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The effects of phytase in nutritionally adequate diets, diets deficient in calcium and phosphorus, and the interactive effects of phytase and *Eimeria acervulina* infection in broiler chicks

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THE EFFECTS OF PHYTASE IN NUTRITIONALLY ADEQUATE DIETS, DIETS
DEFICIENT IN CALCIUM AND PHOSPHORUS, AND THE INTERACTIVE
EFFECTS OF PHYTASE AND *EIMERIA ACERVULINA* INFECTION IN BROILER
CHICKS

A Thesis
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by
Brandy Centrell Watson
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ABSTRACT

Six experiments were conducted to determine the interactive effects of *Eimeria acervulina* (*E. acervulina*) infection and phytase, and the effects of phytase in nutritionally adequate diets and in diets deficient in Ca and available P (aP). Corn-soybean meal (C-SBM) diets were used. In Exp 1, treatments were: 1) C-SBM, 1.0% Ca and 0.45% aP; 2) C-SBM, 0.80% Ca and 0.25% aP; 3) Diet 1 + 600 FTU phytase/kg; 4) Diet 2 + 600 FTU phytase/kg; 5 to 8) Diets 1 to 4 but infected with coccidiosis. Weight gain (ADG), feed intake (ADFI), and gain:feed were reduced ($P < 0.01$) by the coccidial infection and the reduction in Ca and aP. Phytase increased ($P < 0.02$) ADG and ADFI, regardless of the Ca and aP content of the diet or the presence of coccidiosis. Gain:feed was increased by phytase but only in uninfected chicks (phytase x coccidiosis interaction, $P < 0.02$). Phytase increased ($P < 0.02$) bone ash percentage but only in diets deficient in Ca and aP ($P < 0.01$). Experiments 2 and 3 included only treatments 1 to 4 of Exp 1. The reduction in Ca and aP reduced ($P < 0.01$) ADG, ADFI, and gain:feed. Phytase addition increased ($P < 0.02$) ADG and ADFI in diets deficient in Ca and aP and in the nutritionally adequate diets. Experiments 4, 5, and 6 were conducted to determine the effects of phytase on intestinal transit time in broilers. Diets were: 1) C-SBM, 0.9% Ca and 0.35% aP; 2) C-SBM, 0.80% Ca and 0.25% aP + 600 FTU phytase/kg. Transit time on Day 1, but not on Day 7, was faster ($P < 0.03$) in chicks fed phytase. These data indicate that phytase is effective in the presence of a coccidial infection, but it may not be as effective as in uninfected chicks. Furthermore, phytase increases growth in diets deficient in Ca and aP and in diets formulated to be adequate in all nutrients. This increase in growth may be due to a faster transit time of feed through the digestive tract, resulting in a greater feed intake and gain.

CHAPTER 1

INTRODUCTION

Phytase is an enzyme that hydrolyzes P from the phytate molecule. It has an important role in the poultry industry because of its ability to reduce the need for supplemental inorganic P to the diet and to reduce the amount of P excreted into the environment (Coelho, 1999; Kies, 1999). *Eimeria acervulina* infection is also important to the poultry industry. *Eimeria acervulina* is a parasitic protozoa, which invades the duodenum of the chicken (Beaver et al., 1984). *Eimeria acervulina* infection is not usually associated with death of the infected animal, but the cost associated with the disease can have a profound economic effect (Coles et al., 1970; Allen, 2000). Phytase decreases the need for inorganic P additions to the diet, it may also affect transit time of feed through the digestive tract of the chicken, and the efficacy of phytase may be increased by *Eimeria acervulina* infection.

Phytate is found in plants and is the storage form of P (Harland and Oberleas, 1999). Diets fed to chickens are made primarily of plant sources (Ravindran et al., 1999b). Phytate P is an important compound in the poultry industry because the P it contains is unavailable to animals (Harland and Oberleas, 1999; Ravindran et al., 1999b). Based on research done with other enzymes, phytase may decrease transit time of feed through the digestive tract of the chicken, thus increasing feed intake and growth in the chicken (Almirall and Esteve-Garcia, 1994; Almirall et al., 1995).

The cost of vaccinations, as well as the decreased growth associated with *Eimeria acervulina* infection can have economically disastrous implications. We believe that phytase and *Eimeria acervulina* may be interrelated. *Eimeria acervulina*-infection

lowers the duodenal pH of the chicken to a level close to one of the pH optima of phytase (Stephens et al., 1974; Brown and Southern, 1986; Fox et al., 1987). This lower pH may increase the efficacy of phytase in chicks infected with *Eimeria acervulina*.

CHAPTER 2

REVIEW OF LITERATURE

Phytate, myo-inositol 1, 2, 3, 5/4, 6-hexakis, is a compound found in plants that binds P as well as other minerals (Harland and Oberleas, 1999). Phytate acts as a chelating agent; in plants it holds minerals and releases them as the growing plant matures (Harland and Oberleas, 1999). Other roles of phytate besides mineral storage are energy storage, a competitor for ATP allowing for metabolism to be slowed and dormancy induced, “an immobilizer of divalent cations needed for control of cellular processes,” and a regulator of inorganic P concentration (Harland and Oberleas, 1999).

In corn, phytate is found in the germ in a water-soluble form, where as in soybeans, it is found complexed with protein (Harland and Oberleas, 1999).

The enzyme phytase releases P from the phytate molecule (Kies, 1999). The two main phytases are 3-phytase, which is found in microbial sources, and 6-phytase, which is found in plants (Kies, 1999). 3-Phytase hydrolyzes P from the 3-position, while 6-phytase hydrolyzes phytase from the 6-position (Kies, 1999).

Natuphos® phytase is a 3-phytase that is commercially used to free inorganic P from the phytate molecule, thus decreasing the amount of inorganic P addition needed to meet the P needs of animals, and to decrease P excretion into the environment (Coelho, 1999; Kies, 1999). After removing P from the 3-position of the phytate molecule, phytase proceeds to remove P from the 4, 5, and 6, and then the 1-position (Kies, 1999). Phytase has two pH optima (2.5 and 5.5) for releasing P from phytate (Simons et al., 1990).

2.1 LOW CA AND AVAILABLE P AND NUTRITIONALLY ADEQUATE DIETS

The Ca and available P (aP) requirements for chicks 1 to 3 wks of age are 1.0 and 0.45%, respectively (NRC, 1994). Phytase has been shown to be effective in releasing Ca and P in diets deficient in Ca and P (Gordon and Roland, 1998; Cabahug et al., 1999; Sohail and Roland, 1999). Phytase decreases the need for supplemental inorganic P by increasing its bioavailability in the digestive tract (Nys et al., 1999). Leske and Coon (1999) reported that phytase increases the bioavailability of phytate P in corn from 30.8 to 59.0% and the bioavailability of phytate P in soybean meal (SBM) from 34.9 to 72.4%.

Deficiencies of Ca and aP cause decreased average daily gain (ADG), average daily feed intake (ADFI) and gain:feed (Sohail and Roland, 1999; Boling et al., 2000; Johnston and Southern, 2000). Deficiencies also cause a decrease in toe and tibia ash percentage (Sebastian et al., 1996; Johnston and Southern, 2000). In chicks fed diets formulated to be deficient in Ca and aP, phytase addition increases ADG and ADFI (Denbow et al., 1995; Sebastian et al., 1996; Sohail and Roland, 1999; Johnston and Southern, 2000; Yan et al., 2001). The increase in ADG is a result of the increase in feed intake (Denbow et al., 1995; Sebastian et al., 1996); thus, phytase generally does not have an effect on feed efficiency (Denbow et al., 1995; Sebastian et al., 1996; Gordon and Roland, 1997a; Sohail and Roland, 1999; Johnston and Southern, 2000). However, some researchers have reported an increase in feed efficiency in chicks fed diets with added phytase (Broz et al., 1994; Yan et al., 2001). Kornegay et al. (1996) reported an increase in gain:feed in day-old chicks fed diets containing phytase, but only when the diets contained 2.0% non-phytate P; at higher levels of non-phytate P, there was no response in feed efficiency. Gordon and Roland (1998) also reported an increased gain:feed in chickens fed diets with added phytase, but only in corn-SBM diets not supplemented with

inorganic P. Phytase also increases tibia and toe ash percentage in chickens fed diets deficient in Ca and aP (Broz et al., 1994; Denbow et al., 1995; Sebastian et al., 1996; Gordon and Roland, 1997a; Sohail and Roland, 1999; Johnston and Southern, 2000; Yan et al., 2001).

Phytase has been shown to respond positively when the Ca and aP content of the diet is adequate. Waldroup et al. (2000) and Cabahug et al. (1999) reported a positive response in feed efficiency to phytase in chickens fed nutritionally adequate diets. Cabahug et al. (1999) attributed this effect to a positive influence of phytase on protein digestibility. Keshavarz (2000) reported that phytase improved growth performance of pullets fed nutritionally adequate diets during certain phases of a 0- to 18-wk period. Keshavarz (2000) attributed this positive response of phytase in pullets fed nutritionally adequate diets to increased digestibility of certain nutrients that may have limited optimal growth.

Some researchers have not observed a positive response to phytase when feeding chickens a nutritionally adequate diet. Waldroup et al. (2000), who reported a positive response of phytase for feed efficiency, also reported a positive effect of phytase on ADG and tibia ash percentage; however, phytase was more effective when the aP content of the diet was deficient. Sebastian et al. (1997) conducted a study using 1-day-old broiler chicks and reported a greater effect of phytase in diets deficient in Ca and aP.

Cabahug et al. (1999) reported that the positive response of phytase in nutritionally adequate diets might be due to increased protein digestibility. Ravindran et al. (1999a) reported that phytase improved digestibilities of protein and amino acids in feedstuffs; however, the response varied considering the feedstuff and amino acid

evaluated. Ravindran et al. (2001) and other researchers (Yi et al., 1996; Namkung and Leeson, 1999) also reported a positive response of phytase on digestibilities of amino acid for broilers. However, some researchers did not observe a positive response of phytase on amino acid digestibility. Peter and Baker (2001) reported that phytase did not improve growth performance or gain:feed of chicks fed diets deficient in crude protein. Zhang et al. (1999) also reported that phytase did not affect amino acid digestibility in corn-SBM diets fed to broilers. Also, Boling-Frankerbach et al. (2001) reported that phytase did not affect protein efficiency ratio of SBM or corn gluten meal.

2.2 TRANSIT TIME OF DIGESTA

Transit time is the time it takes a meal to pass through the gastrointestinal tract (Almirall and Esteve-Garcia, 1994). Differences in transit time may be another explanation for the response of phytase in nutritionally adequate diets. Transit time of feed through the digestive track is influenced by age (Petersen et al., 1999), temperature (Gordon and Roland, 1997b), viscosity of the diet and enzyme supplementation (Wang et al., 1992; Amirall and Esteve-Garcia, 1994). Viscosity of the diet has a direct effect on transit time; as viscosity decreases so does the transit time of feed through the digestive tract (Almirall and Esteve-Garcia, 1994).

Although, to our knowledge, there are no reports in the literature evaluating the effect of phytase on transit time, the effect of other enzymes on transit time has been studied. The enzyme, β -glucanase, has been shown to decrease viscosity of diets as well as transit time of feed passage through the digestive tract (Wang et al., 1992; Almirall and Esteve-Garcia, 1994; Philip et al., 1995; Svihus et al., 1997a). Supplementation of

other enzymes also may have an effect on transit time. Ritz et al. (1995) reported that the product Avizyme® (contains α -amylase, xylanase, and pectinase) and protease supplemented to a corn-SBM turkey starter diet had no effect on transit time. However, Svihus et al. (1997b) reported that the product Avizyme 1100® (contains protease, xylanase, and β -glucanase) added to a barley-based diet decreases viscosity of the diet.

Almirall et al. (1995) reported that broilers fed diets supplemented with β -glucanase have decreased transit time of feed through the digestive tract. These researchers and others reported that ADFI was significantly higher when β -glucanase (Hesselman and Åman, 1986) and other enzymes (Mannion, 1981) were added to the diet. Transit time and viscosity of the diet are inversely related to ADFI, as transit time decreases, ADFI increases (Almirall and Esteve-Garcia, 1994; Almirall et al., 1995). Almirall and Esteve-Garcica (1994) also reported in young chicks that decreasing the viscosity of the diet decreases the transit time of feed through the digestive tract and results in increased ADFI.

2.3 EIMERIA ACERVULINA

Eimeria acervulina (*E. acervulina*) infection also may have an effect on phytase activity. *E. acervulina*, also known as duodenal coccidia, is a parasitic protozoa that invades the duodenal epithelial cells of the chicken (Beaver et al., 1984). Acute coccidial infection results in decreased ADG, ADFI, and gain:feed (McDonald et al., 1982; Southern and Baker, 1983a; Matthews and Southern, 2000). Kouwenhoven (1972) reported weight loss during acute coccidiosis infection, as well as anorexia and diarrhea. Acute coccidiosis infection also leads to increased morbidity (Hein, 1968), mucosal

epithelial lesions (Kouwenhoven and van der Horst, 1972; Matthews and Southern, 2000), distended segments of the intestines and increased edema (Allen, 1983), decreased villar mucosa and decreased brush border enzymes (Allen, 1987), and bloody droppings and enteritis (Stephens et al., 1974). Chronic coccidiosis infection results in complete anorexia, diarrhea, and death (Morehouse and McGuire 1958; Hein, 1968; Kouwenhoven, 1972).

Coccidiosis has been reported to decrease mineral absorption. Takhar and Farrel (1979a) reported a decrease in total mineral concentration as well as a decrease in Ca and aP concentration during a coccidiosis infection. Willis and Baker (1981), Giraldo et al. (1987), Watkins et al. (1989), and Ward et al. (1990) stated that in chicks, coccidiosis infection decreases tibia ash percentage. According to Giraldo et al. (1987) coccidiosis-infection increases bone P, but this increase may be due to decreased Ca absorption. Turk (1973) also reported decreased Ca absorption in coccidiosis-infected chickens.

Coccidiosis also has been shown to increase absorption of some minerals. Southern and Baker (1982ac, 1983b) reported an increase in Cu, Fe, and Mn absorption. Southern and Baker (1983a) and Czarnecki and Baker (1984) also reported an increase in Cu absorption but only when Cu was fed in excess. Southern and Baker (1983a) also reported a decrease in Zn absorption. Brown and Southern (1985b) stated that Co and Mn absorption as well as tissue mineral concentrations are increased during coccidiosis infection.

Coccidiosis not only affects mineral absorption, but it also has an effect on amino acid, vitamin, and energy absorption. According to Kouwenhoven and van der Horst (1972) coccidial infection decreases vitamin A absorption. Izquierdo and Baker (1988)

reported beneficial effects of coccidiosis infection on growth and feed efficiency of chickens fed diets deficient in lysine. Takhar and Farrel (1979ab) stated that the utilization of metabolizable energy and N retention are decreased in chickens infected with coccidiosis.

Coccidiosis also has an effect on duodenal pH. According to Stephens et al. (1974), Brown and Southern (1986), and Fox et al. (1987) coccidiosis-infected chickens have decreased growth and a lower intestinal pH. The duodenal pH of healthy chicks is 6.0 or greater but is reduced to 5.0 or below in coccidiosis-infected chicks (Stephens et al., 1974; Brown and Southern, 1985a; Fox et al., 1987; Giraldo et al., 1987). The pH in the duodenum of coccidiosis-infected chicks is closer to one of the pH optima of phytase, which are 2.5 and 5.5 (Simons et al., 1990). The lower pH in the intestine of chickens infected with coccidiosis may increase the efficacy of phytase. Han et al. (1998) reported that the addition of 1.5% citric acid, 15% wheat middlings, and 300 FTU of phytase to a corn-SBM diet with a 0.2% reduction in aP was equivalent to feeding a nutritionally adequate diet with added inorganic P. These researchers theorized that acidifying the diet with citric acid gave phytase a better environment to function.

2.4 SUMMARY

In summary, phytase is an enzyme that increases the availability of P and other minerals in corn and SBM-based diets for chickens. Phytase increases ADG, ADFI, and tibia and toe ash percentage of chickens fed diets deficient in Ca and aP. Phytase also increases ADG, ADFI, and gain:feed of chickens fed diets formulated to be adequate in all nutrients. Based on research using other enzymes, phytase may also decrease

viscosity and transit time of feed through the digestive tract of chickens, thus increasing ADFI. This effect may be partially responsible for the increased performance in chickens fed nutritionally adequate diets supplemented with phytase.

E. acervulina is a parasitic protozoa that invades the duodenal epithelial cells, it increases absorption of some minerals, while decreasing absorption of others, it also decreases ADG and feed efficiency. *E. acervulina* infection also decreases the duodenal pH of chickens. This decrease in pH is closer to the pH optimum of phytase. Therefore, the presence of an *E. acervulina* infection may increase the efficacy of phytase.

CHAPTER 3

THE INTERACTIVE EFFECTS OF *EIMERIA ACERVULINA* INFECTION AND PHYTASE FOR BROILER CHICKS

3.1 INTRODUCTION

Eimeria acervulina (*E. acervulina*) is a parasitic protozoa that invades the duodenal epithelial cells of the chicken (Beaver et al., 1984), resulting in mucosal epithelial lesions (Hein, 1968), decreased absorption (Willis and Baker, 1981), decreased feed intake and growth (Matthews and Southern, 2000), diarrhea (Kouwenhoven, 1972), and increased mineral absorption (Southern and Baker, 1982ab, 1983b), and in chronic infection, anorexia and death (Morehouse and McGuire 1958; Hein, 1968; Kouwenhoven, 1972). Coccidiosis also has been shown to lower the pH in the duodenum (Stephens et al., 1974; Brown and Southern, 1986; Fox et al., 1987).

Phytase is an enzyme that increases the availability of P by hydrolyzing P from the phytate molecule (Harland and Oberleas, 1999). The pH optima of phytase to release P from phytate are 2.5 and 5.5 (Simons et al., 1990). The duodenal pH of healthy chicks is much higher, ranging from 5.98 to 6.63 (Stephens et al., 1974; Brown and Southern, (1985a, 1986); Fox et al., 1987; Giraldo et al., 1987). *E. acervulina* has been shown to lower duodenal pH to levels close to one of the pH optima of phytase (Stephens et al., 1974; Brown and Southern, 1986). Therefore, phytase may be more active in *E. acervulina*-infected chicks than in healthy chicks.

Thus, the objective of this experiment was to determine the interactive effects of phytase, *E. acervulina* infection, and Ca and available P (aP) content in the diet.

3.2 MATERIALS AND METHODS

The experiment was approved by the University Animal Care and Use Committee. Two-hundred-forty Ross x Ross male, broiler chicks¹ were allotted to eight treatments in a completely randomized design. Each treatment was replicated six times with five chicks each. The chicks were fed a C-SBM pretest diet, similar to the adequate C-SBM diet in Table 3.1, from 0 to 4 d posthatching. On day 5, after an overnight fast, the chicks were weighed and the heavy and light chicks were removed in order to increase uniformity of the initial weights. The chicks were wing banded and allotted to treatment. They were then fed the experimental diet and the experiment lasted for 10 d. They were housed in an environmentally controlled (32°C) Petersime starter battery² with raised wire floors. Average initial and final BW were 67 and 363 g, respectively. Chicks, feed, and water were checked twice daily, and feed and water were provided on an ad libitum basis throughout the experiment.

A corn-SBM diet, adequate in all nutrients (except Ca and aP) for experimental purposes was used. The diets (Table 3.1) provided 1.26% Lys, and 3,200 kcal ME/kg, and met or exceeded all other nutrient requirements (NRC, 1994). The dietary treatments were: 1) C-SBM, 1.0% Ca and 0.45% aP; 2) C-SBM, 0.80% Ca and 0.25% aP; 3) Diet 1 + 600 FTU phytase/kg; 4) Diet 2 + 600 FTU phytase/kg; 5 to 8) Diets 1 to 4 but infected with 400,000 *E. acervulina* oocysts on Day 0, 3, and 6 of the experiment. The deficient Ca and aP diet contained a 0.2% reduction in Ca and aP in order to give phytase a greater response surface in the event that it was more efficacious in infected chicks. Sand was

¹ Sanderson Farms, McComb, MS 39649.

² Petersime Incubator Company, Gettysburg, Ohio 45328.

Table 3.1. Composition of experimental diet

Ingredients	Control	Low Ca + P
Corn	52.14	52.14
Soybean meal (47.5%CP)	37.99	37.99
Corn oil	5.43	5.43
Monocalcium phosphate	1.55	0.59
Limestone	1.53	1.38
Salt	0.50	0.50
DL- methionine	0.19	0.19
Vitamin premix ^a	0.25	0.25
Mineral premix ^b	0.25	0.25
Choline	0.07	0.07
Rice hulls/phytase	0.10	0.10
Sand	0.00	1.10
Calculated composition		
ME, kcal/kg	3,200	3,200
Crude protein, %	22.97	22.97
Crude fiber, %	7.80	7.80
Lysine, %	1.26	1.26
TSAA, %	0.91	0.91
Tryptophan, %	0.31	0.31
Threonine, %	0.86	0.86
Ca, %	1.00	0.80
P, %	0.71	0.51
aP, %	0.45	0.25

^aProvides per kilogram of diet: vitamin A (vitamin A palmitate), 4,500 IU; vitamin D₃, 450 IU; vitamin E (vitamin E acetate), 50 IU; menadione (menadione sodium bisulfite), 1.5 mg; vitamin B₁₂, 0.02 mg; biotin (d-biotin), 0.6 mg; folacin (folic acid), 6 mg; niacin, 50 mg; thiamin (thiamin•HCl), 13.4 mg.

^bProvides per kilogram of diet: copper (copper sulfate•5 H₂O), 4.0 mg; iodine (potassium iodate), 1.0 mg; iron (ferrous sulfate•7 H₂O), 60 mg; manganese (manganese sulfate•H₂O), 60 mg; selenium (as sodium selenite), 0.1 mg; zinc (zinc sulfate•7 H₂O), 44 mg; calcium (calcium carbonate), 723 mg.

added to the reduced Ca and aP diets in place of the monocalcium phosphate and limestone. Microbial phytase (Natuphos 600³) was added to diets 3 and 4 at 0.1%. The response variables were average daily gain (ADG), average daily feed intake (ADFI), gain:feed, and tibia and toe ash percentage.

At the termination of the experiment, all chicks were killed using CO₂ gas asphyxiation and tibias and central toes were collected from the left leg of each chick. Bones and toes from each pen were pooled and frozen at -40° C until analyzed. The bones and toes were analyzed as previously described (Gray et al., 1998).

3.2.1 *Eimeria acervulina*

Chicks were inoculated by 1 mL crop intubations of water containing 4×10^5 *E. acervulina* oocysts to create an experimental coccidial infection. The chicks were inoculated on Day 0, 3, and 6 of the experiment in order to maintain a constant infection throughout the experimental period. The Parasite Biology and Epidemiology Lab, Beltsville, MD, provided the *E. acervulina* inoculum. The number of oocysts were enumerated prior to each inoculation to insure that the chicks received the correct number of oocysts. Uninfected chicks received a sham inoculation of tap water.

3.2.2 Statistical Analysis

Data were analyzed as a completely randomized design using the GLM procedure of the Statistical Analysis System (SAS Inst. Inc., Cary, NC). The treatments were arranged as a 2 x 2 x 2 factorial, and contrasts were used to determine main effects and interactions. The pen of chicks served as the experimental unit.

³ BASF Corp., Mount Olive, NJ 07828.

3.3 RESULTS

Daily gain, ADFI, and gain:feed were reduced ($P < 0.01$) by the coccidiosis infection and by deficiencies of Ca and aP in the diet (Table 3.2). Phytase increased ($P < 0.01$) ADG and ADFI and the effect was observed in nutritionally adequate diets and also in diets deficient in Ca and aP. Gain:feed was increased by phytase but only in uninfected chicks (phytase x coccidiosis interaction, $P < 0.02$). The results for toe and tibia ash percentage are presented in Figures 3.1 and 3.2, respectively. Toe and tibia ash percentage also were decreased ($P < 0.01$) by the deficiency in Ca and aP. The addition of phytase increased ($P < 0.02$) toe and tibia ash percentage, but phytase had a greater effect in chicks fed deficient diets than in chicks fed nutritionally adequate diets (phytase x Ca and aP interaction, $P < 0.01$). Tibia ash percentage was decreased ($P < 0.01$) by the coccidiosis-infection and by the decrease in Ca and aP in the diet. However, the decrease in tibia ash percentage due to the deficiency of Ca and aP was much more pronounced in healthy chicks than in infected chicks (coccidiosis x Ca and aP interaction, $P < 0.05$). Furthermore, tibia ash percentage was increased by phytase but only in uninfected chicks (phytase x coccidiosis interaction, $P < 0.02$). Phytase increased toe ash percentage of healthy chicks fed a deficient diet, but it had less of an effect in infected chicks fed a deficient Ca and aP diet (coccidiosis x Ca and aP x phytase interaction, $P < 0.08$).

3.4 DISCUSSION

The coccidiosis infection, as well as the reduction in Ca and aP, reduced ADG, ADFI, and gain:feed. These results were as expected and are in agreement with other

Table 3. 2. Effect of phytase and coccidiosis infection in chicks on growth performance and toe and tibia ash percentage^a

Response	Control	Low Ca-aP	PSEM						
Daily gain, g ^{bcd}	33.22	29.36	34.90	32.50	28.17	25.02	27.91	26.75	0.74
Daily feed intake, g ^{bcd}	38.92	35.47	40.31	37.91	34.68	31.70	34.77	33.19	0.78
Gain:feed ^{bce}	853	821	866	857	812	789	803	790	8
Toe ash, % ^{cdfg}	13.45	8.76	12.99	11.63	12.90	9.65	12.95	11.19	0.36
Tibia ash, % ^{bcdegh}	54.55	48.89	55.16	52.93	52.71	48.62	51.55	50.70	0.74

^aData are means of six replicates of five chicks each. Initial and final BW were 67 and 363 g, respectively. Experimental coccidiosis infection was established by 1 mL crop intubation of 4×10^5 sporulated *E. acervulina* oocyst on day 0, 3, and 6 of the experiment. Phytase was added to the diets at 600 FTU of phytase/kg of feed. aP=available P; PSEM=pooled standard error of the mean.

^bCoccidiosis effect, $P < 0.01$.

^cCa and aP effect, $P < 0.01$.

^dPhytase effect, $P < 0.02$.

^ePhytase x coccidiosis interaction, $P < 0.02$.

^fCoccidiosis x Ca and aP x phytase interaction, $P < 0.08$.

^gPhytase x Ca and aP interaction, $P < 0.01$.

^hCoccidiosis x Ca and aP interaction, $P < 0.05$.

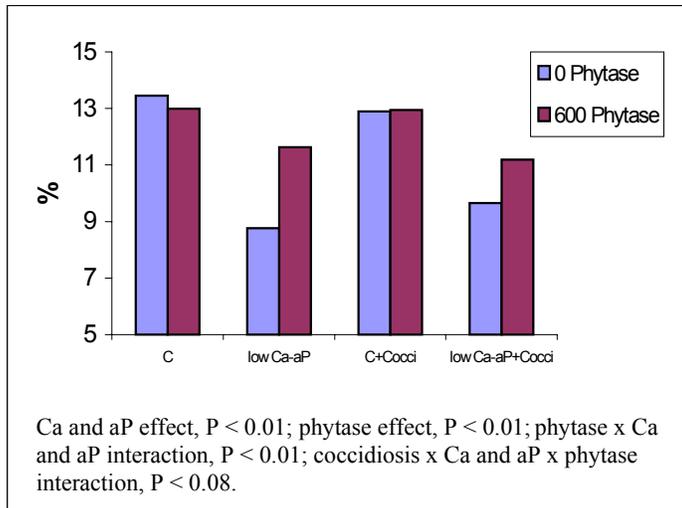


Figure 3.1. Effect of phytase and coccidiosis infection in chicks on central toe ash percentage^a

^aData are means of six replicates of five chicks each. Experimental coccidiosis infection was established by 1 mL crop intubation of 4×10^5 sporulated *E. acervulina* oocyst on day 0, 3, and 6 of the experiment. aP=available P.

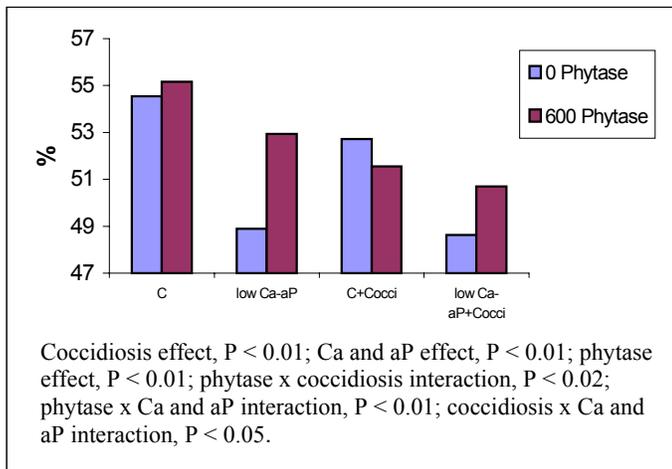


Figure 3.2. Effect of phytase and coccidiosis infection in chicks on tibia ash percentage^a

^aData are means of six replicates of five chicks each. Experimental coccidiosis infection was established by 1 mL crop intubation of 4×10^5 sporulated *E. acervulina* oocyst on day 0, 3, and 6 of the experiment. aP=available P.

studies evaluating the effects of *E. acervulina* (Willis and Baker, 1981; Watkins et al., 1989; Matthews and Southern, 2000), as well as those evaluating the effects of diets deficient in Ca and aP (Boling et al., 2000). Phytase addition increased ADG and ADFI in chicks regardless of the coccidial infection or nutritional adequacy of the diet. The effects of phytase in nutritionally adequate diets are also in agreement with previous research (Cabahug et al., 1999; Keshavarz, 2000).

Phytase was effective in increasing growth and feed intake in the infected and uninfected chicks. However, for gain:feed the magnitude of the response was much greater in healthy chicks than in infected chicks. The negative effect of coccidiosis on gain:feed has been well documented (Southern and Baker, 1982c; Southern and Baker, 1983a; Allen, 2000). Coccidiosis infection leads to epithelial lesions (Kouwenhoven and van der Horst, 1972; Matthews and Southern, 2000), decreased villar mucosa and decreased brush border enzymes (Allen, 1987). These cumulative effects may all contribute to the poor response of gain:feed in chicks infected with coccidiosis.

Tibia ash percentage, but not toe ash percentage, was decreased by the coccidiosis infection. This decrease in tibia ash percentage has previously been reported (Giraldo et al., 1987; Watkins et al., 1989; Ward and Southern, 1990). The Ca and aP deficiency decreased both toe and tibia ash percentage; once again phytase increased toe and tibia ash percentage, but unlike the growth results, phytase was more effective in the diet deficient in Ca and aP.

Phytase was effective in increasing ash percentage in both infected and uninfected chicks. However, for tibia ash percentage, phytase was more effective in healthy chicks. The deficiency of Ca and aP decreased tibia ash percentage more in healthy chicks than in infected chicks. This result is probably due to the fact that gain:feed was decreased in

coccidiosis-infected chicks. The coccidiosis-infected chicks thus consumed more Ca and aP per unit of BW resulting in less of a decrease in tibia ash percentage.

Phytase was more effective in healthy chicks compared with infected chicks for feed efficiency and tibia ash percentage, but phytase seemed equally effective in infected and healthy chicks for ADG and ADFI. Before the experiment it was speculated that phytase would be more effective in coccidiosis-infected chicks than in healthy chicks due to the lower pH of the duodenum of infected chicks. This response was not observed.

The observation that phytase was not more effective in coccidiosis-infected chicks may be due to absorption differences between healthy and infected chicks. Although coccidiosis infection has been reported to increase absorption of some minerals (Southern and Baker, 1982; Brown and Southern, 1985b), it has also been reported to decrease total mineral absorption and absorption of Ca and P (Turk, 1973; Takhar and Farrel, 1979a; Giraldo et al., 1987; Watkins et al., 1989). Possibly, this decreased absorption, along with the decrease in ADFI, were too great to be overcome by phytase additions.

CHAPTER 4

THE EFFECTS OF PHYTASE IN NUTRITIONALLY ADEQUATE DIETS AND IN DIETS DEFICIENT IN CALCIUM AND PHOSPHORUS FOR BROILER CHICKS

4.1 INTRODUCTION

Phytate is a compound that contains bound P and other minerals (Harland and Oberleas, 1999). It is found in most plants, including corn and soybeans (Harland and Oberleas, 1999). Because corn and soybean meal make up a substantial portion of diets for chickens, much of the P in these diets is unavailable for absorption (Harland and Oberleas, 1999; Ravindran et al., 1999b). Therefore, inorganic P must be supplemented to these diets. Phytase is an enzyme that hydrolyzes the release of P from the phytate molecule (Kies, 1999). It has been shown to be effective when the Ca and aP concentration of the diet is reduced, thus reducing the need for inorganic P additions to the diet (Nelson et al., 1971; Denbow et al., 1995; Gordon and Roland, 1998; Sohail and Roland, 1999; Yan et al., 2001). However, the effect of phytase in nutritionally adequate Ca and aP diets has been studied to a much lesser extent and with varied results. Some studies have shown a positive effect or an “extra-phosphoric effect” of phytase (Cabahug et al., 1999; Keshavarz, 2000; Waldroup et al., 2000), while other studies did not see an improvement by phytase in nutritionally adequate diets (Gordon and Roland 1997a; Sebastian et al., 1997). The positive effects of phytase were explained as a nutritional benefit (Cabahug et al., 1999); however, this positive effect may be due to the consistency or viscosity of the diet. Research has shown that higher viscosity diets result in a decrease in feed intake (Almirall et al., 1995). Research also has shown that the addition of enzymes to diets in chickens results in differences in viscosities of the ingesta

(Svihus et al., 1997b) and transit time through the digestive track (Almirall and Esteve-Garcia, 1994).

Therefore the objective of this research was to evaluate the effects of phytase on growth performance, bone ash percentage, and transit time in nutritionally adequate diets as well as in diets deficient in Ca and aP for broiler chicks.

4.2 MATERIALS AND METHODS

4.2.1 Experiments 1 and 2

All experiments were approved by the University Animal Care and Use committee. In each experiment, 240 Ross x Ross, male, broiler chicks¹ were allotted to four dietary treatments in completely randomized designs. Each treatment was replicated twelve times with five chicks each. The chicks were fed a C-SBM pretest diet similar to the adequate C-SBM diet in Table 4.1 from 0 to 6 (Exp 1) or 0 to 4 (Exp 2) d posthatching. On day 6 (Exp 1) or day 4 (Exp 2), after an overnight fast, the chicks were weighed and the heavy and light chicks were removed in order to increase uniformity of the initial weights. The chicks were wing banded and allotted to treatment. They were then fed the experimental diet and the assay periods were 6 to 14 (Exp 1) or 4 to 13 (Exp 2) d. They were housed in an environmentally controlled (32°C), Petersime starter battery² with raised wire floors. Average initial and final BW were 98 and 371 g in Exp 1, and 79 and 369 g in Exp 2. Chicks, feed, and water were checked twice daily, and feed and water were provided on an ad libitum basis throughout each experiment.

¹ Sanderson Farms, McComb, MS 39649.

² Petersime Incubator Company, Gettysburg, Ohio 45328.

Corn-soybean meal (C-SBM) diets adequate in all nutrients (except Ca and aP for experimental purposes) were used. The diets (Table 4.1) provided 1.26% Lys and 3,200 kcal ME/kg and met or exceeded all other nutrient requirements (NRC, 1994). The dietary treatments were: 1) C-SBM, 1.0% Ca and 0.45% aP; 2) C-SBM, 0.80% Ca and 0.25% aP; 3) Diet 1 + 600 FTU phytase/kg; 4) Diet 2 + 600 FTU phytase/kg. Sand was added to the reduced Ca and aP diets in place of the monocalcium phosphate and limestone. Microbial phytase (Natuphos 600³) was added to diets 3 and 4 at 0.1%. In Exp 1, phytase activity of the control diet and the low Ca and aP diet containing phytase were 622 and 635 FTU/kg, respectively. In Exp 2, the phytase activity of the control diet and the low Ca and aP diet containing phytase were 620 and 670 FTU/kg, respectively. The response variables were average daily gain (ADG), average daily feed intake (ADFI), gain:feed (Exp 1 and 2), and tibia and toe ash percentage (Exp 2). Experiment 2 was identical to Exp 1 except that the chicks were fed the pretest diet for 4 d, the experimental period lasted for 8 d and tibia ash data was collected in addition to growth data.

At the termination of the experiment, all chicks were killed using CO₂ gas asphyxiation and tibias and central toes were collected from the right leg of each chick at the end of Exp 2. Bones and toes from each pen were pooled and frozen at -40° C until analyzed. The bones and toes were analyzed as previously described (Gray et al., 1998).

4.2.2 Experiments 3, 4, and 5

The remaining experiments were conducted to evaluate transit time of feed in the intestine of chicks fed diets containing phytase. Ross x Ross chicks⁵, approximately 23 d

³ BASF Corp., Mount Olive, NJ 07828.

⁵ In experiment 3 the source of chicks was Sanderson Farms McComb, MS 39649. In experiment 4 and 5 the source of chicks was ConAgra, Natchitoches, LA 71457.

Table 4.1. Composition of experimental diets

Ingredients	<u>Experiments 1 and 2</u>		<u>Experiments 3, 4, and 5</u>	
	Control	Low Ca + P	Control	Low Ca + P
Corn	52.14	52.14	60.19	60.19
Soybean meal (47.5%CP)	37.99	37.99	31.24	31.24
Corn oil	5.43	5.43	5.01	5.01
Monocalcium phosphate	1.55	0.59	1.08	0.60
Limestone	1.53	1.38	1.43	1.35
Salt	0.50	0.50	0.50	0.50
DL- methionine	0.19	0.19	0.14	0.14
Vitamin premix ^a	0.25	0.25	0.05	0.05
Mineral premix ^b	0.25	0.25	0.25	0.25
Choline	0.07	0.07	0.00	0.00
L-Threonine	0.00	0.00	0.06	0.06
Rice hulls/phytase	0.10	0.10	0.05	0.05
Sand	0.00	1.10	0.00	0.55
Calculated composition				
ME, kcal/kg	3,200	3,200	3,200	3,200
Crude protein, %	22.97	22.97	20.39	20.39
Crude fiber, %	7.80	7.80	2.54	2.54
Lysine, %	1.26	1.26	1.10	1.10
TSAA, %	0.91	0.91	0.79	0.79
Tryptophan, %	0.31	0.31	0.24	0.24
Threonine, %	0.86	0.86	0.81	0.81
Ca, %	1.00	0.80	0.90	0.80
P, %	0.71	0.51	0.61	0.51
aP, %	0.45	0.25	0.45	0.35

^aThe vitamin premix used in Experiments 1 and 2 provides per kilogram of diet: vitamin A (vitamin A palmitate), 4,500 IU; vitamin D₃, 450 IU; vitamin E (vitamin E acetate), 50 IU; menadione (menadione sodium bisulfite), 1.5 mg; vitamin B₁₂, 0.02 mg; biotin (d-biotin), 0.6 mg; folacin (folic acid), 6 mg; niacin, 50 mg; thiamin (thiamin•HCl), 13.4 mg. The vitamin premix used in Experiments 3, 4, and 5 provides per kilogram of diet when added at 0.05%: vitamin A (vitamin A palmitate), 8,000 IU; vitamin D₃, 3000 IU; vitamin E (vitamin E acetate), 25 IU; menadione (menadione sodium bisulfite), 1.5 mg; vitamin B₁₂, 0.02 mg; biotin (d-biotin), 0.1 mg; folacin (folic acid), 1 mg; niacin, 50 mg; pantothenic acid, 15 mg; pyridoxine, 4 mg; riboflavin, 10 mg; and thiamin (thiamin•HCl), 3 mg.

^bThe mineral premix used in Experiments 1 and 2 provides per kilogram of diet: copper (copper sulfate•5 H₂O), 4.0 mg; iodine (potassium iodate), 1.0 mg; iron (ferrous sulfate•7 H₂O), 60 mg; manganese (manganese sulfate•H₂O), 60 mg; selenium (as sodium selenite), 0.1 mg; zinc (zinc sulfate•7 H₂O), 44 mg; calcium (calcium carbonate), 723 mg. The mineral premix used in Experiments 3, 4, and 5 provides per kilogram of diet when added at 0.25%: calcium (calcium carbonate), 704 mg; copper (copper sulfate•5 H₂O), 7.0 mg; iodine (calcium iodate), 1.0 mg; iron (ferrous sulfate• H₂O), 50 mg; manganese (manganese sulfate•H₂O), 100 mg; selenium (as sodium selenite), 0.15 mg; and zinc (zinc sulfate•H₂O), 75 mg.

old, were fed a C-SBM pretest diet similar to the control C-SBM diet used in Exp 1 and 2 (Table 4.1) for 0 to 18 (Exp 3), 0 to 27 (Exp 4), or 0 to 23 (Exp 5) d posthatching. Initial and final BW were 768 and 1,299 g in Exp 3, 1,108 and 1,704 g in Exp 4, and 838 and 1,392 g in Exp 5. Each treatment in each experiment was replicated with 18 chicks. There were nine male and nine female chicks per treatment.

A C-SBM positive control diet (Table 4.1) was formulated to provide 1.10% Lys, 0.9% Ca, and 0.35% aP, and it was adequate in all other nutrients (NRC, 1994). The diets for Exp 3, 4, and 5 were: 1) C-SBM, 0.9% Ca and 0.35% aP; 2) C-SBM, 0.80% Ca and 0.25% aP + 600 FTU phytase/kg. Sand was added to the reduced Ca and aP diets in place of the monocalcium phosphate and limestone. Microbial phytase (Natuphos 600³) was added to diet 2 at 0.1%. The phytase activities of the diet containing phytase in Exp 3, 4, and 5 were 875, 1010, and 368 FTU/kg, respectively.

During the pretest period, chicks were penned in groups of three in Petersime grower batteries². The pretest diet was removed 12 h before the start of each experiment. After 12 h, chicks were randomly allotted to treatments and individually penned. Chicks were then fed the experimental diets. The time the chick first began to eat and the time of first appearance of solid feces was recorded. Transit time was calculated as the difference between these two times (Washburn, 1991). The chicks were once again penned in groups of three. They were then allowed to eat the experimental diet for 7 d, and once again held without feed for 12 h. Transit time was then determined. The response variables were ADG, and transit time on Day 1 and Day 7. Day 1 transit time was not determined for Exp 3. For statistical purposes Exp 3, 4, and 5 were combined.

4.2.3 Statistical Analysis

Data were analyzed as completely randomized designs using the GLM procedure of Statistical Analysis System (SAS Inst. Inc., Cary, NC). In Exp 1 and 2, the dietary treatments were arranged factorially and contrasts were used to determine main effects and interactions. The pen of chicks served as the experimental unit. The data of Exp 3, 4, and 5 were pooled. The model included treatment and experiment. The sex effect, as well as the treatment x sex, and treatment x experiment interactions were not significant ($P > 0.10$) and were dropped from the model. The individual chick served as the experimental unit.

4.3 RESULTS

4.3.1 Experiment 1

Daily gain, ADFI, and gain:feed were decreased ($P < 0.01$) by the Ca and aP deficiency. Phytase increased ($P < 0.01$) ADG and ADFI of chicks fed the Ca and aP deficient diets as well as those fed the nutritionally adequate diet (Table 4.2).

4.3.2 Experiment 2

As in Exp 1, Ca and aP deficiency decreased ($P < 0.01$) ADG, ADFI, and gain:feed. Tibia and toe ash percentages also were decreased ($P < 0.01$) by the Ca and aP deficiency. Phytase increased ($P < 0.01$) ADG, ADFI, and toe and tibia ash percentage of both chicks fed the Ca and aP deficient diets as well as chicks fed the nutritionally adequate diets. However, for toe ash percentage, phytase was more effective in the deficient Ca and aP diet than in the control diet (Ca and aP x Phytase interaction, $P < 0.01$; Table 4.2).

Table 4.2. Effect of phytase on growth performance and tibia and toe ash percentage of chicks fed nutritionally adequate diets and diets deficient in Ca and available P (aP) (Experiments 1 and 2)

Response	Control	Low Ca-aP	Control + Phytase	Low Ca-aP + Phytase	PSEM
<u>Experiment 1^a</u>					
Daily gain, g ^{cd}	34.60	31.59	36.99	33.68	0.55
Daily feed intake, g ^{cd}	40.65	38.35	42.96	40.59	0.48
Gain:feed, g/kg ^c	851	824	861	829	8
<u>Experiment 2^b</u>					
Daily gain, g ^{cd}	30.03	26.66	31.60	29.14	0.69
Daily feed intake, g ^{cd}	37.86	35.32	39.18	38.10	0.60
Gain:feed, g/kg ^c	793	754	807	765	13
Toe ash, % ^{cde}	12.96	9.16	13.12	10.62	0.19
Tibia ash, % ^{cd}	54.87	49.59	56.30	52.13	0.64

^aData are means of 12 replicates of five chicks each. Initial and final BW were 98 and 371 g, respectively. Phytase was added to the diets at 600 FTU of phytase/kg of feed. PSEM=pooled standard error of the mean.

^bData are means of 12 replicates of five chicks each. Initial and final BW were 79 and 369 g, respectively.

^cCa and aP effect, $P < 0.01$.

^dPhytase effect, $P < 0.01$.

^eCa and aP by phytase effect, $P < 0.01$.

4.3.3 Experiment 3, 4, and 5

Transit time on Day 1 was faster ($P < 0.03$; Table 4.3) in chicks fed the diet with added phytase. Transit time on Day 7 tended to be faster in chicks fed phytase, but the effect was not significant ($P > 0.10$). Daily gain was not affected ($P > 0.10$) by treatment.

Table 4.3. Effect of phytase on transit time and daily gain in chicks (combined data of Experiments 3, 4, and 5)^a

Response	Control	Low Ca-aP + Phytase	PSEM
Total min (Day-1) ^{bc}	94.8	78.7	4.8
Total min (Day-7) ^d	113.0	101.1	5.3
Daily gain ^d	80.7	79.4	1.7

^aInitial and final BW were 906 and 1466 g, respectively. Phytase was added to the diets at 600 FTU of phytase/kg of feed. aP=available P; PSEM=pooled standard error of the mean.

^bData are means of 36 replicates of one chick each.

^cPhytase effect, $P < 0.03$.

^dData are means of 54 replicates of one chick each.

4.4 DISCUSSION

As seen in this study and in others, deficiencies of Ca and aP decreased ADG, ADFI, and gain:feed in chicks (Boling et al., 2000; Johnston and Southern, 2000). Phytase addition increased ADG and ADFI in this study, and the increase in ADG was due to an increase in ADFI and with no effect on feed efficiency. This result is in agreement with Denbow et al. (1995) and Sebastian et al. (1996, 1997).

Both tibia ash and toe ash percentages were decreased by the deficiency of Ca and aP, and as expected, phytase addition improved ash percentages. These results are in agreement with Denbow et al. (1995), Kornegay et al. (1996), Waldroup et al. (2000), and Yan et al. (2001).

In this experiment, phytase also had a positive effect on ADG and ADFI in chicks fed a nutritionally adequate diet. This effect has been previously reported by Cabahug et al. (1999) and Keshavarz (2000), although other studies have reported that the response to phytase on ADG and ADFI was more pronounced in the deficient Ca and aP diets (Gordon and Roland, 1997a; Sebastian et al., 1997).

Tibia ash percentage was also improved by phytase in the adequate diets. However, the effect of phytase on toe ash percentage was more pronounced when Ca and aP were reduced in the diet.

Cabahug et al. (1999) referred to this effect of phytase in adequate diets as an “extra-phosphoric effect” and hypothesized that it was due to the “favorable influence of microbial phytase on nitrogen and amino acid digestibilities.” Keshavarz (2000) also reported a positive effect of phytase on ADG of pullets fed nutritionally adequate diets. He reported that the increase in body weight may “have been due to an effect on digestibility of certain ingredients and provided the birds with adequate nutrients that otherwise could have been limiting for optimum growth.” We felt that the positive effect of phytase in nutritionally adequate diets was not due to increased nutrient availability of the diet, because the diets were adequate in all nutrients. The authors hypothesized that it may be due to changes in the viscosity of the diets or transit time through the digestive tract of the chicken.

The positive effect of phytase in nutritionally adequate diets led us to evaluate whether the rate of diet passage may be at least partially responsible for the response. Transit time has been determined to be affected by age (Petersen et al., 1999), temperature (Gordon and Roland, 1997b), viscosity of diet and enzyme supplementation (Wang et al., 1992; Amirall and Esteve-Garcia, 1994). Phytase decreased transit time in

chicks fed diets deficient in Ca and aP on Day 1. There was not a significant effect of phytase on Day 7; however, there was a 10.5% decrease in transit time in chicks fed diets with added phytase. To our knowledge, there are no reports in the literature evaluating the effect of phytase on transit time. However, the effect of β -glucanase and other enzymes on transit time has been extensively studied. β -glucanase decreases transit time (Almirall and Esteve-Garcia, 1994) and viscosity of diets high in β -glucans (Wang et al., 1992; Svihus et al., 1997a).

β -glucanase also has a positive effect on ADG and ADFI (Wang et al., 1992; Almirall and Esteve-Garcia, 1994). Ritz et al. (1995) reported that Avizyme® (contains α -amylase, xylanase, and pectinase) and protease added to a corn and soybean meal diet had no effect on transit time in 0- to 5-wk-old turkeys. However, Avizyme® 1100 (contains protease, xylanase, and β -glucanase) decreases viscosity of barley-based diets (Svihus et al., 1997b).

The results from our experiment suggest that the increase in ADG in chicks fed the phytase diet was due to an increase in feed intake. This increase in feed intake may have been due to a faster transit time in chicks fed diets containing phytase. The chicks fed phytase ate more, and thus gained more weight regardless of the adequacy of the diet.

CHAPTER 5

SUMMARY AND CONCLUSION

The purpose of these experiments were to evaluate the related effects of *Eimeria acervulina* infection and phytase, and the effects of phytase in diets nutritionally adequate and deficient in Ca and available P (aP).

In Exp 1, 2, and 3, the reduction in Ca and aP decreased average daily gain (ADG), average daily feed intake (ADFI), and gain:feed. Phytase increased ADG and ADFI in all three experiments regardless of the Ca and aP adequacy of the diet. Phytase did not have a significant effect on feed efficiency. Phytase also increased toe and tibia ash percentage in chicks fed deficient diets.

In Exp 1, phytase was more effective in healthy chicks than in infected chicks for feed efficiency and tibia ash percentage, but phytase seemed equally effective in infected chicks for ADG and ADFI. However, there was no added effect of phytase for chicks infected with coccidia.

In Exp 4, 5, and 6, transit time of feed through the digestive tract of the chicks was faster in chicks consuming diets containing added phytase on Day 1. On Day 7, there was a 10.5% decrease in transit time. However, this decrease was not significant.

These data indicate that phytase is effective in the presence of a coccidial infection, but it may have a more positive effect in healthy chicks. Furthermore, phytase increases ADG and ADFI in diets deficient in Ca and aP and in diets formulated to be adequate (or excess) in all nutrients for broiler chicks. This increase in ADG and ADFI in chicks fed the nutritionally adequate diet may be due to a faster transit time of feed through the digestive tract, resulting in a greater feed intake and gain.

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