytate phosphorus concentrations on phytase efficacy in broiler chicks. Poult. Sci. 84:1406-1417.

Hurwitz, S., and A. Bar. 1971. Calcium and phosphorus interrelationships in the intestine of the fowl. J. Nutr. 101:677-686.

Jang, D. A., J. G. Fadel, K. C. Klasing, A. J. Mireles, Jr., R. A. Ernst, K. A. Young, A. Cook, and V. Raboy. 2003. Evaluation of low-phytate corn and barley on broiler chick performance. Poult. Sci. 82:1914-1924.

Kwanyuen, P., and J. W. Burton. 2005. A simple and rapid procedure for phytate determination in soybean and soybean products. J. Am. Oil Chemists' Soc. 82:81-85.

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

Leytem, A. B., and R. O. Maguire. 2006. Environmental implications of inositol phosphates in animal manures. Pages 211 to 235 in Inositol Phosphates: Linking Agriculture and the Environment. B. L. Turner, A. E Richardson, and E. J. Mullaney ed. CAB International, Wallingford, UK.

Li, Y. C., D. R. Ledoux, T. L. Veum, V. Raboy, and D. S. Ertl. 2000. Effects of low phytic acid corn on phosphorus utilization, performance, and bone mineralization in broiler chicks. Poult. Sci. 79:1444-1450.

Maguire, R. O., J. T. Sims, W. W. Saylor, B. L. Turner, R. Angel, and T. J. Applegate. 2004. Influence of phytase addition to poultry diets on phosphorus forms and solubility in litters and amended soils. J. Environ. Qual. 33:2306-2316.

Maguire, R. O., Z. Dou, J. T. Sims, J. Brake, and B. C. Joern. 2005. Dietary strategies for reduced phosphorus excretion and improved water quality. J. Environ. Qual. 34:2093-2103.

McGrath, J. M., J. T. Sims, R. O. Maguire, W. W. Saylor, R. Angel, and B. L. Turner. 2005. Broiler diet modification and litter storage: Impacts on phosphorus in litters, soils, and runoff. J. Environ. Qual. 34:1896-1909.

Moeser, A. J., I. B. Kim, E. van Heugten, and T. A. T. G. Van Kempen. 2002. The nutritional value of degermed, dehulled corn for pigs and its impact on the gastrointestinal tract and nutrient excretion. J. Anim. Sci. 80:2629–2638.

National Research Council (NRC). 1994. Nutrient requirements for poultry. 9th revised ed., National Academy Press, Washington, DC.

Nelson, T. S., L. W. Ferrara, and N. L. Storer. 1968a. Phytate phosphorus content of feed ingredients derived from plants. Poult. Sci. 46:1372-1376.

Nelson, T. J., J. J. McGillivray, T. R. Shieh, R. J. Wodzinski, and J. H. Ware. 1968b. Effect of phytate on the calcium requirement of chicks. Poult. Sci. 47:1985-1989.

Nelson, T. S. 1980. Phosphorus availability in plant origin feedstuffs for poultry and swine. Pages 59-84 in Proc. Third International Minerals Conference, Orlando, Fl.

Nelson, T. S., and A. C. Walker. 1963. The biological evaluation of phosphorus compounds. Poult. Sci. 42:95-98.

Penn, C. J., G. L. Mullins, L. W. Zelazny, J. G. Warren, and J. M. McGrath. 2004. Surface runoff losses of phosphorus from Virginia soils amended with turkey manure using phytase and high available phosphorus corn diets. J. Environ. Qual. 33:1431-1439.

Plumstead, P. W., B. A. Lenfestey, H. Romero-Sanchez, J. Brake, and M. R. Bedford. 2004. Comparative efficacy of two thermo-tolerant microbial phytases. 2. Broiler livability and skeletal development. Poult. Sci. 83 (Suppl. 1):1789.

Qian, H., E. T. Kornegay, and D. M. Denbow. 1997. Utilization of phytate phosphorus and calcium as influenced by microbial phytase, cholecalciferol, and the calcium:total phosphorus ratio in broiler diets. Poult. Sci. 76:37-46.

Raboy, V. 2002. Progress in breeding low phytate crops. J. Nutr. 132:503-505.

Raboy, V., D. B. Dickinson, and F. E. Below. 1984. Variation in seed total phosphorus, phytic acid, zinc and calcium, magnesium, and protein among lines of Glycine max and G. soya. Crop Sci. 24:431-434.

Rutherfurd, S. M., T. K. Chung, and P. J. Moughan. 2002. The effect of microbial phytase on ileal phosphorus and amino acid digestibility in the broiler chicken. Br. Poult. Sci. 44:598-606.

Sands, J. S., D. Ragland, J. R. Wilcox, and O. Adeola. 2003. Relative bioavailability of phosphorus in low phytate soybean meal for broiler chicks. Can. J. Anim. Sci. 83:95-100.

SAS Institute. 2004. SAS/STAT User's Guide. Release 9.1. SAS Institute, Inc., Cary, NC.

Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Tech. 59:215-221.

Smith, D. R., P. A. Moore, and D. M. Miles. 2005. Soil extractable phosphorus changes with time after application of fertilizer. I. Litter from poultry fed modified diets. Soil Sci. 170:530-542.

Tamin N. M., and R. Angel. 2003. Phytate phosphorus hydrolysis as influenced by dietary calcium and micro mineral source in broiler diets. J. Agric. Food Chem. 5:4687-4693.

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

Thacker, P. A., B. G. Rossnagel, and V. Raboy. 2003. Phosphorus digestibility in low-phytate barley fed to finishing pigs. Can. J. Anim. Sci. 83:101-104.

Turner, B. L., and A. B. Leytem. 2004. Phosphorus compounds in sequential extracts of animal manures: chemical speciation and a novel fractionation procedure. Environ. Sci. Technol. 38:6101-6108.

Van der Klis, J. D., and H. A. J. Versteegh. 1996. Phosphorus nutrition of poultry. Pages 71-83 in Recent Developments in Poultry Nutrition. P. C. Garnsworthy, J. Wiseman, and W. Haresign ed. Nottingham University Press, Nottingham, UK.

Waldroup, P. W., C. B. Ammerman, and R. H. Harms. 1963a. The availability of phytic acid phosphorus for chicks. 2. Comparison of phytin phosphorus sources. Poult. Sci. 43:426-432.

Waldroup, P. W., C. B. Ammerman, and R. H. Harms. 1963b. The relationship of phosphorus, calcium, and vitamin $D_3$ in the diet of broiler type chickens. Poult. Sci. 42:982-989.

Wilcox, J. R., G. S. Premachandra, K. A. Young, and V. Raboy. 2000. Isolation of high seed inorganic P, low-phytate soybean mutants. Crop Sci. 40: 1601-1605.

Wise, A. 1983. Dietary factors determining the biological activities of phytate. Nutr. Abstr. Rev. 53:791-806.

**Table 3.1.** Analysed composition of three soybean meals (SBM) with different phytate concentrations[1,2].

| SBM source | CP | Lysine | Total P | IP3[2] | IP4[2] | IP5[2] | IP6[3] | Total IP[2] | Phytate P[4] | Phytate P[4] |
|---|---|---|---|---|---|---|---|---|---|---|
| | (g/kg) | | | | | | | | | (% of total P) |
| HP Prolina | 48.96 | 3.09 | 7.80 | 0.99 | 0.73 | 4.71 | 18.17 | 24.60 | 5.12 | 0.66 |
| Commercial | 50.33 | 3.09 | 6.10 | 1.12 | 0.21 | 2.24 | 16.09 | 19.66 | 4.53 | 0.74 |
| Low Phytate | 50.25 | 3.10 | 6.10 | 1.52 | 0.07 | 0.85 | 5.35 | 7.78 | 1.51 | 0.25 |

[1] Abbreviations: High phytate (HP) Crude protein (CP), phosphorus (P), inositol phosphate esters (IP).
[2] Nutrient compositions based on analyzed nutrient values and standardized to 88% dry matter.
[3] IP esters analyzed by HPLC (Kwanyuen and Burton, 2005).
[4] Phytate P represented the phosphorus content as IP6, calculated as 0.2818 x IP6.

**Table 3.2.** Formulation and calculated analyses of the basal diets.

| Ingredients | Soybean Meal Source in Basal Diet[1] | | |
|---|---|---|---|
| | HP Prolina | Commercial | Low Phytate |
| | (%) | | |
| DGDH corn[2] | 45.80 | 46.74 | 46.65 |
| Soybean meal[1] | 40.06 | 39.32 | 36.44 |
| Monocalcium phosphate | 1.21 | 1.09 | 0.59 |
| Limestone[3] | 0.34 – 2.15 | 0.44 – 2.25 | 0.64 – 2.45 |
| Lysine HCl | 0.00 | 0.04 | 0.04 |
| DL Methionine | 0.27 | 0.28 | 0.25 |
| L Threonine | 0.01 | 0.05 | 0.09 |
| Premixes[4] | 0.55 | 0.55 | 0.55 |
| Sodium chloride | 0.50 | 0.50 | 0.50 |
| $TiO_2$ marker | 0.40 | 0.40 | 0.40 |
| Celite marker | 2.00 | 2.00 | 2.00 |
| Soybean oil | 6.20 | 6.20 | 6.20 |
| Filler (washed sand)[3] | 0.10 - 1.91 | 0.43 - 2.24 | 0.76 - 2.56 |
| Water[5] | 0.74 | 0.16 | 3.09 |
| Calculated nutrients[6] | | | |
| Moisture, %[5] | 11.50 | 11.50 | 11.50 |
| ME, kcal/g | 3.06 | 3.06 | 3.06 |
| Crude protein, % | 230.0 | 230.0 | 230.0 |
| Lysine, % | 1.33 | 1.33 | 1.33 |
| Methionine + Cysteine, % | 0.97 | 0.97 | 0.97 |
| Threonine, % | 0.88 | 0.88 | 0.88 |
| Arginine, % | 1.63 | 1.55 | 1.50 |
| Calcium, %[3] | 0.47-1.16 | 0.47-1.16 | 0.47-1.16 |
| Total phosphorus, % | 0.65 | 0.54 | 0.42 |
| NPP, %[7] | 0.35 | 0.35 | 0.35 |
| Calcium: NPP[7] ratio | 1.4 – 3.3 | 1.4 – 3.3 | 1.4 – 3.3 |
| Phytate phosphorus, % | 0.28 | 0.24 | 0.10 |
| Sodium, g/kg | 0.20 | 0.20 | 0.20 |
| DEB, (mEq/kg)[8] | 27.47 | 26.84 | 24.98 |

[1] Three different sources of soybean meal described in Table 3.1 were used in formulation.
[2] Degermed dehulled (DGDH) corn (Moeser et al., 2002).
[3] The inclusion rate of limestone added to the basal diets was varied to provide four different levels of dietary calcium of 0.47%, 0.70%, 0.93%, and 1.16%. Washed sand was used as an inert filler to adjust the volume of each diet formulation to 100%.

**Table 3.2.** Continued.

[4] Premixes provided the following per kg diet: vitamin A, 13,200 IU; vitamin $D_3$, 4,000 IU; vitamin E, 66 IU; vitamin $B_{12}$, 39.6 µg; riboflavin, 13.2 mg; niacin, 110 mg; d-pantothenate, 22 mg; menadione ($K_3$), 4 mg; folic acid, 2.2 mg; thiamine, 4 mg; pyridoxine, 8 mg; d-biotin, 252 µg; selenium (as $Na_2SeO_3$), 0.30 mg; manganese, 120 mg; zinc, 120 mg, iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; cobalt, 1.0 mg; choline chloride 1,200 mg.
[5] The moisture percentage of diets was adjusted to 11.5% with water.
[6] Nutrient compositions calculated from proximate analyses of all ingredients. Final diet composition was confirmed by proximate analyses of all diets (Table 3.3).
[7] Non phytate phosphorus (NPP) calculated as analyzed phosphorus – phosphorus as phytic acid (IP6).
[8] Dietary electrolyte balance (DEB) calculated as mEq (Na + K – Cl).

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**Table 3.3.** Calculated and determined nutrient analyses[1] of dietary treatments.

| | | Soybean Meal Source | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | HP Prolina | | | | Commercial | | | | Low Phytate | | | |
| Nutrients[1] | Diet | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| | | (%) | | | | | | | | | | | |
| CP, calculated | | 23.00 | 23.00 | 23.00 | 23.00 | 23.00 | 23.00 | 23.00 | 23.00 | 23.00 | 23.00 | 23.00 | 23.00 |
| CP, determined | | 23.52 | 23.86 | 22.77 | 23.27 | 23.55 | 23.47 | 23.32 | 22.78 | 24.82 | 23.56 | 22.11 | 23.14 |
| Lys, calculated | | 1.33 | 1.33 | 1.33 | 1.33 | 1.33 | 1.33 | 1.33 | 1.33 | 1.33 | 1.33 | 1.33 | 1.33 |
| Lys, determined | | 1.33 | 1.33 | 1.28 | 1.33 | 1.35 | 1.37 | 1.41 | 1.33 | 1.37 | 1.30 | 1.25 | 1.30 |
| Met + Cys, calculated | | 0.97 | 0.97 | 0.97 | 0.97 | 0.97 | 0.97 | 0.97 | 0.97 | 0.97 | 0.97 | 0.97 | 0.97 |
| Met + Cys, determined | | 0.99 | 0.97 | 0.97 | 0.98 | 0.99 | 0.99 | 1.00 | 1.00 | 0.99 | 0.92 | 0.92 | 0.91 |
| Ca, calculated | | 0.47 | 0.70 | 0.93 | 1.16 | 0.47 | 0.70 | 0.93 | 1.16 | 0.47 | 0.70 | 0.93 | 1.16 |
| Ca, determined | | 0.51 | 0.79 | 0.92 | 1.13 | 0.51 | 0.65 | 0.75 | 1.05 | 0.55 | 0.74 | 1.30 | 1.40 |
| Total P calculated | | 0.65 | 0.65 | 0.65 | 0.65 | 0.54 | 0.54 | 0.54 | 0.54 | 0.42 | 0.42 | 0.42 | 0.42 |
| Total P, determined | | 0.66 | 0.66 | 0.67 | 0.69 | 0.57 | 0.56 | 0.55 | 0.51 | 0.44 | 0.42 | 0.41 | 0.40 |
| Total IP Esters, determined | | 1.17 | 1.22 | 1.10 | 1.12 | 0.90 | 0.92 | 0.92 | 0.90 | 0.42 | 0.41 | 0.40 | 0.44 |
| Phytate P, determined[2] | | 0.28 | 0.29 | 0.26 | 0.27 | 0.24 | 0.24 | 0.24 | 0.23 | 0.10 | 0.10 | 0.10 | 0.11 |
| Non Phytate P, calculated[3] | | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 |
| Non Phytate P, determined[3] | | 0.38 | 0.37 | 0.41 | 0.42 | 0.33 | 0.32 | 0.31 | 0.28 | 0.34 | 0.32 | 0.31 | 0.29 |
| Ca:NPP ratio, calculated | | 0.14 | 0.20 | 0.27 | 0.33 | 0.14 | 0.20 | 0.27 | 0.33 | 0.14 | 0.20 | 0.27 | 0.33 |
| Ca:NPP ratio, determined | | 0.13 | 0.22 | 0.23 | 0.27 | 0.16 | 0.21 | 0.24 | 0.38 | 0.16 | 0.23 | 0.42 | 0.48 |

[1] Abbreviations: High phytate (HP), Crude protein (CP), lysine (Lys), methionine (Met), cysteine (Cys), calcium (Ca), phosphorus (P), inositol phosphate (IP), non phytate P (NPP).
[2] Calculated as the concentration of phytic acid x 0.2818.
[3] Defined as the difference between total P and phytate P.

**Table 3.4.** Main effects of source of soybean meal (SBM) and dietary calcium level on apparent prececal digestibility, absorption, and output of phosphorus, calcium, and phytate P at the distal Ileum.

| | Apparent Prececal Digestibility | | | Apparent Prececal Absorption[1] | | | Output at the Distal Ileum[1] | | |
|---|---|---|---|---|---|---|---|---|---|
| Main Effect | Phosphorus | Calcium | Phytate P | Phosphorus | Calcium | Phytate P | Phosphorus | Calcium | Phytate P |
| SBM | (%) | | | (g/kg DMI) | | | | | |
| HP Prolina | 51.69 [b] | 39.62 | 11.43 | 3.77 [a] | 3.56 | 0.36 [a] | 3.52 [b] | 5.58 | 2.72 [a] |
| Commercial | 52.54 [b] | 40.68 | 13.32 | 3.14 [b] | 3.82 | 0.35 [a] | 2.84 [b] | 5.20 | 2.29 [b] |
| Low Phytate | 65.44 [a] | 42.96 | 12.33 | 3.05 [b] | 3.53 | 0.14 [b] | 1.61 [c] | 5.50 | 0.97 [c] |
| SEM | 1.15 | 3.24 | 3.49 | 0.07 | 0.34 | 0.08 | 0.07 | 0.34 | 0.08 |
| Diet Calcium, % | | | | | | | | | |
| 0.47 | 64.32 [a] | 46.76 | 20.15 [a] | 3.78 [a] | 2.44 [c] | 0.47 [a] | 2.19 [c] | 2.78 [d] | 1.84 [b] |
| 0.70 | 57.85 [b] | 40.59 | 15.09 [ab] | 3.40 [b] | 3.16 [bc] | 0.37 [ab] | 2.57 [b] | 4.62 [c] | 1.97 [ab] |
| 0.93 | 53.55 [c] | 38.41 | 8.61 [b] | 3.14 [c] | 3.97 [ab] | 0.19 [bc] | 2.84 [a] | 6.37 [b] | 2.03 [ab] |
| 1.16 | 50.50 [c] | 38.58 | 5.87 [b] | 2.95 [c] | 4.98 [a] | 0.10 [c] | 3.02 [b] | 7.92 [a] | 2.14 [a] |
| SEM | 1.24 | 3.50 | 3.75 | 0.08 | 0.37 | 0.08 | 0.08 | 0.37 | 0.08 |
| Source of Variation | (Probability > F) | | | | | | | | |
| SBM | <0.001 | 0.571 | 0.854 | <0.001 | 0.803 | 0.002 | <0.001 | 0.803 | <0.001 |
| Calcium | <0.001 | 0.101 | 0.003 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| SBM*Calcium | 0.800 | 0.571 | 0.888 | 0.009 | 0.696 | 0.034 | 0.009 | 0.711 | 0.421 |
| Calcium (Lin) | <0.001 | 0.029 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Calcium (Quad) | 0.069 | 0.232 | 0.713 | 0.067 | 0.630 | 0.975 | 0.067 | 0.630 | 0.787 |

[a-c] Means within the same column with no common superscript differ significantly ($P \leq 0.05$).
[1] Expressed in g / kg of dry matter intake (DMI).

**Table 3.5.** Main effects of source of soybean meal (SBM) and dietary calcium level on retention or output of phosphorus, calcium, and phytate P in the excreta after a 24 h (Collection 1) or 72 h (Collection 2) adaptation period.

| | Collection 1 | | Collection 2 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Percentage retention | | Percentage retention | | Retention per kg DMI[1] | | Excretion per kg DMI[1] | | |
| Main Effect | Phosphorus | Calcium | Phosphorus | Calcium | Phosphorus | Calcium | Phosphorus | Calcium | Phytate P |
| SBM | (%) | | | | (g/kg DMI) | | | | |
| HP Prolina | 32.01 [c] | 47.87 [b] | 38.95 [c] | 38.36 [b] | 2.84 [b] | 3.35 [b] | 4.45 [a] | 5.78 [a] | 2.16 [a] |
| Commercial | 39.37 [b] | 47.10 [b] | 46.89 [b] | 39.83 [b] | 2.80 [b] | 3.39 [b] | 3.17 [b] | 5.62 [b] | 1.69 [b] |
| Low Phytate | 53.78 [a] | 59.40 [a] | 63.37 [a] | 50.22 [a] | 2.95 [a] | 4.35 [a] | 1.70 [c] | 4.67 [b] | 0.56 [c] |
| SEM | 0.80 | 0.81 | 0.49 | 1.25 | 0.03 | 0.12 | 0.03 | 0.12 | 0.065 |
| Diet Calcium, % | | | | | | | | | |
| 0.47 | 37.08 [c] | 48.80 [b] | 43.40 [c] | 50.80 [a] | 2.48 [c] | 2.65 [c] | 3.49 [a] | 2.57 [d] | 1.21 [b] |
| 0.70 | 41.83 [b] | 53.26 [a] | 49.42 [b] | 45.29 [b] | 2.83 [b] | 3.52 [b] | 3.15 [b] | 4.26 [c] | 1.52 [a] |
| 0.93 | 42.80 [ab] | 52.95 [a] | 52.96 [a] | 41.32 [b] | 3.07 [a] | 4.27 [a] | 2.91 [c] | 6.06 [b] | 1.62 [a] |
| 1.16 | 45.16 [a] | 50.80 [ab] | 53.17 [a] | 33.81 [c] | 3.08 [a] | 4.36 [a] | 2.89 [c] | 8.54 [a] | 1.54 [a] |
| SEM | 0.88 | 0.92 | 0.57 | 1.42 | 0.03 | 0.14 | 0.03 | 0.14 | 0.070 |
| Source of Variation | Probability > *F* | | | | | | | | |
| SBM | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.799 | <0.001 | 0.799 | <0.001 |
| Calcium | <0.001 | 0.003 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| SBM*Calcium | 0.363 | 0.004 | <0.001 | 0.215 | <0.001 | 0.126 | <0.001 | 0.118 | 0.483 |
| Calcium (Lin) | <0.001 | 0.159 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Calcium (Quad) | 0.100 | <0.001 | <0.001 | 0.294 | <0.001 | 0.007 | <0.001 | 0.007 | <0.001 |

[a-c] Means within the same column with no common superscript differ significantly ($P \leq 0.05$).

[1] Expressed in g / kg of dry matter intake (DMI).

**Table 3.6.** Select phosphorus analysis of broiler manure from Collection 1 for the high phytate (HP) Prolina, Commercial, and Low Phytate soybean meal (SBM) based diets.

| | Treatment Nutrient Values | | Manure Phosphorus Characteristics | | | | |
|---|---|---|---|---|---|---|---|
| Diet | SBM | Ca[1] | Total P[2] | WSP[3] | WSP:total P[4] | Phytate | Phytate:total P[5] |
| | | (%) | (g kg$^{-1}$) | | (Ratio) | (g kg$^{-1}$) | (Ratio) |
| 1 | HP Prolina | 0.47 | 14.87 | 13.29 | 0.89 | 5.94 | 0.40 |
| 2 | HP Prolina | 0.70 | 14.42 | 12.94 | 0.90 | 6.69 | 0.46 |
| 3 | HP Prolina | 0.93 | 14.5 | 11.57 | 0.80 | 5.67 | 0.39 |
| 4 | HP Prolina | 1.16 | 13.79 | 10.45 | 0.76 | 7.27 | 0.53 |
| 5 | Commercial | 0.47 | 11.54 | 9.98 | 0.87 | 5.33 | 0.46 |
| 6 | Commercial | 0.70 | 10.54 | 8.60 | 0.81 | 5.57 | 0.53 |
| 7 | Commercial | 0.93 | 10.79 | 7.78 | 0.72 | 6.26 | 0.58 |
| 8 | Commercial | 1.16 | 10.76 | 6.50 | 0.60 | 6.37 | 0.59 |
| 9 | Low Phytate | 0.47 | 6.47 | 5.29 | 0.82 | 1.18 | 0.18 |
| 10 | Low Phytate | 0.70 | 6.25 | 4.59 | 0.73 | 1.67 | 0.27 |
| 11 | Low Phytate | 0.93 | 6.32 | 4.32 | 0.68 | 1.84 | 0.29 |
| 12 | Low Phytate | 1.16 | 6.11 | 3.66 | 0.60 | 2.16 | 0.35 |
| | SEM | | 0.16 | 0.15 | 0.01 | 0.23 | 0.01 |
| | Source of Variation | | (Probability > *F*) | | | | |
| | SBM | | <0.0001 | <0.0001 | 0.16 | <0.0001 | <0.0001 |
| | Ca | | 0.0002 | <0.0001 | <0.0001 | 0.002 | <0.0001 |
| | SBM*Ca | | 0.22 | 0.004 | 0.03 | 0.93 | 0.44 |

[1]Ca = the calcium content of the formulated diets.
[2]P = Phosphorus.
[3]WSP = water soluble phosphorus.
[4]WSP:total P = the ratio of water soluble phosphorus to total phosphorus.
[5]Phytate:total P = the ratio of phytate phosphorus to total phosphorus.

**Table 3.7.** Select phosphorus analysis of broiler manure from Collection 2 for the high phytate (HP) Prolina, Commercial, and Low Phytate soybean meal (SBM) based diets.

| | Treatment Nutrient Values | | Manure Phosphorus Characteristics | | | | |
|---|---|---|---|---|---|---|---|
| Diet | SBM | Ca[1] | Total P[2] | WSP[3] | WSP:total P[4] | Phytate | Phytate:Total P[5] |
| | | (%) | (g kg$^{-1}$) | | (Ratio) | (g kg$^{-1}$) | (Ratio) |
| 1 | HP Prolina | 0.47 | 13.90 | 13.00 | 0.94 | 5.07 | 0.36 |
| 2 | HP Prolina | 0.70 | 14.01 | 10.73 | 0.77 | 6.88 | 0.49 |
| 3 | HP Prolina | 0.93 | 13.40 | 7.21 | 0.54 | 7.34 | 0.55 |
| 4 | HP Prolina | 1.16 | 13.10 | 5.73 | 0.44 | 7.36 | 0.56 |
| 5 | Commercial | 0.47 | 10.59 | 9.44 | 0.89 | 4.42 | 0.42 |
| 6 | Commercial | 0.70 | 9.72 | 6.68 | 0.69 | 5.33 | 0.54 |
| 7 | Commercial | 0.93 | 9.74 | 4.32 | 0.44 | 6.05 | 0.62 |
| 8 | Commercial | 1.16 | 9.72 | 3.28 | 0.34 | 5.59 | 0.57 |
| 9 | Low Phytate | 0.47 | 5.38 | 4.18 | 0.78 | 0.92 | 0.17 |
| 10 | Low Phytate | 0.70 | 4.87 | 3.06 | 0.63 | 1.62 | 0.33 |
| 11 | Low Phytate | 0.93 | 4.96 | 2.64 | 0.53 | 2.28 | 0.47 |
| 12 | Low Phytate | 1.16 | 5.08 | 2.44 | 0.48 | 1.94 | 0.38 |
| | SEM | | 0.10 | 0.23 | 0.02 | 0.21 | 0.03 |
| | Source of Variation | | (Probability > *F*) | | | | |
| | SBM | | 0.0002 | <0.0001 | 0.0003 | <0.0001 | 0.04 |
| | Ca | | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| | SBM x Ca | | 0.19 | <0.0001 | 0.0003 | 0.19 | 0.77 |

[1]Ca = the calcium content of the formulated diets.
[2]P = Phosphorus.
[3]WSP = water soluble phosphorus.
[4]WSP:total P = the ratio of water soluble phosphorus to total phosphorus.
[5]Phytate:total P = the ratio of phytate phosphorus to total phosphorus.

**Table 3.8.** Regression parameters for the relationship between water soluble P (WSP; y, g $kg^{-1}$) vs. dietary Ca (x, %) and the water soluble P to total P ratio (WSP:TP; y) versus dietary Ca (x, %) for the high phytate (HP) Prolina, Commercial, or Low Phytate soybean meal (SBM) at Collection 1 and 2.

| SBM | Intercept | Slope | $r^2$ |
|---|---|---|---|
| **Collection 1** | | | |
| **WSP** | | | |
| HP Prolina | 15.56 ± 0.61[a] | -4.29 ± 0.72[a] | 0.70*** |
| Commercial | 12.21 ± 0.50[b] | -4.90 ± 0.58[a] | 0.82*** |
| Low Phytate | 6.29 ± 0.22[c] | -2.25 ± 0.26[b] | 0.84*** |
| **WSP:total P** | | | |
| HP Prolina | 1.02 ± 0.05[a] | -0.22 ± 0.05[b] | 0.52** |
| Commercial | 1.06 ± 0.03[a] | -0.38 ± 0.038[a] | 0.87*** |
| Low Phytate | 0.96 ± -0.03[a] | -0.31 ± 0.04[ab] | 0.84*** |
| **Collection 2** | | | |
| **WSP** | | | |
| HP Prolina | 18.14 ± 1.02[a] | -11.01 ± 1.19[a] | 0.85*** |
| Commercial | 13.32 ± 0.57[b] | -9.06 ± 0.67[a] | 0.92*** |
| Low Phytate | 5.08 ± 0.36[c] | -2.45 ± 0.42[b] | 0.69*** |
| **WSP:total P** | | | |
| HP Prolina | 1.28 ± 0.08[a] | -0.74 ± 0.09[a] | 0.83*** |
| Commercial | 1.26 ± -0.83[a] | -0.82 ± 0.05[a] | 0.94*** |
| Low Phytate | 0.95 ± 0.06[b] | -0.42 ± 0.07[b] | 0.74*** |

[a-c] Means within a treatment and column lacking a common superscript differ significantly ($P < 0.05$).
**, *** denotes model significance at the $P < 0.01$ and $P < 0.001$ levels, respectively.

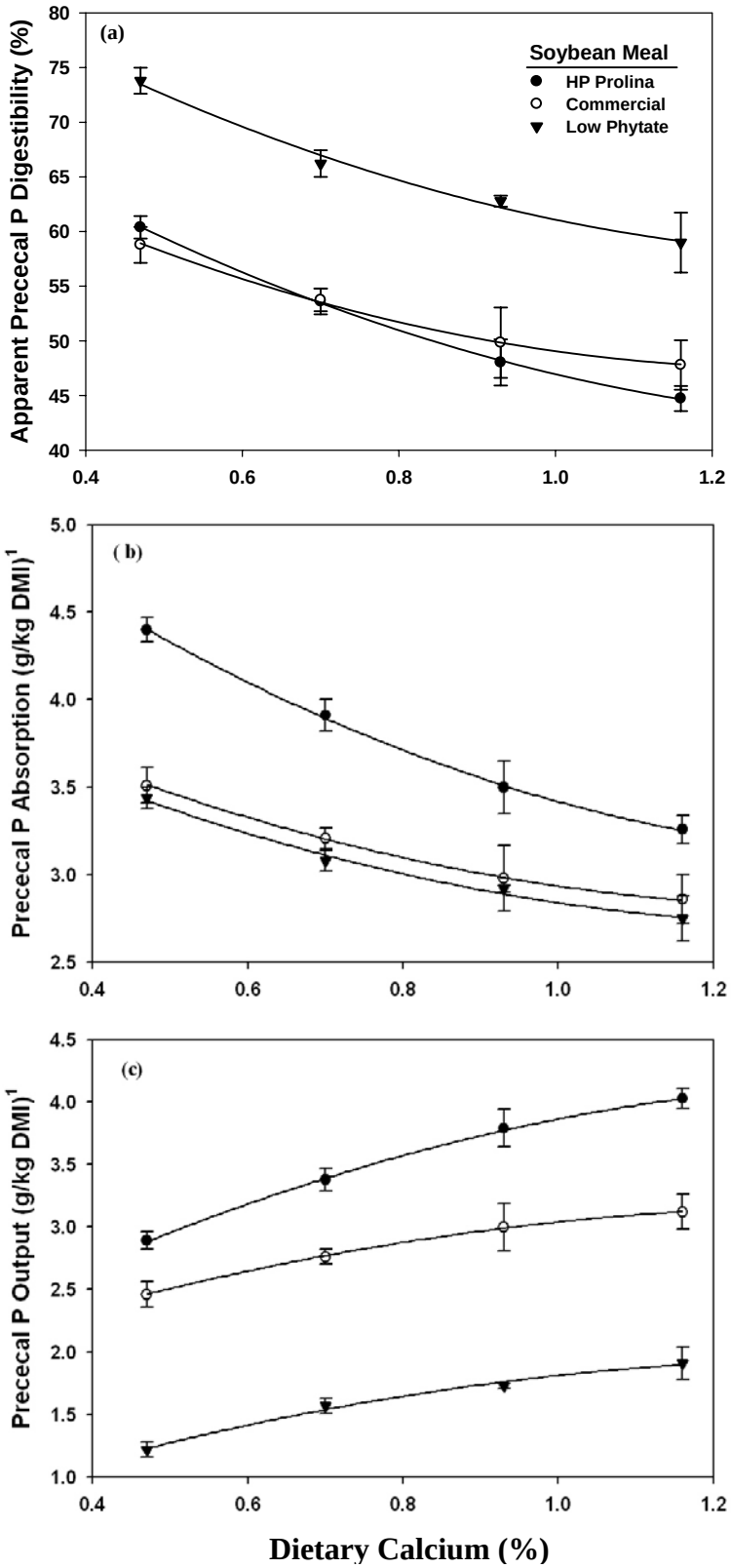


**Figure 3.1 (a-c).** Effects of source of soybean meal with different phytate concentrations and dietary calcium level on apparent prececal digestibility (a), absorption (b), and output (c) of phosphorus (P) at the distal ileum.

[1] Expressed in g / kg of dry matter intake (DMI).

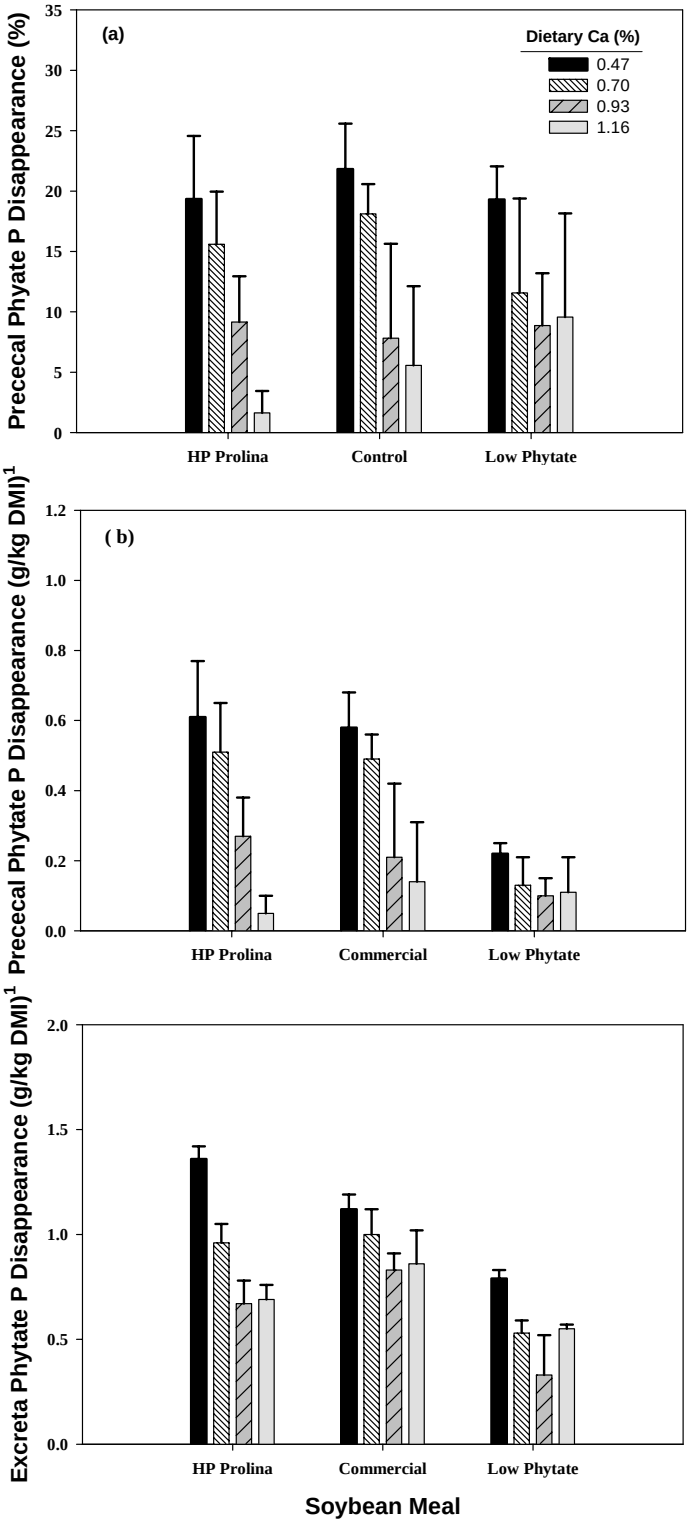


**Figure 3.2 (a-c)** Effects of source of soybean meal with different phytate concentrations and dietary calcium on apparent prececal phytate phosphorus (P) digestibility and disappearance, and total disappearance of phytate P in the excreta from Collection 2.

[1] Expressed in g/kg of dry matter intake (DMI).

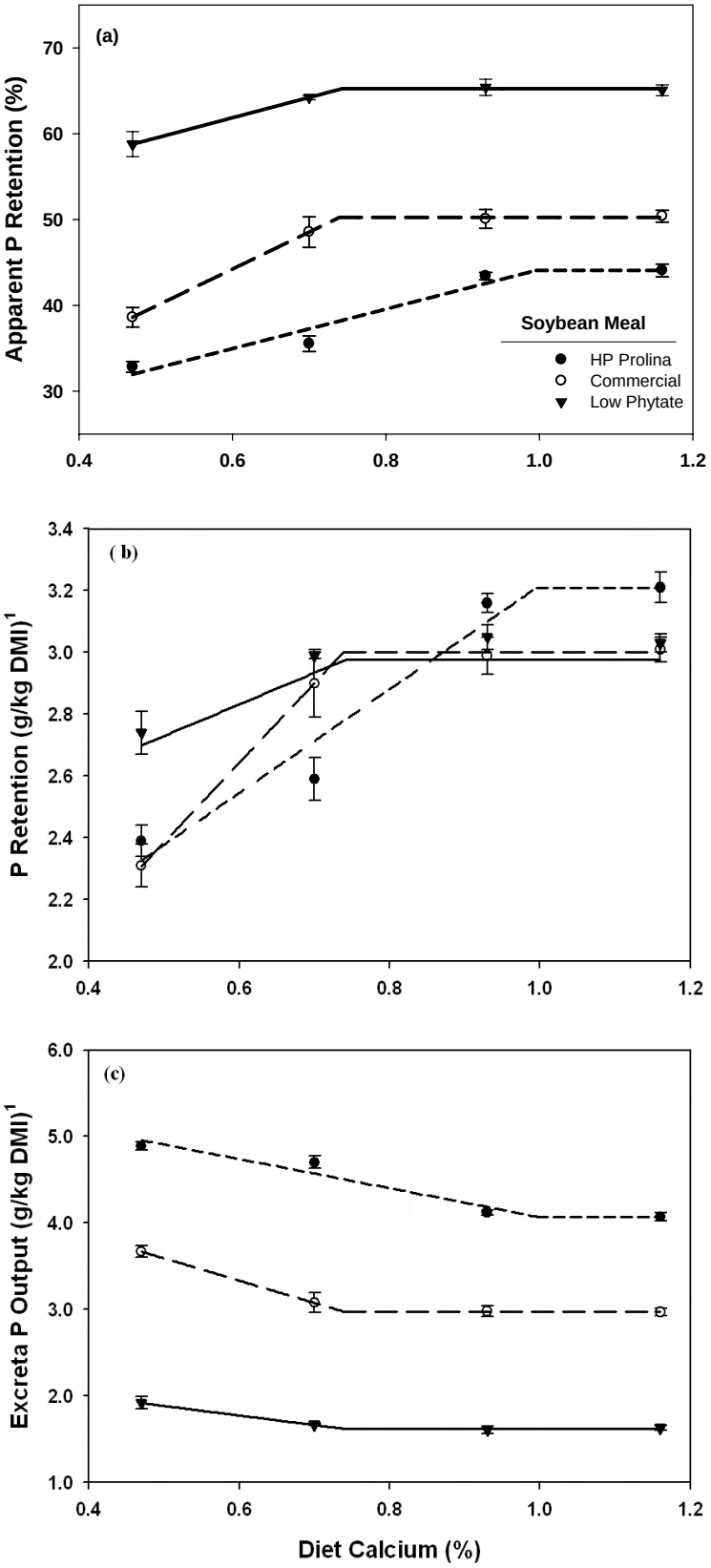


**Figure 3.3 (a-c).** Effects of source of soybean meal with different phytate concentrations and dietary calcium on retention (a, b) and output (c) of phosphorus (P) in the excreta during Collection 2.

[1] Expressed in g / kg of dry matter intake (DMI).

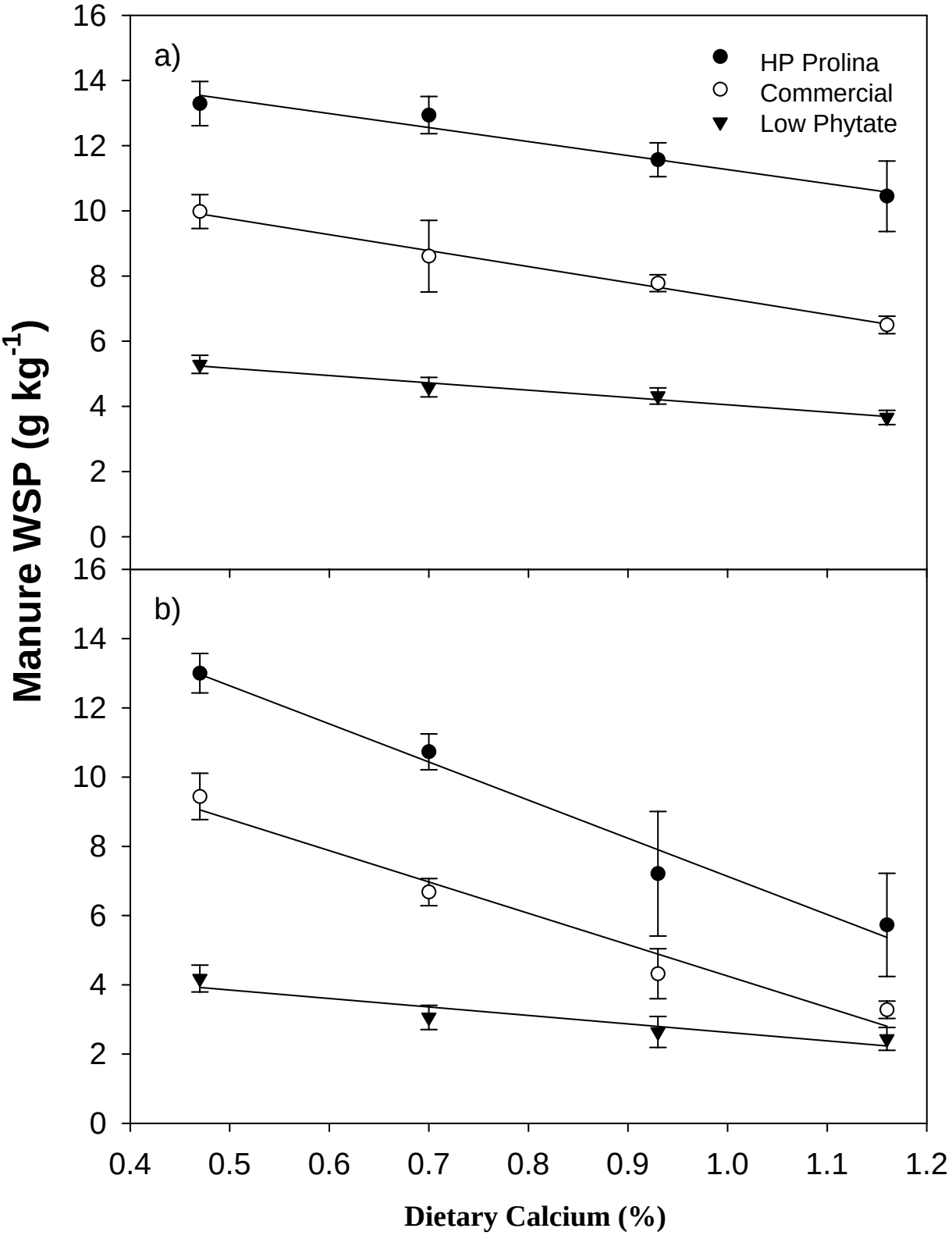


**Figure 3.4 (a,b).** Effects of source of soybean meal with different phytate concentrations and dietary calcium on manure water soluble phosphorus (WSP) for a) Collection 1 (16 d) and b) Collection 2 (20 d).

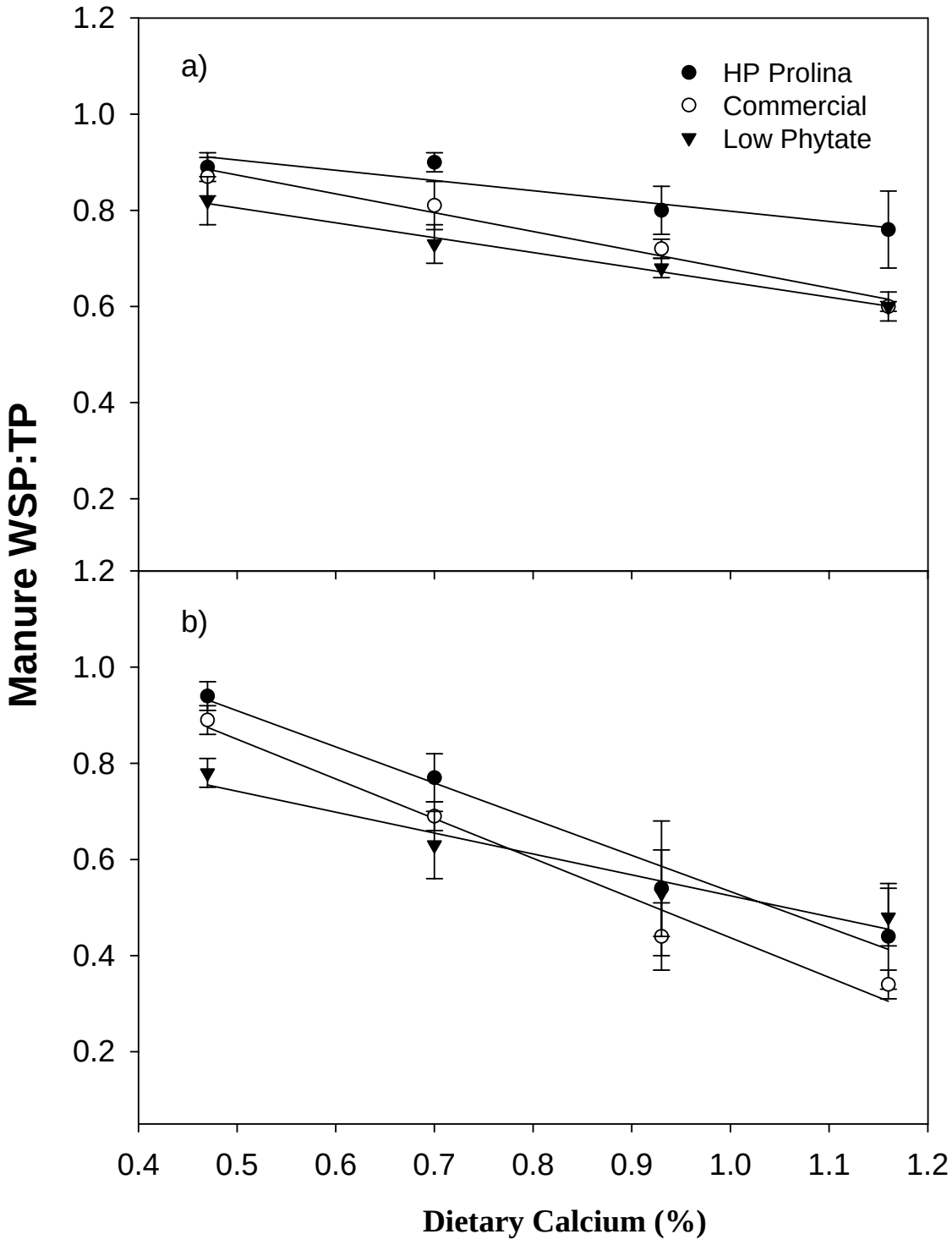


**Figure 3.5 (a,b).** Effects of source of soybean meal with different phytate concentrations and dietary calcium on manure water soluble phosphorus to total phosphorus ratio (WSP:TP) for a) Collection 1 (16 d) and b) Collection 2 (20 d).

# CHAPTER 4

## Interaction of Calcium and Phytate in Broiler Diets. 2. Effects on Apparent Prececal Amino Acid Absorption

**ABSTRACT** Low-phytate (LP) varieties of corn and soybean meal (SBM) have been shown to improve phosphorus (P) utilization and reduce P concentrations in manure from broilers. However, phytate in the diet has also been shown to complex with proteins, amino acids (AA), and di- and tri-valent cations, reducing their availability to animals. The molar ratio of calcium (Ca):phytate in the diet was reported to be the primary factor influencing phytate hydrolysis and the extent whereby phytate reduced the intestinal absorption of other nutrients. Therefore, as the ratio of Ca:phytate could be altered by including LP grains in broiler diets, or by the dietary Ca concentration, this may in-turn also affect the availability of other nutrients such as AA in the diet. To address this, three sources of SBM, namely high-phytate (HP) Prolina, Commercial, and LP SBM were extracted from soybeans that varied in their natural phytate content. Twelve diets were formulated with four levels of dietary Ca ranging from 0.47% to 1.16% and three levels of phytate P ranging from 0.28% in HP Prolina SBM to 0.10% in LP SBM and were fed to Ross 508 broilers from 16 to 21 d of age. Ileal digesta was collected at 21 d of age and samples of feed and digesta were analyzed for Ca, AA, and phytate concentrations and the apparent ileal digestibility coefficients of AA were calculated. No correlation was found between dietary phytate concentration and apparent absorption of AA. However, a significant Ca x SBM source interaction was observed for AA absorption that was not affected by dietary Ca in the HP Prolina SBM diets but was reduced at either low or high dietary Ca in the LP SBM diets. This suggested that the availability of AA in LP SBM diets may be dependent on the dietary Ca concentration.

## INTRODUCTION

Soybean meal (SBM) that contains a high proportion of phosphorus (P) as phytic acid or its salt, phytate, has been typically included as a protein source in most diets fed to poultry. However, phytate has been shown to be not readily digestible in typical corn-soy based diets fed to poultry and a large proportion of phytate P has been found to pass through the small intestine intact (Chapter 3). Undigested phytate entering the duodenum has considerable nutritional implications as at a neutral pH it becomes a strong chelating agent that forms insoluble complexes with tri- and di-valent cations, proteins, free amino acids (AA), and carbohydrates, thereby reducing the availability of these nutrients to poultry (Pallauf and Rimbach, 1997; Lönnerdal et al., 1999). Furthermore, anti-nutritional effects of phytate were also recently shown to be caused by not only reduced dietary nutrient absorption, but also by increased excretion of endogenous nutrients (Cowieson et al., 2004).

The formation of phytate-mineral complexes reduced phytate hydrolysis with the potency of minerals to bind to phytate reported to be in the order of $Zn^{2+} >> Fe^{2+} > Mn^{2+} > Fe^{3+} > Ca^{2+} > Mg^{2+}$ (Maenz et al., 1999). Although Ca ions had a lower affinity to bind to phytate than zinc, iron, or manganese ions, dietary Ca has been shown to have the greatest effect on the extent of phytate hydrolysis in monogastric animals because of its high concentration in practical diets (Taylor, 1965), with the degree of phytate P utilization shown to be dependent on the molar ratio of phytate:Ca in the digesta (Wise, 1983; Tamin and Angel, 2003). As the effect of Ca on phytate hydrolysis was also indirectly influenced by the concentration of phytate available in the small intestine, Fordyce et al. (1987) further suggested the molar ratio of (phytate+Ca): Zn would be a good predictor of the availability of dietary Zn. In consequence, the bioavailability of other nutrients bound to phytate, including

free AA, would presumably also be somewhat dependent on the ratio of phytate and Ca to these nutrients in the diet.

Recently, varieties of corn and SBM have been developed with reductions in their phytate concentration of up to 66% (Bohlke et al., 2005). The inclusion of these low-phytate (LP) grains in diets has been shown to reduce the concentration of phytate in the intestinal lumen (Chapter 3). At a fixed Ca concentration the incorporation of LP grains would also increase the molar ratio of Ca:phytate, while reducing the ratio of (phytate+Ca):AA in the digesta, potentially increasing the digestibility of AA. However, a higher concentration of free ionic Ca not bound to phytate in the digesta has also been shown to potentially antagonize the absorption of certain AA (Hallberg et al., 1991; Shafey and McDonald, 1991). Therefore, the objective of the present study was to investigate effects of dietary Ca on apparent AA absorption in broiler chickens when diets contained graded levels of phytate from SBM.

## MATERIALS AND METHODS

### *Animals and Management*

A resident flock of Ross 344 x 508 broiler breeders supplied eggs that were also incubated and hatched at the institution. Chicks were feather-sexed after hatching and 816 male chicks were permanently identified with neck tags and randomly allocated to 48 cages in 6-tier electrically heated battery brooders. Two battery brooders were located in each of two temperature-controlled brooding rooms. There were 17 chicks per cage and vertical temperature differences between pens were reduced by utilizing only the middle 4 tiers of cages in each battery brooder. Brooding temperatures were initially set at 33 C and reduced

gradually to 25 C by 21 d of age. From 1-15 d all birds had free access to feed and water and received a standard corn-soy broiler starter diet containing 3,150 kcal ME $kg^{-1}$, 23.0% CP, 0.9% Ca, and 0.45% nonphytate P (NPP). At 12 d of age, variation in the body weight (BW) of birds in each pen was reduced by weighing all birds individually and retaining only 13 birds per pen. This resulted in a mean BW of 342 $\pm$ 12 g.

***Dietary Treatments***

Three basal diets were formulated to contain similar nutrient concentrations but analyzed concentrations of phytate P of 0.28, 0.24, and 0.10% (Table 3.3). The range in phytate P was obtained using degermed dehulled corn (DGDH) and one of three sources of SBM, namely high-phytate (HP) Prolina, Commercial, and LP that contained analyzed phytate concentrations of 18.17, 16.09, and 5.35 g $kg^{-1}$, respectively. Four levels of dietary Ca (0.47, 0.70, 0.93, and 1.16%) were obtained in each basal diet by varying the relative inclusion of limestone and washed sand. To reduce effects of the different sources of SBM or added limestone on physical feed texture, the formulated dietary moisture content and inclusion of added soy oil was held constant at 11.5%, and 6.5%, respectively, by varying the inclusion of water and washed sand. All diets also contained 0.4% titanium dioxide ($TiO_2$) as an indigestible marker to allow the determination of digestibility coefficients. Each diet was mixed in two 25 kg batches in a bakery-style vertical mixer and samples from each batch were analyzed for proximate and AA content. At 15 d of age, birds were fasted for 16 h after which the 12 experimental diets were each assigned to four pens of 13 birds each and fed from 16 d to 21 d of age.

***Sample Collection and Analyses***

At 21 d 10 chicks per pen were weighed and killed by cervical dislocation and the terminal 13 cm of ileum removed 3 cm anterior to the ileo-cecal junction. Ileal contents were gently expressed, pooled per cage, and frozen. Feeders for each pen were weighed at the start and end of the 5-d experimental period and the feed intake per bird calculated.

Frozen samples of ileal digesta were lyophilized, and ground prior to analysis. The total concentration of phytate P was determined by acid extraction followed by high performance liquid chromatography (HPLC) analysis (Kwanyuen and Burton, 2005). Amino acid concentrations in the feed, ileal digesta, and excreta were determined by HPLC (University of Missouri Analytical Services Group, Columbia, MO 65211). The $TiO_2$ concentration of the ileal digesta and excreta was based on the method of Short et al. (1996). Briefly, samples were ashed before being digested in 60% (vol/vol) sulfuric acid. The mixture was then incubated with 30% $H_2O_2$ and the absorbance read at 405 nm.

***Calculations and Statistical Analyses***

The apparent percentage prececal nutrient digestibility ($PcND_{\%}$) was calculated using the index method based on the equation:

$$PcND_{\%} = 100-[(TiO_{diet}/ TiO_{out}) \times (Nut_{out}/ Nut_{diet}) \times 100] \quad [1]$$

where $TiO_{diet}$ was the initial $TiO_2$ concentration in the diet; $Nut_{diet}$ was the initial dietary concentration of the nutrient being assayed; and $TiO_{out}$ and $Nut_{out}$ were the respective concentrations of either $TiO_2$ or nutrient (Nut) in the ileal digesta, respectively. To account for differences in the dietary nutrient concentration, the quantity of unabsorbed nutrients in the ileal digesta was expressed per kg of dry matter nutrient intake (DMI).

All data were analyzed using the Mixed Models procedure of SAS (SAS Institute, 2004). There were four replicate cages per treatment arranged in a randomized complete block design with four blocks with two blocks of 12 cages in each room. A cage of birds served as the experimental unit. Data were analyzed as a complete factorial in a mixed model with block as a random effect and fixed effects of SBM (3 levels), dietary Ca (4 levels), and all two-way interactions. Where appropriate, means separation was by Turkeys HSD with an alpha level of 0.05. Orthogonal contrasts were used to assess the significance of linear or quadratic functions to describe the response in the dependent variable to increasing Ca level. Statements of statistical significance were based upon $P < 0.05$ unless otherwise stated.

## RESULTS

The expected and analyzed dietary nutrient concentration was previously reported in Chapter 3, Table 3.3.

The effect of SBM and dietary Ca on the apparent prececal digestibility of indispensable and dispensable AA is shown in Tables 4.1 and 4.2, respectively. Significant interactions of Ca x SBM on apparent digestibility were observed for all AA except methionine, threonine, aspartate, and serine, which suggested that the effects of dietary Ca on apparent AA digestibility may have been influenced by the source of SBM. With the exception of cysteine, increasing dietary Ca had no effect on the apparent digestibility coefficients of AA when diets contained HP Prolina SBM. When diets contained Commercial SBM, small increases in the dietary Ca above 0.46% resulted in improvements of 1.5% to 2.5% in the digestibility of both total indispensable and total dispensable AA, respectively. The increase in AA digestibility with increasing Ca in Commercial SBM could

be described by a quadratic response function with little improvement above 0.70% Ca. When diets contained LP SBM the improvement in AA digestibility was much greater when dietary Ca was increased from 0.46% to 0.70%. However, further increases in the Ca concentration above 0.70% generally decreased AA digestibility. This negative effect of Ca on AA digestibility was not observed when diets contained either HP Prolina or Commercial SBM.

The analyzed concentration of phytate P in HP Prolina, Commercial, and LP SBM diets was 0.28, 0.24, and 0.10%, respectively (Table 3.3). Disappearance of phytate P from the distal ileum was similar between sources of SBM and ranged from 11.43% to 12.33% for the HP Prolina and LP SBM, respectively (Table 4.3). The effect of Ca on phytate P disappearance was similar between sources of SBM and decreased in a stepwise manner from 20.15% to 5.87% when dietary Ca was increased from 0.47% to 1.16%. The low percentage disappearance of phytate P in the small intestine resulted in a high concentration of phytate P remaining in the digesta at the distal ileum that ranged from 2.72 to 0.97 g $kg^{-1}$ DMI for HP Prolina SBM and LP SBM, respectively. Importantly, no negative correlation was observed between the apparent digestibility of dispensable or indispensable AA and the percentage phytate in the diet, the percentage phytate P disappearance, or the ileal phytate concentration.

## DISCUSSION

The development of LP cereal grains and legumes have provided a novel way to study the nutritional and environmental impact of phytate in diets of monogastric livestock (Overturf et al., 2003). In the present study, a 63.6% and 58.2% reduction in dietary phytate

was obtained when LP SBM replaced HP Prolina or Commercial SBM in diets and an associated reduction in P excretion of 62% and 49% was reported in Chapter 3. The improvement in P availability and reduced P excretion was in agreement with previous studies that evaluated LP corn (Li et al., 2000; Waldroup et al., 2000), barley (Li et al., 2001; Overturf et al., 2003), and SBM (Sands et al., 2003) in diets for poultry, swine, and trout. However, while several studies have evaluated effects of LP grains on the availability of P and AA, to our knowledge no investigations have examined the interactive effects of Ca and phytate in diets on AA absorption in broilers.

In the present study there was no improvement in AA absorption when LP SBM was included in diets in place of either Commercial or HP Prolina SBM. This was in agreement with (Adeola, 2005) who reported no improvement in AA availability when LP corn was included in diets. The clear absence of an effect of phytate on AA absorption in the present study was further supported by a lack of any negative correlation between the dietary phytate concentration and apparent absorption of any of the AA measured. Furthermore, while inclusion of LP SBM resulted in a three-fold reduction in phytate concentration at the terminal ileum, no correlation of ileal phytate concentration with apparent AA absorption coefficients was observed.

However, in contrast to these findings, several studies have reported that LP corn and LP SBM (Douglas et al., 2000; Bolke et al., 2005), or phytase (Ravindran et al., 1999; Rutherford et al., 2002; Cowieson et al., 2006) added to diets increased AA availability.

The beneficial effects of phytase or LP diets observed by previous workers were attributed to the negative effect of phytate on the solubility of proteins that resulted from binding of protonized AA groups (lysyl, histidyl, and arginyl residues) with basic phosphate

groups of phytate (Pallauf and Rimbach, 1997) or by formation of ternary protein-metal-phytate complexes (Kamao et al., 2000). The inclusion of phytase (Ravindran et al., 1999; Rutherford et al., 2002; Cowieson et al., 2006), LP corn (Douglas et al., 2000; Bolke et al., 2005), and LP SBM (Adeola, 2005) was further shown to increase the availability of some, but not all AA, being greatest for valine, threonine, isoleucine, and aspartic acid. The beneficial effect of phytase or reduced intestinal phytate concentration from adding LP grains on specific AA, but not others was attributed to a combination of either differential interactions between amino groups and phytate, or due to phytase decreasing the negative effects of phytate on endogenous AA losses (Cowieson et al., 2004; Bohlke et al., 2005; Cowieson et al., 2006). It remains unclear why LP grains were shown to increase AA digestibility in some studies but had no effect in the present study or that of Adeola (2005).

The present study found a significant interaction between SBM source and Ca level for 13 of the 16 AA examined that suggested that AA availability in SBM with different phytate concentrations may be dependent on the concentration of dietary Ca. Effects of dietary Ca on apparent absorption of AA in broiler diets with different concentrations of phytate have not previously been reported. Apparent cysteine absorption in all three sources of SBM was shown to increase when dietary Ca was increased from 0.47% to 0.70% but Ca level had no consistent effect on the absorption of other AA in the HP Prolina SBM. However, when Commercial SBM or LP SBM was included in diets, apparent AA digestibility was again generally lower when diets contained only 0.47% Ca. These results contradicted previous work by Johnston et al. (2004) that reported that reduced dietary concentrations of Ca and P in corn-soy based swine diets increased AA availability to a similar extent as did added phytase. While the results reported in Chapter 3 clearly showed

that more phytate was hydrolyzed at the lowest Ca levels with all sources of SBM, in contrast to the findings by Johnston et al. (2004), there was no associated improvement in apparent AA digestibility.

The reduced AA digestibility found at the lowest Ca concentration of 0.46% was of little practical significance as the dietary Ca requirement at all growth stages of broilers was considerably higher (NRC, 1994). However, the negative effects of Ca concentrations above 0.70% in the LP SBM may be of particular importance as most, if not all, starter and grower diets fed to commercial broilers contain in excess of 0.70% Ca. The negative effects of high Ca on AA digestibility of LP SBM but not HP Prolina or Commercial SBM diets may potentially be caused by a direct antagonism of high concentrations of Ca on AA uptake. Shafey and McDonald (1991) previously reported that excess dietary Ca (2.6%) in broiler diets did not allow for maximum utilization of AA, while a combination of excess dietary Ca and AvP was shown to be more detrimental than excess dietary Ca alone. Although Ca concentrations in that study were considerably higher than the highest level of 1.16% Ca used in the LP SBM diets, the present study suggested that detrimental effects of Ca on AA absorption may be exacerbated when diets contained low levels of phytate. In summary, the negative effects of both low and high Ca concentrations on AA availability in LP SBM diets but not in HP SBM diets, suggested that AA availability in diets with varying phytate concentration may be dependent on the dietary Ca level. This effect of Ca on AA digestibility in LP diets warrants further investigation as LP grains have shown great potential to reduce P excretion from poultry and the use thereof in practical diets can be expected to increase in the future as regulations that limit manure application to land based on its P content become more stringent.

## REFERENCES


Adeola, O. 2005. Metabolisable energy and amino acid digestibility of high-oil maize, low-phytate maize and low-phytate soybean meal for white pekin ducks. Br. Poult. Sci. 46:607-614.

Bohlke, R. A., R. C. Thaler, and H. H. Stein. 2005. Calcium, phosphorus, and amino acid digestibility in low-phytate corn, normal corn, and soybean meal by growing pigs. J. Anim. Sci. 83:2396-2403.

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

Cowieson, A. J., T. Acamovic, and M. R. Bedford. 2006. Supplementation of corn-soy-based diets with an Eschericia coli-derived phytase: Effects on broiler chick performance and the digestibility of amino acids and metabolizability of minerals and energy. Poult. Sci. 85:1389-1397.

Douglas, M. W., C. M. Peter, S. D. Bolling, C. M. Parsons, and D. H. Baker. 2000. Nutritional evaluation of low phytate and high protein corns. Poult. Sci. 79:1586-1591.

Fordyce, E. J., R. M. Forbes, K. R. Robbins, and J. W. Erdman. 1987. Phytate x calcium zinc molar ratios – Are they predictive of zinc bioavailability. J. Food Sci. 52:440-444.

Hallberg, L., M. Brunne, M. Erlandsson, A. S. Sandberg, and L. Rossander-Hulten. 1991. Calcium: effect of different amounts of nonheme- and heme-iron absorption in humans. Am. J. Clin. Nutr. 53:112-119.

Johnston, S. L., S. B. Williams, L. L. Southern, T. B. Bidner, L. D. Bunting, J. O. Mathews, and B. M. Olcott. 2004. Effect of phytase addition and dietary calcium and phosphorus levels on plasma metabolites and ileal and total-tract nutrient digestibilities in pigs. J. Anim. Sci. 82:705-714.

Kamao, M., N. Tsugawa, K. Nakagawa, Y. Kawamoto, K. Fukui, K. Takamatsu, G. Kuwata, M. Imai, and T. Okano. 2000. Absorption of calcium, magnesium, phosphorus, iron, and zinc in growing male rats fed diets containing either phytate-free soybean protein or soybean protein isolate or casein. J. Nutr. Sci. Vitaminol. 46:34-41.

Kwanyuen, P. and J. W. Burton. 2005. A simple and rapid procedure for phytate determination in soybean and soybean products. J. Am. Oil Chem. Soc. 82:81-85.

Li, Y. C., D. R. Ledoux, T. L. Veum, V. Raboy, and D. S. Ertl. 2000. Effects of low phytic acid corn on phosphorus utilization, performance, and bone mineralization in broiler chicks. Poult. Sci. 79:1444-1450.

Li, Y. C., D. R. LeDoux, T. L. Veum, V. Raboy, and K. Zyla. 2001. Low phytic acid barley improves performance, bone mineralization, and phosphorus retention in turkey poults. J. Appl. Poult. Res. 10:178-185.

Lönnerdal, B., L. Jayawickrama, and E. L. Lien. 1999. Effect of reducing the phytate content and of partially hydrolyzing the protein in soy formula on zinc and copper absorption and status in infant rhesus monkeys and rat pups. Am. J. Clin. Nutr. 69:490-496.

Maenz, D. D., C. M. Engele-Schaan, R. W. Newkirk, and H. L. Claasen. 1999. The effect of minerals and mineral chelators on the formation of phytase-resistant and phytase susceptible forms of phytic acid in solution and in a slurry of canola meal. Anim. Feed Sci. Technol. 81:177-192.

National Research Council (NRC). 1994. Nutrient Requirements For Poultry. 9th revised ed. National Academy Press, Washington, D.C.

Overturf, K., V. Raboy, Z. J. Cheng, and R. W. Hardy. 2003. Mineral availability from barley low phytic acid grains in rainbow trout (Oncorhynchus mykiss) diets. Aquaculture Nutr. 9:239-246.

Pallauf, J., and G. Rimbach. 1997. Nutritional significance of phytic acid and phytase. Arch. Anim. Nutr. 50:301-319.

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

Rutherfurd, S. M., T. K. Chung, and P. J. Moughan. 2002. The effect of microbial phytase on ileal phosphorus and amino acid digestibility in the broiler chicken. Br. Poult. Sci. 44:598-606.

Sands, J. S., D. Ragland, J. R. Wilcox, and O. Adeola. 2003. Relative bioavailability of phosphorus in low phytate soybean meal for broiler chicks. Can. J. Anim. Sci. 83:95-100.

SAS Institute. 2004. SAS/STAT User's Guide. Release 9.1. SAS Institute, Inc., Cary, NC.

Shafey, T. M., and M. W. McDonald. 1991. The effects of dietary concentrations of minerals, source of protein, amino-acids, and antibiotics on the growth of and digestibility of amino-acids by broiler chickens. Br. Poult. Sci. 32:535-544.

Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Tech. 59:215-221.

Tamin N. M., and R. Angel. 2003. Phytate phosphorus hydrolysis as influenced by dietary calcium and micro mineral source in broiler diets. J. Agric. Food Chem. 5:4687-4693.

Taylor, T. G. 1965. The availability of the calcium and phosphorus of plant materials for animals. Proc. Nutr. Soc. 24:105-112.

Waldroup, P. W., J. H. Kersey, E. A. Saleh, C. A. Fritts, F. Yan, H. L. Stillborn, R. C. Crum, Jr., and V. Raboy. 2000. Nonphytate phosphorus requirement and phosphorus excretion of broiler chicks fed diets composed of normal or high available phosphate corn with and without microbial phytase. Poult. Sci. 79:1451-1459.

Wise, A. 1983. Dietary factors determining the biological activities of phytate. Nutr. Abstr. Rev. 53:791-806.

**Table 4.1.** Effect of source of soybean meal (SBM) and dietary calcium on apparent prececal digestibility of indispensable amino acids.

| | Treatment Factors | | Apparent Indispensable Amino Acid Digestibility | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Diet | SBM | Calcium | Lys | Met | Thr | Ile | Leu | Val | Phe | His | Arg | Total |
| | | | (%) | | | | | | | | | |
| 1 | HP Prolina | 0.47 | 88.34 [abc] | 92.16 | 80.18 [ab] | 86.94 [ab] | 86.06 [abc] | 84.14 | 86.49 [abc] | 87.00 [abc] | 90.49 [ab] | 86.59 [ab] |
| 2 | | 0.70 | 87.62 [abc] | 92.24 | 79.96 [ab] | 85.69 [ab] | 85.91 [abc] | 82.05 | 85.99 [abc] | 87.09 [abc] | 90.18 [ab] | 85.88 [ab] |
| 3 | | 0.93 | 88.29 [abc] | 93.15 | 81.24 [ab] | 87.34 [ab] | 87.95 [ab] | 84.33 | 87.84 [ab] | 88.50 [ab] | 90.91 [ab] | 87.39 [ab] |
| 4 | | 1.16 | 87.49 [abc] | 93.52 | 80.91 [ab] | 86.34 [ab] | 86.78 [abc] | 83.53 | 86.48 [abc] | 87.72 [abc] | 90.07 [ab] | 86.64 [ab] |
| 5 | Commercial | 0.47 | 88.22 [abc] | 91.80 | 78.64 [b] | 85.44 [ab] | 84.31 [c] | 82.09 | 84.95 [c] | 86.24 [bc] | 89.91 [ab] | 85.37 [ab] |
| 6 | | 0.70 | 88.60 [abc] | 92.33 | 80.69 [ab] | 86.68 [ab] | 85.96 [abc] | 83.77 | 86.46 [abc] | 87.89 [abc] | 90.74 [ab] | 86.69 [ab] |
| 7 | | 0.93 | 88.71 [ab] | 92.60 | 80.69 [ab] | 87.32 [ab] | 86.79 [abc] | 83.62 | 87.09 [abc] | 88.58 [ab] | 90.93 [ab] | 86.99 [ab] |
| 8 | | 1.16 | 88.66 [abc] | 92.53 | 80.40 [ab] | 86.90 [ab] | 86.36 [abc] | 83.96 | 86.49 [abc] | 88.21 [ab] | 90.53 [ab] | 86.80 [ab] |
| 9 | LP Soy | 0.47 | 85.33 [c] | 91.05 | 78.82 [b] | 85.15 [b] | 85.01 [bc] | 81.64 | 85.43 [bc] | 85.43 [c] | 89.41 [b] | 84.89 [b] |
| 10 | | 0.70 | 88.97 [a] | 92.81 | 83.01 [a] | 88.36 [a] | 88.51 [a] | 85.29 | 88.76 [a] | 89.10 [a] | 91.97 [a] | 88.21 [a] |
| 11 | | 0.93 | 86.41 [abc] | 91.48 | 80.24 [ab] | 86.11 [ab] | 86.19 [abc] | 82.54 | 86.56 [abc] | 86.87 [abc] | 89.79 [ab] | 85.87 [ab] |
| 12 | | 1.16 | 85.41 [bc] | 91.12 | 79.32 [ab] | 85.15 [b] | 85.48 [abc] | 81.91 | 85.65 [bc] | 86.39 [abc] | 88.87 [b] | 85.12 [ab] |
| | | SEM | 0.82 | 0.70 | 0.94 | 0.93 | 0.79 | 1.50 | 0.84 | 0.69 | 0.59 | 0.88 |
| | Source of Variation | | Probability > *F* | | | | | | | | | |
| | SBM | | <0.001 | 0.022 | 0.735 | 0.599 | 0.196 | 0.508 | 0.514 | 0.116 | 0.308 | 0.380 |
| | Calcium | | 0.131 | 0.279 | 0.039 | 0.092 | 0.004 | 0.425 | 0.007 | 0.009 | 0.025 | 0.061 |
| | SBM x Calcium | | 0.046 | 0.278 | 0.119 | 0.012 | 0.018 | 0.026 | 0.014 | 0.017 | 0.028 | 0.026 |
| | Calcium (Lin) | | 0.605 | 0.156 | 0.256 | 0.589 | 0.042 | 0.546 | 0.224 | 0.016 | 0.549 | 0.347 |
| | Calcium (Quad) | | 0.034 | 0.223 | 0.014 | 0.014 | 0.002 | 0.146 | <0.001 | <0.001 | 0.004 | 0.014 |

[a-c] Means within the same column with no common superscript differ significantly ($P \leq 0.05$).

[1] Abbreviations: Lysine (Lys), methionine (Met), threonine (Thr), isoleucine, (Ile) leucine (Leu) valine (Val), phenylanaline (phe), histidine (His), agrginine (Arg) high phytate (HP), low phytate (LP).

**Table 4.2.** Effect of source of soybean meal (SBM) and dietary calcium on apparent prececal digestibility of dispensable amino acids.

| | Treatment Factors | | Apparent Dispensable Amino Acid Digestibility | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Diet | SBM | Calcium | Cys | Ser | Tyr | Ala | Asp | Glu | Gly | Total |
| | | | (%) | | | | | | | |
| 1 | HP Prolina | 0.47 | 78.08 [bc] | 83.89 | 86.65 [abc] | 85.27 [abc] | 83.47 [abc] | 88.69 [ab] | 82.03 [abc] | 84.34 [abc] |
| 2 | | 0.70 | 79.35 [abc] | 83.81 | 86.56 [abc] | 85.31 [abc] | 83.39 [abc] | 88.76 [ab] | 82.25 [abc] | 84.53 [abc] |
| 3 | | 0.93 | 82.01 [ab] | 84.50 | 88.11 [ab] | 87.15 [ab] | 84.44 [ab] | 89.79 [ab] | 83.73 [ab] | 86.00 [abc] |
| 4 | | 1.16 | 82.71 [a] | 84.84 | 87.44 [abc] | 86.15 [abc] | 83.52 [abc] | 89.02 [ab] | 83.00 [abc] | 85.61 [abc] |
| 5 | Commercial | 0.47 | 76.10 [c] | 81.59 | 85.31 [bc] | 83.59 [c] | 83.03 [abc] | 88.20 [ab] | 81.00 [abc] | 82.97 [bc] |
| 6 | | 0.70 | 80.93 [ab] | 83.84 | 86.87 [abc] | 85.00 [abc] | 84.86 [a] | 89.55 [ab] | 83.14 [abc] | 85.18 [abc] |
| 7 | | 0.93 | 81.86 [ab] | 83.17 | 87.60 [abc] | 85.76 [abc] | 84.93 [a] | 89.78 [ab] | 83.56 [ab] | 85.52 [ab] |
| 8 | | 1.16 | 81.86 [ab] | 83.42 | 87.14 [abc] | 85.40 [abc] | 84.18 [abc] | 89.34 [ab] | 83.24 [abc] | 85.22 [abc] |
| 9 | LP Soy | 0.47 | 75.87 [c] | 81.57 | 84.99 [c] | 83.80 [bc] | 80.89 [c] | 87.28 [b] | 79.69 [c] | 82.40 [c] |
| 10 | | 0.70 | 81.60 [ab] | 85.73 | 88.40 [a] | 87.36 [a] | 84.90 [a] | 90.23 [a] | 84.34 [a] | 86.37 [a] |
| 11 | | 0.93 | 79.27 [abc] | 83.50 | 86.50 [abc] | 84.79 [abc] | 81.92 [abc] | 87.94 [ab] | 81.54 [abc] | 83.99 [abc] |
| 12 | | 1.16 | 78.45 [bc] | 82.46 | 85.84 [abc] | 84.11 [abc] | 81.16 [bc] | 87.22 [b] | 80.54 [bc] | 83.21 [abc] |
| | | SEM | 1.16 | 1.01 | 0.88 | 0.84 | 0.91 | 0.67 | 0.95 | 0.85 |
| | Source of Variation | | Probability > *F* | | | | | | | |
| | SBM | | 0.016 | 0.154 | 0.188 | 0.089 | <0.001 | 0.025 | 0.041 | 0.072 |
| | Calcium | | <0.001 | 0.063 | 0.003 | 0.021 | 0.010 | 0.014 | 0.003 | 0.002 |
| | SBM x Calcium | | 0.014 | 0.314 | 0.036 | 0.050 | 0.061 | 0.050 | 0.042 | 0.049 |
| | Calcium (Lin) | | <0.001 | 0.231 | 0.021 | 0.111 | 0.647 | 0.469 | 0.061 | 0.025 |
| | Calcium (Quad) | | <0.001 | 0.042 | 0.002 | 0.007 | 0.002 | 0.003 | <0.001 | 0.002 |

[a-c] Means within the same column with no common superscript differ significantly ($P \leq 0.05$).

[1] Abbreviations: Cysteine (Cys), serine (Ser), tyrosine (Tyr), alanine, (Ala) aspartate (Asp) glutamate (Glu), glycine (Gly), high phytate (HP), low phytate (LP).

**Table 4.3.** Main effects of soybean meal (SBM) source and calcium on ileal digestibility and concentrations of phosphorus, Ca, and phytate phosphorus (P) at the terminal ileum.

| | Apparent Ileal Digestibility | | | Ileal Concentrations per kg of DMI | | |
|---|---|---|---|---|---|---|
| Main Effect | Phosphorus | Calcium | Phytate P | Phosphorus | Calcium | Phytate P |
| SBM | (%) | | | (g/kg DMI) | | |
| HP Prolina | 51.69 [b] | 39.62 | 11.43 | 3.52 [b] | 5.58 | 2.72 |
| Commercial | 52.54 [b] | 40.68 | 13.32 | 2.84 [b] | 5.20 | 2.29 |
| LP Soy | 65.44 [a] | 42.96 | 12.33 | 1.61 [c] | 5.50 | 0.97 |
| SEM | 1.15 | 3.24 | 3.49 | 0.07 | 0.34 | 0.08 |
| Calcium Level | | | | | | |
| 0.47 | 64.32 [a] | 46.76 | 20.15 [a] | 2.19 [c] | 2.78 [d] | 1.84 [b] |
| 0.70 | 57.85 [b] | 40.59 | 15.09 [ab] | 2.57 [b] | 4.62 [c] | 1.97 [ab] |
| 0.93 | 53.55 [c] | 38.41 | 8.61 [b] | 2.84 [a] | 6.37 [b] | 2.03 [ab] |
| 1.16 | 50.50 [c] | 38.58 | 5.87 [b] | 3.02 [b] | 7.92 [a] | 2.14 [a] |
| SEM | 1.24 | 3.50 | 3.75 | 0.08 | 0.37 | 0.08 |
| Source of Variation | ----------Probability > *F*---------- | | | | | |
| SBM | <0.001 | 0.571 | 0.854 | <0.001 | 0.803 | <0.001 |
| Calcium | <0.001 | 0.101 | 0.003 | <0.001 | <0.001 | <0.001 |
| SBM x Calcium | 0.800 | 0.571 | 0.888 | 0.009 | 0.711 | 0.421 |
| Calcium (Lin) | <0.001 | 0.029 | <0.001 | <0.001 | <0.001 | <0.001 |
| Calcium (Quad) | 0.069 | 0.232 | 0.713 | 0.067 | 0.630 | 0.787 |

[a-c] Means within the same column with no common superscript differ significantly ($P \leq 0.05$).

[1] Abbreviations: High phytate (HP), low phytate (LP), dry matter intake (DMI).

## SUMMARY AND CONCLUSIONS

The objective of our research was to better understand the basic mechanisms of P metabolism in the intestines of broiler breeders and broilers and develop practical recommendations that would reduce the environmental impact of P in poultry manure and litter. We were able to develop dietary strategies that would safely reduce both total and soluble P fractions in manure and litter from both broiler breeders and broilers.

### *Effects of Diet Amendment on P Excretion and Performance of Broiler Breeders*

Results obtained during the rearing phase of broiler breeders from 10 to 21 wk of age (Chapter 1) showed that the inclusion of phytase at the expense of 0.10% P from dicalcium phosphate had no effect on total P contained in the litter from broiler breeder pullets. However, a reduction in litter total P of 18% was obtained when diet NPP was reduced by 0.10% without added phytase. The lack of an effect of phytase amendment on litter total P was attributed to the recycling of P that had been shown to occur when birds were housed on litter. Analysis of the data from the rearing phase further suggested that the litter WSP:total P ratio was strongly correlated with percentage litter moisture during rearing and was not altered by reduced diet NPP or phytase. During the broiler breeder laying phase from 21 to 64 wk of age the inclusion of phytase at the expense of 0.10% NPP from dicalcium phosphate had no significant impact on total P in the manure when diets contained normal levels of NPP (0.37%). However, reductions in manure total P of 39% and 42% were obtained when diet NPP was reduced to 0.19% without phytase, or to 0.09% with added phytase, respectively. Furthermore, the reductions in diet NPP to 0.19% had no effect on hen-

day egg production or the number of chicks produced per hen-housed, while decreasing diet NPP to 0.09% with added phytase increased hen-day egg production by ~ 3 percentage points. These findings have substantial implications in terms of P nutrition of commercial broiler breeder flocks. In the first instance, a review of the literature has shown that the published recommended dietary specifications for NPP in broiler breeder diets have been largely derived from research conducted on commercial laying hens and broiler breeders housed in cages. As birds in cages were not able to re-cycle P from the litter, the P requirements determined from broiler breeders housed in cages have been overstated. This was confirmed by our results that showed no detrimental effects of feeding a diet containing 0.19% NPP or 0.09% NPP with added phytase on the number of chicks produced per hen-housed. The results obtained in Chapter 1 further suggested that the commercial practice of replacing 0.1% diet NPP with phytase when diets contained high levels of NPP (0.37%) had no benefit on reducing litter total P. However, without detrimentally affecting egg production or the number of chicks produced the manure total P could be reduced by 39% to 42% and WSP by 42% when the NPP in the diet was reduced to 0.19% without phytase or 0.09% with added phytase. The negative effect of reduced diet NPP on fertility that was observed has not previously been reported in other studies that had removed all dicalcium phosphate from broiler breeder diets after 21 wk of age. Therefore, the reduced fertility in the present study may have been associated with the reduced diet NPP during the rearing phase and should be the subject of future research. Furthermore, our calculations showed that the total P excreted during the rearing phase from 0 to 21 wk contributed only 18% of the total P excreted by a broiler breeder to 64 wk of age. Therefore, due the small impact on cumulative P excretion and the apparent high sensitivity to a P deficiency of growing birds from 0 – 21

wk of age our present recommendations were that the NPP levels in rearing diets should not be reduced below current recommendations.

***Effects of Diet Amendment on Total Phosphorus in Litter and Manure from Broilers***

Our research (Chapter 2) confirmed previous findings that showed a linear reduction of 19% to 22% in litter total P when diet AvP was reduced from 0.35% to 0.25%. However, while reduction in dietary AvP to levels as low as 0.25% had positive effects on reducing litter total P and did not affect BW gain or AdjFCR, the higher broiler mortality that occurred when diets contained $\leq$ 0.30% AvP showed that in practice, dietary AvP could not be reduced below 0.35% from as early as 18 d of age. The replacement of 0.10% NPP in diets with phytase had no effect on broiler performance and resulted in a 20% reduction in litter total P. This was consistent with previous reports that showed phytase application in broiler diets to be effective in reducing litter total P without altering broiler performance.

As an additional strategy to reduce P excretion in broilers, Chapter 3 showed that manure total P was reduced by 49% when LP SBM replaced a commercial SBM in diets. Importantly, there was no difference in the total amount of P absorbed by broilers fed either LP or commercial SBM, which showed that the reduced P excretion was a direct result of the higher P digestibility when diets contained LP SBM.

As Ca was known to affect the extent of phytate hydrolysis in broiler diets it was hypothesized in Chapter 3 that the effects of Ca on total P output were dependent on the dietary phytate concentration and may be altered when LP grains were included in diets. Results suggested that the dietary Ca:NPP ratio at which total P output from broilers was minimized was reduced from 2.53:1 to 2.34:1 when the phytate P content of the diets was

decreased from 0.28% to 0.10%. Furthermore, the optimal Ca:NPP ratio at which total P output was minimized in the present study was higher than the Ca:NPP ratio of 2:0:1 to 2.2:1 that has been commonly applied in commercial broiler starter diets. This suggested that total P output in commercial broiler diets could be reduced by simply widening the Ca:NPP in practical broiler diets. However, further research would be required to ascertain the effects of a wider Ca:NPP ratio on long-term broiler performance.

***Effects of Diet Amendment on Water Soluble Phosphorus in Litter and Manure from Broilers***

The results of our research in Chapter 2 showed that the WSP and the WSP:total P ratio in broiler litter was primarily controlled by the dietary Ca:AvP ratio, and that phytase did not affect the concentration of WSP in litter but increased the ratio of WSP:total P in litter by 18%. A review of the literature showed that previous research that had investigated effects of reduced NPP and phytase in broiler diets had not accounted for potential effects of the Ca:AvP ratio that was increased when dietary AvP was reduced at a constant Ca level in the diet. Therefore, the results of the present study may explain why several previous reports that described the effects of phytase on litter WSP had shown conflicting results.

The mechanism whereby the Ca:AvP ratio influenced WSP and the WSP:total P ratio was also examined. The higher WSP in the litter that was found when diets contained a low ratio of Ca:AvP was ascribed to the combined effects of increased phytate hydrolysis that increased P absorption and decreased residual phytate in the litter. However, due to insufficient Ca in the diet the P that was absorbed was not retained and was presumed to be excreted as urinary P, which would be contained in the feces. When the Ca:AvP ratio was

increased the curvilinear reduction in WSP was shown to be the result of an increased proportion of phytate P in the litter and the increased formation of insoluble CaP precipitates.

As the effects of Ca:AvP in Chapter 2 were partially attributed to the effect of Ca on phytate hydrolysis and the molar ratio of Ca:NPP in the intestine, we hypothesized that the reduction in WSP that occurred when Ca was increased was dependent on the dietary phytate concentration and may be altered when LP grains were included in diets for broilers. This was confirmed in Chapter 3 by evidence of a significant interaction of Ca and SBM source. Regression of the response in WSP as a function of Ca level and SBM source showed that the effect of increasing dietary Ca on WSP and the WSP:total P ratio in manure was significantly reduced when diets contained less phytate as a result of including LP SBM in place of a commercial SBM. Importantly, at the calculated Ca:NPP ratio at which total P output was minimized the inclusion of LP SBM reduced WSP in manure by 56% compared to diets that contained commercial SBM. Therefore, the inclusion of LP SBM in diets was able to replace 0.5% added monocalcium phosphate in diets and reduced total manure P and WSP by 49% and 56%, respectively, without reducing the amount of P absorbed at the distal ileum. Future research should investigate the combined effects of including both LP corn and LP SBM in place of conventional sources of corn and SBM in broiler diets as this could be expected to have an even greater effect on P excretion than shown in the present study.

***Effects of Source of SBM and Ca Level in Broiler Diets on Apparent Amino Acid Absorption.***

The results presented in Chapters 2 and 3 had suggested that a wider Ca:AvP ratio was effective in reducing the total P and WSP in litter and manure but the effect was

dependent on the phytate concentration in the diet. However, it was not known how the dietary Ca level and wider ratio of Ca:NPP affected the utilization of dietary amino acids. Results in Chapter 4 showed no consistent effect of Ca level on apparent ileal amino acid absorption when diets contained either high-phytate (HP) Prolina SBM or Commercial SBM. However, amino acid absorption was generally lower in diets with LP SBM at both low (0.47% Ca) and high dietary Ca ($\geq$ 0.93%). As the Ca level of commercial broiler diets was typically between 0.90% and 0.95% these data suggested that amino acid absorption may be impaired when LP SBM was included without reducing the dietary Ca concentration. The combined effects of dietary Ca and LP grains on amino acid absorption warrants further investigation as the commercial viability of including LP grains in diets may be highly dependent on the amino acid digestibility.

In conclusion, reduced NPP diets and phytase were able to reduce total P in manure from broiler breeders by 42%, while the inclusion of phytase in broiler breeder diets was only effective in reducing manure total P when the dietary NPP was reduced closer to the lower NPP requirement of broiler breeders housed in slat-litter laying pens. Total P in litter from broilers was correlated with dietary NPP intake and was also reduced when diets were amended with phytase. However, WSP in litter and manure was shown to be largely controlled by the ratio of Ca:AvP in the diet and the initial dietary phytate level. The inclusion of LP grains was found to be a highly effective strategy to reduce P excretion with reductions of 49% and 56% in total and WSP in manure, respectively. As many poultry producing regions use both total P and WSP as a measure to assess the potential impact of manure application on P losses, the inclusion of LP grains could prove to be a very effective tool to reduce P excretion in broilers. However, to obtain the maximum benefit of LP grains

on P excretion, the Ca:NPP ratio in the diet should be appropriately adjusted, while future research should examine the potentially deleterious effects of high dietary Ca concentrations on amino acid availability of LP grains.