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Response of broiler chickens in the starter and finisher phases to 3 sources of microbial phytase

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ABSTRACT A broiler chicken study was conducted for 42 D to evaluate their responses to 3 commercially available microbial phytases. Growth performance, nutrient digestibility, and bone mineralization at days 21 and 42 posthatching were used as parameters of evaluation. The study was a randomized complete block design with 12 treatments, 8 replicate pens, and 25 birds per pen. Treatments included a positive control (**PC**), a negative control (**NC**) with crude protein (**CP**), non-phytate phosphorus (**P**), and calcium (**Ca**) reduced by 18, 1.5, and 1.8 g/kg, respectively; the NC + 4 levels of phytase A (250, 500, 750, 1,000 FTU/kg), 3 levels of phytase B (250, 500, 750 FTU/kg), and 3 levels of phytase C (500, 750, 1,000 FTU/kg). Broilers fed the NC diet had reduced ($P < 0.05$) performance and digestibility measures at days 21 and 42 relative to the PC. All phytase enzymes improved ($P < 0.05$) BW, gain, feed efficiency, and tibia ash weight and percent. Inclusion of phytase at

the highest levels improved ($P < 0.05$) tibia ash weight by an average of 18.5 and 22% at days 21 and 42, respectively, over the NC. Phytase A linearly improved ($P < 0.05$) the apparent ileal digestibility (**AID**) of DM, Ca, P, copper, and sodium at day 21, and the AID of energy, nitrogen, and all amino acid (**AA**) digestibility at day 42 posthatching. Phytase B linearly ($P < 0.05$) improved BW gain and feed efficiency of birds at day 21 and quadratically improved ($P < 0.05$) the AID of nitrogen and all AA in birds at day 42. Supplementation of birds fed the NC with phytase C linearly improved ($P < 0.05$) the BW gain, feed intake, feed efficiency, and AID of DM, energy, nitrogen, all AA, and all minerals except manganese at day 42. In conclusion, all 3 phytase products improved the growth performance, nutrient and mineral digestibility, and bone mineralization of birds fed diets deficient in nitrogen, Ca, and P similar to or more than birds fed diet adequate in P and CP.

Key words: broilers, digestibility, performance, phytase, tibia ash

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INTRODUCTION

Phosphorus (**P**) utilization inefficiency is of economic importance because of the negative impact on the environment in form of eutrophication and disruption of aquatic life (Edwards and Daniel, 1992). Phytate is a major form of P in cereal grains and oil seeds used in the poultry industry. Owing to the inability of broiler chickens to fully hydrolyze phytate and utilize P from plant-based feeds, there has been an increase in the wastage of P into the soil and in water bodies. Phytase is an enzyme that has been in existence since the 1970s, and it was first used by Nelson et al. (1968) who

postulated that phytase has the ability to hydrolyze phytate in broiler chickens thus releasing P for use by the birds. Phytase can be sourced from either plant-based materials or from microbes such as fungi and bacteria (Nys et al., 1996). Microbial phytases can be 3-phytases (such as from *Aspergillus ficuum*) or 6-phytases (such as from *Escherichia coli*) and are capable of hydrolyzing phytate bonds at carbon 3 or 6, respectively, to release the initial P (Walk et al., 2012). They are mostly heat stable and have been commercialized for use by the poultry and swine industry.

Broiler chickens are rapidly growing meat-type birds with a high demand for P and calcium (**Ca**) necessary to support their skeletal system and other biological functions. Previous studies have found that the supplementation of broiler diets, low in inorganic P, with microbial phytase during the starter and finisher phases improved their BW gain, feed intake (**FI**), feed efficiency, energy, and amino acid (**AA**) digestibility (Simons et al., 1990; Namkung and Leeson, 1999;

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Dilger et al., 2004). Phytase has also been reported to improve bone strength and ash and to improve the digestibility of minerals in broiler chickens (Paiva et al., 2014; Babatunde et al., 2019a,b). However, there is need for more information on the impact of new and different sources of microbial phytase on broiler chickens fed nonphytate P-deficient diets during the starter and finisher phases in the same trial. Thus, in this current trial, we tested the null hypothesis that there is no impact on the response of broiler chickens to different microbial sources of phytase from the starter to grower phase. The objective of this study was to evaluate the response of broiler chickens to 3 different microbial phytase sources using growth performance, nutrient and mineral digestibility, and bone mineralization as response criteria.

MATERIALS AND METHODS

Animal experiments were carried out in accordance with guidelines approved by the Purdue University Animal Care and Use Committee.

Birds, Housing, and Experimental Design

A total of 2,400 Ross 308, day-old male broiler chicks was used for this experiment. They were obtained from a commercial hatchery, tagged, weighed individually, and housed in floor pens within the broiler research barn at the Poultry Unit of the Purdue University Animal Science Research and Education Center. The barn consisted of 4 rooms, with 24 pens each, and radiating from a shared feed storage room. Room temperature was maintained at 35°C for the first week, 32°C for the second week, and 27°C for the last 4 week. Birds were randomly assigned to 96 pens based on a randomized complete block design with 12 dietary treatments, 8 replicates, and 25 birds per pen. Body weight served as the blocking factor.

Experimental Diets and Phytase

Experimental diets were fed over a 2-phase feeding system with the starter phase starting from day 0 to day 21 posthatching and the finisher phase starting at day 21 until day 42 posthatching. The 12 diets were in mash form and consisted of a positive control (PC) formulated to meet or exceed the nutrient requirements of broiler chickens at the starter and finisher phase as stated in the National Research Council recommendation (1994). A negative control (NC) comparable with the PC but with the crude protein (CP) reduced by 18 g/kg, and levels of monocalcium phosphate and limestone manipulated to reduce the concentrations of P and Ca by 1.6 and 1.8 g/kg, respectively. The NC with 3 sources of microbial phytase consisting of 4 graded levels of phytase A (250, 500, 750 and 1,000 FTU/kg; Natuphos E, BASF Corporation, Florham Park, NJ; **Phy A**); 3 graded levels of phytase B (250, 500 and 750 FTU/kg; **Phy B**); and 3

graded levels of phytase C (500, 750, and 1,000 FTU/kg; **Phy C**). Phytase levels were not even for the 3 sources of phytase because of limitations in the amount of treatments that could be accommodated at our facility at the same time. Phytase doses were also based on manufacturers' recommendations. The P replacement values (g/kg diet) of Phy A, B, and C at 500 and 750 FTU/kg diet were 1.49 and 1.83, 1.46 and 1.65, and 1.00 and 1.30, respectively. The ingredient composition and calculated energy and AA and nutrients concentration of the PC and NC at the starter and finisher phase are presented in Table 1. All phytase products were commercially available and are feed additives declared safe for use in avian species. Phytase A is a microbial 6-phytase of which the phytase gene is assembled from a hybrid of phytase-producing bacteria and produced through the fermentation of the fungus *Aspergillus niger*. Similarly, Phy B and Phy C are microbial 6-phytases but produced from the fungi *Trichoderma reesei* and *Aspergillus oryzae*, respectively. The phytase premixes were prepared with ground corn to contain 25 FTU per g of premix and added at 10, 20, 30, or 40 g/kg to the NC diet depending on the concentration of phytase in the diet. One FTU is defined as the activity that liberates 1 μmol of inorganic phosphate from 5.0 mM sodium phytate per minute at 37°C and pH 5.5 (International Union of Biochemistry, 1979). Titanium dioxide was added to the diets as an inert marker at 5 g/kg to determine ileal digestibility using the index method as previously described by Adedokun et al. (2004).

Sample Collection and Chemical Analysis

Birds in each pen were weighed individually on day 21 and 42 posthatching. Similarly, leftover feed was weighed, recorded, and used to determine FI and efficiency of feed utilization. Mortality was recorded throughout the experimental period, and FI per pen was adjusted accordingly. Five median-weight birds from each pen were euthanized on day 21 and 42 by CO₂ asphyxiation, and ileal digesta was collected from the distal two-thirds section of the ileum, flushed with water, pooled by pen, and stored at -20°C until further analyses. The section of the intestine from the Meckel's diverticulum to the ileo-cecal junction was considered as the ileum. The tibiae from the same 5 birds were collected on each day, defatted, weighed, and ashed at 600°C for 24 h in a muffle furnace to determine tibia ash (Adedokun et al., 2004). Tibia ash was reweighed to determine ash weight per bone. Ileal digesta samples were freeze-dried to constant weight and ground in a coffee grinder, whereas experimental diets were ground to pass through a 0.5-mm screen (Retsch ZM 100, GmbH, Haan, Germany) before analysis. Diets and ileal digesta samples were analyzed for DM content by drying in a forced-air oven at 105°C for 24 h (The Precision Scientific Co., Chicago, IL; method 934.01; AOAC, 2006). Gross

Table 1. Ingredients and nutrient composition of starter and finisher diets for broiler chicken.

Item ¹	Starter		Finisher	
	PC	NC	PC	NC
Ingredients, g/kg				
Corn	456.7	529.0	538.6	619.6
Soybean meal, 480 g/kg CP	390.0	338.0	315.0	260.0
Soybean oil	50.0	38.0	48.0	32.2
Limestone	16.5	15.0	16.5	13.0
Monocalcium phosphate	15.3	8.5	11.0	4.2
Salt	3.5	3.5	3.0	3.0
Vitamin mineral premix ²	3.0	3.0	3.0	3.0
Phytase premix A (Phytase A) ³	-	-	-	-
Phytase premix B (Phytase B) ⁴	-	-	-	-
Phytase premix C (Phytase C) ⁵	-	-	-	-
Ground corn	40.0	40.0	40.0	40.0
Titanium dioxide premix ⁶	25.0	25.0	25.0	25.0
Total	1,000.0	1,000.0	1,000.0	1,000.0
Calculated nutrients and energy, g/kg				
CP	229	211	201	181.6
ME, kcal/kg	3,124	3,139	3,198	3,203
Ca	9.9	8.1	9.0	6.5
P	7.1	5.5	6.0	4.4
Phytate P	2.6	2.5	2.5	2.4
Nonphytate P	4.5	3.0	3.5	2.0
Ca:tP	1.4	1.5	1.5	1.5
Ca:nPP	2.2	2.7	2.6	3.2
Amino acids, g/kg				
Arg	16.0	14.0	13.0	12.0
His	6.0	6.0	5.0	5.0
Ile	10.0	9.0	8.0	7.0
Leu	20.0	18.0	18.0	16.0
Lys	13.0	11.0	11.0	9.0
Met	4.0	3.0	3.0	3.0
Cys	4.0	3.0	3.0	3.0
Met + Cys	7.0	7.0	7.0	6.0
Phe	11.0	10.0	10.0	9.0
Tyr	9.0	8.0	8.0	7.0
Phe + Tyr	20.0	18.0	18.0	16.0
Thr	9.0	8.0	8.0	7.0
Trp	3.0	3.0	3.0	2.0
Val	11.0	10.0	9.0	8.0

¹Diets PC = positive control, NC = negative control.

²Supplied the following quantities per kg of diet: vitamin A, 5,484 IU; vitamin D₃, 2,643 ICU; vitamin E, 11 IU; menadione sodium bisulfite, 4.38 mg; riboflavin, 5.49 mg; D-pantothenic acid, 11 mg; niacin, 44.1 mg; choline chloride, 771 mg; vitamin B₁₂, 13.2 µg; biotin, 55.2 µg; thiamine mononitrate, 2.2 mg; folic acid, 990 µg; pyridoxine hydrochloride, 3.3 mg; I, 1.11 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; Se, 300 µg.

³Phytase premix was prepared with Phytase A and corn to contain 25 FTU per g corn. Premix was added to the NC diets (starter and finisher) at the expense of ground corn to supply 250, 500, 750, and 1,000 FTU/kg diet.

⁴Phytase premix was prepared with Phytase B and corn to contain 25 FTU per g corn. Premix was added to the NC diets (starter and finisher) at the expense of ground corn to supply 250, 500, and 750 FTU/kg diet.

⁵Phytase premix was prepared with Phytase C and corn to contain 25 FTU per g corn. Premix was added to the NC diets (starter and finisher) at the expense of ground corn to supply 500, 750, and 1,000 FTU/kg diet.

⁶Prepared as 1 g titanium dioxide added to 4 g corn.

energy was determined in the diet and ileal digesta samples using an isoperibol bomb calorimeter (model 1261; Parr Instrument Co., Moline, IL). The nitrogen content of experimental diets and ileal digesta was determined by combustion method (model FP-2000 nitrogen analyzer; Leco Corp., St. Joseph, MI; method 990.03; AOAC, 2000), and the CP concentration was obtained by multiplying the nitrogen concentration by 6.25. Concentrations of AA in experimental diets and ileal digesta were analyzed (method 982.30 E (a, b, c); AOAC, 2006) at the University of Missouri

Agricultural Experiment Station Chemical Laboratories (Columbia, MO). The concentrations of Ca in the ileal digesta and diet samples were determined by flame atomic absorption spectroscopy after wet digestion using a Varian Spectr.AA 220FS (Varian Australia Pty Ltd., Victoria, Australia) in methods described by Iyayi et al. (2013), whereas the concentration of P in the digested ileal digesta and diet samples was estimated by spectrophotometry with absorbance read at 630 nm (Spark 10 M; Tecan Group Ltd., Männedorf, Switzerland). Sodium (Na), copper (Cu),

zinc (**Zn**), magnesium (**Mg**), and manganese (**Mn**) concentrations in the diet and ileal samples were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories. Concentrations of titanium (**Ti**) in the diet and ileal samples were analyzed as described by Myers et al. (2004).

Calculations and Statistical Analysis

The apparent ileal digestibility (**AID**) of nutrients in the diets was determined using the index method by the following equation (Dilger et al., 2004)

$$\text{AID}(\%) = 100 - [(T_{i1} / T_{i0}) \times (N_o / N_i) \times 100]$$

where T_{i1} is Ti concentration in the diet, T_{i0} is the concentration of Ti in the ileal digesta, N_o is the concentration of nutrients in the ileal digesta, and N_i is the concentration of nutrient in the diet. Concentrations of Ti and nutrients in this equation were expressed as grams per kilogram DM.

Data were analyzed in SAS (9.4; SAS Inst. Inc., Cary, NC.) using the general linear model procedure. Fixed effects included diet type (PC or NC), phytase type (A, B, or C), and phytase level (250, 500, 750, or 1,000 FTU/kg). Polynomial contrasts were used to compare the PC and NC diets and to determine the linear and quadratic effects of phytase supplementation in the NC diets. Proc IML was used to generate contrast coefficients for Phytase C because treatments were unequally

spaced (0, 500, 750, and 1,000 FTU/kg). Statistical significance was set at $P \leq 0.05$.

RESULTS

There was no negative impact of any of the microbial phytase products on health and general well-being of the broiler chickens. Growth performance responses of broiler chickens to phytase from day 0 to 21 and day 21 to 42 posthatching are presented in Table 2. At day 42, the overall BW, BW gain, and feed efficiency of birds fed the PC diet was greater ($P < 0.01$) by 15.6, 15.8, and 10.4%, respectively, relative to birds on the NC diet. There was no difference in FI between birds fed the PC and NC diets at day 21, but birds fed the PC had a greater FI at day 42 as compared with birds on the NC diet. There was no effect of Phy A supplementation on performance of birds from day 0 to 21 posthatching. However, Phy A improved ($P < 0.01$) BW, BW gain, FI, and efficiency in birds from day 21 to 42 posthatching as compared with birds on the NC diets. A quadratic response ($P < 0.01$) was observed with feed efficiency in birds fed diets supplemented with Phy A at day 42. The 500 and 750 FTU/kg dosage elicited a greater feed efficiency in birds (527 and 520 g/kg, respectively) as compared with 1,000 FTU/kg (512 g/kg) during the same period. Birds supplemented with Phy B had increased performance relative to birds on the NC diet with a linear increase ($P < 0.01$) in BW,

Table 2. Effects of supplemental phytase on growth performance (days 0–21 and 21–42) in broiler chickens.¹

Item	Day 0–21				Day 21–42			
	BW (day 21), g	BW gain, g/bird	Feed intake, g/bird	G:F, g/kg	BW (day 42), g	BW gain, g/bird	Feed intake, g/bird	G:F, g/kg
Positive control (PC)	706	658	929	706	2,520	1,794	3,260	551
Negative control (NC)	621	571	925	620	2,126	1,492	3,007	497
NC + Phytase A (FTU/kg)								
250	653	606	909	664	2,291	1,620	3,143	515
500	660	610	926	658	2,359	1,671	3,172	527
750	651	602	890	674	2,350	1,676	3,224	520
1000	658	607	935	650	2,314	1,636	3,195	512
NC + Phytase B (FTU/kg)								
250	619	571	884	646	2,207	1,556	3,022	515
500	639	591	914	648	2,288	1,630	3,142	519
750	651	601	918	656	2,331	1,660	3,220	515
NC + Phytase C (FTU/kg)								
500	654	606	910	666	2,320	1,638	3,173	516
750	657	608	955	635	2,365	1,692	3,233	523
1000	648	599	912	658	2,313	1,644	3,146	523
SEM	11.7	11.7	21.2	11.5	29.7	23.7	37.1	5.2
<i>P</i> -value of contrasts								
PC vs. NC	<0.01	<0.01	0.89	<0.01	<0.01	<0.01	<0.01	<0.01
Phytase A								
Linear	0.06	0.07	0.97	0.07	<0.01	<0.01	<0.01	0.04
Quadratic	0.15	0.11	0.40	0.01	<0.01	<0.01	0.03	<0.01
Phytase B								
Linear	0.05	0.05	0.91	0.05	<0.01	<0.01	<0.01	0.02
Quadratic	0.55	0.69	0.31	0.43	0.52	0.51	0.41	0.05
Phytase C								
Linear	0.07	0.06	0.96	0.07	<0.01	<0.01	<0.01	<0.01
Quadratic	0.14	0.12	0.72	0.27	<0.01	<0.01	<0.01	0.23

¹Data are least squares mean of 8 replicate pens of 25 birds per pen.

BW gain, and feed efficiency at day 21 and 42 post-hatching and a quadratic response ($P < 0.05$) in FI at day 42 posthatching. Supplementation of Phy B in diets at 750 FTU/kg elicited the greatest increase in broiler chicken performance within the group except with feed efficiency at day 42. Supplementation with Phy C elicited a quadratic response ($P < 0.01$) in BW, BW gain, and FI at day 42 posthatching with 750 FTU/kg having the highest response. A linear increase ($P < 0.01$) in feed efficiency of birds at day 42 post-hatching was also observed with Phy C supplementation. Overall growth performance results (day 0–42 posthatching) revealed increased ($P < 0.01$) responses in BW gain, FI, and efficiency in birds fed the PC vs. the NC diets (Table 3). At day 21 posthatching, birds fed the NC diet had a lower ($P < 0.01$) percentage tibia ash and tibia ash weight per bone as compared with birds fed the PC. This decrease ($P < 0.01$) was only observed in tibia ash weight per bone of birds fed the NC diet (2,320 mg/bone) at day 42 posthatching as compared with birds fed the PC diet (3,180 mg/kg; Table 3). There was a quadratic response ($P < 0.05$) in percentage tibia ash at day 21 posthatching with all 3 microbial phytases. However, only Phy C elicited a quadratic response in weight of tibia ash at day 21 posthatching. Phytase A linearly increased ($P = 0.03$) percentage tibia ash at day 42 posthatching, whereas a quadratic response ($P < 0.05$) in the weight of tibia ash was observed with all 3 phytases.

There was no difference in the AID of DM, energy, Ca, Mg, and Mn in birds fed the PC and NC diets at day 21 posthatching. However, birds fed the PC diets had greater AID of P, Na, Cu, and Zn as compared with birds fed the NC diets (Table 4). Supplementation of NC with Phy A quadratically increased ($P < 0.05$) AID of DM, energy, and all minerals except Ca, Cu, and Mn in broiler chickens at day 21 posthatching with 750 FTU/kg eliciting the highest response. However, a quadratic decrease ($P < 0.05$) was observed with Ca digestibility and a linear increase ($P < 0.01$) with Cu digestibility. There was no response in the AID of Ca and Na with the supplementation of Phy B; however, a quadratic response ($P < 0.01$) was observed in the AID of DM and energy peaking at 73.3 and 75.6%, respectively, with supplementation at 500 FTU/kg. Phytase B quadratically improved ($P < 0.01$) the digestibility of P, Zn, and Mg in broiler chickens at day 21 posthatching, with the highest response observed at 500 FTU/kg. Supplementing the NC diet with Phy C at 750 FTU/kg improved the AID of DM, energy, and P by 6, 4, and 16.5%, respectively, when compared with birds fed the NC diet. There was no effect of Phy C on Ca digestibility at day 21 posthatching. However, there was a linear reduction in Ca digestibility at day 42 (Table 5). At day 42 posthatching, birds fed the PC diets had greater digestibility of DM, energy, and all minerals except P, Mg, and Mn relative to birds fed the NC diets. All 3 phytase products linearly increased the AID

Table 3. Effects of supplemental phytase on overall growth performance (days 0–42) and tibia ash in broiler chickens.¹

Item	Day 0–42			Tibia ash (day 21)		Tibia ash (day 42)	
	BW gain, g/bird	Feed intake, g/bird	G:F, g/kg	% Of tibia	Weight, mg/bone	% Of tibia	Weight, mg/bone
Positive Control (PC)	2,451	4,188	586	50.5	1,020	48.7	3,180
Negative Control (NC)	2,063	3,932	525	47.0	760	48.2	2,320
NC + Phytase A (FTU/kg)							
250	2,225	4,052	549	48.3	840	48.4	2,820
500	2,281	4,098	556	49.5	880	49.5	2,880
750	2,278	4,114	554	49.9	920	49.1	2,980
1000	2,243	4,130	543	49.9	960	50.3	3,020
NC + Phytase B (FTU/kg)							
250	2,127	3,905	545	48.4	800	49.3	2,680
500	2,220	4,057	548	49.8	880	50.1	2,840
750	2,261	4,138	546	49.8	900	49.9	2,960
NC + Phytase C (FTU/kg)							
500	2,244	4,083	549	48.8	880	48.8	2,900
750	2,300	4,187	549	48.8	880	48.8	2,860
1000	2,244	4,058	553	49.3	900	49.3	2,960
SEM	30.2	46.5	5.3	0.3	20.0	0.7	60.0
<i>P</i> -value of contrasts							
PC vs. NC	<0.01	<0.01	<0.01	<0.01	<0.01	0.55	<0.01
Phytase A							
Linear	<0.01	<0.01	0.02	<0.01	<0.01	0.03	<0.01
Quadratic	<0.01	0.18	<0.01	<0.01	0.42	0.89	<0.01
Phytase B							
Linear	<0.01	<0.01	<0.01	<0.01	<0.01	0.06	<0.01
Quadratic	0.72	0.27	0.05	<0.01	0.38	0.30	0.04
Phytase C							
Linear	<0.01	0.01	<0.01	<0.01	<0.01	0.28	<0.01
Quadratic	<0.01	0.02	0.13	0.02	0.01	0.99	<0.01

¹Data are least squares mean of 8 replicate pens of 20 birds per pen.

Table 4. Effects of supplemental phytase on apparent ileal digestibility of DM, energy, and minerals in 21-day-old broiler chickens.¹

Item	Apparent ileal digestibility, %								
	DM	Energy	Ca	P	Na	Cu	Zn	Mg	Mn
Positive control (PC)	69.2	72.7	58.4	59.2	-4.2	20.7	18.7	22.4	1.5
Negative control (NC)	68.1	70.6	62.5	51.5	-48.0	-0.3	-20.6	13.6	1.5
NC + Phytase A (FTU/kg)									
250	73.2	74.3	62.6	59.3	-13.0	26.5	15.6	29.4	33.2
500	72.3	74.8	58.1	60.9	-18.2	8.3	25.1	29.9	8.3
750	75.0	76.3	55.8	63.7	-14.3	29.2	40.3	34.6	0.2
1000	71.3	72.8	63.1	63.2	-17.5	25.4	27.3	25.4	22.6
NC + Phytase B (FTU/kg)									
250	71.3	74.1	65.6	60.1	-27.8	9.9	9.2	25.0	10.0
500	73.3	75.6	65.7	62.7	-56.0	30.4	20.9	34.2	-2.3
750	69.7	72.2	62.5	58.4	-24.6	35.5	4.3	20.5	-2.1
NC + Phytase C (FTU/kg)									
500	69.9	72.3	62.6	57.7	-25.8	37.4	29.4	25.4	9.8
750	72.6	74.9	66.5	61.7	-16.7	18.1	22.8	27.9	23.3
1000	71.3	73.5	63.0	59.3	-35.2	18.1	31.7	19.3	37.7
SEM	1.1	1.1	2.1	1.5	7.9	3.3	3.6	3.2	2.0
<i>P</i> -value of contrasts									
PC vs. NC	0.49	0.15	0.17	<0.01	<0.01	<0.01	<0.01	0.06	0.98
Phytase A									
Linear	0.02	0.06	0.40	<0.01	0.02	<0.01	<0.01	<0.01	0.16
Quadratic	<0.01	<0.01	0.04	<0.01	0.03	0.07	<0.01	<0.01	0.81
Phytase B									
Linear	0.15	0.18	0.99	<0.01	0.24	<0.01	<0.01	0.04	0.01
Quadratic	<0.01	<0.01	0.14	<0.01	0.48	0.43	<0.01	<0.01	0.04
Phytase C									
Linear	<0.01	0.01	0.56	<0.01	0.09	<0.01	<0.01	0.06	<0.01
Quadratic	0.44	0.42	0.61	0.05	0.03	<0.01	<0.01	<0.01	<0.01

¹Data are least squares mean of 8 replicate pens of 5 birds per pen.

of DM, energy, and P in broiler chickens at day 42. In addition, Phy A quadratically improved ($P \leq 0.01$) the AID of Cu, Zn, and Mg, whereas decreasing ($P = 0.05$) the digestibility of Mn. Phytase B at 250 FTU/kg improved the AID of Zn, whereas Phy C quadratically improved ($P < 0.01$) the AID of Cu, while linearly decreasing ($P < 0.01$) Mg digestibility when compared with birds fed the NC diets.

Ileal digestibility responses of nitrogen, essential and nonessential AA in broiler chickens at day 21 post-hatching are presented in Tables 6 and 7. The NC diets had a negative impact on birds by reducing ($P < 0.01$) the AID of nitrogen and all AA including Lys, Met, and Thr as compared with birds fed the PC diets at day 21 posthatching. In particular, the AID of nitrogen, Met, Lys, and Thr was reduced by 4, 4.5, 3.4, and 6%, respectively, whereas Cys digestibility was reduced by up to 11% in birds fed the NC diets relative to the PC. A quadratic response ($P < 0.01$) was observed in the AID of nitrogen and all AA with Phy A, as further supplementation at 1,000 FTU/kg elicited a lower improvement when compared with birds fed 750 FTU/kg. Phytase B supplementation elicited a quadratic response ($P < 0.05$) in the digestibility of most AA except Arg, Met, Trp, and Ser as supplementing the NC with Phy B at 500 FTU/kg resulted in the highest improvement on N and AA digestibility. On the other hand, only a linear increase ($P < 0.01$) was observed in nitrogen and all AA digestibility in diets supplemented with Phy C with the highest level of

supplementation (1,000 FTU/kg) resulting in the highest improvement on protein digestibility.

Apparent ileal digestibility of nitrogen, essential and nonessential AA of birds at day 42 posthatching are presented in Tables 8 and 9. There was a reduction ($P < 0.01$) in the digestibility of nitrogen and AA in birds fed the NC diets which was similar to that observed at day 21. All three microbial phytases linearly improved ($P < 0.01$) the digestibility of nitrogen and AA in birds at day 42. There was a quadratic response ($P < 0.05$) with Phy A and Phy B supplementation, with their penultimate doses (i.e., 750 and 500 FTU/kg respectively) having a reduced improvement in protein digestibility as compared with other doses within each phytase enzyme. However, this trend was not observed with Phy C supplementation as a linear relationship was observed generally with all nitrogen and AA digestibility.

DISCUSSION

Microbial phytase is one of the more commonly used feed additives in the poultry industry. Phytases hydrolyze phytate bonds and release P for use by pigs and chickens especially when feeding P-deficient diets (Adeola and Cowieson, 2011). The over-dependence on inorganic sources of P because of the inability of broiler chickens to fully utilize the P inherent in grains and oil seeds has led to wastage and loss of P into the environment (Panda et al., 2007). Phytate present in most plant materials binds minerals such as Ca, Mg, and Zn,

Table 5. Effects of supplemental phytase on apparent ileal digestibility of DM, energy, and minerals in 42-day-old broiler chickens.¹

Item	Apparent ileal digestibility, %								
	DM	Energy	Ca	P	Na	Cu	Zn	Mg	Mn
Positive Control (PC)	74.8	76.3	49.3	49.6	-11.1	14.6	-15.8	25.9	18.1
Negative Control (NC)	71.1	71.9	63.7	53.2	-94.5	-3.1	32.1	29.3	15.1
NC + Phytase A (FTU/kg)									
250	74.0	75.0	56.6	52.2	-59.1	-27.6	10.5	18.7	5.8
500	72.7	74.0	53.7	57.5	-34.8	16.6	21.8	24.7	-0.1
750	74.9	76.2	51.5	60.7	-42.0	7.2	15.8	27.3	7.6
1000	75.4	76.4	50.9	64.0	-27.2	23.3	26.9	26.8	-2.5
NC + Phytase B (FTU/kg)									
250	76.0	77.5	59.8	56.2	-70.7	-22.1	41.3	23.0	13.9
500	73.8	74.6	54.7	62.5	-14.5	38.6	-19.8	28.6	-0.1
750	75.8	77.7	53.8	65.4	-28.1	-23.5	-5.6	27.2	16.2
NC + Phytase C (FTU/kg)									
500	72.8	74.6	57.3	57.5	-76.1	9.8	3.4	21.0	15.7
750	73.7	75.1	52.5	56.3	-50.1	77.9	17.0	25.6	15.0
1000	74.4	75.8	50.6	60.6	-53.5	26.5	44.3	17.8	10.1
SEM	0.9	0.9	2.0	1.4	7.0	1.6	2.6	1.7	1.6
<i>P</i> -value of contrasts									
PC vs. NC	<0.01	<0.01	<0.01	0.08	<0.01	<0.01	<0.01	0.17	0.17
Phytase A									
Linear	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.53	0.50	<0.01
Quadratic	0.69	0.46	0.09	0.24	<0.01	<0.01	<0.01	0.01	0.05
Phytase B									
Linear	<0.01	<0.01	<0.01	<0.01	<0.01	0.97	<0.01	0.95	0.14
Quadratic	0.10	0.17	0.47	0.98	0.01	<0.01	0.34	0.16	<0.01
Phytase C									
Linear	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.03	<0.01	0.06
Quadratic	0.92	0.52	0.91	0.78	0.67	<0.01	<0.01	0.99	0.05

¹Data are least squares mean of 8 replicate pens of 5 birds per pen.

rendering them unavailable for use by animals. Phytate forms complexes with carbohydrates and proteins and thus, prevents the optimum use of energy and AA by broiler chickens (Selle et al., 2000). With phytase supplementation, broilers extract the P in corn-soybean meal-based diets, especially because P is an important component of various biological processes including skeletal formation and energy transactions. In this study, birds were fed a NC diet deficient in P, Ca, and nitrogen throughout their lifecycle resulting in the decreased growth performance observed in these birds. This is consistent with previous studies where broiler chickens fed nutrient-deficient diets at the starter or finisher phases had lower growth rate, feed intake, and efficiency as compared with birds fed a complete diet (Broz et al., 1994; Santos et al., 2008). Supplementation with any of the microbial phytase products improved the growth performance of broiler chickens at the starter and finisher phases, showing that birds were able to extract nutrients from the otherwise deficient diets and convert them into body mass as observed with Shaw et al. (2011) and Babatunde et al. (2019a). Microbial phytase with a pH range of 2.5 to 5.5 has been shown to be active in the crop and upper section of the digestive tract (Dersjant-Li et al., 2015). Sommerfeld et al. (2018) reported an increase in the disappearance of phytate and its isomers and an increase in the appearance of *myo*-inositol in the crop and distal ileum with phytase supplementation. Thus, with the residence time of digesta in the crop and gizzard, phytase can begin the process of hydrolyzing

phytate and releasing nutrients for use by the birds. In addition, improvements in weight gain and feed efficiency by phytase supplementation could be FI-based, as feed consumption was significantly improved in broiler chickens receiving phytase during the finisher phase. Phytase supplementation improved tibia ash percentage and weight in birds at day 21 and 42 posthatching. This is consistent with studies reporting that birds fed P-deficient diets supplemented with phytase at various levels had improved bone mineralization (Yonemochi et al., 2000; Adedokun et al., 2004; Walk et al., 2012). Phosphorus and Ca are major components of the skeletal system as they are mainly stored in the bones as hydroxyapatite. It is therefore not unexpected that birds fed diets deficient in these minerals would have lower tibia ash and bone mineralization as reported in the current study. It is also expected that with supplementation of phytase, P and Ca bound-up in phytate complexes would be made available for use by the broiler chickens. In this study however, phytase supplementation had minimal effect on percentage tibia ash at day 42 as compared with birds at day 21 posthatching. This could be because of an age factor where younger birds in the starter phase require more deposition of Ca and P in the bones to support the rapid gain in BW characterized by the finisher phase of broiler chickens. It could also be because of the tibia bone used to estimate bone mineralization in older birds. Scholey and Burton (2017) suggested that the femur bone may be more appropriate in estimating bone ash

Table 6. Effects of supplemental phytase on apparent ileal digestibility of indispensable AA in 21-day-old broiler chickens.¹

Item ²	Apparent ileal digestibility, %											
	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	P + T	M + C
Positive control (PC)	92.1	86.4	84.7	86.8	87.3	89.7	86.7	78.9	90.0	83.7	86.4	81.2
Negative control (NC)	90.8	83.4	81.2	84.3	84.1	85.7	83.8	74.2	88.0	79.7	83.5	75.1
NC + Phytase												
A (FTU/kg)												
250	92.8	87.2	85.8	88.0	88.2	89.7	87.7	80.8	90.3	84.5	87.4	81.8
500	92.3	86.6	84.8	87.1	87.1	89.0	87.0	78.6	90.2	83.2	86.6	80.7
750	93.3	88.7	87.3	89.5	89.7	91.0	89.2	82.7	92.0	86.2	88.7	83.9
1000	92.3	87.4	85.3	87.4	87.8	89.1	87.3	79.9	89.8	83.8	87.1	81.0
NC + Phytase												
B (FTU/kg)												
250	91.9	86.0	84.1	86.6	86.9	87.9	86.3	77.9	88.8	82.5	86.0	79.1
500	92.1	87.1	85.1	87.7	87.7	89.1	87.4	80.0	90.1	84.0	87.1	81.0
750	92.5	86.6	84.6	87.2	87.2	88.6	87.0	79.0	89.8	83.2	86.7	80.0
NC + Phytase												
C (FTU/kg)												
500	92.7	86.3	84.2	86.9	86.9	88.5	86.7	78.4	88.9	82.6	86.7	79.8
750	92.1	86.6	84.3	87.1	87.0	88.5	87.0	78.9	89.5	83.0	86.5	80.2
1000	92.7	87.1	85.1	87.8	87.9	89.5	87.5	79.8	90.4	83.6	87.3	81.1
SEM	0.5	0.6	0.7	0.6	0.6	0.7	0.6	0.9	0.5	0.8	0.6	1.1
<i>P</i> -value of contrasts												
PC vs. NC	0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01
Phytase A												
Linear	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Quadratic	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Phytase B												
Linear	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Quadratic	0.47	0.02	0.02	0.02	<0.01	0.06	0.02	0.01	0.36	0.02	0.02	0.02
Phytase C												
Linear	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.002	<0.01	<0.01	<0.01
Quadratic	0.21	0.19	0.30	0.27	0.30	0.43	0.20	0.27	0.57	0.33	0.13	0.24

¹Data are least squares mean of 8 replicate pens of 5 birds per pen.

²P + T = Phenylalanine + Tyrosine, M + C = Methionine + Cysteine.

in broiler chickens at wk 6, as mineralization was still occurring in the femur as compared with the tibia bone. However, there could have been a carryover effect on tibia ash weight from day 21 as birds at day 42 post-hatching had increased tibia ash weight per bone with phytase supplementation.

There has been consistency in reports on the effects of phytase supplementation in mineral digestibility in broiler chickens, and this current study was not an exception. Apparent ileal digestibility of P has been used as a tool to indicate the efficacy of phytase in broiler chickens (Ravindran et al., 2000). In this study, supplementation of broiler diets with phytase (A, B, or C) improved the ileal digestibility of P at the starter and finisher phases, consistent with the report from Woyengo and Nyachoti (2011). There was no effect of Phy B and C on Ca digestibility in the starter phase; however, a quadratic decrease was observed with Phy A. There has been variability in reports on the effects of phytase supplementation on Ca digestibility. Olukosi et al. (2013) and Babatunde et al. (2019a) reported an improvement in Ca digestibility in birds raised until day 21 and fed diets deficient in P but supplemented with phytase. Other reports from Walk et al. (2012) and Amerah et al. (2014) indicated no effects of phytase supplementation on Ca digestibility. However, during the finisher phase in the current trial, the AID

of Ca was reduced by phytase supplementation in birds as indicated by results from day 42. Although differing from reports of previous studies, it is possible that the reduced utilization of Ca with phytase supplementation may have been as a result of the high Ca:P ratio in the NC diet. Birds may have attempted to regulate the homeostasis of P and Ca by reducing the utilization of Ca in the diet, in response to the increased availability of P and even Ca from phytate complexes in the diet. However, more studies may be required to validate this theory. Phytate, which is negatively charged in the intestines, has been known to bind strongly with positively charged metallic ions such as Fe²⁺, Zn²⁺, Ca²⁺, Cu²⁺, and Mg²⁺ forming insoluble chelates and hindering the utilization of these minerals to animals (Barrientos and Murphy, 1996). Similarly, these metallic cations share similar transporters at the brush border membrane of the intestine; thus, with the increasing release of these cations by phytase, there could be antagonistic relationships that negatively alter the digestibility of these minerals (Maenz et al., 1999). In the current study, phytase supplementation improved the overall digestibility of Cu, Zn, Mn, and Mg. Although, there were a lot of variabilities in the effects within each of the phytase products, results were in accordance with Sebastian et al. (1996) who reported an improvement in Cu and Zn utilization with phytase supplementation in male broiler

Table 7. Effects of supplemental phytase on apparent ileal digestibility of nitrogen, dispensable and total AA in 21-day-old broiler chickens.¹

Item	Apparent ileal digestibility, %									
	Nitrogen	Ala	Asp	Cys	Glu	Gly	Pro	Ser	Tyr	Total AA
Positive Control (PC)	82.0	85.3	84.5	72.7	90.2	80.8	85.1	84.3	85.8	85.8
Negative Control (NC)	78.5	82.2	81.7	64.5	88.5	76.4	81.8	80.5	83.1	82.8
NC + Phytase A (FTU/kg)										
250	82.2	86.4	86.4	73.9	91.5	82.3	86.2	85.9	87.0	87.0
500	82.9	85.3	85.5	72.6	90.8	81.2	85.4	84.4	86.0	86.1
750	84.9	88.0	87.7	77.0	92.4	84.4	87.7	87.1	87.9	88.4
1000	82.6	85.8	85.7	73.2	91.1	81.9	85.6	85.2	87.0	86.5
NC + Phytase B (FTU/kg)										
250	81.0	84.6	84.4	70.6	90.3	80.4	84.5	83.4	85.6	85.4
500	82.6	86.2	85.5	72.8	91.0	81.9	85.9	84.9	86.6	86.5
750	81.1	85.4	85.1	71.3	90.7	81.7	85.3	85.3	86.3	86.1
NC + Phytase C (FTU/kg)										
500	80.7	85.0	85.1	71.0	90.7	80.3	84.7	83.9	86.8	85.7
750	81.7	85.3	84.8	72.2	90.4	80.8	85.2	84.1	85.9	85.8
1000	82.7	86.2	85.9	73.1	91.3	81.6	86.0	85.1	86.9	86.6
SEM	0.8	0.7	0.7	1.5	0.5	0.9	0.7	0.7	0.6	0.6
<i>P</i> -value of contrasts										
PC vs. NC	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Phytase A										
Linear	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Quadratic	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Phytase B										
Linear	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Quadratic	0.01	0.03	0.02	0.01	0.03	0.02	0.02	0.08	0.03	0.02
Phytase C										
Linear	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Quadratic	0.92	0.39	0.20	0.21	0.32	0.24	0.41	0.33	0.07	0.28

¹Data are least squares mean of 8 replicate pens of 5 birds per pen.

chickens. Similarly, [Nourmohammadi et al. \(2012\)](#) reported an increase in Mg digestibility by up to 6% in broiler chickens fed diets supplemented with phytase but did not report any effect on Zn digestibility. Minerals play an important role in the physiological and biochemical state of poultry, and any improvement in availability is beneficial for maximal development and health. However, the reduction in the availability of some trace minerals observed in this trial did not appear to hinder growth performance metrics like weight gain or feed efficiency.

In the current study, energy, DM, and CP digestibility were improved with phytase supplementation in accordance with reports from [Namkung and Leeson \(1999\)](#), where AME was improved by 2.3%, and from [Shirley and Edwards \(2003\)](#) who reported a 5% increase in AME when birds were supplemented with phytase (Phy A) at 750 FTU/kg. There have been varying reports on the benefits of phytase addition to protein utilization, and this has stemmed from several factors including dietary levels of nPP and Ca, inert markers used, dietary electrolyte balance, or the inherent digestibility of AA ([Selle and Ravindran, 2007](#)). It has been known that phytate forms complexes with proteins including basic AA such as Lys, His, and Arg in the proventriculus section of the gastrointestinal tract. These complexes are usually not hydrolyzed by pepsin because of the alteration in the nature of the proteins by phytate ([Selle et al., 2000](#)). It is also possible for phytate to

increase the flowrate of basal endogenous losses, thus reducing the apparent ileal digestibility of AA ([Cowieson et al., 2004](#)). Therefore, phytase can partially resolve this situation by hydrolyzing phytate in the crop before it reaches the acidic environment of the proventriculus conducive for the formation of the phytate-protein complex. In the current study, all 3 microbial phytases improved the ileal digestibility of AA including the first 3 limiting AA (Lys, Met, and Thr) in poultry. Similarly, digestibility of all other AA, including the essential and nonessential, were improved by phytase supplementation and in agreement with previous studies ([Namkung and Leeson, 1999](#); [Ravindran et al., 2001](#); [Onyango et al., 2005](#)). This improvement in the utilization of AA, together with enhancement of P utilization are co-responsible for the greater BW and BWG of birds fed the phytase supplemented diets. Amino acids are the building blocks of protein synthesis in the body of broiler chickens, and they are a major component of various biochemical reactions, hormones, enzymes, neurotransmitters, etc. and are required in the formation of cells, tissues, muscle, and meat ([Wu, 2009](#)). Therefore, an increased utilization of AA from diets should result in improved growth performance of broiler chickens characterized by the rapid accumulation of meat over a relatively short period. The supply of AA, P, and other nutrients also has to be optimum to attain the genetic potential of the modern broiler chicken. However, considering that quadratic responses were observed

Table 8. Effects of supplemental phytase on apparent ileal digestibility of indispensable AA in 42-day-old broiler chickens.¹

Item ²	Apparent ileal digestibility, %											
	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	P + T	M + C
Positive Control (PC)	92.0	87.9	85.1	87.1	85.9	87.3	87.3	79.3	90.6	83.5	87.4	82.0
Negative Control (NC)	88.9	82.7	80.1	82.0	81.0	81.0	82.1	70.8	84.0	78.2	82.4	73.6
NC + Phytase A (FTU/kg)												
250	91.2	86.0	84.0	85.9	84.8	86.0	85.9	75.9	87.7	82.3	85.9	79.7
500	91.3	86.4	84.1	85.8	84.6	85.4	86.2	75.8	88.9	82.0	86.3	79.2
750	90.8	85.8	83.7	85.6	84.1	86.1	85.8	75.7	88.5	81.8	85.9	80.4
1000	91.3	86.5	84.3	86.8	84.7	86.3	86.6	76.8	89.3	82.7	86.8	80.4
NC + Phytase B (FTU/kg)												
250	92.1	87.7	85.6	87.4	86.6	88.2	87.5	78.0	88.3	83.9	87.4	81.8
500	91.0	86.1	83.6	85.8	84.6	85.1	85.8	75.7	88.2	81.7	85.9	79.1
750	92.0	87.4	85.1	87.1	85.6	87.1	87.1	77.7	89.3	83.3	87.4	81.8
NC + Phytase C (FTU/kg)												
500	91.2	85.9	83.9	86.1	84.8	86.2	86.3	76.2	87.1	82.1	86.3	79.2
750	91.9	87.2	84.9	87.2	85.5	87.8	87.2	78.1	88.7	83.2	87.3	81.9
1000	92.4	88.2	86.5	87.7	87.5	88.7	88.0	78.6	89.4	85.1	87.3	82.5
SEM	0.4	0.6	0.7	0.6	0.8	0.8	0.6	0.9	0.5	0.7	0.6	0.8
<i>P</i> -value of contrasts												
PC vs. NC	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Phytase A												
Linear	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Quadratic	<0.01	<0.01	<0.01	0.02	0.02	<0.01	<0.01	0.02	<0.01	0.03	<0.01	<0.01
Phytase B												
Linear	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Quadratic	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Phytase C												
Linear	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Quadratic	0.25	0.45	0.61	0.08	0.73	0.14	0.08	0.12	0.37	0.79	0.02	0.15

¹Data are least squares mean of 8 replicate pens of 5 birds per pen.

²P + T = Phenylalanine + Tyrosine, M + C = Methionine + Cysteine.

with AA digestibility, it could mean that phytase supplementation beyond a certain dose may reduce AA digestibility. Thus, any improvements in growth or meat accumulation observed with phytase supplementation at that dosage may be ascribed to improvements in the utilization of minerals or other nutrients and not necessarily on AA digestibility. Further research may be needed to prove this theory.

With the improvement in the AID of P observed during the starter phase, the additionally released P contributed to the greater performance of birds. Broiler chickens fed the NC diet (with P concentration of 5.5 g/kg) during the starter phase had a 51.5% AID of P. Thus, about 2.8 g/kg of P was utilized from the diet, leaving 2.7 g/kg of P unavailable to birds. On average, the supplementation of the NC diets with Phy A, B, or C pushed the utilization of P up to 3.4, 3.3, and 3.2 g/kg, respectively, whereas reducing the concentration of unavailable P in the diets to 2.1, 2.2, and 2.3 g/kg, respectively. The starter phase has been postulated to be of great importance in the lifecycle of broiler chickens because of the rapid growth and development of organs and tissues characterized by this phase (Babatunde et al., 2019b). Phosphorus being an important component of cells, tissues, and the skeletal system would be invaluable in this phase. Therefore, the further infusion of P because of the supplementation of phytase in the diets could have been responsible for the improved performance of birds observed in the starter phase. Similarly, tibia ash of birds was significantly improved with

the addition of phytase in NC diets during the starter phase, thus supporting the theory of increased AID of P improving the BWG of birds at this phase. The overall increase in the BWG observed in birds fed diets supplemented with phytase is because of a combination of factors including the improvement in the AID of energy, CP, P, and other minerals during the entire lifecycle and the increase in FI during the finisher phase.

In conclusion, broiler chickens fed P-deficient diets supplemented with microbial phytases were able to increase their productivity in terms of growth performance, energy and nutrient utilization, and bone mineralization. As expected, all 3 phytases examined liberated nutrients bound to phytate for improved performance of broiler chickens. However, the shape of the dose response curve, particularly between 0 and 500 FTU/kg, was unique for each phytase. Therefore, it is important to understand the specific phytase so that dosing can be optimized to fit the situation. Microbial phytase proves effective in improving broiler chicken production while reducing environmental concerns regardless of the species of microbes responsible for producing the phytase.

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Table 9. Effects of supplemental phytase on apparent ileal digestibility of nitrogen, dispensable and total AA in 42-day-old broiler chickens.¹

Item	Apparent ileal digestibility, %									
	Nitrogen	Ala	Asp	Cys	Glu	Gly	Pro	Ser	Tyr	Total AA
Positive Control (PC)	83.1	85.1	85.5	76.4	90.4	82.1	86.8	85.7	87.5	86.3
Negative Control (NC)	78.5	79.8	80.7	65.9	87.2	75.4	81.2	78.8	82.8	81.2
NC + Phytase A (FTU/kg)										
250	81.8	83.5	84.3	73.1	89.9	79.8	84.7	83.2	85.9	84.9
500	81.0	83.3	84.5	72.7	89.8	80.1	84.9	83.4	86.5	84.9
750	82.1	83.6	84.1	74.6	89.7	79.5	85.0	83.3	86.0	84.6
1000	82.7	84.7	84.5	74.4	90.3	80.4	85.8	84.0	87.1	85.4
NC + Phytase B (FTU/kg)										
250	83.6	85.7	85.8	75.2	91.0	81.9	86.2	84.9	87.2	86.4
500	82.5	83.6	84.3	73.2	89.8	79.7	84.9	83.0	86.0	84.7
750	82.7	85.1	85.3	76.4	90.6	81.4	86.2	85.1	87.7	86.0
NC + Phytase C (FTU/kg)										
500	81.0	84.2	84.4	71.8	90.0	79.8	84.6	83.8	86.3	84.9
750	83.5	85.4	85.8	75.9	90.9	81.4	86.2	85.3	87.5	86.1
1000	82.4	85.9	86.5	76.2	91.3	82.4	86.4	85.3	86.1	86.8
SEM	0.7	0.7	0.6	1.0	0.4	0.7	0.6	0.6	0.6	0.6
<i>P</i> -value of contrasts										
PC vs. NC	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Phytase A										
Linear	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Quadratic	0.18	0.08	<0.01	<0.01	0.01	<0.01	0.01	<0.01	0.02	<0.01
Phytase B										
Linear	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Quadratic	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	<0.01
Phytase C										
Linear	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Quadratic	0.14	0.10	0.18	0.20	0.13	0.29	0.12	0.01	<0.01	0.18

¹Data are least squares mean of 8 replicate pens of 5 birds per pen.

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REFERENCES

- Adedokun, S. A., J. S. Sands, and O. Adeola. 2004. Determining the equivalent phosphorus released by an *Escherichia coli*-derived phytase in broiler chicks. *Can. J. Anim. Sci.* 84:437–444.
- Adeola, O., and A. J. Cowieson. 2011. Board-invited review: Opportunities and challenges in using exogenous enzymes to improve non-ruminant animal production. *J. Anim. Sci.* 89:3189–3218.
- Amerah, A. M., P. W. Plumstead, L. P. Barnard, and A. Kumar. 2014. Effect of calcium level and phytase addition on ileal phytate degradation and amino acid digestibility of broilers fed corn-based diets. *Poult. Sci.* 93:906–915.
- AOAC International. 2000. *Official Methods of Analysis*, 17th ed. AOAC Int., Gaithersburg, MD.
- AOAC International. 2006. *Official Methods of Analysis*, 18th ed. AOAC, Gaithersburg, MD.
- Babatunde, O. O., A. J. Cowieson, J. W. Wilson, and O. Adeola. 2019a. Influence of age and duration of feeding low-phosphorus diet on phytase efficacy in broiler chickens during the starter phase. *Poult. Sci.* 98:2588–2597.
- Babatunde, O. O., A. J. Cowieson, J. W. Wilson, and O. Adeola. 2019b. Impact of age and feeding length on phytase efficacy during the starter phase of broiler chickens. *Poult. Sci.* 98:6742–6750.
- Barrientos, L. G., and P. P. N. Murphy. 1996. Conformational studies of myo-inositol phosphates. *Carbohydr. Res.* 296:39–54.
- Broz, J., P. Oldale, A. H. Perrin-Voltz, G. Rychen, J. Schulze, and C. S. Nunes. 1994. Effects of supplemental phytase on performance and phosphorus utilisation in broiler chickens fed a low phosphorus diet without addition of inorganic phosphates. *Br. Poult. Sci.* 35:273–280.
- Cowieson, A. J., T. Acamovic, and M. R. Bedford. 2004. The effects of phytase and phytic acid on the loss of endogenous amino acids and minerals from broiler chickens. *Br. Poult. Sci.* 45:101–108.
- Dersjant-Li, Y., A. Awati, H. Schulze, and G. Partridge. 2015. Phytase in non-ruminant animal nutrition: a critical review on phytase activities in the gastrointestinal tract and influencing factors. *J. Sci. Food Agric.* 95:878–896.
- Dilger, R. N., E. M. Onyango, J. S. Sands, and O. Adeola. 2004. Evaluation of microbial phytase in broiler diets. *Poult. Sci.* 83:962–970.
- Edwards, D. R., and T. C. Daniel. 1992. Environmental impacts of on-farm poultry waste disposal—a review. *Bioresour. Technol.* 41:9–33.
- International Union of Biochemists (IUB). 1979. Enzyme nomenclature. Pages 247 in *Recommendations of the Nomenclature Committee of the International Union of Biochemistry*. Academic Press, New York.
- Iyayi, E. A., F. Fru-Nji, and O. Adeola. 2013. True phosphorus digestibility of black-eyed pea and peanut flour without or with phytase supplementation in broiler chickens. *Poult. Sci.* 92:1595–1603.
- Maenz, D. D., C. M. Engle-Schaan, R. W. Newkirk, and H. L. Classen. 1999. The effect of minerals and mineral chelators on the formation of phytase-resistant and phytase susceptible forms of phytic acid in solution and in a slurry of canola meal. *Anim. Feed Sci. Technol.* 81:177–192.
- Myers, W. D., P. A. Ludden, V. Nayigihugu, and B. W. Hess. 2004. Technical note: a procedure for the preparation and quantitative analysis of samples for titanium dioxide. *J. Anim. Sci.* 82:179–183.
- Namkung, H., and S. Leeson. 1999. Effect of phytase enzyme on dietary nitrogen-corrected apparent metabolisable energy and the ileal digestibility of nitrogen and amino acids in broiler chicks. *Poult. Sci.* 78:1317–1319.
- Nelson, T. S., T. R. Schieh, R. J. Wodzinski, and J. H. Ware. 1968. The availability of phytate phosphorus in soybean meal before and after treatment with a mold phytase. *Poult. Sci.* 47:1842–1848.
- Nourmohammadi, R., S. M. Hosseini, H. Farhangfar, and M. Bashtani. 2012. Effect of citric acid and microbial phytase enzyme on ileal digestibility of some nutrients in broiler chicks fed corn-soybean meal diets. *Ital. J. Anim. Sci.* 11:36–40.
- National Research Council. 1994. *Nutrient Requirements of Poultry*, 9th rev. ed. Natl. Acad. Press, Washington, DC.

- Nys, Y., D. Frapin, and A. Pointillart. 1996. Occurrence of phytase in plants, animals, microorganisms. Pages 213–226 in *Phytase in Animal Nutrition and Waste Management*. M. B. Coelho and E. T. Kornegay eds. BASF Corporation, Mount Olive, NJ.
- Olukosi, O. A., C. Kong, F. Fru-Nji, K. M. Ajuwon, and O. Adeola. 2013. Assessment of a bacterial 6-phytase in the diets of broiler chickens. *Poult. Sci.* 92:2101–2108.
- Onyango, E. M., M. R. Bedford, and O. Adeola. 2005. Efficacy of an evolved *Escherichia coli* phytase in diets of broiler chicks. *Poult. Sci.* 84:248–255.
- Paiva, D., C. Walk, and A. Mcelroy. 2014. Dietary calcium, phosphorus, and phytase effects on bird performance, intestinal morphology, mineral digestibility, and bone ash during a natural necrotic enteritis episode. *Poult. Sci.* 93:2752–2762.
- Panda, A. K., S. V. R. Rao, M. V. L. N. Raju, S. S. Gajula, and S. K. Bhanja. 2007. Performance of broiler chickens fed low non-phytate phosphorus diets supplemented with microbial phytase. *J. Poult. Sci.* 44:258–264.
- Ravindran, V., S. Cabahug, G. Ravindran, P. H. Selle, and W. L. Bryden. 2000. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorous levels: effects on apparent metabolizable energy, nutrient digestibility and nutrient retention. *Br. Poult. Sci.* 41:193–200.
- Ravindran, V., P. H. Selle, G. Ravindran, P. C. H. Morel, A. K. Kies, and W. L. Bryden. 2001. Microbial phytase improves performance, apparent metabolizable energy, and ileal amino acid digestibility of broilers fed a lysine-deficient diet. *Poult. Sci.* 80:338–344.
- Santos, F. R., M. Hruby, E. E. M. Pierson, J. C. Remus, and N. K. Sakomura. 2008. Effect of phytase supplementation on nutrient digestibility and performance in broiler chicks. *J. Appl. Poult. Res.* 17:191–201.
- Scholey, D. V., and E. J. Burton. 2017. The effect of bone choice on quantification of mineralization in broiler chickens up to 6 weeks of age. *J. Appl. Poult. Res.* 26:485–490.
- Sebastian, S., S. P. Touchburn, E. R. Chavez, and P. C. Lague. 1996. The effects of supplemental microbial phytase on the performance and utilization of dietary calcium, phosphorus, copper, and zinc in broiler chickens fed corn-soybean diets. *Poult. Sci.* 75:729–736.
- Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition. *Anim. Feed Sci. Technol.* 135:1–41.
- Selle, P. H., V. Ravindran, R. A. Caldwell, and W. L. Bryden. 2000. Phytate and phytase: consequences for protein utilization. *Nut. Res. Rev.* 13:255–278.
- Shaw, A. L., J. B. Hess, J. P. Blake, and N. E. Ward. 2011. Assessment of an experimental phytase enzyme product on live performance, bone mineralization, and phosphorus excretion in broiler chickens. *J. Appl. Poult. Res.* 20:561–566.
- Shirley, R. B., and H.M. Edwards, Jr. 2003. Graded levels of phytase past industry standards improves broiler performance. *Poult. Sci.* 82:671–680.
- Simons, P. C. M., H. A. J. Versteegh, A. W. Jongbloed, P. A. Kemme, P. Slump, K. D. Bos, M. G. E. Wolters, R. F. Beudeker, and G. J. Verschoor. 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. *Br. J. Nutr.* 64:525–540.
- Sommerfeld, V., S. Kunzel, M. Schollenberger, I. Kuhn, and M. Rodehutschord. 2018. Influence of phytase or myo-inositol supplements on performance and phytate degradation products in the crop, ileum, and blood of broiler chickens. *Poult. Sci.* 97:920–929.
- Walk, C. L., E. K. Addo-Chidie, M. R. Bedford, and O. Adeola. 2012. Evaluation of a highly soluble calcium source and phytase in the diets of broiler chickens. *Poult. Sci.* 91:2255–2263.
- Woyengo, T. A., and C. M. Nyachoti. 2011. Review: supplementation of phytase and carbohydrases to diets for poultry. *Can. J. Anim. Sci.* 91:177–192.
- Wu, G. 2009. Amino acids: metabolism, functions, and nutrition. *Amino Acids* 37:1–17.
- Yonemochi, C., H. Takagi, M. Hanazumi, S. Hijikuro, K. Koide, T. Ina, and T. Okada. 2000. Improvement of phosphorus availability by dietary supplement of phytase in broiler chicks. *Jpn. Poult. Sci.* 37:154–161.