Evaluation of the responses of broiler chickens to varying concentrations of phytate phosphorus and phytase. II. Grower phase (day 12–23 post hatching)

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ABSTRACT A randomized complete block design study used 768 male broiler chickens to investigate the effects of phytate P (**PP**) and a novel consensus bacterial phytase variant (**PhyG**) concentration on growth performance, bone mineralization, apparent ileal digestibility (**AID**), and total tract retention (**TTR**) of nutrients in broiler chickens. Treatments were arranged in a 1 + 3 × 5 factorial with a nutrient-adequate positive control diet (**PC**) with 2.8 g PP/kg, 3 nutrient-reduced negative control diets (NC: PC minus 88 kcal/kg ME, 0.8 g/kg dig. Lys, 2.0 g/kg available P, 2.0 g/kg Ca and 0.5 g/kg Na) with varying PP (g/kg) levels, mainly from rice bran, at 2.3 (NC1), 2.8 (NC2), or 3.3 (NC3) and 5 PhyG doses at 0, 500, 1,000, 2,000, or 4,000 FTU/kg. All treatments had 6 replicate cages with 8 birds/cage. A commercial starter diet was fed from d 0 to 12 and the experimental diets from d 12 to 23 post hatching. Birds fed the NC2 diet without phytase had lower ($P < 0.01$) BW, BW gain, and feed intake (**FI**) as compared with birds fed the PC with the same PP level. With increasing phytate, there was a decrease ($P < 0.05$) in BW, BW gain, and FI. Phytase increased ($P < 0.01$) BW and feed efficiency of broiler chickens. An interaction ($P < 0.05$) between PP and phytase concentrations was observed on the AID of Met, Cys, and Thr. Linear decrease ($P < 0.01$) in the AID and TTR of P and Ca with increasing PP concentrations were observed. Phytase supplementation increased ($P \leq 0.05$) the AID of P, Ca, and all AA. The TTR of P, Ca, and Zn was linearly increased ($P < 0.01$) by 112, 123, and 46%, respectively, when birds fed NC diets with 0 and 4,000 FTU/kg were compared. In conclusion, phytate reduced the growth performance and nutrient utilization of broiler chickens from d 12 to 23 post hatching while phytase ameliorated these negative effects.

Key words: broiler chickens, growth performance, nutrient utilization, phytase, phytate

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INTRODUCTION

Phytate phosphorus (**PP**) is the main form of P in most cereal grains and oilseeds fed to poultry. However, it is not well utilized by birds due to poor digestion and hydrolysis of the bonds that hold P tightly in the phytate complex. Similarly, the negatively charged phytate forms complexes with some positively charged molecules in the intestinal tract thus reducing their digestibility (Woyengo and Nyachoti, 2013). Exogenous phytase is widely used in poultry diets to hydrolyze PP and release P and other nutrients for use by birds (Dilger et al., 2004; Gautier et al., 2018; Babatunde et al., 2020a). Several fungal and bacterial phytases have been produced and used commercially. However, there is continuous research on creating improved phytases with better efficiency in utilizing most of the P available in feedstuff, as well as improving the utilization of other nutrients. To test the efficacy of these phytase products in poultry production, trials must be carried out to determine the nutrient matrix in birds. The evaluation of parameters such as growth performance, bone mineralization, and nutrient utilization have proved valuable in determining the efficiency of phytase in improving P bioavailability and utilization of other nutrients such as AA and energy for broiler chickens (Ravindran et al., 2000, 2001; Babatunde et al., 2020a). The information from these trials will help to determine the contribution of available nutrients from phytase and the knowledge can be used in the formulation of diets that accurately meet the requirements of broiler chickens while reducing the waste of nutrients to the environment and encouraging sustainability.

Varying concentrations of phytate is a factor that can be used to estimate the efficiency of a new phytase

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product since PP is the substrate on which phytase acts on. From previous studies, we know that the PP in rice bran is more difficult to hydrolyze by phytase as compared to other feedstuffs such as canola meal, wheat, or soybean meal (Leske and Coon, 1999; Almeida et al., 2017). Thus, it seems appropriate to test a new phytase product using the most challenging PP source, hereby giving an opportunity to properly estimate its efficacy. Its ability to efficiently hydrolyze the PP from rice bran may encourage the use of other high PP ingredients in broiler chicken nutrition.

Commercial broiler chicken production generally occurs in 3 growth phases that differ mainly in the nutrient requirements of birds and in the biological and physiological states of birds. The starter phase of broiler chickens is characterized by the rapid growth and development of tissues, organs, and the skeletal system. Thus, the evaluation of phytase efficacy in this phase is of great importance (Babatunde et al., 2019a). Previous work from our lab evaluated the responses of birds to varying levels of a novel consensus bacterial phytase variant (**PhyG**) and PP in broiler chickens in the starter phase (Babatunde et al., 2021). However, it is also important to evaluate the responses of broiler chickens to this novel phytase in the grower phase. The age of birds has been known to influence the utilization of nutrients and efficacy of phytase in broiler chickens (Batal and Parsons, 2002; Babatunde et al., 2019a,b). Similarly, the grower phase prepares broiler chickens for the rapid deposition of meat in the finisher phase. Thus, a proper evaluation of PhyG in this phase allows nutritionists to determine the nutrient contribution of the phytase enzyme especially in the presence of varying concentrations of PP.

Therefore, this study aimed to evaluate the responses of broiler chickens in the grower phase (d 12−23 post hatching) to varying levels of PP concentrations and a novel consensus bacterial phytase variant (PhyG) using growth performance, bone mineralization, apparent ileal digestibility (**AID**), and total tract retention (**TTR**) of energy and nutrients as the response criteria. The null hypothesis tested in this study was that there was no effect of PP and phytase on responses of broiler chickens in the grower phase.1

MATERIALS AND METHODS

All protocols of animal experiments were reviewed and approved by the Purdue University Animal Care and Use Committee.

Birds, Experimental Design, and Diets

From d 0 to 12 post hatching, male Cobb 500 birds were individually tagged, housed in temperature-controlled battery cages (model SB 4T, Alternative Design Manufacturing, Siloam Springs, AR) and fed a commercial starter diet formulated to meet or exceed the requirements of broiler chickens (NRC, 1994). On d 12 post hatching, birds were weighed and allotted to one of 16 dietary treatments with 6 replicate cages and 8 birds per cage for a total of 768 birds. Treatments were arranged as a 1 + 3 × 5 factorial in a randomized complete block design with BW as the blocking factor. Experimental diets consisted of a nutrient-adequate positive control diet (**PC**) with 2.8 g PP/kg, 3 nutrient-reduced negative control (NC: PC minus 88 kcal/kg ME, 0.8 g/kg dig. Lys, 2.0 g/kg available P, 2.0 g/kg Ca and 0.5 g/kg Na) diets with varying PP (g/kg) levels, mainly from rice bran, at 2.3 (NC1), 2.8 (NC2), or 3.3 (NC3) and 5 PhyG doses at 0, 500, 1,000, 2,000, or 4,000 FTU/kg (Table 1). All diets were formulated based on analyzed nutrient values in feed ingredients. The PC diet had the same PP concentration as the NC2 diets. The phytate concentrations in diets were adjusted through the addition of rice bran and polished rice. The

Table 1. Ingredient composition of experimental diets fed to broiler chickens at grower phase (d 12−23 post hatching), g/kg as-fed basis.

	Diet[1,2]			
Item	PC	NC1	NC2	NC3
Ingredients, g/kg				
Corn	531.1	479.0	476.0	473.0
Soybean meal, 480 g/kg CP	325.9	276.2	272.8	269.7
Soybean oil	15.8	9.1	10.0	8.0
Rice, polished	26.6	80.4	45.0	18.1
Rice bran	30.5	7.2	46.4	84.5
Soy hulls	1.4	38.8	41.5	39.4
Meat and bone meal	11.0	12.2	11.0	9.8
Limestone	11.0	9.6	9.9	10.2
Monocalcium phosphate	9.9	0.0	0.0	0.0
Salt	3.1	2.7	2.7	2.7
Vitamin-mineral premix[3]	3.0	3.0	3.0	3.0
DL-Methionine	3.0	2.9	2.9	3.0
L-Lysine·HCl	2.1	2.8	2.8	2.7
L-Threonine	0.7	0.9	0.9	0.8
L-Tryptophan	0.0	0.2	0.1	0.1
Phytase premix[4]	-	50.0	50.0	50.0
Titanium dioxide premix[5]	25.0	25.0	25.0	25.0
Total	1,000	1,000	1,000	1,000
Calculated nutrients and energy, g/kg				
CP	209.1	192.4	193.0	193.6
ME, kcal/kg	3,050.0	2,962.2	2,962.2	2,962.2
Ca	8.4	6.4	6.4	6.4
P	6.6	4.1	4.6	5.1
Phytate-P	2.8	2.3	2.8	3.3
Non-phytate P	3.8	1.8	1.8	1.8
Na	1.7	1.2	1.2	1.2
dig. Lys	11.2	10.5	10.5	10.5
dig. Met	5.8	5.4	5.5	5.5
dig. Thr	7.3	6.7	6.7	6.7

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

[2]Each NC diet had 5 levels of phytase including 0, 500, 1,000, 2,000, and 4,000 phytase units (FTU)/kg.

[3]Supplied the following quantities per kg of diet: vitamin A, 5,484 IU; vitamin D_3, 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 B_{12}, 13.2 μg; biotin, 55.2 μg; thiamine mononitrate, 2.2 mg; folic acid, 990 μg; pyridoxine hydrochloride, 3.3mg; I, 1.11 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; Se, 300 μg.

[4]Each premix contained 1 g of phytase product prepared with 99 g of corn. Adding 50 g of premix/kg of NC diet supplied 0, 500, 1,000, 2,000, or 4,000 FTU, respectively.

[5]Prepared as 1 g titanium dioxide added to 4 g corn.

PhyG phytase (Danisco Animal Nutrition [IFF], The Netherlands) used in the NC diets was expressed in *Trichoderma reesei* and developed to have a broad pH profile, higher intrinsic thermostability, and faster hydrolysis in the upper gastrointestinal tract. The phytase was prepared as a premix with ground corn and included at 50 g/kg to provide the targeted phytase doses. Titanium dioxide, which is an indigestible marker, was included into all diets at 5 g/kg but in a corn premix at 25 g/kg. All experimental diets were fed ad libitum to broiler chickens from d 12 to 23 post hatching. Water was freely available. Mortality and general health of birds were monitored daily.

Sample Collection and Chemical Analyses

On d 23 post hatching, BW of all birds and feed intake (**FI**) per cage were recorded and used to determine the BW gain and gain to feed ratio. Birds were euthanized by CO_2 asphyxiation and dissected to collect the digesta from the distal two-thirds of the ileum which is that section of the intestines from the Merkel's diverticulum to the ileocecal junction. The ileal digesta was flushed with distilled water into plastic containers, pooled by cage and stored at −20°C until lyophilized. Excreta collected during the last 3 days of the experimental period, was dried in a forced air oven at 56°C for 7 d. The left tibia of 4 median weight birds per cage were collected and processed to determine percentage bone ash and ash weight per bone as described by Ogunwole et al. (2017). Dried ileal digesta was ground using a coffee grinder while diet and dried excreta samples were ground using centrifugal grinder (Retsch ZM 200 GmbH, Haan, Germany). All ground samples were passed through a 0.5-mm screen. Diets, ileal digesta, and excreta samples were analyzed for dry matter (**DM**) by placing in a drying oven for 24 h at 105°C (The Precision Scientific Co., Chicago, IL; method 934.01; AOAC, 2006). An isoperibol bomb calorimeter (Parr 1261; Parr 105 Instrument Co., Moline, IL) was used to determine the gross energy in diets, ileal digesta and excreta samples using benzoic acid as the calibration standard. The nitrogen content of diets, ileal, and excreta samples were determined by the combustion method (TruMac N; LECO Corp., St. Joseph, MI; method 990.03; AOAC, 2000) using EDTA as a calibration standard. Nitrogen values were multiplied by a factor of 6.25 to estimate the CP contents. The University of Missouri Experiment Station Chemical Laboratories (Columbia, MO) carried out the amino acid (**AA**) analyses in the ingredients, diet, and ileal digesta samples using methods described by the AOAC [method 982.30 E (a, b, c); 2006]; and Ti analysis in diets, ileal digesta, and excreta samples (Short et al., 1996). Phosphorus and Ca concentrations in the diets, ileal and excreta samples were determined after wet ash digestion with nitric and hydrochloric acid using methods described by Babatunde et al. (2019a). While Zn concentrations in the samples were determined by flame atomic absorption spectrometry using a Varian Spectr. AA 220FS (Varian Australia Pty Ltd., Victoria, Australia) with absorbance read at 214 nm. Phytase activity in diets was analyzed by DuPont Feed Technical Service (Brabrand, Denmark) using methods previously described by Engelen et al. (1994).

Calculation and Statistical Analyses

The AID and TTR of nutrients in the ileal digesta and excreta were determined using the index method described by Adeola (2001):

$$\text{AID or TTR, } \% = 100 - [(Ti_I/Ti_o) \times (N_o/N_I) \times 100]$$

Where Ti_I is Ti concentration in the diets, Ti_O is the Ti concentration in the ileal digesta or excreta, N_O is the concentration of a nutrient in the ileal digesta or excreta and N_I is the concentration of a nutrient in the diets. The concentration of Ti and nutrients in this equation was expressed as g/kg of DM. The AID of energy and the apparent metabolizable energy (**AME**; kcal/kg DM) of the diet was calculated as a product of the coefficient and gross energy concentrations (kcal/kg DM) in the diet. The nitrogen-corrected AME (**AMEn**) was calculated by correcting for zero N retention using a factor of 8.22 kcal/g N.

Data were analyzed using the general linear model procedure of SAS (SAS Inst. Inc., Cary, NC) as 1 + 3 × 5 factorial arrangements of treatments with PC, phytate, phytase, and their interactions as fixed effects and replicate blocks as random effects. Polynomial contrasts were used to compare the PC and NC2 (0 FTU/kg) diets which had similar PP content, and to determine the linear and quadratic effects of PP and phytase doses in the NC diets. Cage served as the experimental unit for all analyses. Statistical significance was set at $P \leq 0.05$.

RESULTS

The analyzed nutrients and phytase activity in experimental diets were similar with calculated values and within acceptable ranges (Table 2). All birds were healthy throughout the experimental period and only 0.4% mortality was recorded in this trial. There was a decrease ($P < 0.01$) in the BW, BW gain, FI, and gain to feed ratio of birds fed the NC2 without phytase as compared with birds fed the PC (Table 3). There was no interaction between phytate and phytase dose concentrations on growth performance indices in broiler chickens. However, there was a linear reduction ($P < 0.05$) in the BW and FI of birds with increasing PP contents in the NC diets. The respective reduction in BW gain and feed efficiency of broiler chickens were 6 and 3.3% as PP increased from 2.3 to 3.3 g/kg. There was a quadratic increase ($P < 0.01$) in BW, BW gain, FI, and feed efficiency of birds with phytase supplementation from 0 to 4,000 FTU/kg. The BW gain was increased by 25.6% while the feed efficiency was increased by 11.9% with phytase supplementation from 0 to 4,000 FTU/kg.

Table 2. Analyzed energy and nutrients of experimental diets fed to broiler chickens at grower phase (d 12−23 post hatching), g/kg as-fed basis.

Item	Diet[1,2]			
	PC[3]	NC1[4]	NC2[5]	NC3[6]
Energy and nutrients, g/kg				
DM	900	885	886	891
GE, kcal/kg	4,090.1	3,890.0	3,956.3	3,987.1
CP	202.6	182.9	185.9	182.2
Phytate P	2.6	2.0	3.1	3.7
P	6.8	4.2	4.6	5.2
Ca	9.5	7.3	7.3	7.2
Zn	0.01	0.01	0.02	0.02
Arg	13.4	12.1	12.7	12.8
His	5.2	4.8	5.0	5.0
Ile	8.9	8.3	8.6	8.6
Leu	16.7	15.7	16.3	16.1
Lys	13.0	11.8	13.0	12.2
Met	5.1	5.3	5.8	5.6
Phe	10.1	9.2	9.6	9.6
Thr	8.2	7.4	7.7	7.6
Trp	2.5	2.4	2.3	2.4
Val	9.7	9.2	9.5	9.6
Ala	10.0	9.4	9.8	9.8
Asp	20.3	18.7	19.5	19.1
Cys	3.0	3.0	3.1	3.1
Glu	35.1	32.2	33.5	33.0
Gly	8.9	8.4	8.7	8.6
Pro	11.0	10.3	10.7	10.7
Ser	8.6	7.8	8.1	7.9
Tyr	7.0	6.3	6.6	6.6
Total AA	199.8	185.5	193.9	191.4

[1]PC, positive control; NC, negative control.
[2]NC1, NC2, and NC3 had 5 levels of phytase inclusion (0, 500, 1,000, 2,000, and 4,000 phytase units [FTU]/kg); analyzed values for each NC are average of 5 diets.
[3]PC had an analyzed phytase activity of 233 phytase units/kg.
[4]NC1 diets had analyzed phytase activities of 181, 817, 1,420, 2,393, and 4,170 FTU/kg respectively.
[5]NC2 diets had analyzed phytase activities of 200, 623, 1,219, 1807, and 5,060 FTU/kg respectively.
[6]NC3 diets had analyzed phytase activities of 210, 684, 1,403, 2,486, and 4,641 FTU/kg respectively.

There was a decrease ($P < 0.01$) in the percentage tibia ash and the ash weight per bone of birds fed the NC2 without phytase versus the PC (Table 3). There was no interaction between phytate and phytase and no effect of phytate on tibia ash properties. However, there was a quadratic response ($P < 0.01$) of phytase on tibia ash properties with a difference of 642 mg ash/bone between birds fed the NC without phytase and birds fed the NC with 4,000 FTU/kg. Similarly, percent tibia ash was increased by 23% with phytase supplementation. Compared with birds fed the PC, there was a decrease ($P < 0.05$) in the AID of DM, energy, P, and Ca of birds fed the NC2 without phytase (Table 4). There was no interaction between phytate and phytase on the AID of DM, energy, nitrogen, P, Ca, and Zn. There was a linear reduction ($P < 0.01$) in the AID of DM, energy, and P by 2.9, 2.9, and 28.8%, respectively, as PP increased from 2.3 to 3.3 g/kg in the NC diets. Phytase mitigated the effects of phytate on DM, energy, and P by increasing ($P < 0.01$) their AID by 3.8, 3.6, and 96%, respectively. Similar increases ($P < 0.05$) in the AID of nitrogen, Ca, and Zn were also observed in birds fed diets with added phytase. Within birds fed each NC diet, the inclusion of phytase from 500 to 4,000 FTU/kg increased the AID of P by 31−83, 37−105, and 39−105% over each respective NC1, NC2, and NC3 diets without phytase (Figure 1).

The AID of some indispensable AA such His, Phe, Thr, and Trp were lower ($P \leq 0.05$) in birds fed the NC2 without phytase as compared with the PC (Table 5). There was an interaction ($P < 0.05$) between phytate and phytase on the AID of Met and Thr with linear increases in birds fed NC1 and NC2 diets with phytase but a quadratic increase in birds fed the NC3 with phytase. An interaction was also observed for AID

Table 3. Main effects of phytate and phytase concentrations on growth performance and bone mineralization of broiler chickens fed experimental diets at grower phase (d 12−23 post hatching).

Diet[1]	Phytate P, g/kg	Phytase, FTU/kg	Final BW (g)	BW gain, g/bird	Feed intake, g/bird	G:F, g/kg	Tibia ash weight, mg/bone	Tibia ash, %	No. of replicates
PC[2]	2.8	0	1021	704	971	725	2,192	48.7	6
NC2	2.8	0	845	531	873	608	1,517	42.2	6
	Main effect phytate								
	2.3		958	644	941	684	1,945	47.0	30
	2.8		933	620	930	666	1,959	47.3	30
	3.3		920	606	916	661	1,905	46.3	30
	Main effect phytase								
		0	850	536	862	621	1,508	40.5	18
		500	934	620	930	668	1,889	46.5	18
		1,000	952	638	941	680	2,003	48.5	18
		2,000	963	650	945	688	2,132	49.1	18
		4,000	986	673	968	695	2,150	49.8	18
SEM[3]			11.94	11.90	17.47	12.44	36.56	0.75	
P values									
PC vs. NC2 (0 FTU/kg)			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	
Phytate × Phytase			0.33	0.32	0.48	0.48	0.62	0.17	
Phytate linear			<0.01	<0.01	0.02	<0.01	0.09	0.14	
Phytate quadratic			0.38	0.41	0.88	0.34	0.09	0.14	
Phytase linear			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	
Phytase quadratic			<0.01	<0.01	0.01	<0.01	<0.01	<0.01	

[1]PC, positive control; NC, negative control: formulated without inorganic P, with reduction of 88 kcal/kg ME, 0.8 g/kg dig. Lys, 2.0 available P, 2.0 g/kg Ca, and 0.5 g/kg Na vs. PC diet.
[2]PC contains 2.8 g/kg phytate and 0 FTU/kg, formulated with higher ME, dig AA, adequate in P and Ca.
[3]SEM = standard error of mean (for the simple effects).

Table 4. Main effects of phytate and phytase concentrations on apparent ileal digestibility (%) of DM, energy, and nutrients in broiler chickens fed experimental diets at grower phase (d 12−23 post hatching).

Diet[1]	Phytate P, g/kg	Phytase, FTU/kg	DM	Energy	Nitrogen	P	Ca	Zn	No. of replicates
PC[2]	2.8	0	67.0	71.8	76.2	60.7	50.3	30.6	6
NC2	2.8	0	63.5	66.4	73.0	32.1	33.3	20.5	6
	Main effect phytate								
	2.3		66.0	69.3	75.3	58.6	43.7	26.7	30
	2.8		64.4	67.5	75.9	50.0	41.7	24.3	30
	3.3		64.1	67.3	74.8	41.7	37.9	22.7	30
	Main effect phytase								
		0	63.8	66.9	72.4	32.7	33.2	21.1	18
		500	64.5	67.3	74.5	44.2	37.8	24.0	18
		1,000	65.3	68.0	75.3	52.1	42.1	24.9	18
		2,000	64.5	68.6	76.6	57.4	44.9	26.0	18
		4,000	66.2	69.3	77.9	64.1	47.5	27.0	18
SEM[3]			0.95	0.89	1.25	3.10	5.01	3.72	
P values									
PC vs. NC2 (0 FTU/kg)			0.01	<0.01	0.08	<0.01	0.02	0.06	
Phytate × Phytase			0.91	1.00	0.61	0.94	1.00	1.00	
Phytate linear			<0.01	<0.01	0.49	<0.01	0.07	0.10	
Phytate quadratic			0.19	0.11	0.20	0.94	0.75	0.85	
Phytase linear			0.01	<0.01	<0.01	<0.01	<0.01	0.05	
Phytase quadratic			0.91	0.80	0.68	0.07	0.61	0.65	

[1]PC, positive control; NC, negative control: formulated without inorganic P, with reduction of 88 kcal/kg ME, 0.8 g/kg dig. Lys, 2.0 available P, 2.0 g/kg Ca, and 0.5 g/kg Na vs PC diet.
[2]PC contains 2.8 g/kg phytate and 0 FTU/kg, formulated with higher ME, dig AA, adequate in P and Ca.
[3]SEM = standard error of mean (for the simple effects).

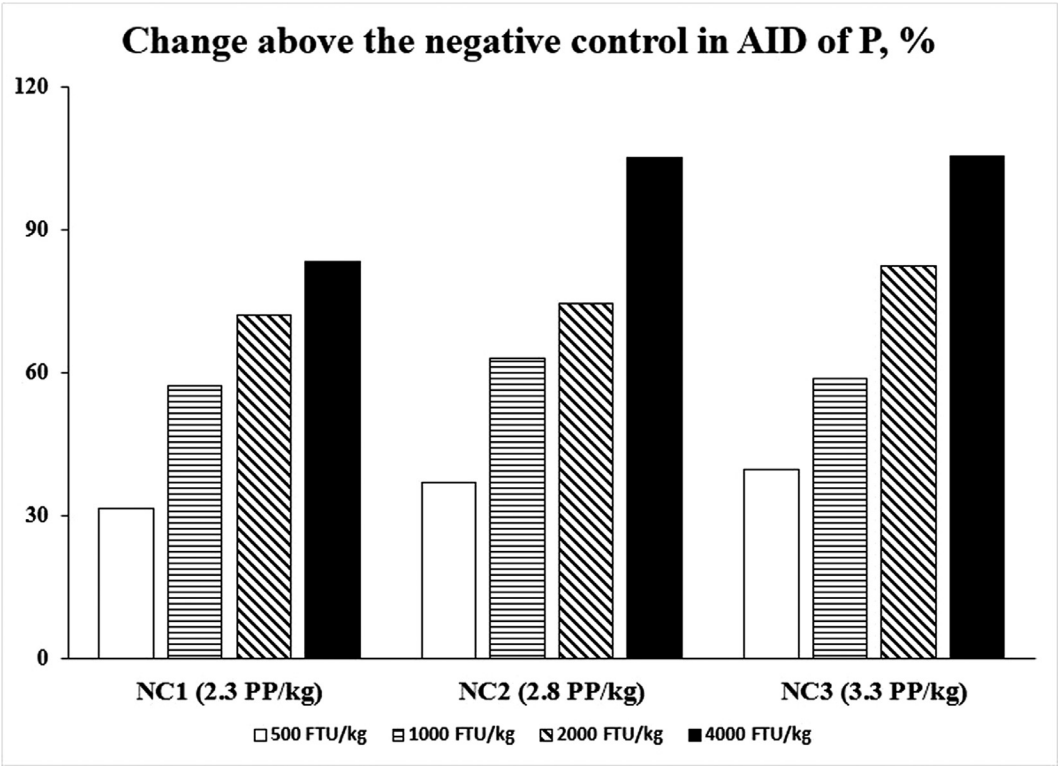

Figure 1. The efficacy of phytase (PhyG) on the apparent ileal digestibility (AID) of P relative to the phytate P (PP) concentration in each of the NC diets. The percentage difference values were derived by subtracting the AID of P in the NC diets (i.e., NC1, NC2, or NC3) with 0 FTU/kg from 500, 1,000, 2,000, or 4,000 FTU/kg diet, in each block within each NC diet.

Cys ($P < 0.01$), there were linear increments in birds fed the NC diets containing 2.3 and 2.8 g PP/kg with added phytase, and an increase, which plateaued at 2,000 FTU/kg, in the NC diet containing 3.3 g PP/kg. There was a quadratic response ($P \leq 0.05$) in the AID of Lys and Phe with increases in phytate levels. With phytase addition, there was a linear increase ($P < 0.01$) in the AID of all indispensable AA. When comparing birds fed the NC2 without phytase and the PC, there was a decrease ($P < 0.01$) in the AID of the total AA and all dispensable AA except Asp (Table 6). There was a quadratic response ($P \leq 0.05$) in the AID of total AA and all dispensable AA except Ala, Pro, Tyr with increases in the PP concentration. Linear increase ($P < 0.01$) in the AID of Ala, Asp, Cys, Glu, Gly, Tyr, and total AA, and a quadratic response ($P < 0.05$) with the

Table 5. Effect of phytate and phytase concentrations on apparent ileal digestibility (%) of indispensable AA in broiler chickens fed experimental diets at grower phase (d 12−23 post hatching).

Diet[1]	Phytate P, g/kg	Phytase, FTU/kg	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	No. of replicates
PC[2]	2.8	0	84.4	79.8	76.6	78.5	81.3	89.6^{DEFG}	78.7	72.6^{BCD}	82.8	72.9	6
NC1	2.3	0	82.7	74.7	73.5	75.1	78.6	88.0^{G}	75.6	66.6^{GH}	80.1	69.3	6
		500	83.7	76.8	75.5	77.5	79.4	89.0^{EFG}	77.7	69.0^{EFG}	80.9	71.3	6
		1,000	86.6	79.3	78.2	79.7	82.3	89.2^{EFG}	80.1	71.7^{CDE}	83.1	73.9	6
		2,000	86.5	78.9	78.8	80.1	81.9	89.7^{DEFG}	80.7	71.8^{CDE}	82.3	73.9	6
		4,000	88.0	81.4	81.3	82.0	83.3	91.2^{ABCD}	83.3	75.4^{AB}	84.5	77.0	6
NC2	2.8	0	83.0	75.7	74.3	75.8	80.0	88.4^{FG}	76.0	68.0^{FG}	79.1	70.3	6
		500	84.1	77.2	75.9	77.8	81.1	90.6^{BCDE}	77.8	70.2^{DEF}	79.7	71.6	6
		1,000	85.5	79.2	77.8	79.8	82.2	89.1^{EFG}	80.1	71.0^{CDEF}	81.3	73.0	6
		2,000	87.3	81.5	79.8	81.5	83.8	91.9^{ABC}	81.9	75.7^{AB}	82.4	75.9	6
		4,000	89.4	83.2	82.7	83.6	86.0	92.0^{AB}	84.2	75.8^{AB}	84.8	78.6	6
NC3	3.3	0	80.3	73.4	71.2	73.3	74.9	84.7^{H}	72.8	63.9^{H}	77.8	66.8	6
		500	82.5	76.0	73.6	75.8	78.4	89.3^{EFG}	75.4	66.5^{GH}	81.1	70.0	6
		1,000	84.8	79.2	77.6	79.0	80.4	90.2^{CDEF}	79.0	71.7^{CDE}	83.6	73.4	6
		2,000	88.5	83.5	82.1	82.9	84.2	92.6^{A}	83.5	77.2^{A}	84.1	78.3	6
		4,000	87.3	81.3	79.4	81.1	82.5	91.2^{ABCD}	81.6	74.3^{ABC}	84.3	75.6	6
	Main effect of phytate												
	2.3		85.5	78.2	77.4	78.9	81.1	89.4	79.5	70.9	82.2	73.1	30
	2.8		85.9	79.4	78.1	79.7	82.6	90.4	80.0	72.1	81.5	73.9	30
	3.3		84.7	78.7	76.8	78.4	80.1	89.6	78.5	70.7	82.2	72.8	30
	Main effect of phytase												
		0	82.0	74.6	73.0	74.7	77.8	87.0	74.8	66.1	79.0	68.8	18
		500	83.4	76.7	75.0	77.0	79.6	89.6	77.0	68.6	80.6	71.0	18
		1,000	85.7	79.3	77.9	79.5	81.6	89.5	79.8	71.5	82.6	73.4	18
		2,000	87.5	81.3	80.3	81.5	83.3	91.4	82.0	74.9	82.9	76.0	18
		4,000	88.2	81.9	81.1	82.2	83.9	91.5	83.1	75.2	84.5	77.1	18
SEM[3]			0.76	0.97	1.09	1.00	1.01	0.65	0.96	1.25	0.88	1.19	
P values													
PC vs. NC2 (0 FTU/kg)			0.18	<0.01	0.13	0.06	0.36	0.19	0.05	0.01	<0.01	0.12	
Phytate × Phytase			0.11	0.11	0.14	0.36	0.12	<0.01	0.14	0.04	0.35	0.12	
Phytate linear			0.10	0.46	0.33	0.46	0.12	0.68	0.09	0.81	0.99	0.75	
Phytate quadratic			0.07	0.09	0.10	0.06	<0.01	0.01	0.05	0.06	0.14	0.17	
Phytase linear			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	
Phytase quadratic			0.29	0.11	0.23	0.09	0.22	0.03	0.18	0.16	0.39	0.41	

[A-H]Simple effect means within a column with different superscripts differ ($P < 0.05$).

[1]PC, positive control; NC, negative control: formulated without inorganic P, with reduction of 88 kcal/kg ME, 0.8 g/kg dig. Lys, 2.0 available P, 2.0 g/kg Ca, and 0.5 g/kg Na vs. PC diet.

[2]PC contains 2.8 g/kg phytate and 0 FTU/kg, formulated with higher ME, dig AA, adequate in P and Ca.

[3]SEM = standard error of mean (for the simple effects).

AID of Pro and Ser with phytase supplementation were noted.

A difference ($P < 0.05$) in the AID of P, Ca, and Zn in birds fed the NC2 without phytase versus birds fed the PC was observed (Table 7). There was no interaction between phytate and phytase concentrations on the TTR of nutrients and the metabolizable energy. A linear decrease ($P \leq 0.01$) in the TTR of DM, nitrogen, P, and Ca was observed in birds fed diets with increasing PP content. With phytase supplementation, the AME and AMEn were linearly increased ($P < 0.01$) by 113 and 110 kcal/kg DM intake, respectively. Similarly, the TTR of DM and Zn were linearly increased ($P < 0.01$) by 2.1 and 45.5%, respectively, while a quadratic response ($P < 0.01$) was observed with the TTR of P and Ca with phytase supplementation.

DISCUSSION

The importance of P in the nutrition of broiler chickens cannot be overemphasized due to its role in skeletal development and in several biochemical reactions necessary to support life (Leeson and Summers, 2001). Inadequate supply of P in the diets of broiler chickens will usually result in reduced growth and development of birds as observed in the current study. The inability of broiler chickens to utilize the P in PP and the absence of inorganic P in the NC2 diet resulted in the reduced BW, BW gain, FI, and feed efficiency of birds fed the NC2 diet without phytase. This observation is consistent with previous studies where insufficient available P in the NC diet resulted in reduced growth performance (Walters et al., 2019; Babatunde et al., 2020a). However, it should also be noted that the NC2 diet without phytase was deficient in non-phytate P (**nPP**), energy and other nutrients such as CP, AA, Ca, and Na as compared with the PC. This deficiency in addition to the inadequate available P would have contributed to the lowered growth observed in birds fed the NC2 without phytase. The negative effect of increasing concentrations of phytate on growth performance was expected and has been reported by Cabahug et al. (1999). In addition, feeding the low PP diet (NC1) without phytase supplementation to birds resulted in reduced growth performance. This diet will be typical of a corn-soybean meal-based commercial diet that has been formulated to be low in P, however, with additional P-supplying

Table 6. Effect of phytate and phytase concentrations on apparent ileal digestibility (%) of dispensable and total AA in broiler chickens fed experimental diets at grower phase (d 12−23 post hatching).

Diet[1]	Phytate P, g/kg	Phytase, FTU/kg	Ala	Asp	Cys	Glu	Gly	Pro	Ser	Tyr	Total AA	No. of replicates
PC[2]	2.8	0	77.4	75.1	66.0^{DE}	83.3	71.2	77.5	78.1	79.9	78.2	6
NC1	2.3	0	73.0	71.9	56.5^{H}	80.5	63.4	72.0	70.7	74.8	74.2	6
		500	75.9	74.0	60.8^{FG}	82.0	66.5	75.3	73.8	76.8	76.3	6
		1,000	78.0	77.3	63.2^{EF}	84.6	70.3	77.4	76.8	80.0	78.8	6
		2,000	78.3	77.6	63.6^{EF}	84.7	70.4	77.4	76.9	79.6	78.9	6
		4,000	79.9	80.2	71.1^{ABC}	86.3	73.0	79.9	79.8	82.3	81.3	6
NC2	2.8	0	74.4	73.0	58.2^{GH}	81.1	65.6	73.6	72.2	76.3	75.2	6
		500	76.1	74.3	63.2^{EF}	82.4	67.4	75.2	75.0	78.0	76.8	6
		1,000	78.0	77.0	65.7^{DE}	84.4	70.2	77.7	77.3	79.4	78.6	6
		2,000	79.5	79.1	69.4^{BCD}	85.8	72.3	79.1	80.5	81.0	80.7	6
		4,000	82.0	81.1	73.4^{AB}	87.6	75.1	81.0	81.1	83.3	82.7	6
NC3	3.3	0	71.8	69.7	55.7^{H}	78.6	62.3	71.3	67.4	73.3	72.0	6
		500	75.0	71.2	58.3^{GH}	80.7	66.4	74.1	72.0	76.0	74.8	6
		1,000	77.4	76.1	68.0^{CD}	83.7	69.4	77.4	76.6	79.2	78.2	6
		2,000	80.9	80.8	74.4^{A}	87.0	74.7	81.1	80.9	83.3	82.3	6
		4,000	79.5	78.1	69.2^{CD}	85.3	71.9	79.6	79.5	81.7	80.3	6
	Main effect of phytate											
	2.3		77.0	76.2	63.0	83.6	68.7	76.4	75.6	78.7	77.9	30
	2.8		78.0	76.9	66.0	84.2	70.1	77.3	77.2	79.6	78.8	30
	3.3		76.9	75.2	65.1	83.0	68.9	76.7	75.3	78.7	77.5	30
	Main effect of phytase											
		0	73.0	66.1	56.8	80.0	63.8	72.3	70.1	74.8	73.8	18
		500	75.7	68.6	60.8	81.7	66.8	74.9	73.6	76.9	76.0	18
		1,000	77.8	71.5	65.6	84.2	70.0	77.5	76.9	79.6	78.5	18
		2,000	79.6	74.9	69.1	85.8	72.5	79.2	79.4	81.3	80.6	18
		4,000	80.5	75.2	71.2	86.4	73.3	80.2	80.1	82.4	81.4	18
SEM[3]			1.10	0.98	1.50	0.74	1.21	0.94	1.04	0.93	0.96	
P values												
PC vs. NC2 (0 FTU/kg)			0.05	0.14	<0.01	0.04	<0.01	<0.01	<0.01	0.01	0.03	
Phytate × Phytase			0.60	0.07	<0.01	0.12	0.23	0.29	0.06	0.10	0.13	
Phytate linear			0.87	0.11	0.03	0.22	0.76	0.62	0.63	0.99	0.51	
Phytate quadratic			0.09	0.03	0.02	0.03	0.05	0.15	<0.01	0.08	0.04	
Phytase linear			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	
Phytase quadratic			0.12	0.12	0.13	0.06	0.06	0.04	0.01	0.15	0.13	

$^{A-H}$Simple effect means within a column with different superscripts differ ($P < 0.05$).

[1]PC, positive control; NC, negative control: formulated without inorganic P, with reduction of 88 kcal/kg ME, 0.8 g/kg dig. Lys, 2.0 available P, 2.0 g/kg Ca, and 0.5 g/kg Na vs PC diet.

[2]PC contains 2.8 g/kg phytate and 0 FTU/kg, formulated with higher ME, dig AA, adequate in P and Ca.

[3]SEM = standard error of mean (for the simple effects).

ingredients such as soyhulls, polished rice, rice bran, and meat and bone meal. In the current trial, we aimed to test the efficacy of the new phytase enzyme in birds fed diets of commercial or practical importance which explains why diets from low to high PP were included in the trial.

Broiler chickens are known to have difficulties in hydrolyzing the phytic acid bonds that tightly bind P in most cereals and oilseeds (Babatunde et al., 2020b). Phytate also forms complexes with other divalent minerals such as Ca and Zn, AA, and even enzymes such as carbohydrases and proteases necessary to digest starch and proteins, respectively (Selle and Ravindran, 2007; Cowieson et al., 2011). Thus, increasing the concentration of PP in the diets without phytase supplementation should reduce the availability of several nutrients necessary to support the growth of broiler chickens. In addition, PP from rice bran has been reported to be particularly difficult to hydrolyze as compared to other feed ingredients (Almeida et al., 2017), hereby reducing the accessibility of nutrients to birds. The inclusion of phytase in the diets mitigated the negative effects of low nPP on growth performance in birds regardless of the PP content and as observed with several studies (Ravidran et al., 2008; Powell et al., 2011; Leyva-Jimenez et al., 2019). Phytase increases the bioavailability of P and other nutrients by hydrolyzing the phytate complex and releasing nutrients necessary for growth in broiler chickens.

When comparing birds fed the PC and the NC2 without phytase but with similar PP levels, we observe the impact of available P on bone mineralization. Broiler chickens were able to utilize the inorganic P in the PC for mineral deposition to the bones as compared with birds fed the NC2 with limited access to the P in phytate. There was no effect of increasing PP on tibia ash properties. This observation was surprising as we expected that the increase in relatively unavailable PP will adversely affect the deposition of P and Ca in bones particularly as birds grow older. However, the PP effect was across all phytase dose levels and not just the NC without phytase, hence, phytase would have increased the available P and tibia ash thus negating the debilitating effect of phytate. Similarly, birds may have utilized the available P in the diets to meet their bone mineralization requirements. It is possible that on d 23, birds may still be young enough such that the requirement of P and Ca for skeletal development may have been met despite the absence of inorganic P and the increase in PP content of the NC diets. Furthermore, birds were fed

Table 7. Main effects of phytate and phytase concentrations on total tract retention (%) of DM, energy, and nutrients in broiler chickens fed experimental diets at grower phase (d 12−23 post hatching).

Diet[1]	Phytate P, g/kg	Phytase, FTU/kg	DM	AME, kcal/kg DMI	AMEn, kcal/kg DMI	Nitrogen	P	Ca	Zn	No. of replicates
PC[2]	2.8	0	73.9	3,377	3,183	70.9	59.3	42.6	32.7	6
NC2	2.8	0	72.4	3,294	3,108	68.2	25.4	20.4	22.2	6
	Main effect of phytate									
	2.3		73.5	3,335	3,149	68.1	58.4	46.6	30.3	30
	2.8		72.9	3,359	3,169	69.3	47.1	36.3	31.0	30
	3.3		72.4	3,350	3,158	70.5	36.9	30.9	27.4	30
	Main effect of phytase									
		0	72.1	3,291	3,104	68.2	27.6	21.3	22.4	18
		500	72.6	3,316	3,128	68.8	43.9	35.1	29.2	18
		1,000	72.7	3,358	3,169	69.3	51.7	40.8	31.1	18
		2,000	73.6	3,372	3,179	70.6	55.4	45.0	32.5	18
		4,000	73.6	3,403	3,214	69.4	58.7	47.5	32.6	18
SEM[3]			0.72	3.14	2.83	1.41	2.17	2.95	3.43	
P values										
PC vs. NC2 (0 FTU/kg)			0.14	0.07	0.07	0.19	<0.01	<0.01	0.03	
Phytate × Phytase			0.99	0.91	0.84	1.00	0.07	0.97	1.00	
Phytate linear			0.01	0.46	0.65	<0.01	<0.01	<0.01	0.18	
Phytate quadratic			0.89	0.36	0.31	0.98	0.64	0.14	0.26	
Phytase linear			<0.01	<0.01	<0.01	0.11	<0.01	<0.01	<0.01	
Phytase quadratic			0.91	0.82	0.90	0.35	<0.01	<0.01	0.06	

[1]PC, positive control; NC, negative control: formulated without inorganic P, with reduction of 88 kcal/kg ME, 0.8 g/kg dig. Lys, 2.0 available P, 2.0 g/kg Ca, and 0.5 g/kg Na vs PC diet.
[2]PC contains 2.8 g/kg phytate and 0 FTU/kg, formulated with higher ME, dig AA, adequate in P and Ca.
[3]SEM = standard error of mean (for the simple effects).

P-sufficient diets from d 0 to 11 and may have had P reserves that prevented the depression of the P status of birds and the decrease of tibia ash significantly in the short duration of the experimental period. Subsequently, further studies may be required to evaluate the effect of increasing PP on the bone mineralization of much older birds that is, at the finishing phase. The positive effect of PhyG on tibia ash in birds on d 23 post hatching was similar to the previous study with birds on d 11 post hatching (Babatunde et al., 2021). However, when comparing both studies, the effect of the NC diet without phytase on tibia ash seemed to be more evident in the younger birds as compared with the older birds (37.2 vs. 40.5%). Similarly, the efficacy of phytase on tibia ash in terms of percentage improvement was more evident in the younger birds as compared with birds in the current study when phytase was included at 4,000 FTU/kg (23 vs. 29%). Therefore, we speculate that younger birds may be more sensitive to P deficiency for bone mineralization as well as to phytase supplementation. However, this does not dispute the positive influence of PhyG or other phytases on bone mineralization regardless of the growth phase of broiler chickens (Augspurger and Baker, 2004; Manobhavan et al., 2016; Babatunde et al., 2020a).

Differences in the concentration of nPP and other nutrients between the PC and the NC2 diet without phytase was responsible for the lowered AID of DM, energy, P and Ca observed in broiler chickens. In the current study, increased PP concentrations hindered the AID of DM, energy and P but had no effect on the AID of nitrogen, Ca, and Zn. From previous studies, we know that phytate is able to hinder the utilization of several nutrients (Ravindran et al., 2000; Babatunde et al., 2019b), however, the sensitivity of birds to the presence of phytate and its effect on digestibility of nutrients may be influenced by several factors including age of birds, phytate source, and solubility, etc. Batal and Parsons (2002) previously reported that age of broiler chickens could influence the utilization of nutrients. Similarly, previous work from our lab has reported the impact of age on the AID of P and Ca in broiler chickens, with younger birds being more sensitive to P deficiency (Babatunde et al., 2019a,b). It is possible that the increased demand for energy and nutrients in older birds impacted the sensitivity of birds to the higher phytate content in the diet thus, reducing the digestibility of DM and energy in addition to P.

The addition of phytase improved the AID of DM, energy, nitrogen, P, and Ca in agreement with previous studies (Dersjant-Li and Kwakernaak, 2019; Babatunde and Adeola, 2021; Dersjant-Li et al., 2020). In addition, PhyG improved the AID of Zn which was not the case in younger birds from the previous study (Babatunde et al., 2021). Age of birds may have played a role in the response of birds with regards to Zn absorption and utilization in the presence of phytase. Although Zn is required in minor quantities as compared with other minerals, birds may require more Zn to support biochemical reactions and the immune system as they grow older. The efficacy of phytase on the AID of P was apparent and seemed to be dependent on the PP content of the diet. When comparing birds fed the NC1 diet (2.3 g PP/kg) and the NC3 diet (3.3 g PP/kg) with similar phytase concentrations (4,000 FTU/kg), the AID of P was improved by 83 and 105%, respectively. This indicates that an increase in the PP content of diets potentially increases the substrate pool from which phytase could act upon. Thus, increasing the bioavailability of nutrients particularly P and Ca to broiler chickens.

Even though the P in rice bran is more tightly bound as compared to other ingredients, PhyG was able to extract the P in the PP complex even as the phytate concentrations increased. Therefore, this should encourage the use of feed ingredients with high PP content in the grower phase of broiler chickens if diets are properly supplemented with PhyG. In addition, the effect of high doses of phytase was evident as the AID of P was improved by over 100% in some instances. This agrees with several studies where higher doses of phytase allowed for an increased degradation of PP and supported the growth of broiler chickens beyond the use of the low 500 FTU/kg dose (Lee et al., 2017; Sommerfeld et al., 2018; Dersjant-Li and Kwakernaak, 2019).

The effect of phytase on the AID of Met, Thr, and Cys was influenced by the phytate content of the diet resulting in an interaction. At the highest PP levels, the AID of most AA was lower in the NC as compared with the NC in the lower PP levels. However, the improvement of phytase above the NC on some digestible AA such as Met and Cys was larger at high PP levels as compared with lower PP levels. Generally, phytase improved the digestibility of all AA both linearly and quadratically. However, significant linear responses were observed on the AID of most AA when phytase was added from 500 to 4,000 FTU/kg in diets, while quadratic responses were observed with digestible Met, Ser, and Pro. As observed with previous studies, the extra-phosphoric effect of PhyG on AA utilization was evident as phytase improved not just the P utilization but was able to counter the antinutritive effects of PP on digestible AA (Truong et al., 2015; Christensen et al., 2020; Dersjant-Li et al., 2020). In agreement with previous studies, phytase inclusion improved the AID of all dispensable and indispensable AA (Dersjant-Li and Kwakernaak, 2019; Babatunde et al., 2020a).

There was an effect of phytate on the TTR of nitrogen, P, and Ca in birds at the grower phase but not on the AME and AMEn. We cannot readily explain the increase in the TTR of nitrogen with increasing PP content mainly because there was no effect of phytate on the AID of nitrogen. However, we can speculate that with increasing PP content, a significant amount of unhydrolyzed phytate and its derivatives may have reached the cecum of birds. This may have supported the degradation of phytate by microbes and increased the amount of nutrients such as microbial nitrogen released and retained in birds. An estimation of the microbial population and activity in the post-ileal region of the gut of broiler chickens in response to increasing phytate concentrations may be important information in further studies. In agreement with other trials (Ravindran et al., 2000; Ravindran et al., 2001; Santos et al., 2008) there was an increase in the AME of birds with phytase inclusion. The age of birds may have an impact the effect of phytase on energy utilization. Older birds may require more energy in supporting growth and development and may be more sensitive to the activity of phytase in energy deficient diets. Similarly, phytase improved the TTR of DM, P, Ca, and Zn in agreement with Sebastian et al. (1996) and Babatunde et al. (2019a,b).

In conclusion, broiler chickens in the grower phase generally responded negatively to the presence of phytate in the diets. However, the inclusion of PhyG in diets of broiler chickens increased growth performance, bone mineralization, digestibility and retention of energy, AA, P, Ca, and other nutrients in the grower phase regardless of phytate levels. Lastly, inclusion of high doses of PhyG extends extra-phosphoric benefits on broiler production beyond the traditional dosage and may be considered during the grower phase of production if benefits outweigh the financial implications.

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DISCLOSURES

A. Bello and Y. Dersjant-Li are employees of Danisco Animal Nutrition (IFF).

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