Evaluation of Microbial Phytase in Broiler Diets[1]

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ABSTRACT Two trials were conducted to evaluate the efficacy of a new microbial phytase (Phyzyme XP) for broiler chicks. Trial 1 used 192 8-d-old male broilers in a 14-d trial to assess growth and nutrient utilization. Dietary treatments (221.9 g/kg CP) included a positive control [5.0 g/kg nonphytate P (NPP)], negative control (1.2 g/kg NPP), and negative control plus 500 or 1,000 phytase units/kg of diet. Phytase addition increased weight gain, feed intake, feed efficiency, and tibia and toe ash (linear, $P < 0.01$) with tibia ash also responding quadratically ($P < 0.05$). Apparent ileal digestibility of P (linear and quadratic, $P < 0.05$), tryptophan, and valine (linear, $P < 0.05$) also increased. Linear and quadratic responses were observed for retention of DM, nitrogen, P, and several amino acids ($P < 0.05$) with added phytase. Trial 2 utilized 576 1-d-old male broilers over a 42-d period to evaluate growth performance. Diets were formulated for starter (222.7 g/kg CP) and grower (201.5 g/kg CP) phases and included a positive control (starter and grower, 5.0 and 3.8 g/kg NPP, respectively); negative control (starter and grower, 2.4 and 1.8 g/kg NPP, respectively); negative control plus 500, 750, or 1,000 phytase units/kg; and negative control plus 500 phytase units/kg of Natuphos phytase. Phytase increased weight gain and feed intake (starter, grower, overall) as well as feed efficiency during the starter period (linear, $P < 0.05$). Feed intake was also improved during the grower period and overall (quadratic, $P < 0.05$). Tibia and toe ash of birds fed for the first 21 d increased (linear, $P < 0.05$) with tibia ash also increasing quadratically ($P < 0.05$). Overall, tibia and toe ash were improved due to phytase addition (linear and quadratic, $P < 0.05$). In conclusion, this microbial phytase, derived from *Escherichia coli* and expressed in *Schizosaccaromyces pombe*, elicited improved growth performance, bone mineralization, and P utilization in broiler chicks.

(*Key words*: broiler, growth, microbial phytase, nutrient utilization)

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INTRODUCTION

A significant portion of the P in mature cereal grains and oilseeds is present as phytate P as this is the main storage form of plant P and inositol (Erdman, 1979; Maga, 1982). Nonruminant animals have insufficient phytase to effectively digest phytate (Nelson, 1971), hence inorganic P is often added to their diets. The excretion of P in animal manure has received the greatest attention as potential environmental pollutants of agricultural origin. Phosphates are not highly soluble in water but accumulate in soils that may eventually result in runoff and, along with N, lead to eutrophication of surface waters, a condition that is detrimental to aquatic animals. The poor digestive utilization of phytic acid P by monogastric animals and its consequences on diet cost, environment, and digestibility of minerals and proteins have led to extensive research efforts directed toward improving phytate digestion. Research has demonstrated definitively that phytase has merit as a tool for minimizing P excretion by increasing P availability and subsequent utilization (Ravindran et al., 1995a). The currently available feed-grade phytase products have room for improvement in efficacy. The search for novel phytase preparations, with relatively greater potency in intestinal phytate hydrolysis and better heat stability, is an ongoing process. In accordance with this goal, the objective of this study was to evaluate the efficacy of a microbial phytase on growth performance and nutrient utilization when fed to broiler chicks in corn-soy based diets.

MATERIALS AND METHODS

Phytases

The experimental microbial phytase was derived from *Escherichia coli* and expressed in *Schizosaccaromyces pombe*. This new phytase product (Phyzyme XP) was produced

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Abbreviation Key: FTU = phytase unit; NPP = nonphytate P.

by Danisco Animal Nutrition[3] as a 6-phytase, which initiates phosphate hydrolysis at the 6-position of the phytate molecule, and had an analyzed enzyme activity of 500 phytase units/g. Enzymatic activity of phytase was determined by the method of Engelen et al. (1994). The experimental phytase was compared to the commercially available Natuphos[4] phytase that was derived from *Aspergillus niger*. The Natuphos phytase was a 3-phytase and had an enzyme activity of 5,000 phytase units/g. One phytase unit (FTU) is defined as the quantity of enzyme required to liberate 1 μmol of inorganic P/min, at pH 5.5, from an excess of 15 μM sodium phytate at 37°C (International Union of Biochemistry, 1979).

Trial 1

In this trial, the microbial phytase was evaluated based on nutrient utilization and growth performance over a 14-d period. To attain this objective, 1-d-old male broiler chicks (Ross 308) were obtained from a local hatchery, wing-banded, and reared in electrically heated battery brooders maintained at 35°C from d 1 to 7 with continuous fluorescent lighting. During this time, birds had ad libitum access to water and a standard broiler starter diet containing 221.9 g/kg crude protein, 3,229 kcal/kg ME_n, 10.0 g/kg Ca, and 5.0 g/kg nonphytate P (NPP).

At 8 d of age, chicks were weighed individually, and 192 birds were assigned to 4 diets such that the average weight across dietary treatments was similar with 6 birds per cage and 8 replicate cages per treatment. Birds were provided ad libitum access to water and dietary treatments from d 8 to 22, and battery temperatures were maintained at 32 and 27°C from d 8 to 14 and d 15 to 21, respectively. Individual BW of birds and feed consumption per cage were recorded at 8 and 22 d of age; mortality was monitored on a daily basis. Bird management and handling procedures used in this trial were approved by the Purdue Animal Care and Use Committee.

Dietary Treatments. All diets were corn-soybean meal-based and formulated to meet or exceed NRC recommendations for the 0- to 21-d-old broiler (NRC, 1994) with the exception of dietary P in the negative control diet. Experimental diets consisted of (1) positive control (5.0 g/kg NPP); (2) negative control (1.2 g/kg NNP, no source of inorganic P); (3) negative control plus 500 FTU/kg microbial phytase; and (4) negative control plus 1,000 FTU/kg microbial phytase. Composition of the positive and negative control diets are presented in Table 1. All 4 experimental diets contained 3.0 g/kg chromic oxide as an indigestible marker to quantify nutrient digestibility and retention values.

Sample Collection and Analysis. Excreta samples were collected from beneath each cage between d 17 and 21 and dried in a forced-air oven at 55°C for 5 d. At 22 d of age, birds were euthanized by carbon dioxide asphyxiation and ileal digesta were collected by dissecting a segment of the ileum defined as extending from Meckel's diverticulum to the ileocecal junction. Contents of this segment were squeezed into a plastic container, pooled per cage of 6 birds, and subsequently lyophilized. In addition, the left tibia and left toe from individual birds were excised, sealed in plastic bags, and stored at –4°C pending further analysis.

Duplicate proximate analyses were performed on all diet, excreta, and ileal samples. Dry matter content was determined by drying the samples at 100°C for 24 h. Diets, excreta, and ileal samples were analyzed for amino acids by HPLC [AOAC 982.30 E (a, b, c), 2000] and for calcium, phosphorus, and chromium [nitric/perchloric wet ash, AOAC 968.08D(b)] by inductively coupled plasma atomic emission spectroscopy (AOAC 990.08, 2000) at the University of Missouri Experiment Station Chemical Laboratory. Nitrogen content of diets, excreta, and ileal samples was determined by the combustion method[5] and energy content by adiabatic bomb calorimetry.[6] For determination of bone ash content of tibia and toe samples, these tissues were dried overnight at 100°C, extracted in ether for 6 h, and ashed in a muffle furnace for 18 h at 600°C.

Calculations. Apparent nutrient retention and digestibility values were calculated by using the index method with the equation:

$$ANV_x = [1 - [(C_i/C_o) \times (X_o/X_i)]] \times 100$$

where ANV_x = the apparent nutrient digestibility or retention value for ileal or excreta samples, respectively, expressed as a percentage; C_i = the concentration of chromic oxide present in the diet; C_o = the concentration of chromic oxide present in the ileal or excreta output; X_o = the nutrient concentration present in the ileal or excreta output; and X_i = the nutrient concentration present in the diet. All values for C_i, C_o, X_o, and X_i are expressed as a percentage of dry matter.

Though incidence of mortality was low, performance criteria (weight gain, feed intake, and gain:feed) were adjusted according to the number of bird-days. This value is defined as the number of birds alive in each pen multiplied by the number of days without incidence of mortality.

Trial 2

To further evaluate the efficacy of the microbial phytase, a growth performance trial was conducted over a 42-d period. Five hundred seventy-six 1-d-old male broiler chicks (Ross 308) were wing-banded, individually weighed, and assigned to 48 floor pens with 12 chicks per pen. Birds were allotted to 6 dietary treatments such that the average initial weight across diets was similar with 8 replicate pens per treatment. Management and handling procedures were approved by the Purdue Animal Care and Use Committee.

[3]Danisco Animal Nutrition, Marlborough, UK.
[4]BASF, Mt. Olive, NJ.
[5]Model FP2000, LECO Corp., St. Joseph, MI.
[6]Model 1261, Parr Instrument Co., Moline, IL.

TABLE 1. Composition (g/kg) of positive and negative control diets (as-fed basis)

Ingredient	Trial 1 Starter PC[1]	Trial 1 Starter NC	Trial 2 Starter PC	Trial 2 Starter NC	Trial 2 Grower PC	Trial 2 Grower NC
Corn	529.7	529.7	538.7	538.7	613.0	613.0
Soybean meal	364.7	364.7	364.7	364.7	308.0	308.0
Corn oil	50.0	50.0	50.0	50.0	35.0	35.0
Limestone	11.6	10.4	11.6	10.8	13.0	11.7
Dicalcium phosphate	20.0	0.0	20.0	6.0	14.0	3.5
Salt	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin-mineral premix[2]	3.0	3.0	3.0	3.0	3.0	3.0
DL-Methionine	2.0	2.0	2.0	2.0	1.0	1.0
Chromic oxide premix[3]	15.0	15.0	—	—	—	—
Corn starch	0.0	21.2	6.0	20.8	9.0	20.8
Calculated composition						
Crude protein	221.9	221.9	222.7	222.7	201.5	201.5
ME_n, kcal/kg	3,146	3,233	3,201	3,261	3,179	3,227
Calcium	10.0	5.1	10.0	6.6	9.0	6.2
Total P	7.7	3.9	7.7	5.1	6.4	4.4
Nonphytate P	5.0	1.2	5.0	2.4	3.8	1.8
Analyzed composition[4]						
Crude protein	231.0	231.5	212.5	217.9	186.9	188.3
Calcium	8.3	5.8	13.2	9.8	12.7	9.6
Total P	6.7	3.7	9.1	5.4	7.4	5.0

[1]PC = positive control; NC = negative control.

[2]Provided per kilogram of diet: vitamin A, 11,343 IU; vitamin D_3, 5,688 IU; vitamin E, 37.8 IU; riboflavin, 5.66 mg; D-pantothenic acid, 20.8 mg; niacin, 75.6 mg; choline, 574 mg; vitamin B_{12}, 21.3 μg; biotin, 280 μg; thiamine, 1.89 mg; folic acid, 940 μg; pyridoxine, 2.8 mg; I, 1.8 mg; Mn, 107.2 mg; Cu, 6.6 mg; Fe, 42.9 mg; Zn, 107.2 mg; Se, 260 μg.

[3]Chromic oxide premix (20%): 1 g of chromic oxide added to 4 g of corn.

[4]Proximate analyses were performed in duplicate.

The feeding program consisted of a starter (d 1 to 22) and grower phase (d 22 to 43). Compositions of the positive and negative control diets are presented in Table 1 for both feeding phases; all diets met or exceeded NRC recommendations (NRC, 1994) with the exception of P in the negative control diet. Experimental diets consisted of 1) positive control, 2) negative control diet (contained 52% less NPP than positive control), 3) negative control plus 500 FTU/kg microbial phytase, 4) negative control plus 750 FTU/kg microbial phytase, 5) negative control plus 1,000 FTU/kg microbial phytase, and 6) negative control plus 500 FTU/kg Natuphos phytase. The positive and negative control diets contained 5.0 and 2.4 g/kg NPP, respectively, in the starter phase and 3.8 and 1.8 g/kg NPP, respectively, in the grower phase.

Bird weights and feed consumption were measured on a pen basis every 7 d, beginning when the chicks were 8 d old. On d 22 and 43 of the trial, 2 birds were selected from each pen (representative of the mean BW) and euthanized via carbon dioxide asphyxiation. The left tibia and toe were excised from individual birds for bone ash determination as described in trial 1. Mortality was recorded on a daily basis. Again, growth data were adjusted according to the number of bird-days as described for the first trial.

Statistical Analysis

Data were analyzed as a randomized complete block design using the GLM procedure of SAS (SAS, 2001). Pen served as the experimental unit for all nutrient balance and growth performance variables with the exception of bone ash data, which were examined per individual bird. Orthogonal polynomial contrasts were used to determine significant linear and quadratic responses of nutrient balance and growth performance to phytase supplementation of the negative control diet in each trial. Additionally, contrasts were used to determine significant phytase effects between positive and unsupplemented negative control diets in both trials as well as between the experimental and Natuphos phytase products in trial 2.

RESULTS

Analysis of phytase activity in experimental diets indicated slightly higher than expected results but values were within an acceptable range. Phyzyme XP enzyme activities in trial 1 were 662 and 1,158 FTU/kg in diets with formulated activities of 500 and 1,000 FTU/kg, respectively. In trial 2, phytase activities were 496, 877, and 1,108 FTU/kg in diets formulated to contain 500, 750, and 1,000 FTU/kg Phyzyme XP during the starter feeding phase, and 500, 848, and 1,195 FTU/kg in diets with identically formulated activities during the grower feeding phase. Diets formulated to contain 500 FTU/kg Natuphos phytase had analyzed phytase activities of 456 and 434 FTU/kg during the starter and grower feeding phases, respectively. Mortality during each trial was minimal and not associated with dietary treatment, as presented for trials 1 and 2 in Tables 2 and 3, respectively.

TABLE 2. Efficacy of microbial phytase on growth performance of male broiler chicks,[1] trial 1

	Diet[2]				
	PC	NC	NC + 500 FTU/kg Phyzyme XP	NC + 1,000 FTU/kg Phyzyme XP	SD
Initial weight, g	123	123	123	123	0.1
Final weight, g[3,4]	637	569	596	651	42.9
Weight gain, g[3,4]	514	445	472	528	42.9
Feed intake, g[3,4]	791	713	750	786	54.5
Gain:feed, g:kg[4]	649	623	629	671	29.5
Mortality, %	0.0	0.0	2.1	0.0	
n[5]	8	8	8	8	

[1]Broiler chicks were on trial from 8 to 22 d of age.

[2]PC = positive control; NC = negative control; FTU = phytase units.

[3]Contrast of positive versus unsupplemented negative control diets ($P < 0.01$).

[4]Linear response to graded levels of Phyzyme XP phytase ($P < 0.01$).

[5]Cage served as the experimental unit with 6 chicks per cage and 8 replicate cages per diet.

Growth Performance

Results for trials 1 and 2 showing the effect of microbial phytase on growth performance are presented in Tables 2 and 3, respectively. In each study, weight gain and feed intake were different between the positive and negative control diets ($P < 0.05$). There was a linear increase in weight gain, feed intake, and feed efficiency ($P < 0.01$) due to phytase addition over the 14-d period in trial 1. The addition of microbial phytase to the negative control diet improved each growth criterion over the first 21 d in trial 2 (linear, $P < 0.05$). Only weight gain (linear, $P < 0.05$) and feed intake (linear and quadratic, $P < 0.05$) increased due to phytase inclusion from d 22 to 43 and over the entire 42-d period of trial 2. Weight gain, feed intake, and feed efficiency reached a plateau with phytase supplementation at 750 FTU/kg diet in trial 2, regardless of feeding period. There were no differences in growth performance ($P > 0.10$) between Phyzyme XP and Natuphos phytase products during trial 2.

Tibia and Toe Ash

Figure 1 presents the ash content of left tibia and toe samples for trial 1. Both tibia and toe ash increased with supplementation of microbial phytase (linear, $P < 0.01$) to the negative control diet, ranging from 42 to 47% for tibia and 10 to 12% for toe samples. Additionally, a quadratic response was observed for tibia ash ($P < 0.05$). Tibia and toe ash taken from birds at day 22 in trial 2 (Table

TABLE 3. Efficacy of microbial phytase on growth performance of male broiler chicks,[1] trial 2

	Diet[2]						
	PC	NC	NC + 500 FTU/kg Phyzyme XP	NC + 750 FTU/kg Phyzyme XP	NC + 1,000 FTU/kg Phyzyme XP	NC + 500 FTU/kg Natuphos	SD
Body weight, g							
Day 1	48	48	48	48	48	48	0.1
Day 22[3,4]	726	660	715	746	746	696	44.3
Day 43[3,4]	2,247	2,053	2,183	2,294	2,257	2,185	127.7
Weight gain, g							
Days 1 to 22[3,4]	678	612	667	697	698	647	44.3
Days 22 to 43[3,4]	1,521	1,393	1,468	1,548	1,511	1,490	108.0
Overall[3,4]	2,198	2,005	2,134	2,246	2,209	2,137	127.7
Feed intake, g							
Days 1 to 22[3,4]	932	855	923	934	937	907	50.2
Days 22 to 43[3,4,5]	2,791	2,575	2,751	2,862	2,803	2,724	139.0
Overall[3,4,5]	3,723	3,430	3,674	3,796	3,740	3,631	179.1
Gain:Feed, g:kg							
Days 1 to 22[4]	727	715	721	746	744	713	16.0
Days 22 to 43	545	541	534	541	540	547	28.5
Overall	591	585	581	591	591	589	19.9
Mortality, %	3.1	5.2	2.1	4.2	8.3	3.1	
n[6]	8	8	8	8	8	8	

[1]Broiler chicks were on trial from 1 to 43 d of age.

[2]PC = positive control; NC = negative control; FTU = phytase units.

[3]Contrast of positive vs. unsupplemented negative control diets ($P < 0.05$).

[4]Linear response to graded levels of Phyzyme XP phytase ($P < 0.05$).

[5]Quadratic response to graded levels of Phyzyme XP phytase ($P < 0.05$).

[6]Pen served as the experimental unit with 12 chicks per floor pen and 8 replicate pens per diet.

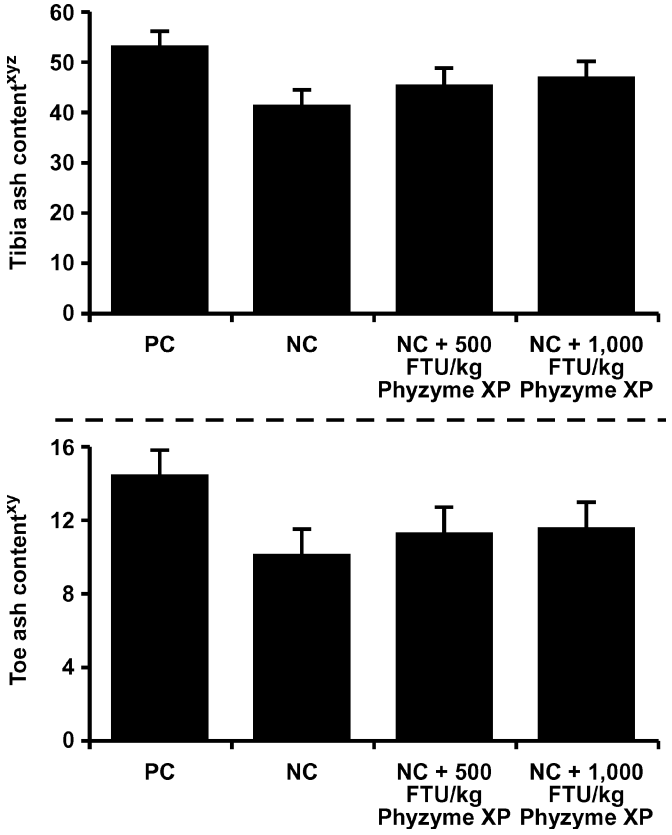

FIGURE 1. Efficacy of microbial phytase on tibia and toe ash content (%), trial 1. PC = positive control; NC = negative control; FTU = phytase units. Cage served as the experimental unit with 6 chicks per cage and 8 replicate cages per diet. Error bars represent standard deviations. Broiler chicks were on trial from 8 to 22 d of age. [X]Contrast of positive versus unsupplemented negative control diets ($P < 0.01$). [Y]Linear response to graded levels of Phyzyme XP phytase ($P < 0.01$). [z]Quadratic response to graded levels of Phyzyme XP phytase ($P < 0.05$).

4) exhibited an increasing response to phytase addition (linear, $P < 0.05$) with ash contents similar to those of trial 1. Tibia ash of birds determined at d 22 also showed a quadratic response to phytase level ($P < 0.05$). Tibia and toe ash additionally exhibited linear and quadratic responses to phytase supplementation over the entire 42-d period ($P < 0.05$). Bone mineralization was greater in both tibia and toe samples of birds fed the positive control diet compared with those fed the negative control diet during each trial ($P < 0.01$). Additionally, tibia ash of birds fed Phyzyme XP phytase-supplemented diets from d 1 to 22 was greater than that of birds fed Natuphos-supplemented diets ($P < 0.01$). However, differences between these 2 phytase products were not found in toe ash of birds fed during this period or in tibia and toe ash of birds fed for the full 42-d period ($P > 0.10$).

Apparent Nutrient Digestibility and Retention

The effects of microbial phytase supplementation on apparent ileal nutrient digestibilities from trial 1 are presented in Table 5. Addition of graded levels of phytase to corn-soy diets deficient in P resulted in both linear ($P < 0.05$) and quadratic ($P < 0.01$) improvements in apparent ileal P digestibility. Apparent ileal digestibilities of tryptophan and valine also increased (linear, $P < 0.05$), but nitrogen digestibility was not affected by phytase addition ($P > 0.05$). Also, apparent ileal digestibilities of dry matter, gross energy, calcium, and all other amino acids were not influenced by addition of microbial phytase ($P > 0.10$). Differences in apparent ileal digestibilities between the positive and negative control diets were observed for nitrogen, gross energy, calcium, and all amino acids except aspartic acid, glutamic acid, lysine, and phenylalanine ($P < 0.05$). For each affected nutrient and gross energy mentioned above, with the exception of calcium, birds fed the positive control diet exhibited greater apparent ileal digestibilities compared with those fed the negative control diet.

Nutrient retention values from trial 1 are shown in Table 6. Increasing phytase levels resulted in improved retention of nitrogen, phosphorus, arginine, histidine, iso-

TABLE 4. Efficacy of microbial phytase on tibia and toe ash content[1] of male broiler chicks, trial 2

	Diet[2]						
	PC	NC	NC + 500 FTU/kg Phyzyme XP	NC + 750 FTU/kg Phyzyme XP	NC + 1,000 FTU/kg Phyzyme XP	NC + 500 FTU/kg Natuphos	SD
Tibia ash							
Day 22[3,4,5,6]	52.0	44.5	49.0	49.4	49.3	46.2	2.08
Day 43[3,5,6]	54.3	48.5	51.9	52.7	52.0	50.6	2.86
Toe ash							
Day 22[3,5]	13.3	10.4	10.7	11.9	11.8	10.7	1.57
Day 43[3,5,6]	12.8	10.8	12.2	12.5	11.7	11.6	1.54
n[7]	16	16	16	16	16	16	

[1]Expressed as a percentage of fat-extracted, dried sample weight. Broiler chicks were on trial 1 to 43 d of age.

[2]PC = positive control; NC = negative control; FTU = phytase units.

[3]Contrast of positive versus unsupplemented negative control diets ($P < 0.01$).

[4]Contrast of Phyzyme XP versus Natuphos phytase products ($P < 0.01$).

[5]Linear response to graded levels of Phyzyme XP phytase ($P < 0.05$).

[6]Quadratic response to graded levels of Phyzyme XP phytase ($P < 0.05$).

[7]Individual bird served as the experimental unit with 2 birds of mean pen weight selected from each of 8 replicate pens per diet.

TABLE 5. Efficacy of microbial phytase on apparent ileal digestibilities (%) of dry matter, nitrogen, gross energy, phosphorus, calcium, and amino acids for male broiler chicks,[1] trial 1

	Diet[2]				
	PC	NC	NC + 500 FTU/kg Phyzyme XP	NC + 1,000 FTU/kg Phyzyme XP	SD
Dry matter	71.1	69.2	71.6	71.3	2.31
Nitrogen[3]	89.1	84.8	87.1	88.1	2.98
Gross energy[3]	70.6	67.4	69.4	69.7	2.73
Phosphorus[4,5]	50.7	53.1	68.8	65.8	5.02
Calcium[3]	48.9	71.1	74.1	69.5	6.28
Amino acids					
Indispensable					
Arginine[3]	90.5	88.5	90.1	90.4	1.96
Histidine[3]	86.8	84.0	85.6	85.9	2.20
Isoleucine[3]	83.8	80.6	82.9	83.5	2.77
Leucine[3]	84.9	82.1	83.5	84.0	2.52
Lysine	89.2	87.2	88.7	90.2	2.73
Methionine[3]	92.2	88.9	88.9	90.6	2.20
Phenylalanine	84.9	82.5	84.1	84.6	2.36
Threonine[3]	79.0	75.4	76.7	76.9	3.03
Tryptophan[3,4]	84.8	79.2	81.9	82.3	2.83
Valine[3,4]	82.4	78.3	80.9	81.8	2.88
Dispensable					
Alanine[3]	82.9	79.0	80.5	81.3	2.99
Aspartic acid	85.2	83.6	85.0	85.4	2.26
Cysteine[3]	77.4	71.2	71.0	72.7	4.58
Glutamic acid	89.8	88.2	89.2	89.8	1.87
Glycine[3]	80.7	76.8	78.9	79.1	2.70
Proline[3]	84.8	80.8	83.1	82.8	2.13
Serine[3]	85.5	82.7	83.5	83.7	2.49
Tyrosine[3]	85.7	82.3	84.2	84.9	2.81
n[6]	8	8	8	8	

[1]Broiler chicks were on trial from 8 to 22 d of age.

[2]PC = positive control; NC = negative control; FTU = phytase units.

[3]Contrast of positive versus unsupplemented negative control diets ($P < 0.05$).

[4]Linear response to graded levels of Phyzyme XP phytase ($P < 0.05$).

[5]Quadratic response to graded levels of Phyzyme XP phytase ($P < 0.01$).

[6]Cage served as the experimental unit with 6 chicks per cage and 8 replicate cages per diet.

leucine, methionine, phenylalanine, tryptophan, valine, aspartic and glutamic acids, proline, and tyrosine (linear, $P < 0.05$). Dry matter, nitrogen, phosphorus, arginine, aspartic acid, cysteine, and proline additionally exhibited a quadratic response to phytase supplementation ($P < 0.05$). Differences in apparent nutrient retention were observed between the positive and negative control diets for dry matter, gross energy, phosphorus, calcium, arginine, aspartic acid, glutamic acid, lysine, proline, serine, and tryptophan ($P < 0.05$). In contrast to apparent ileal digestibility values, apparent retention values of the affected nutrients and gross energy were higher for birds fed the negative control diet compared with those fed the positive control diet with the exception of tryptophan.

DISCUSSION

The combined objective of the 2 trials presented herein was to evaluate the efficacy of a microbial phytase on growth performance and nutrient metabolism of broiler chicks as fed in corn-soy diets. Supplementation of P-deficient diets with microbial phytase significantly improved growth performance, bone mineralization, and nutrient utilization. This was interpreted as a phytase-induced release of phytate-bound P. The ability of phytase to improve P availability by hydrolyzing phytate-bound P in poultry diets is well documented (Broz et al., 1994; Coelho and Kornegay, 1996; Kornegay et al., 1996; Qian et al., 1996, 1997). Increased utilization of P from phytate can therefore reduce supplementation of diets with inorganic P sources while maintaining normal growth of the bird. Many researchers have observed an improvement, due to dietary phytase supplementation, in BW gain and feed intake during the first 21 d of age (Broz et al., 1994; Sebastian et al., 1996; Cabahug et al., 1999), whereas others reported no effect (Perney et al., 1993; Boling-Frankenbach et al., 2001). These contrasting results may be due to a number of factors including phytase source, ingredients (type, source, phytate content), and dietary characteristics (processing, Vitamin D_3 level, Ca:P ratio) (Ravindran et al., 1995a).

In the current study, phytase supplementation increased BW gain, feed intake, and feed efficiency in both trials during the starter phase. Improvements in feed efficiency were 1.0 and 7.7% with phytase supplementation of 500 and 1,000 FTU/kg, respectively, in trial 1, and 0.9 and 4.1%, respectively, at the corresponding phytase levels in trial 2 when compared with the negative control diet. These improvements were due to larger increases in weight gain rather than feed intake, suggesting the

TABLE 6. Efficacy of microbial phytase on apparent retention (%) of dry matter, nitrogen, gross energy, phosphorus, calcium, and amino acids for male broiler chicks,[1] trial 1

	Diet[2]				
	PC	NC	NC + 500 FTU/kg Phyzyme XP	NC + 1,000 FTU/kg Phyzyme XP	SD
Dry matter[3,4]	68.6	70.9	72.5	71.3	1.29
Nitrogen[4,5]	51.9	52.5	58.2	56.4	2.97
Gross energy[3]	72.5	73.8	74.9	74.2	1.07
Phosphorus[3,4,5]	40.6	54.5	66.4	68.6	3.87
Calcium[3]	46.5	60.7	64.7	67.3	5.54
Amino acids					
Indispensable					
Arginine[3,4,5]	93.1	93.7	94.4	94.4	0.37
Histidine[5]	89.9	89.8	90.7	90.7	0.80
Isoleucine[5]	88.3	88.6	90.0	90.1	1.22
Leucine	88.9	89.4	90.2	90.3	0.93
Lysine[3]	90.2	91.2	91.9	91.9	0.77
Methionine[5]	93.0	92.8	92.9	93.5	0.59
Phenylalanine[5]	89.0	89.7	90.5	90.7	0.89
Threonine	82.8	83.5	84.4	83.9	1.20
Tryptophan[3,5]	90.1	89.2	89.9	90.1	0.77
Valine[5]	86.9	87.0	88.7	88.6	1.28
Dispensable					
Alanine	84.2	84.7	85.7	85.5	1.55
Aspartic acid[3,4,5]	88.6	89.7	90.6	90.5	0.69
Cysteine[4]	81.4	81.2	82.7	81.4	1.58
Glutamic acid[3,5]	91.9	92.6	93.3	93.2	0.54
Proline[3,4,5]	87.2	86.0	87.6	87.3	0.94
Serine[3]	87.4	89.5	90.1	89.8	0.89
Tyrosine[5]	89.9	90.3	91.0	91.3	0.74
n[3]	8	8	8	8	

[1]Broiler chicks were on trial from 8 to 22 d of age.

[2]PC = positive control; NC = negative control; FTU = phytase units.

[3]Contrast of positive versus unsupplemented negative control diets ($P < 0.05$).

[4]Quadratic response to graded levels of Phyzyme XP phytase ($P < 0.05$).

[5]Linear response to graded levels of Phyzyme XP phytase ($P < 0.05$).

[6]Cage served as the experimental unit with 6 chicks per cage and 8 replicate cages per diet.

broiler could more efficiently utilize dietary P with phytase supplementation. Throughout the 42-d growing period in trial 2, improvements in BW gain and feed intake were observed, reaching a plateau with phytase supplementation of 750 FTU/kg. However, a phytase response in feed efficiency was seen only for the starter feeding phase, as similarly reported by Sohail (1999).

Microbial phytase increased tibia and toe ash content in both trials, supporting the observed improvements in growth performance noted by others (Denbow et al., 1995). Both tibia (Broz et al., 1994) and toe (Denbow et al., 1995) ash contents observed in the current study were similar to those reported in the literature. Ravindran et al. (1995b) showed that bone mineralization criteria are more sensitive indicators of P status in the bird than are growth criteria. Hence, as P is a major component of the skeletal system, this observation reiterates that bone mineralization requirements are met before growth when P nutrition is the focus.

Results from the current study suggest that microbial phytase-mediated hydrolysis of phytate-bound P is responsible for the observed growth improvements. However, weight gain and feed efficiency were numerically greater in birds fed the negative control diet supplemented with 1,000 FTU/kg Phyzyme XP compared with the positive control diet, possibly highlighting an additional role of phytase in growth promotion. Hydrolysis and subsequent utilization of phytate-associated nutrients including proteins, lipids, carbohydrates, and minerals (including divalent cations such as Cu^{2+}, Zn^{2+}, and Ca^{2+}) may be involved in these growth improvements (Ravindran et al., 1995a). In the current study, apparent ileal dry matter, nitrogen, and calcium digestibilities did not respond to phytase addition whereas growth performance and bone mineralization were concurrently affected. Apparent ileal amino acid digestibilities of broiler chicks in this study were consistent with reported values (Namkung and Leeson, 1999; Zhang et al., 1999). Only 2 indispensable amino acids, tryptophan and valine, were shown to be affected by phytase addition and improvements in the digestibility of these 2 amino acids was less than 2 percentage units. In a study by Sebastian et al. (1997), only improvements in apparent ileal digestibility of methionine and phenylalanine were seen. This, taken with the fact that pH is likely a contributing factor in the formation of phytate-protein complexes within the gizzard-proventriculus of the chicken (Adeola and Sands, 2003), suggests that this microbial phytase may not have liberated sufficient quantities of non-P nutrients to affect digestibility.

The product of percentage P digestibility (53%) and analyzed P (3.70 g/kg) in the negative control diet gives digested P at 1.96 g/kg; the difference—undigested P at 1.74 g/kg—would be available for hydrolysis by the added microbial phytase. Given that the addition of 500 or 1,000 units of microbial phytase to the negative control diet reduced digested P from 1.74 to between 1.15 and 1.27 g/kg, it follows that the added microbial phytase hydrolyzed approximately 30% of the undigested P. The efficiency of the hydrolytic process is dependent on several factors such as phytase activity, form and location of phytate in feedstuffs, and conditions in the gastrointestinal tract (Adeola and Sands, 2003). This dependency raises more research questions in the improvement of the efficiency of hydrolysis of the phytate molecule.

In slight contrast, apparent nutrient retentions of dry matter, nitrogen, and many of the amino acids were affected by phytase supplementation. The average improvement in combined indispensable amino acid retention with addition of 1,000 FTU/kg microbial phytase was 1.0% when compared with the negative control diet, with 7 of these 10 amino acids being statistically affected by phytase supplementation. At the same time, higher amino acid retention values were observed for the negative vs. positive control diet, which was opposite that of apparent ileal amino acid digestibilities. This observation suggests that the negative control was adequate in dietary nutrients other than P and that the bird is more efficient at extracting P in diets deficient in this nutrient. In this case, the influence of hindgut microbial fermentation of these nutrients may have played a small but significant role. Degradation or modification of dietary amino acids by the microflora could reduce amino acid levels in excreta output and therefore artificially inflate retention values as there is little nutrient absorption past the distal ileum. This was deduced on the basis of the observation that the release of nutrients other than P by microbial phytase was more evident in retention measures than in digestibility. The most affected nutrient was calcium, which has been shown by others to be improved by phytase addition (Sebastian et al., 1996; Zanini and Sazzad, 1999). As the pH conditions within the intestinal tract are favorable for phytate-divalent cation complex formation, microbial phytase hydrolysis of phytate likely ameliorated these complexes and therefore allowed greater calcium utilization. Also, this observation is likely a response to the increased retention of P as an attempt by the bird to maintain a favorable ratio of these 2 nutrients for physiological normality.

In conclusion, the new microbial phytase evaluated in these 2 trials was shown to improve growth performance, bone mineralization, and P utilization in male broiler chicks fed corn-soy based diets up to 6 weeks of age. As this phytase product was derived from *Escherichia coli* and expressed in *Schizosaccaromyces pombe*, it was important to determine the efficacy with which it liberated phytate-bound P in typical broiler diets. Phyzyme XP was able to overcome the 52% reduction in nonphytate P resulting from decreased inclusion of an inorganic P source. In comparison with the unsupplemented negative control diet, this microbial phytase was able to liberate approximately 30% more P from the undigested phytate-P as calculated from both nutrient digestibility and retention and thereby significantly reduce the excretion of P by the broiler chick.

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