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**THE EFFECT OF DIETARY PHOSPHORUS LEVEL AND PHYTASE
SUPPLEMENTATION ON GROWTH PERFORMANCE, BONE BREAKING
STRENGTH, AND PHOSPHORUS EXCRETION IN BROILERS**

A Thesis

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
Master of Science

in

The Interdepartmental Program in
Animal, Dairy, and Poultry Sciences

by

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ABSTRACT

This research was conducted to determine the effects of feeding different P levels with and without phytase supplementation on broiler growth performance, bone breaking strength (BBS), and P excretion. An experiment with 4 trials was conducted with 7,840 Ross x Ross straight run broilers. For each trial, 1,960 broilers were allotted on d 0 to 4 treatments with 7 replications per treatment with 70 broilers per replication. The broilers were fed a 4 period feeding program consisting of starter (0 to 14 d), grower (14 to 32 d), finisher (32 to 41 d), and withdrawal (41 to 50 d) periods. For each trial, the same pen was used continuously for each treatment/replication combination, and the litter was not removed between trials. Broilers were fed a control diet (0.43, 0.40, 0.36, or 0.32% nonphytate P (nPP) in the starter, grower, finisher, and withdrawal periods, respectively), a low Ca and P (LCaP) diet with a 0.05% reduction in nPP in each period, and these two diets supplemented with 600 phytase units/kg (nPP was reduced by 0.094% in diets with phytase). Diet did not affect ($P > 0.10$) broiler performance in the starter or withdrawal periods. Generally, both phytase addition and the LCaP diet decreased some aspects of growth performance during the grower and finisher periods. There was no main effect of phytase on BBS, but BBS was decreased in the broilers fed the LCaP diet with phytase addition (nPP x Phy, $P < 0.01$) in the grower period, and BBS was decreased in finisher ($P < 0.02$) and withdrawal ($P < 0.01$) periods for broilers fed the LCaP diet. Total P (TP), soluble P (SP), and reactive soluble P (RSP) were decreased ($P < 0.04$) in the litter of broilers fed the LCaP diets. Total P was decreased ($P < 0.01$) in the litter of broilers fed phytase, but SP and RSP were not affected ($P > 0.10$) by phytase. These data indicate that phytase supplementation at 600 phytase units/kg reduces growth in some periods, has no effect on BBS, and that phytase reduces TP but not SP or RSP in the litter.

CHAPTER 1 INTRODUCTION

Phosphorus is an essential nutrient for plants and animals and is critically important to the production of poultry. However, there is a concern worldwide regarding the quantity of P that is released into the environment generated from the waste of commercial poultry production. This concern rises from the fact that P contributes to the eutrophication of water. This enrichment of surface waters by plant nutrients, according to Withers et al. (1995) is a form of pollution that restricts the potential use of affected water. This problem has led to vigorous research to reduce the quantity of P as a byproduct of the poultry industry.

Recent research has reported substantial differences in the nonphytate phosphorus (nPP) requirement of broilers compared to those published by the NRC (1994). Waldroup et al. (2000) reported that the nPP requirement for the starter phase ranges from 0.37 to 0.39%. Angel et al. (2000a,b) determined the nPP requirement to be between 0.32 and 0.28% nPP (0.80% Ca) for the grower period (18 to 32 d), between 0.24 and 0.19% nPP (0.70% Ca) for the finisher period (32 to 42 d), and 0.11% nPP (0.61% Ca) in the withdrawal period (42 to 49 d). Dhandu and Angel (2003) determined the nPP requirement in a 4 period system and reported a requirement of 0.20% nPP for the finisher period and 0.16% nPP in the withdrawal period. These nPP requirement results are considerably lower than the recommendation in the NRC (1994). These data suggest that it is possible to reduce the P concentration of the diet in an effort to reduce the quantity of P in the litter, without reducing broiler growth performance, therefore, reducing the potential for P pollution. Researchers are in agreement that a reduction of supplemental P in the diet results in a decrease in total P in broiler litter; however there is the need to establish this response in a 4 period feeding program.

Researchers also are in general agreement that the supplementation of phytase to the diet of broilers can replace approximately 0.1% dietary P. However, the data are inconsistent as to the effect of phytase on the soluble P (SP) concentration in litter runoff water during rainfall simulation (Delaune et al., 2001). This is a major concern because the soluble P in the litter is readily available to aquatic plants, and it is often considered the key factor contributing to accelerated eutrophication of surface waters (Pote, 2000).

Although considerable improvements have been made in recent years in the reduction of P in poultry excreta, there are many areas of research that need to be conducted. Therefore, the objectives of this research were to investigate the effect of lowering the P concentration and the addition of phytase in the diet on growth performance, bone breaking strength (BBS) and P in the litter of broilers in a 4 period feeding program.

CHAPTER 2 LITERATURE REVIEW

Phosphorus

Phosphorus makes up 1% of the weight of broilers, with about 80% found in bones and 20% in soft tissue and blood (Waldroup, 1985). The bulk of P in the bone is present as hydroxyapatite, which provides important structural support to the skeleton. Because of the need for rapid skeleton development in fast growing broilers, there is a requirement for P in the diet of broilers. Apart from the structural role of P, P also has numerous other physiological functions. A deficiency of P may result in reduced growth performance and increased mortality. Therefore, a failure to provide adequate P in the diets of broilers can lead to serious problems within the poultry industry.

The quantity of P required by the broiler is dependent on a number of factors; the most important of which is age of the broiler. It has been established that as the broiler gets older, the requirement for P decreases. The present requirement guideline recommended for P by the NRC (1994) entails 3 periods, starter (0 to 3 wk) 0.45% nPP, grower (3 to 6 wk) 0.35% nPP, and finisher (6 to 8 wk) 0.30% nPP. The industry however, is generally utilizing a 4 period feeding program (0 to 14 d, 14 to 32 d, 32 to 41 d, and 41 to 49 d). Researchers are constantly working on the requirement for P for the different periods of growth using different variables to establish a requirement. However, there are some differences between the recommended requirement by the NRC (1994), which is based on research published from 1952 to 1982 and recent research that is suggesting a lower P requirement than those recommended.

Limiting Dietary Phosphorus Level

The most significant single factor that affects fecal P content is dietary P level. Diets can be supplemented with microbial phytase, but dietary P level is more important. Without adequate

dietary P, no strategy works. There is no doubt that P content in the excreta can be decreased by lowering dietary P level. However, reducing dietary P level without reducing bird performance must be based on a good understanding of the broiler's requirement for P, because P is one of the most critical nutrients for broilers. The increase in mortality and other adverse effects on broiler breeders, broilers, and laying hens from 1990 to 1993 in the Netherlands were largely due to a reduction in dietary P level, which was made in an effort to minimize P pollution (van Tuijl, 1998). Numerous studies have been conducted to determine the P requirement of broilers for the first 3 or 4 wk of age; however, minimal research is available after this time, when feed consumption and P excretion is the greatest. Numerous researchers have reported P concentrations that they considered to be nearer to the broiler requirement than the NRC (1994) recommended values for the grower, finisher, and withdrawal periods. A brief review of recent research on the P requirement is outlined for the respective periods.

Starter Period (0 to 21 d)

Waldroup et al. (2000) reported that the nPP requirement was greatest for maximum tibia ash and ranges from 0.39% for normal yellow dent corn to 0.37% for high available P corn. Brugelli et al. (1999) reported a nPP requirement of 0.31% for weight gain to 0.43% for tibia ash. Boling-Frankenbach et al. (2001) reported a requirement of 0.41% when using tibia ash as the response variable. These are generally lower than the NRC (1994) recommended value of 0.45% for this period.

Grower Period (21 to 42 d)

Yan et al. (2001) reported that nPP levels of 0.33, 0.186, and 0.163% were required for tibia ash, BW gain, and gain to feed respectively for 3 to 6 wk old broilers. Dhandu and Angel (2003) reported a requirement of 0.20% nPP for broilers 32 to 42 d of age using tibia ash as the

response variable. Angel et al. (2000a) reported that the nPP requirements for maximizing bird performance, BBS, and bone ash range from 0.32 to 0.28% for the grower period. These values are below the recommended 0.35% nPP by the NRC (1994).

Finisher Period (42 to 63d)

Dhandu and Angel (2003) reported a nPP requirement of 0.16% based on tibia ash weight for broilers 42 to 49 d. Yan et al. (2003) reported that the level of nPP needed to optimize tibia ash was 0.31, 0.23, and 0.22% for 49, 56, and 63 d respectively. Ling et al. (2000) reported a requirement of 0.26 and 0.18% in the finisher period and 0.19 and 0.14% in the withdrawal period to maximize BBS and bone ash for broilers in a 4 period feeding program. These recent values are generally below the NRC (1994) 0.30% nPP recommended requirement. The industry tends to feed a higher quantity of P than recommended by the NRC (1994) and recent research findings.

Sources of Phosphorus

Dietary P in a typical broiler diet is obtained from organic material (plant and animal feedstuffs) and supplemental inorganic P. The P from plant material can be classified into 2 separate groups; organically bound P present as salts of phytic acid and P present in other forms such as nPP (Waldroup, 1999). Generally it is accepted that the broiler is not able to fully utilize the organically bound P because they have little if any of the phytase enzyme that is needed to break down phytate. A typical corn-soybean meal diet contains 0.2% of its P bound as phytate (Edwards 1991); therefore, even though such a diet would contain enough total P to meet the need of the broiler, the phytate bound portion is partially unavailable. This unavailability of P results in the need for supplementation with inorganic P. The bioavailability of P from animal and inorganic sources is considered to be variable (Waldroup, 1999).

Vander der Klis and Versteegh (1996) reported that the bioavailability of P in animal byproducts ranges from 65 to 74% and that the P in inorganic phosphates ranges from 55 to 92% (Table 2.1). Gomes et al. (1993) reported that the relative availability for monoammonium and monocalcium phosphates were 81 and 82%, respectively. The values of Gomes et al. (1993) were based on the 100% availability of P from dicalcium phosphate. Waldroup (1999) indicated that supplemental P makes up approximately 60% of the diet of broilers; therefore, the type of P source that is fed will impact the quantity of P in the excreta.

Table 2.1 Availability of P in some animal feed material and commercial phosphates measured in 3 wk old broilers (Van der Klis and Versteegh, 1996)

Sources of P	Total P %	Available P % of total
Bone meal	7.6	59
Fish meal	2.2	74
Meat meal	2.9	65
Meat and bone meal	6.0	66
Calcium sodium phosphate	18.0	59
Dicalcium phosphate (anhydrous)	19.7	55
Dicalcium phosphate (hydrous)	18.1	77
Monocalcium phosphate	22.6	84
Mono-dicalcium phosphate	21.3	79
Monosodium phosphate	22.4	92

Phytase Supplementation

The supplementation of broiler diets with microbial phytase, which is an enzyme that breaks down phytate (Gibson and Ullah, 1990) is well documented. To date, many studies utilizing phytase have reduced dietary P by approximately 0.1% overall relative to the control diet. Mitchell and Edwards (1996) indicated that the total P requirement for 0 to 21-d old broilers was reduced by 0.1% nPP by the supplementation of 600 phytase units/kg diet. Waldroup et al. (2000) reported that the supplementation of phytase releases approximately 50% of the P bound as phytate. These researchers using 0 to 21 d old broilers reported that the P requirement was reduced from 0.39% to 0.29% in diets with yellow dent corn, and reduced from 0.37 to 0.32% in high available P corn. Yan et al. (2003) reported that supplementation of broiler diets with 800 phytase units/kg diet reduced the nPP requirement from 0.31 to 0.15% for 49-d old broilers, and from 0.23 and 0.22 to 0.10% for broilers 56 and 63 d respectively. While this reduction has been demonstrated in many phytase studies, the majority of these have focused on broilers 21 d of age, with the results transferred to later stages.

Response of BW to phytase has been variable. Some researchers have shown significant increases in BW with phytase supplementation (Huff et al., 1998; Yan et al., 2000). Huff et al. (1998) fed broilers a control diet consisting of 0.45, 0.43, and 0.40% nPP for 0 to 21, 21 to 42, and 42 to 49 d, respectively. They then reduced the nPP levels by 0.1% in diets with phytase. The levels of nPP fed by these researchers are higher than the recommended requirement by the NRC (1994). They reported a 4% increase in BW with phytase supplementation over the control diet. These researchers did not adjust the diet for energy or AA that phytase might release. This could account for the increase in BW observed by these researchers. Yan et al. (2000) reported

an increase in 56-d BW with phytase supplementation to diets containing 0.15% nPP, but they reported no change in BW with diets that were at the NRC (1994) recommended levels of nPP. Watson et al. (2005) reported an increase in average daily gain (ADG) and average daily feed intake (ADFI) for broilers in the starter period with phytase addition to diets adequate (0.45% nPP) and deficient (0.25% nPP) in nPP. Other researchers have demonstrated that phytase supplementation has no effect on BW (Applegate et al., 2003; Miles et al., 2003; and Shelton et al., 2004). Applegate et al. (2003) reported no effect with phytase supplementation on BW, ADFI, or G:F. The control diet utilized by these researchers consisted of 0.48, 0.35, 0.31, and 0.30% nPP for the starter (0 to 17 d), grower (17 to 31 d), finisher (31 to 42 d), and withdrawal (42 to 49 d) periods, respectively. The nPP content was reduced by 0.1% for the starter and grower period and by 0.06% for the finisher and withdrawal periods when phytase was added. Miles et al. (2003) formulated to the NRC (1994) recommended requirement levels of nPP for broilers in the starter (1 to 21 d) and grower (22 to 42 d) periods, and they reduced the nPP levels by 0.10% in diets with added phytase. They reported no difference in BW for broilers with phytase supplementation. Shelton et al. (2004) formulated diets to 0.45, 0.41, and 0.35% nPP for the starter (0 to 15), grower (16 to 35), and finisher (36 to 42 d) periods, respectively. These researchers utilized the phytase nutrient matrix values for Ca, nPP, ME, and AA in formulating some diets but not others for broilers in the floor pen study. They reported that there was no difference in final BW, ADG, ADFI, and G:F between the broilers fed the control diet and those fed the diets formulated with the phytase nutrient matrix values. They concluded that using the phytase nutrient matrix values in formulating diets for broilers had no negative effect on broiler growth performance when diets were formulated to meet the AA and ME needs of broilers. Angel et al. (2005) in a series of 3 EXP reduced the nPP concentration with the addition of

phytase by 0.1% for the diets formulated to meet the NRC (1994) recommended requirement (0.45, 0.35, 0.35, and 0.30% nPP for starter, grower, finisher, and withdrawal periods, respectively) and 0.06% for the University of Maryland recommended requirement (0.45, 0.31, 0.23, and 0.18% nPP) and reported no differences in BW, ADFI, and feed to gain for the diets with and without phytase supplementation. Therefore, from published data, phytase supplementation seems to have no negative effect on growth performance and tends to be dependent on the level of nPP in the diet.

Researchers have reported varying results of phytase supplementation on BBS in broilers. Perney et al. (1993) showed an improved BBS by the addition of dietary phytase but not by increasing levels of P when diets were formulated to contain 0.32, 0.38, and 0.44% nPP and phytase levels of 250, 500, and 750 units/kg of diet, respectively. Huff et al. (1998) reported no significant or consistent effect of phytase on BBS. However, as discussed earlier, the level of nPP fed by Huff et al. (1998) in the grower and finisher periods exceeds the NRC (1994) recommendation. Sohail et al. (1999) formulated diets to contain 0.225 and 0.325% nPP and 3 levels of phytase (0, 300, and 600 phytase units/kg diet) for broilers 4 to 6 wk of age. They reported that broilers fed diets with added phytase had much higher BBS (41.05 kg) than from broilers not fed phytase (34.35 kg), and that broilers fed different levels of nPP responded differently to supplemental phytase. They concluded that the effect of supplemental phytase on BBS was greater at the lower levels of nPP. Many researchers have also shown that bone ash percentage is increased by phytase supplementation for broilers (Qian et al., 1996; Sebastian et al., 1996). However, bone ash was not affected by phytase when dietary P was sufficient. Some other bone characteristics also have been used to evaluate the effects of phytase on P utilization;

tibia shear force and tibia shear stress (Denbow et al., 1995), tibia breaking stress (Perney et al., 1993), and tibia density (Sohail et al., 1999). Although most of the measurements were affected by phytase supplementation, they are generally considered not as sensitive as tibia ash.

Phosphorus Excretion

About 50 to 80% of P in plant-based feed ingredients is bound by phytate, which is poorly available for broilers and most other nonruminants. Also, the bioavailability of inorganic P is not 100%. Therefore, the portion of P that cannot be utilized or exceeds the requirement will be excreted by broilers. Growing concerns about the high P levels of soils treated with broiler litter and eutrophication of waterways resulting in the broiler producing areas will no doubt bring about legislation limiting land application of broiler litter based on its P content.

The phytate-bound portion of broiler diets has received a lot of attention in relation to the problems of P excretion; the same attention also should be directed towards the utilization of the inorganic P portion of the diet. In a typical broiler starter diet, about two-thirds of the dietary nPP comes from P supplements and/or animal proteins. Waldroup et al. (2000) suggested that at least half of the P in the excreta comes from the mineral P supplement, either from undigested P or from exogenous excretion of P. Although limited studies have been done on the actual digestibility of P supplements in broilers, these data suggest that most P supplements are no more than 70% digestible. Therefore, it is likely that the source of P used to supplement poultry feeds may be a major factor in the reduction of P excretion. Skinner et al. (1992) and Yan et al. (2003) reported that during the later stages of broiler production, there is minimal need for supplemental P, provided adequate skeletal development has been supported in the younger chick. This practice however, is not being done as the poultry industry continues to supplement with greater levels of P than is suggested by the research.

Litter Phosphorus

Edwards and Daniel (1993) reported that 80 to 90% of P in runoff from pastures receiving poultry litter was dissolved inorganic P, also referred to as reactive soluble P (RSP), which is immediately available for biological uptake (Sonzogni et al., 1982) by aquatic organisms. It has been assumed that the use of phytase, which decreases total P in the diet, would decrease P in the litter. However, recent results tend to be controversial. DeLaune et al. (2001) reported that phytase supplementation increased SP in the runoff water. Other researchers however, have not reported the same response. Previous research by Foster (2003) reported that phytase supplementation reduced TP by up to 10%. Foster (2003) reported no reduction in RSP or SP, either in the litter itself or in runoff from the litter obtained from broilers fed phytase when subjected to simulated rainfall. Applegate et al. (2003) reported a decrease of 24.4% and 35.6% in TP and SP, respectively, when diets were supplemented with phytase with a 0.1% reduction in nPP. Shelton et al. (2004) reported a 13.16% reduction in TP when diet contained phytase. Research therefore, has been inconsistent on the effect of phytase on TP, SP, RSP.

CHAPTER 3 MATERIALS AND METHODS

All methods used in this EXP regarding animal care were approved by the Louisiana State University (LSU) Agricultural Center Animal Care and Use Committee. An EXP was conducted with 7,840 Ross x Ross 508 commercial broilers from House of Raeford (Gibbsland, LA). This EXP consisted of 4 trials. For each trial in the EXP, 1,960 broilers were allotted on the day of hatch to 4 treatments with 7 replications per treatment with 70 broilers per replication. Each trial lasted 50 d. The broilers were housed in 1.52 x 3.05-m pens at the LSU Poultry Farm in one room of a tunnel ventilated house equipped with cool cells and fans. The pens contained 12 to 14 cm of fresh litter for the first trial. Litter was raked and a top dressing of fresh litter was applied before the start of each of the remaining 3 trials. For each trial, the same pen was used continuously for each treatment/replication combination, and the litter was not removed between trials. A lighting schedule similar to commercial conditions was used in this study. Lighting was via incandescent lighting and hours of light for a 24 h period consisted of 4 d of 24 h light, followed by 5 d of 20 h of light, 6 d of 18h of light, and 16 h of light for the remainder of the project. The temperature in the house was maintained at 29 to 32°C for the first week and was decreased by 5°C every week until the house temperature was 21 to 24 °C, weather permitting. Feed and water were offered for ad libitum consumption throughout the experimental period. Feed was fed in a mash form via a feed tray for the first week and then by 2 hanging tube feeders (43 cm diameter) per pen. Water was provided via 1 automatic waterer with 9 nipples in each pen. The broilers were fed a 4 period feeding program consisting of starter (0 to 14 d), grower (14 to 32 d), finisher (32 to 41 d), and withdrawal (41 to 50 d) periods. The dietary treatments (Table 3.1) were in a 2 x 2 factorial arrangement.

Table 3.1. Non phytate P (nPP) concentrations in treatments for the 4 growth periods ¹

Treatment	Starter (0 to 14 d)	Grower (14 to 32 d)	Finisher (32 to 41 d)	Withdrawal (41 to 50 d)
Control	0.429	0.399	0.359	0.320
Low Ca + P	0.379	0.349	0.309	0.270
Control + phytase ²	0.335	0.305	0.265	0.276
Low Ca P + phytase ²	0.285	0.255	0.215	0.176

¹ A Ca to nPP ratio of 2.22 was maintained for all diets.

² Phytase was added at 600 phytase unit/kg of diet (Natuphos 1200, BASF Corporation, Mount Olive, NJ).

For the starter period, the diets were formulated to contain 3,034 kcal ME/kg, 1.26% total Lys, and 0.91% TSAA (Table 3.2). For the growing period, the diets were formulated to contain 3,116 kcal ME/kg, 1.14% total Lys, and 0.86% TSAA (Table 3.3). For the finishing period, the diets were formulated to contain 3,160 kcal ME/kg, 0.96% total Lys, and 0.78% TSAA (Table 3.4). For the withdrawal period, the diets were formulated to contain 3,192 kcal ME/kg, 0.87 total Lys, and 0.66% TSAA (Table 3.5). All other nutrients met or exceeded the requirement (NRC, 1994). Natuphos 1200 was included in diets 3 and 4 at 0.05% to provide 600 phytase units/kg diet. The nPP were reduced by 0.094% in the diets with added phytase for all growth periods. The phytase nutrient matrix values (Table 3.6) evaluated by Shelton et al. (2004) were utilized in the diets with phytase to account for the nutrient sparing effect of phytase on ME and AA.

Table 3.2. Composition of diets for starter period (0 to 14 d)

Ingredient	Control	LCaP ¹	Control + Phy ²	LCaP + Phy ^{2,3}
Corn	54.67	55.47	56.21	57.02
Soybean meal (47.5%)	37.76	37.69	37.63	37.56
Tallow	3.23	2.93	2.30	2.00
Monocalcium phosphate	1.44	1.20	0.99	0.75
Limestone	1.29	1.09	1.22	1.02
BMD + 3 nitro ⁴	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50
Mineral premix ⁵	0.25	0.25	0.25	0.25
DL–Met	0.19	0.19	0.19	0.19
Biocox ⁶	0.05	0.05	0.05	0.05
Ethoxyquin ⁷	0.05	0.05	0.05	0.05
Vitamin premix ⁸	0.05	0.05	0.05	0.05
L-Thr	0.02	0.02	0.02	0.02
Phytase ⁹	-	-	0.05	0.05
Calculated or analyzed composition				
Crude protein	22.71	22.74	22.99	23.02
ME, kcal/kg	3,034	3,034	3,034	3,034
Lys	1.26	1.26	1.30	1.30
TSAA	0.91	0.91	0.92	0.92
Thr	0.89	0.89	0.88	0.88

Trp	0.31	0.31	0.31	0.31
Formulated Ca	0.95	0.84	0.95	0.84
Analyzed Ca	1.24	1.08	1.03	0.94
Formulated total P	0.69	0.64	0.60	0.55
Analyzed total P	0.68	0.63	0.61	0.56
nPP ¹⁰	0.43	0.38	0.43	0.38

¹ LCaP diet reduced in P by 0.05% from the control.

² Phy = Phytase.

³ LCaP + Phy diet reduced in P by 0.094% from the LCaP diet.

⁴ Bacitracin methylene disacicylate + 3-nitro-4 hydroxyphenylarsonic acid from Nutra Blend, Neosho, MO.

⁵ Provides the following per kilogram of diet: Fe, 50 mg; Mn, 100 mg; Cu, 7 mg; Se, 0.15 mg; Zn 75 mg; I, 1 mg, as ferrous sulfate monohydrate, manganese sulfate, copper sulfate, sodium selenite, zinc sulfate, ethylenediamine dihydriodide, respectively with calcium carbonate as the carrier.

⁶ Biocox provides 132.3g/kg salinomycin sodium.

⁷ Ethoxyquin provides 66.6% 6-ethoxy 1, 2 dihydro-2,2,4-trimethylquionine.

⁸ 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.

⁹ Naptuphos (BASF Corporation, Mt Olive, NJ) 1200 provides 600 phytase unit/kg diet.

¹⁰ nPP = nonPhytate P.

Table 3.3 . Composition of diets for grower period (14 to 32 d).

Ingredient	Control	LCaP ¹	Control + Phy ²	LCaP + Phy ^{2,3}
Corn	58.58	59.39	60.12	60.39
Soybean meal (47.5%)	33.37	33.30	33.23	33.16
Tallow	3.91	3.61	2.98	2.68
Monocalcium phosphate	1.33	1.09	0.88	0.64
Limestone	1.18	0.99	1.11	0.917

BMD + 3 nitro ⁴	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50
Mineral premix ⁵	0.25	0.25	0.25	0.25
DL-Met	0.19	0.18	0.19	0.18
Biocox ⁶	0.05	0.05	0.05	0.05
Ethoxyquin ⁷	0.05	0.05	0.05	0.05
Vitamin premix ⁸	0.05	0.05	0.05	0.05
L-Thr	0.04	0.04	0.04	0.04
Choline chloride ⁹	0.005	0.004	0.004	0.003
Phytase ¹⁰	-	-	0.05	0.05

Calculated or analyzed composition

Crude protein	20.96	21.00	21.24	21.27
ME, kcal/kg	3,116	3,116	3,116	3,116
Lys	1.14	1.14	1.14	1.14
TSAA	0.86	0.86	0.86	0.86
Thr	0.83	0.83	0.83	0.83
Trp	0.28	0.28	0.28	0.28
Formulated Ca	0.89	0.78	0.89	0.78
Analyzed Ca	1.24	0.92	1.03	0.88
Formulated total P	0.65	0.60	0.56	0.51
Analyzed total P	0.71	0.59	0.56	0.53
nPP ¹¹	0.40	0.35	0.40	0.35

¹ LCaP diet reduced in P by 0.05% from the control.

² Phy = Phytase.

³ LCaP + Phy diet reduced in P by 0.094% from the LCaP diet.

⁴ Bacitracin methylene disacylate + 3-nitro-4 hydroxyphenylarsonic acid from Nutra Blend, Neosho, MO.

⁵ Provides the following per kilogram of diet: Fe, 50 mg; Mn, 100 mg; Cu, 7 mg; Se, 0.15 mg; Zn 75 mg; I, 1 mg, as ferrous sulfate monohydrate, manganese sulfate, copper sulfate, sodium selenite, zinc sulfate, ethylenediamine dihydriodide, respectively with calcium carbonate as the carrier.

⁶ Biocox provides 132.3g/kg salinomycin sodium.

⁷ Ethoxyquin provides 66.6% 6-ethoxy 1, 2 dihydro-2,2,4-trimethylquionine.

⁸ 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.

⁹ Contains 600,000 mg/kg of choline.

¹⁰ Naptuphos (BASF Corporation, Mt Olive, NJ) 1200 provides 600 phytase unit/kg diet.

¹¹ nPP = nonPhytate P.

Table 3.4. Composition of diets for finisher period (32 to 41 d).

Ingredient	Control	LCaP ¹	Control + Phy ²	LCaP + Phy ^{2,3}
Corn	66.17	66.98	67.72	68.53
Soybean meal (47.5%)	26.62	26.55	26.48	26.41
Tallow	3.41	3.11	2.48	2.18
Monocalcium phosphate	1.18	0.94	0.73	0.49
Limestone	1.05	0.86	0.98	0.79
BMD + 3 nitro ⁴	0.50	0.50	0.50	0.50

Salt	0.50	0.50	0.50	0.50
Mineral premix ⁵	0.25	0.25	0.25	0.25
DL–Met	0.12	0.12	0.12	0.12
Biocox ⁶	0.05	0.05	0.05	0.05
Ethoxyquin ⁷	0.05	0.05	0.05	0.05
Vitamin premix ⁸	0.05	0.05	0.05	0.05
L-Thr	0.01	0.01	0.01	0.01
Choline chloride ⁹	0.03	0.03	0.03	0.03
Phytase ¹⁰	-	-	0.05	0.05

Calculated or analyzed composition

Crude protein	18.34	18.38	18.62	18.65
ME, kcal/kg	3,160	3,160	3,160	3,160
Lys	0.96	0.96	0.96	0.96
TSAA	0.73	0.73	0.73	0.73
Thr	0.70	0.70	0.70	0.70
Trp	0.24	0.24	0.24	0.24
Formulated Ca	0.80	0.69	0.80	0.69
Analyzed Ca	0.98	0.85	0.85	0.70
Formulated total P	0.60	0.55	0.51	0.46
Analyzed total P	0.58	0.53	0.50	0.50
nPP ¹¹	0.36	0.31	0.36	0.31

¹ LCaP diet reduced in P by 0.05% from the control.

² Phy = Phytase.

³ LCaP + Phy diet reduced in P by 0.094% from the LCaP diet.

⁴ Bacitracin methylene disacylate + 3-nitro-4 hydroxyphenylarsonic acid from Nutra Blend,

Neosho, MO.

⁵ Provides the following per kilogram of diet: Fe, 50 mg; Mn, 100 mg; Cu, 7 mg; Se, 0.15 mg; Zn 75 mg; I, 1 mg, as ferrous sulfate monohydrate, manganese sulfate, copper sulfate, sodium selenite, zinc sulfate, ethylenediamine dihydriodide, respectively with calcium carbonate as the carrier.

⁶ Biocox provides 132.3g/kg salinomycin sodium.

⁷ Ethoxyquin provides 66.6% 6-ethoxy 1, 2 dihydro-2,2,4-trimethylquionine.

⁸ 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.

⁹ Contains 600,000 mg/kg of choline.

¹⁰ Naptuphos (BASF Corporation, Mt Olive, NJ) 1200 provides 600 phytase unit/kg diet.

¹¹ nPP = nonPhytate P.

Table 3.5. Composition of diets for withdrawal period (41 to 50 d).

Ingredient	Control	LCaP ¹	Control + Phy ²	LCaP + Phy ^{2,3}
Corn	70.77	71.58	72.31	73.12
Soybean meal (47.5%)	23.17	23.10	23.04	22.97
Tallow	2.97	2.68	2.04	1.74
Monocalcium phosphate	1.01	0.77	0.56	0.32
Limestone	1.09	0.90	1.02	0.83
Salt	0.50	0.50	0.50	0.50
Mineral premix ⁴	0.25	0.25	0.25	0.25
DL–Met	0.08	0.08	0.08	0.08
Ethoxyquin ⁵	0.05	0.05	0.05	0.05
Vitamin premix ⁶	0.05	0.05	0.05	0.05
Choline chloride ⁷	0.04	0.04	0.04	0.04

Phytase ⁸	-	-	0.05	0.05
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Calculated or analyzed composition

Crude protein	17.07	17.11	17.35	17.39
ME, kcal/kg	3,192	3,192	3,192	3,192
Lys	0.87	0.87	0.87	0.87
TSAA	0.66	0.66	0.66	0.66
Thr	0.64	0.64	0.64	0.64
Trp	0.21	0.21	0.21	0.21
Formulated Ca	0.71	0.60	0.71	0.60
Analyzed Ca	0.87	0.70	0.73	0.60
Formulated total P	0.55	0.50	0.46	0.41
Analyzed total P	0.56	0.50	0.50	0.50
nPP ⁹	0.32	0.27	0.32	0.27

¹ LCaP diet reduced in P by 0.05% from the control.

² Phy = Phytase.

³ LCaP + Phy diet reduced in by 0.094% from the LCaP diet.

⁴ Provides the following per kilogram of diet: Fe, 50 mg; Mn, 100 mg; Cu, 7 mg; Se, 0.15 mg; Zn 75 mg; I, 1 mg, as ferrous sulfate monohydrate, manganese sulfate, copper sulfate, sodium selenite, zinc sulfate, ethylenediamine dihydriodide, respectively with calcium carbonate as the carrier.

⁵ Ethoxyquin provides 66.6% 6-ethoxy 1, 2 dihydro-2,2,4-trimethylquionine.

⁶ 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.

⁷ Contains 600,000 mg/kg of choline.

⁸ Naptuphos (BASF Corporation, Mt Olive, NJ) 1200 provides 600 phytase unit/kg diet.

⁹ nPP = nonPhytate P.

Table 3.6. Nutrient matrix values of Natuphos 1200 for broilers ¹

Nutrient	Value	Amount provided in the diet
Available P, %	188	0.094
Calcium, %	188	0.094
Crude protein, %	427	0.214
Lys, %	29	0.015
Met, %	5	0.003
Cys, %	10	0.005
TSAA, %	15	0.008
Trp, %	5	0.003
Thr, %	24	0.012
Val, %	26	0.013
Ile, %	22	0.011
Leu, %	33	0.017
Arg, %	16	0.008
Phe, %	21	0.011
His, %	11	0.006
ME, kcal/kg	61,937	30.969

¹ Naptuphos 1200 was added at 0.05% of the diet, which provided 600 phytase units/kg. Amino acids are on a true digestible basis.

At the end of each growth period, all birds and feeders were weighed to determine ADG, ADFI, and gain:feed; also 6 broilers were randomly selected, killed by CO₂ asphyxiation, and the left tibia was removed and frozen for subsequent determination of BBS. Bone breaking strength was determined by using a HD 250 Texture Machine (Texture Technologies Corporation, Scarsdale, NY) fitted with a 3 point bend rig with a load cell capacity of 25 (starter period) or 250 kg (grower, finisher, and withdrawal periods) and a cross-head speed of 100 mm/min. Litter was collected at the termination of the EXP from 9 locations within each pen to determine TP (Bender and Wood, 2000), SP (Pote, 2000; Self-Davis and Moore, 2000), and RSP (Pote, 2000).

Data were analyzed by analysis of variance procedures appropriate for a randomized complete block design (Steel and Torrie, 1980) using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The model included trial and trial by treatment interaction. The trial by treatment interaction was significant for most variables, but in general, they were due to changes in magnitude. Contrast statements were included to examine phytase, nPP, and phytase x nPP interaction as a 2 x 2 factorial arrangement of treatments. The pen of broilers was the experimental unit for all data.

CHAPTER 4 RESULTS

Diet did not affect ($P > 0.10$) broiler performance in the starter period. During the grower period, phytase decreased ($P < 0.01$ to 0.05), ADG, ADFI, gain to feed (G:F), and BW, and the LCaP diet decreased ($P < 0.03$ to 0.08) ADFI and G:F. Phytase decreased ADG and G:F more in broilers fed the control diet than in those fed the LCaP diet (nPP x Phy, $P < 0.10$). During the finisher period, phytase decreased ADFI ($P < 0.10$) and BW ($P < 0.01$), and the decrease in ADFI was greater in broilers fed the control diet than in those fed the LCaP diet (nPP x Phy, $P < 0.01$). During the withdrawal period, diet did not affect ($P < 0.10$) growth performance. The overall data showed no effect ($P < 0.1$) of the LCaP diet on growth performance. However, phytase supplementation decreased ($P < 0.02$ to 0.05) ADG, ADFI, and G:F. The overall effect of phytase was a 1.6% decrease ($P < 0.07$) in final BW (Table 4.1).

Bone breaking strength was not affected ($P > 0.10$) by phytase supplementation, but BBS was decreased in the finisher ($P < 0.02$), and withdrawal ($P < 0.01$) periods for broilers fed the LCaP diet. Bone breaking strength in the grower period was decreased in the broilers fed the LCaP with the addition of phytase (nPP x Phy, $P < 0.01$; Table 4.2).

Total P, SP, and RSP were reduced in the litter of broilers fed the LCaP diets ($P < 0.01$ to 0.04). Total P was decreased ($P < 0.01$) in the litter of broilers fed phytase by 23%, but SP and RSP were not affected ($P > 0.1$) by phytase supplementation (Table 4.3).

These data indicate that phytase supplementation at 600 phytase units/kg of the diet reduces growth in the grower period, has no negative effect on BBS during any period, and that phytase reduces the TP concentration of poultry litter by 25%, but phytase does not affect the concentration of SP in the litter. These data also indicate that a 0.05% reduction in P and Ca

from the control diet had no negative effect on overall growth performance, but decreases BBS in the finisher and withdrawal periods, and that the LCaP and LCaP+phy diets resulted in a 14 and 32% reduction respectively, of TP excreted.

Table 4.1 Effects of low Ca, P and phytase supplementation on broiler growth performance for starter (0 to 14 d), grower (14 to 32 d), finisher (32 to 41 d), and withdrawal (41 to 50 d) periods.¹

Item	Dietary treatments				SEM	Contrasts <i>P > F</i>		
	Control	Low Ca +P	Control + phy ²	Low Ca+ P +phy ²		nPP	Phy	nPP x Phy
<i>Starter (0 to 14 d)</i>								
BW, g	354.5	352.4	346.3	345.0	7.80	0.83	0.34	0.96
ADG ³ , g	22.47	22.34	21.91	21.79	0.49	0.81	0.29	0.99
ADFI ⁴ , g	30.00	30.35	30.31	30.35	0.73	0.79	0.84	0.83
G:F ⁵	0.750	0.737	0.725	0.719	0.02	0.64	0.31	0.87
<i>Grower (14 to 32 d)</i>								
BW, g	1,376.1	1,382.5	1,343.5	1,355.5	8.43	0.28	<0.01	0.74
ADG, g	57.45	56.98	55.15	56.11	0.43	0.56	<0.01	0.10
ADFI, g	93.52	93.96	91.61	93.37	0.63	0.08	0.05	0.30
G: F	0.615	0.607	0.603	0.601	0.002	0.03	<0.01	0.10
<i>Finisher (32 to 41 d)</i>								
BW, g	1,972.1	1,954.6	1,918.1	1,936.0	13.23	0.99	0.01	0.18
ADG, g	59.55	56.96	57.45	58.15	0.97	0.33	0.64	0.1
ADFI, g	141.55	137.27	135.82	138.60	1.33	0.57	0.10	0.01
G: F	0.420	0.415	0.423	0.417	0.005	0.33	0.49	0.93

Withdrawal (41 to 50 d)

BW, g	2,492.8	2,472.5	2,437.9	2,447.5	21.89	0.81	0.07	0.50
ADG, g	67.45	66.70	65.48	65.20	1.48	0.72	0.25	0.88
ADFI, g	173.10	171.64	170.16	171.90	2.13	0.95	0.53	0.45
G: F	0.388	0.387	0.383	0.378	0.005	0.51	0.16	0.72

Overall (0 to 50 d)

ADG, g	46.92	46.52	45.30	45.96	0.45	0.78	0.02	0.24
ADFI, g	91.78	91.24	89.34	90.98	0.68	0.42	0.05	0.11
G:F	0.511	0.510	0.507	0.505	0.002	0.37	0.03	0.88

¹ Data are the mean of 4 trials with a total of 28 replications with 70 birds per replicate pen for starter period, 64 birds per replicate pen for grower period, 58 birds per replicate pen for finisher period, and 52 birds per replicate pen for withdrawal period.

² Phy = phytase.

³ ADG = Average daily gain.

⁴ ADFI = Average daily feed intake.

⁵ G:F = Gain to feed ratio.

Table 4.2 Effects of low Ca, P, and Phytase supplementation on bone breaking strength (kg) for starter (0 to 14 d), grower (14 to 32 d), finisher (32 to 41 d) and withdrawal (41 to 50 d) periods.¹

Item	Dietary treatments				SEM	Contrasts <i>P > F</i>		
	Control	Low Ca P	Control + phy ²	Low Ca P +phy ²		P	Phy	P x Phy
Starter	10.21	9.73	9.63	8.89	0.44	0.19	0.14	0.77
Grower	33.39	34.14	34.14	31.49	0.61	0.12	0.12	0.01
Finisher	38.47	36.01	36.87	35.83	0.75	0.02	0.23	0.35
Withdrawal	39.61	36.53	39.61	37.52	0.77	<0.01	0.52	0.52

¹ Data are the mean of 4 trials with 28 replications of 6 birds per replicate pen for the starter, grower, finisher, and withdrawal periods.

² Phy = phytase.

Table 4.3 Effects of low Ca, P, and phytase supplementation on P level in litter.¹

Item	Dietary treatments				SEM	Contrasts <i>P > F</i>		
	Control	Low Ca P	Control +phy ²	Low Ca P +phy ²		nPP	Phy	nPP x Phy
TP, mg/kg ³	14,876	12,726	11,085	10,099	353	<0.01	<0.01	0.11
RSP, mg/kg ⁴	1,628	1,357	1,469	1,372	84.9	0.04	0.41	0.32
SP, mg/kg ⁵	1,666	1,441	1,632	1,443	84.8	0.02	0.85	0.83
SP, % TP ⁶	11.27	11.30	14.72	14.32	0.76	0.80	<0.01	0.78

¹ Data are means of 7 replications from 4 groups of broilers. All calculations are on a DM basis.

² Phy= phytase.

³ TP = Total P.

⁴ RSP = Reactive Soluble P.

⁵ SP = Soluble P.

⁶ Soluble P as a percent of total P.

CHAPTER 5 DISCUSSION

Diet did not affect broiler performance in the starter period. These data are in agreement with published results (Applegate et al., 2003; Angel et al., 2005). However, Yan et al., 2000 reported an increase in BW at 21 d for broilers with the addition of phytase. The results obtained in this EXP for the starter period supported the reduction of nPP from the NRC (1994) recommended values. These results indicated that a 0.05% reduction of the nPP concentration from 0.43 to 0.38% in the LCaP diets and a further 0.094% reduction in nPP with phytase supplementation had no negative effect on broiler growth performance. This in agreement with the requirement of 0.39% nPP reported by Waldroup et al (2000) and the 0.31% reported by Brugelli et al. (1999). These data suggest that the nPP concentration required to maximize growth performance in the starter period is lower than the NRC (1994) recommended level of 0.45% nPP. During the grower period, phytase decreased ADG, ADFI, G:F, and BW. This general decrease in growth performance during the grower period is not consistent with reported results from Angel et al. (2005), who reported no difference in growth performance in broilers when the nPP concentration of the diet was reduced by 0.07% from 0.33 to 0.26%. Also Yan et al. (2003) reported that a reduction of 0.05% from 0.20% nPP with the addition of phytase resulted in no difference in growth performance between broilers fed phytase. However, Yan et al. (2000) reported that when dietary P level was reduced from the NRC (1994) recommended nPP level of 0.35 to 0.275% when supplemented with phytase, BW of broilers was decreased by phytase; however a reduction of 0.15% nPP benefited from phytase supplementation. The results reported by Yan et al. (2000) indicates that the level of dietary P influences the effect of phytase supplementation on growth performance, the results are similar to the results obtained in the

present research, where phytase decreased ADG and G:F more in broilers fed the control diet than in those fed the LCaP. The levels of nPP fed in the grower period (0.40, 0.35, 0.31, and 0.26%) are higher than the levels reported by Angel et al. (2000a), Yan et al. (2001), and Dhandu and Angel (2003) to maximize broiler growth performance (0.28, 0.186, and 0.20% nPP respectively). The start of the growth period in this research also differs from others in that the growth period started at d 14 and not at d 17 or 18. However, Shelton et al. (2004) started the grower period at d 15 and reported no difference in growth performance of broilers using the same value for phytase as this research. This effect of reduced growth performance with the addition of phytase to diets high in P indicates that the effect of phytase supplementation is dependent on the level of dietary P fed, with the lower levels of dietary P receiving the positive benefits. The lower BW in broilers fed phytase in the finisher and withdrawal periods seems to be a carry-over effect from the grower period because there was no difference in ADG and G:F for these periods. However, during the finisher period, phytase supplementation decreased ADFI, and this decrease was greater in broilers fed the control diet than in those fed the LCaP diet. This response again seems to be due to the level of 0.26% nPP fed in this period; this level of nPP is higher than the 0.16% reported by Dhandu and Angel (2003) and the 0.23% reported by Yan et al. (2003). The overall data indicate that phytase supplementation decreased final BW by 1.6% compared with broilers not fed phytase. Yan et al. (2000) reported a decrease of 3% in 56 d old broilers fed phytase with a reduction of 0.075% in dietary P from the NRC recommended values, and a 5.7% increase for the broilers whose diet was reduced by 0.15% dietary P. The increase in BW was not observed in broilers fed phytase in the LCaP diet. A possible explanation is that the increase in availability of AA and ME from phytase was used in the formulation of diets for this EXP, but they were not considered in the work of Yan et al.

(2000). These data indicate that the effect of phytase supplementation is dependent on the concentration of dietary P.

Bone breaking strength was not affected by phytase supplementation in any period of growth. This response indicates that the release of nPP by phytase was adequate for normal bone development. This response also indicates that the decrease in growth performance is not a result of P deficiency, because BBS would be expected to be the first variable affected by phytase supplementation. Bone breaking strength was decreased in the finisher and withdrawal periods for broilers fed the LCaP diet. This indicates that the level of nPP fed was inadequate to maximize BBS. Ling et al. (2000) reported that nPP levels of 0.26 and 0.19% were needed to maximize BBS in the finisher and withdrawal periods, respectively. Bone breaking strength in the grower period was decreased in broilers fed the LCaP with the addition of phytase, which indicates that the 0.26% nPP fed in this diet was inadequate to maximize BBS. This nPP level is below the 0.28 to 0.32% nPP reported by Angel et al. (2000a) to maximize BBS in the grower period.

Total P, SP, and RSP were reduced by 14.5, 13.5, and 16.6% respectively in the litter of broilers fed the diets with LCaP. This indicates that if broilers are fed closer to requirement for P, then the concentration of P in the litter would be decreased, resulting in a reduction in the potential of litter P to pollute waterways. Total P was decreased in the litter of broilers fed phytase by 25.4% in the control diet and 20.6% in the LCaP diet, but SP and RSP were not affected by phytase supplementation. These data are similar to those reported by Applegate et al. (2003) and Shelton et al. (2003). The increase in SP in runoff from litter of broilers fed phytase reported by DeLaune et al. (2001) was not observed in this research. These data indicate that there is a positive correlation between the concentration of P in the diet and TP in the litter.

In summary, these data indicate that phytase supplementation at 600 phytase unit/kg reduces growth in the grower period, has no negative effect on BBS during any period, and that phytase reduces the TP concentration of poultry litter, but it does not affect the concentration of SP in the litter. These data also indicate that a 0.05% reduction in P and Ca from the control nPP levels of 0.43, 0.40, 0.36 and 0.32 for the starter, grower, finisher, and withdrawal periods respectively, has no negative effect on overall growth performance, but decreases BBS in the finisher and withdrawal periods.

CHAPTER 6 CONCLUSION

Dietary nPP level can be reduced from the levels fed in this EXP (0.43, 0.40, 0.36, and 0.36% for the starter, grower, finisher, and withdrawal periods, respectively) by 0.05% without negatively affecting broiler growth performance. However, the reduction in BBS during the finisher and withdrawal periods must be investigated further to establish the BBS that is required to minimize loss during processing.

Total P, SP, and RSP were reduced in the litter of broilers fed the LCaP and phytase supplemented diets. Soluble P concentrations in litter were not affected by phytase supplementation.

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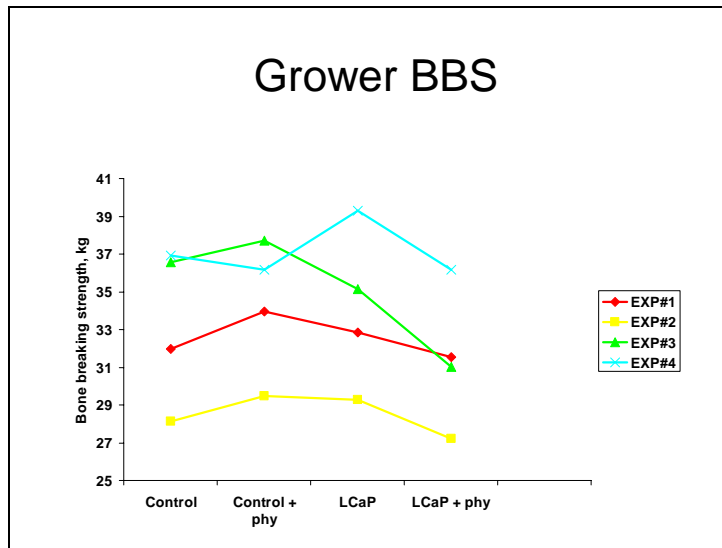
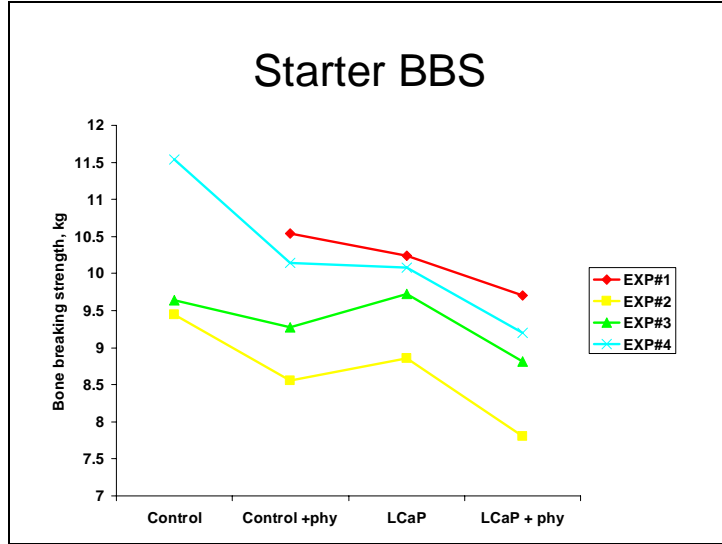
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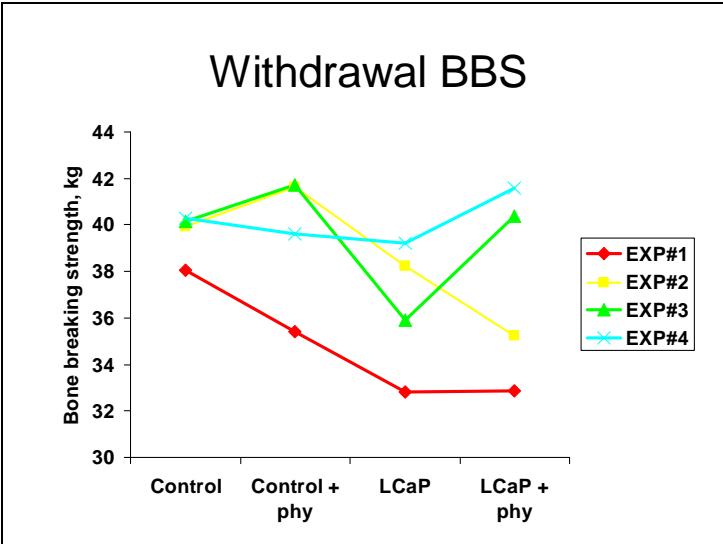
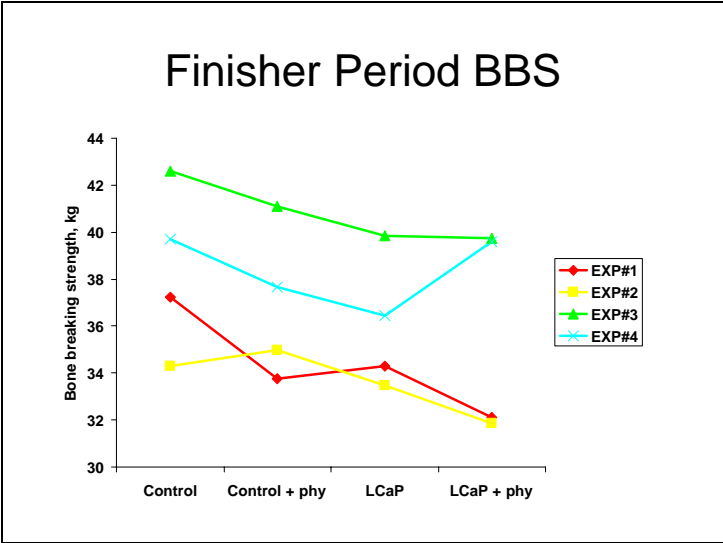
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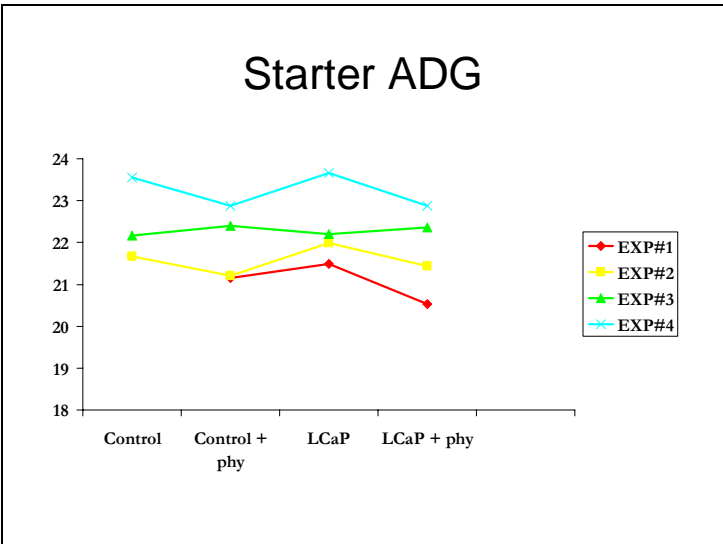
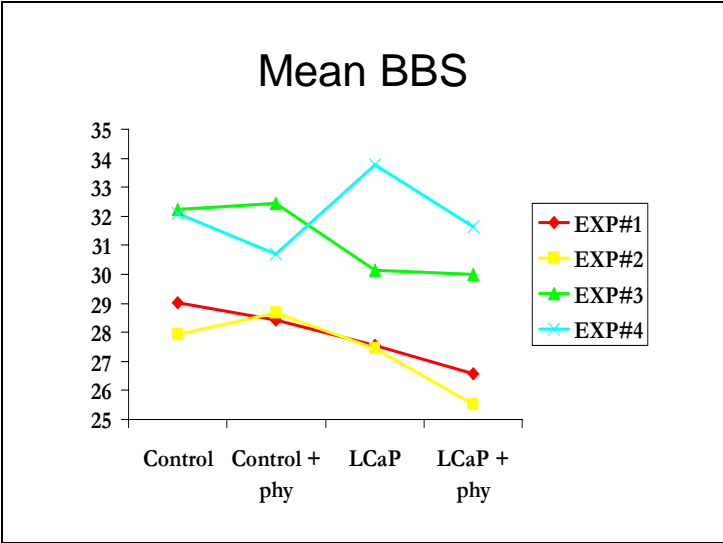
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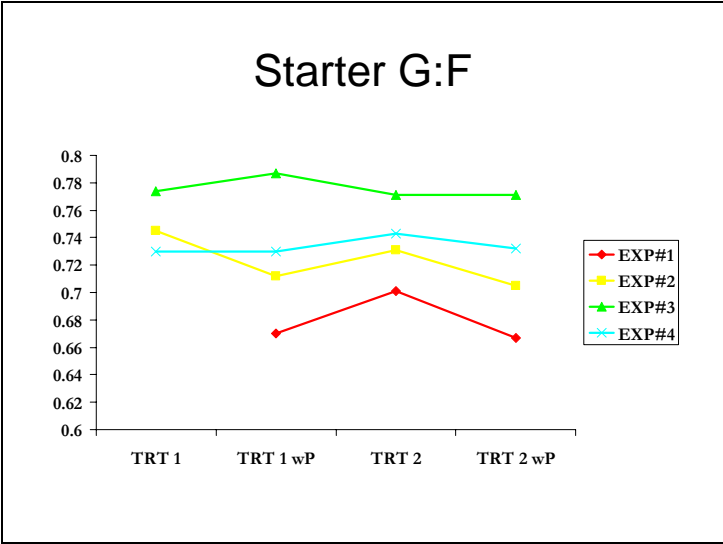
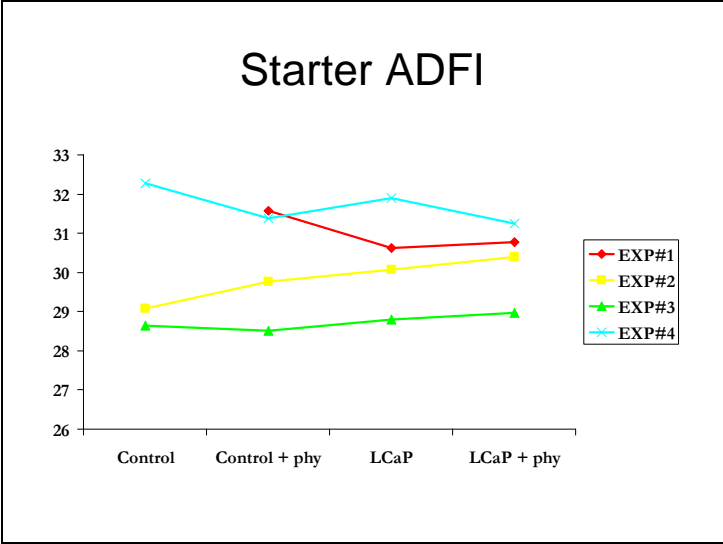
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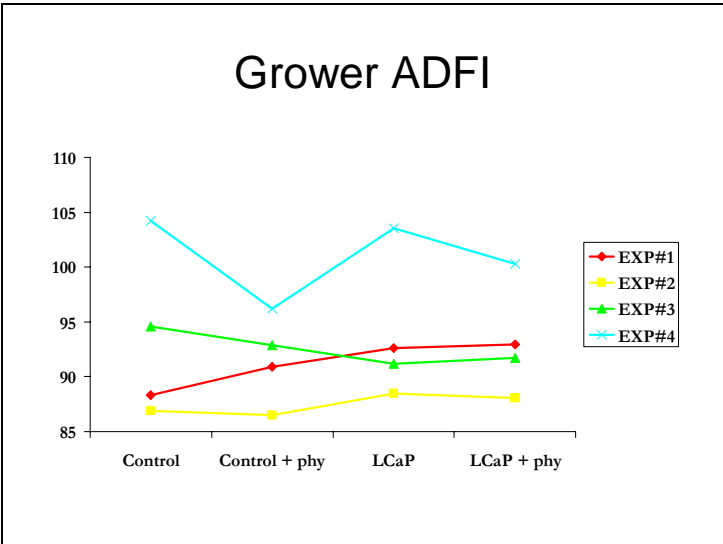
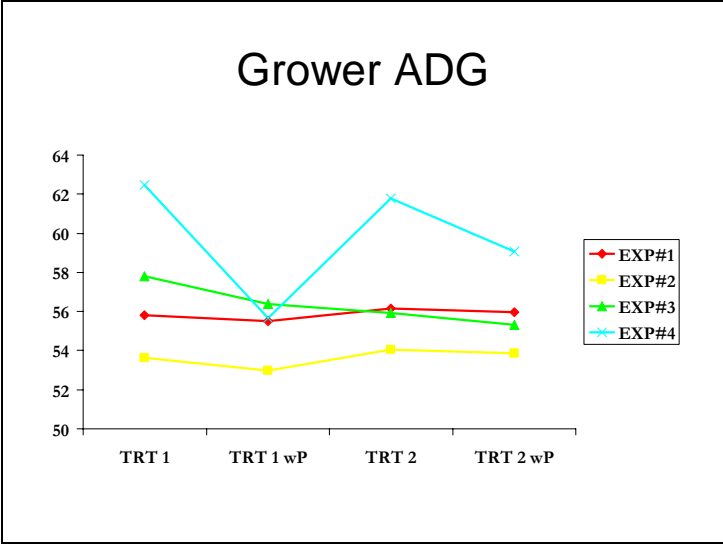
APPENDIX A
GRAPHS OF FOUR TRIALS

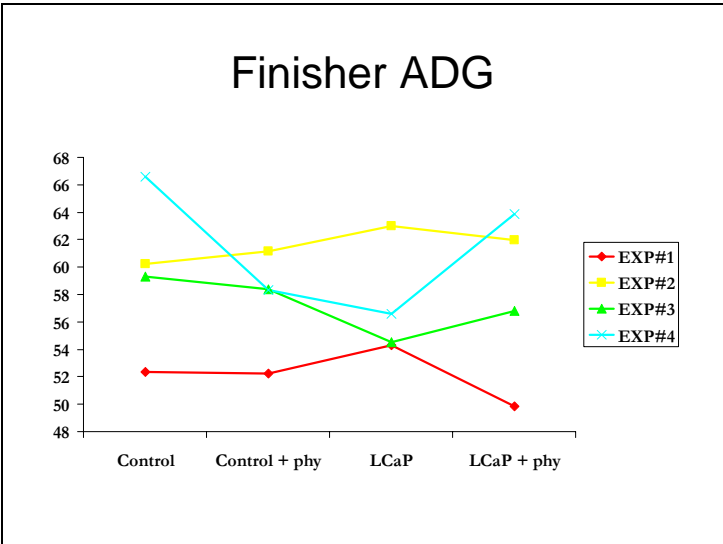
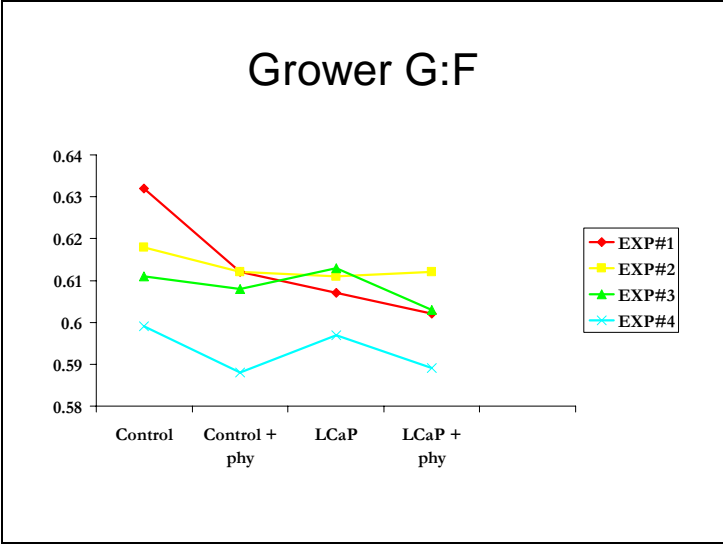


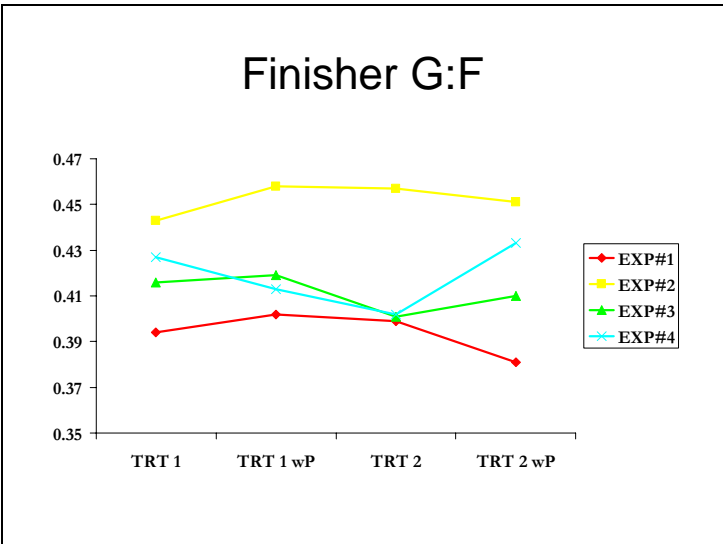
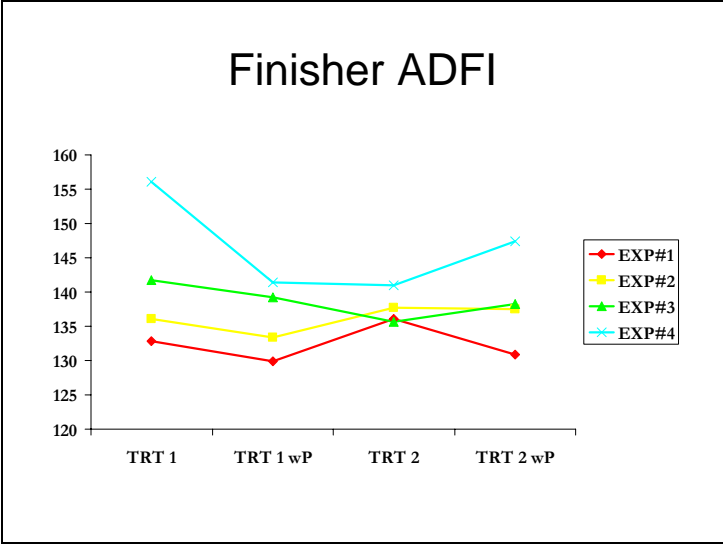


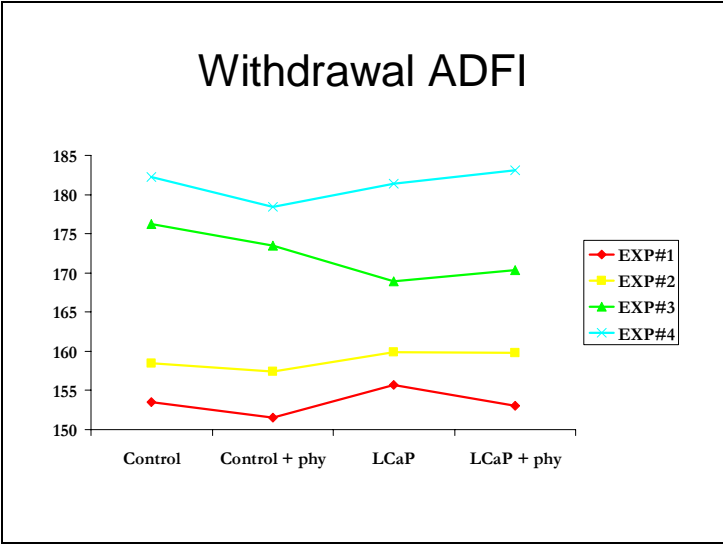
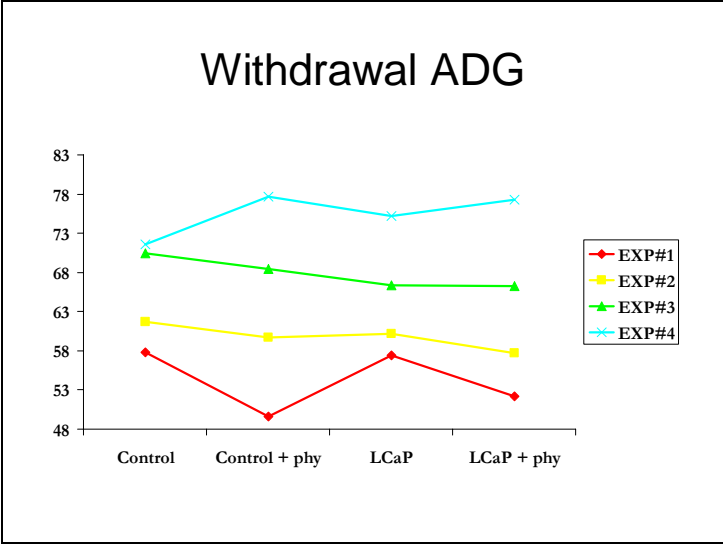


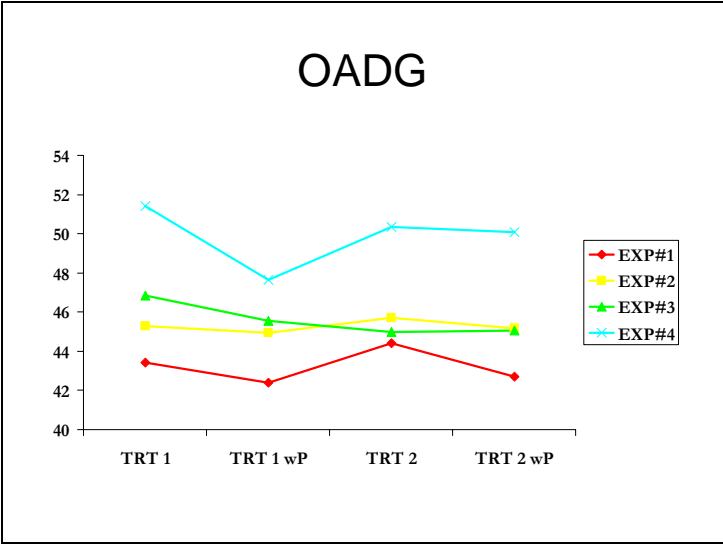
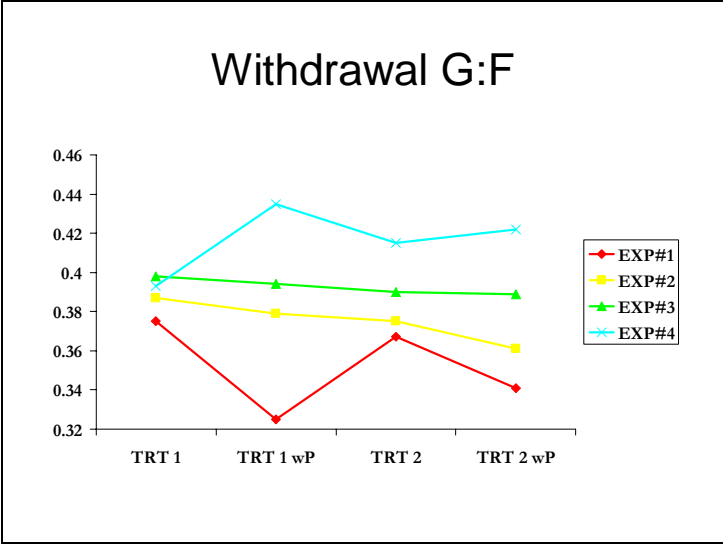


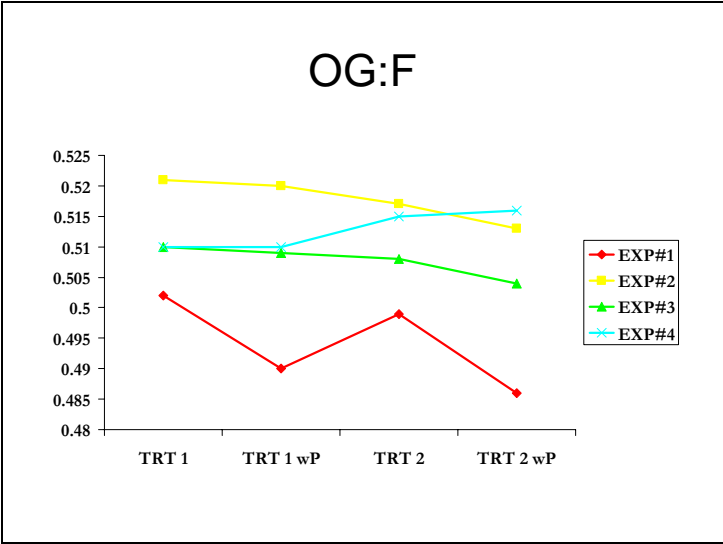
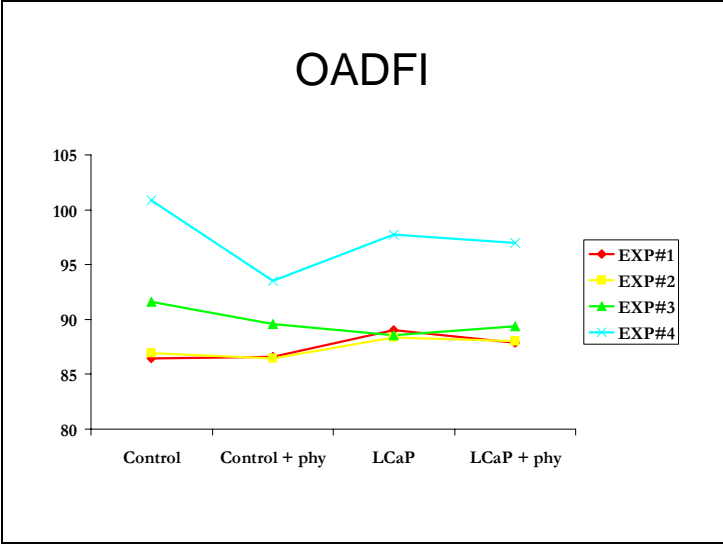












APPENDIX B
TIMELINE AND TEMPERATURE

Table B.1. Start date for each trial and the temperature at the start of each period.

Trial	Date started	Temperature at the start of each period °C			
		Starter	Grower	Finisher	Withdrawal
1	6/9/04	32	32	27	26
2	8/12/04	31	32	28	25
3	10/21/04	32	30	28	23
4	1/25/05	27	25	24	23

VITA

Syrena Campbell was born September 23, 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 the 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 at the Louisiana State University studying in non-ruminant nutrition and is presently a candidate for a Master of Science degree in animal science.