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The Interactive Effects of Phosporus and Calcium on Phosphorus Requirement, Utilization, and Phytase Efficacy in Poultry

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THE INTERACTIVE EFFECTS OF PHOSPHORUS AND CALCIUM ON PHOSPHORUS
REQUIREMENT, UTILIZATION, AND PHYTASE EFFICACY IN POULTRY

A Dissertation
Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy
in
The Interdepartmental Program in
the School of Animal Sciences

by
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“All things are possible if you only believe”

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ABSTRACT

This research was conducted with broilers to investigate the interactive effects of P and Ca on P requirement, digestibility, utilization, and phytase efficacy. Each treatment had a minimum of 6 replications. One experiment with 3 trials was conducted to identify the nonphytate P (nPP) requirement of 0 to 14 d old broilers. The nPP levels fed ranged from 0.30 to 0.60% with a Ca:nPP of 2.22:1 or 1.9:1. Requirement estimates for 0 to 14 d ranged from 0.52 to 0.60% nPP. One experiment with 2 trials was conducted to identify the nPP requirement of 14 to 28 d old broilers. The nPP levels fed ranged from 0.25 to 0.55% with a Ca:nPP of 1.9:1, 2.22:1, or 2.5:1 or set Ca at 0.90%. The requirement estimate was 0.45% nPP. An experiment with 2 trials was conducted to estimate the optimal Ca:nPP for 0 to 18 d old broilers and to investigate Ca and P digestibility and utilization at varying Ca:nPP. The nPP levels fed ranged from 0.30 to 0.50% with Ca:nPP of 1.9:1, 2.2:1, or 2.5:1 (trial 1) and 0.50% nPP with Ca:nPP of 1.5:1, 1.7:1, 1.9:1, or 2.1:1 (trial 2). Optimum Ca:nPP was 1.9:1 for 0 to 18 d old broilers. Digestibility and utilization of Ca and P decreased with increasing nPP and Ca:nPP. Two experiments were conducted to investigate any effect of starter nPP on growth performance and bone characteristics in subsequent growth phases. Feeding below the nPP requirement for 0 to 14 d resulted in better adaptation to a lower nPP in the grower phase. Feeding above the nPP requirement for 0 to 21 d resulted in a decrease in ADG at 35 d and G:F at 49 d. One experiment was conducted to compare the relative bioavailability of 2 *Escherichia coli* phytases. Retained P was higher for broilers fed Finase, which was more bioavailable than Optiphos. One experiment was conducted to investigate the influence of Ca level on the efficacy of phytase. Calcium level did not influence phytase efficacy. The results of these experiments suggest that dietary Ca influences the utilization of P but does not affect phytase efficacy.

CHAPTER ONE

INTRODUCTION

Phosphorus is an essential nutrient for poultry. However, with changing economical conditions, management practices, and environmental concerns, there is a constant need to re-evaluate the P requirement for broilers.

The recommendation for the P requirement outlined by the NRC (1994) is applicable to a 3 phase feeding system; however, within the poultry industry, a 4 or 5 phase system is more common. This change in management practice is an attempt to reduce the quantity of P excreted, to decrease feed cost, and to feed the growing broiler more closely to its requirement. There is an increased cost associated with mixing feed for these shorter phases. This cost must be reduced by feeding as close to the P and AA requirements as possible without negatively affecting production. O'Rourke et al. (1952) suggested that as the broiler increased in age, the requirement for P decreased. Recent research published after the NRC (1994) has shown a substantial decrease in the P requirement compared with the recommendation by the NRC (1994); however, the majority of this research was done with the 3 phase feeding system. Therefore, there is the need to establish the P requirement for shorter periods of time with an increased number of feeding phases.

Calcium sources are generally inexpensive; therefore, there is not as much emphasis on its level in complete diets. However, the Ca:P has an effect on the ability of broilers to utilize the available P from feed ingredients. Therefore, a better understanding of the effect of Ca on P retention is essential in maximizing the use of P in feed ingredients. Also, the effect of Ca on the ability of phytase to release P from phytate has been questioned; however, the data in the literature is inconsistent. Therefore, there is the need to establish the effect of dietary Ca level on phytase efficacy.

The ability of broilers to benefit from excess P in the early stages of growth or to compensate for P deficiency in any phase of growth can provide useful information to poultry production practitioners in making management decisions that will positively influence production. There is some indication that feeding above the P requirement in the early phase of growth, when the quantity of feed consumed by the broiler is relatively small, may positively influence growth performance in the subsequent phases of growth. Also, broilers have shown the ability to compensate to some degree for deficiency of P; the usefulness of this effect needs further investigation.

The need to reduce feed cost, environmental pollution, and to better manage poultry nutrition has created the need for more investigation into P digestibility and utilization. Therefore, the objectives of this research are to establish P requirements for shorter feeding phases, identify the Ca:nPP that optimizes growth performance, study the effect of Ca level on phytase efficacy, and to investigate possible carryover and compensatory growth of broilers with regard to dietary P level.

CHAPTER 2 REVIEW OF LITERATURE

PHOSPHORUS REQUIREMENT

The most important factor that determines the quantity of P required by the broiler is age. The NRC (1994) recommends P feeding levels based on 3 feeding phases. The recommended P levels are 0.45, 0.35, and 0.30% nonphytate P (nPP) for 0 to 3, 3 to 6, and 6 to 8 wk, respectively. These recommendations are based on research published for the period 1952 to 1982. Recent research that evaluates similar feeding phases has shown markedly lower P requirements than those recommended by NRC (1994). A recent summary of the P requirement of broilers based on published data concluded that there is a lot of variability in the suggested P requirement (Angel, 2008). Some of this variability can be attributed to the criteria used for identifying the requirement. The requirement is generally higher for bone breaking strength (BBS) and tibia ash (ASH) than for growth performance.

Starter Phase (0 to 21 d)

In a recent review (Angel, 2008), 20 data sets were evaluated within this age range. The average requirement for nPP based on growth performance was 0.35 and 0.36% for males and straight run broilers, respectively, with no data for females. The average requirement was higher when bone responses (tibia or toe ash) were evaluated. The requirement estimates were 0.39 and 0.40% nPP for males and straight run broilers, respectively, with no data for females. Two data sets provided information on broilers to 14 d. Waldroup and Fritts (2003) reported that the nPP requirement of 0 to 14 d old broilers using growth performance as the response criteria was 0.40% nPP, while Perney et al. (1993) reported a lower value of 0.32% nPP for 4 to 14 d old broilers. However, when tibia and toe ash were used as the response criteria, the nPP requirement reported was higher at 0.44% nPP (Perney et al., 1993). If the industry continues to decrease the

time within feeding phases, clearly there is the need for more information on the P requirement for broilers younger than 21 d.

Grower Phase (21 to 42 d)

Five data sets were included in the review by Angel (2008) for 21 to 42 d. The average requirement based on growth performance was 0.30% nPP. When bone responses (tibia or toe ash) were utilized, the requirement was 0.31% nPP. Also, most of the information was obtained with male broilers. There was no information present on 14 to 28 d broilers, limiting the ability of producers to be more flexible in choosing growth phases. Both Moran and Todd (1994) and Chen and Moran (1995) using growth performance and bone responses reported 0.45 and 0.40% nPP requirement for 21 to 42 d old broilers. The average requirement was greatly influenced by reports of 0.19% (Yan et al., 2001) and 0.15% nPP (Dhandu and Angel, 2003) using growth performance as criteria. There is obviously greater variability within this growth phase than in the starter phase. Some of this variability may be attributed to the level of P fed in the starter phase. However, this is generally overlooked as researchers state that broilers were fed to meet or exceed the NRC (1994) recommendation. However, NRC recommendations cover specific time periods. Therefore, there is the need to further investigate responses to P within this growth period.

CALCIUM AND PHOSPHORUS INTERRELATIONSHIP

Growth performance of poultry is dependent on the ability of the broiler to utilize minerals, especially Ca and P, for bone formation. Over time, numerous researchers have highlighted the relationship between the level of Ca and P in the diet and their respective availability. An imbalance of either mineral will affect the availability of the other. This interrelationship has resulted in the general acceptance that both Ca and P are required in some kind of ratio to each other. The correct ratio of these minerals is somewhat subjective and will

vary based on a number of factors. This variability is further compounded by the differences within the literature of the fraction of dietary P that is at ratio to Ca. Caution must be taken to identify whether authors are utilizing total P, nPP, or in some cases, available P (aP) in establishing the correct ratio. This is a great source of confusion in reviewing the literature and can lead to incorrect conclusions when comparing different results. Therefore, no attempt will be made to compare results within this section; emphasis will be placed on summarizing what is generally accepted presently.

Because the majority of both Ca (99%) and P (80%) within the body of a chick is found in the bone, researchers generally report some bone response variable as response criteria in Ca and P studies. Holmes et al. (1931) investigated the effect of varying dietary Ca:P on the Ca:P of the tibia of growing chicks. They utilized varying Ca:P ranging from 1.10:1 to 5.16:1. They reported that the different dietary treatments resulted in differences in growth performance at 9 wk but no difference in the Ca:P of the tibia, which averaged 2.02:1, 2.03:1, and 2.03:1 at 3, 6, and 9 wk, respectively. The fairly constant ratio of deposition of Ca and P was regardless of the BW of the broiler, Ca:P in the diet, or with the quantity of ash. This research provides some support to the 2.2:1 dietary ratio recommended by the NRC (1994); however, this ratio is based on the available portion of P within the diet and not on total P.

Hurwitz and Bar (1971) investigated the interrelationship between Ca and P level in the diet with a constant dietary P level of 0.66% and varying levels of Ca (1.11, 0.71, and 0.31) in 4 wk old White Rock chicks. They reported an increase in soluble Ca with increasing Ca in the diet in all sections of the small intestine. Soluble P increased from the stomach to the duodenum, decreased in the jejunum and upper ileum, with an increase in the lower ileum. However, this difference in soluble P was not affected by dietary Ca. The higher dietary Ca resulted in an increase in total P in all areas of the small intestine but not in the stomach. Hurwitz and Bar

(1971) further evaluated the effect of changing dietary P (0.42, 0.56, and 0.70%) in diets with a set level of Ca at 1.00%. Increasing dietary P did not increase soluble P, but soluble Ca was increased in all segments within the intestine except in the duodenum with increasing dietary P. This positive relationship of an increase in soluble Ca with increasing P was related to an increased absorption of Ca with the lower P diets in the duodenum. However, no explanation was given for the observation that increasing P or Ca in the diet did not affect soluble P in the small intestine. They concluded that lower dietary levels of either Ca or P resulted in reduced concentrations of both minerals in the small intestine. This response implies that more of one mineral is absorbed at a low inclusion level of the other mineral. They also reported a constant relationship of 2:1 total Ca to P within the small intestine supporting the suggestion that there is a chemical relationship between Ca and P within the small intestine and not at the site of absorption.

The aforementioned research within this section utilized reports that supplemented practical levels of either Ca or P. However, consideration should be given to reports wherein there is an imbalance of either mineral. In general, high dietary Ca negatively affects growth performance of chicks. Because poultry producers supplement diets with Ca, it is critical to identify the Ca level that can be tolerated by broiler chicks, and to determine what influence, if any, is exerted by dietary P on the growth performance of broilers fed high dietary Ca. Shafey and McDonald (1990) conducted a series of experiments in which they fed varying levels of Ca or P on Ca tolerance of broiler chicks. In the first experiment, they held aP constant at 0.50% and varied Ca (1.06, 1.61, 2.12, 2.57, and 3.04%); they also kept Ca constant in 4 other treatments and varied aP (0.75, 1.00, 1.25, and 1.50%). Dietary Ca starting at 2.12% reduced the concentration of P in the tibia and plasma. Body weight gain was reduced by levels of Ca at or above 2.57%. Neither plasma Ca nor P concentrations were affected by levels higher than 0.50%

aP. However, BW gain was reduced and FCR was increased by 1.00% aP. Increasing aP to 0.76% with adequate Ca did not influence growth but increased FCR. Calcium:aP above 4:1 with adequate aP and below 1.4:1 with adequate Ca negatively affected growth performance. Dietary Ca levels of up to 2.12% with adequate aP could be tolerated by growing chicks. Similar to Hurwitz and Bar (1971), they reported that increasing dietary P level with adequate Ca resulted in no change in P retention. However, high levels of Ca with adequate aP reduced growth rate and calcification of the tibia. This response indicates that high levels of dietary Ca affect the retention of P.

Al-Masri (1995) investigated the influence of dietary Ca and P on absorption and endogenous excretion of P utilizing different Ca:P (1:1, 1.5:1, 2:1, and 2.5:1). These ratios were achieved by increasing Ca and keeping supplemental P constant (1.23% dicalcium phosphate). Increasing the Ca:P decreased the absorption of P. However, endogenous excretion of P was decreased with increasing dietary Ca. This response resulted in an increased in the percentage of P retained, but because the absorption of P was reduced, the total P retained by the whole body was reduced by increasing dietary Ca. This implies that the high rate of endogenous P excretion at the lower level of Ca resulted in a higher availability of P and that increasing dietary Ca decreased the absorption of P and endogenous P loss.

Clearly, there is evidence that the dietary Ca level affects the utilization of dietary P, even more than the dietary level of P affects P utilization. Therefore, investigation into the optimal Ca:P will serve to enhance utilization of P, and reduce environmental pollution and feed cost.

PHYTASE

Phytases are enzymes (phosphatases) that hydrolyze phosphate groups from the phytate molecule. Phytate is myo-inositol hexaphosphate, IP6, and it occurs as the storage form of P in plants. A large proportion of the P in animal feed is bound to phytate, and this P is partially

unavailable to nonruminants. The unavailability of P from phytate is due to very little endogenous phytase activity in nonruminants, and the phytase activity of various plant ingredients is relatively low. This unavailability of plant P led to development of phytase enzymes that are generally classified as 3- or 6- phytases based on the carbon atom position of the first phosphate group that is hydrolyzed. Phytase can remove up to 5 inorganic P molecules from each phytate molecule. Phytases are not all the same and each has varying characteristics that enable them to work at different pH and also on the quantity of P that they can release (Selle and Ravindran, 2007).

The use of phytase to hydrolyze phytate P in poultry nutrition is well established. However, according to Selle and Ravindran (2007), less than 35% of the phytate within diets for broilers is hydrolyzed by phytase enzymes when ileal disappearance is considered. This lack of complete hydrolysis leaves an area for improvement in developing new enzymes and establishing how common ingredients supplemented to poultry diets affect phytase efficacy in the hydrolysis of phytate to provide inorganic P.

CALCIUM EFFECT ON PHYTASE EFFICACY

Calcium supplementation is critical to poultry production; however, because sources of Ca, namely limestone and oyster shell flour, are inexpensive compared with other mineral sources, there has been little emphasis on determining the Ca requirement. The effect of Ca on the ability of phytase to hydrolyze phytate P is now receiving more attention. Applegate et al. (2003) reported that 0.90% dietary Ca reduced intestinal phytase activity by 9% and phytate P hydrolysis by 11.9% compared with broilers fed 0.40% Ca. This study was conducted with 2 different strains of broilers (Ross 308 and Hubbard × Peterson); both strains responded similarly to Ca level with regard to reducing intestinal phytase activity and apparent ileal phytate P hydrolysis. Tamim et al. (2004) utilized in vitro and in vivo techniques to investigate phytate P

hydrolysis by both a 3- and 6-phytase at varying Ca levels. At pH 6.5, the lowest Ca inclusion of 0.1% reduced phytate P hydrolysis (in vitro). They then fed 0 or 500 FTU of a 3- or 6-phytase along with 0 and 0.5% supplemented Ca. Ileal phytate P disappearance was decreased by 43.8% when Ca supplementation was increased from 0 to 0.5%. This decrease was coupled with reductions of P and Ca absorption by 38.5 and 12.7%, respectively, when Ca was increased in the diet. The 3-phytase increased phytate P disappearance by 33.5%; the improvement was less for the 6-phytase. Also, Ca absorption was increased by phytase addition and increasing Ca in the diet. This study provides useful information; however, it contained two major limitations. The first of which is that the dietary treatments were fed for only 30 h. Secondly, the level of 0.5% supplemental Ca is below the inclusion rate in the industry. This leaves the question of how broilers would respond over a longer time period with practical dietary Ca inclusion.

Driver et al. (2005) investigated the effect of Ca and nPP level on phytase efficacy. They utilized 4 levels of Ca (0.38, 0.58, 0.78, and 0.98%) and 4 levels of nPP (0.2, 0.3, 0.4, and 0.50%). Growth and bone response to phytase was greatest at the low nPP and high Ca levels, and these responses decreased when Ca was reduced and nPP was increased. The data highlight the complication associated with determining a single inorganic P equivalency for phytase. Clearly, they demonstrated that phytase efficacy is related to the level of Ca and nPP in the diets fed to broilers.

Because phytase is presently releasing less than 40% of the P bound to phytate, it is important to see if this can be increased by providing more information on the effect of Ca level on the efficacy of phytase using practical levels of Ca and P.

ADAPTATION TO DEFICIENT OR EXCESS DIETARY PHOSPHORUS OR CALCIUM

Some animals have shown the ability to grow normally on deficient levels of nutrients. The mechanisms involved in these adaptive processes are hard to explain and limit the application to practical situations. For example, Yan et al. (2005) investigated feeding deficient Ca and P levels in the starter phase on growth performance, bone characteristics, and nutrient absorption in the grower phase. They fed 2 levels of Ca and nPP in the starter phase, adequate and low. Then they divided the broilers on the adequate diet in the grower phase into an adequate and low Ca and P for that period. Broilers on the low starter continued to get a low Ca and nPP in the grower phase. For the starter phase, they reported a lower BW and ASH for broilers fed the low Ca and P diet; however, by the end of the grower phase, there was no difference in response variables. The broilers fed the low Ca and P diets for the starter phase absorbed more Ca and P in the grower phase than broilers fed the adequate diet. The ability of the broilers to compensate for Ca and P deficiency was clear; however, the mechanism whereby the broilers were able to increase absorption is still not known.

Broilers have also shown the ability to recover from high levels of dietary Ca. Ewing et al. (1995) reported on 2 flocks of broilers diagnosed with rickets, based on signs of lameness at 5 and 6 d. These broilers were fed very high Ca:P (7.7:1 or 3.5:1) due to a feed mill error; however, the feed was replaced at d 7, and at the time the broilers were processed, they performed similar to unaffected broilers. Therefore, broilers are able to overcome severe rickets caused by excess Ca in the first week of growth.

The ability of broilers to overcome moderate deficiencies of Ca or P and recover from excessive dietary Ca might prove beneficial to poultry producers, not only in salvaging flocks but in managing nutrition to reduce P in excreta. Research within this area is limited; therefore,

more benefit from this ability is still not realized. Unanswered questions regarding any benefit of feeding more Ca and P in the starter phase when feed intake is low on broiler growth performance needs further investigation.

CONCLUSIONS

There is a vast amount of literature on Ca and P, which highlights the importance of these minerals to poultry production. However, the need to reduce feed cost and environmental pollution from poultry waste will benefit from a reevaluation of the P requirement at shorter feeding intervals to match more closely with what is presently practiced within the poultry industry. Because phytase is presently releasing less than 40% of the P bound to phytate, it is important to see if this can be increased by providing more information on the effect of Ca level on the efficacy of phytase using practical levels of Ca and P. Investigation into modification of dietary Ca and P levels to increase understanding of mechanisms of adaptation will add to the vast amount of literature and provide alternatives to poultry management.

CHAPTER 3

NONPHYTATE PHOSPHORUS REQUIREMENT OF BROILERS

INTRODUCTION

The increase in demand for phosphates to be used as fertilizers for crop production has resulted in nearly a fivefold increase in the price of phosphate sources. This price increase along with environmental regulations has forced the poultry industry to find alternatives to reduce the quantity of inorganic phosphate supplemented to poultry diets. The need to feed closer to the P requirement has caused nutritionists to re-evaluate the P requirement of broilers for different stages of growth. Angel (2008), in a review of the literature including only research published after 1992, reported average requirements from hatch to 21 d of age to be 0.35 and 0.36% nonphytate P (nPP) for males and straight run broilers, respectively, based on growth performance, and 0.39 and 0.40% nPP based on bone response variables. For broilers 21 to 42 d of age, the average nPP requirement was reported as 0.30 and 0.31% based on growth performance and bone response variables, respectively. These values are lower than those recommended by the NRC (1994).

Apart from the limited data to evaluate the P requirement of broilers, the length of the growth phases is another area of concern. The NRC (1994) recommendation is based on a feeding schedule of 3 feeding phases while the industry is utilizing a 4 or 5 phase system. Because the P requirement of the broiler chick, as a proportion of the diet, changes with age (O'Rourke et al., 1952), it would be beneficial to have more phase changes to feed broilers closer to their requirement. However, there is limited published data to cover shorter growth periods.

The objective of this research was to determine nPP requirement for broilers from 0 to 7 d, 0 to 14 d, and 14 to 28 d to facilitate more options for producers in feeding closer to the nPP requirement.

MATERIALS AND METHODS

All methods used in these experiments regarding animal care were approved by the Louisiana State University Agricultural Center Animal Care and Use Committee. Two experiments (5 trials) were conducted with Ross x Ross 708 broilers. The broilers were housed in 0.76 x 3.05 m pens at the Louisiana State University Agricultural Center Poultry farm in one tunnel ventilated room with cool cells and fans. The pens contained 15 cm of fresh litter for each trial. The lighting schedule was similar to that used in the poultry industry. Hours of light for a 24 h period consisted of 4 d of 24 h of light, followed by 5 d of 20 h of light, 6 d of 18 h of light, and 16 h of light from d 15 onward where appropriate. Weather permitting, the temperature in the house was maintained at 29 to 32°C for the first 7 d post hatch and was decreased by 2°C every 7 d until temperature ranged from 21 to 24°C. Broilers were given access to feed in mash form for ad libitum consumption throughout each experimental period. Feed was fed via a feed tray for the first 7 d and then via one hanging feeder with a diameter of 43 cm. Water was provided via one automatic waterer with 6 nipples. The broilers were fed in a phased feeding program consisting of starter (0 to 14 d) and grower (14 to 28 d) where appropriate.

At the end of each trial, all broilers and feeders were weighed for the determination of ADG, ADFI, and G:F. At the end of each experimental period, 6 broilers were randomly selected, killed by CO₂ asphyxiation, and the left tibia was removed, cleaned of adhering tissue, and frozen for determination of bone breaking strength (BBS), bone ash percentage (ASH), and bone Ca and P concentrations. Bone breaking strength was determined using an HD 250 Texture Machine (Texture Technologies Corporation, Scarsdale, NY) fitted with a 3-point bend rig with a load cell capacity of 25 kg (0 to 14 d) or 250 kg (14 to 28 d) and a cross-head speed of 100 mm/min. Bone response variables are reported on a pen basis and not for the individual broiler.

Bone ash percentage was determined after ethanol and ether extraction under reflux for 36 h each, followed by at least 36 h of ashing at 550°C in a muffle furnace.

Diets were corn soybean meal (C-SBM) based and formulated for the starter phase to contain 3,025 kcal of ME/kg, 1.42% total Lys, and 1.07% total TSAA. For the grower phase, diets were formulated to contain 3,150 kcal of ME/kg, 1.24% total Lys, and 0.95% total TSAA (Table 3.1). All other nutrients met or exceeded the NRC (1994) recommended requirement except P where appropriate. Dietary total Ca and P were determined via inductively coupled plasma-emission spectroscopy after microwave (MarsXpress, CEM Corporation, Matthews, NC) digestion of 0.5 g of sample with 7 mL HNO₃ and 1 mL HCl.

Experiment 1 was conducted to determine the nPP requirement of 0 to 7 d (trials 1 and 2) or 0 to 14 d old broilers (trials 1, 2, and 3). Three trials were conducted utilizing 2,100 broilers each for trials 1 and 2 and 1,470 broilers for trial 3. For trial 1, the levels of nPP ranged from 0.30 to 0.50% in 0.05% increments with Ca:nPP of 2.22:1. Each treatment was replicated 12 times (8 males and 4 females) with 35 broilers per replicate pen with average initial and final BW of 35 and 362 g, respectively. Trial 2 was similar to trial 1 except the nPP levels ranged from 0.40 to 0.60% and broilers average initial and final BW were 36 and 316 g, respectively. Trial 3 had 7 treatments with nPP ranging from 0.35 to 0.60% in 0.05 increments for treatments 1 to 6 with Ca:nPP of 1.9:1 and treatment 7 was 0.50% nPP with Ca:nPP of 2.22:1. Each treatment was replicated 6 times (3 males and 3 females) with 35 broilers per replicate pen with average initial and final BW of 40 and 410 g, respectively.

Experiment 2 was conducted to determine the nPP requirement of 14 to 28 d old broilers. Two trials were conducted utilizing 2,100 and 1,890 broilers for trials 1 and 2, respectively. Broilers were fed a common diet consisting of 0.50% nPP and 0.95% Ca for the starter phase (0 to 14 d). For trial 1, the levels of nPP ranged from 0.25 to 0.50% with Ca:nPP of 1.9:1 and 0.25

to 0.40% nPP with Ca set at 0.90%. Trial 2 consisted of 3 levels of nPP (0.35, 0.45, and 0.55) and 3 Ca:nPP (1.9:1, 2.2:1, and 2.5:1). For each trial, treatments were replicated 6 times (3 males and 3 females) with 35 broilers per replicate pen with average initial and final BW of 415 and 807 g and 362 and 882 g for trials 1 and 2, respectively.

Data were analyzed by ANOVA procedures appropriate for randomized block designs (Steel and Torrie, 1980) using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The pen of broilers served as the experimental unit and sex was the blocking factor. There were no sex \times treatment interactions; therefore, it was removed from the model. Orthogonal contrasts were used to determine treatment differences. Individual treatment differences were obtained using the PDIFF procedure in SAS. Treatment differences were considered significant at $\alpha = 0.10$. The Proc NLIN procedure of SAS (broken line analysis) was used to determine the nPP requirement using growth and bone response variables (Robbins et al., 2006).

RESULTS

For most variables measured, there was a sex effect in that males grew faster, were more efficient and had greater bone response than females; however, there were no sex \times treatment interactions.

In trial 1 of experiment 1 (Table 3.2), increasing the nPP level from 0.30 to 0.50% had no effect ($P > 0.10$) on growth performance for the 0 to 7 d period. However, BBS, ash wt, and ASH linearly increased ($P = 0.01$) as nPP increased. From 0 to 14 d, ADG and ADFI increased ($P = 0.02$) with increasing levels of nPP; however, G:F was not affected by nPP level. Bone breaking strength increased linearly ($P = 0.01$) and quadratically ($P = 0.03$) with increasing levels of nPP. The ash wt and ASH increased linearly ($P = 0.01$) with increasing levels of nPP.

Table 3.1. Percentage composition of basal diets for 0 to 14 and 15 to 28 day old broilers¹

Experiments	1			2		
	0 to 14 d			0 to 14 d	15 to 28 d	
Trials	1	2	3	1 and 2	1	2
Ingredients						
Corn	52.18	50.49	50.80	52.21	57.83	55.57
Soybean meal (47.5%)	38.87	39.13	39.62	39.50	32.72	32.92
Poultry fat	3.36	3.92	3.69	3.20	4.38	5.16
Monocalcium phosphate	0.78	1.26	1.02	1.73	0.60	1.08
Limestone	0.69	1.09	0.59	1.06	0.32	0.63
BMD + 3 nitro ²	0.50	0.50	0.50	0.50	0.50	0.50
NaCl	0.50	0.50	0.50	0.50	0.50	0.50
DL–Met	0.34	0.34	0.33	0.33	0.28	0.29
Mineral premix ³	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix ⁴	0.25	0.25	0.25	0.25	0.25	0.25
Biolys	0.25	0.24	0.20	0.20	0.20	0.20
L-Thr	0.13	0.13	0.12	0.12	0.06	0.06
Biocox ⁵	0.05	0.05	0.05	0.05	0.05	0.05
Ethoxyquin ⁶	0.05	0.05	0.05	0.05	0.05	0.05
Choline chloride ⁷	0.05	0.05	0.05	0.05	0.05	0.05
Nutrient composition						
CP (%)	23.27	23.25	23.47	23.53	20.70	20.61
ME (kcal/kg)	3,025	3,025	3,025	3,025	3,150	3,150
Lys (%)	1.43	1.43	1.43	1.43	1.24	1.24
TSAA (%)	1.07	1.07	1.07	1.07	0.95	0.95
Thr (%)	1.00	1.00	1.00	1.00	0.83	0.83
Trp (%)	0.28	0.28	0.29	0.29	0.25	0.25
Ca (%)	0.67	0.89	0.67	0.95	0.48	0.67
Nonphytate P ⁸ (%)	0.30	0.40	0.35	0.50	0.25	0.35

¹As fed basis.

(Table 3.1 continued)

²Bacitracin methylene disalicylate + 3-nitro-4 hydroxyphenylarsonic acid (Nutra Blend, Neosha, MO).

³Provided the following per of kilogram diet: Fe, 50 mg; Mn, 100 mg; Cu, 7 mg; Se, 0.15 mg; Zn, 75 mg; I, 1 mg; as ferrous sulfate, manganese sulfate, copper sulfate, sodium selenite, zinc sulfate, and calcium iodate, respectively, with calcium carbonate as the carrier.

⁴Provided the following per kilogram of diet: vitamin A, 8,000 IU; vitamin D₃, 3,000 IU; vitamin E, 25 IU; vitamin K, 1.5 IU; riboflavin, 10 mg; pantothenic acid, 15 mg; niacin, 50 mg; vitamin B₁₂, 0.02 µg; biotin, 0.1 µg; folic acid, 1 mg; pyridoxine, 4 mg; and thiamin, 3 mg.

⁵Biocox provides 132.3 g/kg salinomycin sodium (Roche, Parsippany, NJ).

⁶Provided 66.6% 6-ethoxy-1,2-dihydro-2,2,4-trimethylquionine (Novus International, St Louis, MO).

⁷Provided 600,000 mg/kg of choline chloride.

⁸The nonphytate P levels were calculated based on NRC (1994).

In trial 2 of experiment 1 (Table 3.3), ADG was quadratically increased ($P = 0.08$) during the 0 to 7 d period and ADFI was linearly increased ($P = 0.06$) for the 0 to 14 d period as nPP increased from 0.40 to 0.60%. For the period 0 to 7 d, BBS, ash wt, and ASH linearly increased as nPP level increased; however, for the 0 to 14 d period, the responses in BBS and ASH were quadratic ($P = 0.01$ to $P = 0.03$).

No requirement estimate was achieved for the 0 to 7 d period using broken line analysis, but responses seemed to plateau at 0.45% nPP in trial 1 and at 0.55% nPP in trial 2. The estimates of an nPP requirement for 0 to 14 d old broilers by broken line analysis (Table 3.4) of the bone responses varied depending on the method used. The one-slope procedure resulted in an estimated requirement of 0.52% nPP for BBS and ASH; however, the quadratic broken line resulted in a higher estimate of 0.57% nPP.

In trial 3 of experiment 1 (Table 3.5), ADG was linearly increased ($P = 0.01$) with increasing levels of nPP; however, ADFI and G:F were not affected. Bone breaking strength and ASH increased quadratically ($P = 0.02$ to $P = 0.6$) with increasing levels of nPP. No estimate was achieved for any response during the 0 to 14 d period using broken line analysis. However, ADG and the bone response variables seemed to plateau at 0.50% nPP. There was no difference in growth performance or bone responses for broilers fed 0.50% nPP at Ca:nPP of 1.9 and 2.2.

In trial 1 of experiment 2 (Table 3.6), ADG, ADFI, and G:F were not affected by increasing the nPP from 0.25 to 0.50% at a Ca:nPP of 1.9:1; however, ADG and ADFI were linearly increased ($P = 0.01$) with the set Ca of 0.90%. There were Ca method (set vs ratio) by linear nPP interaction for ADG ($P = 0.01$), ADFI ($P = 0.04$), and G:F ($P = 0.09$). Broilers fed 0.25 and 0.30% nPP with the 0.90% Ca had lower growth performance compared with broilers fed the same levels of nPP with Ca:nPP of 1.9:1; however, at 0.35 and 0.40% nPP, broilers fed the set Ca of 0.90% had greater growth performance. Bone breaking strength, ash weight, and

ASH were linearly increased ($P < 0.01$ to $P = 0.04$) with increasing levels of nPP at Ca:nPP of 1.9:1; however, ash weight and ASH linearly ($P < 0.01$) increased with the set Ca of 0.90%. There was a Ca method by linear nPP interaction ($P = 0.01$) for ash wt in that broilers fed 0.25 and 0.30% nPP with 0.90% Ca had lower ash weight compared with those fed Ca:nPP 1.9:1 at the same levels of nPP; however, at 0.35 and 0.40% nPP broilers fed the set Ca of 0.90% had higher ash wt.

In trial 2 of experiment 2 (Table 3.7 and 3.8), increasing nPP from 0.35 to 0.55% in 0.10% increments linearly decreased ADG ($P = 0.06$) and ADFI ($P = 0.06$) but had no effect on G:F. Increasing dietary nPP resulted in a linear increase in ASH ($P = 0.02$) and ash wt ($P = 0.02$) but had no effect on BBS. Dietary Ca:nPP had no effect on the response variables measured.

DISCUSSION

Experiment 1 was conducted to estimate the nPP requirement for broilers from 0 to 7 d and from 0 to 14 d to facilitate more options for producers in feeding closer to the nPP requirement. In trial 1, feeding 0.30 to 0.50% nPP did not affect growth performance of 0 to 7 d old broilers; however, BBS, ash wt, and ASH increased with increasing levels of nPP. For 0 to 14 d old broilers, ADG, ADFI, BBS, ash wt, and ASH increased with increasing levels of nPP; however, BBS seemed to plateau at 0.45% nPP. No broken line or quadratic estimate was possible due to the continuous response to increasing dietary nPP. In trial 2, the level of nPP was increased with levels ranging from 0.40 to 0.60% nPP. For 0 to 7 d, no estimate was possible using broken line analysis; however, bone responses seemed to plateau at 0.55% nPP. Broken line analysis on data for 0 to 14 d using one slope analysis resulted in an estimate of 0.52% nPP for BBS and ASH, while one slope quadratic analysis resulted in an estimate of 0.57% nPP.

The nPP requirement estimates of 0.52 and 0.57% nPP obtained for 0 to 14 d old broilers in this experiment are similar to results reported by Runho et al. (2001). They reported available P (aP) requirements of 0.56 and 0.58% for male and female broilers, respectively, using ASH as a response criteria. However, these requirement estimates are higher than the 0.45% nPP presently recommended by the NRC (1994) and the average estimate of 0.39% calculated from a recent review of literature (Angel, 2008). Both NRC and the review of literature use data for 0 to 21 d old broilers. The higher requirement estimate we obtained may be attributed to the younger (0 to 14 d) age of the broilers. The P requirement of the broiler has been shown to decrease with age (O'Rourke et al., 1952). This decrease in P requirement with age was further supported by a continuous response to nPP levels for 0 to 7 d old broilers but not in the 0 to 14 d old broilers. This response would indicate that the nPP requirement for the 0 to 7 d old broiler is higher than for 0 to 14 d old broiler. Data for 0 to 14 d old broilers are limited, Fritts and Waldroup (2006) reported that growth performance of broilers fed 0.40% nPP for 0 to 14 d was similar to those fed 0.45% nPP; however, no bone response was considered. This estimate is similar to our findings in that growth performance of broilers was not affected by the levels of nPP fed. The lower requirement for growth is further supported by Perney et al. (1993) with an estimate of 0.32% and 0.44% nPP for growth and ASH, respectively for broilers 4 to 14 d of age.

Trial 3 was conducted to reevaluate the nPP requirement estimate at a lower level of Ca using Ca:nPP of 1.9:1 instead of the 2.2:1 used in trials 1 and 2. No estimate was possible using broken line or quadratic analysis. However, BBS and ASH seemed to plateau at 0.50% nPP (Figs. 3.1 and 3.2) and were similar to the reference diet at 0.50% nPP at Ca:nPP of 2.2:1. Therefore, these data indicate that the nPP requirement estimate for 0 to 14 d old broilers is 0.50% nPP with Ca:nPP of both 2.2:1 and 1.9:1.

Table 3.2. Growth performance, bone breaking strength, and tibia ash of 0 to 14 day old broilers fed varying levels of nonphytate P (nPP), experiment 1, trial 1¹

Treatment	nPP (%)					SEM	Sex ³	P value ²	
	0.30	0.35	0.40	0.45	0.50			Linear	Quadratic
<i>0 to 7 d</i>									
ADG (g)	14.55	14.57	14.55	14.68	14.85	0.16	0.01	NS	NS
ADFI (g)	15.40	15.31	14.78	16.12	15.00	0.42	NS	NS	NS
G:F (g:g)	0.952	0.957	0.986	0.923	0.994	0.03	0.05	NS	NS
BBS ⁴ (kg)	2.00	2.15	2.39	2.63	2.63	0.06	0.01	0.01	NS
Ash wt (g)	0.47	0.52	0.58	0.64	0.65	0.02	NS	0.01	NS
ASH ⁵ (%)	35.89	37.46	39.96	41.17	41.87	1.68	NS	0.01	NS
<i>0 to 14 d</i>									
ADG (g)	23.16	23.26	23.31	23.34	23.76	0.17	0.01	0.02	NS
ADFI (g)	28.01	28.27	28.15	28.96	28.92	0.34	0.01	0.02	NS
G:F (g:g)	0.827	0.823	0.828	0.807	0.822	0.01	NS	NS	NS
BBS (kg)	8.21	9.78	11.14	12.17	12.40	3.61	0.03	0.01	0.03
Ash wt (g)	1.69	1.92	2.08	2.22	2.35	0.05	0.01	0.01	NS
ASH (%)	44.75	46.75	47.67	49.60	50.52	0.47	NS	0.01	NS

¹Data are means of 12 replications (8 males and 4 females) with 35 broilers per replicate pen. Average initial and final BW of broilers were 35 and 362 g, respectively.

²NS = not significant, P > 0.10.

³Sex = blocking factor.

⁴BBS = bone breaking strength.

⁵ASH = percentage tibia ash.

Table 3.3. Growth performance, bone breaking strength, and tibia ash of 0 to 14 day old broilers fed varying levels of nonphytate P (nPP), experiment 1, trial 2¹

Treatment	nPP (%)					SEM	Sex ³	P value ²	
	0.40	0.45	0.50	0.55	0.60			Linear	Quadratic
<i>0 to 7 d</i>									
ADG (g)	13.93	13.69	13.48	13.73	13.78	0.17	0.01	NS	0.08
ADFI (g)	16.86	17.71	16.30	16.96	16.86	0.27	0.01	NS	NS
G:F (g:g)	0.828	0.773	0.828	0.810	0.819	0.01	NS	NS	NS
BBS ⁴ (kg)	2.13	2.08	2.15	2.40	2.40	0.07	0.01	0.01	NS
Ash wt (g)	0.39	0.40	0.41	0.45	0.46	0.01	0.03	0.01	NS
ASH ⁵ (%)	47.17	47.56	48.63	49.20	49.59	0.31	0.01	0.01	NS
<i>0 to 14 d</i>									
ADG (g)	22.62	22.34	22.52	22.61	22.66	0.23	0.01	NS	NS
ADFI (g)	31.11	32.71	31.57	32.56	32.32	0.41	0.01	0.06	NS
G:F (g:g)	0.728	0.684	0.714	0.696	0.701	0.01	NS	NS	NS
BBS (kg)	8.81	9.60	11.32	11.68	11.38	0.30	0.01	0.01	0.01
Ash wt (g)	1.53	1.63	1.91	1.91	1.96	0.05	0.01	0.01	0.03
ASH (%)	49.72	51.18	52.18	52.90	52.68	0.26	NS	0.01	0.01

¹Data are means of 12 replications (8 males and 4 females) with 35 broilers per replicate pen. Average initial and final BW of broilers were 36 and 316 g, respectively.

²NS = not significant, P > 0.10.

³Sex = blocking factor.

⁴BBS = bone breaking strength.

⁵ASH = percentage tibia ash.

Table 3.4. Estimate of the nonphytate P (nPP) requirement for 0 to 14 day old broilers using broken-line regression of bone responses in experiment 1, trial 2¹

Items ²	One-slope, straight	One-slope, quadratic
BBS (kg)	0.52	0.57
Tibia ash wt (g)	0.55	0.60
ASH ³ (%)	0.52	0.57

¹Data are means of 12 replications with 6 tibias per replicate pen.

²BBS = bone breaking strength, ASH = percentage tibia ash.

Table 3.5. Growth performance, bone breaking strength, and tibia ash of 0 to 14 day old broilers fed varying levels of nonphytate P (nPP), experiment 1, trial 3¹

Item	Ca:nPP 1.9:1						2.2:1	SEM	Sex ³	P value ²	
	nPP (%)									Linear	Quadratic
	0.35	0.40	0.45	0.50	0.55	0.60	0.50				
ADG (g)	25.99	26.08	26.17	26.96	26.60	27.04	26.34	0.39	0.01	0.01	NS
ADFI (g)	35.25	34.81	34.97	35.51	35.73	35.17	35.33	0.74	0.01	NS	NS
G:F (g:g)	0.737	0.752	0.749	0.760	0.745	0.771	0.747	0.012	NS	NS	NS
BBS ⁴ (kg)	9.81	10.31	11.14	12.64	12.30	12.42	12.42	0.40	NS	0.01	0.06
Ash wt (g)	1.72	1.85	2.05	2.24	2.38	2.44	2.25	0.09	NS	0.01	NS
ASH ⁵ (%)	49.67	51.22	51.72	52.84	52.93	53.32	52.87	0.23	NS	0.01	0.02

¹Data are means of 6 replications (3 males and 3 females) with 35 broilers per replicate pen. Average initial and final BW of broilers were 40 and 410g, respectively.

²NS = not significant, P > 0.10.

³Sex = blocking factor.

⁴BBS = bone breaking strength.

⁵ASH = percentage tibia ash.

Table 3.6. Growth performance, bone breaking strength, and tibia ash of 14 to 28 day old broilers fed varying levels of nonphytate P (nPP), experiment 2, trial 1¹

Item	Ca ²	nPP (%)						SEM	P value ³				
		0.25	0.30	0.35	0.40	0.45	0.50		Linear nPP (set and ratio Ca) ⁴	Linear nPP (ratio Ca) ⁵	Linear nPP (set Ca) ⁶	Ca (set vs ratio) ²	Linear nPP ⁴ x Ca ²
ADG (g)	Ratio	57.46	59.04	57.90	55.99	55.62	57.88						
ADG (g)	Set	56.98	56.18	58.41	61.00			1.19	NS	NS	0.01	NS	0.01
ADFI (g)	Ratio	87.68	90.53	88.67	87.23	86.76	89.61						
ADFI (g)	Set	86.39	86.90	88.43	91.39			1.44	NS	NS	0.01	NS	0.04
G:F (g:g)	Ratio	0.656	0.652	0.652	0.642	0.641	0.645						
G:F (g:g)	Set	0.659	0.646	0.660	0.667			0.01	NS	NS	NS	NS	0.09
BBS ⁷ (kg)	Ratio	24.60	23.78	24.77	27.10	26.73	28.71						
BBS (kg)	Set	25.62	27.08	27.09	28.26			1.20	0.04	0.01	NS	0.03	NS
Ash wt (g)	Ratio	6.42	6.76	6.74	7.30	7.07	7.44						
Ash wt (g)	Set	5.96	6.61	7.24	7.72			0.20	0.01	0.01	0.01	NS	0.01
ASH ⁸ (%)	Ratio	53.15	53.90	53.63	54.34	54.65	55.18						
ASH (%)	Set	52.63	53.46	54.31	54.43			0.34	0.01	0.01	0.01	NS	NS

¹Data are means of 6 replications (3 males and 3 females) with 35 broilers per replicate pen. Average initial and final BW of broilers were 415 and 844 g, respectively.

²Ca was either ratio to nPP at 1.9:1 or set at 0.90%.

³NS = not significant, P > 0.10.

⁴Linear nPP utilized nPP levels of 0.25 to 0.40% with both Ca:nPP of 1.9:1 and set Ca of 0.90%.

⁵Linear nPP utilized nPP levels of 0.25 to 0.50% with Ca:nPP of 1.9:1.

⁶Linear nPP utilized nPP levels of 0.25 to 0.40% with set Ca of 0.90%.

⁷BBS = bone breaking strength.

⁸ASH = percentage tibia ash.

Table 3.7. Growth performance, bone breaking strength, bone weight, and tibia ash of 14 to 28 day old broilers fed varying levels of nonphytate P (nPP), experiment 2, trial 2¹

Treatment		P value ^{2,3}											
Ca:nPP	1.9:1			2.2:1			2.5:1			SEM	Sex ⁴	Linear	Quadratic
nPP (%)	0.35	0.45	0.55	0.35	0.45	0.55	0.35	0.45	0.55			nPP	nPP
ADG (g)	61.57	61.30	60.91	61.75	59.09	59.15	60.82	59.77	58.76	1.12	0.01	0.06	NS
ADFI (g)	100.41	90.81	90.37	95.11	94.79	92.69	93.09	90.78	89.86	3.04	NS	0.04	NS
G:F (g:g)	0.614	0.681	0.675	0.651	0.636	0.638	0.654	0.664	0.656	0.02	0.01	NS	NS
BBS ⁵ (kg)	29.73	31.61	31.09	31.46	31.30	32.61	30.55	32.62	33.05	1.41	0.01	NS	NS
Ash wt (g)	7.19	7.60	7.38	7.42	7.54	7.88	7.17	7.43	8.01	0.25	0.01	0.02	NS
ASH ⁶ (%)	53.26	53.56	54.29	53.78	53.88	54.77	53.65	54.10	54.26	0.44	NS	0.02	NS

¹Data are means of 6 replications (3 males and 3 females) with 35 broilers per replicate pen. Average initial and final BW of broilers were 362 and 882 g, respectively.

²NS = not significant, P > 0.10.

³Ca:nPP not significant, P > 0.10.

⁴Sex = blocking factor.

⁵BBS = bone breaking strength.

⁶ASH = percentage tibia ash.

Table 3.8. Main effects of dietary nonphytate P (nPP) on growth performance, bone breaking strength, bone weight, and tibia ash of 14 to 28 day old broilers, experiment 2, trial 2¹

Main effects	P value ²						
	nPP (%)	0.35	0.45	0.55	SEM	Sex ³	Linear nPP
ADG (g)	61.38	60.05	59.61	1.12	0.01	0.06	NS
ADFI (g)	96.20	92.13	90.97	3.04	NS	0.04	NS
G:F (g:g)	0.640	0.660	0.656	0.02	0.01	NS	NS
BBS ⁴ (kg)	30.58	31.84	32.25	1.41	0.01	NS	NS
Ash wt (g)	7.26	7.52	7.76	0.25	0.01	0.02	NS
ASH ⁵ (%)	53.56	53.85	54.39	0.44	NS	0.02	NS

¹Data are means of 18 replications (9 males and 9 females) with 35 broilers per replicate pen. Average initial and final BW of broilers were 362 and 882 g, respectively.

²NS = not significant, P > 0.10.

³Sex = blocking factor.

⁴BBS = bone breaking strength.

⁵ASH = percentage tibia ash.

Experiment 2 was conducted to estimate the nPP requirement of 14 to 28 d old broilers. The levels of nPP fed ranged from 0.25 to 0.50% at Ca:nPP of 1.9:1. These levels of nPP were chosen based on the assumption that the P requirement decreases with age. No requirement estimate was possible using broken line or quadratic analysis. Both BBS and ASH linearly increased with increasing levels of nPP. Reference diets fed with a set level of 0.90% Ca with nPP levels ranging from 0.25 to 0.40% also showed linear increases in ADG, ADFI, ash wt, and ASH. Broilers fed the 0.90% Ca had greater growth and bone responses at the higher nPP levels of 0.35 and 0.40% nPP compared with those fed the Ca:nPP of 1.9:1. Therefore, trial 2 was conducted to investigate these higher levels of nPP (0.35, 0.45, and 0.55) at 3 Ca:nPP (1.9:1, 2.2:1, and 2.5:1). Daily gain and ADFI were decreased as nPP increased. However, ASH and ash and bone wt increased as level of nPP increased. The growth performance and BBS data indicate a requirement estimate of no greater than 0.45 % nPP, which agrees with the data of Rao et al. (1999) who recommended an nPP requirement of 0.44% for weight gain. Rao et al. (1999) also predicted nPP requirement of 0.74% aP for optimizing tibia ash based on regression coefficients. This higher level of nPP for ASH was also implied by our data.

In summary, data from these experiments suggest an nPP requirement for 0 to 14 d old broilers of 0.50% and not greater than 0.45% for broilers 14 to 28 d old.

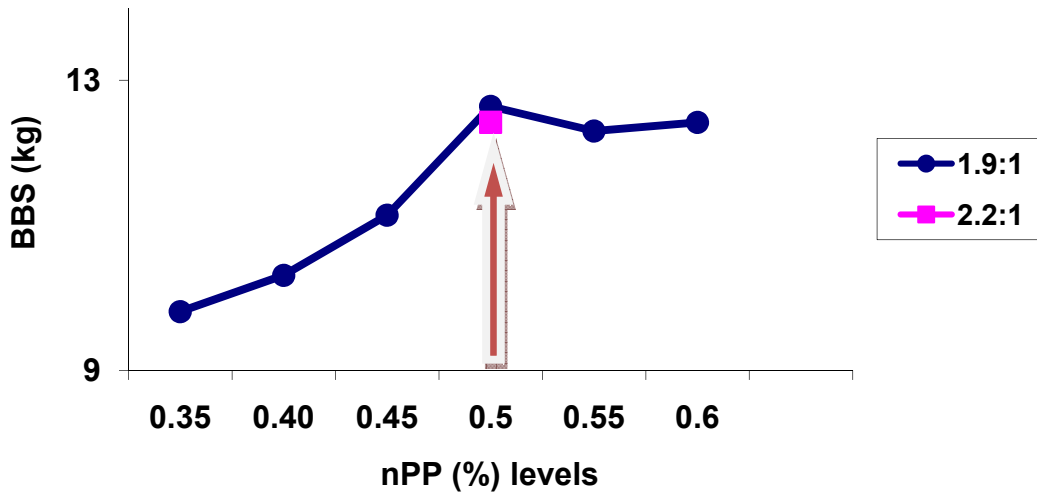


Figure 3.1. Bone breaking strength (BBS) of 0 to 14 day old broilers fed varying levels of nonphytate P (nPP) with Ca:nPP of 1.9:1, experiment 1, trial 3. SEM = 0.40.

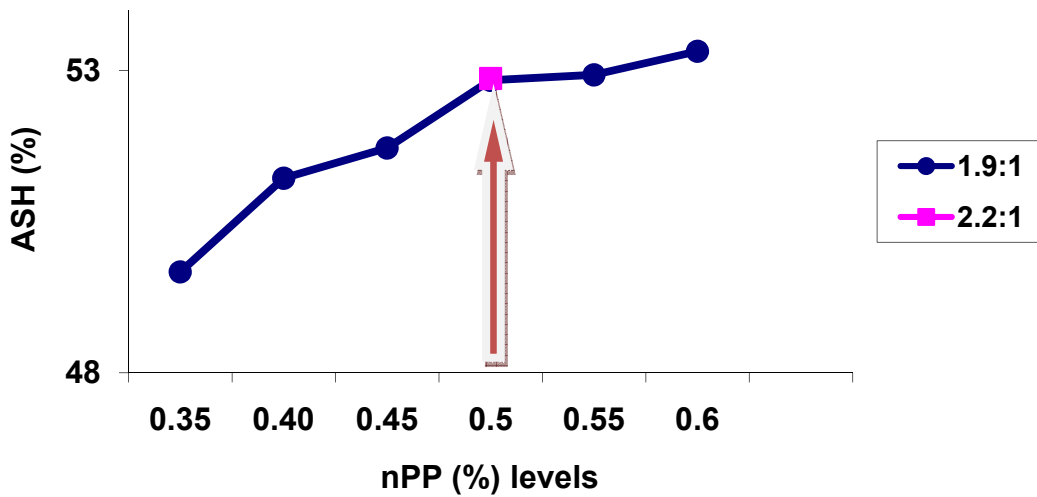


Figure 3.2. Percentage tibia ash (ASH) of 0 to 14 day old broilers fed varying levels of nonphytate P (nPP) with Ca:nPP of 1.9:1, experiment 1, trial 3. SEM = 0.23.

CHAPTER 4

THE EFFECT OF CALCIUM AND PHOSPHORUS LEVELS ON CALCIUM AND PHOSPHORUS AVAILABILITY AND OPTIMAL CALCIUM PHOSPHORUS RATIO

INTRODUCTION

The supplementation of Ca and P to poultry diets is critical for skeleton growth, which is of importance to successful poultry production. Presently, the poultry industry is faced with making critical decisions because of the increasing cost of P sources. The cost of Ca is less than the cost of P; therefore, less attention is given to Ca. However, it is generally accepted that the level of Ca in diets affects the availability of both Ca and P.

The majority of the P in poultry diets is in the form of phytate P, which is generally unavailable to poultry. This unavailable source of P recently has received a lot of attention. The commercial availability of phytase; an enzyme that is used to hydrolyze P from phytate, has led to investigation into optimizing the hydrolysis of phytate. The level of Ca to total P, available P (aP), or nonphytate P (nPP) in the diet has been shown to affect phytate P hydrolysis (Angel et al., 2002). Plumstead et al. (2008) recommended an optimum ratio of Ca:nPP of 2.53:1, 2.40:1, and 2.34:1 for diets containing 0.28, 0.24, and 0.10% phytate, respectively. They reported a decrease in P digestibility with increasing dietary Ca.

Apart from the high level of dietary Ca decreasing the availability of P from phytate P, Ca level also affects the quantity of P absorbed by the broiler chick. Al-Masri (1995) reported that increasing dietary Ca level resulted in decreased P absorption and retention. However, increasing Ca had a greater effect on P absorption than on P retention. This response is attributable to a decrease in endogenous secretion of P in broilers with reduced P absorption at the high level of dietary Ca. The unavailability of P in high Ca diets stems from an increase in insoluble P in the intestine of broilers. Hurwitz and Bar (1971) reported that high dietary Ca

resulted in an increase in total P in all sections of the small intestine but not the stomach; however, this increase was not seen in soluble P. The increase in insoluble Ca and P confirmed the precipitation of a chemical with a constant ratio of Ca to P in the small intestine. This increase in insoluble P would imply that absorption of P is influenced by dietary Ca. Low dietary levels of either mineral resulted in less of the other mineral in the intestine, implying that more of one mineral is absorbed at a lower level of the other.

The essentiality of Ca and P to skeleton development is critical to poultry development; therefore, decreasing Ca to facilitate P utilization must be carefully investigated. Therefore, the objectives of this research were to identify the Ca to P ratio that would optimize performance in 0 to 18 d and 14 to 28 d old broilers, as well as to evaluate the effect of either mineral at varying ratios on their respective digestibility and utilization.

MATERIALS AND METHODS

All methods used in these experiments regarding animal care were approved by the Louisiana State University Agricultural Center Animal Care and Use Committee. Two experiments were conducted with Ross x Ross 708 broilers.

Diets were corn soybean meal (C-SBM) based and formulated for the starter phase (0 to 18 d in experiment 1 and 0 to 14 d in experiment 2) to contain 3,150 kcal of ME/kg (experiment 1) and 3,025 kcal of ME/kg (experiment 2), 1.43% total Lys, and 1.07% total TSAA. The diets for the grower phase (14 to 28 d: experiment 2) were formulated to contain 3,150 kcal of ME/kg, 1.24% total Lys, and 0.95% total TSAA (Table 4.1). Diets were adequate in all nutrients (NRC, 1994) except for Ca and nPP where appropriate. Diets were formulated to meet the highest level of Ca and P, and sand was used to replace Ca and P to achieve the treatment diets. Feed in mash form and water were provided for ad libitum consumption throughout the experimental periods.

Experiment 1 consisted of two 18 d trials. For each trial, broilers were housed in environmentally controlled Petersime brooder batteries (Petersime Incubator Co., Gettysburg, OH) with pen size of 38 x 100 cm and raised wire floors. Continuous fluorescent lighting was provided for the duration of each trial. Broilers were weighed, wing banded, and randomly allotted on the day of hatch to dietary treatment.

In experiment 1 trial 1, seven hundred and twenty broilers with average initial and final BW of 35 and 550 g, respectively were utilized. Five levels of nPP ranging from 0.30 to 0.50% in 0.05% increments and 3 Ca:nPP (1.9:1, 2.2:1, and 2.5:1) were utilized, resulting in 15 treatment diets in a 5×3 factorial arrangement of treatments. Each treatment diet was replicated with 6 pens (3 males and 3 females) of 8 broilers per replicate pen. In trial 2, one hundred and forty four male broilers with an average initial and final BW of 41 and 641 g, respectively, were utilized. Dietary treatments consisted of 4 Ca:nPP (1.5:1, 1.7:1, 1.9:1, and 2.1:1) at a set nPP level of 0.50%, resulting in 4 treatment diets. Each treatment diet was replicated with 6 pens of 6 broilers per replicate pen.

In experiment 1 trial 1, on d 14, 0.50% chromic oxide was added to the treatment diets. Excreta were collected on d 16, 17, and 18 and samples pooled by pen and day for calculation of Ca and P utilization. Ileal samples were collected from the 8 broilers per pen and pooled by replicate pen to determine apparent ileal digestibility of Ca and P. Ileal samples were collected from the Meckels diverticulum to 1 cm proximal to the ileal cecal junction. Calcium, P, and Cr contents of the diets, excreta, and ileal samples were determined after acid hydrolysis. Dietary Ca and total P were determined via inductively coupled plasma-emission spectroscopy after microwave (MarsXpress, CEM Corporation, Matthews, NC) digestion of 0.5 g of sample with 7 mL HNO₃ and 1 mL HCl. Chromium was determined after digestion on a hot plate of a 0.5 g sample with a 70:30 mixture of perchloric and nitric acid. Apparent P and Ca digestibility (ileal

digesta) and utilization (excreta) were calculated as $[100 - ((\text{Marker}_{\text{diet}} \times \text{Nutrient}_{\text{sample}} / \text{Marker}_{\text{sample}} \times \text{Nutrient}_{\text{diet}}) \times 100)]$.

In experiment 2, the broilers were housed in 0.76 x 3.05 m pens at the Louisiana State University Agricultural Center Poultry Farm in one tunnel ventilated room with cool cells and fans. The pens contained 15 cm of fresh litter at the start of the experiment. The lighting schedule was similar to that used in the poultry industry. Hours of light for a 24 h period consisted of 4 d of 24 h of light, followed by 5 d of 20 h of light, 6 d of 18 h of light, and 16 h of light from d 15 onward where appropriate. Weather permitting, the temperature in the house was maintained at 29 to 32°C for the first 7 d post hatch and was decreased by 2°C every 7 d until temperature ranged from 21 to 24°C. Feed was fed via a feed tray for the first 7 d and then a hanging feeder with a diameter of 43 cm. Water was provided via one automatic waterer with 6 nipples.

In experiment 2, six hundred broilers were fed a common diet for the starter phase (0 to 14 d) and were allotted to treatment on d 14. Dietary treatment consisted of a reference diet consisting of 0.35% nPP and Ca:nPP of 2.57:1 and 3 levels of nPP (0.25, 0.35, and 0.45%) and 3 Ca:nPP (1.6:1, 1.9:1, and 2.2:1). Each treatment diet was replicated with 6 pens of 10 broilers per replicate pen with average initial and final BW of 410 and 966 g, respectively.

At the end of each trial in both experiments, all broilers and feeders were weighed for the determination of ADG, ADFI, and G:F. Broilers were selected (all 8 for experiment 1 trial 1 and all 6 for experiment 1 trial 2 and 6 randomly for experiment 2), killed by CO₂ asphyxiation and the left tibia was removed, cleaned of adhering tissue, and frozen for determination of bone breaking strength (BBS), bone ash percentage (ASH), and bone Ca and P concentrations. Bone breaking strength was determined using an HD 250 Texture Machine (Texture Technologies Corporation, Scarsdale, NY) fitted with a 3-point bend rig with a load cell capacity of 25 kg (0 to

18 d) or 250 kg (14 to 28 d) and a cross-head speed of 100 mm/min. Bone response variables were reported on a pen basis and not for individual broilers. Bone ash percentage was determined after ethanol and ether extraction under reflux for 36 h each followed by at least 36 h of ashing at 550°C in a muffle furnace.

Data were analyzed by ANOVA procedures appropriate for randomized block designs (Steel and Torrie, 1980) using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The pen of broilers served as the experimental unit and sex was the blocking factor. There were no sex \times treatment interactions; therefore, it was removed from the model. Orthogonal contrasts were used to determine treatment differences. Individual treatment differences were obtained using the PDIFF procedure in SAS. Treatment differences were considered significant at $\alpha = 0.10$.

RESULTS

In trial 1 of experiment 1 (Table 4.2) for the growth period 0 to 14 and 0 to 18 d, increasing the P level of the diet from 0.30 to 0.50% nPP increased ADG (nPP linear, $P = 0.021$ and 0.001 for 0 to 14 and 0 to 18 d, respectively) and G:F (nPP linear, $P = 0.003$ and 0.03 for 0 to 14 and 0 to 18 d, respectively). Daily gain and ADFI plateaued at 0.40% nPP regardless of the Ca:nPP resulting in quadratic effects ($P = 0.027$ to 0.051). Increasing the Ca:nPP from 1.9 to 2.5 linearly decreased ADG ($P < 0.001$) and ADFI ($P = 0.002$); however, G:F was not affected. The decrease in ADG and ADFI for 0 to 18 d was greater at the lower level of nPP at the Ca:nPP of 2.5:1 (nPP linear \times Ca:nPP, $P = 0.032$ and 0.056 , respectively). Bone breaking strength and ASH (Table 4.3) increased up to 0.45% nPP with no difference between 0.45 and 0.50% nPP (nPP quadratic, $P < 0.001$). Increasing the dietary Ca:nPP level had no effect on BBS or ASH. However, there was a nPP linear \times Ca:nPP quadratic interaction for BBS ($P = 0.09$) in that BBS increased with increasing levels of nPP at Ca:nPP of 1.9:1 and 2.2:1 but not at Ca:nPP of 2.5:1.

Table 4.1. Percentage composition of basal diet for 0 to 18 and 14 to 28 day old broilers¹

Experiments	1		2	
	0 to 18 d		0 to 14 d	
	1	2	1	
Ingredients				
Corn	48.85	50.00	50.80	57.90
Soyabean meal (47.5%)	39.96	39.69	39.62	32.72
Poultry fat	5.72	5.36	3.69	4.35
Monocalcium phosphate	0.78	1.74	1.73	0.59
Limestone	0.62	0.72	1.06	0.12
DL-Met	0.34	0.34	0.34	0.28
NaCl	0.50	0.50	0.50	0.50
Mineral premix ²	0.25	0.25	0.25	0.25
Vitamin premix ³	0.25	0.25	0.25	0.25
Biolys	0.20	0.20	0.20	0.20
L-Thr	0.12	0.12	0.12	0.06
Choline chloride ⁴	0.05	0.05	0.05	0.05
BMD + 3 nitro ⁵	----	----	0.50	0.50
Biocox ⁶	----	----	0.05	0.05
Ethoxyquin ⁷	----	----	0.05	0.05
Sand ⁸	2.37	0.79	0.79	2.13
Nutrient composition				
CP (%)	23.46	23.44	23.47	20.70
ME (kcal/kg)	3,150	3,150	3,025	3,150
Lys (%)	1.43	1.43	1.43	1.24
TSAA (%)	1.07	1.07	1.07	0.95
Ca (%)	0.57	0.75	0.95	0.40
Nonphytate P ⁹ (%)	0.30	0.50	0.50	0.25

¹As fed basis.² Provided the following per of kilogram diet: Fe, 50 mg; Mn, 100 mg; Cu, 7 mg; Se, 0.15 mg; Zn, 75 mg; I, 1 mg; as ferrous sulfate, manganese sulfate, copper sulfate, sodium selenite, zinc sulfate, and calcium iodate, respectively, with calcium carbonate as the carrier.

(Table 4.1 continued)

³Provided the following per kilogram of diet: vitamin A, 8,000 IU; vitamin D₃, 3,000 IU; vitamin E, 25 IU; vitamin K, 1.5 IU; riboflavin, 10 mg; pantothenic acid, 15 mg; niacin, 50 mg; vitamin B₁₂, 0.02 µg; biotin, 0.1 µg; folic acid, 1 mg; pyridoxine, 4 mg; and thiamin, 3 mg.

⁴Provided 600,000 mg/kg of choline chloride.

⁵Bacitracin methylene disalicylate + 3-nitro-4 hydroxyphenylarsonic acid (Nutra Blend, Neosha, MO).

⁶Biocox provides 132.3 g/kg salinomycin sodium (Roche, Parsippany, NJ).

⁷Provided 66.6% 6-ethoxy-1,2-dihydro-2,2,4-trimethylquionine (Novus International, St Louis, MO).

⁸Sand was replaced by limestone and monocalcium phosphate to achieve the desired dietary Ca and P levels.

⁹The nonphytate P levels were calculated based on NRC (1994).

In trial 1 of experiment 1 (Table 4.4, Figure 4.1), increasing nPP level of the diet resulted in a decrease ($P > 0.001$) in both apparent excreta Ca utilization (ADCa) and ileal Ca digestibility (ADICa). Increasing nPP level of the diet resulted in a decrease ($P < 0.001$) in both apparent excreta P utilization (ADP) and ileal P digestibility (ADIP). However, there was no difference between P digestibility and utilization at 0.45 and 0.50% nPP resulting in a quadratic effect ($P = 0.002$ and 0.021 for ADP and ADIP, respectively). Increasing Ca:nPP (Figure 4.2), resulted in a decrease in both ADCa and ADICa at Ca:nPP of 2.2:1 (quadratic, $P = 0.05$ and 0.07 , respectively). Increasing Ca:nPP, resulted in a decrease ($P < 0.001$) in ADIP; however, there was a quadratic effect ($P = 0.006$) on ADP wherein at Ca:nPP of 2.2:1 ADP increased then decreased at Ca:nPP of 2.5:1.

In trial 2 of experiment 1 (Table 4.5), increasing the Ca level of the diets from 0.75 to 1.05% had no effect on growth performance. However, BBS ($P = 0.095$) and ASH ($P = 0.024$) linearly increased with increasing dietary Ca (Table 4.6).

In experiment 2 (Table 4.7), increasing the nPP level from 0.25 to 0.45% nPP and the Ca:nPP from 1.6:1 to 2.2:1 had no effect on growth performance. Bone breaking strength, ASH, bone wt, and ash wt increased with increasing levels of nPP (linear nPP, $P < 0.001$). The effect of nPP level on bone and ash wt was also quadratic ($P = 0.083$ and 0.077). Similarly, there was an increase in both BBS ($P < 0.001$), ASH ($P = 0.009$), and ash wt ($P = 0.044$) with increasing Ca:nPP from 1.6:1 to 2.2:1.

DISCUSSION

Experiment 1 was conducted to identify the optimal Ca:nPP for 0 to 18 d old broilers and the effect of varying Ca:nPP and nPP levels on digestibility and utilization of Ca and P. Feed efficiency increased with increasing levels of nPP; however, ADG and ADFI seemed to plateau at 0.40% nPP. Increasing the Ca:nPP from 1.9:1 to 2.5:1 resulted in a decrease in ADG and

Table 4.2. Growth performance of 0 to 18 day old broilers fed varying levels of nonphytate P (nPP) and Ca:nPP, experiment 1, trial 1¹

Treatment		0 to 14 d			0 to 18 d		
Ca:nPP	nPP	ADG	ADFI	G:F	ADG	ADFI	G:F
	(%)	(g)	(g)	(g:g)	(g)	(g)	(g:g)
1.9:1	0.30	21.77	27.29	0.799	28.29	36.25	0.781
	0.35	21.12	26.81	0.789	28.39	36.04	0.791
	0.40	23.34	28.82	0.810	29.93	38.17	0.785
	0.45	23.26	28.38	0.821	29.94	37.79	0.794
	0.50	22.85	27.93	0.818	30.41	37.76	0.806
2.2:1	0.30	21.58	26.95	0.802	28.11	35.94	0.783
	0.35	22.13	27.88	0.794	28.91	37.63	0.769
	0.40	22.24	27.58	0.806	29.65	37.05	0.801
	0.45	22.03	27.29	0.807	28.72	36.39	0.789
	0.50	21.73	26.58	0.818	28.36	35.58	0.797
2.5:1	0.30	20.04	25.24	0.794	26.24	33.56	0.782
	0.35	21.16	26.46	0.800	27.85	35.68	0.781
	0.40	21.68	27.08	0.800	28.12	36.43	0.772
	0.45	21.52	26.73	0.805	28.29	35.72	0.792
	0.50	21.27	26.42	0.805	28.21	35.78	0.789
SEM		0.56	0.70	0.008	0.565	0.873	0.010
<i>nPP main effect</i>							
	0.30	21.13	26.49	0.798	27.55	35.25	0.782
	0.35	21.47	27.05	0.794	28.38	36.45	0.780
	0.40	22.42	27.83	0.805	29.23	37.22	0.786
	0.45	22.27	27.47	0.811	28.93	36.63	0.792
	0.50	21.95	26.98	0.814	28.99	36.37	0.797
<i>Ca:nPP main effect</i>							
	1.9:1	22.47	27.85	0.807	29.39	37.20	0.791
	2.2:1	21.94	27.26	0.805	28.75	36.52	0.788
	2.5:1	21.13	26.39	0.801	27.74	35.43	0.783
Contrasts					P > F ²		

(Table 4.2 continued)

nPP linear	0.021	NS	0.003	0.001	NS	0.03
nPP quadratic	0.051	0.036	NS	0.027	0.027	NS
Ca:nPP linear	<0.001	0.002	NS	<0.001	0.002	NS
Ca:nPP quadratic	NS	NS	NS	NS	NS	NS
nPP linear x Ca:nPP linear	NS	NS	NS	NS	NS	NS
nPP linear x Ca:nPP quadratic	NS	NS	NS	0.032	0.056	NS

¹Data are means of 6 replications with 8 broilers per replicate pen. Average initial and final BW were 35 and 550 g, respectively, and the experiment lasted from 0 to 18 d post-hatching.

²NS = not significant, $P > 0.10$.

Table 4.3. Bone breaking strength and tibia ash of 0 to 18 day old broilers fed varying levels of nonphytate P (nPP) and Ca:nPP, experiment 1, trial 1¹

Treatment			
Ca:nPP	nPP (%)	BBS ² (kg)	ASH ³ (%)
1.9:1	0.30	11.92	48.87
	0.35	13.62	50.45
	0.40	15.00	51.28
	0.45	17.42	52.18
	0.50	19.05	52.68
2.2:1	0.30	12.40	49.49
	0.35	13.95	50.30
	0.40	16.78	51.56
	0.45	17.44	51.93
	0.50	17.38	52.61
2.5:1	0.30	11.03	49.51
	0.35	13.75	50.42
	0.40	15.84	51.26
	0.45	18.36	52.83
	0.50	17.44	52.62
SEM		0.64	0.28
<i>nPP main effect</i>			
	0.30	11.78	49.29
	0.35	13.77	50.39
	0.40	15.87	51.37
	0.45	17.74	52.31
	0.50	17.97	52.64
<i>Ca:nPP main effect</i>			
1.9:1		15.40	51.09
2.2:1		15.59	51.18
2.5:1		15.28	51.33
Contrasts		P > F ⁴	

(Table 4.3 continued)

nPP linear	<0.001	<0.001
nPP quadratic	0.007	0.011
Ca:nPP linear	NS	NS
Ca:nPP quadratic	NS	NS
nPP linear x Ca:nPP linear	NS	NS
nPP linear x Ca quadratic	0.090	NS

¹Data are means of 6 replications with tibia collected from 8 broilers per replicate pen. Average initial and final BW were 35 and 550 g, respectively.

²BBS = bone breaking strength.

³ASH = percentage tibia ash.

⁴NS = not significant, $P > 0.10$.

Table 4.4. Apparent digestibility and utilization of Ca and P of 0 to 18 day old broilers fed varying levels of nonphytate P (nPP) and Ca:nPP, experiment 1, trial 1¹

Ca:nPP	nPP (%)	ADCa ²	ADICa	ADP	ADIP
1.9:1	0.30	58.65	59.10	54.57	56.32
	0.35	62.20	45.93	50.88	47.90
	0.40	59.19	51.55	49.64	50.07
	0.45	50.33	46.46	47.38	46.83
	0.50	45.80	39.65	45.98	44.44
2.2:1	0.30	51.93	48.77	55.36	51.40
	0.35	51.39	41.06	51.92	45.72
	0.40	48.05	40.65	50.57	43.89
	0.45	50.96	37.38	48.72	44.24
	0.50	44.73	34.98	48.46	42.56
2.5:1	0.30	60.51	55.31	54.50	46.71
	0.35	54.27	50.63	52.96	45.38
	0.40	52.07	38.79	50.37	41.49
	0.45	51.92	38.31	37.24	36.13
	0.50	46.10	33.68	48.29	39.39
SEM		4.22	5.29	1.18	1.61
Contrasts				P > F ³	
nPP linear		<0.001	<0.001	<0.001	<0.001
nPP quadratic		NS	NS	0.002	0.021
Ca:nPP linear		NS	NS	NS	<0.001
Ca:nPP quadratic		0.050	0.068	0.006	NS
nPP linear x Ca:nPP linear		NS	NS	NS	NS
nPP linear x Ca:nPP quadratic		NS	NS	NS	NS

¹ Data are means of 6 replications with 8 broilers per replicate. Ileal samples were collected and pooled from 8 broilers per replicate pen. Average initial and final BW were 35 and 550 g, respectively, and the experiment lasted from 0 to 18 d post-hatching.

² ADCa = Apparent Ca utilization, ADICa = Apparent ileal Ca digestibility, ADP = Apparent P utilization, and ADIP = Apparent ileal P digestibility.

³ NS = not significant, P > 0.10.

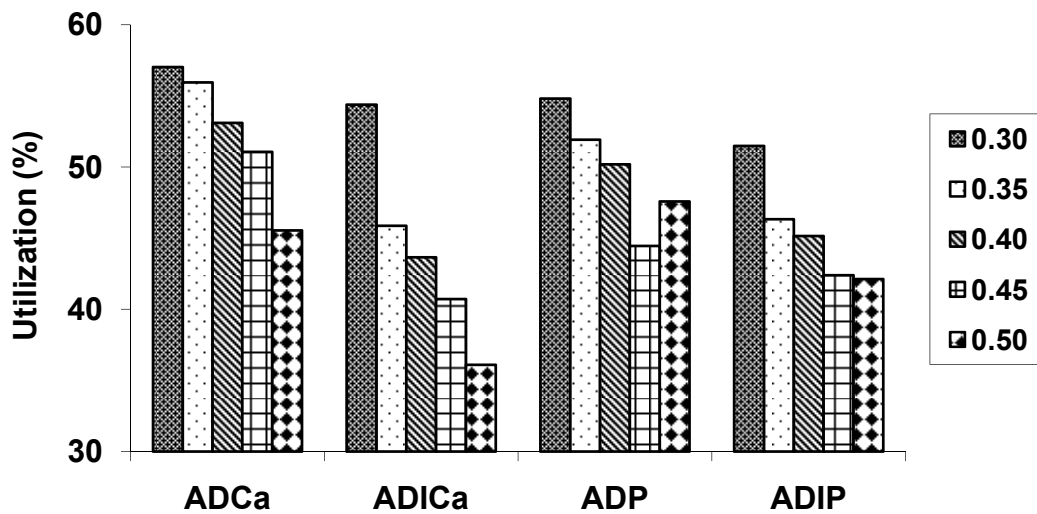


Figure 4.1. Main effect of nonphytate P (nPP) on apparent Ca and P digestibility and utilization of 0 to 18 day old broilers fed varying levels of nPP and Ca:nPP, experiment 1, trial 1. ADCa = apparent Ca utilization (SEM = 4.22), ADICa = apparent ileal Ca digestibility (SEM = 5.29), ADP = apparent P utilization (SEM = 1.18), and ADIP = apparent ileal P digestibility (SEM = 1.61).

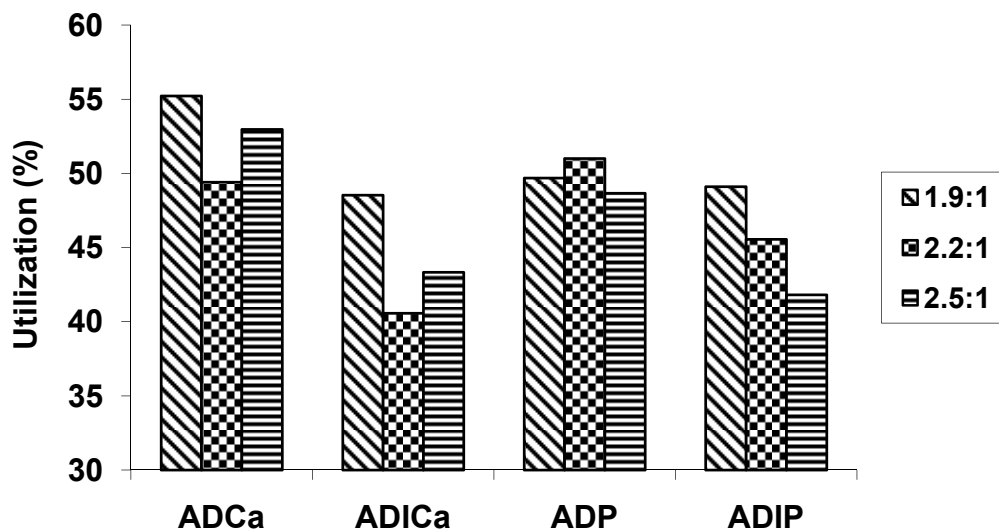


Figure 4.2. Main effect of Ca ratio to nonphytate P (nPP) on apparent Ca and P digestibility and utilization of 0 to 18 day old broilers fed varying levels of nPP and Ca:nPP, experiment 1, trial 1. ADCa = apparent Ca utilization (SEM = 4.22), ADICa = apparent ileal Ca digestibility (SEM = 5.29), ADP = apparent P utilization (SEM = 1.18), and ADIP = apparent ileal P digestibility (SEM = 1.61).

Table 4.5. Growth performance of 0 to 18 day old broilers fed 0.50% nonphytate P (nPP) with varying levels of Ca, experiment 1, trial 2¹

Treatment		0 to 14 d			0 to 18 d		
Ca:nPP	Ca	ADG	ADFI	G:F	ADG	ADFI	G:F
	(%)	(g)	(g)	(g:g)	(g)	(g)	(g:g)
1.5:1	0.75	27.57	33.70	0.818	32.94	42.29	0.779
1.7:1	0.85	27.74	33.76	0.821	33.70	42.89	0.786
1.9:1	0.95	27.89	33.22	0.840	33.96	41.89	0.811
2.1:1	1.05	26.33	32.34	0.832	32.58	41.91	0.794
SEM		0.61	0.61	0.01	0.74	0.71	0.01
Contrasts				P > F ²			
Ca:nPP linear		NS	NS	NS	NS	NS	NS
Ca:nPP quadratic		NS	NS	NS	NS	NS	NS

¹Data are means of 6 replications with 6 broilers per replicate pen. Average initial and final BW were 41 and 641 g, respectively, and the experiment lasted from 0 to 18 d post-hatching.

²NS = not significant, P > 0.10.

Table 4.6. Bone breaking strength and tibia ash of 0 to 18 day old broilers fed 0.50% nonphytate P (nPP) with varying levels of Ca, experiment 1, trial 2¹

Treatment		0 to 18d	
Ca:nPP	Ca	BBS ²	ASH ³
1.5:1	0.75	19.29	50.42
1.7:1	0.85	20.50	51.24
1.9:1	0.95	21.59	51.29
2.1:1	1.05	21.31	51.53
SEM		0.89	0.30
Contrasts		P > F ⁴	
Ca:nPP linear		0.095	0.024
Ca:nPP quadratic		NS	NS

¹Data are means of 6 replications with 6 broilers per replicate pen. Average initial and final BW were 41 and 641 g, respectively, and the experiment lasted from 0 to 18 d post-hatching.

²BBS = bone breaking strength.

³ASH = percentage tibia ash.

⁴NS = not significant, P > 0.10.

Table 4.7. Growth performance and bone characteristics of 14 to 28 day old broilers fed varying levels of nonphytate P (nPP) and Ca:nPP, experiment 2¹

Ca:nPP	nPP (%)	ADG (g)	ADFI (g)	G:F (g)	BBS ² (kg)	ASH ³ (%)	Bone wt (g)	Ash wt (g)
2.57:1	0.35	65.44	108.48	0.602	33.22	53.25	14.96	7.96
1.6:1	0.25	64.34	103.53	0.619	21.94	50.81	11.69	5.94
	0.35	66.80	104.95	0.636	25.23	51.57	13.86	7.15
	0.45	67.17	105.69	0.635	30.71	52.95	15.63	8.28
1.9:1	0.25	67.10	104.50	0.641	27.02	51.30	12.37	6.34
	0.35	67.85	105.89	0.639	27.66	52.69	14.26	7.52
	0.45	65.50	104.61	0.625	32.50	53.16	15.75	8.39
2.2:1	0.25	66.86	107.14	0.623	25.72	51.28	12.14	6.22
	0.35	65.12	102.84	0.632	31.68	52.76	14.87	7.84
	0.45	64.92	103.56	0.626	32.83	53.31	15.58	8.31
SEM		1.24	1.69	0.008	1.15	0.30	0.38	0.20
<i>nPP main effect</i>								
	0.25	66.1	105.06	0.628	24.89	51.13	12.07	6.17
	0.35	66.59	104.56	0.636	28.19	52.34	14.33	7.50
	0.45	65.86	104.62	0.629	32.01	53.14	15.65	8.33
<i>Ca:nPP main effect</i>								
	1.6:1	66.10	104.72	0.630	25.96	51.78	13.73	7.12
	1.9:1	66.93	105.00	0.635	29.06	52.38	14.13	7.42
	2.2:1	65.63	104.51	0.627	30.08	52.45	14.20	7.46
Contrast					P > F ⁴			
nPP linear		NS	NS	NS	<0.001	<0.001	<0.001	<0.001
nPP quadratic		NS	NS	NS	NS	NS	0.083	0.077
Ca:nPP linear		NS	NS	NS	<0.001	0.009	NS	0.044
Ca:nPP quadratic		NS	NS	NS	NS	NS	NS	NS

¹Data are means of 6 replications with 10 broilers per replicate pen. Average initial and final BW were 410 and 966 g, respectively, and the experiment lasted from 0 to 28 d post-hatching.

²BBS = bone breaking strength.

³ASH = percentage tibia ash.

⁴NS = not significant, P > 0.10.

ADFI; this decrease in growth response was greater at the lower levels of nPP at Ca:nPP 2.5:1 (Figures 4.3 and 4.4). These results indicate that a Ca:nPP of 1.9:1 for 0 to 18 d old broilers optimizes growth performance. A similar response was observed in BBS as nPP increased at Ca:nPP of 2.2:1 and 2.5:1, but BBS plateaued at 0.45% nPP. For bone response variables, increasing the Ca:nPP did not affect ASH; however, BBS was increased with increasing nPP levels at Ca:nPP of 1.9:1 (Figure 4.5). Therefore, the Ca:nPP that optimize bone responses seems to be 0.50% nPP at Ca:nPP of 1.9:1 or 0.45% nPP at Ca:nPP of 2.2:1 or 2.5:1. The Ca:nPP of 1.9:1 is lower than what is presently recommended by the NRC (1994). Also, in a recent review, Angel (2008) reported a Ca:nPP of 2.54:1 and 2.58:1 for males and straight run broilers, respectively, based on nPP requirements of 0.35 and 0.36% nPP and Ca requirement of 0.89 and 0.93%. The results from our experiment do not agree with the summary of Angel (2008) because at Ca:nPP of 2.5:1, there was a decrease in ADG and ADFI compared with broilers fed Ca:nPP of 1.9:1 and 2.2:1.

The linear decrease in growth performance with increasing Ca:nPP might be a direct result of the decrease in digestibility and utilization of P and Ca. Digestibility and utilization of P and Ca decreased with increasing levels of nPP; however, P digestibility and utilization were not different at 0.45 and 0.50% nPP. Ileal digestibility of P was decreased with increasing Ca:nPP, while Ca digestibility and utilization were greatest at Ca:nPP of 1.9:1. This general decrease in P and Ca digestibility and utilization with increasing Ca:nPP was even more detrimental at the higher Ca:nPP because the Ca and P intake were reduced. The decrease in P digestibility observed in this experiment with increasing Ca:nPP is similar to that reported by Plumstead et al. (2008) who reported a decrease in apparent prececal P digestibility with increasing dietary Ca. However, they concluded that Ca:nPP ranging from 2.3:1 to 2.5:1 is the optimum range of ratio that resulted in the highest P retention when phytate P was increased from 0.10 to 0.28%. This is

higher than the 1.9:1 Ca:nPP identified in this experiment; however, the level of nPP fed by Plumstead et al. (2008) was 0.35% compared with a wider range fed in our experiment. The lower nPP fed by Plumstead et al. (2008) could account for the difference in Ca absorption resulting in a higher Ca:nPP. Hurwitz and Bar (1971) reported that a greater absorption of Ca occurs with a low P diet, increasing the retention of P and reducing P excretion. This response was also supported by our data in that increasing the nPP levels resulted in a decrease in Ca digestibility and utilization. This decrease in digestibility and utilization is as a result of increased precipitation of an insoluble Ca and P as outlined by Hurwitz and Bar (1971). Therefore, the level of dietary nPP must be taken into account when comparing the optimum Ca:nPP.

Identification of the optimum Ca:nPP for 14 to 28 d old broiler was not possible based on the growth response obtained in this experiment, because increasing the Ca:nPP from 1.6:1 to 2.2:1 had no effect on growth performance. However, ASH, BBS, and ash wt increased with increasing Ca:nPP. The bone response data indicate that the Ca:nPP is above the 1.9:1 identified for broilers 0 to 18 d of age.

In summary, the Ca:nPP for 0 to 18 d old broilers to optimize growth, digestibility, and utilization of P and Ca is 1.9:1. Broilers 14 to 28 d had no response in growth to Ca:nPP fed; however, bone responses continue to increase with the highest Ca:nPP of 2.2:1.

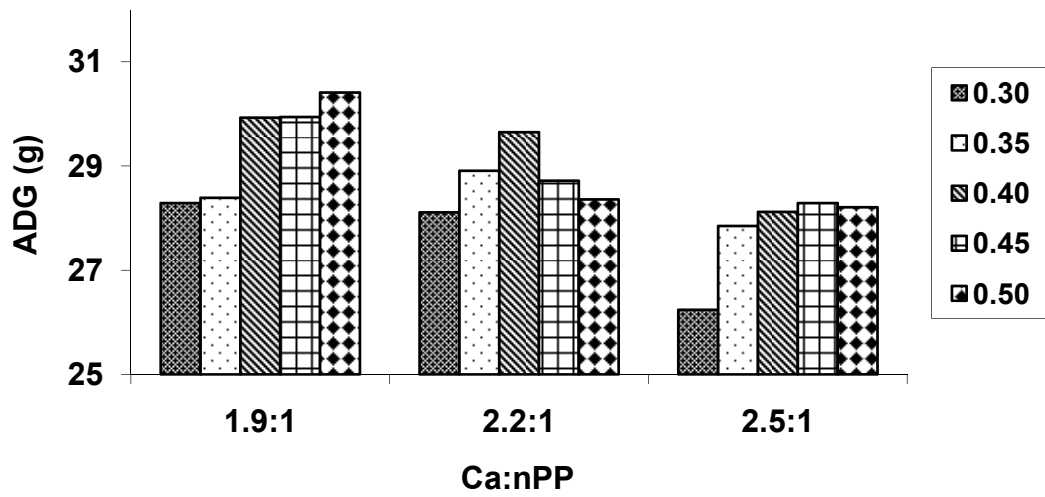


Figure 4.3. Interaction effect of nonphytate P (nPP) level and Ca:nPP on ADG of 0 to 18 day old broilers. SEM = 1.24.

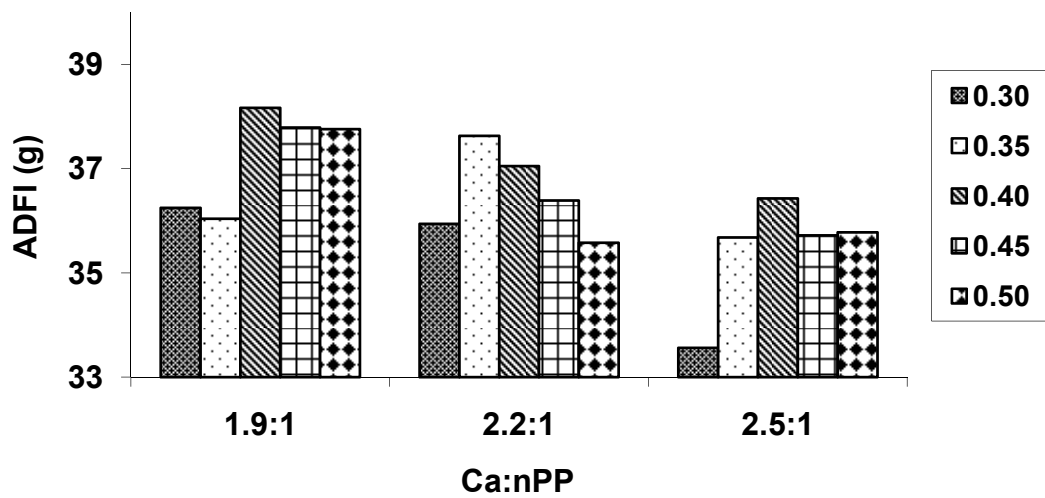


Figure 4.4. Interaction effect of nonphytate P (nPP) level and Ca:nPP on ADFI of 0 to 18 day old broilers. SEM = 1.69.

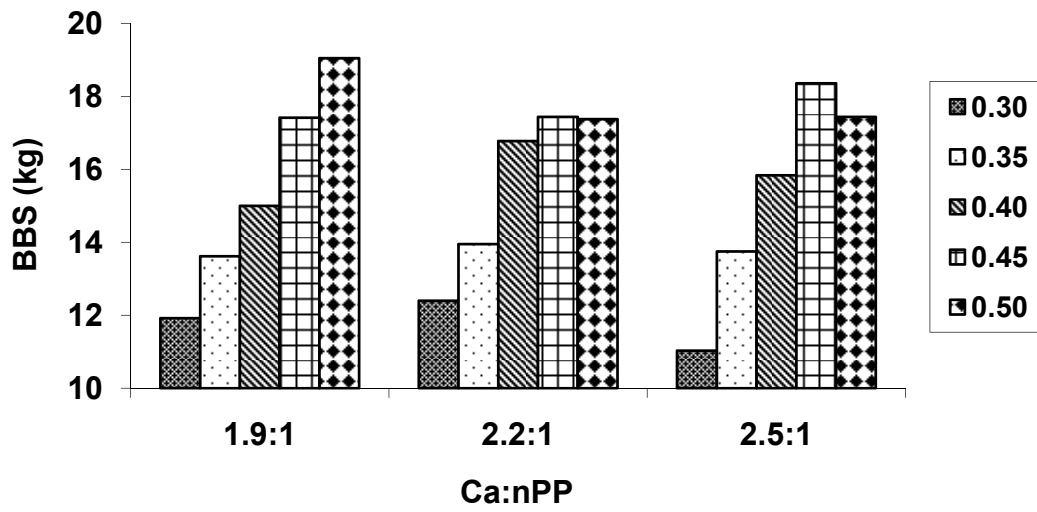


Figure 4.5. Interaction effect of nonphytate P (nPP) level and Ca:nPP on bone breaking strength (BBS) of 0 to 18 day old broilers. SEM = 1.15.

CHAPTER 5

COMPENSATORY AND CARRYOVER EFFECTS OF FEEDING VARIOUS LEVELS OF NONPHYTATE PHOSPHORUS ON BROILER GROWTH PERFORMANCE AND BONE CHARACTERISTICS

INTRODUCTION

The ability of animals to adapt to the deficiency of some nutrient without any negative effect on growth performance has received very little attention. This is mainly due to a lack of knowledge on the mechanisms responsible for these adaptations. Therefore, any practical benefit that could be achieved from this phenomenon is not realized. This area provides potential, with the need to develop different feeding strategies, to reduce feed cost and environmental pollution from P. For example, Yan et al. (2005) investigated the feeding of diets deficient in Ca and P in the starter phase on growth performance, bone characteristics, and nutrient absorption in the grower phase. They reported that reduced BW from feeding the diets deficient in Ca and P observed on d 18 was not present at the end of the grower study. The broilers fed the low Ca and P diets were able to compensate for the dietary deficiency in the starter phase by increasing absorption of Ca and P in the grower phase. Also, retention of P has been shown to increase in situations where high Ca interferes with P absorption. To achieve increased retention of P, the broiler chicks reduced endogenous secretion of P (Hurwitz and Bar, 1971).

Feeding above the P requirement in the early phase of growth, when the quantity of feed consumed by the broiler is relatively small, may positively influence growth performance in subsequent phases of growth. The ability of the broiler chick to benefit from this feeding strategy provides more of a safety margin for producers, but these feeding strategies have little or no scientific support. Investigation into the ability of broilers to benefit from excess P in the early stages of growth or to compensate for P deficiency in any phase of growth will provide useful

information to poultry production practitioners in making management decisions that will positively influence production.

The purpose of this research was to investigate the effect of feeding marginally deficient or excessive P in the starter phase on the growth performance and bone response of broiler chicks in the subsequent feeding period.

MATERIALS AND METHODS

All methods used in these experiments regarding animal care were approved by the Louisiana State University Agricultural Center Animal Care and Use Committee. Two experiments were conducted with Ross x Ross 708 broilers. The broilers were housed in 0.76 x 3.05 m pens (experiment 1) and 1.52 x 3.05 m pens (experiment 2) at the Louisiana State University Agricultural Center Poultry farm in one tunnel ventilated room with cool cells and fans. The pens contained 15 cm of fresh litter for each experiment. The lighting schedule was similar to that used in the poultry industry. Hours of light for a 24 h period consisted of 4 d of 24 h of light, followed by 5 d of 20 h of light, 6 d of 18 h of light, and 16 h of light from d 15 onward where appropriate. Weather permitting, the temperature in the house was maintained at 29 to 32°C for the first 7 d post hatch and was decreased by 2°C every 7 d until temperatures ranged from 21 to 24°C. Broilers were given access to feed in mash form for ad libitum consumption throughout each experimental period. Feed was fed via a feed tray for the first 7 d and then via one (experiment 1) or 2 (experiment 2) hanging feeders with a diameter of 43 cm. Water was provided via one automatic waterer with 6 (experiment 1) or 9 (experiment 2) nipples. The broilers were fed in a phased feeding program consisting of starter (0 to 14 d, experiment 1; 0 to 21 d, experiment 2), grower (14 to 28 d, experiment 1; 21 to 35 d, experiment 2), and finisher (35 to 49 d, experiment 2).

At the end of each growth phase, all broilers and feeders were weighed for the determination of ADG, ADFI, and G:F. Also, 6 broilers were randomly selected, killed by CO₂ asphyxiation, and the left tibia was removed, cleaned of adhering tissue, and frozen for determination of bone breaking strength (BBS), tibia ash percentage (ASH), and bone Ca and P concentrations. Bone breaking strength was determined using an HD 250 Texture Machine (Texture Technologies Corporation, Scarsdale, NY) fitted with a 3-point bend rig with a load cell capacity of 25 (0 to 14 d) or 250 kg (14 to 28 d) and a cross-head speed of 100 mm/min. Bone response variables were reported on a pen basis and not for individual broilers. Bone ash percentage was determined after ethanol and ether extraction under reflux for 36 h each followed by at least 36 h of ashing at 550°C in a muffle furnace.

Diets (Tables 5.1 and 5.2) were corn-soybean meal (C-SBM) based and formulated for the starter phase to contain 3,025 kcal of ME/kg, 1.43% Lys, and 1.07% TSAA. For the grower phase, diets were formulated to contain 3,150 kcal of ME/kg, 1.24% Lys, and 0.95% TSAA. For the finisher phase, diets were formulated to contain 3,200 kcal of ME/kg, 1.06% Lys, and 0.83% TSAA. All other nutrients met or exceeded the NRC (1994) recommended requirement except Ca and P where appropriate. Dietary Ca and total P were determined via inductively coupled plasma-emission spectroscopy after microwave (MarsXpress, CEM Corporation, Matthews, NC) digestion of 0.5 g of sample with 7 mL HNO₃ and 1 mL HCl.

In experiment 1, two thousand one hundred broilers with an average initial and final BW of 36 and 1,140 g, respectively, were allotted to 5 dietary treatments with nonphytate P (nPP) levels ranging from 0.40 to 0.60% and Ca:nPP of 2.2:1. Each treatment had 12 replications (8 males and 4 females) of 35 broilers in the starter phase. For the grower phase, 6 replicate pens (4 males and 2 females) of 23 broilers per pen from each starter treatment were fed either 0.30 or 0.35% nPP (Table 5.1).

In experiment 2, one thousand twenty eight broilers with an average initial and final BW of 37 and 2,812 g, respectively, were allotted to either 0.50 or 0.60% nPP with Ca:nPP of 1.9:1 for the starter phase (0 to 21 d). Each treatment had 12 replications (4 males and 8 females) of 35 broilers in the starter phase. For the grower phase, broilers from each treatment in the starter phase were divided into 2 groups and fed either 0.30 or 0.35% nPP resulting in 4 dietary treatments with 6 replications (2 males and 4 females) of 26 broilers per replicate pen. Broilers were fed a common diet in the finisher phase adequate in all nutrients (Table 5.2).

Data were analyzed by ANOVA procedures appropriate for a randomized block design (Steel and Torrie, 1980) using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The pen of broilers served as the experimental unit and sex was the blocking factor. There were no sex \times treatment interactions; therefore, it was removed from the model. Orthogonal contrasts were used to determine treatment differences which were considered significant at $\alpha = 0.10$.

RESULTS

In experiment 1 (Table 5.3), increasing nPP from 0.40 to 0.60% nPP in the starter phase had no effect on ADG or G:F; however, there was a linear increase ($P = 0.06$) in ADFI. Bone breaking strength and ASH were linearly increased ($P = 0.01$) by increasing nPP level. Feeding either 0.30 or 0.35% nPP in the grower phase had no effect on ADG or ADFI; however, G:F was increased ($P = 0.04$) with the 0.35% nPP. There was also an increase in BBS ($P < 0.01$) and ASH ($P = 0.01$) for the broilers fed 0.35% nPP in the grower phase. The level of nPP fed in the starter phase influenced ADG (starter nPP \times grower nPP, $P = 0.05$, Figure 5.1) in the grower phase in that the broilers fed the lower levels of nPP (0.40, 0.45, and 0.50) in the starter phase gained more when fed 0.30% nPP in the grower phase, while broilers fed the higher levels of nPP (0.55 and 0.60) in the starter phase gained more when fed 0.35% nPP in the grower phase. A similar

Table 5.1. Percentage composition of diets for 0 to 14 and 14 to 28 day old broilers, experiment 1¹

Ingredient	0 to 14 d	14 to 28 d	
		0.30% nPP	0.35% nPP
Corn	52.18	60.91	60.13
Soybean meal (47.5%)	38.87	31.83	31.90
Poultry fat	3.36	3.46	3.73
Monocalcium phosphate	0.78	0.83	1.07
Limestone	0.69	0.73	0.93
BMD + 3 nitro ²	0.50	0.50	0.50
NaCl	0.50	0.50	0.50
DL-Met	0.34	0.28	0.29
Mineral premix ³	0.25	0.25	0.25
Vitamin premix ⁴	0.25	0.25	0.25
Biolys	0.25	0.25	0.07
L-Thr	0.13	0.06	0.05
Biocox ⁵	0.05	0.05	0.05
Ethoxyquin ⁶	0.05	0.05	0.05
Choline chloride ⁷	0.05	0.05	0.05
Sand ⁸	2.44	-----	-----
Nutrient composition			
CP (%)	23.27	20.57	20.53
ME (kcal/kg)	3,025	3,150	3,150
Lys (%)	1.43	1.24	1.24
TSAA (%)	1.07	0.95	0.95
Thr (%)	1.00	0.83	0.83
Trp (%)	0.28	0.24	0.24
Ca (%)	0.67	0.67	0.78
Nonphytate P ⁹ (%)	0.30	0.30	0.35

¹As fed basis.²Bacitracin methylene disacicylate + 3-nitro-4 hydroxyphenylarsonic acid (Nutra Blend, Neosha, MO).

(Table 5.1 continued)

³ Provides the following per of kilogram diet: Fe, 50 mg; Mn, 100 mg; Cu, 7 mg; Se, 0.15 mg; Zn, 75 mg; I, 1 mg; as ferrous sulfate, manganese sulfate, copper sulfate, sodium selenite, zinc sulfate, and calcium iodate, respectively, with calcium carbonate as the carrier.

⁴ Provides the following per kilogram of diet: vitamin A, 8,000 IU; vitamin D₃, 3,000 IU; vitamin E, 25 IU; vitamin K, 1.5 IU; riboflavin, 10 mg; pantothenic acid, 15 mg; niacin, 50 mg; vitamin B₁₂, 0.02 µg; biotin, 0.1 µg; folic acid, 1 mg; pyridoxine, 4 mg; and thiamin, 3 mg.

⁵ Biocox provides 132.3 g/kg salinomycin sodium (Roche, Parsippany, NJ).

⁶ Provided 66.6% 6-ethoxy-1,2-dihydro-2,2,4-trimethylquionine (Novus International, St Louis, MO).

⁷ Provided 600,000 mg/kg of choline chloride.

⁸ Sand was replaced by varying levels of monocalcium phosphate and limestone to achieve the desired levels of Ca and nonphytate P (nPP) in individual diets.

⁹ The nPP levels were calculated based on NRC (1994).

Table 5.2. Percentage composition of diets for 0 to 21, 21 to 35, and 35 to 49 day old broilers, experiment 2¹

Ingredient	nPP (%) Levels				
	Starter		Grower		Finisher
	0.50	0.60	0.30	0.35	0.35
Corn	50.80	50.80	59.09	58.83	65.69
Soybean meal (47.5%)	39.62	39.62	32.62	32.64	26.09
Poultry fat	3.69	3.69	3.94	4.03	3.83
Monocalcium phosphate	1.73	2.21	0.83	1.07	1.12
Limestone	1.06	1.37	1.34	1.25	1.15
Sand	0.79	0.00	0.00	0.00	0.00
BMD + 3 nitro ²	0.50	0.50	0.50	0.50	0.50
NaCl	0.50	0.50	0.50	0.50	0.50
DL-Met	0.33	0.33	0.28	0.28	0.22
Mineral premix ³	0.25	0.25	0.25	0.25	0.25
Vitamin premix ⁴	0.25	0.25	0.25	0.25	0.25
Biolys	0.20	0.20	0.20	0.20	0.20
L-Thr	0.12	0.12	0.06	0.06	0.05
Biocox ⁵	0.05	0.05	0.05	0.05	0.05
Ethoxyquin ⁶	0.05	0.05	0.05	0.05	0.05
Choline chloride ⁷	0.05	0.05	0.05	0.05	0.05
Calculated composition					
CP (%)	23.47	23.47	20.75	20.74	18.16
ME (kcal/kg)	3,025	3,025	3,150	3,150	3,200
Lys (%)	1.43	1.43	1.24	1.24	1.06
TSAA(%)	1.07	1.07	0.95	0.95	0.83
Thr (%)	1.00	1.00	0.83	0.83	0.72
Trp (%)	0.29	0.29	0.25	0.25	0.21
Ca (%)	0.95	1.14	0.90	0.90	0.85
Nonphytate P ⁸ (%)	0.50	0.60	0.30	0.35	0.35

¹ As fed basis.

(Table 5.2 continued)

²Bacitracin methylene disacicylate + 3-nitro-4 hydroxyphenylarsonic acid (Nutra Blend, Neosha, MO).

³ Provides the following per of kilogram diet: Fe, 50 mg; Mn, 100 mg; Cu, 7 mg; Se, 0.15 mg; Zn, 75 mg; I, 1 mg; as ferrous sulfate, manganese sulfate, copper sulfate, sodium selenite, zinc sulfate, and calcium iodate, respectively, with calcium carbonate as the carrier.

⁴ Provides the following per kilogram of diet: vitamin A, 8,000 IU; vitamin D₃, 3,000 IU; vitamin E, 25 IU; vitamin K, 1.5 IU; riboflavin, 10 mg; pantothenic acid, 15 mg; niacin, 50 mg; vitamin B₁₂, 0.02 µg; biotin, 0.1 µg; folic acid, 1 mg; pyridoxine, 4 mg; and thiamin, 3 mg.

⁵ Biocox provides 132.3 g/kg salinomycin sodium (Roche, Parsippany, NJ).

⁶ Provided 66.6% 6-ethoxy-1,2-dihydro-2,2,4-trimethylquionine (Novus International, St Louis, MO).

⁷ Provided 600,000 mg/kg of choline chloride.

⁸ The nonphytate P (nPP) levels were calculated based on NRC (1994).

tendency was observed for G:F (starter nPP x grower nPP, $P = 0.11$, Figure 5.2). There was also a linear increase (starter nPP, $P = 0.03$) in ASH at the end of the grower phase based on the levels of nPP fed in the starter phase.

In experiment 2 (Table 5.4), the starter phase was increased in length from 0 to 14 d to 0 to 21 d. Feeding 0.60% nPP resulted in a decrease ($P = 0.03$) in ADG. However, broilers fed 0.60% nPP had increased ASH ($P < 0.01$) compared with those fed 0.50% nPP. During the grower phase, broilers fed 0.50% nPP in the starter phase had higher ADG ($P = 0.05$) and G:F ($P = 0.08$) compared with those fed 0.60% nPP. Similarly, broilers fed 0.30% nPP in the grower phase had higher ADG ($P = 0.05$) and G:F ($P = 0.07$) compared with those fed 0.35% nPP. However, BBS was higher ($P = 0.03$) at the end of the grower phase in broilers fed 0.60% nPP in the starter phase. The ASH in the grower phase was not influenced by the starter nPP fed; however, ASH was higher ($P < 0.01$) in broilers fed 0.35% nPP in the grower phase compared with those fed 0.30% nPP. During the finisher phase when a common diet was fed, there was no difference in growth performance and bone response variables. However, in the overall growth data, G:F was higher ($P = 0.02$) for broilers fed 0.50% nPP in the starter phase than for those fed 0.60% nPP.

DISCUSSION

Feeding higher than the P requirement in the starter phase may have beneficial effects on broilers in subsequent phases. Within our laboratory, we have estimated the nPP requirement for 0 to 7 d and 0 to 14 d old broilers to be not greater than 0.55% and 0.50%, respectively. Therefore, for the starter phase, 2 levels of nPP (0.40 and 0.45%) below the requirement and 2 levels above the requirement (0.55 and 0.60) were fed along with the 0.50% nPP requirement estimate. The broilers were then fed 0.30 and 0.35% nPP for the grower phase (14 to 28 d).

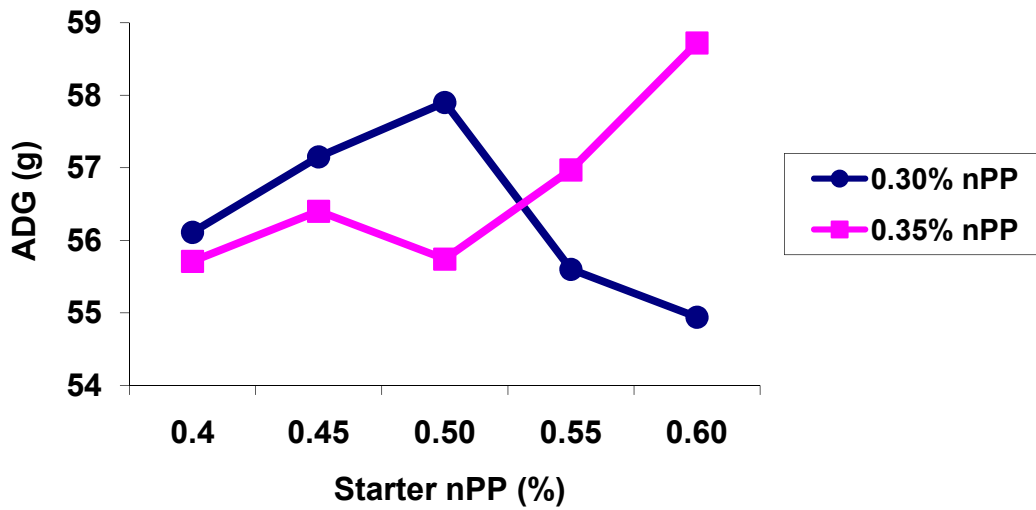


Figure 5.1. Interaction effect of starter nonphytate P (nPP) level on ADG of 0 to 28 day old broilers fed varying levels of nPP in the starter and high and low levels of nPP in the grower phase, experiment 1. SEM = 1.36.

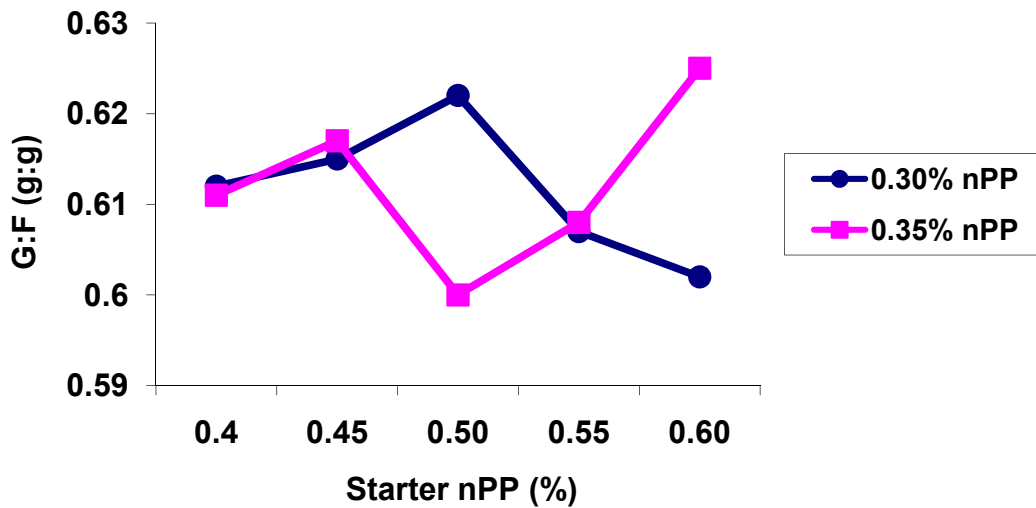


Figure 5.2. Interaction effect of starter nonphytate P (nPP) level on G:F of 0 to 28 day old broilers fed varying levels of nPP in the starter and high and low levels of nPP in the grower phase, experiment 1. SEM = 0.01.

Table 5.3. Growth performance and bone characteristics of 0 to 28 day old broilers fed varying levels of nonphytate P (nPP) for 0 to 14 days and a high and low nPP for 14 to 28 days, experiment 1¹

	Treatment										SEM	P value ²		
	0 to 14 d		0.40		0.45		0.50		0.55			0.60		Starter nPP
ADG (g)	22.62	22.32	22.52	22.61	22.66	0.23	NS	-----	-----					
ADFI (g)	31.11	32.71	31.57	32.56	32.32	0.41	0.06	-----	-----					
G:F (g:g)	0.728	0.684	0.714	0.696	0.701	0.01	NS	-----	-----					
BBS ³ (kg)	8.81	9.60	11.32	11.68	11.38	0.30	0.01	-----	-----					
ASH ⁴ (%)	49.72	51.18	52.18	52.90	52.68	0.26	0.01	-----	-----					
14 to 28 d														
Grower nPP (%)	0.30	0.35	0.30	0.35	0.30	0.35	0.30	0.35	0.30	0.35				
ADG (g)	56.11	55.71	57.15	56.40	57.90	55.74	55.60	56.97	54.94	58.72	1.36	NS	NS	0.05
ADFI (g)	91.66	91.08	92.85	91.26	92.92	92.83	91.63	93.83	91.24	93.81	1.69	NS	NS	NS
G:F (g:g)	0.612	0.611	0.615	0.617	0.622	0.600	0.607	0.608	0.602	0.625	0.01	NS	0.04	0.11
BBS (kg)	25.12	29.41	24.78	27.79	23.42	27.71	25.85	28.51	24.78	29.57	1.43	NS	<0.01	NS
ASH (%)	51.29	52.22	51.40	52.25	51.81	52.43	51.69	52.68	51.79	52.60	0.24	0.03	<0.01	NS

¹Data are means of 12 (8 males and 4 females) and 6 (4 males and 2 females) replications with 35 and 23 broilers per replicate pen for the starter and grower phases, respectively. Average initial and final BW were 36 and 1,140 g, respectively, and the experiment lasted from 0 to 28 d post-hatching.

²NS = not significant, P > 0.10.

³BBS = bone breaking strength.

⁴ASH = percent tibia ash.

Table 5.4. Growth performance and bone characteristics of 0 to 49 day old broilers fed 2 levels of nonphytate P (nPP) for 0 to 21 days, a high and low nPP for 21 to 35 days, and a common diet for 35 to 49 days, experiment 2¹

	Treatment				SEM	P- value ²	
	0 to 21 d		21 to 35 d			Starter nPP	Grower nPP
Starter nPP (%)	0.50		0.60				
ADG (g)	36.10		35.46		0.19	0.03	-----
ADFI (g)	40.52		41.05		0.70	NS	-----
G:F (g:g)	0.893		0.867		0.019	NS	-----
BBS ³ (kg)	19.55		20.96		0.63	NS	-----
Bone wt (g)	8.73		8.99		0.18	NS	-----
Ash wt (g)	4.58		4.79		0.10	NS	-----
ASH ⁴ (%)	52.45		53.29		0.26	< 0.01	-----
21 to 35 d							
Grower nPP (%)	0.30	0.35	0.30	0.35			
ADG (g)	70.23	67.55	67.67	66.60	0.88	0.05	0.05
ADFI (g)	119.58	116.49	116.59	116.67	1.03	NS	NS
G:F (g:g)	0.587	0.579	0.579	0.569	0.005	0.08	0.07
BBS (kg)	30.61	30.56	33.02	33.18	1.08	0.03	NS
Bone wt (g)	19.28	18.95	19.37	19.16	0.37	NS	NS
Ash wt (g)	10.34	10.31	10.46	10.45	0.21	NS	NS
ASH (%)	53.63	54.41	54.00	54.56	0.18	NS	< 0.01
35 to 49 d							
Finisher nPP (%)	0.35						
ADG (g)	87.12	87.52	87.90	89.33	1.36	NS	NS
ADFI (g)	240.01	239.18	244.29	242.82	5.14	NS	NS
G:F (g:g)	0.362	0.365	0.359	0.367	0.008	NS	NS
BBS (kg)	37.26	36.62	37.51	39.77	1.23	NS	NS
Bone wt (g)	31.07	31.49	30.20	31.68	0.75	NS	NS
Ash wt (g)	17.58	18.04	17.23	18.02	0.42	NS	NS
ASH (%)	56.60	57.34	57.08	56.90	0.24	NS	NS

(Table 5.4 continued)

Overall

0 to 49 d

ADG (g)	57.01	56.95	56.43	56.17	0.59	NS	NS
ADFI (g)	107.12	106.71	107.64	107.26	0.89	NS	NS
G:F (g:g)	0.532	0.533	0.523	0.523	0.004	0.02	NS

¹Data are means of 12 (4 males and 8 females), 6 (2 males and 4 females), and 6 (2 males and 4 females) replications with 35, 26, and 20 broilers per replicate pen for the starter, grower, and finisher phases, respectively. Average initial and final BW were 37 and 2,812 g, respectively, and the experiment lasted from 0 to 49 d post-hatching.

²NS = not significant, $P > 0.10$.

³BBS = bone breaking strength.

⁴ASH = percent tibia ash.

Broilers fed at or below 0.50% nPP in the starter phase had higher ADG when fed 0.30% nPP in the grower phase while broilers fed above the requirement at 0.55 or 0.60% nPP in the starter phase had higher ADG when fed 0.35% in the grower period (Fig. 5.1). Feed efficiency was similar for broilers fed 0.30 and 0.35% nPP in the grower phase; however, broilers fed 0.50% nPP in the starter phase had higher G:F when fed 0.30% nPP in the grower phase, while broilers fed 0.60% nPP in the starter phase had higher G:F when fed 0.35% nPP in the grower phase (Fig. 5.2).

The effect on growth performance of nPP levels fed in the starter phase on the grower phase indicates that broilers fed lower levels of nPP in the starter phase are better able to adapt to a lower level of nPP in the grower phase than those fed a higher level of nPP in the starter phase. Skinner and Waldroup (1992) reported similar findings with regard to BBS when they fed broilers high, medium, and low levels of Ca and nPP for 42 d, then removed the dicalcium phosphate during the finisher phase. They reported that there was no negative effect on BBS in broilers fed the low Ca and nPP during the starter and grower phase when the dicalcium phosphate was removed during the finisher phase. However, the broilers fed high levels of Ca and nPP in the starter and grower phase had reduced BBS when dicalcium phosphate was removed from the finisher diet.

The ASH at 28 d was independently influenced by levels of nPP fed in both phases with ASH increasing with increases in dietary nPP. This finding was similar to Driver et al. (2006) who reported that the integrity of the tibia was more dependent on the Ca and nPP levels fed to broilers in the early stages of growth.

Because broilers fed above the nPP requirement had higher ADG, G:F, and ASH when fed 0.35% in the grower phase, experiment 2 was conducted to evaluate any benefit that could be

achieved by feeding nPP above the requirement for an extended starter phase (0 to 21 d). Results indicate that broilers fed 0.60% nPP in the starter phase or 0.35% nPP during the grower phase had decreased ADG and G:F. There was no difference in growth performance at d 49; however, broilers fed the 0.60% nPP in the starter phase had a decrease in overall G:F. Moran and Todd (1994) also reported an advantage of low P diets fed in the early phase on G:F in the last 2 wk of a 8 wk study. This improvement in efficiency can be attributed to an increase in ADG at the lower levels of nPP fed with similar ADFI. Bone breaking strength in the grower phase was higher for broilers fed the 0.60% nPP in the starter phase and ASH was higher for broilers fed 0.35% in the grower phase; however, no benefit was observed for feeding either the 0.60% nPP in the starter phase or 0.35% nPP in the grower phase at d 49. This response indicates that broilers fed 0.50% nPP in the starter phase and 0.30% nPP in the finisher phase were able to compensate for the reduced BBS and ASH in the grower phase.

In summary, the results indicate that there is benefit to feeding higher than the nPP requirement for 0 to 14 d; however, this response was dependent on feeding a higher level of nPP in subsequent phases. Also, this benefit was not achieved when the starter phase was extended to 21 d. To successfully reduce nPP level in the grower or finisher phase, it seems more beneficial to feed at or slightly below the nPP requirement during the 0 to 14 d phase. Broilers seem better able to cope with lower levels of nPP in subsequent phases if fed lower levels of nPP in the starter phase. This response could prove beneficial to producers in lowering the dietary nPP levels in later phases where broilers consumed more P, reducing feed cost and environmental pollution.

CHAPTER 6

A COMPARISON OF THE EFFECT OF TWO *E. COLI* DERIVED PHYTASES ON BROILER GROWTH PERFORMANCE AND BONE BREAKING STRENGTH AND THE EFFECT OF DIETARY CALCIUM ON PHYTASE EFFICACY

INTRODUCTION

Phosphorus is essential to poultry production, and the use of phytase to improve the availability of phytate P in poultry diets has increased since phytase became available in 1991. Phytases are enzymes (phosphatases) that hydrolyze the phosphate group from phytate. Phytate is myo-inositol hexaphosphate; IP₆, occurs as the storage form of P in plants and a large proportion of the P in animal feed is bound to phytate. Phytate P is considered partially unavailable to non-ruminants because they have very little intestinal phytase activity and the phytase activity of various plant ingredients is relatively low. This resulted in the commercial development of phytase enzymes that are generally classified as 3- or 6- phytases based on the carbon atom position of the first phosphate group that gets hydrolyzed. The theory presently is that phytase can remove up to 5 inorganic P molecules from each phytate molecule. Phytases are not identical, and each has characteristics that enable them to work at different pH and also on the quantity of P that they release. The first commercial phytase was derived from the fungus *Aspergillus niger* (Natuphos, BASF) and it entered the market in 1991; it has been followed by phytases derived from *Escherichia coli* (Optiphos, JBS United, Inc). More recently, a phytase that was genetically modified to show heat resistance has shown some promise, but it is still not commercially available in the US (Finase, ABVista).

The evaluation of the various exogenous phytases has shown inconsistency in the quantity of inorganic P they release. The quantity of phytate P and the level of Ca within the test diets are 2 possible reasons for this inconsistency. Calcium has been shown to bind with phytate

making it resistant to enzyme action. Selle et al. (2009) in evaluating the effect of Ca interaction with phytate and phytase, concluded that the dietary levels of Ca must be kept at a minimum to facilitate phytase activity.

The objective of this research was to compare the efficacy of 2 *Escherichia coli* phytases and to investigate the effect of Ca level in the diet on the efficacy of one of these enzymes.

MATERIALS AND METHODS

All methods used in these experiments regarding animal care were approved by the Louisiana State University Agricultural Center Animal Care and Use Committee. Two experiments were conducted with Ross x Ross 308 (experiment 1) or 708 (experiment 2) broilers housed in environmentally controlled Petersime brooder batteries (Petersime Incubator Co., Gettysburg, OH) with a pen size of 38 x 100 cm and raised wire floors. Continuous fluorescent lighting was provided for the duration of each experiment. Broilers were weighed, wing banded, and randomly allotted on the day of hatch to dietary treatment. All diets were corn-soybean meal (C-SBM) based and formulated to 1.26% total Lys and all other nutrients met or exceeded the requirements for 0 to 21 d old broilers (NRC, 1994) except for Ca and nonphytate P (nPP) where appropriate. Diets were formulated to meet the highest level of Ca and nPP and sand was used to replace the sources of Ca and P to achieve the treatment diets. Feed in mash form and water were provided for ad libitum consumption throughout the experiments (Table 6.1).

In experiment 1, six hundred and forty Ross x Ross 308 broilers with an average initial and final BW of 49 and 605 g, respectively, were allotted to 10 dietary treatments with 8 replications (3 replicates of male and 5 of female) of 8 broilers per replicate pen. The treatments were 1) negative control (NC) containing 0.20% nPP, 2 to 4) NC + 0.083, 0.167, or 0.250% nPP from monocalcium phosphate, 5 to 7) NC + 100, 200, or 500 FTU of Finase, and 8 to 10) NC +

100, 200, or 500 FTU Optiphos. The positive control (PC) diet contained 0.45% nPP. All diets contained 1% Ca resulting in a Ca:nPP of 2.2:1 in the PC. The diets contained 1% acid insoluble ash (AIA, Perma-Guard Inc.) and 0.50% chromic oxide for the determination of apparent P and Ca utilization and retention. Apparent P and Ca utilization of excreta samples were calculated as $[100 - ((\text{Marker}_{\text{diet}} \times \text{Nutrient}_{\text{sample}} / \text{Marker}_{\text{sample}} \times \text{Nutrient}_{\text{diet}}) \times 100)]$. Retained P (rP) was calculated as P consumed – P excreted.

In experiment 2, two hundred and eighty eight male Ross x Ross 708 broilers with an average initial and final BW of 37 and 705 g, respectively, were allotted to 8 treatments with 6 replications of 6 broilers per replicate pen. The treatments were 1) PC with 0.45% nPP and 1% Ca, 2) NC with 0.20% nPP and 0.67% Ca, 3) NC + 0.33% Ca, 4) NC + 0.66% Ca, 5 to 8) were treatments 1 to 4 with 500 FTU of Optiphos.

The broilers were weighed on d 0 and 18 (experiment 1) or on d 0 and 21 (experiment 2) for the determination of ADG, ADFI, and G:F. Excreta were collected on d 14 to 18 (experiment 1) and samples pooled by pen. In experiment 1, eight broilers from each pen were killed by CO₂ asphyxiation and the left tibia was removed, cleaned of adhering tissue, and frozen for determination of bone breaking strength (BBS). Bone breaking strength was determined using an HD 250 Texture Machine (Texture Technologies Corporation, Scarsdale, NY) fitted with a 3-point bend rig with a load cell capacity of 250 kg and a cross-head speed of 100 mm/min. Feed and excreta samples were analyzed for AIA, Cr, Ca, and P, for the calculation of Ca and P utilization. Acid insoluble ash determination was conducted by a modified method of Scott and Boldaji (1997) wherein the quantity of acid was increased to 30 mL. Dietary Ca and total P were determined via inductively coupled plasma-emission spectroscopy after microwave (MarsXpress, CEM Corporation, Matthews, NC) digestion of a 0.5 g sample with 7 mL HNO₃

and 1 mL HCl. Chromium was determined after digestion on a hot plate of a 0.5 g sample with a 70:30 mixture of perchloric and nitric acid. Dietary phytase was determined by the method of Chen (1996).

Data were analyzed by ANOVA procedures appropriate for a randomized block (experiment 1) or a completely randomized (experiment 2) design (Steel and Torrie, 1980) using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Sex was the blocking factor in experiment 1; the model included sex and sex \times treatment interaction; sex \times treatment interaction was not significant; therefore, it was removed from the model. Relative bioavailability was obtained using the multiple linear regression slope ratio assay (Littell et al., 1995). The pen of broilers served as the experimental unit. Orthogonal contrasts were used to determine treatment differences, which were considered significant at $\alpha = 0.10$.

RESULTS

In experiment 1, the results of the analysis of the diets for Ca, P, and phytase (Table 6.2) agree closely with the formulated values. Relative bioavailability was calculated using formulated phytase values of 100, 300, and 500 FTU.

In experiment 1 (Table 6.3), ADG, and ADFI were increased ($P < 0.001$) with the addition of inorganic P. The effect on ADG and ADFI was quadratic ($P < 0.001$) as there was no difference between broilers fed 0.367 and 0.45% nPP. Feed efficiency was decreased from 0.20 to 0.283% nPP but increased from 0.283 to 0.450% nPP resulting in a quadratic effect ($P = 0.013$) of inorganic P. Phytase addition linearly increased ADG (quadratic, $P < 0.001$) and ADFI (quadratic, $P < 0.001$); however, there was no difference between broilers fed 300 and 500 FTU for ADG ($P = 0.041$) and ADFI ($P = 0.016$). The Finase phytase increased ADG ($P = 0.003$) and ADFI ($P = 0.002$) more than Optiphos.

Table 6.1. Percentage composition of basal diet for 0 to 18 (experiment 1) and 0 to 21 (experiment 2) day old broilers¹

Ingredient	Experiment 1		Experiment 2	
	Low P	Positive control	Low Ca and P	Positive control
Corn	50.95	50.95	52.32	52.32
Soybean meal (47.5% CP)	37.34	37.34	37.22	37.22
Soy oil	5.92	5.92	5.48	5.48
Monocalcium phosphate	0.33	1.52	0.33	1.52
Limestone	1.95	1.48	1.48	1.48
NaCl	0.50	0.50	0.50	0.50
Trace minerals ²	0.25	0.25	0.25	0.25
Vitamins ³	0.25	0.25	0.25	0.25
DL-Met	0.20	0.20	0.20	0.20
Choline chloride ⁴	0.05	0.05	0.05	0.05
L-Thr	0.04	0.04	0.42	0.42
Chromic oxide	0.50	0.50	0.50	0.50
Fossil shell flour ⁵	1.00	1.00	0.00	0.00
Sand ⁶	0.72	0.00	0.55	0.55
Calculated composition				
ME (kcal/kg)	3,200	3,200	3,200	3,200
CP (%)	22.21	22.21	22.17	22.17
Lys (%)	1.26	1.26	1.26	1.26
TSAA (%)	0.91	0.91	0.91	0.91
Trp (%)	0.27	0.27	0.27	0.27
Thr (%)	0.88	0.88	0.88	0.88
Ca (%)	1.00	1.00	0.67	1.00
P (%)	0.47	0.72	0.47	0.72
Nonphytate P ⁷ (%)	0.20	0.45	0.20	0.45

¹As fed basis.

² Provides the following per of kilogram diet: Fe, 50 mg; Mn, 100 mg; Cu, 7 mg; Se, 0.15 mg; Zn, 75 mg; I, 1 mg; as ferrous sulfate, manganese sulfate, copper sulfate, sodium selenite, zinc sulfate, and calcium iodate, respectively, with calcium carbonate as the carrier.

(Table 6.1 continued)

³Provides the following per kilogram of diet: vitamin A, 8,000 IU; vitamin D₃, 3,000 IU; vitamin E, 25 IU; vitamin K, 1.5 IU; riboflavin, 10 mg; pantothenic acid, 15 mg; niacin, 50 mg; vitamin B₁₂, 0.02 µg; biotin, 0.1 µg; folic acid, 1 mg; pyridoxine, 4 mg; and thiamin, 3 mg.

⁴Contains 600,000 mg/kg of choline chloride.

⁵ Fossil shell flour to provide acid insoluble ash from Perma-Guard Inc.

⁶The level of sand was changed based on the level of inclusion of monocalcium phosphate, limestone, and the phytase products.

⁷The nonphytate P (nPP) levels were calculated based on NRC (1994).

Bone breaking strength was increased ($P < 0.001$) with increasing addition of inorganic P; however, the effect was also quadratic ($P = 0.013$). Phytase supplementation increased ($P < 0.001$) BBS. The magnitude of increase in BBS was influenced by the source ($P < 0.001$) and the level ($P < 0.001$) of phytase. The increase at each level of phytase was different for the phytase sources ($P = 0.088$). At 100 FTU, BBS was similar for Finase and Optiphos, but at 300 and 500 FTU, BBS was increased more by Finase than by Optiphos (Figure 6.1).

Retained P was linearly and quadratically increased ($P < 0.001$) by increasing dietary inorganic P. Phytase addition increased rP ($P < 0.001$); however, Finase supplementation resulted in higher rP ($P = 0.038$) than Optiphos (Table 6.3).

Inorganic P released (Table 6.2) by the 2 sources of phytase was calculated using a regression line created by the formulated values of inorganic P from monocalcium phosphate. The slope, intercept, and R^2 obtained for the regression lines were 61.95, 27.03, and 1.00; 90.21, 35.39, and 0.97; 74.19, 9.29, and 0.99; for ADG, ADFI, and BBS, respectively. Inorganic P release from 500 FTU of Optiphos ranged from 0.065 to 0.072 while the range for Finase was 0.092 to 0.101.

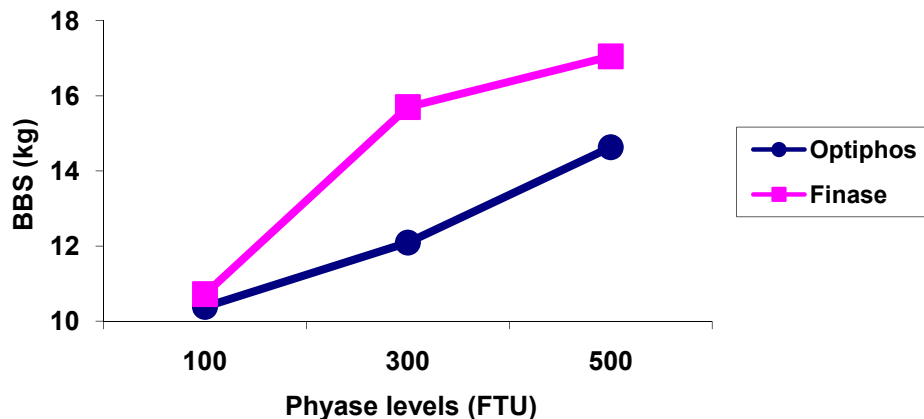


Figure 6.1. Interaction effect of phytase level and source on bone breaking strength (BBS) of 0 to 18 day old broilers fed varying levels and sources of phytase, experiment 1. SEM = 0.74.

Table 6.2. Inorganic P released by varying sources of phytase¹

Diet	Formulated phytase	Analyzed phytase	ADG	ADFI	BBS ²
Optiphos					
	100	110	-0.005	0.002	0.015
	300	327	0.057	0.055	0.038
	500	502	0.065	0.068	0.072
Finase					
	100	101	0.024	0.031	0.019
	300	333	0.076	0.086	0.086
	500	511	0.101	0.092	0.105

¹Regression line was created using added values of P; 0, 0.083, and 0.167% from monocalcium phosphate. Slope, intercept, and R² for regression lines are as follows:

ADG; 61.95, 27.03, and 1.00

ADFI; 90.21, 35.39, and 0.97

BBS; 74.19, 9.29, and 0.99.

²BBS = bone breaking strength.

Table 6.3. Growth performance, bone breaking strength, and retained P of 0 to 18 day old broilers fed varying levels and sources of phytase¹

Diet	ADG (g)	ADFI (g)	G:F (g:g)	BBS ² (kg)	rP ³ (g)
0.200% nPP ⁴	27.05	34.67	0.780	8.95	1.81
0.283% nPP	32.20	44.26	0.732	16.12	2.61
0.367% nPP	37.40	49.74	0.752	21.34	3.11
0.450% nPP	37.64	48.69	0.773	24.71	3.38
100 FTU Optiphos	26.76	35.57	0.753	10.38	1.91
300 FTU Optiphos	30.55	40.36	0.757	12.09	2.13
500 FTU Optiphos	31.10	41.53	0.749	14.63	2.30
100 FTU Finase	28.57	38.13	0.751	10.72	2.00
300 FTU Finase	31.76	43.13	0.736	15.70	2.35
500 FTU Finase	33.29	43.68	0.764	17.05	2.39
SEM	0.68	0.95	0.014	0.74	0.07
<i>Contrasts</i>				P > F ⁵	
Inorganic P linear	<0.001	<0.001	NS	<0.001	<0.001
Inorganic P quadratic	<0.001	<0.001	0.013	0.013	0.001
Optiphos vs Finase	0.003	0.002	NS	<0.001	0.038
Phy linear	<0.001	<0.001	NS	<0.001	<0.001
Phy quadratic	0.041	0.016	NS	NS	NS
Phy linear x Optiphos vs Finase	NS	NS	NS	NS	NS
Phy quadratic x Optiphos vs Finase	NS	NS	NS	0.088	NS

¹Data are means of 8 replications (3 males and 5 females) with 8 broilers per replicate pen. Average initial and final BW were 49 and 605 g, respectively.

²BBS = bone breaking strength.

³rP = P retention (total grams for the 18 d period) calculated using values for the formulated P level in diets.

⁴nPP = nonphytate P.

⁵NS = not significant, P > 0.10.

⁶Phy = phytase.

Table 6.4. Multiple linear regression equations and validation for multiple linear regressions using the slope ratio assay for growth performance, bone breaking strength, and retained P based on added phytase concentrations

	ADG	ADFI	G:F	BBS ¹	rP ²
Multiple Linear					
Regression Equations					
Intercept	26.82	35.29	0.76	9.18	1.83
Slope					
Optiphos	0.0089	0.0128	-0.00002	0.0105	0.0009
Finase	0.0135	0.0190	-0.00002	0.0171	0.0012
R-square	0.54	0.57	0.02	0.71	0.51
RBV³					
Optiphos (%)	65.47	67.52	112.84	61.10	72.58
Validation⁴					
Average slope ⁵	<0.001	<0.001	0.357	<0.001	<0.001
Different slope ⁶	0.009	0.008	0.924	<0.001	0.052
Intercept ⁷	0.297	0.401	0.626	0.926	0.599
Curvature ⁸	0.221	0.025	0.100	0.099	0.193

¹BBS = bone breaking strength.

²rP = P retention (total grams for the 18 d period) calculated using values for the formulated P concentration in diets.

³RBV = relative bioavailability.

⁴Validation values reported are $P > F$.

⁵If average slope is significant, then average of the 3 slopes are different from zero.

⁶If different slope is significant, then the linear regressions have different slopes.

⁷If intercept is significant, then the linear regressions do not have equal intercepts.

⁸If curvature is significant, this indicates the failure of linear regression to fit the nonzero supplemental levels.

The relative bioavailability of Optiphos in relation to Finase was evaluated using the slope ratio assay for growth performance, BBS, and rP (Table 6.4). Values for the relative bioavailability of Optiphos relative to Finase ranged from 61.10% (BBS) to 112.84% (G:F).

Calcium and P utilizations were calculated using the analyzed and expected values for both chromic oxide and AIA (Table 6.5). To calculate expected utilization, the average analyzed values for Cr and AIA were used along with formulated values for Ca and P. Expected values for AIA for diets 1 to 4 will not be reported because the inclusion of monocalcium phosphate to replace sand resulted in a decrease in AIA. Data will only be discussed for Ca and P utilization calculated using the expected Cr values. Calcium utilization decreased at 0.283% nPP and 0.367% nPP but increased at 0.450% nPP resulting in a quadratic effect ($P = 0.057$) of inorganic P on Ca utilization. Phosphorus utilization, was linearly decreased ($P < 0.001$) by increasing dietary inorganic P. Optiphos supplementation resulted in higher ($P = 0.094$) Ca utilization. Increasing the level of both phytases from 100 to 300 FTU decreased Ca utilization ($P = 0.110$); however, Ca utilization at 300 FTU was not different from that at 500 FTU resulting in a quadratic effect ($P = 0.061$). Phytase increased P utilization ($P = 0.082$). The increase in P utilization with phytase supplementation was not dependent on the source of phytase as both Finase and Optiphos had average P utilization of 63.67%.

In experiment 2 (Table 6.6), increasing Ca from 0.67 to 1.33% linearly decreased ADG ($P < 0.001$) and ADFI ($P < 0.001$); however, there were quadratic effects for ADFI, G:F, and mortality ($P = 0.008$ to $P = 0.067$). Supplementation with 500 FTU of Optiphos increased ADG ($P < 0.001$), ADFI ($P < 0.001$), and decreased mortality ($P = 0.054$). However, phytase supplementation increased ADG (Ca \times Phytase $P = 0.003$, Figure 6.2), ADFI (Ca \times Phytase $P = 0.002$, Figure 6.3) and decreased mortality (Ca \times Phytase $P = 0.058$, Figure 6.4) more in broilers

fed the higher level of Ca. Mortality was below 6% for all dietary treatments except for broilers fed 1.33% Ca without phytase, which had a mortality of 13.89%. Growth performance of broilers fed 0.20% nPP at all levels of Ca without phytase were all lower than broilers fed the positive control of 0.45% nPP and 1% Ca.

Bone breaking strength, bone wt, tibia ash wt, and ASH decreased ($P < 0.001$ to $P = 0.003$) with increasing level of Ca (Table 6.7). However, the decrease in ASH was quadratic ($P = 0.090$) as there was no difference in ASH of broilers fed 1.00 or 1.33% Ca. Phytase supplementation increased ($P < 0.001$) BBS, bone wt, ash wt, and ASH. The increase in bone wt ($P = 0.006$, Figure 6.5), ash wt ($P = 0.008$, Figure 6.6), and ASH ($P = 0.026$, Figure 6.7) with phytase supplementation was greater in broilers fed the higher levels of Ca.

DISCUSSION

Experiment 1 was conducted to compare the efficacy of 2 *Escherichia coli* derived phytases. Three levels of each phytases were added to a negative control diet that contained 0.20% nPP, along with 4 levels of monocalcium phosphate, which were utilized to establish regression equations to estimate the inorganic P released by both Finase and Optiphos. The increase in growth and bone response to the addition of monocalcium phosphate and the varying levels of both phytases indicated that the negative control diet containing 0.20% nPP provided a response surface to compare both phytases. Finase and Optiphos supplementation increased ADG and ADFI; this increase in growth performance was greater for Finase than for Optiphos phytase. However, there was no difference in growth performance of broilers fed 300 and 500 FTU regardless of phytase source. Bone breaking strength was increased by phytase supplementation; however, the magnitude of increase at the varying inclusion levels of phytase was greater for Finase than for Optiphos phytase.

Table 6.5. Calcium and P utilization for 0 to 18 day old broilers fed different levels of phytase¹

Treatments	Ca utilization (%)				P utilization (%)			
	Analyzed ²		Expected ³		Analyzed ²		Expected ³	
	AIA	Cr	AIA ⁴	Cr	AIA	Cr	AIA ⁴	Cr
0.200% nPP	52.8	52.7	-----	53.1	57.6	57.6	-----	61.6
0.283% nPP	44.7	45.3	-----	45.9	59.6	60.0	-----	59.3
0.367% nPP	45.5	45.5	-----	49.8	50.4	50.7	-----	54.6
0.450% nPP	56.1	52.4	-----	50.3	58.2	54.6	-----	53.7
100 FTU Optiphos	52.0	42.7	57.2	55.3	59.9	52.1	64.7	63.2
300 FTU Optiphos	45.4	44.4	50.1	47.4	61.5	60.8	64.2	62.2
500 FTU Optiphos	43.8	50.4	53.9	50.1	62.6	67.1	68.2	65.6
100 FTU Finase	49.1	49.1	52.5	49.2	58.0	58.1	64.6	62.1
300 FTU Finase	53.2	49.9	52.2	47.3	63.7	61.1	67.8	64.5
500 FTU Finase	46.7	44.1	53.2	47.9	62.9	61.3	67.8	64.4
SEM	1.76	2.10	1.68	2.02	1.51	1.41	1.47	1.32
<i>Contrasts</i>					<i>P > F⁵</i>			
Inorganic P linear	NS	NS	-----	NS	NS	0.005	-----	<0.001
Inorganic P quadratic	<0.001	0.001	NS	0.057	0.061	NS	NS	NS
Optiphos vs Finase	0.074	NS	NS	0.094	NS	NS	NS	NS
Phy ⁶ level linear	0.004	NS	NS	NS	0.015	<0.001	0.025	0.082
Phy level quadratic	NS	NS	0.038	0.061	NS	NS	NS	NS
Phy linear × Optiphos × Finase	0.101	0.003	NS	NS	NS	NS	<0.001	NS
Phy quadratic × Optiphos × Finase	0.013	NS	NS	NS	NS	NS	NS	NS

¹Data are means of 8 replications (3 males and 5 of females) with 6 chicks per replicate pen. Average initial and final BW were 49 and 605 g, respectively.

²Utilization calculated using analyzed AIA or Cr values, and the analyzed Ca or P value in the feed.

³Utilization calculated using an average analyzed AIA or Cr value, and the formulated Ca or P value in the feed.

⁴Ca and P utilization calculated for treatment 1 to 4 using average AIA for P and Ca was ignored. It was observed that with increasing levels of monocalcium phosphate that replaced sand, AIA values decreased.

⁵NS = not significant, P > 0.10.

⁶Phy = phytase.

Table 6.6. Growth performance of 0 to 21 day old broilers fed low levels of nonphytate P (nPP) with varying levels of Ca and with and without phytase (Phy) supplementation¹

Item	ADG (g)	ADFI (g)	G:F (g:g)	Mortality (%)
Treatments				
0.45% nPP, 1% Ca	35.58	46.48	0.765	0.00
0.20% nPP, 0.67% Ca	33.48	43.19	0.775	5.56
0.20% nPP, 1% Ca	26.70	35.57	0.752	2.78
0.20% nPP, 1.33% Ca	21.98	28.53	0.770	13.89
0.45% nPP, 1% Ca + Phy	36.41	46.25	0.789	2.78
0.20% nPP, 0.67% Ca + Phy	35.97	45.74	0.787	5.56
0.20% nPP, 1% Ca + Phy	34.12	45.65	0.748	0.00
0.20% nPP, 1.33% Ca + Phy	29.27	38.32	0.764	2.78
SEM	0.73	1.01	0.010	2.67
Ca level main effect				
0.67	34.73	44.47	0.781	5.56
1.00	30.41	40.61	0.750	1.39
1.33	25.63	33.43	0.767	8.34
Phy main effect				
- Phy	27.39	35.76	0.766	7.41
+ Phy	33.12	43.24	0.766	2.78
Contrast ²			P > F ³	
Phy	<0.001	<0.001	NS	0.054
Ca level linear	<0.001	<0.001	NS	NS
Ca level quadratic	NS	0.067	0.008	0.030
Phy x Ca level linear	0.003	0.002	NS	0.058

¹Data are the means of 6 replications with 6 broilers per replicate pen. Average initial and final BW were 37 and 705 g, respectively, and the experiment lasted from 0 to 21 d posthatching.

²Diets with 0.45% nPP were excluded from main effects analysis.

³NS = not significant, P > 0.10.

Table 6.7. Bone characteristics of 0 to 21 day old broilers fed low levels of nonphytate P (nPP) with varying levels of Ca and with and without phytase (Phy) supplementation¹

Item	BBS ² (kg)	Bone wt (g)	Ash wt (g)	ASH ³ (%)
Treatments				
0.45% nPP, 1% Ca	23.08	9.17	4.84	52.72
0.20% nPP, 0.67% Ca	14.71	6.38	3.05	47.94
0.20% nPP, 1% Ca	10.52	4.97	2.28	45.76
0.20% nPP, 1.33% Ca	9.28	3.56	1.64	46.18
0.45% nPP, 1% Ca + Phy	25.22	9.35	4.98	53.23
0.20% nPP, 0.67% Ca + Phy	19.98	8.02	4.09	51.02
0.20% nPP, 1% Ca + Phy	20.85	8.37	4.29	51.22
0.20% nPP, 1.33% Ca + Phy	16.82	7.06	3.58	50.73
SEM	0.88	0.32	0.16	0.28
Ca level main effect				
0.67	17.35	7.20	3.57	49.48
1.00	15.69	6.67	3.29	48.49
1.33	13.05	5.31	2.61	48.46
Phy main effect				
- Phy	11.50	4.97	2.32	46.63
+ Phy	19.22	7.82	3.99	50.99
Contrast ⁵			P > F ⁵	
Phy	<0.001	<0.001	<0.001	<0.001
Ca level linear	<0.001	<0.001	<0.001	0.003
Ca level quadratic	NS	NS	NS	0.090
Phy x Ca level linear	NS	0.006	0.008	0.026

¹Data are the means of 6 replications with 6 broilers per replicate pen. Average initial and final BW were 37 and 705 g, respectively, and the experiment lasted from 0 to 21 d posthatching.

²BBS = bone breaking strength.

³ASH = percent tibia ash.

⁴Diets with 0.45% nPP were excluded from main effects analysis.

⁵NS = not significant, P > 0.10.

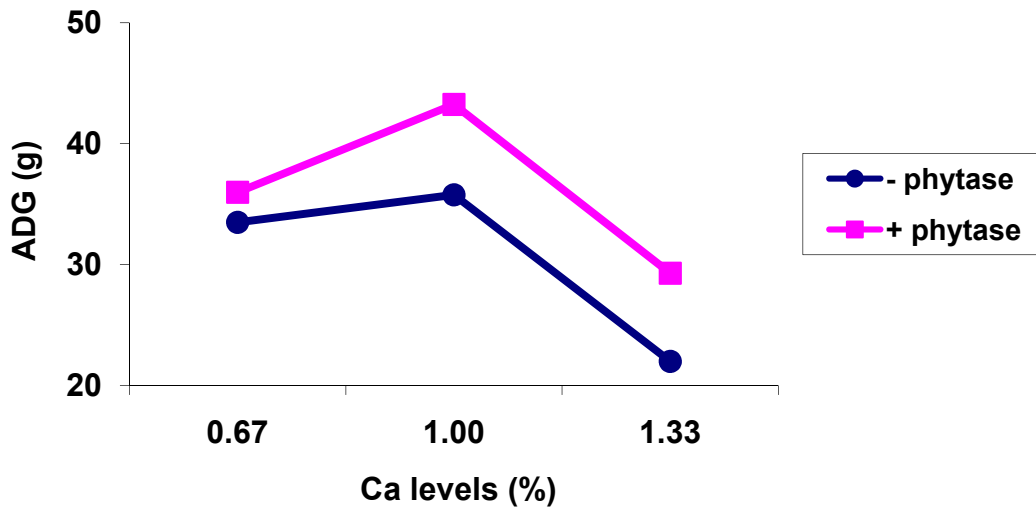


Figure 6.2. Interaction effect of Ca level and phytase on ADG of 0 to 21 day old broilers fed low levels of nonphytate P (nPP) with varying levels of Ca (0.67, 1.00, or 1.33%), experiment 2. SEM = 0.73.

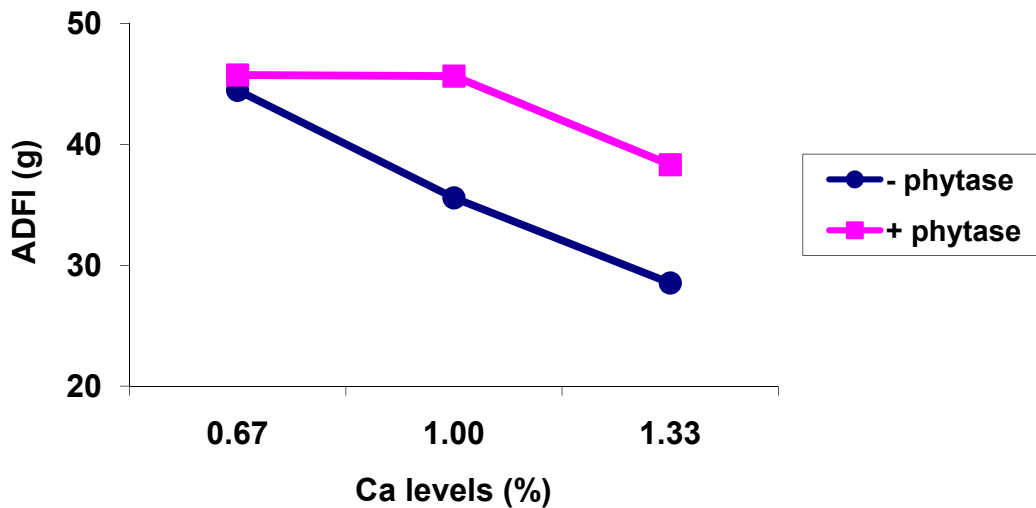


Figure 6.3. Interaction effect of Ca level and phytase on ADFI of 0 to 21 day old broilers fed low levels of nonphytate P (nPP) with varying levels of Ca (0.67, 1.00, or 1.33%), experiment 2. SEM = 1.01.

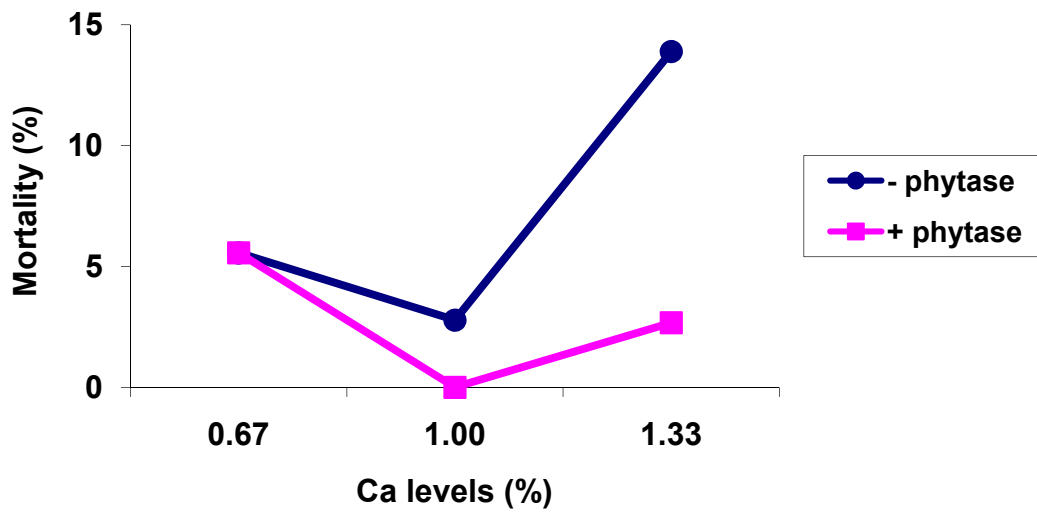


Figure 6.4. Interaction effect of Ca level and phytase on mortality of 0 to 21 day old broilers fed low levels of nonphytate P (nPP) with varying levels of Ca (0.67, 1.00, or 1.33%), experiment 2. SEM = 2.67.

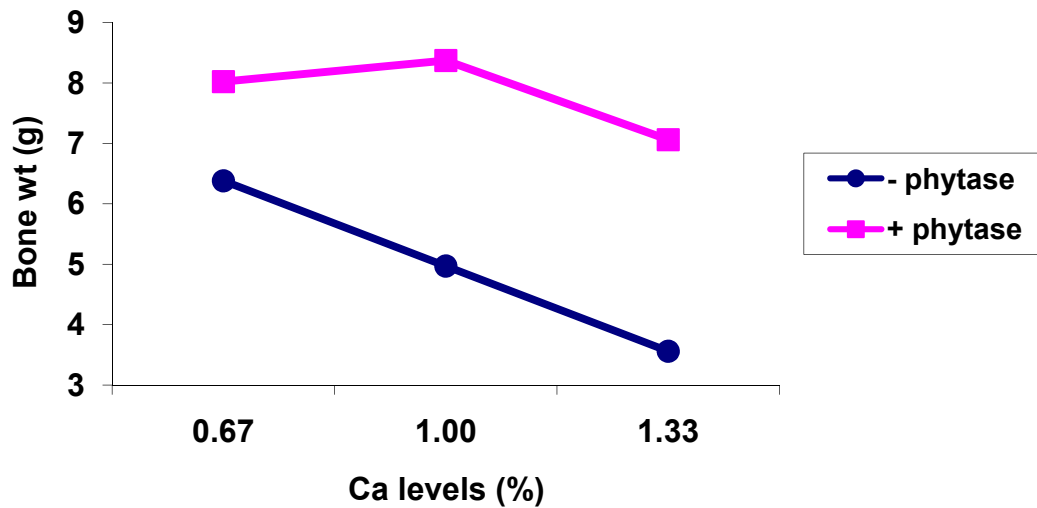


Figure 6.5. Interaction effect of Ca level and phytase on bone weight of 0 to 21 day old broilers fed low levels of nonphytate P (nPP) with varying levels of Ca (0.67, 1.00, or 1.33%), experiment 2. SEM = 0.32.

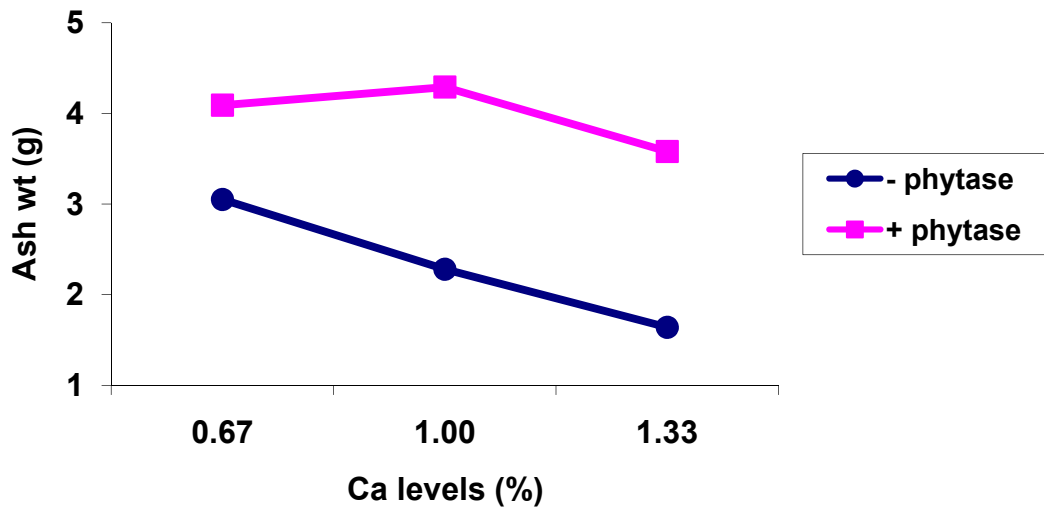


Figure 6.6. Interaction effect of Ca level and phytase on ash weight of 0 to 21 day old broilers fed low levels of nonphytate P (nPP) with varying levels of Ca (0.67, 1.00, or 1.33%), experiment 2. SEM = 0.16.

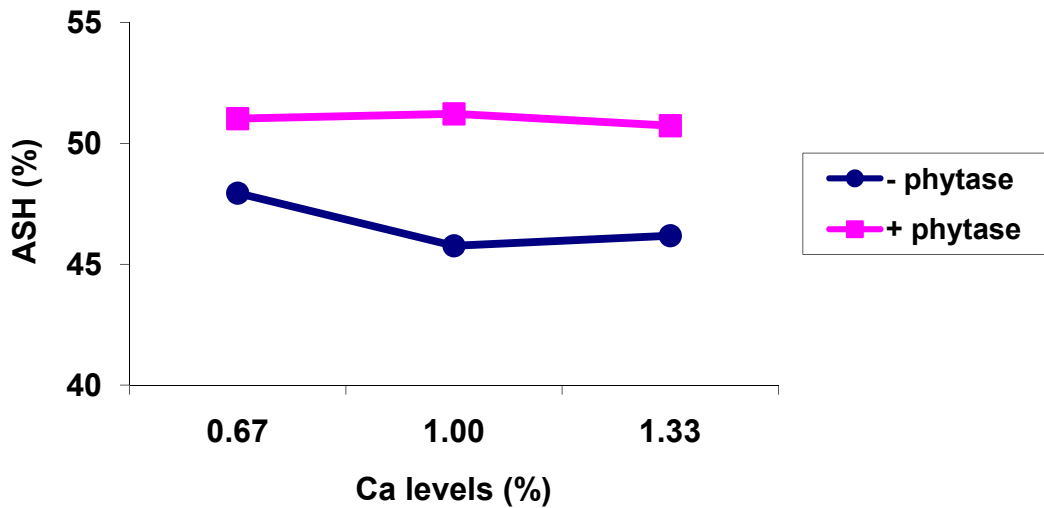


Figure 6.7. Interaction effect of Ca level and phytase on percent tibia ash (ASH) of 0 to 21 day old broilers fed low levels of nonphytate P (nPP) with varying levels of Ca (0.67, 1.00, or 1.33%), experiment 2. SEM = 0.28.

This increase in growth performance with phytase supplementation to a P inadequate diet is well documented in the literature (Selle and Ravindran, 2007). A large portion of this increase is attributed to the increase in aP to the animal. That broilers fed the Finase product had higher ADG, ADFI, and BBS, supports the data that Finase released more inorganic P than Optiphos with values ranging from 0.065 to 0.072 for Optiphos and 0.092 to 0.105 for Finase. The results of the release of inorganic P by both phytases are in general agreement with reports by various researchers summarized by Selle and Ravindran (2007). They reported that 805 FTU of phytase is equivalent to 1.05 g/kg of inorganic P in diets containing an average of 0.44% total P and 0.24% phytate P.

The average utilization of P was calculated to be similar (63.67%) for both Finase and Optiphos; however, Optiphos showed a tendency for higher average utilization of Ca than Finase (51 vs 48%). The utilization data are not consistent with the data that indicate Finase released more P than Optiphos. However, the quantity of rP was greater for broilers fed Finase than for those fed Optiphos, which indicates the higher feed intake of the broilers fed Finase was responsible for the differences in growth and BBS, and not the utilization of P.

In experiment 1, broilers fed the Optiphos supplemented diets had lower RBV and rP compared with broilers fed Finase; however, they had similar P utilization. Again, this response may be attributed to a lower feed intake of broilers fed Optiphos than those fed Finase. Broilers fed Optiphos also had a greater utilization of Ca. High dietary levels of Ca have been shown to decrease feed intake. Therefore, one possible reason for the difference in performance of broilers fed Optiphos vs those fed Finase could be the greater release by Optiphos of dietary Ca that negatively affected the performance of broilers fed Optiphos. Therefore, experiment 2 was conducted to investigate the effect of dietary Ca level on Optiphos efficacy. Three levels of dietary Ca were used, 0.67, 1.00, and 1.33%, which represent 0.33% below and above the 1%

used in experiment 1. The results indicated that increasing the Ca level without phytase supplementation decreased growth performance and bone characteristics. Optiphos supplementation at 500 FTU increased ADG, ADFI, and all bone response variables measured at the higher levels of dietary Ca; however, feed efficiency was not affected by phytase supplementation, which indicates that improvement in growth and bone responses was as a result of an increased feed intake. This result is not in agreement with what is presently accepted according to Selle et al. (2009). They reported that dietary Ca level had a negative effect on phytase efficacy. The interactions observed wherein Optiphos increased growth and bone responses more at the higher levels of Ca indicate that the high levels of dietary Ca used in this experiment did not affect the efficacy of Optiphos; however, the lower level of Ca limited the response to phytase in that insufficient Ca was present to utilize the P released by Optiphos. Hence, no difference in growth performance of the broilers fed the low level of Ca with phytase supplementation was observed. This response is in agreement with results of Driver et al. (2005) who reported a greater increase in growth performance and bone characteristics of broilers fed 0.80% Ca compared with those fed 0.47% Ca with phytase supplementation.

In summary, from the results of both experiments, there are differences in benefits obtained from phytases derived from *Escherichia coli*, and these differences can be attributed to feed intake. Increased dietary Ca level decreased feed intake but did not affect the efficacy of Optiphos.

CHAPTER 7

SUMMARY AND CONCLUSIONS

This research was conducted with broilers to establish P requirements for shorter feeding phases, identify the Ca:nPP that optimizes growth performance, investigate possible carryover and compensatory growth of broilers with regards to dietary P level, to compare the efficacy of 2 phytase sources, and to study the effect of Ca level on phytase efficacy.

One experiment with 3 trials was conducted to identify the nonphytate P (nPP) requirement of 0 to 14 d old broilers. Requirement estimates were 0.52, 0.55, and 0.52% nPP for bone breaking strength (BBS), tibia weight, and percentage of tibia ash (ASH), respectively.

One experiment with 2 trials was conducted to identify the nonphytate P (nPP) requirement of 14 to 28 d old broilers. Requirement estimates were not greater than 0.45% nPP.

One experiment with 2 trials was conducted to estimate the optimal Ca:nPP for 0 to 18 d old broilers and to investigate Ca and P utilization when varying Ca:nPP. The optimum Ca:nPP was 1.9:1 for 0 to 18 d old broilers. Utilization of Ca and P decreased with increasing nPP, and Ca utilization decreased at Ca:nPP of 2.2:1 but increased at 2.5:1.

One experiment was conducted to estimate the optimal Ca:nPP for 14 to 28 d old broilers. The optimum Ca:nPP for 14 to 28 d old broilers seemed to be above the 1.9:1 for 0 to 14 d old broilers.

Two experiments were conducted to investigate the effect of dietary starter phase nPP level on broiler growth performance and bone characteristics in subsequent growth phases. Feeding below the nPP requirement for 0 to 14 d resulted in better adaptation to a lower nPP in later phases. Feeding above the nPP requirement for 0 to 21 d resulted in a decrease in ADG at 35 d and G:F at 49 d.

One experiment was conducted to compare the relative bioavailability of 2 *Escherichia coli* phytase products. Supplementation of Finase resulted in greater growth performance, bone characteristics, and rP than broilers fed Optiphos.

One experiment was conducted to investigate the influence of Ca level on the efficacy of phytase. Results indicate that the higher dietary Ca levels fed did not influence phytase efficacy.

In conclusion, the nPP requirement of the broiler decreases with age; however, when utilizing shorter feeding phases, the nPP requirement to optimize bone characteristics is higher than the current recommendation. Utilization of Ca and P decrease as dietary inclusion increases, and an excess of either mineral will decrease the utilization of the other. Therefore, feeding as close as possible to the P and Ca requirement will increase efficiency. Feeding below the P requirement in the starter phase seems to be more beneficial to reducing dietary P in later phases than feeding above the requirement. The efficacy of phytase was not affected by higher levels of dietary Ca but was limited by the lower levels of Ca. Further research is needed to evaluate the exact mechanism by which broilers adapt to early marginal deficient P.

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**APPENDIX
LIST OF ABBREVIATIONS**

Items	Abbreviation
Acid insoluble ash	AIA
Apparent Ca utilization	ADCa
Apparent ileal Ca digestibility	ADICa
Apparent ileal P digestibility	ADIP
Apparent P utilization	ADP
Ash percentage	ASH
Available P	aP
Average daily feed intake	ADFI
Average daily gain	ADG
Body weight	BW
Bone breaking strength	BBS
Corn	C
Crude protein	CP
Feed conversion ratio	FCR
Gain:feed	G:F
Metabolizable energy	ME
Negative control	NC
Nonphytate P	nPP
Phytase	Phy
Phytase unit	FTU

Positive control	PC
Relative bioavailability	RBV
Retained P	rP
Soybean meal	SBM
Total sulfur amino acids	TSAA

VITA

Syrena Campbell was born in September 1970, in St Catherine, Jamaica. She graduated from St. Jago High School in the spring of 1990. She married Joseph Powell in the fall of 1990; the union produced three daughters Shantel, Jossel, and Tyler. Syrena worked for six years as a Laboratory Technician at the National Research Station in St. Catherine, Jamaica. She graduated from the College of Agriculture, Science, and Education with an Associates of Science degree in general agriculture in the spring of 1996. She then worked with the Ministry of Education in St. Catherine, Jamaica, as a Teacher of Chemistry, at the Old Harbour High School. Syrena obtained a diploma in education (Honors) in the fall of 2002 from the College of Agriculture, Science, and Education. She then transferred to Louisiana State University where she graduated in the fall of 2003 with a Bachelor of Science degree (*Summa Cum Laude*) in animal, dairy, and poultry sciences. Syrena continued in graduate school studying in nonruminant nutrition at Louisiana State University. She obtained a Master of Science degree in animal science in the fall of 2005. Syrena worked as a Research Associate for the LSU AgCenter and continued to study. She is presently a candidate for a doctoral degree in animal science.