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The non-phosphorus effects of dietary phytase in swine and poultry

Jason Layne Shelton
Louisiana State University and Agricultural and Mechanical College

THE NON-PHOSPHORUS EFFECTS OF DIETARY PHYTASE IN SWINE AND POULTRY

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Jason Layne Shelton
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ABSTRACT

This research was conducted to determine the non-phosphorus effects of phytase in diets for swine and poultry. An experiment was conducted to determine the effect of phytase addition on energy availability and protein and fat deposition in growing pigs. Results from this experiment indicated that phytase had small positive effects on energy availability, protein deposition, and fat deposition. In this study, 23 of 29 response variables were at least numerically increased with phytase addition. Two experiments were conducted to determine the accuracy of the energy and amino acid matrix values for Natuphos 1200 in broilers from 0 to 21 or 0 to 42 d posthatching. Results from these experiments indicated that the nutrient matrix values for phytase are accurate, and that they can be used in diet formulations for broilers. Experiments also were conducted to determine the effect of phytase addition to diets with or without the trace mineral premix in nursery, growing, and finishing pigs and in commercial broilers. Results indicated that phytase can replace the trace mineral premix in swine diets. Phytase addition had no negative effect on growth performance or pork quality, and it had minimal effects on carcass traits. In broilers, removing the trace mineral premix had no effect on growth performance but decreased bone breaking strength, and adding phytase did not reverse this response. This research indicates that phytase addition has little effect on carcass traits or meat quality in swine and poultry. When formulating swine diets with phytase, the trace mineral premix can be removed with no negative effects on growth performance or pork quality. However, more research is needed to determine the effect of phytase addition in diets without the trace mineral premix in broilers, because the addition of phytase did not overcome the decrease in bone breaking strength.

CHAPTER 1

INTRODUCTION

Phytate, an anionic acid with strong anti-nutritional properties, has been shown to bind to cations including Ca, Zn, Cu, Pb, Mn, Mg, Co, and Fe (Oberleas and Harland, 1996). Phytate is found in many common feed ingredients in varying amounts, and it can decrease nutrient availability in diets containing these ingredients. The main anti-nutritional effect of phytate is that phytate P is essentially unavailable for digestion and absorption by nonruminants (Nelson, 1967), but it also has negative effects on other nutrients. Phytate has been shown to negatively affect digestive enzymes (Deshpande and Cheryan, 1984; Caldwell, 1992) and protein (Okubo et al., 1976), AA (Cosgrove, 1969), and carbohydrate (Thompson and Yoon, 1984) availability.

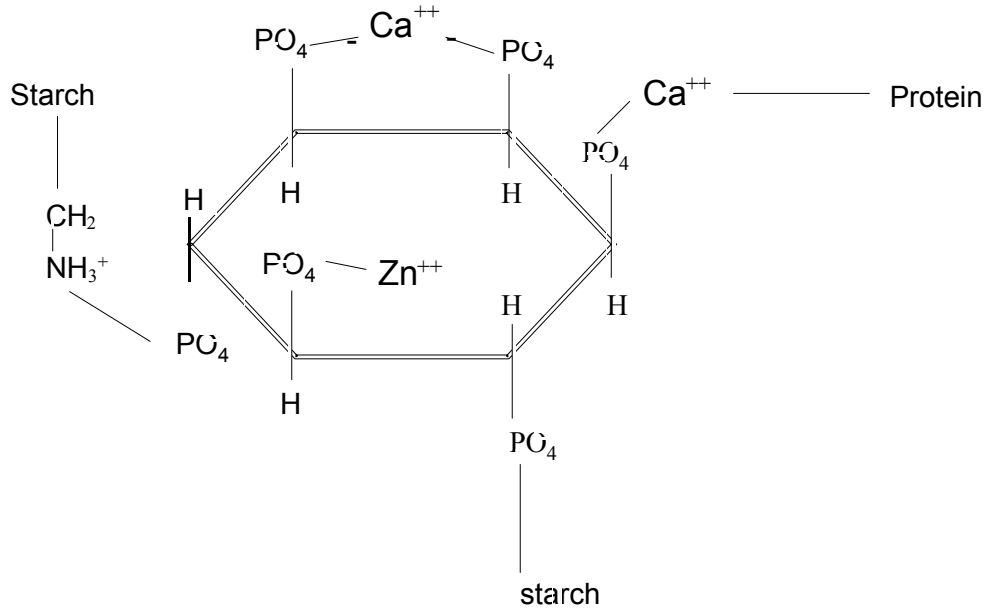

Figure 1.1. Structure of phytate

Phytase is an enzyme that breaks down phytate. It is produced in very limited amounts by mammals, but it is produced by bacteria and yeasts. Most research with phytase has been conducted to determine its effect on dietary P and Ca availability. The addition of microbial phytase to diets for nonruminants has been shown to increase the availability of P (Nelson et al., 1968; Cromwell et al., 1991), as well as other minerals (Biehl et al., 1995; Radcliffe et al., 1995; Simpson and Wise, 1990), protein and amino acids (Johnston, 2000; Johnston et al., 2004), carbohydrates (Johnston et al., 2004), and energy (Rojas and Scott, 1969). By decreasing the amount of protein, amino acids, and trace minerals excreted from swine and poultry, phytase can have positive effects on the environment. Also, the effect of phytase on minerals, protein, carbohydrates, and energy may result in an effect on carcass traits or pork quality.

In order to fully utilize and receive the benefits of the addition of phytase to swine and poultry diets, its effect on nutrients other than P and Ca need to be more clearly determined. Therefore, the objective of this research was to determine the effect of microbial phytase addition to swine and poultry diets on energy, protein, AA, and trace mineral availability, and its effects on carcass traits and meat quality.

CHAPTER 2

REVIEW OF LITERATURE

Phytate

Phytate (myo-inositol 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate) is an anionic acid with anti-nutritional properties, which is present in many livestock feed ingredients (Table 2.1). The main anti-nutritional effect of phytate is that phytate P is essentially unavailable for digestion and absorption by nonruminants (Nelson, 1967). Phytate also has negative effects on other nutrients. Phytate may influence starch digestibility through interaction with amylase, proteins associated with starch, Ca (which catalyzes amylase activity), or with starch itself (Sharma et al., 1978; Deshpande and Cheryan, 1984; Thompson and Yoon, 1984; Thompson et al., 1987; Johnston et al., 2004). Phytate also has been shown to form complexes with both dietary and digestive proteins (Okubo et al., 1976; Hartman, 1979, Satterlee and Abdul-Kadir, 1983) and to inhibit trypsinogen (Caldwell, 1992); thus affecting the protein nutritional quality of feed ingredients. Phytate has been shown to form complexes with trace minerals and decrease their availability (Maddaiah et al., 1964; Vohra, 1965; Davies and Nightengale, 1975; Erdman, 1979; Ravindran et al., 1995), but the order of affinity that trace minerals bind to phytate varies. Maddaiah et al. (1964) reported that phytic acid affects Zn > Cu > Co > Mn, but Vohra (1965) indicated that phytate forms complexes with Cu > Zn > Ni > Co > Mn > Fe.

Phytase is an enzyme that breaks down phytate (Gibson and Ullah, 1990) allowing the release of nutrients bound to phytate and increasing their availability for digestion and utilization. There is ample research on the effect of phytase on P and Ca availability (Johnston, 2000; Kornegay and Verstegen, 2001; Pond and Lei, 2001), so these effects will not be covered in this review.

Table 2.1. The concentration of phytic acid in common feed ingredients

Feedstuff	g/kg
Corn	7.44
Sorghum	7.44
Wheat	5.67
Soybean meal	16.67
Canola meal	26.24
Cottonseed meal	32.98
Sunflower meal	27.30
Wheat middlings	27.66
Rice polishings	54.96

Data obtained from Ravindran et al. (1999a).

Energy

Because of the aforementioned effect of phytate on protein, starch, and minerals, the addition of phytase to the diet may have positive effects on energy availability for swine and poultry. Research has indicated that phytase may have positive effects on energy availability in diets for chicks (Rojas and Scott, 1969; Miles and Nelson, 1974; Namkung and Leeson, 1999; Ravindran et al., 1999b, 2000, 2001; Camden et al., 2001; Lan et al., 2002; Selle et al., 2003b; Shirley and Edwards, 2003). However, other reports have indicated that phytase has no effect on energy availability (Biehl and Baker, 1997; Ledoux et al., 2001; Murai et al., 2002). Selle et al. (2003c) reported that adding phytase to wheat-sorghum-SBM based diets increased chick apparent metabolizable energy (ME) in one experiment but had no effect in two others. Johnston and Southern (2000a) indicated that phytase (600 phytase units/kg) provided 45.7 kcal of ME/kg in C-SBM diets for

chicks. However, the ME matrix values for Natuphos indicates it provides 31 and 24 kcal/kg for starter and growing-finishing broilers, respectively. The ME matrix values for Natuphos indicates it provides 23 and 26 kcal/kg for starter and growing-finishing pigs, respectively. There is less research on the effects of phytase on energy availability in pigs. Murry et al. (1997) reported no effect on digestible energy when 700 phytase units/kg of diet were added to pearl-millet-soybean meal (SBM)-based diets but an increase in digestible energy when 1,000 phytase units/kg of diet were added. Johnston et al. (2004) reported that phytase addition increased apparent ileal gross energy digestibility in pigs with no effect on total-tract energy digestibility. O'Doherty et al. (1999) indicated that adding phytase to finishing pig diets increased energy digestibility. However, Murry (1994), O'Quinn et al. (1997), Gebert et al. (1999b), Walz and Pallauf (2002), and Sauer et al. (2003) reported that adding phytase had no effect on energy digestibility in pigs.

From the review of the literature, phytase has had variable effects on energy digestibility, but some reports have suggested possible modes of action other than its effect on minerals and protein digestibility. Phytase may increase energy availability by increasing the availability of carbohydrates. Johnston et al. (2004) reported that adding phytase to growing pig diets increased starch digestibility and plasma levels of glucose and insulin 30 min after a meal. However, Nava et al. (2001) and Walz and Pallauf (2002) indicated that phytase addition to chick or swine diets, respectively, had no effect on plasma glucose concentration. In the study by Walz and Pallauf (2002), phytase was added to low crude protein (CP) diets. Some reports indicate that phytase may increase energy digestibility by increasing fat digestion. In chicks, fat digestion has been shown to be increased with phytase addition (Um et al., 2000; Lim et al., 2001). Helander et al. (1996) indicated that

phytase addition to a pea-based diet for growing pigs increased crude fat and carbohydrate digestibility, but Brady et al. (2003) and Johnston et al. (2004) reported that phytase addition to growing-finishing pig diets had no effect on fat digestibility.

The positive effects of phytase on energy may be due to additive effects on fat, starch, protein, and minerals; however, it is yet to be determined if this increase in energy will lead to an increase in protein or fat deposition in pigs or chicks.

Protein, Nitrogen, and Amino Acids

Phytase has the potential to improve the environment by allowing a decreased excretion of P and N, which in excess, can be major pollutants from animal waste. Because of this, it is important to determine the effect of phytase on protein digestibility, which will allow nutritionists to formulate diets closer to the animal's requirement, thus reducing N in the excreta. Phytase may increase the availability of protein in swine and poultry diets, not only by breaking the phytate-protein complex, but also by reducing the amount of inorganic P added to the diet. Nasi (1990) reported that adding inorganic P to the diet for growing pigs reduced N digestibility.

Research reporting the effect of phytase addition on N (CP) availability is shown in Table 2.3. Phytase also has been shown to have variable effects on the protein efficiency ratio (PER), which can be used as a measure of protein quality of common feed ingredients. Boling-Frankenback et al. (2001) indicated no effect of phytase supplementation on the PER of soybean meal (SBM), corn gluten meal, canola meal, casein, cottonseed meal, peanut meal, wheat bran, wheat middlings, rice bran, defatted rice bran, or meat and bone meal in chicks. In that study, adding Ca and P diet, but it did not affect the PER relative to those fed a positive control

Table 2.2. Effect of phytase on nitrogen digestibility

Reference	phytase units	Effect	Diet/Feedstuff	Comments
Nursery pigs				
Yang et al. (2001)	500	NE	C-SBM	
Sands et al. (2001)	600	NE	C-SBM-whey	Normal and high aP corn
Yi et al. (1996c)	350 to 1,400	NE	SBM-cornstarch:dextrose	Different Ca:aP ratios
Kornegay and Qian (1996)	350 to 1,400	↑	C-SBM	Increase only at 1,400 FTU fed at 0.32% aP
Kwon et al. (2002)	Not available	NE	C-SBM	
Murry et al. (1997)	700,1,000	↑	pearl millet-SBM	Low P diets
Valencia andChavez. (2002)	1,000	↑	C-SBM	Low P diets
Growing-finishing pigs				
Bruce and Sundstøl (1995)	500, 750	NE	oat-SBM	
Fandrejewski et al. (1997)	1,000	↑	rapeseed, C, wheat, barley	
Ketaren et al. (1993)	1,000	↑	SBM-sucrose	
Helander et al. (1996)	1,000	↑	high pea diets	
Han et al. (1997)	1,000, 1,200	↑	C-SBM-rapeseed	Wean to finish pigs
Li et al. (1999)	750	↑	C-SBM-rapeseed-cottonseed	Different Ca levels
O'Quinn et al. (1997)	300, 600	NE	sorghum-SBM	
Murry (1994)	500	NE	C-SBM	
Brady et al. (2002)	750	NE	barley-wheat-SBM	Different dietary P levels
Brady et al. (2002)	750	↑	barley-wheat-SBM	Different dietary Ca levels

(table continued)

Mroz et al. (1994)	800	↑	C-tapioca-SBM	
Omogbenigun et al. (2003)	500	NE	C-SBM	Low P diets
Valaja et al. (1998)	250,500	NE	semipurified diets	
O'Doherty et al. (1999)	300,600	↑	barley-wheat-SBM-rapeseed	Different Ca and P levels
Brady et al. (2003)	500, 750, 1,000	↑	barley-C-SBM	Two phytases were used
Li et al. (1998)	750	↑	C-SBM	
Sauer et al. (2003)	500	NE	barley-canola meal	
Chicks				
Lan et al. (2002)	250, 500, 750, 1,000	↑	C-SBM-fishmeal	0 to 42 d
Um et al. (2000)	600	NE	C-SBM-corn gluten meal	0 to 35 d
Selle et al. (2003b)	600, 800	NE	Sorghum-SBM-meat and bone	7 to 25 d
Yi et al. (1996b)	350, 750, 1,050	↑	SBM-semipurified	21 d old
Zanini and Sazzad (1999)	500	↑	C-SBM	
Paik et al. (2000)	600	NE	C-SBM-C-gluten meal	Different aP levels
Murai et al. (2002)	1,000	↑	barley-isolated soy protein	Different P levels
Shirley and Edwards (2003)	93.75 to 1,200	↑	C-SBM	Low P diets
Piao et al. (1999)	500	↑	C-SBM-fishmeal	0 to 42 d
Valaja et al. (2001)	750	↑	C-SBM	Two phytases were used
Singh et al. (2003)	750	↑	C-SBM-rice-groundnut	0 to 42 d
Ravindran et al. (1999b)	5,000	↑	wheat-casein	35 to 42 d
Ravindran et al. (2000)	400, 800	↑	wheat-sorghum-SBM	7 to 25 d
Lim et al. (2001)	500	↑	C-SBM-C-gluten meal	Different dietary aP levels

aP = available P; C = Corn; CP = crude protein; NE = no effect; P = phosphorus; SBM = soybean meal.

diet. Also, Selle et al. (2003b) reported that adding phytase to wheat-sorghum based diets did not affect PER in 7 to 25 d old chicks but increased PER in 0 to 42 d old chicks. Peter and Baker (2001) indicated that phytase addition to low CP-SBM-based diets had no effect on PER of chicks.

As mentioned earlier, phytate has the potential to form complexes with amino acids (AA), thus making them unavailable. Because of this, research has been conducted on the effects of phytase on AA availability in swine and poultry. Rutherford et al. (2002) and Ravindran et al. (1999a) reported that phytase addition increased the availability of most AA in corn, SBM, wheat, rice bran, rapeseed, sorghum, cottonseed meal, canola meal, and sunflower meal for chicks. In the study by Rutherford et al. (2002) the availability of all AA were at least numerically increased with phytase addition. Johnston and Southern (2000b) reported no effect of phytase addition on overall AA digestibility in chicks, but the addition of phytase to low Ca and P diets did increase the availability of Lys, Ile, and Leu. Ravindran et al. (1999b) indicated that Lys, Thr, and Ser digestibilities were increased in chicks fed diets with added phytase. Namkung and Leeson (1999) indicated that phytase addition to chick diets increased total AA and non-essential AA ileal digestibility, and specifically increased the availability of Val and Ile. Also, Ravindran et al. (2000) reported that adding phytase to low, medium, or high phytic acid diets for chicks increased AA digestibility, and as expected, phytase had more of an effect in diets with high phytic acid. Sebastian et al. (1997) reported that phytase had a greater effect on AA digestibility in female chicks (increased digestibility of all AA except for Lys, Met, Phe, and Pro) relative to male chicks. Johnston et al. (2004) reported that adding phytase to diets for growing pigs increased total AA digestibility relative to those fed a control diet. Mroz et al. (1994) reported that adding phytase to pig diets increased apparent total tract digestibility of AA (all AA were numerically increased except for Cys and Pro). Murry et al. (1997) reported that adding

phytase to pig diets with two different levels of P increased apparent digestibility of Lys, Leu, Ile, and Val. However, some reports in swine indicate no effect of phytase addition on AA digestibility. Valaja et al. (1998) indicated that adding phytase to a semi-purified diet for finishing pigs had no effect on apparent total tract or ileal AA digestibility. Also, Omegbenigun et al. (2003) reported no effect on phytase addition to low P diets on essential AA digestibility (except for His) in early-weaned pigs.

The aforementioned research has been conducted with phytase addition to diets that are adequate in CP, but because of the increased interest in reducing the amount of P and N in animal waste, phytase addition in low CP diets could have a major impact on the environment. Phytase has been added to swine and poultry diets with low CP and/or AA levels with varying effects on AA availability. Some studies indicate an increase in AA digestibility (Biehl and Baker, 1996; Biehl and Baker, 1997; Kornegay et al., 1998; Radcliffe et al., 1999; Zhang et al., 1999; Ravindran et al., 2000; Ravindran et al., 2001; Camden et al., 2001; Ledoux et al., 2001; Selle et al., 2003a; Selle et al., 2003c) while others have reported no effect (Zhang et al., 1999; Peter et al., 2000; Brumm, 2001a; Grandhi, 2001; Traylor et al., 2001; Walz and Pallauf, 2002).

To properly formulate diets with phytase, we must know the correct amount of Lys that is released by phytase. Research has shown that phytase provides 0.023% (Johnston and Southern, 1999) or 0.064% (Ravindran et al., 2001) Lys for chicks, and 0.03% Lys for 48-kg pigs (Radcliffe et al., 1999). Also, phytase has been shown to provide 0.76% CP for finishing pigs (Zhang and Kornegay, 1999) and 0.52% CP for 48-kg pigs (Radcliffe et al., 1999). However, for Natuphos, the matrix value used for CP and Lys are underestimated. For chicks, the CP and Lys matrix values are 0.21 and 0.014% and 0.24 and 0.016% for starter and grower-finisher broilers, respectively. For swine, the CP and Lys matrix values

are 0.28 and 0.013% and 0.31 and 0.014% for starter and growing-finishing pigs, respectively.

Trace Minerals

More research has been conducted on the effect of phytase on minerals than on energy and protein. Because of the aforementioned effect of phytate on trace mineral availability, adding phytase may increase their availability for swine and poultry. The trace minerals normally added to swine and poultry diets are Zn, Cu, Mn, Fe, Se, and I. No research could be found on the effect of phytase on Se or I availability.

Most research has indicated that the addition of phytase increases the digestibility and(or) the availability of Zn in diets for pigs and chicks. Phytase increased Zn digestibility and availability in nursery pigs (Lei et al., 1993; Adeola, 1995; Adeola et al., 1995; Hargrave et al., 2000; Valencia and Chavez, 2002), growing-finishing pigs (Nasi, 1990; Gebert et al., 1999b; Walz and Pallauf, 2002; Brady et al., 2003), and chicks (Biehl et al., 1995; Zanini and Sazzad, 1999; Paik et al., 2000; Lim et al., 2001; Lan et al., 2002; Viveros et al., 2002). However, some research has indicated that phytase addition has no effect on Zn digestibility in pigs (Murry, 1994) or chicks (Roberson and Edwards, 1994; Sebastian et al., 1996a; Um et al., 2000) or that it decreases Zn digestibility in growing-finishing pigs (Gebert et al., 1999a). Phytase also may increase Zn levels in certain tissues of pigs and chicks. Phytase has increased plasma Zn levels in pigs and chicks in some studies (Lei et al., 1993; Pallauf et al., 1994; Murry et al., 1997; Mohanna and Nys, 1999; Paik et al., 2000; Lan et al., 2002), but not in others (Roberson and Edwards, 1994; Murry and Lewis, 1995; Sebastian et al., 1996a; Viveros et al., 2002; Williams et al., 2004). Phytase has increased Zn level in bone of pigs and chicks in some studies (Roberson and Edwards, 1994; Biehl et al., 1995; Yi et al., 1996a; Mohanna and Nys, 1999; Zanini and Sazzad, 1999; Lan et al., 2002; Walz and Pallauf, 2002; Zacharias et al., 2003) but not in another (Sebastian et al.,

1996a). Yi et al. (1996a) indicated that phytase addition increased liver Zn level of chicks, and Mohanna and Nys (1999) indicated that phytase addition increased feather Zn levels in chicks, but Walz and Pallauf (2002) indicated that phytase addition had no effect on muscle Zn levels. Spears et al. (2001) indicated that phytase can provide 10-20 ppm Zn in growing-finishing pig diets and Mohanna and Nys (1999) indicated that phytase could replace 14 ppm inorganic Zn in chick diets.

Research has indicated that adding phytase increased the retention of Cu in nursery pigs (Adeola, 1995; Adeola et al., 1995; Valencia and Chavez, 2002), growing-finishing pigs (Nasi, 1990; Gebert et al., 1999b), and chicks (Sebastian et al., 1996b; Um et al., 2000; Lim et al., 2001; Lan et al., 2002). However, in other studies Cu retention or availability was not affected in growing-finishing pigs (Murry, 1994; Gebert et al., 1999a) or chicks (Sebastian et al., 1996a; Biehl et al., 1997) or decreased in growing-finishing pigs (Brady et al., 2003) or chicks (Aoyagi and Baker, 1995). Adding phytase has variable effects on tissue Cu concentration. Murry et al. (1997) and Spears et al. (2001) reported that phytase addition did not affect tissue Cu levels in pigs. Murry and Lewis (1995) reported no effect on blood Cu levels in growing pigs fed diets with phytase (Murry and Lewis, 1995). In chicks, Sebastian et al. (1996a) and Paik et al. (2000) reported that plasma Cu concentration was increased with the addition of phytase to chick diets, but in the study by Sebastian et al. (1996a) bone Cu levels were not affected. Sebastian et al. (1996b) reported that adding phytase had no effect on plasma or bone Cu levels in chicks. Also, Lan et al. (2002) indicated that phytase decreased bone Cu level in chicks, and Aoyagi and Baker (1995) indicated that phytase had no effect or decreased bile Cu levels in two studies.

The addition of phytase has been shown to increase the retention of Fe for pigs (Gebert et al., 1999b; Valencia and Chavez, 2002) and chicks (Paik et al., 2000, Um et al., 2000). However, other reports have indicated that phytase has no effect on Fe digestibility

or availability in pigs (Nasi 1990; Murry, 1994; Gebert et al., 1999a) or chicks (Sebastian et al., 1996a; Biehl et al., 1997). Stahl et al. (1999) indicated that phytase addition increased Fe levels in blood and increased blood packed cell volume in young pigs, and Paik et al. (2000) indicated that adding phytase increased serum levels of Fe in chicks. Zacharias et al. (2003) indicated that phytase addition decreased Fe levels in the serum but increased Fe levels in the liver of growing-finishing pigs. Walz and Pallauf (2002) indicated that Fe level in the bone was decreased when phytase was added to the diet of finishing pigs.

There is less research on the effects of phytase addition on Mn retention in pigs and chicks. Windisch and Kirchgessner (1996) and Lan et al. (2002) indicated that phytase addition increased Mn retention in pigs and chicks, respectively. However, Adeola (1995) and Adeola et al. (1995) reported no effect of phytase addition on the retention of Mn for nursery pigs. Also, Sebastian et al. (1996a) indicated that adding phytase to chick diets had no effect on retention of Mn, and at dietary levels of 1.00% Ca, phytase addition decreased Mn retention. Brady et al. (2003) reported that phytase addition decreased Mn retention in growing-finishing pigs. Little research has been conducted on the effect of phytase on tissue Mn levels. Biehl et al. (1995), Mohanna and Nys (1999), and Lan et al. (2002) indicated that phytase addition increased tibia Mn levels in chicks.

The variability of the effect of phytase on trace minerals in these studies could be due to the ingredients used, level of phytase in the diet, and the level of minerals in the diet. Phytase will have less of an effect in diets that contain ingredients that have low levels of phytate relative to those that have high levels of phytate. Also, adding phytase to diets with trace mineral levels above the animal's requirement make the effect of phytase on trace minerals more difficult to see. Research is still needed to determine if phytase releases enough Zn, Cu, Fe, and Mn so that the trace mineral premix can be removed from the diet with no negative effect on the animal.

Carcass Traits and Meat Quality

Because phytase may affect the availability of minerals, fat, starch, proteins, and AA in diets for swine and poultry, it may have an effect on carcass traits and meat quality. The addition of phytase has had variable effects on carcass traits. O'Quinn et al. (1997) reported a decreased dressing percentage when 300 phytase units/kg of diet were added to diets for swine but not when 500 phytase units/kg of diet were added (Ca and P were decreased by 0.08%). However, O'Doherty et al. (1999) reported an increase in dressing percentage when phytase was added to pig diets, but other reports have indicated no effect of phytase supplementation on dressing percentage (Liu et al., 1998; Brumm, 2001b; Grandhi, 2001; Rienstra et al., 2001; Brady et al., 2002; Walz and Pallauf, 2002). Wittman et al. (1994), O'Quinn et al. (1997), and Peter et al. (2001) indicated no effect on carcass lean percentage when phytase was added to the diet. Ketaren et al. (1993) and Fandrejewski et al. (1999) reported no effect on carcass DM, fat, or protein concentration, but Ketaren et al. (1993) reported that phytase addition increased protein deposition in pigs. Rienstra et al. (2001) indicated that pigs fed phytase had an increased longissimus muscle area, but Harper et al. (1997), O'Doherty et al. (1999), Lindemann et al. (2000), and Walz and Pallauf (2002) reported no effect of phytase supplementation on longissimus muscle area. Zhang and Kornegay (1999) reported an increase in tenth-rib backfat thickness in pigs fed diets with added phytase; however, Ketaren et al. (1993), Wittman et al. (1994), Harper et al. (1997), O'Quinn et al. (1997), Liu et al. (1998), O'Doherty et al. (1999), Peter et al. (2001), Rienstra et al. (2001), Brady et al. (2002), Walz and Pallauf (2002), and Lindemann et al. (2000) reported no effect of phytase addition on backfat thickness. In poultry, research on the effects of phytase on carcass traits is lacking. Adding phytase to poultry diets has resulted in no effect on percentage carcass yield (Huytghebaert, 1996; Vetesi et al., 1998; Scheideler and Ferket, 2000; Attia et al., 2003). However, Taghipour

and Pirzadeh (2002) indicated an increase in breast yield and leg weight resulting from phytase addition.

Studies have shown that phytase has little effect on pork quality. O'Quinn et al. (1997) indicated no effect on cook loss or sensory traits when phytase was added and Gebert et al. (1999c) reported no effect of phytase addition on water holding capacity. However, Rienstra et al. (2001) reported that pigs fed phytase had a decreased drip loss. Gebert et al. (1999c) reported a decrease in 45-min pH and a paler longissimus dorsi when pigs were fed phytase, but Wittman et al. (1994) and Walz and Pallauf (2002) indicated that phytase addition had no effect on pork color. Harper et al. (1997) indicated that adding phytase to pig diets had no effect on firmness or marbling, but Rienstra et al. (2001) reported that pigs fed phytase had a decreased marbling but no effect on intramuscular fat, color, or firmness.

Conclusion

Phytase has positive effects on energy availability in swine and poultry, even though more research has been conducted with poultry. However, there is little research as to whether this increase in energy results in an increase in protein or fat deposition. Research has shown that phytase also has positive effects on CP and AA digestibility, which may have a major impact on the environment. Increasing the availability of CP and AA in diets for animals will lead to a decreased amount of N in the waste. This effect, along with phytase reducing the amount of P in the waste, can result in reduced levels of nutrients applied to the land when used for fertilizer. The effect of phytase on AA availability is a much-debated topic in swine and poultry nutrition. Phytase seems to have small positive effects on AA. However, this small increase in availability often leads to no statistical effect on individual AA availability but increases when the AA availability is expressed as total AA, essential AA, or non-essential AA. There is still a need to determine how much and to what

degree AA are affected by dietary phytase addition. Phytase increases the availability of both Zn and Cu in most studies in swine and poultry; however, its effect on Mn and Fe are more variable. This variablility could be due to the lower affinity that phytate has for these minerals. The positive effects phytase has on energy, protein, and trace minerals do not lead to any major effects on carcass traits or pork quality. Most of the results on carcass traits and pork quality are variable and may be due to the reduction in inorganic P supplementation rather than the addition of phytase.

CHAPTER 3

EFFECT OF PHYTASE ON ENERGY AVAILABILITY, AND LIPID AND PROTEIN DEPOSITION IN GROWING SWINE *

Introduction

The addition of phytase to corn-soybean meal (C-SBM) diets for swine has been shown to increase the availability of Ca and P (Jongbloed et al., 1992; Radcliffe et al., 1995, Kemme et al., 1997). Phytase also has been shown to increase energy and AA availability in diets for nonruminants (Namkung and Leeson, 1999; Radcliffe et al., 1999; Ravindran et al., 1999b), but there is much less data on the AA and energy effects than on the Ca and P effects. Furthermore, most of this research has been done in poultry rather than swine. Johnston (2000) reported that phytase increased gross energy digestibility in pigs fed a C-SBM diet. Williams (2001) reported that phytase increased starch digestibility in pigs fed C-SBM diets and plasma glucose concentration in pigs after a meal (Williams et al., 2001). However, not all of the data indicate that phytase affects AA and energy availability. Biehl and Baker (1997) reported no effect of phytase on true nitrogen-corrected metabolizable energy in cecectomized roosters fed dehulled-SBM.

Thus, the objective of these experiments was to determine the effect of phytase on energy utilization, and protein and lipid deposition in growing pigs fed a C-SBM diet.

Materials and Methods

All methods used in these experiments were approved by the Louisiana State University (LSU) Agricultural Center Animal Care and Use Committee.

Two experiments were conducted to determine the effect of microbial phytase supplementation in C-SBM diets on energy availability, and protein and lipid deposition in growing swine.

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Experiment 1

General. Forty-eight crossbred barrows from the LSU Agricultural Center Swine Farm with an average initial and final BW of 26.4 and 52.0 kg, respectively, were used in this experiment. The pigs were individually penned in 1.1 × 3.7-m pens with solid concrete floors at the LSU Veterinary School Swine Facility, and they were allotted to four treatments in a 2 x 2 factorial arrangement. Corn-soybean meal diets were fed at two energy levels (2.9 and 3.2 x maintenance [M]) with and without the addition of 500 phytase units/kg of diet (Table 3.1). Maintenance intake was considered to be 106 kcal of ME/kg of BW $^{0.75}$ (NRC, 1998). Defatted rice bran (10%) was added to the diets to decrease the energy content. Natuphos® 600 (BASF Corporation, Mount Olive, NJ) was included in the diet at 0.083%, which added 500 phytase units/kg of diet. Actual analysis of the diets for phytase indicated that the diets provided 535 phytase units/kg of diet. The diets were formulated with the NRC model to contain 115% of the Ca, available P (aP), and Lys requirement for 20-kg pigs gaining 300 g of lean tissue per day (NRC, 1998). All other AA met or exceeded the ratio to Lys calculated using the NRC model (NRC, 1998). The diets also contained an equal ME:Lys ratio. On d 21 of the experiment, the diets were reformulated to contain 115% of the Ca, aP, and Lys requirement for 35-kg pigs gaining 300 g of lean tissue per day (NRC, 1998). Otherwise the diets were as previously described. Analyzed AA and mineral contents of corn, SBM, and defatted rice bran were used to formulate diets (Table 3.2). The AA composition of corn, SBM, and rice bran was determined after acid hydrolysis (AOAC, 1990), whereas total sulfur AA content was determined after performic acid oxidation followed by acid hydrolysis (AOAC, 1990). Tryptophan content was determined after alkaline hydrolysis (AOAC, 1990). The mineral composition of the feed sources was determined by inductively coupled plasma emission spectroscopy (Model Optima 3000, Perkin Elmer, Norwalk, CT 06859) after digestion in nitric acid and peroxide.

Table 3.1. Composition of the basal diets in Experiment 1 [a]

Ingredient, %	20-kg [a]	35-kg [b]
Corn	55.76	62.81
Soybean meal, 47.5% CP	31.18	24.40
Defatted rice bran	10.00	10.00
Monocalcium phosphate	0.92	0.67
Limestone	1.13	1.11
Salt	0.40	0.40
Vitamin premix [c]	0.38	0.38
Trace mineral premix [d]	0.10	0.10
Selenium premix [e]	0.05	0.05
Rice hulls	0.083	0.083
Calculated Composition		
ME, kcal/kg	3,170	3,182
NE, kcal/kg	1,965	1,997
CP, %	20.79	18.62
Lys, %	1.15	0.97
Sulfur AA, %	0.70	0.66
Trp, %	0.28	0.24
Thr, %	0.75	0.69
Ca, %	0.77	0.68
P, %	0.79	0.73
P available, %	0.32	0.26

[a] The diet with added phytase was similar to the basal diet but contained: rice hulls, 0%; phytase, 0.083%; limestone, 1.05%; monocalcium phosphate, 0.45%; sand, 0.55%. Natuphos® 600 provided 535 phytase units/kg of diet. (table continued)

[b] The diet with added phytase was similar to the basal diet but contained: rice hulls, 0%; phytase, 0.083%; limestone, 1.03%; monocalcium phosphate, 0.19%; sand, 0.55%. Natuphos® 600 provided 535 phytase units/kg of diet.
[c] Vitamin premix provided the following per kilogram of diet: vitamin A, 8,267 IU; vitamin D_3, 2,480 IU; vitamin E, 66 IU; menadione (as menadione pyrimidinol bisulfite complex), 6.2 mg; riboflavin, 10 mg; Ca d-pantothenic acid, 37 mg; niacin, 66 mg; vitamin B_{12}, 45 µg; d-biotin, 331 µg; folic acid, 2.5 mg; pyridoxine, 3.31 mg; thiamine, 3.31 mg; vitamin C, 83 mg.
[d] Trace mineral premix provided the following per kilogram of diet: Zn, 127 mg; Fe, 127 mg; Mn, 20 mg; Cu, 12.7 mg; I, 0.80 mg, as zinc sulfate, ferrous sulfate, manganese sulfate, copper sulfate, ethylenediamine dihydroiodide, respectively, with calcium carbonate as the carrier.
[e] Provided 0.3 mg Se per kilogram of diet.

Table 3.2. Mineral and amino acid content (%) of ingredients used in Experiment 1

Item	Corn	Soybean meal	Defatted rice bran
Thr	0.26	1.89	0.59
Met	0.17	0.66	0.34
Cys	0.19	0.17	0.36
Ile	0.27	2.26	0.59
Phe	0.37	2.38	0.74
His	0.23	1.27	0.50
Lys	0.26	2.97	0.79
Arg	0.39	3.39	1.42
Trp	0.07	0.68	0.24
Val	0.35	2.27	0.89
Ca	0.02	0.44	0.55
P	0.27	0.64	2.43

Pigs were individually fed at 0600 and 1700. Feed was placed in plastic feeders and water was added to the feed. The pigs were weighed weekly for calculation of the next week's feed allowance and for determination of ADG and gain:feed. Feed intake was

determined by the following equation: (106 BW in kg $^{0.75}$) $\times$ 2.9 or 3.2 M level / ME content of the diet (NRC, 1998). Water was provided for ad libitum consumption throughout the experiment.

Blood Analysis. On d 0 and 14 of the experiment, blood was taken from all pigs 2 h after initiation of feeding for determination of plasma urea N (PUN) levels. Blood also was collected on d 28 from all pigs at 0 and 30 min after initiation of feeding for determination of glucose concentrations. Blood was collected 30 min after initiation of feed based on data of Williams et al. (2001). Blood was collected via the anterior vena cava and placed into 7-mL tubes containing 17.5 mg sodium fluoride and 14.0 mg potassium oxalate (Monoject, Sherwood Medical, St. Louis, MO). The samples were then centrifuged at 1,500 $\times$ g at 4°C for 30 min. After centrifugation, the plasma from each sample was collected and frozen until analysis. Plasma urea N concentrations were determined by the methods of Laborde et al. (1995). Glucose concentrations were determined by a spectrophotometric procedure (Sigma, 1989a).

Ultrasound and carcass evaluation. At the beginning of the experiment, eight pigs with an average BW of 27.7 kg were slaughtered by exsanguination following electrical stunning at the LSU Agricultural Center Meats Laboratory for determination of initial body composition and initial organ weights and composition. Ultrasound backfat and longissimus muscle area (LMA) measurements were determined on d 35 of the experiment. These measurements were made by a single technician using a real-time ultrasound (Aloca 500 [12.5-cm and 3.5-MHz probe], Coremetrics Medical Systems, Wallingford, CT). On each of d 35 and 42 of the experiment, six pigs per treatment were slaughtered. Hair, hoof coverings, and tails were removed from the carcass, but the heads remained on the carcass. Carcass measurements and values from total body electrical conductivity

(TOBEC; Model MQI-27: Meat Quality Inc., Springfield, IL) analysis (for calculation of total protein and fat content in the carcass) were obtained from the left side of all carcasses after cooling at 2°C for 20 h. The linear carcass measurements included dressing percentage, LMA, average backfat, tenth-rib fat depth, and carcass length. Longissimus muscle area was determined by tracing the longissimus muscle surface area at the tenth-rib. Tenth-rib fat depth was determined by measuring the fat thickness at the tenth-rib, three quarters of the lateral length of the longissimus muscle, perpendicular to the outer skin surface. Average backfat was determined by averaging the backfat thickness at the first and last rib and last-lumbar vertebra.

Three pigs per treatment from each of the slaughter groups on d 35 and 42 of the experiment were used for determination of body composition by grinding and chemical analyses. Separate slaughter days were necessary because not all 48 pigs could be processed in one day. Organs (stomach, small intestine, large intestine, heart, lungs, liver+gallbladder, kidneys, spleen, and pancreas) were collected, individually weighed, and frozen together for further analysis. The stomach and intestines were cleaned before weighing. Total blood for the 24 pigs used for determination of body composition was collected, weighed, and a 10-ml sample was taken. The blood was then centrifuged and frozen as previously described for further analysis of plasma protein concentration (Sigma, 1989b). The response variables derived from the chemical body composition data included the weight, percentage, and daily accretion rate of protein, fat, and ash in the carcass and viscera, retained energy (RE) in the carcass and viscera, heat production (HP), net energy for production (NE_p), net energy for maintenance (NE_m), viscera weights, viscera weights as a percentage of final body weight, and viscera energy expenditure.

On the day after taking carcass measurements, the left sides of the 24 carcasses were transferred to the LSU Muscle Foods Laboratory, partially frozen by placing them in a

– 40°C freezer for 8 h, and ground once through a 1.91-cm plate and twice through a 0.79-cm plate using a Weiler grinder (Model 878, Weiler and Co., Inc., Whitewater, WI). The ground samples were sealed and frozen for later analyses. On the day after grinding the carcasses, the organs were combined and ground through a 0.32-cm plate using a Butcher Boy grinder (Model TCA 32, Lasar Mfg. Company, Inc., Los Angeles, CA), mixed in a single-phase induction motor mixer (Serial No. 4173W 3, Howell Electronic Motors Co., Howell, MI), sealed, and frozen for later analyses. All samples were homogenized at speed 3 (Brinkman Homogenizer Model PT 10/35, Brinkman Instrument, Westbury, NY) into a paste before analysis.

Prediction equations for the determination of protein and fat using readings from TOBEC were obtained from the 24 pigs whose body composition was determined by grinding and subsequent analysis. The equations were developed using similar methodology to that of Higbie et al. (2002). The equations used carcass TOBEC readings (PMA, phase maximum average) and linear carcass measurements. The prediction equation to estimate kilograms of dry matter in pork carcasses was: {[-3.07825 + (0.67334 × left side cold carcass weight, kg) + (-0.12883 × PMA) + (0.33835 × tenth-rib fat depth, cm)] × 2}, [R^2 = 0.96; root mean square error (RMSE) = 0.20]. The prediction equation to estimate kilograms of protein was: {[1.13269 + (0.17516 × left side cold carcass weight, kg) + (0.00232 × PMA) + (-0.05636 × tenth-rib fat depth, cm) + (0.02049 × temperature, °C) + (0.00022369 × LMA, cm^2) + (-0.01552 carcass length, cm)] × 2}, (R^2 = 0.91; RMSE = 0.12). The prediction equation to estimate kilograms of fat was: {[-1.81944 + (0.41374 × left side cold carcass weight, kg) + (-0.11177 × PMA) + (0.08470 × tenth-rib fat depth, cm) + (-0.00809 × LMA, cm^2) + (-0.28161 × temperature, °C)] × 2}, (R^2 = 0.85; RMSE = 0.22). Percentages of DM, protein, and fat using TOBEC prediction equations were determined by

the following equations: (kilograms of dry matter / hot carcass weight) × 100; (kilograms of protein / hot carcass weight) × 100; (kilograms of fat / hot carcass weight) × 100. To determine total body weight at grinding, the following equation was used: {left side carcass weight at grind × [(left side cold carcass weight + right side cold carcass weight) / left side cold carcass weight]}. Protein and fat deposition were determined by the following equation: [(final protein or fat content, g - initial protein or fat content, g) / days on trial]. Initial protein and fat content were based on actual analysis of the eight pigs slaughtered at the beginning of the experiment. Percentage of protein or fat increase was determined by the following equation: [(final kilograms of protein or fat - initial kilograms of protein or fat) / initial kilograms of protein or fat] x 100.

The DM, CP, fat, and ash content were determined on the left side of the carcass and on the organ fraction. Crude protein was determined using a Bran Luebbe Auto Analyzer 3 Digital Colorimeter (Serial No. 9521423, Buffalo Grove, IL). Fat was determined using a CEM (Model AVC-80, Matthews, NC) with a solvent recovery system (Model AEF-81, Matthews, NC). Dry matter was determined by weighing a 5.0-g sample and placing it into a drying oven at 100°C for 24 h. Ash was determined by placing the samples into an oven and ashing for 12 h at 550°C.

Retained energy was calculated using three methods. Retained energy was calculated as energy retained in protein and in fat from TOBEC prediction equations and also from actual carcass analysis using values of 5.66 and 9.46 Mcal/kg for protein and fat, respectively (Ewan, 2001). Furthermore, GE was determined on samples of carcass and viscera of the initial and final pigs by a bomb calorimeter (Model 1341 Plain Jacket Calorimeter, Parr Instrument Co., Moline, IL). Retained energy was calculated using the following equation: [energy in the final pigs (Mcal) - energy in the initial pigs (Mcal)]. Net

energy for production was calculated using the following equation: [RE (Mcal) / kg of feed consumed].

Net energy for M was estimated by the following equation (Just, 1982): 78 kcal/kg of BW in kg $^{0.75}$. Total HP was estimated by the following equation (Noblet et al., 1994): [(360 $\times$ final BW in kg $^{0.42}$) + (0.25 $\times$ ME)]. Organ energy expenditures were calculated based on total HP of each pig. The portal-drained viscera (gastrointestinal tract, spleen, and pancreas), liver, heart, lungs, and kidneys were estimated to represent 22.5, 22.5, 10.0, 2.5, and 12.0% of total heat production, respectively (Barcroft, 1947; Bard, 1961; Wade and Bishop, 1962; Forster, 1964; Milnor, 1968; Neutze et al., 1968; Smith and Baldwin, 1974; Canas et al., 1982; Thomson et al., 1995; Yen, 1997). Organ energy expenditure per gram of organ was calculated as (energy expenditure of the organ / organ weight in grams) for pigs fed the C-SBM diet at 3.2 $\times$ M. Organ energy expenditures for all pigs were then calculated as [organ energy expenditure per gram of organ (as determined for the C-SBM diet fed at 3.2 $\times$ M) $\times$ organ weight in grams] as previously described by Knowles et al. (1998).

Statistical Analysis. Data were analyzed by analysis of variance procedures appropriate for a completely randomized design using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). The statistical model included treatment. For the growth, blood, and carcass measurement data, initial BW was used as a covariate. Day-0 PUN concentration was used as a covariate for d-14 PUN concentration. Contrast statements were included to examine phytase, energy, and phytase $\times$ energy effects as a 2 $\times$ 2 factorial arrangement of treatments. The pig was the experimental unit for all data.

Experiment 2

General. Sixty-four crossbred barrows and gilts from the LSU Agricultural Center Swine Farm with an initial and final BW of 23.3 and 47.4 kg were used in this experiment. They were allotted to two treatments in a randomized complete block design on the basis of weight and ancestry. There were eight replications (five replications of barrows and three replications of gilts) per treatment and four pigs per replication. The two dietary treatments (Table 3.3) were: 1) C-SBM control, and 2) C-SBM with reduced Ca and aP with 500 phytase units/kg of diet. Defatted rice bran (10%) was included in the diets to decrease the ME content. Natuphos® 1200 (BASF Corporation, Mount Olive, NJ) was analyzed to contain 1530 phytase units/kg. It was added to the diet at 0.033% to provide 500 phytase units/kg of diet. Actual analysis of the diet indicated that the diet contained 475 phytase units/kg diet. The diets were formulated to contain 1.02% Lys, 0.65% Ca, and 0.25% aP (Ca and aP were reduced by 0.10% in the diet with added phytase). All other AA met or exceeded the ratio to Lys calculated using the NRC model (NRC, 1998). All pigs were weighed on d 14 and at termination of the experiment (d 28) to determine ADG, ADFI, and gain:feed. Pigs were allowed ad libitum access to feed and water throughout the experiment.

Table 3.3. Composition of the treatment diets in Experiment 2

Ingredients, %	Control	Phytase
Corn	60.88	60.88
Soybean meal, 47.5% CP	26.01	26.01
Defatted rice bran	10.00	10.00
Limestone	1.05	0.97
Monocalcium phosphate	0.60	0.13
Sodium bentonite	0.50	0.50

(table continued)

Salt	0.40	0.40
Vitamin premix[a]	0.38	0.38
Trace mineral premix[b]	0.10	0.10
Se premix[c]	0.05	0.05
Rice hulls	0.033	-
Phytase[d]	-	0.033
Sand	-	0.55
Calculated Composition		
ME, kcal/kg	3,173	3,173
CP, %	19.21	19.21
Lys, %	1.02	1.02
Sulfur AA, %	0.66	0.66
Thr, %	0.72	0.72
Trp, %	0.23	0.23
Ca, %	0.65	0.55
P, %	0.72	0.62
Available P, %	0.25	0.25

[a]Provides the following per kilogram of diet: vitamin A, 8,267 IU; vitamin D_3, 2,480 IU; vitamin E, 66 IU; menadione (as menadione pyrimidinol bisulfite complex), 6.2 mg; riboflavin, 10 mg; Ca-d-pantothenic acid, 37 mg; niacin, 66 mg; vitamin B_{12}, 45 µg; d-biotin, 331 mg; folic acid, 2.5 mg, pyridoxine, 3.31 mg, thiamin, 3.31 mg; and vitamin C, 83 mg.
[b]Provides the following per kilogram of diet: Zn, 127 mg; Fe, 127 mg; Mn, 20 mg; Cu, 12.7 mg; I, .80 mg, as zinc sulfate, ferrous sulfate, manganese sulfate, copper sulfate, ethylenediamine dihydroiodide, respectively, with calcium carbonate as the carrier.
[c] Provided 0.3 mg Se per kilogram of diet.
[d] Natuphos® 1200 provided 475 phytase units/kg of diet.

Ultrasound and Carcass Evaluation. Ultrasound backfat and LMA measurements were determined at the initiation and termination of the experiment as described in Exp. 1.

Longissimus muscle area difference was determined by the following equation: (final LMA – initial LMA). Tenth-rib fat depth difference was determined by the following equation: (final tenth-rib fat depth – initial tenth-rib fat depth).

Statistical Analysis. Data were analyzed by analysis of variance procedures appropriate for a completely randomized block design using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). The statistical model included treatment, replication, and sex. There were no treatment x sex interactions so this term was removed from the model. The pen of pigs was the experimental unit for all data.

Results

Experiment 1

Growth Performance and Blood Metabolites. Daily gain was increased ($P < 0.01$) in pigs fed at 3.2 × M compared with pigs fed at 2.9 × M, but gain:feed was not affected ($P > 0.10$) by diet (Table 3.4). Pigs fed the diets at 3.2 × M had an increased ($P < 0.09$) PUN, but phytase addition eliminated the increase in PUN (phytase x energy, $P < 0.06$). Pigs fed the diets with added phytase had an increase ($P < 0.08$) in fasting plasma glucose.

Carcass Traits. Actual tenth-rib fat depth and ultrasound LMA were increased ($P < 0.05$) in pigs fed phytase (Table 3.4). Average backfat ($P < 0.05$) and carcass length ($P < 0.01$) were increased in pigs fed at 3.2 × M relative to those fed at 2.9 × M. No other linear carcass measurements were affected ($P > 0.10$) by treatment.

Ultrasound and Carcass Evaluation. Initial carcass composition and organ weights from the eight pigs slaughtered at the beginning of the experiment are shown in Table 3.5. Carcass composition of the pigs on treatment using actual chemical analysis is shown in Table 3.6. Fat percentage in the carcass, kilograms of fat in the carcass, fat deposition in the carcass, fat deposition in the carcass + viscera, RE in the carcass and carcass +

Table 3.4. Effect of phytase and metabolizable energy intake on growth performance, plasma metabolites, and carcass composition of growing pigs in Experiment 1[a]

	Energy, kcal	2.9 x M		3.2 x M		
Item	Phytase, units/kg	0	500	0	500	SEM
ADG, kg [b]		0.61	0.63	0.71	0.73	0.02
ADFI (as-fed basis), kg [b]		1.40	1.41	1.58	1.59	0.01
Gain:feed		0.44	0.45	0.45	0.46	0.01
PUN, mmol/L[c]		3.28	3.39	3.90	3.36	0.17
Fasting glucose, mmol/L[d,e]		4.36	4.44	4.24	4.48	0.10
Fed glucose, mmol/L [e]		4.46	4.47	4.41	4.46	0.18
Actual						
Loin muscle area, cm^2		25.87	26.51	26.09	26.74	0.93
Tenth-rib fat, cm [f]		0.69	0.84	0.77	0.89	0.06
Average backfat, cm [g]		1.12	1.09	1.30	1.14	0.05
Carcass length, cm [h]		64.10	64.64	66.10	66.20	0.52
Dressing percentage		76.12	76.13	76.51	75.32	0.57
Ultrasound						
Loin muscle area, cm^2 [f]		20.09	20.86	19.88	21.30	0.47
Tenth-rib fat, cm		1.01	0.94	1.01	1.03	0.04

[a] Data are means of 12 pigs per treatment. Average initial and final BW were 26.4 and 52.0 kg, respectively.
[b] Energy, $P < 0.01$.
[c] Energy, $P < 0.09$; phytase x energy, $P < 0.06$. PUN = plasma urea N measured on blood taken on d 14, 2 h after feeding (PUN taken on d 0 was used as a covariate for the data).
[d] Phytase, $P < 0.08$.
[e] Fasting glucose = glucose concentration measured on blood taken on d 28 at time 0 before initiation of feeding; fed glucose = glucose concentration measured on blood taken on d 28 at time 30 min after initiation of feeding.
[f] Phytase, $P < 0.05$.
[g] Energy, $P < 0.05$.
[h] Energy, $P < 0.01$.

Table 3.5. Initial body composition of growing pigs in Experiment 1[a]

Item	Component	SE
Carcass weight, kg [b]	19.4	0.5
Heart, g	140.2	7.5
Spleen, g	48.8	6.0
Pancreas, g	68.0	6.9
Lung, g	306.6	18.8
Liver + gall bladder, g	650.6	22.7
Kidney, g	127.5	6.2
Stomach, g	220.6	6.3
Small intestine, g	879.1	41.9
Large intestine, g	633.5	38.0
Total intestine, g	1,512.6	80.0
Carcass		
DM, %	32.5	1.2
Protein, %	54.2	1.1
Protein, g	3,413.4	115.0
Fat, %	31.8	1.0
Fat, g	2,009.0	129.2
Ash, %	11.0	0.3
Ash, g	694.8	27.4
Calculated energy, Mcal/kg [c]	38.3	1.6
Gross energy, Mcal/kg [d]	40.6	2.2
Organ		
Organ weight, g	3,134.9	88.6

(table continued)

DM, %	20.5	1.1
Protein, %	68.7	0.3
Protein, g	442.4	17.0
Fat, %	17.0	0.9
Fat, g	110.2	8.2
Ash, %	5.8	0.2
Ash, g	37.2	1.4
Calculated energy, Mcal/kg [c]	3.6	1.8
Gross energy, Mcal/kg [d]	4.4	1.9
Carcass + organ		
Calculated energy, Mcal/kg [c]	41.9	1.8
Gross energy, Mcal/kg [d]	45.1	2.0

[a] Data are means of eight pigs with an average BW of 27.67 kg. Protein, fat, and ash percentages are based on DM basis.
[b] Carcass weight is based on weight of carcass at grinding.
[c] Calculated energy level determined by protein and fat content of carcass and organs by using the values 5.66 and 9.46 Mcal/kg for protein and fat, respectively.
[d] Energy level determined by GE analysis on carcass and organs by bomb calorimetry.

Table 3.6. Effect of phytase and metabolizable energy intake on protein, fat, and ash accretion of growing pigs in Experiment 1[a]

	Energy, kcal	2.9 x M		3.2 x M		
Item	Phytase, units/kg	0	500	0	500	SEM
Carcass						
Dry matter, %		34.5	35.1	35.8	35.6	0.6
Protein, %		54.0	54.4	52.3	54.2	1.5
Protein, kg [b]		6.7	7.1	7.3	7.4	0.3
Protein deposition, g/d		85.4	94.7	101.1	105.0	8.2

(table continued)

Fat, % [c]	32.3	33.5	35.6	36.0	1.3
Fat, kg [c]	4.0	4.4	5.1	5.0	0.4
Fat deposition, g/d [c]	52.0	61.3	78.7	78.2	10.0
Ash, %	9.6	8.4	9.5	8.9	0.7
Ash deposition, g/d	13.4	10.3	16.2	14.3	2.8
RE, Mcal [c,d]	37.0	43.0	50.9	50.6	4.7
Viscera					
Dry matter, %	23.2	22.9	24.0	23.3	0.4
Protein, %	66.0	63.9	63.0	63.3	1.3
Protein deposition, g/d	5.9	5.4	7.1	5.7	0.8
Fat, %	24.6	26.5	27.5	28.1	1.4
Fat deposition, g/d [b]	3.7	4.2	5.4	4.9	0.7
Ash, %	4.4	4.5	4.5	4.5	0.1
Ash deposition, g/d	0.23	0.28	0.30	0.25	0.05
RE, Mcal [d]	2.6	2.7	3.5	3.0	0.4
Plasma protein g/L	61.4	60.0	64.2	60.8	1.4
Carcass + viscera					
Protein deposition, g/d	92.5	101.4	110.3	112.0	8.9
Fat deposition, g/d [c]	55.7	65.5	84.0	83.1	10.4
Ash deposition, g/d	14.6	11.6	17.0	14.6	3.1
Heat production, Mcal [c]	3.14	3.21	3.41	3.39	0.07
NE_m, Mcal/kg	1.45	1.50	1.55	1.53	0.04
NE_p, Mcal/kg	0.75	0.83	0.88	0.90	0.07
RE, Mcal [c,d]	39.8	46.0	54.8	53.9	5.0
RE, Mcal [b,e]	39.7	46.0	52.1	51.1	4.7

(table continued)

[a] Data are means of six pigs per treatment. All percentages are on dry matter basis. RE = retained energy; NE_p = net energy for production.
[b] Energy, $P < 0.10$.
[c] Energy, $P < 0.05$.
[d] Calculated energy level determined by protein and fat content of carcass and organs by using the values 5.66 and 9.46 Mcal/kg for protein and fat, respectively.
[e] Determined by GE analysis on carcass and viscera by bomb calorimetry.

viscera (determined by protein and fat analysis), and HP were increased ($P < 0.05$) in pigs fed at 3.2 × M compared with pigs fed at 2.9 × M. Fat deposition in the viscera, kilograms of protein in the carcass, and RE (determined by GE analysis) also were increased ($P < 0.10$) in pigs fed at 3.2 × M relative to those fed at 2.9 × M.

Carcass composition using prediction equations developed with TOBEC analysis is shown in Table 3.7. Fat deposition, percentage of fat increase, and RE were increased ($P < 0.09$) in pigs fed at 3.2 x M.

Organ data. Liver + gallbladder weight was increased ($P < 0.10$) in pigs fed at 3.2 × M, but only in pigs not fed phytase (phytase × energy, $P < 0.06$; Table 3.8). Spleen weight was increased by phytase in pigs fed at 2.9 × M but decreased by phytase in pigs fed at 3.2 × M (phytase × energy, $P < 0.06$). Stomach weight was increased ($P < 0.10$) in pigs fed at 3.2 × M relative to those fed at 2.9 × M. Heart, lung, kidney, and small and large intestines were not affected by treatment ($P > 0.10$). Liver + gallbladder energy expenditure (Table 3.8) was increased in pigs fed at 3.2 × M but only in pigs not fed phytase (phytase × energy, $P < 0.06$).

Liver + gallbladder weight as a percentage of final BW was increased in pigs fed at 3.2 x M, but only in pigs not fed phytase (phytase x energy, $P < 0.09$; Table 3.8). Total intestine weight as a percentage of final BW was decreased ($P < 0.04$) in pigs fed phytase at either M level.

Experiment 2

In Exp. 2, diet did not affect (P > 0.10) ADG, ADFI, gain:feed, initial or final LMA, initial or final tenth-rib fat depth, and LMA or tenth-rib fat depth difference (Table 3.9).

Table 3.7. Effect of phytase and metabolizable energy intake on protein and fat accretion using total body electrical conductivity data in Experiment 1[a]

	Energy, kcal	2.9 x M		3.2 x M		
Item	Phytase, units/kg	0	500	0	500	SEM
Carcass						
DM, %		36.0	36.5	36.7	36.8	0.6
Protein, %		52.5	51.6	50.9	50.7	0.9
Protein, kg		7.0	7.1	7.3	7.3	0.2
Protein deposition, g/d		92.5	96.3	103.0	103.1	5.7
Fat, %		34.4	34.6	35.3	35.2	0.9
Fat, kg		4.6	4.8	5.2	5.2	0.3
Fat deposition, g/d [b]		67.3	71.8	81.8	82.6	6.8
RE, Mcal [b,c]		44.7	47.2	52.1	52.0	3.5
Percentage protein increase		77.2	86.3	87.7	91.0	5.0
Percentage fat increase [b]		98.7	129.2	131.5	145.1	13.6

[a] Data are means of 12 pigs per treatment. All percentages are on a dry matter basis. RE = retained energy.
[b] Energy, P < 0.09.
[c] Calculated energy level determined by protein and fat content of carcass and organs by using the values 5.66 and 9.46 Mcal/kg for protein and fat, respectively.

Table 3.8. Effect of phytase and metabolizable energy intake on viscera weights of growing pigs in Experiment 1[a]

	Energy, kcal	2.9 x M		3.2 x M		
Item	Phytase, units/kg	0	500	0	500	SEM
Heart, g		215.3	216.3	218.1	231.9	12.3
% [b]		0.44	0.42	0.41	0.44	0.02
kcal/d		336.6	338.1	341.0	362.6	19.3
Lung, g		537.5	530.3	619.4	503.4	52.8
%		1.08	1.02	1.15	0.95	0.09
kcal/d		72.8	71.8	85.2	68.2	7.4
Liver + gall bladder, g [c,d,e]		848.1	860.2	972.1	851.7	30.8
%		1.73	1.67	1.81	1.61	0.03
kcal/d [c,d,e]		669.4	679.0	767.2	672.3	24.3
Kidney, g		192.9	179.6	194.8	208.2	10.4
%		0.39	0.35	0.36	0.40	0.02
kcal/d		405.3	377.2	409.2	437.2	21.8
Spleen, g [d,e]		64.1	76.4	80.4	75.0	4.3
%		0.13	0.15	0.15	0.14	0.01
Pancreas, g		81.9	85.8	84.1	91.2	3.6
%		0.17	0.17	0.16	0.17	0.01
Stomach, g [d]		363.8	385.7	430.3	391.7	18.6
%		0.74	0.75	0.81	0.74	0.03
Small intestine, g		963.3	983.9	1,095.5	1020.2	65.3
%		1.96	1.91	2.04	1.92	0.11

(table continued)

Large intestine, g	1,050.4	1,126.2	1,191.1	1,122.2	74.8
%	2.34	2.18	2.23	2.12	0.13
Total intestine, g	2,113.7	2,110.1	2,286.6	2,142.4	83.7
% [c]	4.30	4.10	4.27	4.04	0.10
Gastrointestinal tract, g	2,623.4	2,657.9	2,881.6	2,700.2	101.7
kcal/d [f]	695.7	704.6	767.2	714.8	27.5

[a] Data are means of six pigs per treatment.
[b] Expressed as a percentage of final BW.
[c] Phytase, $P < 0.10$.
[d] Energy, $P < 0.10$.
[e] Phytase x energy, $P < 0.06$.
[f] Gastrointestinal tract includes entire gastrointestinal tract, spleen, and pancreas.

Table 3.9. Effect of diet on growth performance and ultrasound measurements in Experiment 2[a]

Item	Control	Phytase	SEM
Overall			
ADG, kg	0.85	0.84	0.02
ADFI (as-fed basis), kg	1.92	1.85	0.04
Gain:feed	0.443	0.452	0.008
Ultrasound measurements			
Initial LMA, cm^2	10.48	10.44	0.12
Final LMA, cm^2	17.28	17.58	0.25
Initial tenth-rib fat, cm	0.91	0.87	0.03
Final tenth-rib fat, cm	1.40	1.43	0.04
LMA difference, cm^2	6.83	7.11	0.21
Tenth-rib fat difference, cm	0.49	0.56	0.05

[a] Data are means of eight replicates of four pigs per replicate. Average initial and final BW were 23.3 and 47.4 kg, respectively. LMA = longissimus muscle area.

Discussion

Growth performance was not affected by phytase supplementation in pigs limit fed (Exp. 1) or in pigs with ad libitum access to feed (Exp. 2). However, ADG was increased in growing pigs fed the higher energy level (Exp. 1).

In Exp. 1, PUN levels were increased in pigs fed the higher energy level but not in pigs fed phytase. This response indicates that phytase may have more of an effect on PUN levels in pigs fed closer to ad libitum intake. Fasting plasma glucose levels were increased in pigs fed phytase in Exp. 1, which agrees with the results of Williams et al. (2001). However, there was no effect of phytase on glucose levels 30 min after initiation of feeding, which does not agree with Williams et al. (2001). Williams et al. (2001) reported that plasma glucose peaked in blood 30 min after initiation of feeding; however, it may have peaked at a different time in the present study, which may explain the difference in results. The increase in fasting glucose concentration by phytase may be explained by the positive effect of phytase on carbohydrate digestion and absorption (Williams et al., 2001); however, we would have expected more of a response 30 min after the initiation of feeding. Phytate may influence starch digestibility through interaction with amylase enzyme, proteins associated with starch, Ca (which catalyzes amylase activity), or with starch itself (Deshpande and Cheryan, 1984; Thompson and Yoon, 1984).

O'Quinn et al. (1997) reported no effect in finishing pigs on tenth-rib fat depth, last-rib backfat thickness, LMA, or dressing percentage when phytase was added at 500 phytase units/kg of diet to sorghum-SBM-based diets. However, Johnston (2000) reported an increase in tenth-rib fat depth in growing-finishing pigs fed C-SBM diets with the addition of phytase, indicating a possible increase in energy availability. Data from Exp. 1 showed no differences in dressing percentages, actual LMA, or ultrasound tenth-rib fat depth in pigs fed phytase. In Exp. 1, actual tenth-rib fat depth and ultrasound LMA were increased in

pigs fed phytase, but in Exp. 2, ultrasound tenth-rib fat depth and LMA were not affected in pigs fed phytase. In the study by O'Quinn et al. (1997) and our Exp. 2, pigs were allowed ad libitum access to feed, but in Exp. 1, pigs were limit fed at 2.9 or 3.2 x M. The difference in the data of our Exp. 1 and the data of O'Quinn et al. (1997) may be due to the different levels of energy restriction.

Protein and fat deposition in the carcass were increased in pigs fed the higher energy level. Assuming that only energy is limiting in the diet, protein deposition increases linearly with energy intake (de Lange et al., 2001). Campbell et al. (1983) and Bikker et al. (1995) also reported an increase in protein and fat deposition in pigs fed an increased energy level. Retained energy also was increased in pigs fed the higher energy level, which was to be expected because of the increase in protein and fat deposition. The data from TOBEC prediction equations mimicked the data from grinding the pigs in that protein and fat deposition and RE were increased when pigs were fed the higher energy level.

The protein percentage in the carcass from chemical or TOBEC analyses was not affected in pigs fed phytase, but there tended to be an increase in protein and fat deposition in the carcass and viscera in pigs fed phytase. In a comparative slaughter experiment, Ketaren et al. (1993) reported that phytase increased protein deposition in 20-kg pigs fed diets composed of SBM and sucrose and offered ad libitum. As in our Exp. 1, Ketaren et al. (1993) reported numerical increases in fat and energy deposition. Furthermore, they reported that efficiency of both protein and energy retention was increased by the addition of phytase. O'Quinn et al. (1997) reported no effect on CP or percentage fat or lean in pigs fed increasing levels of phytase. However, in their experiment, the pigs were allowed ad libitum access to feed while the pigs in our experiment were limit fed.

Organ weight and organ energy expenditures also were determined in our experiment. Spleen weight was increased when pigs were fed at 3.2 x M with no added

phytase. However, energy level did not affect spleen weight as a percentage of final BW. Energy level also increased pancreas, stomach, and liver + gallbladder weight. Liver + gallbladder weight was decreased in pigs fed phytase. This response was due to the weight of the liver + gallbladder in the pigs fed at 3.2 x M with no added phytase. Viveros et al. (2002) reported a decreased liver weight, expressed as g/100 g BW, in broiler chicks fed diets with low nonphytate P levels and supplemented with phytase.

Energy expenditure in the liver + gallbladder was increased in pigs fed at 3.2 x M with no added phytase. Furthermore, spleen, stomach, and intestine weights were all numerically decreased when phytase was added to the diets of pigs fed at 3.2 x M. The organ data suggest that phytase may affect the NE_m of pigs. For example, the liver accounts for approximately 22.5% (Smith and Baldwin, 1974) of total HP in pigs; thus, reducing liver weight will reduce the NE_m.

Phytase supplementation significantly affected only a few of the response variables in Exp. 1. However, when looking at growth performance, actual linear carcass measurements, protein and fat deposition (actual chemical analysis and TOBEC)], and RE (actual chemical analysis and TOBEC), phytase supplementation numerically increased 19 of 24 response variables when pigs were fed at either energy level. Similarly, in Exp. 2 four of the five response variables measured were numerically increased by phytase. When the experiments are combined, 23 out of 29 response variables were numerically increased when phytase was added to the diet. Early reports by Rojas and Scott (1969) and Miles and Nelson (1974) reported increased apparent ME yields for chicks fed diets with supplemental phytase. Similarly, Ravindran et al. (2000) reported that phytase increased apparent ME in diets for chicks. Data on the effects of phytase on energy availability in pigs are more limited. Johnston (2000) reported an increase in apparent ileal GE digestibility in 50-kg pigs, but O'Quinn et al. (1997) reported no increase in ileal GE digestibility in 50- to

80-kg pigs. However, total tract digestibility of GE was not affected in the studies by Johnston (2000) or O'Quinn et al. (1997). In the experiments by Johnston (2000) and O'Quinn et al. (1997), older pigs were used compared with the pigs used in our experiments. Also, the diet compositions were different in the experiments. The experiment by O'Quinn et al. (1997) used sorghum-SBM diets but our experiments and the one by Johnston (2000) used C-SBM diets. As mentioned above, Ketaren et al. (1993) reported a numerical increase in energy deposition in 20-kg pigs fed diets with added phytase, which agrees with our results in Exp. 1.

These experiments indicate that growth, protein deposition, fat deposition, and retained energy are increased when energy level is increased in the diet for growing swine. Furthermore, because phytase numerically increased the majority of the response variables measured, more research is needed to determine the effect of phytase on energy availability for swine.

CHAPTER 4

EVALUATION OF THE NUTRIENT MATRIX VALUES OF PHYTASE IN BROILERS *

Introduction

Phytate has been shown to bind to cations including Ca, Zn, Cu, Pb, Mn, Mg, Co, and Fe (Oberleas and Harland, 1996). It also has been shown to have negative effects on digestive enzymes (Caldwell, 1992), and protein (Okuba et al., 1976), AA (Cosgrove, 1996), and carbohydrate (Thompson and Yoon, 1984) availability. Phytate is found in feed ingredients such as corn (C) and soybean meal (SBM), and it can cause a decrease in nutrient availability in diets containing these ingredients.

Microbial phytase has been shown to increase the availability of phytate P for swine and poultry (Cromwell et al., 1991; Qian et al., 1996). Phytase also has been shown to increase energy (Namkung and Leeson, 1999; Ravindran et al., 1999b) and AA (Sebastian et al., 1997; Ravindran et al., 1999b; Johnston, 2000) digestibility in diets for poultry. The amount of Ca and available P (aP) that phytase releases has been studied extensively (Denbow et al., 1995; Mitchell and Edwards, 1996; Gordon and Roland, 1998) and the values range from approximately 0.09 to 0.10%. However, to fully realize the economic potential of phytase, the amount of metabolizable energy (ME) and AA released by phytase needs to be evaluated. The nutrient matrix values for phytase indicates the amount of a nutrient (Ca, P, ME, or AA) that will be released when phytase is added to the diet. Having correct nutrient matrix values allows for more accurate formulation of diets that include phytase. These formulations allow the producer to add less Ca, P, AA, and ME in diets for poultry; thus, reducing the cost of feed. For example, adding phytase in the diets for poultry can reduce the amount of limestone, monocalcium phosphate, SBM, crystalline AA, and fat added in the diets.

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As a result of the aforementioned effects of phytase on nutrient availability, phytase also may have positive effects on the environment. This increased availability results in less P and N in the feces and less to be placed on soil when poultry waste is used as a fertilizer. In order to fully utilize and receive the benefits of the addition of phytase to the diets the objective of these experiments (EXP) was to evaluate the nutrient matrix values for phytase in broilers.

Materials and Methods

General

All methods used in these EXP were approved by the Louisiana State University Agricultural Center (LSU) Animal Care and Use Committee. Two EXP were conducted with Ross x Ross commercial broilers from Sanderson Farms, McComb, MS (EXP 1) or ConAgra Poultry Company, Nachitoches, LA (EXP 2) to evaluate the accuracy of the nutrient matrix values for Natuphos® 1200 (Table 4.1).

Experiment 1

In EXP 1, 288 broilers were fed the same diet (C-SBM in Table 4.2) adequate in all nutrients from 0 to 4 d posthatching (NRC, 1994). They were held overnight without feed and water on the day before allotment to treatment. The broilers were then weighed, wingbanded, and allotted to treatments in a completely randomized design. There were seven replications (four male and three female) with six broilers per replication. The initial and final BW were 72 and 574 g, respectively, and the EXP lasted 14 d. The treatments were: 1) C-SBM control diet, 2) C-SBM diet deficient in AA, 3) C-SBM diet deficient in AA but with added phytase at 600 phytase units/kg using the matrix values for Ca, aP, and AA, 4) as Diet 3 with no added phytase, 5) C-SBM diet low in ME, 6) C-SBM diet low in ME but with added phytase at 600 phytase units/kg using the matrix values for Ca, aP, and ME, 7) as Diet 6 with no added phytase. They were housed in thermostatically controlled starter

Table 4.1. Nutrient matrix values of Natuphos® 1200 for broilers[a]

Nutrient	value	amount provided in the diet
Available phosphorus, %	188	0.094
Calcium, %	188	0.094
Crude protein, %	427	0.214
Lysine, %	29	0.015
Methionine, %	5	0.003
Cystine, %	10	0.005
Sulfur amino acids, %	15	0.008
Tryptophan, %	5	0.003
Threonine, %	24	0.012
Valine, %	26	0.013
Isoleucine, %	22	0.011
Leucine, %	33	0.017
Arginine, %	16	0.008
Phenylalanine, %	21	0.011
Histidine, %	11	0.006
ME, kcal/kg	61,937	30.969

[a] Natuphos® 1200 (BASF Corporation, Wyandotte, MI) added at 0.05% of the diet, which provides 600 phytase units/kg. Amino acids are on a true digestible basis.

batteries with raised wire floors and continuous lighting. Feed and water were offered ad libitum throughout the experimental period. At the end of the experiment, all broilers were weighed individually and pen feed intake was measured.

Diets 1 to 4 (Table 4.2) evaluated the AA matrix values of Natuphos® 1200. Diet 1 (Basal) was adequate in all nutrients. Diet 2 (LAA) was deficient in AA, providing 0.82%

true digestible lysine (Lys) and all other AA met or exceeded the ratio to Lys (Baker, 1997). Diet 3 (LAA+Phy) was similar to Diet 2 but formulated with phytase nutrient matrix values for AA, Ca, and aP. Diet 4 (LAA–Phy) was similar to Diet 3 but with supplemental Ca and P to reach adequate levels and no added phytase. Diet 1 and Diets 5 to 7 evaluated the energy matrix value of Natuphos® 1200 (Table 4.2). Diet 5 (LME) was low in energy, providing 2,937 kcal of ME/kg. Diet 6 (LME+Phy) was similar to Diet 5 but formulated with phytase nutrient matrix values for energy, Ca, and aP. Diet 7 (LME–Phy) was similar to Diet 6 but with supplemental Ca and P to reach adequate levels and no added phytase. In all diets, vitamins and minerals met or exceeded the requirement for broilers from 0 to 21 d posthatching (NRC, 1994). The control and the low energy diets were formulated to provide 1.12% true digestible Lys and all other AA met or exceeded the ratio to Lys (Baker, 1997). Natuphos® 1200 (BASF Corporation, Wyandotte, MI) was included in the diet at 0.05%, which provided 600 phytase units/kg. Actual analysis of diets indicated that phytase provided 670 phytase unit/kg for Diet 3 and 855 phytase units/kg for Diet 6. All diets were C-SBM and produced in mash form.

Data were analyzed by ANOVA procedures appropriate for completely randomized designs (Steel and Torrie, 1980). For ease of presentation, the data were analyzed as two EXP. The AA subset included broilers fed Diets 1, 2, 3, and 4. The ME subset included broilers fed Diets 1, 5, 6, and 7. For the AA subset, treatment and sex were included in the model. There were no treatment x sex interactions so it was removed from the analysis. For the energy subset, treatment, sex, and the treatment x sex interaction were included in the model. Pen of broilers was the experimental unit for all data.

Experiment 2

In EXP 2, 1,575 broilers were allotted on d 0 to three treatments with 10 replications (five male and five female) per treatment and 50 (male) or 55 (female) broilers per

Table 4.2. Composition of diets in Experiment 1[A]

Diet	1	2	3	4	5	6	7
Ingredient	Basal	LAA	LAA+Phy	LAA-Phy	LME	LME+Phy	LME-Phy
Corn	52.47	66.53	67.23	67.23	56.72	56.72	56.72
Soybean meal (47.5% CP)	36.85	24.83	24.24	24.24	36.53	36.53	36.53
Soy oil	6.46	4.34	4.24	4.24	1.00	1.00	1.00
Monocalcium phosphate	1.52	1.61	1.16	1.61	1.51	1.06	1.51
Limestone	1.66	1.72	1.65	1.72	1.66	1.59	1.66
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mineral premix[B]	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix[C]	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Choline chloride[D]	-	0.04	0.04	0.04	-	-	-
DL-methionine	0.19	0.08	0.08	0.07	0.18	0.18	0.18
Rice hulls	0.05	0.05	-	0.05	0.05	-	0.05
Phytase[E]	-	-	0.05	-	-	0.05	-
Cornstarch	-	-	-	-	1.55	0.78	0.78
Sand	-	-	0.51	-	-	1.29	0.78
Calculated composition[F]							
ME, kcal/kg	3,200	3,200	3,200	3,200	2,937	2,937/(2,906)	2,906

(table continued)

Crude protein, %	22.44	17.74	17.73/(17.51)	17.51	22.65	22.65	22.65
Lysine, %	1.12	0.82	0.82/(0.81)	0.81	1.12	1.12	1.12
Sulfur amino acids, %	0.81	0.59	0.59/(0.58)	0.58	0.81	0.81	0.81
Threonine, %	0.76	0.60	0.60/(0.59)	0.59	0.76	0.76	0.76
Valine, %	0.96	0.77	0.77/(0.76)	0.76	0.97	0.97	0.97
Tryptophan, %	0.24	0.18	0.18/(0.18)	0.18	0.24	0.24	0.24
Isoleucine, %	0.86	0.66	0.66/(0.65)	0.65	0.86	0.86	0.86
Calcium, %	1.00	1.00	1.00/(0.91)	1.00	1.00	1.00/(0.91)	1.00
Available phosphorus, %	0.45	0.45	0.45/(0.45)	0.45	0.45	0.45	0.45
Total phosphorus, %	0.72	0.70	0.70/(0.61)	0.70	0.73	0.73/(0.64)	0.73

[A] Amino acids formulated on a true digestible basis. CP = crude protein; LAA = corn-soybean meal (C-SBM) diet low in AA; LAA+Phy = C-SBM diet low in AA but with 600 FTU of phytase/kg of diet; LAA-Phy = the LAA+Phy diet without phytase but with adequate Ca and aP; LME = C-SBM diet low in ME; LME+Phy = C-SBM diet low in ME but with 600 FTU of phytase/kg of diet; LME-Phy = the LME+Phy diet without phytase but with adequate Ca and aP.

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

[C] Provides the following per kilogram of diet: vitamin A (retinyl palmitate), 8,000 IU; vitamin D_3, 3,000 IU; vitamin E (DL-α-tocopherol acetate), 25 IU; vitamin K, 1.5 IU; riboflavin, 10 mg; pantothenic acid, 15 mg; niacin, 50 mg; vitamin B_{12}, 0.02 µg; biotin, 0.1 µg; folic acid, 1 mg; pyridoxine, 4 mg; thiamin, 3 mg.

[D] Contains 600,000 mg/kg of choline.

[E] Phytase (as Natuphos® 1200) provided 600 FTU/kg. Actual analysis was 670 FTU/kg for Diet 3 and 855 FTU/kg for Diet 6.

[F] Numbers not in parentheses are the calculated composition using the analyzed values for the ingredients and the nutrient matrix values for phytase. Numbers in parentheses are the calculated composition using the analyzed values for ingredients but as if phytase was not added.

replication. The initial and final BW were 44 and 2,203 g, respectively and the EXP lasted 42 d. The treatments were: 1) C-SBM control diet, 2) C-SBM diet with added phytase at 600 phytase units/kg using the nutrient matrix values for Ca, aP, and ME, 3) C-SBM diet with added phytase at 600 phytase units/kg using the nutrient matrix values for Ca, aP, ME, and AA. The broilers were housed in 1.52 x 3.05-m pens at the LSU Poultry Farm in one room of a ventilated tunnel house equipped with cool cells and fans. The lighting system consisted of 3 d of 24-h light, followed by 16 hr of light and 8 hr of dark for the remainder of the project. The broilers were conditioned to the dark period over 3 d by increasing the periods of dark until 8 hr of dark were reached. The temperature in the house was 31 to 33°C for the first week and was dropped each week until 24 to 27°C was reached.

The litter was reused after raising one group of broilers and topped with 6 to 8 cm of new shavings before the experiment began. Feed and water were offered ad libitum throughout the experimental period. The broilers were fed a three phase feeding program consisting of starting (0 to 15 d), growing (16 to 35 d), and finishing (36 to 42 d) periods. At the end of EXP 2, all broilers were weighed by pen and pen feed intake was measured.

Natuphos® 1200 was included in the diet at 0.037%, which added 600 phytase units/kg of diet (actual analysis of the Natuphos® 1200 indicated an activity of 1,620 phytase units/kg). Actual analysis of diets indicated that phytase provided 878 phytase units/kg for Diet 2 and 600 phytase units/kg for Diet 3. Corn and SBM were analyzed for AA, Ca and P and their values were used in diet formulations (Table 4.3). The AA composition (Table 4.3) of corn and soybean meal was determined after acid hydrolysis (AOAC, 1990), whereas Met and Cys content were determined after performic acid oxidation followed by acid hydrolysis (AOAC, 1990). Tryptophan content was determined after alkaline hydrolysis (AOAC, 1990). The mineral composition (Table 4.3) of corn and

soybean meal was determined by inductively coupled plasma emission spectroscopy (Model Optima 3000, Perkin Elmer, Norwalk, CT 06859) after digestion in nitric acid and peroxide. All diets were formulated using Agristat values. During the starting period (0 to 15 d), the diets (Table 4.4) were formulated to provide 3,075 kcal/kg, 0.95% Ca, 0.45% aP, 1.25% total Lys, and 0.94% total sulfur AA (TSAA). During the growing period (16 to 35 d), the diets were formulated to provide 3,150 kcal/kg, 0.85% Ca, 0.41% aP, 1.11% total Lys, and 0.87% TSAA. During the

Table 4.3. Mineral and amino acid content (%) of the ingredients used in Experiment 2

Item	Corn	Soybean meal
Calcium	0.02	0.37
Phosphorus	0.29	0.62
Lysine	0.34	3.03
Methionine	0.21	0.68
Cystine	0.22	0.79
Tryptophan	0.08	0.69
Threonine	0.35	1.81
Valine	0.45	2.31
Isoleucine	0.32	2.16
Leucine	1.06	3.63
Arginine	0.51	3.48
Phenylalanine	0.48	2.41
Histidine	0.27	1.32

Table 4.4. Diet composition for the starter phase in Experiment 2 [a]

Diet	1	2	3
Ingredient	Control[b]	ME[b]	ME/AA[b]
Corn	58.53	60.21	60.88
Soybean meal (47.5% CP)	33.38	33.19	32.62
Tallow	3.52	2.55	2.45
Monocalcium phosphate	1.56	1.11	1.11
Limestone	1.15	1.09	1.09
Salt	0.50	0.50	0.50
BMD+3 [c]	0.50	0.50	0.50
Mineral premix [d]	0.25	0.25	0.25
Vitamin premix [e]	0.05	0.05	0.05
Choline chloride [f]	0.14	0.14	0.14
DL-methionine	0.20	0.19	0.19
L-lysine•HCl	0.05	0.05	0.05
Monteban [g]	0.08	0.08	0.08
Rendox [h]	0.05	0.05	0.05
Rice hulls [i]	0.04	-	-
Phytase [i]	-	0.04	0.04
Calculated composition [j]			
ME, kcal/kg	3,075	3,075/(3,044)	3,075/(3,044)
Crude protein, %	21.33	21.38	21.37/(21.16)
Lysine, %	1.25	1.25	1.25/(1.24)

(table continued)

Sulfur amino acids, %	0.94	0.94	0.94/(0.93)
Tryptophan, %	0.28	0.28	0.27/(0.27)
Threonine, %	0.81	0.81	0.80/(0.79)
Calcium, %	0.95	0.95/(0.86)	0.95/(0.86)
Available phosphorus, %	0.45	0.45/(0.45)	0.45/(0.45)
Phosphorus, %	0.71	0.61/(0.52)	0.61/(0.52)

[a] Abbreviations used: ME = metabolizable energy; AA = amino acid. The ME diet used the phytase matrix values for ME. The ME/AA diet used the phytase matrix values for ME and AA.
[b] The control diets during the growing and finishing phases were similar to the starter diet but contained the following: corn, 63.91 and 71.64%; soybean meal, 28.03 and 21.68%; tallow, 3.82 and 3.19%; limestone, 1.00 and 1.03%; and monocalcium phosphate, 1.41 and 1.16%; DL-Met, 0.18 and 0.13%; monteban, 0.08 and 0%; BMD + 3 nitro, 0.50 and 0%, respectively. The diets using the phytase nutrient matrix values for ME during the growing and finishing phases were similar to the starter diet but contained the following: corn, 65.79 and 73.52%; soybean meal, 27.82 and 21.47%; tallow, 2.74 and 2.10%; limestone, 0.93 and 1.06%; and monocalcium phosphate, 0.90 and 0.65%; DL-Met, 0.17 and 0.13%; monteban, 0.08 and 0%; BMD + 3 nitro, 0.50 and 0%, respectively. The diets using the phytase nutrient matrix values for AA and ME during the growing and finishing phases were similar to the starter diet but contained the following: corn, 66.54 and 74.24%; soybean meal, 27.17 and 20.86%; tallow, 2.63 and 2.00%; limestone, 0.93 and 1.06%; and monocalcium phosphate, 0.91 and 0.66%; DL-Met, 0.17 and 0.12%; monteban, 0.08 and 0%; BMD + 3 nitro, 0.50 and 0%, respectively.
[c] Provides per kilogram of diet: bacitracin methylene disalicylate (BMD), 0.022g and 3-nitro-4-hydroxyphenylarsonic acid, 0.025g. Nutra Blend Corporation, Neosha, MO 64850.
[d] Provides the following per kilogram of diet: Fe, 50 mg; Mn, 100 mg; Cu, 7 mg; Se, 0.15 mg; Zn, 75; I, 1 mg, as ferrous sulfate monohydrate, manganese sulfate, copper sulfate, sodium selenite, zinc sulfate, ethylenediamine dihydroiodide, respectively with calcium carbonate as the carrier.
[e] Provides the following per kilogram of diet: vitamin A (retinyl palmitate), 8,000 IU; vitamin D_3, 3,000 IU; vitamin E (DL-α-tocopherol acetate), 25 IU; vitamin K, 1.5 IU; riboflavin, 10 mg; pantothenic acid, 15 mg; niacin, 50 mg; vitamin B_{12}, 0.02 g; biotin, 0.1 µg; folic acid, 1 mg; pyridoxine, 4 mg; thiamin, 3 mg.
[f] Contains 600,000 mg/kg of choline.
[g] Active ingredient is 0.825 g per kg narsin.
[h] Used as an anitoxidant (Kemin Industries, Des Moines, IA 50301).
[li] Phytase (Natuphos® 1200) provided 878 phytase units/kg in Diet 2 and 600 phytase units/kg in Diet 3.
[j] Numbers not in parentheses are the calculated composition using the analyzed values for the ingredients and the nutrient matrix values for phytase. Numbers in parentheses are the calculated composition using the analyzed values for ingredients but as if phytase was not added.

finishing period (36 to 42 d), the diets were formulated to provide 3,200 kcal/kg, 0.77% Ca, 0.35% aP, 0.94% total Lys, and 0.76% TSAA. All nutrients met or exceeded the requirement (NRC, 1994) for broilers. Because the AA matrix for phytase is based on digestible AA values, Diet 3 was formulated to meet the true digestible Lys percentage as in Diets 1 and 2. All diets were C-SBM and produced in mash form.

At the termination of the EXP, final weights were taken, and six broilers per pen were randomly selected for processing. The broilers were held without feed for 12 hr then transported to the LSU Muscle Foods Laboratory. The broilers were slaughtered by severing the jugular vein. The broilers were then scalded, defeathered, eviscerated, and placed in an aerated chill tank (ice and water). After the broilers were chilled to 6 to 8° C, they were removed and allowed to drain for at least 15 min and then weighed. The broilers were then deboned and individual breast weights were recorded. After the chilled breast weight was recorded, the individual breasts were placed into a poultry meat tray that contained two absorbent pads, sealed, and placed in a refrigerator (4 to 6° C). After approximately 24 hr, the breasts were removed from the tray, blotted with a paper towel, and weighed. The left tibia from each broiler was removed for determination of bone ash percentage. Bone ash percentage was determined after refluxing in ethanol and ether for 36 h each using a Soxhlet Extracting Apparatus (Lab Glass, Vineland, NJ) and placing in an ashing oven at 550° C for 24 h. In addition, litter samples were taken from nine locations within each pen at the termination of the experiment to determine total P (Bender and Wood, 2000) and soluble P (Pote, 2000; Self-Davis and Moore, 2000) in waste from the broilers fed phytase compared with those fed a conventional diet.

Data were analyzed by ANOVA procedures appropriate for a completely randomized design (Steel and Torrie, 1980). There were no treatment x sex interactions so this

parameter was removed from the model. Final BW was used as a covariate for the carcass data.

Results and Discussion

Experiment 1

Table 4.5 presents the performance data of the AA portion of EXP 1. Daily gain and gain:feed were decreased ($P < 0.03$) in broilers fed the diet deficient in AA with or without added phytase relative to those fed the control diet. Daily feed intake was decreased ($P < 0.03$) in broilers fed the diet deficient in AA with no added phytase but adequate in Ca and aP (Treatment 4) relative to those fed the C-SBM control. Table 4.6 presents the performance data of the ME portion of EXP 1. Daily gain and gain:feed were decreased ($P < 0.04$) in broilers fed the diet low in energy with or without added phytase relative to those fed the control diet. Broilers fed the diets with phytase using the nutrient matrix for ME or AA resulted in similar growth performance compared with broilers fed the deficient diets. Removing the phytase from the diets low in AA or ME did not decrease the AA or ME content low enough to cause a significant decrease in growth performance in the broilers.

Table 4.5. Growth performance of broilers in the amino acid portion in Experiment 1[a]

Treatment	ADG	ADFI	GF
1. C-SBM control diet	41.35[b]	51.39[b]	0.805[b]
2. C-SBM deficient in AA	33.86[c]	49.02[b]	0.691[c]
3. C-SBM diet deficient in AA but with 600 phytase units/kg of diet	33.24[c]	48.98[b]	0.679[c]
4. Diet 3 without phytase but adequate in Ca and aP	33.08[c]	48.59[c]	0.682[c]
SEM	0.67	0.82	0.009

[a] Data are means of seven replications of six broilers per replication with an initial and final BW of 72 and 574 g. AA = amino acid; ADG = average daily gain; ADFI = average daily feed intake; GF = gain:feed; C-SBM = corn-soybean meal; FTU = phytase units; aP = available phosphorus. The Ca and aP was reduced by 0.094% in the diet with phytase.
[b,c] Data in columns with different superscripts differ, P < 0.03.

Table 4.6. Growth performance of broilers in the energy portion in Experiment 1[a]

Treatment	ADG	ADFI	GF
1. C-SBM control diet	41.26[b]	51.36	0.804[b]
5. C-SBM low in energy	38.08[c]	51.87	0.735[c]
6. C-SBM low in energy but with 600 phytase units/kg of diet	39.26[c]	52.63	0.746[c]
7. Diet 6 without phytase but adequate in Ca and aP	38.79[c]	52.66	0.737[c]
SEM	0.61	0.91	0.007

[a] Data are means of seven replications of six broilers per replication with an initial and final BW of 72 and 574 g. ADG = average daily gain; ADFI = average daily feed intake; GF = gain:feed; C-SBM = corn-soybean meal; FTU = phytase units; aP = available phosphorus. The Ca and aP was reduced by 0.094% in the diet with phytase.
[b,c] Data in columns with different superscripts differ, P < 0.04.

Experiment 2

Diet did not affect (P > 0.05) final BW, daily gain, daily feed intake, gain:feed, mortality, or tibia ash percentage when broilers were fed the control diet or the diets with added phytase (Table 4.7). This response suggests that the nutrient matrix values used for phytase are accurate when growth performance and tibia ash are used as response variables. The nutrient matrix values for phytase used in this EXP included Ca, aP, ME, and AA. The availability of calcium (Mroz et al., 1994; Radcliffe, et al., 1995), aP (Cromwell et al., 1991; Qian et al., 1996), energy (Namkung and Leeson, 1999; Ravindran et al., 1999b), and AA (Sebastian et al., 1997; Ravindran et al., 1999a; Johnston, 2000) has been

reported to be increased in broilers when phytase is supplemented to the diet. Diet did not affect (P > 0.05) live weight, eviscerated weight, chill weight, carcass yield, moisture gain due to chill, breast weight as a percentage of live weight, or 24-h moisture loss in

Table 4.7. Effect of phytase on growth performance of 42 d-old broilers in Experiment 2[a]

Diet	1	2	3	
Item	Control	ME	ME/AA	SEM
Final weight, g	2,219.7	2,200.9	2,188.8	13.2
Average daily gain, g	51.7	51.3	51.0	0.3
Feed intake, g	94.1	92.8	92.0	1.0
Gain:feed, g:g	0.549	0.550	0.552	0.005
Mortality, chicks/replication [B]	0.60	1.00	0.80	0.25
Tibia ash, %	57.27	58.02	57.12	0.56

[a] Data are means of ten replicates of 50 or 55 broilers per replicate. Average initial BW was 44 g. ME = metabolizable energy; AA = amino acids. The ME diet used the phytase matrix values for ME. The ME/AA diet used the phytase matrix values for ME and the AA.
[B] Mortality was analyzed using the square-root transformation of (y + 0.5). Treatment means are actual means from original data.

broilers fed the control diet or diets with added phytase using the nutrient matrix values (Table 4.8). Broilers fed the diets with phytase using the matrix values for ME, Ca, and aP had a decreased (P < 0.03) breast weight as a percentage of chill weight compared with those fed the diet with phytase using the matrix values for ME, AA, Ca, and aP. This response in breast weight was unexpected because the diet using the matrix values for ME, Ca, and aP actually had more AA in the diet than the diet using the nutrient matrix values for ME, AA, Ca, and aP.

There was no effect on moisture loss in the breast meat when broilers were fed the diets with phytase. This response disagrees with a report by Rienstra et al. (2001) who

reported a decrease in drip loss of loin chops when phytase was added to diets for swine. On the other hand, Gebert et al. (1999c) reported no effect on water holding capacity when phytase was added to diets for swine.

Table 4.8. Effect of phytase on carcass traits of 43 d-old broilers in Experiment 2[a]

Diet	1	2	3	
Item	Control	ME	ME/AA	SEM
Live weight, kg	2.20	2.15	2.17	0.03
Eviscerated weight, kg	1.58	1.53	1.55	0.02
Chill weight, kg	1.62	1.57	1.58	0.02
Carcass yield, % [b]	72.1	71.8	71.3	0.3
Moisture gain due to chill, % [c]	2.54	2.35	2.32	0.27
24-h moisture loss, % [d]	1.10	1.07	1.05	0.08
Breast weight PLW, % [e]	13.0	12.7	13.1	0.14
Breast weight PCW, % [e]	17.6[f,g]	17.4[g]	17.9[f]	0.18

[a] Data are means of ten replicates of six broilers per replicate. Average initial and final BW were 44 and 2,203 g, respectively. The growth trial lasted 42 d. The broilers were processed on d 43 after a 12-h fast. ME = metabolizable energy; AA = amino acids. Average initial BW was 44 g. ME = metabolizable energy; AA = amino acids. The ME diet used the phytase matrix values for ME. The ME/AA diet used the phytase matrix values for ME and the AA.
[b] Carcass yield calculated as eviscerated weight divided by live weight times 100.
[c] Moisture gain calculated as chill weight minus eviscerated weight divided by eviscerated weight times 100.
[d] Moisture loss calculated as 24-h breast weight minus initial breast weight divided by initial breast weight times 100.
[e] PLW = percentage of live weight; PCW = percentage of chill weight.
[f,g] Means in a row with different superscripts differ, $P < 0.03$.

Total P, soluble P, and inorganic soluble P in the litter were decreased ($P < 0.03$) in the litter of broilers fed the diets with added phytase relative to those fed the control diet (Table 4.9). DeLaune et al. (2001) reported an increase in soluble P in the litter of broilers

fed phytase. However, it is well documented that phytase increases P retention in broilers (Leske and Coon, 1999; Murry et al., 1997; Qian et al., 1997). In addition, our data agree with data by Moore et al. (1998) who reported numerical decreases (not always significant) in both total and soluble phosphorus content in litter from broilers fed diets with added phytase compared with litter from broilers fed diets without added phytase.

Table 4.9. Effect of phytase on phosphorus levels in the litter in Experiment 2[a]

	1	2	3	
Item	Control	ME	ME/AA	SEM
Total P, mg/kg	14,295[b]	12,928[c]	12,411[c]	233
Inorganic soluble P, mg/kg	1,563[b]	1,348[c]	1,223[c]	93
Total soluble P, mg/kg	1,881[b]	1,664[c]	1,525[c]	90

[a] Data are means of ten replicates. Litter samples were taken from nine locations within each pen at the termination of the experiment. ME = metabolizable energy; AA = amino acids. Average initial BW was 44 g. ME = metabolizable energy; AA = amino acids. The ME diet used the phytase matrix values for ME. The ME/AA diet used the phytase matrix values for ME and the AA.
[b,c] Phytase, $P < 0.03$.

In EXP 1, reducing the AA or ME concentrations in the diets for broilers decreased growth performance. The addition of phytase did not affect growth performance of broilers fed diets deficient in AA or ME. In EXP 2, using the nutrient matrix values for phytase in formulating C-SBM diets for commercial broilers resulted in similar growth performance, carcass traits, meat quality, and tibia ash percentage compared with broilers fed a conventional C-SBM diet. Phytase addition to broiler diets reduces the levels of total and soluble P in the litter. The nutrient matrix values presented in these EXP are accurate and can be used in formulating diets for commercial broilers that incorporate Natuphos® 1200.

CHAPTER 5

EFFECT OF MICROBIAL PHYTASE, LOW CALCIUM AND PHOSPHORUS, AND REMOVING THE DIETARY TRACE MINERAL PREMIX ON CARCASS TRAITS, PORK QUALITY, PLASMA METABOLITES, AND TISSUE MINERAL CONTENT IN GROWING-FINISHING PIGS

Introduction

Feed represents approximately 65% of the production costs of market pigs; thus, reducing the cost of feed is important to the swine industry. Kim et al. (1997) and Mavromichalis et al. (1999) indicated that trace mineral premixes (TMP) could be deleted in diets for finishing pigs with no deleterious effects on growth, carcass characteristics, or meat quality. However, there has been little research conducted on removing the TMP for the entire growing-finishing period, and most research deleting the TMP during the finishing phase also deleted the vitamin premix (Edmonds and Arentson, 2001; Shaw et al., 2002).

Phytate has the potential to form insoluble salts with Ca, Fe, Zn, Mn, Cu, and Co (Vohra et al., 1965), which may reduce the availability of these minerals. Lei et al. (1993) and Adeola et al. (1995) reported that Zn bioavailability and retention were improved with the addition of phytase in the diet for weanling pigs. Furthermore, Spears et al. (2001) reported that pigs fed phytase with no supplemental Zn performed as well as those fed supplemental Zn. Therefore, phytase addition to diets for pigs may reduce or eliminate the need for TMP supplementation.

If phytase is to be used in diets for growing-finishing pigs, effects on carcass traits and meat quality need to be determined. To date, there have been varying effects of phytase addition on carcass traits and pork quality. Rienstra et al. (2001) reported that pigs fed phytase had an increased longissimus muscle area (LMA) and a decreased marbling and drip loss. Gebert et al. (1999c) reported that phytase addition resulted in a paler

longissimus muscle and a decreased 45-min pH, and O'Quinn et al. (1997) reported a decreased dressing percentage when phytase was added to diets for swine.

Therefore, the objectives of this experiment were to determine the effects of phytase addition, reduced Ca and available P (aP), and removing the TMP on growth performance, plasma metabolites, carcass traits, pork quality, and tissue mineral content in growing-finishing pigs.

Materials and Methods

All methods used in this experiment were approved by the Louisiana State University Agricultural Center Animal Care and Use Committee.

General. One hundred twenty crossbred (Yorkshire x Landrace and Yorkshire x Duroc) gilts and barrows with an average initial and final BW of 22 and 109 kg, respectively, were used in this experiment. The pigs were allotted to five dietary treatments based on weight and gender with three replications of barrows and three replications of gilts with four pigs per replicate pen in a randomized complete block design. Ancestry was equalized within treatment as much as possible. The pigs were housed in total confinement in 1.5 x 3.0-m pens with concrete-slatted floors.

The dietary treatments were as follows: 1) corn-soybean meal (C-SBM) positive control; 2) C-SBM with reduced Ca and aP; 3) C-SBM with reduced Ca and aP with phytase (Natuphos 1200; BASF Corporation, Mount Olive, NJ) to provide 500 phytase units/kg of diet; 4) Diet 1 without the TMP supplementation; and 5) Diet 3 without the TMP supplementation (Table 5.1). Actual analysis indicated that Diet 3 provided 877 phytase units/kg of diet and Diet 5 provided 883 phytase units/kg of diet. To test the effect of reduced Ca and aP with and without phytase the following dietary treatments were used: 1) C-SBM positive control diet; 2) C-SBM with reduced Ca and aP; 3) C- SBM with reduced Ca and aP with phytase. To test the effects of phytase addition in diets with and without the

TMP the following diets were used: 1) C-SBM positive control diet; 3) C-SBM diet with reduced Ca and aP with phytase; 4) C-SBM diet without the TMP supplementation; and 5) C-SBM diet with reduced Ca and aP with phytase but without the TMP supplementation.

A four-phase growing-finishing feeding program was used and all pigs within a replication were switched from one phase to the next at the same time. Diets for this experiment were formulated to provide 0.97, 0.88, 0.79, and 0.68% total Lys for gilts and 0.97, 0.78, 0.68, and 0.60% total Lys for barrows for weight ranges of 20 to 43, 43 to 66, 66 to 89, and 89 to 113 kg, respectively. All AA met a minimum of 105% of the AA requirement according to each growth phase for gilts or barrows gaining 325 grams of lean per day as calculated using the NRC model (1998). The diets also were formulated to contain 0.60% Ca and 0.24% aP from 20 to 43 kg, 0.53% Ca and 0.19% aP from 43 to 66 kg, 0.48% Ca and 0.17% aP from 66 to 89 kg, and 0.45% Ca and 0.15% aP from 89 to 113 kg. The Ca and aP were reduced by 0.10% in the low Ca and aP diets and the diets with added phytase. The aP was only reduced to 0.054% for barrows and 0.058% for gilts from 89 to 113 kg. The AA and mineral values for corn and SBM were based on the NRC (1998). The pigs were weighed at the end of each growth phase for calculation of ADG, ADFI, and gain:feed. Treatment diets and water were provided for ad libitum consumption throughout the 98-d experiment.

Blood Metabolites. On d 26 of the experiment, the feeders were removed from the pen at 1600. On d 27 at 0800, blood was collected via the anterior vena cava and placed into 7-mL tubes containing 17.5 mg sodium flouride and 14.0 mg potassium oxalate (Monoject, Sherwood Medical, St. Louis, MO). The samples were then centrifuged at 1500 x *g* and 4°C for 45 min. After centrifugation, the plasma from each sample was collected and frozen until analysis. Glucose concentrations were determined by a spectrophotometric

Table 5.1. Compositon of the basal diets for the early-growing phase.

Item	Control [a]	Low Ca and available P [b]	No trace minerals
Ingredient			
Corn	70.90	70.90	70.90
Soybean meal, 48% CP	25.89	25.89	25.89
Monocalcium phosphate	0.81	0.33	0.81
Limestone	0.97	0.90	0.97
Salt	0.40	0.40	0.40
Sodium bentonite	0.50	0.50	0.50
Vitamin premix [c]	0.38	0.38	0.38
Trace mineral premix [d]	0.10	0.10	-
Rice hulls	0.05	0.05	0.05
Phytase [e]	-	-/+	-/+
Sand	-	0.55	0.10
Calculated Composition			
ME, kcal/kg	3,298	3,298	3,298
CP, %	18.18	18.18	18.18
Lys, %	0.97	0.97	0.97
Sulfur AA, %	0.62	0.62	0.62
Trp, %	0.21	0.21	0.21
Thr, %	0.68	0.68	0.68
Ca, %	0.60	0.50	0.60
Available P, %	0.24	0.14	0.24
Cu, mg/kg	20.00	20.00	7.30

(table continued)

I, mg/kg	0.80	0.80	0.00
Fe, mg/kg	288.00	249.70	160.90
Mn, mg/kg	37.03	36.41	17.03
Zn, mg/kg	154.00	154.00	26.99
Se, mg/kg	0.42	0.42	0.12

[a] The control diets for barrows during the late-growing, early-finishing, and late-finishing phases were similar to the early-growing diet but contained the following: corn, 77.60, 81.56, and 84.72%; soybean meal, 19.40, 15.58, and 12.52%; limestone, 0.92, 0.85, and 0.82%; and monocalcium phosphate, 0.61, 0.54, and 0.46%, respectively. The control diets for gilts during the late-growing, early-finishing, and late-finishing phases were similar to the early-growing diet but contained the following: corn, 74.22, 77.81, and 81.71%; soybean meal, 22.82, 19.38, and 15.57%; limestone, 0.90, 0.83, and 0.81%; and monocalcium phosphate, 0.59, 0.52, and 0.44%, respectively.

[b] The low Ca and available P diets for barrows during the late-growing, early-finishing, and late-finishing phases were similar to the early growing diets but contained the following: limestone, 0.84, 0.77, and 0.74%; and monocalcium phosphate, 0.14, 0.06, and 0.002%, respectively. The low Ca and available P diets for gilts during the late-growing, early-finishing, and late-finishing phases were similar to the early-growing diet but contained the following: limestone, 0.82, 0.75, and 0.72%; and monocalcium phosphate, 0.12, 0.04, and 0.004%, respectively.

[c] Vitamin premix provided the following per kilogram of diet: vitamin A, 8,267 IU; vitamin D_3 2,480 IU; vitamin E, 66 IU; menadione (as menadione pyrimidinol bisulfite complex) 6.2 mg; riboflavin, 10 mg; Ca d-pantothenic acid, 37 mg; niacin, 66 mg; vitamin B_{12}, 45 µg; d-biotin, 331 µg; folic acid, 2.5 mg; pyridoxine, 3.31 mg; thiamine, 3.31 mg; vitamin C, 83 µg.

[d] Trace mineral premix provided the following per kilogram of diet: Zn, 127 mg; Fe, 127 mg; Mn, 20 mg; Cu, 12.7 mg; I, 0.80 mg; Se, 0.30 mg, as zinc sulfate, ferrous sulfate, manganese sulfate, copper sulfate, calcium iodate, sodium selenite, respectively with calcium carbonate as the carrier.

[e] Phytase replaced rice hulls in the diets with added phytase. Natuphos 1200 provides 500 phytase units/kg of diet. The Ca and aP was reduced by 0.10% when phytase was added.

procedure (Sigma, 1989). Blood samples were collected on d 27 because this is the approximate time that Shelton et al (2003b) and Johnston et al. (2004) reported changes in plasma glucose in pigs fed phytase. At slaughter, blood was collected during exsanguination for determination of hematocrit percentage (Adams Autocrit Centrifuge, Papsippanny, N.J).

Carcass Evaluation. On the day after the growth trial ended, three pigs per replicate pen were randomly selected and slaughtered by exsanguination after electrical stunning at the Louisiana State University Agricultural Center Meats Laboratory. Conventional carcass measurements and values from total body electrical conductivity (Model MQ1-27: Meat Quality Inc., Springfield, IL) were determined as described by Matthews et al. (2001a). The NPPC (1991) equation that assumes 5% estimation for intramuscular fat and compensates for unequal BW was used to evaluate percentage acceptable quality lean and kilograms of carcass lean. Leaf fat and the liver and kidneys were individually weighed at slaughter. After a 20-h chill at 2° C, the left front foot was removed and frozen.

Pork Quality. Pork quality measurements were taken from the left side of the carcass after a 20-h chill at 2° C as described by Matthews et al. (2001a,c). Drip loss was determined by a suspension method (Shelton et al., 2003a). Cooking loss and shear force also were determined on a fresh chop taken from the 10th-rib as outlined by Matthews et al. (2001c). Shear force was determined on three cores using an HD 250 Texture Machine (Texture Technologies Corporation, Scarsdale, NY) fitted with a Warner-Bratzler head with a load cell capacity of 25 kg and a cross-head speed of 100 mm/min. One additional chop was taken from the 9th-rib, deboned, external fat removed, and frozen for 90 d and thaw and cook loss were determined.

Tissue Ash Determination and Mineral Content. The 3rd and 4th metacarpal bones from the left foot of each pig were removed and manually cleaned of adhering tissue. The 4th metacarpal bone was broken using a HD 250 Texture Machine fitted with three point bend rig with a load cell capacity of 250 kg and cross-head speed of 100 mm/min and a span over which the bone was set of 1.5 cm. Fat was removed from the 3rd metacarpal bone by a 36-h Soxhlet extraction in ethyl alcohol followed by a 36-h extraction with diethyl ether, and then dried at 100° C.

A 20-g sample of kidney and liver was taken at slaughter and frozen for subsequent determination of ash percentage. A 5-mL sample of bile was taken for determination of

mineral content after drying at 100° C for 24 h. The liver sample also was analyzed for mineral content. A 1.27-cm chop from the 8th-rib was taken, homogenized, and frozen for subsequent determination of ash percentage and mineral content. Dry matter of the liver, kidney, and longissimus muscle was determined by weighing a 5.0-g sample and drying at 100° C for 24 h. Percentage ash was determined by placing the samples into a muffle furnace and ashing for 12 (liver, kidney, and longissimus muscle) or 36 h (3rd metacarpal bone) at 550° C. The ash samples were dissolved in nitric acid, and mineral content was determined by inductively coupled plasma emission spectroscopy (Model Optima 3000, Perkin Elmer, Norwalk, CT).

Statistical Analysis. Data were analyzed by analysis of variance procedures appropriate for a randomized complete block design using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). The statistical model included treatment, replication, and sex. The treatment x sex interaction was significant only for the bile mineral content data, and it was removed from the model for all other data. To test the effect of reduced Ca and aP with and without phytase the following dietary treatments were used: 1) C-SBM positive control diet; 2) C-SBM with reduced Ca and aP; 3) C-SBM with reduced Ca and aP with phytase. Treatment differences for pigs fed these three diets separated by the PDIFF option of SAS. To test the effects of phytase addition in diets with and without the TMP the following diets were used: 1) C-SBM positive control diet; 3) C-SBM diet with reduced Ca and aP with phytase; 4) C-SBM diet without the TMP supplementation; and 5) C-SBM diet with reduced Ca and aP with phytase but without the TMP supplementation. These four diets were analyzed by contrast statements to evaluate phytase, TMP, and phytase x TMP effects as a 2 x 2 factorial arrangement of treatments. Treatment differences were considered significant at $\alpha = 0.10$. Final BW was used as a covariate for the carcass data. The pen of pigs was the experimental unit for all data.

Results

Growth Performance

Diet did not affect ADFI or gain:feed (Table 5.2).

Low Ca and aP with or without Phytase. Daily gain was decreased (P = 0.02) in pigs fed the low Ca and aP diet, but adding phytase to the low Ca and aP diet increased (P = 0.02) ADG equal to that of pigs fed the control diet. This response was seen during all phases of growth.

Phytase x Trace Mineral. Growth performance was not affected in pigs fed diets with or without the TMP and with or without phytase. This response was seen during all phases of growth.

Blood metabolites

Low Ca and aP with or without Phytase. Hematocrit percentage was increased (P = 0.10) in pigs fed the low Ca and aP diet with phytase relative to those fed the low Ca and aP diet (Table 5.2). These dietary treatments did not affect fasting plasma glucose level of pigs.

Phytase x Trace Mineral. Fasting plasma glucose concentration was increased (P = 0.09) in pigs fed the diets without the TMP. Hematocrit percentage of pigs was not affected by these diets. Diet did not affect longissimus muscle area, average backfat thickness, lean gain per day, leaf fat, ham butt-face fat thickness, NPPC (National Pork Producers Council) percentage of acceptable quality lean, kilograms of carcass fat-free lean, percentage of carcass fat-free lean, kilograms of carcass fat, percentage of carcass fat, carcass lean:fat ratio, kilograms of ham fat-free lean, percentage of ham fat-free lean, kilograms of ham fat, or percentage of ham fat (Table 5.3).

Table 5.2. Effect of reducing calcium and available phosphorus, removing the trace mineral premix, and adding phytase in diets for growing-finishing pigs on growth performance and blood metabolites[a]

Item Diets	Control	Low Ca and aP	Low Ca and aP+ PHY	No TMP	No TMP + PHY	SEM
Growth performance						
ADG, kg	0.91[e]	0.83[f]	0.89[e]	0.88	0.88	0.02
ADFI, kg	2.93	2.78	2.90	2.82	2.82	0.07
Gain:feed ratio	0.31	0.30	0.31	0.31	0.31	0.01
Blood components						
Hematocrit, %[b]	38.20[e,f]	37.56[f]	38.92[e]	38.53	37.17	0.71
Glucose, mmol/L [c,d]	0.243	0.246	0.246	0.261	0.256	0.008

[a] Data are means of three replications of barrows and three replications of gilts with four pigs per replicate pen. Average initial and final BW were 22 and 109 kg, respectively. Average daily feed intake and gain:feed are on an as-fed basis. TMP = trace mineral premix; PHY = phytase; aP = available P. Treatment differences for the pigs fed the control diet, the low Ca and aP diet, or the low Ca and aP diet with phytase were separated by the PDIFF procedure. Contrast statements also were included to examine phytase, TMP, and phytase x TMP effects as a 2 x 2 factorial arrangement of treatments that included the control diet, the low Ca and aP diet with phytase, the control diet without the TMP, and the low Ca and aP diet with phytase but without the TMP.
[b] Hematocrit was measured on blood taken from pigs on the day of slaughter.
[c] Fasting glucose measured on d 27.
[d] Mineral, P = 0.09.
[e,f] Means with different superscripts differ, P = 0.02.

Carcass evaluation

Low Ca and aP with or without Phytase. Final BW, hot carcass weight, dressing percentage, and NPPC kilograms of carcass lean were decreased (P = 0.10) but liver and kidney weights, and liver and kidney weight as a percentage of final BW were increased (P = 0.10) in pigs fed the low Ca and aP diet, but adding phytase reversed these responses (P

= 0.10). Carcass length of pigs was increased (P = 0.10) in pigs fed the low Ca and aP diets with phytase relative to pigs fed the low Ca and aP diet without phytase.

Phytase x Trace Mineral. Tenth-rib backfat thickness was increased (P = 0.03) but carcass length and ham weight were decreased (P = 0.03) in pigs fed the diets without the TMP. Liver weight and liver weight as a percentage of final BW were increased in pigs fed diets without the TMP but this response was reversed by phytase (mineral, phytase, and phytase x mineral, P = 0.06).

Pork Quality

Diet did not affect color, marbling, Commission internationale de l'Eclairage (CIE) a* value, CIE b* value, 45-min pH, 24-h temperature or pH, cook loss or shear force of a fresh chop, and thaw loss, cook loss, or total loss of a frozen (thawed) chop (Table 5.4).

Low Ca and aP with or without Phytase. Drip and total loss of a fresh chop were decreased (P = 0.09) in pigs fed the low Ca and aP diet relative to pigs fed the control diet. The CIE L* value was decreased (P = 0.09) in pigs fed the low Ca and aP diet with phytase. Shear force of a frozen chop was decreased (P = 0.09) in pigs fed the low Ca and aP diet relative to the low Ca and aP diet with added phytase.

Phytase x Trace Mineral. Forty-five-min temperature was increased (P = 0.07) in pigs fed the diets with added phytase.

Tissue ash determination and mineral content

Many mineral analyses were not affected by diet. Unaffected tissue mineral concentrations (means $\pm$ pooled SEM, DM basis) were representative of 30 replications of three pigs per replicate pen (Nor shown in Tables 5.5 or 5.6). All values are on a DM basis. Bile: K, 0.37 $\pm$ 0.02%; Mg, 0.051 $\pm$ 0.002%; Na, 4.22 $\pm$ 0.22%. Muscle: ash 4.78 $\pm$ 0.10%; K, 1.20 $\pm$ 0.11%; Mg, 0.10 $\pm$ 0.01%; Na, 0.40 $\pm$ 0.05%. Liver: ash, 5.34 $\pm$ 0.04%; Mg, 0.058 $\pm$ 0.004%; Na, 0.50 $\pm$ 0.07%. Kidney: ash, 6.35 $\pm$ 0.09%.

Table 5.3. Effect of reducing calcium and available phosphorus, removing the trace mineral premix, and adding phytase in diets for growing-finishing pigs on carcass characteristics [a]

Item Diets	Control	Low Ca and aP	Low Ca and aP+ PHY	No TMP	No TMP + PHY	SEM
Conventional Measurements						
Final BW, kg	112.3[f]	104.7[g]	110.8[f]	109.1	108.1	1.4
Hot carcass weight, kg	80.2[f]	79.0[g]	80.0[f]	80.0	79.9	0.3
Longissimus muscle, cm^2	44.0	42.6	43.1	45.5	44.4	0.9
10th-rib backfat, cm [b]	1.67	1.87	1.65	1.83	1.93	0.08
Average backfat, cm	2.53	2.66	2.60	2.56	2.59	0.08
Carcass length, cm [b]	80.9[f,g]	79.7[f]	81.0[g]	79.6	80.0	0.44
Dressing percentage	73.6[f]	72.4[g]	73.4[f]	73.4	73.3	0.3
Lean gain per day, g	356	343	350	344	346	8
Liver, g [b,c]	1,539[f]	1,662[g]	1,509[f]	1,660	1,522	26
% final BW [b,c]	1.42[f]	1.52[g]	1.39[f]	1.52	1.40	0.02
Kidney, g	359.1[f]	419.1[g]	376.4[f]	366.6	370.5	12.4
% final BW	0.33[f]	0.38[g]	0.35[f]	0.34	0.34	0.01
Leaf fat, g	1,013	1,061	1,059	1,112	1,005	51
Ham weight, kg [b]	10.03	9.93	10.02	9.85	9.79	0.08
Ham butt-face fat, cm	1.49	1.55	1.53	1.58	1.63	0.06
NPPC [d]						
Percentage acceptable quality lean	55.83	54.68	55.64	55.71	54.96	0.53
Total carcass lean, kg	44.72[f]	43.15[g]	44.47[f]	44.54	43.82	0.46
TOBEC[e]						

(table continued)

Carcass fat-free lean, kg	42.45	42.10	42.03	41.73	41.71	0.41
Carcass fat-free lean, %	52.99	53.41	52.57	52.20	52.28	0.51
Carcass fat, kg	20.94	21.24	21.18	21.18	21.61	0.51
Carcass fat, %	26.08	26.78	26.38	26.38	27.00	0.60
Carcass lean:fat ratio	2.04	2.03	2.02	2.01	1.97	0.06
Ham fat-free lean, kg	6.50	6.46	6.56	6.45	6.37	0.10
Ham fat-free lean, %	64.87	65.05	65.43	65.46	65.01	0.62
Ham fat, kg	1.97	1.95	1.93	1.91	1.94	0.05
Ham fat, %	19.58	19.59	19.25	19.40	19.87	0.59

[a] Data are means of three replications of barrows and three replications of gilts with three pigs per replicate pen. Average final BW was 110 kg. TMP = trace mineral premix; TOBEC = total body electrical conductivity; PHY = phytase; aP = available P. Treatment differences for the pigs fed the control diet, the low Ca and aP diet, or the low Ca and aP diet with phytase were separated by the PDIFF procedure. Contrast statements also were included to examine phytase, TMP, and phytase x TMP effects as a 2 x 2 factorial arrangement of treatments that included the control diet, the low Ca and aP diet with phytase, the control diet without the TMP, and the low Ca and aP diet with phytase but without the TMP. Final BW was used as a covariate for the carcass data.

[b] Mineral, P = 0.03.

[c] Phytase, P = 0.01; phytase x minerals, P = 0.06.

[d] Calculated using the equation described by NPPC (1991), which uses a 5% estimation for intramuscular fat and compensates for unequal BW.

[e] Data taken from pigs using TOBEC equations (Higbie et al., 2002).

[f,g] Means with different superscripts differ, P = 0.10

Table 5.4. Effect of reducing calcium and available phosphorus, removing the trace mineral premix, and adding phytase in diets for growing-finishing pigs on pork quality [a]

Item Diets	Control	Low Ca and aP	Low Ca and aP+ PHY	No TMP	No TMP + PHY	SEM
Color	2.33	2.39	2.43	2.33	2.44	0.14
Marbling	1.72	1.63	1.56	1.42	1.69	0.16
CIE L*	52.99[f]	52.02[f,g]	50.45[g]	51.97	51.85	0.99
CIE a*	5.51	5.81	5.58	5.32	5.68	0.40
CIE b*	10.51	10.27	10.03	10.15	10.47	0.42
45-min temperature, °C [b,c]	37.54	37.66	37.64	36.74	38.00	0.34
45-min pH [b]	6.14	6.22	6.21	6.21	6.14	0.06
24-h temperature, °C [b]	0.97	1.11	0.99	1.10	1.10	0.04
24-h pH [b]	5.73	5.69	5.70	5.64	5.68	0.04
Fresh chop [d]						
Drip loss, %	3.64[f]	2.20[g]	2.79[f,g]	3.15	2.56	0.46
Cook loss, %	22.56	21.00	20.87	21.10	22.01	0.85
Total loss, %	26.19[f]	23.20[g]	23.65[f,g]	24.25	24.56	1.15
Shear force, kg	4.57	4.16	4.30	4.04	4.16	0.19
Frozen chop [e]						

(table continued)

Thaw loss, %	16.98	15.63	16.05	16.20	16.03	0.69
Cook loss, %	17.41	17.96	17.33	18.28	16.63	0.71
Total loss, %	34.39	33.60	33.38	34.48	32.66	1.08
Shear force, kg	3.17[f,g]	2.89[g]	3.22[f]	3.35	3.17	0.13

[a] Data are means of three replications of barrows and three replications of gilts with three pigs per replicate pen. TMP = trace mineral premix; PHY = phytase; aP = available P; Commission internationale de l'Eclairage = CIE. Treatment differences for the pigs fed the control diet, the low Ca and aP diet, or the low Ca and aP diet with phytase were separated by the PDIFF procedure. Contrast statements also were included to examine phytase, TMP, and phytase x TMP effects as a 2 x 2 factorial arrangement of treatments that included the control diet, the low Ca and aP diet with phytase, the control diet without the TMP, and the low Ca and aP diet with phytase but without the TMP.

[b] The pH and temperature measurements were taken in the longissimus muscle between the 10th- and 11th-ribs.

[c] Phytase, P = 0.07.

[d] Drip, cook, and total losses were determined on a fresh 10th rib chop. Shear force was determined approximately 24 h after cooking using a HD 250 (Texture Technologies Corporation, Scarsdale, NY) fitted with a Warner-Bratzler head.

[e] Thaw, cook and total losses were determined on a thawed 9th rib chop that had been frozen for 90 d. Shear force was determined approximately 24 h after cooking using a HD 250 (Texture Technologies Corporation, Scarsdale, NY) fitted with a Warner-Bratzler head.

[f,g] Means with different superscripts differ, P = 0.09.relative to those fed the control diet

Table 5.5. Effect of reducing calcium and available phosphorus, removing the trace mineral premix, and adding phytase in diets for growing-finishing pigs on mineral levels in the bone and muscle [a]

Item Diets	Control	Low Ca and aP	Low Ca and aP+ PHY	No TMP	No TMP + PHY	SEM
Bone						
Strength, kg[b]	172.2[f]	114.9[g]	160.5[f]	162.8	163.7	6.73
Ash, %	63.90[f]	61.73[g]	63.53[f]	63.28	63.35	0.25
Ca, %	25.7	24.8	24.3	25.7	24.6	0.8
P, %	11.7	11.1	11.1	11.7	11.2	0.4
Cu, ppm [c]	9.55	9.96	11.35	14.30	6.49	2.03
Fe, ppm	91.4	102.3	99.3	86.9	98.2	9.1
K, % [d]	0.124[f]	0.110[g]	0.114[f,g]	0.119	0.113	0.004
Mg, % [d]	0.43[f]	0.37[g]	0.39[f,g]	0.43	0.41	0.01
Mn, ppm	9.09[f]	7.86[g]	8.44[f,g]	8.35	7.90	0.42
Na, % [d]	0.78	0.74	0.73	0.78	0.73	0.02
Zn, ppm [c,e]	350.4[f]	326.1[f,g]	293.2[g]	227.3	267.9	26.1
Muscle						
Ca, %	0.020	0.019	0.021	0.019	0.019	0.001
P, %	0.89	0.88	0.88	0.88	0.88	0.01
Cu, ppm [d]	2.00[f]	2.04[f,g]	2.31[g]	1.78	2.15	0.13
Fe, ppm	21.7	20.4	21.6	20.8	20.4	0.7
Mn, ppm	0.41	0.40	0.40	0.44	0.40	0.01
Zn, ppm [e]	56.40	54.42	53.43	51.16	51.04	1.76

(table continued)

[a] Data are the means of three replications of barrows and three replications of gilts with three pigs per replicate pen. All values are on a DM basis. TMP = trace mineral premix; PHY = phytase; aP = available P. Treatment differences for the pigs fed the control diet, the low Ca and aP diet, or the low Ca and aP diet with phytase were separated by the PDIFF procedure. Contrast statements also were included to examine phytase, TMP, and phytase x TMP effects as a 2 x 2 factorial arrangement of treatments that included the control diet, the low Ca and aP diet with phytase, the control diet without the TMP, and the low Ca and aP diet with phytase but without the TMP.
[b] Bone strength was determined using a HD 250 Texture Machine (Texture Technologies Corporation, Scarsdale, NY) fitted with a three point bend rig with a load cell capacity of 250 kg and cross-head speed of 100 mm/min.
[c] Phytase x mineral, P = 0.05.
[d] Phytase, P = 0.09.
[e] Mineral, P = 0.08.
[f,g] Means with different superscripts differ, P = 0.10.

Table 5.6. Effect of reducing calcium and available phosphorus, removing the trace mineral premix, and adding phytase in diets for growing-finishing pigs on mineral levels in the bile and liver [a]

Item	Diets	Control	Low Ca and aP	Low Ca and aP+ PHY	No TMP	No TMP + PHY	SEM
Bile							
Ca, %		0.23	0.23	0.23	0.24	0.23	0.01
P, %		0.37	0.39	0.40	0.44	0.43	0.02
Cu, ppm [b]		16.88[e]	21.52[f]	14.87[e]	5.77	3.97	1.41
Fe, ppm [b,c]		16.41	18.04	16.36	15.90	13.63	1.22
Mn, ppm [b,c,d]		4.22[e]	6.29[f]	4.40[e]	9.24	6.34	0.53
Zn, ppm		61.90	50.05	54.27	53.55	57.16	10.18
Liver							
Ca, %		0.018	0.019	0.019	0.020	0.019	0.001
P, % [c]		1.35[e,f]	1.31[f]	1.38[e]	1.33	1.36	0.02

(table continued)

K, % [b]	0.75	0.74	0.76	0.77	0.78	0.01
Na, %	0.49[e]	0.53[f]	0.50[e,f]	0.50	0.47	0.02
Cu, ppm [c,d]	20.61	21.26	20.77	20.77	15.82	0.81
Fe, ppm	226.3	239.9	219.7	206.9	186.5	17.1
Mn, ppm [b,c,d]	8.72	9.50	9.04	12.41	10.38	0.41
Zn, ppm [b,c]	209.6[e]	246.2[f]	251.0[f]	119.9	145.1	15.0

[a] Data are the means of three replications of barrows and three replications of gilts with three pigs per replicate pen. All values are on a DM basis. TMP = trace mineral premix; PHY = phytase; aP = available P. Treatment differences for the pigs fed the control diet, the low Ca and aP diet, or the low Ca and aP diet with phytase were separated by the PDIFF procedure. Contrast statements also were included to examine phytase, TMP, and phytase x TMP effects as a 2 x 2 factorial arrangement of treatments that included the control diet, the low Ca and aP diet with phytase, the control diet without the TMP, and the low Ca and aP diet with phytase but without the TMP.
[b] Mineral, P = 0.08.
[c] Phytase, P = 0.09.
[d] Phytase x mineral, P = 0.05.
[e,f] Means with different superscripts differ, P = 0.10.

Low Ca and aP with or without Phytase. Bone strength and ash percentage were decreased in pigs fed the low Ca and aP diet, but adding phytase reversed the response (P = 0.10; Table 5.5). Copper and Mn levels in the bile and Na and Zn levels in the liver were increased in pigs fed the low Ca and aP diet (Table 5.6). Adding phytase reversed these responses in Cu and Mn levels in the bile (P = 0.10). Copper level in the muscle and Zn level in the liver were increased (P = 0.10) and Zn level in the bone was decreased (P = 0.10) in pigs fed the diet with phytase added to the low Ca and aP diet relative to those fed the control diet. Phosphorus level in the liver was increased (P = 0.10) in pigs fed the diet with phytase added to the low Ca and aP diet relative to those fed the low Ca and aP diet. Potassium, Mg, and Mn levels in the bone were decreased (P = 0.10) in pigs fed the low Ca and aP diet relative to pigs fed the control diet.

Phytase x Trace Mineral. Removing the TMP in the diet decreased (P = 0.08) Cu and Fe levels in the bile, Zn level in the muscle and liver but increased (P = 0.09) Mn levels in the bile and liver and K level in the liver. Adding phytase to the diets decreased (P = 0.09) Fe level in the bile and Na, Mg, and K levels in the bone, but increased (P = 0.09) Cu level in the muscle and P and Zn levels in the liver. Manganese level in the bile and liver was increased when the TMP was removed from the diet and the addition of phytase partially reversed the response (phytase x mineral, P = 0.05). Copper levels in the bone and liver were decreased by phytase addition to the diet with the TMP removed (phytase x mineral, P = 0.05). Zinc level in the bone was decreased in pigs fed the diet without the TMP but the effect was much greater in pigs not fed phytase. (phytase x mineral, P = 0.05).

Discussion

Low Ca and aP with or without Phytase

Harper et al. (1997), O'Quinn et al. (1997), and Rienstra et al. (2001) reported that dietary phytase addition overcame the negative effects of reduced levels of dietary Ca and P on growth performance of pigs. Similarly, Kornegay and Qian (1996), Harper et al. (1997), and O'Quinn et al. (1997) reported a similar effect of phytase in reduced Ca and P diets on bone strength and bone ash percentage. Our data agree with these reports.

The effect of phytase in reduced Ca and P diets on carcass traits has been inconsistent. O'Quinn et al. (1997) reported a decreased dressing percentage when 300 phytase units/kg of diet were added to the diets of swine but not when 0 or 500 phytase units/kg of diet were added (the Ca and P was reduced by 0.08% in diets with phytase). Rienstra et al. (2001) indicated that pigs fed phytase had an increased LMA, and Harper et al. (1997) indicated that adding phytase to swine diets had no effect on carcass length, backfat thickness, or LMA. Our data indicate that reduced Ca and P diets have negative effects on carcass traits of pigs, but the addition of phytase reversed these responses.

Gebert et al. (1999c) reported a paler longissimus dorsi when pigs were fed phytase, which the authors attributed to an increase in the availability of trace minerals, mainly Fe and Cu. Also, Berg (2001) indicated that supranutritional levels of Cu may result in less desirable (paler) pork color. However, in our study, Cu level in the muscle was increased when phytase was added to the diet, but the chops were darker (CIE L* was decreased). Gebert et al. (1999c) reported that adding phytase to pig diets resulted in a decreased 45-min pH, but we observed no effect of phytase on 45-min pH. Harper et al. (1997) indicated that adding phytase to pig diets had no effect on firmness or marbling, which agrees with our data. However, Rienstra et al. (2001) reported that pigs fed phytase had a decreased marbling and drip loss. In our study, there were numerical decreases in drip loss, thaw loss, and cook loss (fresh and frozen chops) in pigs fed diets with phytase and the diet with reduced levels of Ca and P. The effect of phytase on moisture loss may be due to the reduction in monocalcium phosphate in the diet, which has been shown to decrease cooking loss (Mavromichalis et al., 1999).

Phytase x Trace Mineral

Research has shown that removing the TMP only during the finishing period does not affect growth performance of swine (Kim et al., 1997; Mavromichalis et al., 1999). However, weight ranges for these studies were 70 to 112 kg and 86 to 116 kg, respectively, while in our study, the weight range was 22 to 109 kg. Other research has shown that removing the vitamin premix and TMP had no effect on growth performance in swine (Edmonds and Arentson, 2001; Shaw et al., 2002) or poultry (Skinner et al., 1992; Deyhim and Teeter, 1993; Christmas et al., 1995). Research could not be found in the literature on removing the TMP at the start of the growing period (22 to 109 kg). However, Spears et al. (2001) indicated that removing the Zn and Cu for 22 to 91-kg pigs had no negative effect on growth performance. Even though we observed no adverse effect of removing the TMP on

growth performance, on commercial farms there are many stressors that can increase the requirement for TMP including temperature, stocking density, and degree of contamination (Cunha, 1977; Stahly et al., 1997).

Stahl et al. (1999) reported that phytase overcame the reduced hematocrit percentage that resulted from Fe deficient diets. In our study, hematocrit percentage was not affected when pigs were fed diets with or without the TMP or with or without phytase. This lack of response could be due to the high Fe levels in the diets even with the removal of the TMP. Johnston et al. (2004) and Shelton et al. (2003b) reported an increase in fasting glucose levels when phytase was added to diets for growing pigs, but we observed no effect of phytase on fasting glucose. The increase in fasting glucose with the removal of the TMP from the diet that we observed may be due to an interaction of one or more of the trace minerals that were removed with another mineral such as Cr, which has been shown to affect glucose metabolism (Matthews et al., 2001b)

Kim et al. (1997) and Mavromichalis et al. (1999) reported no effect on carcass traits when the TMP was removed from diets for finishing pigs. Also, Skinner et al. (1992) indicated that removing the TMP from poultry diets from 42 to 49 d had no effect on dressing or abdominal fat percentage. Deyhim and Teeter (1993) indicated that removing the TMP from poultry diets from 28 to 49 d had no effect on dressing percentage and breast and fat pad weight as a percentage of carcass weight. The negative effects (increased tenth rib backfat and decreased carcass length and ham weight) seen in our study could be due to the length of time the TMP was removed from the diets, which was much longer than in previous research. Adding phytase did not reverse the negative effects in carcass traits observed with the removal of the TMP.

Shelton et al. (2003b) reported a decreased liver weight in growing pigs fed diets with phytase at 3.2 x maintenance level of growth, but TMP were included in the diet in that

study. Our results do not agree with Deyhim and Teeter (1993) who indicated that liver weight as a percentage of carcass weight was not affected when chicks were fed diets without the TMP from 28-49 d. In our study, reducing the Ca and P or removing the TMP resulted in an increase in the liver weight of pigs, but phytase reversed the response. This response indicates that the increase in liver weight may result from a reduction in dietary mineral (macro or micro) levels.

Kim et al. (1997) and Mavromichalis et al. (1999) reported that removing the TMP in the diets of growing-finishing pigs had no effect on pork quality. Tian et al. (2001) reported no effect on pork quality when finishing pigs were fed diets with 50% of the requirement for Zn, Fe, Mn, Cu, I, and Se. These data agree with our study. Even though in our study there were no negative effects on pork quality when the TMP was removed, its effect on human nutrition needs to be determined because reductions in tissue trace minerals may potentially have negative effects on human health.

Edmonds and Arentson (2001) and Shaw et al. (2002) indicated that removing the TMP had no effect on Cu, Fe, or Zn levels in the longissiumus muscle. In both studies, the vitamin premix was deleted and in the study by Shaw et al. (2002), two-thirds of the dicalcium phosphate was deleted. Furthermore, in these studies, the treatment diets were only fed during the finishing phase, whereas in our study, the treatment diets were fed during the growing and finishing phases. These data do not agree with our data, which indicated that removing the TMP had variable effects on tissue mineral concentration, which may have been caused by the length of time of feeding in our study.

Phytate has the potential to bind with some trace minerals, such as Zn, Cu, Mn, Mo, Co, Mg, and Fe; thus, reducing their availability (Vohra et al., 1965; Erdman, 1979; Ravindran et al., 1995), thus trace mineral levels may be able to be reduced in diets with phytase addition. Our data agree with data of Adeola et al. (1995) who indicated that Zn,

Cu, and P absorption and retention were increased when phytase was added to the diet of pigs, but Mg absorption and retention were not affected by phytase supplementation. Also, Stahl et al. (1999) reported that phytase addition to the diet of young pigs increased Fe availability, but in our study, Fe level in the bile was decreased and Fe levels in other tissues were not affected.

The effect of phytase supplementation to diets with and without the TMP on Mn indicate there may be an interaction between Zn and Mn, because when Zn level was decreased by removing the TMP, Mn level in the liver was increased, but when Zn levels were increased by adding phytase to the diet, Mn level decreased. The same effect regarding Mn occurred in the bile but there was only a numerical increase in Zn from the phytase addition (53.6 ppm Zn with the TMP removed and 57.2 ppm Zn with phytase added to that diet). Adeola et al. (1995) indicated that supplementing pig diets with Zn decreased Mg and Mn absorption and retention. Mohanna and Nys (1999) found no improvement in Mn retention by microbial phytase supplementation in broiler chicks, but Windisch and Kirchgessner (1996) indicated that phytase addition increased Mn retention by 3.0% in pigs. The effects seen regarding the Cu levels may be explained by the interaction of Zn and Cu. It has been documented that increasing the Zn level in the diet will decrease the availability of dietary Cu for pigs (Blakeborough and Salter, 1987) and rats (Frimpong and Magee, 1989), and the levels of Zn were increased in bone and liver when phytase was added to the diet.

Removing the TMP in the diets of pigs during the growing-finishing periods resulted in no effect on growth performance or pork quality components measured but some negative effects on carcass traits. Furthermore, removing the TMP will decrease the amount of trace minerals in pork. The effects of reducing trace mineral concentrations in pork and its effect on human nutrition need to be further investigated.

CHAPTER 6

EFFECT OF MICORBIAL PHYTASE ADDITION WITH OR WITHOUT THE TRACE MINERAL PREMIX IN NURSERY, GROWING, AND FINISHING PIGS

Introduction

Research has been conducted on the effects of removing the trace mineral premix (TMP) in diets for swine; however, most of the research used growing or finishing pigs. Kim et al. (1997) and Mavromichalis et al. (1999) indicated that the TMP could be removed from the diet of finishing pigs with no negative effects on growth, carcass characteristics, or pork quality. Shelton et al. (2004b) reported similar results on growth performance and pork quality with growing-finishing pigs (22 to 109 kg), but there were some negative effects on carcass traits. However, we are not aware of research on removing the TMP in diets for nursery pigs.

Phytate forms insoluble salts with Fe, Zn, Mn, and Cu (Vohra et al., 1965). Phytase, an enzyme that breaks down phytate (Gibson and Ullah, 1990), has been shown to increase the absorption and retention of Zn and Cu (Lei et al., 1993; Adeola et al., 1995). Therefore, phytase may be able to replace the TMP in swine diets and result in equal growth performance relative to pigs fed diets with the TMP.

Thus, the objective of these experiments was to determine the effect of phytase with and without the TMP in diets for nursery, growing, and finishing pigs on growth performance, bone ash percentage and strength, and tissue mineral concentrations.

Materials and Methods

General

Methods used in these experiments related to animal care were approved by the Louisiana State University (LSU) Agricultural Center Animal Care and Use Committee.

Two experiments were conducted with Yorkshire x Landrace and Yorkshire x Duroc barrows and gilts to determine the effect of phytase with and without the TMP in diets for nursery, growing, and finishing pigs. During the nursery period, pigs were housed in total confinement in an environmentally controlled modular building in 0.97 × 1.47-m pens on hard plastic slotted floors and an under-floor flush system. During the growing period, pigs were housed in total confinement in 1.2 x 2.4-m pens with metal-slatted floors. During the finishing period, pigs were housed in total confinement in an open-sided finishing barn with 1.5 x 3.0-m pens with concrete-slatted floors. Pigs and their environment were monitored twice daily.

Natuphos 1200 (BASF Corporation, Mount Olive, NJ) when included in the diet was added at 0.033%, which provided 500 phytase units/kg of diet. Analysis of the Natuphos 1200 indicated an activity of 1,515 phytase units/kg of premix. The Ca and available P (aP) were reduced by 0.10% in all diets with phytase. Values for the AA and minerals for all ingredients were taken from NRC (1998). All diets and water were provided for ad libitum consumption and the feed was in mash form.

Experiment 1

Nursery period. Two hundred and eight barrows and gilts with an average initial BW of 5.5 kg were used in these experiments. They were weaned at an average age of 18 d, and the treatment diets were started on the day of weaning. The pigs were allotted to treatments on the basis of weight in randomized complete block designs. Ancestry was equalized within treatment as much as possible. There were three replications of gilts and five replications of barrows with six or seven pigs per replicate pen.

The four dietary treatments in a 2 x 2 factorial arrangement (Table 6.1) were: 1) corn-soybean meal (C-SBM) control diet, 2) C-SBM diet with 500 phytase units/kg of diet, 3)

C-SBM diet without the TMP, 4) C-SBM diet without the TMP and with 500 phytase units/kg of diet. Actual analysis of the diets with phytase indicated that Diet 2 provided 609 phytase units/kg of diet and Diet 4 provided 602 phytase units/kg of diet. The diets were formulated to contain 1.60, 1.40, and 1.20% total Lys; 0.90, 0.80, and 0.70% Ca; and 0.55, 0.40, and 0.32% aP for Phases I, II, and III, respectively. All other AA met or exceeded the ratio to Lys (NRC, 1998). The nursery phase consisted of a Phase I (7 d), Phase II (14 d), and Phase III (13 d) period.

Table 6.1. Composition of the control diets for the nursery and early-growing in Experiments 1 and 2 [a]

Item	Phase I[b]	Phase II[b]	Phase III[b]	Early-growing[c,d]
Ingredient				
Corn	36.78	46.85	63.01	73.63
Soybean meal, 48% CP	24.53	31.38	26.35	23.04
Whey	15.00	10.00	-	-
Lactose	5.00	-	-	-
AP920[e]	5.00	-	-	-
Fishmeal	6.00	5.00	5.00	-
Dry fat[f]	4.00	3.00	2.00	-
Monocalcium phosphate	0.64	0.57	0.52	0.83
Limestone	0.73	0.68	0.66	0.99
Salt	0.25	0.50	0.50	0.40
Sodium bentonite	0.50	0.50	0.50	0.50
Vitamin premix[g]	0.50	0.50	0.50	0.38

(table continued)

Trace mineral premix[h]	0.10	0.10	0.10	0.10
Lysine•HCl	-	-	-	0.10
Anitbiotic[i]	0.75	0.75	0.75	-
Flavor[j]	0.08	0.08	0.08	-
DL-methionine	0.06	0.01	-	-
Choline chloride	0.05	0.05	-	-
Rice hulls[k]	0.033	0.033	0.033	0.033
Calculated Composition				
ME, kcal/kg	3,286	3,389	3,372	3,297
CP, %	24.19	22.13	20.86	17.15
Lys, %	1.60	1.40	1.20	0.97
Sulfur AA, %	0.91	0.78	0.72	0.59
Trp, %	0.32	0.28	0.24	0.19
Thr, %	1.07	0.92	0.80	0.74
Ca, %	0.90	0.80	0.70	0.60
P, %	0.78	0.69	0.62	0.54
Available P, %	0.55	0.40	0.32	0.24
Zn, ppm	157.4	161.0	160.2	152.9
Cu, ppm	21.32	22.23	20.41	19.52
Fe, ppm	303.0	297.0	276.2	285.5
Mn, ppm	38.17	40.66	39.60	38.25
I, ppm	0.80	0.80	0.80	0.80

[a] During Phase I, II, and III, the diets were fed with and without the trace mineral premix, which was replaced by sand. During the growing-finishing period, the diet with phytase was

(table continued)

fed with and without the trace mineral premix, which was replaced by sand.
[b] The diets with phytase during Phases I, II, and III were the same as the control diets but contained: limestone, 0.65, 0.61, and 0.59%; monocalcium phosphate, 0.166, 0.09, and 0.05%, respectively. Limestone and monocalcium phosphate were replaced by sand in the diets with added phytase.
[c] The control diets during the late-growing, early-finishing, and late-finishing phases were similar to the early-growing diet but contained: corn, 77.00, 80.59, and 84.50%; soybean meal, 19.97, 16.53, and 12.72%; limestone, 0.92, 0.84, and 0.82%; monocalcium phosphate, 0.61, 0.53, and 0.46%, respectively.
[d] The diets with phytase during the late-growing, early-finishing, and late-finishing phases were the same as the control diet but contained: limestone, 0.84, 0.77, and 0.74%; monocalcium phosphate, 0.13, 0.06, and 0%, respectively. Limestone and monocalcium phosphate were replaced by sand in the diets with added phytase.
[e] AP920, American Protein Corporation, Ames, IA.
[f] Provided 99% crude fat, Fat Pak 100, Milk Specialties Co., Dundee, IL.
[g] Vitamin premix contained the following per kilogram of premix and was added at 0.50% for the nursery phases and 0.38% for the growing and finishing phases: vitamin A, 2,204,600 IU; vitamin D_3, 661,400 IU; vitamin E, 17,600 IU; menadione (as menadione pyrimidinol bisulfite complex), 1,660 mg; riboflavin, 2,600 mg; Ca d-pantothenic acid, 10,000 mg; niacin, 17,600 mg; vitamin B_{12}, 12,200 µg; d-biotin, 88,200 µg; folic acid, 660 mg; pyridoxine, 882 mg; thiamin, 882 mg; vitamin C, 22,000 mg.
[h] Trace mineral premix provided the following per kilogram of diet: Zn, 127 mg; Fe, 127 mg; Mn, 20 mg; Cu, 12.7 mg; I, 0.80 mg; Se, 0.30 mg, as zinc sulfate, ferrous sulfate, manganese sulfate, copper sulfate, calcium iodate, sodium selenite, respectively, with calcium carbonate as the carrier.
[i] Neo-Terra 10/10 contains oxytetracycline hydrochloride, and neomycin sulfate (Nutrablend Inc, Neosho, MO).
[j] Dried Strawberry, Feed Flavors, Inc., Wheeling, IL.
[k] Phytase replaced rice hulls in the diet to provide 500 phytase units/kg of diet.

At the end of Phase III, two pigs from each pen were randomly selected and the posterior section of the tails were removed using a stericut tail docker (Sharpvet; Cotran Corp., Portsmouth, RI) for the determination of bone mineral concentration. The tails were autoclaved at 121°C for 30 min to facilitate removal of muscle, skin, and connective tissue. The coccygeal bones were then dried at 110 °C for 24 h. A dry weight was determined and bones then were ashed at 500 °C for 24 h. Ash samples were solubilized with 20% nitric acid, heated, and then diluted to a known volume. Mineral content was determined by inductively coupled plasma emission spectroscopy (Model Optima 3000, Perkin Elmer, Norwalk, CT).

Growing-finishing period. At the end of Phase III, the 72 gilts were continued on the project. The gilts were re-allotted within treatment on the basis of weight in a randomized complete block design. There were four (treatments 3 and 4) or five (treatments 1 and 2) replications with four gilts per replicate pen. Their BW at the end of Phase III was 16.2 kg and the final BW was 111.6 kg. The dietary treatments were the same as in the nursery period except that pigs fed the diet without the TMP and without phytase during the nursery period were fed the control diet during the growing-finishing period. Skin lesions occurred on 26 of 52 pigs, and it was apparent that many of these pigs would have died without a diet change. A four-phase growing-finishing program was used and diets (Table 6.1) were formulated to provide 0.97, 0.88, 0.79, and 0.68% total Lys for weight ranges of 20 to 43, 43 to 66, 66 to 89, and 89 to 112 kg, respectively. All AA met a minimum of 105% of the AA requirement according to each growth phase for gilts gaining 325 grams of lean per day as calculated using the NRC model (NRC, 1998). The diets also were formulated to contain 0.60% Ca and 0.24% aP in the early-growing phase, 0.53% Ca and 0.19% aP in the late-growing phase, 0.48% Ca and 0.17% aP in the early-finishing phase, and 0.45% Ca and 0.15% aP in the late-finishing phase. The Ca and aP levels were reduced by 0.10% in the diets with added phytase. The aP level was only reduced to 0.053% during the late-finishing period. Actual analysis of the diets for phytase indicated that Diet 2 provided 575 phytase units/kg of diet and Diet 4 provided 470 phytase units/kg of diet.

On the day after the growth trial ended, two pigs per replicate pen were randomly selected and slaughtered by exsanguination after electrical stunning. At slaughter, liver and kidneys were weighed, sampled (approximately 20 g), and the samples were frozen at -20 °C until analysis. Also, samples of bile, pancreas, and longissimus muscle (between the 9th and 10th ribs) were taken and frozen at -20 °C until analysis. Samples of liver, kidney, pancreas, and muscle were analyzed for dry ash percentage and mineral content as

previously described for the coccygeal bones at the end of the nursery phase. Mineral content of bile was determined after drying in an oven at 100° C for 24-h and digesting in nitric acid and hydrogen peroxide. Mineral content of the liver, kidney, pancreas, and muscle samples was determined on the ash after dissolving in nitric acid and hydrogen peroxide as previously described. The third and fourth metacarpal bones from the left foot of each pig were removed and manually cleaned of adhering tissue. The third metacarpal was used to determine bone ash percentage and bone mineral content, as previously described (except the bones were ashed for 36 h), after being extracted of fat (Soxhlet, Labglass, Vineland, NJ) and redried. The fourth metacarpal was broken using a HD 250 Texture Machine (Texture Technologies Corporation, Scarsdale, NY) fitted with three point bend rig with a load cell capacity of 250 kg and cross-head speed of 100 mm/min and a span over which the bone was set of 1.5 cm.

Experiment 2

Nursery period. During the nursery period, Exp. 2 was conducted exactly as Exp. 1 with these exceptions. One hundred eight-five barrows and gilts with an average initial BW of 5.4 kg were used. The pigs were allotted to the same four dietary treatments as in Exp. 1, but had three replications of gilts and three replications of barrows with six or seven pigs per replicate pen. However, the diet without the TMP and without phytase had 12 replications (six gilt and six barrow) because at the end of the nursery period, six replicate pens were fed the diet with the TMP while the other six replicate pens were fed the diet without the TMP but with phytase. Actual analysis of the diets with phytase indicated that Diet 2 provided 646 phytase units/kg of diet and Diet 4 provided 631 phytase units/kg of diet. At the end of Phase III, tails were removed from two pigs per pen for determination of coccygeal bone mineral content as previously described.

Early-growing period. At the end of Phase III, pigs (initial and final BW of 16 and 22 kg, respectively) were moved to a growing facility and fed until growth performance of pigs fed the diets without the TMP was similar to the growth of pigs fed the positive control diet. Three replications of gilts and three replications of barrows fed the diet without the TMP and without phytase for the nursery period were fed the control diet for the early-growing period. The other three replications of gilts and three replications of barrows fed the diet without the TMP and without phytase for the nursery period were fed the diet without the TMP but with phytase for the early-growing period. The diets (Table 6.1) were formulated as previously described for the early-growing period in Exp. 1.

Statistical Analysis

Data were analyzed by analysis of variance procedures appropriate for a randomized complete block design using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). For the nursery data in Exp. 1 and 2, the statistical model included treatment, replication, and sex. There were no treatment x sex interactions so it was removed from the model. Orthogonal contrasts appropriate for a 2 x 2 factorial arrangement of treatments were used to determine treatment effects. For the growing-finishing data in Exp. 1 and the early-growing data in Exp. 2, the statistical model included treatment and replication and treatments were compared with the PDIFF option in SAS. Treatment differences were considered significant at alpha = 0.10. The pen of pigs served as the experimental unit for all data.

Results

Experiment 1

Nursery period. Daily gain and ADFI were decreased (P = 0.05) during Phases II, III, and overall in pigs fed the diets without the TMP (Table 6.2). Daily gain during Phase III and overall and ADFI during Phase II, III, and overall were increased (P = 0.02) in pigs fed

diets with phytase. Daily gain during Phase III and overall and ADFI during Phases II, III, and overall were decreased in pigs fed the diet without the TMP, but phytase reversed these responses (phytase x TMP, P = 0.02). Gain:feed was not affected by diet. Skin lesions occurred on 26 of 52 pigs fed the diets without the TMP and without phytase.

Table 6.2. Effect of phytase addition with or without the trace mineral premix on growth performance during the nursery period in Experiment 1[a]

Item	Control	+ Phytase	No TMP	No TMP + phytase	SEM
Phase I (0 to 7 d)					
ADG, g	122	141	141	150	15
ADFI, g	177	195	211	211	15
Gain:feed ratio, g/kg	665	725	667	692	53
Phase II (8 to 21 d)					
ADG, g [b]	345	342	292	323	11
ADFI, g [b,c,d]	538	539	462	546	17
Gain:feed ratio, g/kg	641	635	626	603	27
Phase III (21 to 34 d)					
ADG, g [b,c,d]	457	440	339	468	20
ADFI, g [b,c,d]	812	819	609	819	30
Gain:feed ratio, g/kg	566	540	551	573	15
Overall (0 to 34 d)					
ADG, g [b,c,d]	342	338	277	350	11
ADFI, g [b,c,d]	566	575	465	575	15
Gain:feed ratio, g/kg	605	588	591	608	13

(table continued)

[a] Data are means of eight replications of six or seven pigs per replicate pen. Average initial and final BW were 5.5 and 16.2 kg, respectively. Average daily feed intake and gain:feed ratio are on an as-fed basis. TMP = trace mineal premix.
[b] TMP, P = 0.05.
[c] Phytase, P = 0.02.
[d] Phytase x TMP, P = 0.02.

Unaffected coccygeal bone mineral concentrations (means ± pooled SEM, DM basis) represent 32 replications of two pigs per replicate pen (not shown in Table 6.3): K, 0.66 ± 0.06%; Mg, 0.68 ± 0.03%. The Zn and Na levels in the coccygeal bones were decreased (P = 0.09) in pigs fed the diets without the TMP (Table 6.3). The Fe and Zn levels were increased (P = 0.03) and ash percentage was decreased (P = 0.03) in the coccygeal bones of pigs fed the diets with phytase. Coccygeal bone ash percentage was not affected in pigs fed the diet with the TMP and phytase, but it was decreased in the bones of pigs fed the diet without the TMP and with phytase (phytase x TMP, P = 0.10).

Growing-finishing period. There was no effect on overall growth performance in pigs fed any of the dietary treatments (Table 6.4).

Table 6.3. Effect of phytase addition with or without the trace mineral premix on ash percentage and mineral concentrations in the coccygeal bones of nursery pigs in Experiment 1 [a]

Item	Control	+ Phytase	No TMP	No TMP + phytase	SEM
Exp. 1					
Ash, % [b,c]	37.87	37.23	39.15	36.50	0.53
Ca, %	35.39	35.25	36.04	35.42	0.33
P, %	23.37	22.75	21.65	22.27	0.98
Na, % [d]	1.88	1.84	1.73	1.77	0.06
Cu, ppm	1.40	1.41	1.48	1.53	0.26

(table continued)

Mn, ppm	2.04	2.60	1.79	2.12	0.35
Fe, ppm [b]	111.65	123.78	94.04	117.07	7.57
Zn, ppm [b,d]	250.51	274.86	100.28	152.98	11.38
Exp. 2					
Ca, % [c]	35.02	36.40	36.25	36.23	0.38
P, %	16.82	17.00	17.14	17.02	0.14
Cu, ppm [d]	3.28	3.99	2.75	2.81	0.36
Mn, ppm [c]	2.72	4.34	4.07	3.30	0.36
Fe, ppm	109.1	124.8	117.7	111.1	8.6
Zn, ppm [b,d]	308.67	382.47	191.12	270.36	20.48

[a] Data are the means of eight (Exp. 1) or six (Exp. 2) replications of two pigs per replicate pen. In Exp. 2, the treatment without the TMP and without phytase had 16 replications. Ash percentage and mineral concentrations are based on a DM basis. TMP = trace mineral premix.
[b] Phytase, P = 0.03.
[c] Phytase x TMP, P = 0.10.
[d] TMP, P = 0.09.

Table 6.4. Effect of phytase addition and previous trace mineral consumption on growth performance during the growing-finishing period in Experiment 1 [a]

	Nursery phase diets				
				No TMP	
	Control	+ Phytase	No TMP	+ Phytase	
	Grower phase diets				
				No trace minerals	
Item	Control	+ Phytase	Control	+ Phytase	SEM
Final BW	112.6	110.3	114.0	111.3	2.7
ADG, kg	0.74	0.73	0.75	0.71	0.02
ADFI, kg	2.43	2.20	2.31	2.21	0.22
Gain:feed ratio, kg:kg	0.33	0.33	0.32	0.32	0.02

(table continued)

[a] Data are means of four (without the TMP/control and without the TMP and with phytase treatments) or five (control and with phytase treatments) replications of four pigs per replicate pen. Initial and final BW were 16.2 and 111.6 kg. Average daily feed intake and gain:feed ratio are on an as-fed basis. There was no effect of treatment, $P \geq 0.10$. TMP = trace mineral premix.

The effects of diet on tissue ash percentage and mineral concentrations are presented in Table 6.5. Many tissue mineral concentrations were not affected by diet (not shown in Tables 6.5 and 6.6). Unaffected tissue mineral concentrations (means $\pm$ pooled SEM, DM basis) represent 18 replications of two pigs per replicate pen. Bile: Na, 4.46 $\pm$ 0.14%. Muscle: ash, 4.72 $\pm$ 0.12%; K, 0.99 $\pm$ 0.04%; Mg, 0.085 $\pm$ 0.002%. Kidney: weight, 366.8 $\pm$ 21.0 g; ash, 5.37 $\pm$ 0.25%; K, 0.75 $\pm$ 0.04%; Mg, 0.067 $\pm$ 0.001%; Na, 1.05 $\pm$ 0.04%. Pancreas: ash, 5.46 $\pm$ 0.41%; K, 0.69 $\pm$ 0.07%; Mg, 0.067 $\pm$ 0.003%. Liver: weight 1,716 $\pm$ 115 g; Na, 0.78 $\pm$ 0.05%. Bone ash percentage, Ca, P, Mg, and Na levels in the bone, Na levels in the loin muscle and pancreas, and K and Mg levels in the liver were decreased ($P = 0.10$); while Ca level in the kidney and pancreas and Zn level in the pancreas were increased ($P = 0.10$) in pigs fed the diet with the TMP and with phytase relative to those fed the control diet. Potassium level in the bone, Na levels in the loin muscle and pancreas, and Cu level in the pancreas were decreased ($P = 0.10$), and Cu levels in the bile and kidney and Ca level in the kidney were increased ($P = 0.10$) in pigs fed the diet without the TMP and phytase for the nursery period and the control diet for the growing-finishing period relative to those fed the control diet. Bone strength, Ca, P, K, Mg, and Na levels in the bone, Cu levels in the bile, loin muscle, and pancreas, and Na level in the pancreas were decreased ($P = 0.10$) and Mn levels in the bile and bone, Ca level in the

kidney, and liver ash percentage were increased (P = 0.10) in pigs fed the diet without the TMP but with phytase relative to those fed the control diet.

Table 6.5. Effect of phytase addition and previous trace mineral consumption on bile, kidney, pancreas, and liver mineral concentrations in Experiment 1 [a]

	Nursery phase diets				
				No TMP	
	Control	+ Phytase	No TMP	+ Phytase	
	Grower phase diets				
				No TMP	
Item	Control	+ Phytase	Control	+ Phytase	SEM
Bile					
Ca, %	0.31	0.28	0.28	0.31	0.03
P, %	0.57	0.52	0.51	0.54	0.02
K, %	0.411[b,c]	0.432[b]	0.402[c]	0.410[b,c]	0.010
Mg, %	0.049[b,c]	0.052[b]	0.047[c]	0.052[b,c]	0.002
Cu, ppm	8.01[b]	10.82[b,c]	13.58[c]	2.88[d]	1.22
Zn, ppm	86.4	77.0	97.9	129.4	11.5
Fe, ppm	19.57[b,c]	18.25[b,c]	14.97[c]	20.68[b]	1.77
Mn, ppm	8.76[b]	9.12[b]	6.18[b]	12.97[c]	1.37
Kidney					
Ca, %	0.057[b]	0.067[c]	0.066[c]	0.069[c]	0.003
P, %	1.00[b,c]	0.98[b]	1.02[b,c]	1.03[c]	0.02
Cu, ppm	13.24[b,d]	15.07[b,c]	17.42[c]	11.12[d]	1.25

(table continued)

Zn, ppm	98.6	108.4	125.5	118.3	4.9
Fe, ppm	221.2	227.0	208.7	206.0	22.4
Mn, ppm	5.62	5.66	5.42	5.84	0.26
Pancreas					
Ca, %	0.058[b]	0.070[c]	0.064[b,c]	0.054[b]	0.004
P, %	1.10	1.05	1.06	1.03	0.07
Na, %	0.71[b]	0.57[c]	0.52[c]	0.52[c]	0.04
Zn, ppm	111.9[b]	161.5[c]	123.7[b]	98.8[b]	13.9
Cu, ppm	4.26[b]	3.91[b,c]	3.70[c]	3.46[c]	0.19
Fe, ppm	66.4	61.6	57.3	60.5	3.3
Mn, ppm	6.51	6.39	5.81	6.37	0.77
Liver					
Ash, %	5.72[c]	5.57[c]	5.39[c]	6.11[d]	0.15
Ca, %	0.038	0.038	0.038	0.043	0.003
P, %	1.13[b,c]	1.02[c]	1.05[c]	1.22[b]	0.06
K, %	0.81[b]	0.69[c]	0.77[b,c]	0.82[b]	0.05
Mg, %	0.061[b,d]	0.054[c]	0.058[b,c]	0.067[d]	0.003
Cu, ppm	19.6	19.0	17.5	17.9	2.6
Zn, ppm	187.6	183.6	191.8	180.8	16.5
Fe, ppm	486.4	469.8	499.8	441.9	55.4
Mn, ppm	11.8	9.3	9.7	11.8	1.1

[a] Data are the means of four (no trace mineral/control and no trace mineral + phytase treatments) or five (control and + phytase treatments) replications of two pigs per replicate pen. Ash percentage and mineral concentrations are based on a DM basis. TMP = trace mineral premix.

[b,c,d] Means within a row without a common superscript differ, P = 0.10.

Table 6.6. Effect of phytase addition and previous trace mineral consumption on bone and loin muscle mineral concentrations in Experiment 1 [a]

	Nursery phase diets				
	Control	+ Phytase	No TMP	No TMP + Phytase	
	Grower phase diets				
Item	Control	+ Phytase	Control	No TMP + Phytase	SEM
Bone					
Strength, kg	151.2[c,d]	135.7[c,e]	165.1[d]	118.1[e]	9.6
Ash, %	62.75[c,e]	61.45[d]	63.21[e]	61.92[c,d]	0.48
Ca, %	26.39[c]	25.72[d]	26.98[c]	25.71[d]	0.26
P, %	10.70[c]	10.36[d]	10.79[c]	10.35[d]	0.09
K, %	0.080[c]	0.072[c,d]	0.069[d]	0.066[d]	0.004
Mg, %	0.40[c]	0.38[d]	0.40[c]	0.38[d]	0.01
Na, %	0.68[c]	0.66[d]	0.70[c]	0.65[d]	0.01
Zn, ppm	301.7	301.4	275.8	257.1	46.0
Cu, ppm	9.15	8.89	8.31	8.27	1.31
Fe, ppm	82.3	65.2	89.7	87.2	9.9
Mn, ppm	0.28[c]	0.34[c,d]	0.28[c]	0.41[d]	0.04
Loin muscle					
Ca, %	0.046[c,d]	0.040[c,d]	0.050[d]	0.038[c]	0.004
P, %	0.72	0.73	0.70	0.73	0.02
Na, %	0.50[c]	0.43[d]	0.40[d]	0.45[c,d]	0.02

(table continued)

Zn, ppm	56.9	63.0	62.4	57.0	5.1
Cu, ppm	1.56[c]	1.47[c,d]	1.22[c,d]	1.14[d]	0.14
Fe, ppm	18.63	17.23	16.48	16.72	1.0
Mn, ppm	0.49	0.43	0.49	0.45	0.08

[a] Data are the means of four (no trace mineral/control and no trace mineral + phytase treatments) or five (control and + phytase treatments) replications of two pigs per replicate pen. Ash percentage and mineral concentrations are based on a DM basis. TMP = trace mineral premix.
[b] Bone strength was measured using a HD 250 Texture Machine (Texture Technologies Corporation, Scarsdale, NY) fitted with a three point bend rig and a load cell capacity of 250 kg and cross-head speed of 100 mm/min.
[c,d,e] Means within a row without a common superscript differ, P = 0.10.

Experiment 2

Nursery period. Daily gain during Phase II and overall and ADFI during Phases II, III, and overall were decreased and gain:feed during Phase III was increased (P = 0.10) in pigs fed the diet without the TMP (Table 6.7). Daily gain during Phases I and II and gain:feed during Phase II were decreased in pigs fed the diet without the TMP, but phytase reversed these responses (phytase x TMP, P = 0.10).

Table 6.7. Effect of phytase addition with or without the trace mineral premix on growth performance during the nursery period in Experiment 2 [a]

Item	Control	+ Phytase	No TMP	No TMP + phytase	SEM
Phase I (0 to 7 d)					
ADG, g [b]	130	129	116	149	9
ADFI, g	212	218	201	231	12
Gain:feed ratio, g/kg	617	587	557	658	32
Phase II (8 to 21 d)					

(table continued)

ADG, g [b,c]	322	326	256	301	13
ADFI, g [c]	543	553	478	526	18
Gain:feed ratio, g/kg [b]	593	568	537	573	16
Phase III (21 to 34 d)					
ADG, g	472	462	462	464	14
ADFI, g [c]	889	872	806	816	24
Gain:feed ratio, g/kg [c]	533	532	573	568	15
Overall (0 to 34 d)					
ADG, g [c]	340	333	306	332	10
ADFI, g [c]	599	598	545	576	14
Gain:feed ratio, g/kg	568	558	564	575	11

[a] Data are means of eight replications (the treatment without the trace mineral premix and without phytase had 16 replications) of six or seven pigs per replicate pen. Average initial and final BW were 5.4 and 16.3 kg, respectively. Average daily feed intake and gain:feed ratio are on an as-fed basis. TMP = trace mineral premix.
[b] Phytase x TMP, P = 0.10.
[c] TMP, P = 0.10.

Unaffected coccygeal bone mineral concentrations (means ± pooled SEM, DM basis) represent 30 replications of two pigs per replicate pen (not shown in Table 6.3): Ash, 39.2 ± 0.7%; K, 0.44 ± 0.04%; Mg, 0.72 ± 0.01%; Na, 2.14 ± 0.05 ppm. Phytase addition and removing the TMP increased Ca concentration in the coccygeal bones, but the effect was not additive (phytase x TMP, P = 0.10). The Cu and Zn levels in the coccygeal bones were decreased (P = 0.09) in pigs fed the diets without the TMP. The Zn level in the coccygeal bones was increased (P = 0.03) in pigs fed the diets with added phytase. Adding phytase increased the Mn level in the coccygeal bones of pigs fed the diet with the TMP but

decreased the Mn level in the coccygeal bones of pigs fed the diet without the TMP (phytase x TMP, P = 0.10).

Early-growing period. During the early-growing period, ADG and gain:feed were decreased (P = 0.07) in pigs fed the diet without the TMP and phytase relative to pigs fed the diet without the TMP for the nursery period and that diet with phytase added during the early-growing period (Table 6.8). Gain:feed was decreased (P = 0.07) in pigs fed the diet with the TMP and phytase during the nursery and early-growing periods relative to pigs fed the diet without the TMP for the nursery period and that diet with added phytase for the early-growing period.

Table 6.8. Effect of phytase addition and previous trace mineral consumption on growth performance during the early-growing phase in Experiment 2 [a]

	Nursery phase diets					
	Control	+ Phytase	No TMP	No TMP	No TMP + Phytase	
	Early-growing phase diets					
Item	Control	+ Phytase	Control	No TMP + Phytase	No TMP + Phytase	SEM
ADG, g	0.46[b,c]	0.44[b,c]	0.46[b,c]	0.49[b]	0.42[c]	0.02
ADFI, g	1.09	1.06	1.06	1.05	1.10	0.03
Gain:feed, g/kg	0.42[b,c]	0.41[c]	0.43[b,c]	0.48[b]	0.39[c]	0.03

[a] Data are means of eight replications (the treatment without the TMP and without phytase had 16 replications) of six or seven pigs per replicate pen. Average initial ad final BW were 16 and 22 kg. Average daily feed intake and gain:feed ratio are based on an as-fed basis. TMP = trace mineral premix.
[b,c,] Means within a row without a common superscript differ, P = 0.07.

Discussion

We are not aware of data on removing the TMP from the diets of nursery pigs; however, studies have shown that removing the TMP from the diets for finishing or growing-finishing pigs results in no effect on growth performance (Kim et al., 1997; Mavromichalis et al., 1999, Shelton et al., 2004b), but in our study removing the TMP reduced growth performance during the nursery period and adding phytase reversed this response. In Exp. 1, during the growing-finishing period, overall growth performance of gilts was not affected by diet indicating that phytase may be able to replace the TMP for pigs for the entire growth period.

In our experiments, 26 out of 52 (Exp. 1) and 4 out of 37 (Exp. 2) pigs that were fed the diet without the TMP and without phytase developed skin lesions starting at the end of Phase II. Feeding pigs diets deficient in Zn can lead to parakeratosis (Luecke, 1984). Skin problems did not develop on pigs fed the diet without the TMP and with phytase, indicating that phytase released enough Zn to overcome signs of parakeratosis. However, in Exp. 2 the skin problems that developed in Phase II were not evident by the end of Phase III and growth performance during Phase III was not affected by diet, indicating that pigs in Exp. 2 overcame the mineral deficiency during Phase III. This response could have been due to a slightly increased ADFI for the pigs in Exp. 2 (465 and 545 g/d in pigs fed the diet without the TMP for Exp. 1 and 2, respectively).

Shelton et al. (2004b) reported that adding phytase or removing the TMP during the growing-finishing period had no effect on bone strength or bone ash percentage relative to pigs fed a control diet, but in the current study, bone strength was decreased in gilts fed the diet without the TMP and with phytase. In the present study, phytase activity in the diet without the TMP and with phytase was below the 500 phytase units/kg of diet (actual analysis indicated that the activity was 370 and 306 phytase units/kg of diet for the early-

and late-finishing periods, respectively) needed to release the 0.10% Ca and aP. This decreased activity may have caused the decreased bone strength, and the bone Ca and P concentrations were decreased in these pigs relative to those fed the control diet.

Shelton et al. (2004b) reported an increase in liver weight in growing-finishing pigs fed diets without the TMP or with reduced Ca and P, but adding phytase reduced liver weight to that of the pigs fed the control diet. In our study, liver weight was numerically increased in pigs fed the diet without the TMP and without phytase for the nursery period followed by the control diet for the growing-finishing periods (1,828 and 1,700 g for pigs fed the diet without the TMP for the nursery period and the control diet for the growing-finishing period relative to those fed the diet without the TMP and with phytase for the nursery and growing-finishing periods, respectively). This response indicates that reducing the trace minerals in the diet may result in an increase in liver weight.

Kornegay and Qian (1996), O'Quinn et al. (1997), and Shelton et al. (2004b) reported that adding phytase (300 to 1,400 phytase units) to low Ca and P diets increased bone ash percentage equal to that of a control diet. Shelton et al. (2004b) also reported that adding phytase to diets without the TMP had no effect on bone ash percentage. These data agree with our study.

The authors have no explanation for the increase in coccygeal bone ash percentage in pigs fed the diet without the TMP and without phytase (Exp. 1), but the Ca level in the coccygeal bones also was increased in pigs fed diets without the TMP and without phytase. Adding phytase reversed these responses. These responses were not seen in Exp. 2.

Feeding diets with added phytase or without the TMP to nursery pigs had variable effects on mineral content in the coccygeal bones. In both experiments, feeding diets without the TMP resulted in a decrease in Zn level in the coccygeal bones, and adding phytase reversed this response, which agrees with previous research that indicated that

phytase increases the availability of Zn in diets for pigs (Lei et al., 1993; Adeola et al., 1995; Spears et al., 2001). In Exp. 1, Fe level in the coccygeal bones was increased when phytase was added to the diet, but in Exp. 2, Fe level in the coccygeal bones was not affected by phytase addition, and tissue Fe concentrations were not affected in growing-finishing gilts fed the diet without the TMP and with phytase for the growing-finishing period. Stahl et al. (1999) reported that phytase addition increased Fe availability in young pigs. The differences in Fe concentrations between our experiments in the coccygeal bones of nursery pigs may be explained by the slight increase in ADFI in Exp. 2. The Cu levels in the coccygeal bones were decreased in pigs fed the diet without the TMP in Exp. 2 but not affected in Exp. 1. Adding phytase had no effect on Cu level in the coccygeal bones, which disagrees with Adeola et al. (1995) who reported that the addition of phytase increased Cu absorption and retention. In Exp. 2, Mn level in the coccygeal bones was increased when phytase was added to the control diet. Removing the TMP from the diet increased the bone Mn level relative to the control diet and adding phytase decreased bone Mn level. These responses indicate that there may be a Zn by Mn interaction, as discussed by Shelton et al. (2004b). However, this response was not seen in Exp. 1.

Tissue Zn concentrations were not affected in gilts fed the diet without the TMP and with phytase for the growing-finishing period. Removing the TMP and adding phytase to the diets of growing-finishing gilts resulted in a decrease in Cu level in the bile and muscle relative to those fed the control diet. Shelton et al. (2004b) reported a decrease in Cu level in the bile and liver and numerical decreases in the Cu level in the bones of pigs fed a diet without the TMP and with phytase relative to a control diet for the growing-finishing periods. Edmonds and Arentson (2001) and Shaw et al. (2002) indicated that removing the vitamin and TMP during the finishing period had no effect on Cu, Fe, or Zn concentrations in the longissiumus muscle.

Removing the TMP and adding phytase to the diets of growing-finishing gilts also resulted in a decrease in Ca, P, Na, Mg, and K levels in the bone, and Na level in the pancreas and an increase in Mn levels in the bile and bone relative to those fed the control diet. As mentioned earlier, the phytase activity was lower in the diet without the TMP and with phytase that was fed for the early- and late-finishing periods, which may have caused the decrease in the Ca and P concentrations in the bone. Shelton et al. (2004b) reported a decrease in the Na, Mg, and K concentrations in the bone of pigs fed a diet without the TMP and with phytase for the growing-finishing periods.

These data indicate that removing the TMP in the diets for nursery pigs results in negative growth performance. Adding phytase to the diets without the TMP resulted in growth performance equal to that of pigs fed the control diet. Adding phytase and(or) removing the TMP had variable effects on tissue mineral concentrations.

CHAPTER 7

EFFECT OF PHYTASE ADDITION WITH OR WITHOUT THE TRACE MINERAL PREMIX ON GROWTH PERFORMANCE AND TISSUE MINERAL CONCENTRATION IN COMMERCIAL BROILERS

Introduction

Research has been conducted on the effects of removing the trace mineral premix (TMP) in diets for swine. Kim et al. (1997), Mavromichalis et al. (1999), and Shelton et al. (2004b) indicated that the TMP could be removed from the diet of growing and finishing pigs with no negative effects on growth performance.

In broilers, removing the TMP during the finisher period (after 42 d) has resulted in no negative effects on growth performance, bone variables, or carcass traits (Christmas et al., 1995; Skinner et al., 1992; Waldroup et al., 1968). Also, Deyhim and Teeter (1993) indicted that removing the TMP from poultry diets from 28 to 49 d had no effect on growth performance or carcass traits. However, we are not aware of research on removing the TMP for broilers from the day of hatch to market weight.

Vohra et al. (1965) indicated that phytate has the potential to form insoluble salts with Ca, Fe, Zn, Mn, Cu, and Co, which may reduce the availability of these minerals. Phytase, an enzyme that breaks down phytate (Gibson and Ullah, 1990), has been shown to increase the absorption and retention of Zn and Cu (Lei et al., 1993; Adeola et al., 1995). Therefore, phytase may be able to replace the TMP in broiler diets and result in equal growth performance relative to broilers fed diets with the TMP.

Therefore, the objectives of this experiment were to determine the effects of phytase addition and removing the TMP on growth performance, bone breaking strength, and tissue mineral content in broilers fed to market weight.

Materials and Methods

All methods used in these EXP regarding animal care were approved by the Louisiana State University Agricultural Center (LSU) Animal Care and Use Committee.

An EXP was conducted with 1,260 Ross x Ross commercial broilers from House of Raeford (Gibsland, LA). They were allotted on the day of hatch to four treatments with six replications (three male and three female) per treatment and 50 (male) or 55 (female) broilers per replication. The initial and final BW were 40 and 2,275 g, respectively, and the EXP lasted 43 d. The broilers were housed in 1.52 x 3.05-m pens at the LSU Poultry Farm in one room of a ventilated tunnel house equipped with cool cells and fans. The pens contained 12 to 14 cm of fresh litter. The lighting system consisted of 3 d of 24-h light, followed by 16 hr of light and 8 hr of dark for the remainder of the project. The broilers were conditioned to the dark period over 3 d by increasing the periods of dark until 8 hr of dark were reached. The temperature in the house was 31 to 33°C for the first week and was dropped each week until 24 to 27°C was reached. Feed and water were offered ad libitum throughout the experimental period and the feed was fed in mash form.

The dietary treatments (Table 7.1) were arranged in a 2 x 2 factorial and were as follows: 1) corn-soybean meal (C-SBM); 2) C-SBM with reduced Ca and aP with phytase to provide 600 phytase units/kg of diet (Natuphos 1200; BASF Corporation, Mount Olive, NJ); 3) Diet 1 without the TMP supplementation; and 4) Diet 2 without the TMP supplementation. The broilers were fed a three phase feeding program consisting of starting (0 to 15 d), growing (15 to 36 d), and finishing (36 to 43 d) periods. The diets were formulated using Agristat values. For the starter period, the diets were formulated to contain 3,035 kcal/kg, 0.89% Ca, 0.45 % aP, 1.25% total Lys, and 0.94% total sulfur AA. For the growing period, the diets were formulated to contain 3,117 kcal/kg, 0.82% Ca, 0.40% aP, 1.14% total Lys, and 0.88% total sulfur AA. For the finishing period, the diets were formulated to contain

3,155 kcal/kg, 0.80% Ca, 0.36% aP, 0.98% total Lys, and 0.78% total sulfur AA. All other nutrients met or exceeded the requirement (NRC, 1994). The Ca and aP was reduced by 0.10% in the diets with added phytase. Natuphos 1200 was included in the diet at 0.03%, which added 600 phytase units/kg of diet (actual analysis of Natuphos 1200 indicated that it contained 1,750 phytase units/kg of premix). Actual analysis of diets indicated that phytase provided 420 of phytase units/kg of diet for Diet 2 and 697 of phytase units/kg of diet for Diet 4. All other nutrients met or exceeded their requirements (NRC, 1994).

Table 7.1. Composition of the starter diets [a]

Item	Control[b]	+ phytase[c]	No TMP	No TMP + phytase
Ingredient, %				
Corn	56.06	56.06	56.06	56.06
Soybean meal (47.5%)	35.97	35.97	35.97	35.97
Tallow	3.27	3.27	3.27	3.27
Monocalcium phosphate	1.55	1.08	1.55	1.08
Limestone	1.26	1.18	1.26	1.18
BMD + 3 nitro [d]	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50
Mineral premix [e]	0.25	0.25	-	-
DL–methionine	0.24	0.24	0.24	0.24
Choline chloride [f]	0.14	0.14	0.14	0.14
Ethoxyquin	0.10	0.10	0.10	0.10
Bio-Cox 60 [g]	0.03	0.03	0.03	0.03

(table continued)

Vitamin premix[h]	0.05	0.05	0.05	0.05
Lysine·HCl	0.05	0.05	0.05	0.05
Rice hulls	0.03	-	0.03	-
Phytase[i]	-	0.03	-	0.03
Sand	-	0.55	0.25	0.80
Calculated composition				
CP,%	22.40	22.40	22.40	22.40
ME, kcal/kg	3,035	3,035	3,035	3,035
Lys, %	1.25	1.25	1.25	1.25
Sulfur AA, %	0.94	0.94	0.94	0.94
Thr, %	0.84	0.84	0.84	0.84
Trp, %	0.30	0.30	0.30	0.30
Ca, %	0.89	0.79	0.89	0.79
P, %	0.71	0.61	0.71	0.61
aP, %	0.45	0.45	0.45	0.45
Mn, ppm	124.05	121.94	24.05	21.94
Fe, ppm	301.20	275.60	251.20	225.60
Zn, ppm	107.98	106.00	32.98	31.00
Cu, ppm	14.31	13.78	7.31	6.78
I, ppm	1.00	1.00	-	-

[a] TMP = trace mineral premix; aP = available P.
[b] The control diets during the grower and finisher periods were similar to the starter diet but contained the following: corn, 59.70 and 66.80%; soybean meal, 31.94 and 25.90%; tallow, 3.97 and 3.32%; limestone, 1.18 and 1.41%; monocalcium phosphate, 1.34 and 1.19%; DL-Met, 0.22 and 0.18; and Bio-Cox 60, 0.03 and 0%, respectively.
[c] The diets with phytase during the grower and finisher periods were similar to the control

(table continued)

diet but contained the following: corn, 59.70 and 66.80%; soybean meal, 31.94 and 25.90%; tallow, 3.97 and 3.32%; limestone, 1.11 and 1.34%; monocalcium phosphate, 0.87 and 0.71%; DL-Met, 0.22 and 0.18; and Bio-Cox 60, 0.03 and 0%, respectively.
[d] Bacitracin methylene disalicylate (4.4 g/kg premix) and 3-nitro-4-hydroxyphenylarsonic acid (5.0 g/kg premix) from Nutra Blend, Neosha, Mo.
[e] Provides the following per kg 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 dihydroiodide, respectively, with calcium carbonate as the carrier.
[f] Contains 600,000 mg/kg of choline.
[g] Bio-Cox 60 is salinomycin sodium (132.3 g/kg premix) from Nutra Blend, Neosha, MO.
[h] Provides the following per kilogram of diet: vitamin A, 8,000 IU; vitamin D_3, 3,000 IU; vitamin E, 25 IU; vitamin K, 1.5 IU; riboflavin, 10 mg; pantothenic acid, 15 mg; niacin, 50 mg; vitamin B_{12}, 0.02 μg; biotin, 0.1 μg; folic acid, 1 mg; pyridoxine, 4 mg; thiamin, 3 mg.
[i] In the diets with phytase, phytase replaced rice hulls to provide 600 phytase units/kg of diet.

At the end of each growth period all birds and feeders were weighed to determine average daily gain (ADG), average daily feed intake (ADFI), and gain:feed, and six broilers were randomly selected, killed by CO_2 asphixiation, and the left tibia was removed and frozen for subsequent determination of bone breaking strength and bone ash percentage. At the end of the 43-d growth trial, samples of bile, liver, pancreas, and breast muscle were taken, pooled by pen, and frozen for determination of mineral content. The breast muscles, livers, and pancreas from each pen were pooled, homogenized, and frozen for subsequent determination of mineral content. Bone breaking strength was determined by using a HD 250 Texture Machine (Texture Technologies Corporation, Scarsdale, NY) fitted with a three point bend rig with a load cell capacity of 25 (starter phase) or 50 (grower and finisher phases) kg and a cross-head speed of 100 mm/min. After determination of bone breaking strength, fat was removed from the tibias by a 36-h Soxhlet extraction in ethyl alcohol followed by a 36-h extraction with diethyl ether, and then dried at 100° C for 24 h. Bone ash percentage was determined by placing the bones in a muffle furnace and ashing for 36 h at

550° C. Samples of liver, pancreas, bile, and muscle were dried for 24-h at 100°C and mineral content was determined on the dry samples after being digested in nitric acid and hydrogen peroxide by inductively coupled plasma emission spectroscopy (Model Optima 3000, Perkin Elmer, Norwalk, CT).

Data were analyzed by analysis of variance procedures appropriate for a completely randomized design (Steel and Torrie, 1980) using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). There were no treatment x sex interactions so this parameter was removed from the model. There was a wing effect for muscle mineral levels so it was kept in the model for this response variable, but it was removed from the model for all other variables. Contrast statements were included to examine phytase, TMP, and phytase x TMP effects as a 2 x 2 factorial arrangement of treatments. The pen of broilers was the experimental unit for all data.

Results

Daily gain and ADFI were not affected ($P > 0.10$) by diet during any period of growth (Table 7.2). Gain:feed during the grower period and in the overall data was increased ($P < 0.07$) in broilers fed the diets with phytase. Mortality was increased ($P < 0.08$) during the grower period in broilers fed the diets with phytase.

Bone breaking strength was not affected ($P > 0.10$) by diet during the starter period but was decreased ($P < 0.08$) during the grower and finisher periods in broilers fed diets without the TMP (Table 7.3). Bone ash percentage during the starter period was decreased ($P < 0.06$) in broilers fed diets with phytase and decreased ($P < 0.08$) during the grower period in broilers fed diets without the TMP.

Table 7.2. The effect of phytase with and without the trace mineral premix on growth performance in broilers[a]

Item	Control	+ phytase	No TMP	No TMP + phytase	SEM
Starter (0 to 15 d)					
ADG, g	22.83	22.87	23.29	23.37	0.28
ADFI, g	32.21	32.91	32.97	32.70	0.50
Gain:feed, g:g	0.709	0.697	0.707	0.715	0.010
Mortality, chicks/replication	0.67	0.67	0.67	0.50	0.29
Grower (15 to 36 d)					
ADG, g	65.54	66.14	64.54	66.23	0.86
ADFI, g	109.38	108.62	109.67	108.73	1.60
Gain:feed, g:g[b]	0.599	0.609	0.588	0.609	0.004
Mortality, chicks/replication[b]	0.17	0.83	0.00	0.67	0.25
Finisher (37-43 d)					
ADG, g	73.05	73.64	71.88	74.79	2.21
ADFI, g	153.41	151.23	152.56	157.67	3.78
Gain:feed, g:g	0.478	0.487	0.471	0.475	0.007
Mortality, chicks/replication	0.33	0.00	0.00	0.00	0.17
Overall (0 to 43 d)					
Final BW, g	2,254	2,291	2,249	2,307	32
ADG, g	51.55	52.24	51.20	52.64	0.73
ADFI, g	85.43	84.88	85.95	85.84	1.21
Gain:feed, g:g[b]	0.603	0.615	0.596	0.613	0.003
Mortality, chicks/replication	1.17	1.50	0.67	1.17	0.40

[a] Data are means of six replications with an initial BW of 40.0 g
[b] Phytase, $P < 0.07$.

Table 7.3. The effect of phytase with and without the trace mineral premix on bone strength and ash percentage in broilers[a]

Item	Control	+ phytase	No TMP	No TMP + phytase	SEM
Starter (0 to 15 d)					
Bone strength, kg	10.73	10.98	10.89	10.11	0.40
Bone ash, %[c]	55.39	54.22	54.48	54.28	0.34
Grower (15 to 36 d)					
Bone strength, kg[d]	37.56	35.40	34.52	33.15	1.29
Bone ash, %[d]	56.31	56.43	56.15	55.36	0.30
Finisher (37-43 d)					
Bone strength, kg[d]	36.15	39.20	35.88	33.98	1.48
Bone ash, %	57.06	57.02	57.77	56.81	0.51

[a] Data are means of six replications of six broilers per replicate pen. Bone ash percentage is on a DM basis.
[b] Bone strength was determined using a HD 250 Texture Machine (Texture Technologies Corporation, Scarsdale, NY) fitted with a three point bend rig with a load cell capacity of 25 kg for the starter period and 50 kg for the grower and finisher periods and cross-head speed of 100 mm/min.
[c] Phytase, $P < 0.06$.
[d] TMP, $P < 0.08$.

Unaffected tissue mineral concentrations (means ± pooled SEM, DM basis) represent 24 replications of six broilers per replicate pen (data not shown in Table 7.4): Bile: Mg, 0.04 ± 0.01; Ca, 0.64 ± 0.10%; P, 0.51 ± 0.10%; K, 0.44 ± 0.05%. Muscle: Ca, 0.04 ± 0.01%; P, 1.06 ± 0.02%; K, 1.53 ± 0.03%; Na, 0.16 ± 0.02%. Liver: Ca, 0.12 ± 0.01%; P, 1.19 ± 0.03%; K, 1.15 ± 0.04%; Na, 0.59 ± 0.04%. Pancreas: Mg, 0.11 ± 0.01; Ca, 0.06 ±

0.01%; P, 1.68 ± 0.17%; K, 1.22 ± 0.13%; Na, 0.30 ± 0.03%. Removing the TMP from the diet decreased (P < 0.09) bile and liver Cu levels, muscle Mg level, and pancreas Mn level (Table 7.4). Adding phytase to the diet increased (P < 0.10) muscle Zn level and pancreas Mn level, but decreased liver Mg level. Adding phytase to the diet with the TMP reduced muscle Cu level, but adding phytase to the diet without the TMP increased muscle Cu level (phytase x TMP, P < 0.07). Adding phytase to the diet with the TMP increased bile Na level, but adding phytase to the diet without the TMP decreased bile Na level (phytase x TMP, P < 0.07).

Table 7.4. Effect of phytase with and without the trace mineral premix on mineral levels in the bile, muscle, liver, and pancreas in broilers[a]

				No TMP	
Item	Control	+ phytase	No TMP	+ phytase	SEM
Bile					
Na, %[c]	4.50	4.93	4.90	4.11	0.31
Cu, ppm[b]	24.24	21.31	16.95	16.32	1.81
Mn, ppm	2.34	3.72	1.90	2.28	0.89
Fe, ppm	16.44	19.71	18.72	17.55	2.90
Zn, ppm	254.68	291.36	280.80	233.18	81.17
Muscle					
Mg, %[b]	0.138	0.142	0.134	0.137	0.002
Cu, ppm[c]	2.42	1.74	1.60	2.78	0.32
Mn, ppm	0.25	0.29	0.20	0.26	0.08
Fe, ppm	63.35	42.85	46.34	43.08	8.40

(table continued)

Zn, ppm [d]	52.02	62.44	44.11	56.46	6.46
Liver					
Mg, %[d]	0.068	0.062	0.067	0.065	0.002
Cu, ppm[b]	12.41	12.10	11.39	11.23	0.41
Mn, ppm	10.52	9.65	8.18	9.62	0.70
Fe, ppm	619.1	566.0	480.8	593.8	62.5
Zn, ppm	102.55	100.85	116.14	107.42	7.36
Pancreas					
Cu, ppm	4.20	5.35	3.77	4.12	0.54
Mn, ppm[b,d]	6.12	8.56	4.75	6.43	0.94
Fe, ppm	59.91	81.80	69.40	74.97	9.35
Zn, ppm	146.21	171.57	114.08	153.33	21.27

[a] Data are the means of six replications of six broilers per replicate pen. All values are on a DM basis.
[b] TMP, $P < 0.09$.
[c] Phytase x TMP, $P < 0.07$.
[d] Phytase, $P < 0.10$.

Discussion

Research has indicated that removing the TMP in finisher diets (after 42 d) for broilers had no effect on growth performance (Waldroup et al., 1968; Skinner et al., 1992; Christmas et al., 1995). Also, Deyhim and Teeter (1993) reported that removing the TMP from broiler diets from 28 to 49 d had no effect on growth performance. However, research on removing the TMP from broiler diets on the day of hatch to market weight could not be found. In pigs, Kim et al. (1997), Mavromichalis et al. (1999), and Shelton et al. (2004b) reported that removing the TMP from the diet of growing and finishing pigs had no effect on growth performance. These data agree with our data. However, Shelton et al. (2004a)

reported that growth performance was decreased in chicks fed diets with no supplemental Zn from 0 to 18 d posthatching, but the chicks in that study were raised in stainless steel starter batteries, whereas the chicks in our study were raised in floor pens. Raising chicks in floor pens may lead to increased nutrient recycling relative to chicks raised in starter batteries. Also in our study, chicks were fed from galvanized feeders, which may have increased the amount of Zn available to the broilers. In our study, broilers fed diets with added phytase (either the control diet or the diet without the TMP) during the grower period and the overall period had an increased gain:feed. This agrees with Watson (2001) who reported that phytase addition to diets adequate in all nutrients increased growth performance in 0 to 21 d old chicks. Cabahug et al. (1999) referred to the positive 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. Also, commercial type diets are low in energy and Johnston and Southern (2000a) and Shelton et al. (2003) reported that phytase increased energy availability for chicks and pigs, respectively.

Shelton et al. (2004a) reported that bone strength was decreased in chicks fed diets with no supplemental Zn, but as previously mentioned, the chicks in that study were raised in stainless steel starter batteries, whereas in our study, the chicks were raised in floor pens. Also, unpublished data in our lab has shown that removing Mn and Cu from the diet of chicks raised in starter batteries did not affect bone strength or bone ash percentage. In our study, bone strength was decreased during the growing and finishing periods and adding phytase did not reverse this response. In pigs, removing the TMP and adding phytase to the diet during the growing-finishing periods had no effect on bone strength or bone ash percentage (Shelton et al., 2004b).

Shelton et al. (2004b) reported that removing the TMP in growing-finishing pig diets reduced the level of Zn in bone, liver, and muscle, which does not agree with our study.

Phytase has been shown to increase tissue Zn levels (Yi et al., 1996a; Mohanna and Nys, 1999). In our study, adding phytase to the diets with or without the TMP increased Zn level in the muscle and tended to increase Zn level in the pancreas (130 vs 162 ppm). In the studies by Yi et al. (1996a) and Mohanna and Nys (1999), liver Zn levels were increased by phytase addition, but in our study, liver Zn level was not affected.

The authors are unable to explain the decrease in muscle Cu level when phytase was added to the diet with the TMP. Shelton et al. (2004b) reported that adding phytase to pig diets without the TMP decreased bile, liver, and bone Cu levels but increased muscle Cu level. In our study, bile Cu level was decreased when the TMP was removed from the diet and adding phytase did not reverse this response. Phytase has been shown to decrease bone Cu level in chicks (Lan et al., 2002). However, other research has reported that phytase addition increased the retention of Cu (Sebastian et al., 1996b; Um et al., 2000; Lim et al., 2001). The Cu level in the pancreas or liver was not affected by diet in our study, which agrees with reports by Sebastian et al. (1996a) and Biehl et al. (1997).

Zacharias et al. (2003) indicated that phytase addition increased liver Fe level, which does not agree with our study. Tissue Fe levels were not affected in our study by removing the TMP or by adding phytase. Shelton et al. (2004b) reported that phytase addition did not affect liver, bone, or muscle Fe levels, but decreased bile Fe levels. Sebastian et al. (1996b) and Biehl et al. (1997) reported that phytase addition had no effect on Fe retention in chicks. However, Paik et al. (2000) and Um et al. (2000) reported an increase in Fe retention in chicks fed phytase.

Lan et al. (2002) reported that phytase addition increased Mn retention in chicks. Shelton et al. (2004b) reported that removing the TMP increased bile and liver Mn levels and adding phytase to the diet reduced Mn levels, which does not agree with our study. However, Sebastian et al. (1996a) reported that adding phytase had no effect on Mn retention (and at dietary levels of 1.00% Ca decreased Mn retention) in chicks. Our study

indicates that removing the TMP decreased the Mn level in the pancreas and adding phytase increased the pancreas Mn level.

This study indicates that removing the TMP in diets for broilers had no effect on growth performance, decreased bone strength during the grower and finisher periods, and had variable effects on tissue mineral concentrations. Adding phytase increased gain:feed and had variable effects on tissue mineral levels.

CHAPTER 8

SUMMARY AND CONCLUSIONS

The purpose of this research was to determine the non-phosphorus effects of phytase in swine and poultry. Specifically, the effect of phytase on energy availability, amino acid availability, carcass traits, pork quality, and trace mineral availability were determined. In the first experiment, phytase numerically increased the majority of the response variables measured indicating that phytase has small positive effects on energy availability and protein and fat deposition. However, more research is needed to determine the mode of action that phytase uses to increase energy availability. Results from the second experiment indicated that the energy, amino acid, calcium, and available phosphorus matrix values for phytase (Natuphos 1200) in formulating corn-soybean meal diets for commercial broilers are accurate and resulted in similar growth performance, carcass traits, meat quality, and tibia ash percentage compared with broilers fed a conventional corn-soybean meal diet. Furthermore, adding phytase to broiler diets reduced the levels of total and soluble P in the litter. Results from the third, fourth, and fifth experiments indicated that phytase can replace the trace mineral premix in pigs but not in broilers. Removing the trace mineral premix in the diets of nursery pigs resulted in a greater negative effect on growth performance and tissue mineral concentration relative to removing the trace mineral premix in growing-finishing pigs. In broilers, removing the trace mineral premix had no negative effect on growth performance but did decrease bone breaking strength, and adding phytase did not reverse this response. Removing the trace mineral premix will decrease the amount of trace minerals in meat and adding phytase does not supply enough of the trace minerals to equal that provided by the premix; thus the effects of reducing trace mineral concentrations in pork and its effect on human nutrition need to be further investigated.

Most of the research with phytase has been conducted to determine its effect on P and Ca in swine and poultry. However, phytate affects other nutrients and to get the full economic and environmental effect of phytase, its effect on these nutrients is important. This research indicates that phytase positively affects energy, amino acids, and trace minerals in swine and poultry without negatively affecting carcass traits or meat quality.

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LETTERS OF PERMISSION

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Jason Shelton
Research Associate
Department of Animal Sciences
105 J. B. Francioni Bldg.
Louisiana State University
Baton Rouge, LA 70803
(225)578-3448

VITA

Jason Shelton was born November 16, 1972, in Sherman, Texas. Jason lived most of his life in Delhi, Louisiana. He graduated from Riverfield Academy in the spring of 1991 and began college at Louisiana State University. In the spring of 1996 he received a Bachelor of Science degree in zoology. After working for two years he was accepted into graduate school in the fall of 1998 and began studying in nonruminant nutrition. In the spring of 2000, he received his Master of Science degree in animal science. Currently, Jason is a candidate for his doctoral degree in animal science.