Evaluation of the responses of broiler chickens to varying concentrations of phytate phosphorus and phytase. I. Starter phase (day 1−11 post hatching)

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ABSTRACT Growth performance, tibia ash, apparent ileal digestibility (**AID**), and total tract retention (**TTR**) of nutrients responses of broiler chickens fed diets containing varying concentrations of phytate P (**PP**) and a novel consensus bacterial 6-phytase variant (PhyG) from d 1 to 11 post hatching were evaluated with 1,152 broiler chicks. Diets were a nutrient-adequate positive control diet (**PC**) with 2.8 g PP/kg or one of 15 nutrient-reduced negative control (NC: PC minus 88 kcal/kg ME, 0.8 g/kg dig. Lys, 2.0 g/kg available P, 1.8 g/kg Ca and 0.5 g/kg Na) diets with 3 PP (g/kg) levels, mainly from rice bran, at 2.3 (NC1), 2.8 (NC2), or 3.3 (NC3) and 5 PhyG supplementation at 0, 500, 1,000, 2,000, or 4,000 FTU/kg in a $1 + 3 \times 5$ factorial. All treatments had 6 replicate cages with 12 birds per cage. Despite comparable PP levels, birds fed the PC diet had greater ($P \leq 0.01$) body weight (**BW**), feed intake (**FI**), tibia ash, AID of energy, AA, P, and Ca as compared with birds fed the NC2 without phytase. There was no interaction between PP and phytase for all responses. Increasing PP concentrations linearly decreased ($P < 0.01$) BW, FI, AID, and TTR of P and Ca. With phytase supplementation, there was a quadratic response ($P < 0.05$) in BW, FI, tibia ash, and a linear increase ($P < 0.05$) in the AID of energy, nitrogen, and all the measured AA. Increasing phytase dose from 0 to 4,000 FTU/kg increased ($P < 0.01$) AID of P and Ca by 88 and 18%, respectively. There was also a quadratic response ($P \leq 0.05$) on TTR of P and Ca with increasing phytase dose. In conclusion, increasing levels of PP reduced growth performance and most nutrient utilization responses of broiler chickens while phytase supplementation positively impacted the responses of broiler chickens during d 1 to 11 post hatching.

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

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INTRODUCTION

Phosphorus is of utmost importance in broiler chickens due to its role in growth and development. The use of phytase to improve the productivity of broiler chickens through the release of P from feed ingredients has been established by several studies (Coelho and Kornegay, 1999; Adeola and Cowieson, 2011; Babatunde and Adeola, 2021). The efficacy of phytase in broiler chickens can be determined by evaluating the nutrient matrix in birds through responses such as mineral utilization, and bone mineralization. In poultry production, microbial phytases are commonly used instead of fungal phytases due to their proven efficacy as established by several studies (Cabahug et al., 1999; Dilger et al., 2004; Babatunde et al., 2020a). However, the drive for higher production efficiency and environmental sustainability in broiler industry necessitates the need for phytase with improved phosphoric and extraphosphoric efficacies. Thus, new generation phytases are developed and tested by evaluating their improved efficacy to allow for higher reductions in dietary nutrient.

In most cereals and oilseeds, the majority of P is bound in a complex as phytate P (**PP**). This inhibits its utilization by broiler chickens, however, phytases hydrolyze the phytate complex and release P and other nutrients for use by the birds (Namkung and Leeson, 1999). By reducing the density of dietary energy, protein, and minerals, the efficacy of phytases to degrade phytate and increase nutrients availability to maintain optimum productivity of broiler chickens can be evaluated (Walk and Olukosi, 2019). Phytate is regarded as an antinutritional factor. Thus, several feed ingredients with high PP content such as canola meal or rice bran are not conventionally used in diets fed to broiler chickens relative to corn and soybean

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meal in the United States. However, to properly test the efficacy of a new phytase product, it may be important to evaluate in birds fed diets with varying PP content.

The accessibility of PP to enzymatic hydrolysis is not the same for all feed ingredients. Previous studies have reported differences in the hydrolysis of PP and in the impact of phytase on P utilization from various feed ingredients (Leske and Coon, 1999; Almeida et al., 2017). The PP in rice bran was reported to be the most difficult to hydrolyze even in the presence of phytase when compared with those from other ingredients like canola meal, soybean meal, sunflower meal, or wheat probably due to its high stability (Leske and Coon, 1999; Almeida et al., 2017). Thus, using rice bran as the source of PP should be a more effective test of a new phytase product as compared with other PP sources. In addition, evaluating the phytate-degrading efficiency of phytase in rice bran-containing diets will provide an insight into the phytase's effectiveness. The high efficacy of phytase to degrade rice bran PP may also encourage the use of ingredients with high PP content in broiler diets, thereby reducing the complete dependence on corn and soybean meal and potentially reducing the variable production cost.

Lastly, it has been observed from previous work in our lab that broiler chickens during the first 2 wk post hatching were able to utilize P more efficiently than at an older age, and that phytase was more efficacious during this period when mineral digestibility was considered (Babatunde et al., 2019a,b). The starter phase in broiler chickens is characterized by rapid growth and development of tissues, muscles, and bones that form the foundation for the deposition of meat in the latter phases (Batal and Parsons, 2002). Thus, the adequate supply of nutrients including P in this phase is important. Most digestibility studies are carried out with birds at d 21 post hatching with limited studies reporting digestibility in younger broiler chickens. Hence, evaluating the potency of a new phytase to efficiently degrade rice bran PP during the starter period may give a clear understanding of the efficacy of the phytase in broiler chickens.

Therefore, the objective of this study was to evaluate the effects of PP concentrations from rice bran and varying doses of a novel consensus bacterial phytase variant (**PhyG**) on broiler chickens in the starter phase (d 1−11 post hatching) using growth performance, bone mineralization, apparent ileal digestibility (**AID**), and total tract retention (**TTR**) of energy and nutrients as response criteria. This study tested the null hypothesis that there was no effect of PP and phytase concentrations on responses of broiler chickens in the starter phase.

MATERIALS AND METHODS

Birds and Management

Male Cobb 500 broiler chicks were individually tagged and housed in temperature-controlled battery cages (model SB 4T, Alternative Design Manufacturing, Siloam Springs, AR) located at the poultry unit of the Purdue Animal Science Research and Experimental Station. Birds were given free access to water via water nipples and were fed a commercial starter mash diet formulated to meet or exceed the nutrient requirements of growing broiler chicks (Cobb, 2013) for 1 d as they could not be allocated to dietary treatments at d 0 due to transportation stress. Subsequently, birds were weighed and randomly allotted to treatments and the experimental diets were fed ad libitum for 10 d (d 1−11 post hatching). Housing temperature and humidity, general well-being of birds, and mortality were observed and recorded daily. All protocols of animal experiments were reviewed and approved by the Purdue University Animal Care and Use Committee.

Experimental Design and Diets

This experiment was conducted as a randomized complete block design with dietary treatments arranged as a $1 + 3 \times 5$ factorial and BW used as the blocking factor. Diets were a nutrient-adequate positive control diet (**PC**) with 2.8 g PP/kg or one of 15 nutrient-reduced negative control (NC: PC minus 88 kcal/kg ME, 0.8 g/kg dig. Lys, 2.0 g/kg available P, 1.8 g/kg Ca and 0.5 g/kg Na) diets with 3 PP (g/kg) levels, mainly from rice bran, at 2.3 (NC1), 2.8 (NC2), or 3.3 (NC3) and 5 PhyG supplementation at 0, 500, 1,000, 2,000, or 4,000 FTU/kg in a $1 + 3 \times 5$ factorial. The non-phytate P (**nPP**) content of the PC and NC diets were 4.3 and 2.3 g/kg, respectively, while the PC contained similar PP content as the NC2 diets. Levels of phytate and phytase used in this trial were those commonly found in commercial or experimental diets. The phytase used in this trial was a novel consensus bacterial 6-phytase variant originating from *Buttiauxella* spp but expressed in *Trichoderma reesei* (PhyG; Danisco Animal Nutrition, The Netherlands) and screened for a broad pH profile but higher activity in the acidic sections of the gut. Phytase was prepared as a premix with ground corn and included at 50 g/kg of the phytase diets. The PP contents of the NC diets were adjusted through the addition of rice bran and polished rice while soy hulls were used as filler. Energy and all other nutrients in the PC diet met or exceeded the nutrient requirements of broiler chicks as recommended by Cobb (2013). Titanium dioxide was included into all diets at 5 g/kg as an indigestible marker.

Sample Collection and Chemical Analyses

Excreta was collected twice daily during the last 3 d of the experimental period from pans lined with wax paper and placed under the cages. After collection, excreta was dried in a forced air oven at 56°C for 7 d and stored until analysis. On d 11 post hatching, individual BW of birds and feed intake (**FI**) per cage were recorded and used to determine the BW gain and gain to feed ratio (**G:F**). Furthermore, all birds were euthanized by CO_2 asphyxiation and dissected to collect digesta from the distal

two-thirds of the ileum (intestinal section from the Merkel's diverticulum to the ileocecal junction). Ileal digesta was flushed using distilled water into plastic containers, pooled by cage and stored at −20°C until they were freeze dried. The left tibia of 4 median weight birds per cage were collected, defatted using a Soxhlet extractor, weighed and ashed in a muffle furnace at 600°C for 24 h to determine bone ash as described by Ogunwole et al. (2017). Tibia ash was then reweighed to determine ash weight per bone. Dried ileal digesta and excreta samples were ground using a coffee and centrifugal (Retsch ZM 200 GmbH, Haan, Germany) grinder, respectively, and 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). Gross energy of diets, ileal digesta, and excreta samples was determined by an isoperibol bomb calorimeter using benzoic acid as the calibration standard (Parr 1261; Parr 105 Instrument Co., Moline, IL). Nitrogen content of samples were determined by combustion methods (TruMac N; LECO Corp., St. Joseph, MI; method 990.03; AOAC, 2000) using EDTA as a calibration standard and 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 AA analyses in the ingredients, diets, and ileal digesta samples. Ground samples were hydrolyzed by 6 M HCl (or BaOH for Trp analysis) at 110°C for 24 h under nitrogen atmosphere. However, samples used for the analysis of Met and Cys were oxidized by performic acid before acid hydrolysis. The concentrations of AA in samples were analyzed by a high-performance liquid chromatography after post-column derivatization (method 982.30 E [a, b, c]; AOAC, 2006). Titanium concentration in the diets, ileal digesta, and excreta samples were determined using methods previously described by Short et al. (1996) at the University of Missouri Experiment Station Chemical Laboratories (Columbia, MO). Following the wet ash digestion of samples by hydrochloric and nitric acid, P concentration was determined by spectrophotometry with absorbance read at 630 nm (Spark 10 M; Tecan Group Ltd., Männedorf, Switzerland), while Ca and Zn concentrations were determined by flame atomic absorption spectrometry using a Varian Spectr. AA 220FS (Varian Australia Pty Ltd., Victoria, Australia) with absorbance read at 425 and 214 nm, respectively. Phytase activity in diets was analyzed by DuPont Feed Technical Service (Brabrand, Denmark) using methods previously described by Engelen et al. (1994) with modifications from Christensen et al. (2020).

Calculation and Statistical Analyses

The AID and TTR of nutrients in the ileal digesta and excreta were determined using the index method according to the following equation:

$$\textit{AID or TTR}, \% = 100 - [(Ti_I/Ti_O) \times (N_O/N_I) \times 100]$$

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) 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 as previously described by Zhang and Adeola (2017).

Data was analyzed using the general linear model procedure of SAS (SAS Inst. Inc., Cary, NC) as $1 + 3 \times 5$ factorial arrangements of treatments with PC, phytate, phytase, their interactions as fixed effects and replicate blocks as random effects. Using PROC IML to generate appropriate contrast coefficients, polynomial contrasts were used to compare the PC and NC2 (0 FTU/kg and equivalent phytate-P as the PC), and to determine the linear and quadratic effects of PP and phytase concentrations in the NC diets. Cage served as the experimental unit for all analyses. Statistical significance was set at $P \leq 0.05$ and a trend was set at $0.05 < P \leq 0.1$.

RESULTS

All birds were healthy throughout the experimental period. Analyzed nutrients and phytase activity in experimental diets were similar with calculated values and within acceptable ranges (Table 2).

Broiler chickens fed the PC diet had a higher ($P < 0.05$) BW, BW gain, FI, and G:F than birds fed the NC2 without phytase (Table 3). There was no PP level × phytase dose interaction for any of the growth performance and bone ash responses. There was a linear reduction ($P \leq 0.01$) in BW, BW gain, and FI by 4.5, 5.0, and 4.1%, respectively, as PP concentrations increased from 2.3 to 3.3 g/kg in the NC diets. There was no effect of phytate on feed efficiency. With phytase supplementation (from 0 to 4,000 FTU/kg), there was a quadratic response ($P < 0.05$) with BW and FI, and a linear increase ($P < 0.01$) in the BW gain of broiler chickens. Similarly, the G:F of broiler chickens was linearly increased ($P < 0.01$) from 703 to 771 g/kg with the addition of phytase. Birds fed the NC2 diet without phytase had lower ($P < 0.01$) tibia ash (%) and tibia ash weight per bone (mg/bone) as compared with birds fed the PC diet (Table 3). Similarly, there was no significant effect of phytate on tibia ash properties. There was a quadratic response ($P < 0.01$) in tibia ash properties, with increases in percentage tibia ash from 37.2 to 47.9% and in tibia ash weight from 289 to 453 mg/bone as phytase concentration increased from 0 to 4,000 FTU/kg.

The AID of DM, energy, P, and Ca was lower ($P < 0.01$) in birds fed the NC2 without phytase as compared with birds fed the PC (Table 4). Similarly, the AID of

Table 1. Ingredient composition of experimental diets fed to broiler chickens at starter phase (d 1−11 post hatching), g/kg as-fed basis.

	Diet[1,2]			
Item	PC	NC1	NC2	NC3
Ingredients, g/kg				
Corn	504.3	438.1	434.8	433.6
Soybean meal, 480g/kg CP	322.8	308.0	302.7	297.2
Soybean oil	10.4	5.0	5.0	5.0
Rice, Polished	33.0	80.8	49.4	15.9
Rice bran	34.6	3.1	42.5	82.0
Soy hulls	13.6	46.1	47.7	49.1
Meat and bone meal	21.2	22.4	21.2	20.2
Limestone	9.8	8.8	9.1	9.3
Monocalcium phosphate	10.2	0.0	0.0	0.0
Salt	3.7	2.5	2.5	2.5
Vitamin-mineral premix[3]	3.0	3.0	3.0	3.0
DL-Methionine	3.6	3.0	3.1	3.1
L-Lysine.HCl	3.2	2.7	2.7	2.7
Threonine	1.7	1.3	1.3	1.2
L-Tryptophan	0.0	0.1	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	215.2	209.7	209.7	209.7
ME, kcal/kg	2950.0	2862.2	2862.2	2862.2
Ca	9.0	7.2	7.2	7.2
P	7.1	4.6	5.1	5.6
Phytate-P	2.8	2.3	2.8	3.3
Non-phytate P	4.3	2.3	2.3	2.3
Na	1.7	1.2	1.2	1.2
dig. Lys	12.2	11.4	11.4	11.4
dig. Met	6.4	5.7	5.8	5.8
dig. Thr	8.3	7.6	7.6	7.6

[1]Abbreviations: NC, negative control; PC, positive 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 99g 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.

Table 2. Analyzed energy and nutrients of experimental diets fed to broiler chickens at starter phase (d 1−11 post hatching), g/kg as-fed basis.

	Diet[1,2]			
Item	PC[3]	NC1[4]	NC2[5]	NC3[6]
Energy and nutrients, g/kg				
DM	890	888	889	884
GE, kcal/kg	4,016	3,940	3,942	3,958
CP	209.1	206.5	206.9	205.8
Phytate P	2.7	2.0	3.0	3.7
P	7.2	4.6	5.2	5.6
Ca	10.0	8.5	8.5	8.6
Zn	0.01	0.01	0.01	0.01
Arg	13.3	13.9	13.7	13.6
His	5.2	5.3	5.3	5.2
Ile	9.0	9.3	9.2	9.0
Leu	16.7	17.2	16.9	16.8
Lys	13.4	13.5	13.6	13.3
Met	6.0	5.9	5.7	5.6
Phe	10.0	10.3	10.1	10.1
Thr	8.7	8.7	8.6	8.6
Trp	2.5	2.6	2.6	2.7
Val	10.0	10.3	10.2	10.1
Ala	10.0	10.4	10.4	10.4
Asp	20.0	21.1	20.8	20.5
Cys	3.2	3.2	3.2	3.1
Glu	34.7	35.9	35.1	34.6
Gly	9.1	9.9	9.7	9.9
Pro	11.2	11.5	11.3	11.4
Ser	8.2	8.6	8.5	8.5
Tyr	6.7	7.1	6.9	6.8
Total AA	201.0	208.3	205.4	203.9

[1]Abbreviations: NC, negative control; PC, positive 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 226 phytase units/kg.
[4]NC1 diets had analyzed phytase activities of 200, 849, 1,332, 2,349, and 4,493 FTU/kg, respectively.
[5]NC2 diets had analyzed phytase activities of 184, 879, 1,244, 2,004, and 4,517 FTU/kg, respectively.
[6]NC3 diets had analyzed phytase activities of 202, 714, 1,070, 3,093, and 5,143 FTU/kg, respectively.

nitrogen in birds fed the NC2 diet without phytase was 9.3% lower ($P < 0.05$) than that of birds fed the PC diet. There was no interaction between phytate and phytase on the AID of DM, energy, nitrogen, P, Ca, and Zn. Similarly, there was no effect of phytate concentration on the AID of DM, energy, nitrogen, and Zn. However, there was linear decrease ($P < 0.01$) in the AID of P and Ca with increasing PP concentrations. There was a linear increase ($P \leq 0.05$) in the AID of DM, energy, and nitrogen with phytase supplementation. Likewise, the AID of P and Ca was linearly increased ($P < 0.01$) by 88.5 and 18.0%, respectively, with the addition of phytase in the NC diets. Within each NC diet, and in comparison with the NC diets without phytase, the percentage difference in the AID of P was higher with increasing phytase levels (Figure 1). In addition, the increase in the percentage difference of the AID of P was higher in the NC3 diets with 3.3 g PP/kg diet.

There was a significant difference ($P < 0.05$) in the AID of some indispensable AA including His, Met, Thr, Trp, and Val between birds fed the PC diet and the NC2 diet without phytase (Table 5). There was no interaction between PP levels and phytase dose on the AID for any of the indispensable AAs. Although, there was no significant effect of phytate on the AID of most indispensable AA, however, a quadratic trend ($P < 0.1$) was observed with the AID of Arg, His, Leu, Phe, and Val while a quadratic response ($P < 0.05$) was observed with the AID of Met, Thr, and Trp. The lowest AID of all indispensable AA was observed in NC2 diets with 2.8 g PP/kg. When phytase was added to the NC diets, there was a linear increase ($P < 0.01$) in the AID of all indispensable AA, with the highest improvements observed with the AID of Thr, Phe, and Trp (7.6, 7.0, and 7.0%, respectively), and the least improvement observed with the AID of Arg (4.8%). For effects on the dispensable AA, birds fed the PC had a higher ($P < 0.05$) AID of Cys, Pro, and Tyr as compared with birds fed the NC2 diet without phytase (Table 6). There was no interaction between phytate and phytase concentrations on any of the AID of dispensable AA. However, a quadratic

Table 3. Effect of phytate and phytase concentrations on growth performance and bone mineralization of broiler chickens fed experimental diets at starter phase (d 1–11 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	267	214	272	785	420	48.1	6
NC2	2.8	0	215	168	238	704	297	38.3	6
Main effect phytate									
	2.3		247	197	265	743	389	44.6	30
	2.8		243	194	260	745	395	45.1	30
	3.3		236	187	254	737	374	44.1	30
Main effect phytase									
		0	214	166	236	703	289	37.2	18
		500	237	190	258	735	377	44.5	18
		1,000	245	194	262	742	397	46.0	18
		2,000	251	202	267	758	412	47.1	18
		4,000	262	213	276	771	453	47.9	18
SEM[3]			5.02	5.40	5.13	14.58	14.66	0.70	
P values									
PC vs. NC2 (0 FTU/kg)			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	
Phytate × Phytase			1.00	1.00	1.00	1.00	0.98	0.35	
Phytate linear			<0.01	0.01	<0.01	0.49	0.10	0.32	
Phytate quadratic			0.62	0.45	0.73	0.50	0.10	0.07	
Phytase linear			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	
Phytase quadratic			0.02	0.07	0.03	0.36	<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 g/kg available P, 1.8 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 is for interaction effects.

trend ($P < 0.1$) and a quadratic response ($P \leq 0.05$) was observed in the AID of all dispensable AA except Glu with increasing PP concentrations. The lowest AID of all dispensable AA was observed in NC2 diets with 2.8 g PP/kg. With the addition of phytase, there was a linear increase ($P < 0.01$) in the AID of all dispensable and total AA.

Birds fed the NC2 diet without phytase had lower ($P < 0.05$) AME, AMEn, and TTR of P and Ca as compared with birds fed the PC diet (Table 7). There was no interaction between phytate and phytase concentrations on the metabolizable energy and the TTR of nutrients. Increasing PP concentrations in NC diets, linearly increased ($P < 0.05$) the TTR of nitrogen while reducing ($P < 0.01$) the TTR of P and Ca. There was no effect of phytate on AME, AMEn, and TTR of DM and Zn. A linear increase ($P < 0.05$) in the TTR of DM, nitrogen and Zn, and a quadratic response ($P \leq 0.05$) with the AMEn and TTR of P and Ca was observed with increasing phytase supplementation.

DISCUSSION

The use of phytase and its benefits on broiler production have been growing over the years (Sebastian et al., 1996; Selle and Ravindran, 2007; Babatunde et al., 2020b). It is probably the enzyme class that has shown the highest consistency in its ability to improve the growth performance, nutrient utilization, or bone mineralization of poultry and swine, while efficiently extracting nutrients from feedstuff (Adedokun et al., 2015; Babatunde et al., 2019b). Although phytase is an established enzyme, there is always the opportunity to improve the product or the application in poultry and swine. To thoroughly investigate the efficiency of a new phytase product on broiler production, experimental trials that examine their effects on nutrient matrices are important. Growth performance, utilization of nutrients particularly with minerals such as P and Ca, and bone mineralization of broiler chickens are important parameters necessary to investigate the efficacy of the phytase enzyme. Furthermore, the PP factor is another tool used to investigate the efficacy of phytase. To counter the negative effects of phytate, there arises the need for phytase to hydrolyze phytate quickly and completely in the upper section of the gastrointestinal tract thus, reducing the antinutritive effect of phytate and promoting the extra-phosphoric effects of the phytase (Dersjant-Li et al., 2020). Phytase degrades phytate in a stepwise manner in the gut and releases bound nutrients for use by birds while reducing the amount of minerals such as P wasted through excreta and into the environment. In theory, it is assumed that phytase will hydrolyze most of the phytate content in the diet however, the accessibility of phytate differ among feed ingredients. Leske and Coon (1999) and Almeida et al. (2017) observed that the accessibility of phytate in rice bran was lower than in canola meal, soybean meal, or sunflower meal. Thus, including rice bran as the source of PP and varying its

Table 4. Effect of phytate and phytase concentrations on apparent ileal digestibility (%) of dry matter (DM), energy, and nutrients in broiler chickens fed experimental diets at starter phase (d 1−11 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	67.6	71.2	71.3	62.2	68.5	27.8	6
NC2	2.8	0	60.5	63.9	64.7	35.2	54.5	22.1	6
	Main effect phytate								
	2.3		62.5	66.8	69.7	55.6	64.5	25.3	30
	2.8		62.9	65.7	68.2	49.8	59.2	25.5	30
	3.3		67.6	65.6	68.7	42.7	50.6	24.0	30
	Main effect phytase								
		0	61.7	64.5	65.9	33.0	52.2	22.7	18
		500	62.8	65.4	67.8	45.9	56.5	24.5	18
		1,000	63.0	66.2	69.8	50.5	58.7	25.1	18
		2,000	64.1	67.0	70.1	55.2	61.4	26.2	18
		4,000	63.7	67.1	70.8	62.2	61.6	26.2	18
SEM[3]			1.43	1.36	2.05	3.24	2.54	6.68	
P values									
PC vs. NC2 (0 FTU/kg)			<0.01	<0.01	0.03	<0.01	<0.01	0.54	
Phytate × Phytase			0.87	1.00	1.00	0.86	0.94	1.00	
Phytate linear			0.33	0.16	0.46	<0.01	<0.01	0.75	
Phytate quadratic			0.25	0.47	0.38	0.72	0.22	0.81	
Phytase linear			0.05	<0.01	<0.01	<0.01	<0.01	0.47	
Phytase quadratic			0.49	0.63	0.35	0.10	0.16	0.84	

[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 g/kg available P, 1.8 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 is for interaction effects.

concentration in the diet, should provide a challenging but adequate substrate from which phytase can hydrolyze while serving as a good tool for evaluating the efficacy of the phytase. Previous studies have reported the efficacy of phytase to improve utilization of nutrients as the PP content of diets was increased (Ravindran et al., 2000, 2006; Liu et al., 2008)

In the current trial and in agreement with several studies (Sebastian et al., 1996; Babatunde et al 2019a, b), there was a difference in the growth performance of birds fed the PC diet and the NC2 diet. Although both diets had similar PP content, they differed in their nPP content with the NC2 diet having the lower concentration. The impact of the unavailability of the P in phytate was evident in birds fed the NC2 diet without phytase as they had lower BW, BW gain, and feed efficiency as compared with birds fed the PC diet. Broiler chicks have been known to perform poorly when

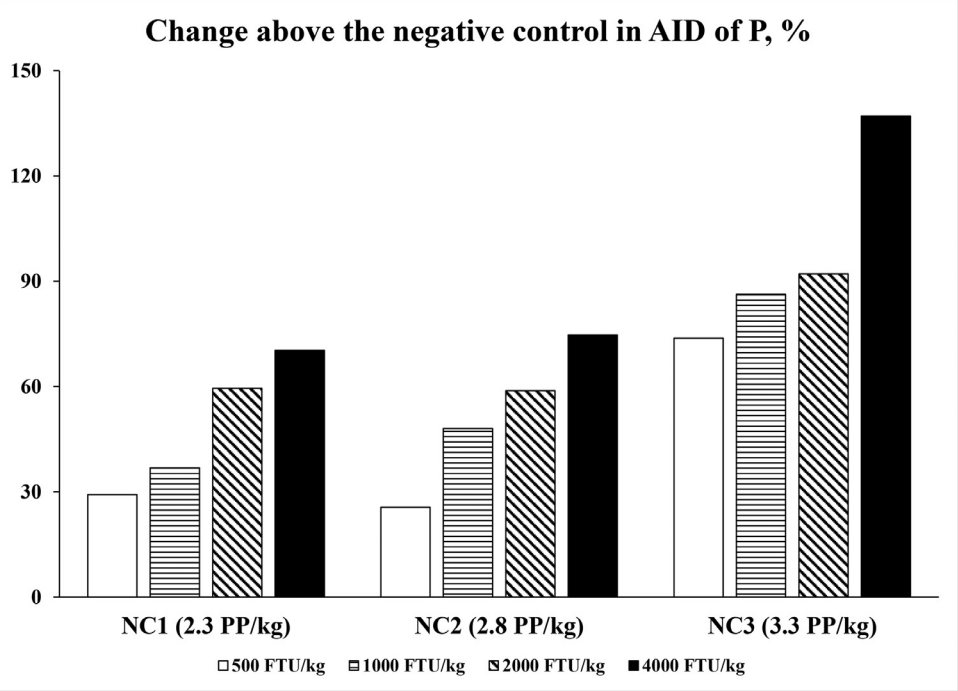

Figure 1. The efficacy of phytase (PhyG) on the apparent ileal digestibility (AID) of P relative to the phytate P (PP) concentration in each of the NC diets. The percentage difference values were derived by subtracting the AID of P in the NC diets (i.e., NC1, NC2, or NC3) with 0 FTU/kg from 500, 1,000,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

Table 5. Effect of phytate and phytase concentrations on apparent ileal digestibility (%) of indispensable AA in broiler chickens fed experimental diets at starter phase (d 1−11 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	80.3	75.2	72.0	73.2	76.2	86.8	73.7	68.6	77.9	67.7	6
NC2	2.8	0	78.3	70.6	68.0	69.9	72.4	80.9	70.5	62.0	74.3	62.6	6
	Main effect phytate												
	2.3		82.1	74.4	73.2	74.1	76.9	86.1	75.0	67.2	77.0	68.4	30
	2.8		80.7	73.2	71.6	72.8	75.6	84.6	73.4	65.4	76.4	66.4	30
	3.3		81.7	74.7	72.6	74.1	76.2	85.6	74.7	67.7	79.2	67.7	30
	Main effect phytase												
		0	79.7	72.0	70.1	71.3	74.1	82.6	71.9	64.4	75.4	65.4	18
		500	80.4	73.1	71.1	72.5	75.2	84.4	72.9	65.9	75.0	66.4	18
		1,000	81.6	74.1	72.8	73.8	76.4	86.3	74.7	66.9	77.5	67.8	18
		2,000	82.3	75.0	73.5	74.8	76.9	86.7	75.6	67.4	79.3	68.5	18
		4,000	83.5	76.2	74.7	75.9	78.6	87.2	76.9	69.3	80.7	69.5	18
SEM[3]			1.11	1.34	1.51	1.44	1.53	0.94	1.38	1.71	1.24	1.73	
P values													
PC vs. NC2 (0 FTU/kg)			0.20	0.02	0.07	0.10	0.08	<0.01	0.10	0.01	0.04	0.04	
Phytate × Phytase			0.65	0.60	0.40	0.68	0.73	0.44	0.58	0.98	0.48	0.41	
Phytate linear			0.63	0.75	0.55	1.00	0.48	0.37	0.72	0.63	0.01	0.57	
Phytate quadratic			0.06	0.06	0.13	0.09	0.26	0.02	0.06	0.04	0.01	0.09	
Phytase linear			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	
Phytase quadratic			0.84	0.94	0.85	0.87	0.91	0.05	0.92	0.92	0.25	0.86	

[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 g/kg available P, 1.8 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 is for interaction effects.

deficient of P and other nutrients as they play a huge role in supporting growth and development particularly at the starter phase (Babatunde et al., 2019a). In the presence of increased PP concentrations in the diets, a negative impact was observed in the growth performance of broiler chicks. Cabahug et al. (1999) observed a similar decline in growth performance of broiler chickens when dietary PP was increased from 2.9 to 4.4 g/kg using rice pollards. This observation was expected as phytate is known to bind nutrients in its complex and prevent their use by birds. Thus, the higher the PP content in the diet the lower the nutrient availability to the birds, which will explain why birds fed diets with the highest PP (3.3 g PP/kg) from rice bran had the lowest performance. However, the use of PhyG mitigated the negative impacts of PP on the growth performance of birds indicating its extraphosphoric effects especially at high doses. Previous studies have shown that inclusion of phytase improves the growth performance of broiler chicks fed diets deficient in nutrients in the starter phase (Kiarie et al., 2015; Babatunde et al., 2019a,b; Dersjant-Li et al., 2020). Dietary phytase up to 4,000 FTU/kg

Table 6. Effect of phytate and phytase concentrations on apparent ileal digestibility (%) of dispensable and total AA in broiler chickens fed experimental diets at starter phase (d 1−11 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	71.1	70.9	61.0	79.1	66.1	73.1	68.6	74.8	73.5	6
NC2	2.8	0	67.5	68.4	49.8	77.1	61.9	68.8	66.1	71.0	69.8	6
	Main effect phytate											
	2.3		72.0	73.0	57.4	80.5	66.7	72.9	70.0	75.4	74.2	30
	2.8		70.4	71.2	55.9	79.2	64.6	71.5	68.5	73.9	72.8	30
	3.3		72.1	72.3	58.7	80.0	67.2	73.2	70.2	75.3	74.0	30
	Main effect phytase											
		0	69.5	70.2	53.2	78.2	64.3	70.5	67.4	72.4	71.5	18
		500	70.8	70.8	55.3	78.8	66.2	72.1	68.1	73.6	72.6	18
		1,000	71.4	72.4	57.4	80.0	66.1	72.4	69.2	75.0	73.9	18
		2,000	72.4	73.0	59.1	80.7	66.6	73.6	70.7	76.1	74.5	18
		4,000	73.5	74.5	61.7	81.7	67.5	74.3	72.5	77.2	75.7	18
SEM[3]			1.60	1.38	2.03	1.10	1.64	1.34	1.57	1.28	1.40	
P values												
PC vs. NC2 (0 FTU/kg)			0.12	0.20	<0.01	0.20	0.07	0.02	0.25	0.04	0.07	
Phytate × Phytase			0.58	0.39	0.53	0.59	0.27	0.66	0.82	0.61	0.68	
Phytate linear			0.92	0.46	0.30	0.49	0.66	0.70	0.81	0.94	0.80	
Phytate quadratic			0.07	0.05	0.06	0.11	0.01	0.04	0.06	0.04	0.09	
Phytase linear			<0.01	<0.01	<0.01	<0.01	0.02	<0.01	<0.01	<0.01	<0.01	
Phytase quadratic			0.97	0.77	0.90	0.88	0.71	0.76	0.44	0.87	0.88	

[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 g/kg available P, 1.8 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 is for interaction effects.

Table 7. Effect of phytate and phytase concentrations on total tract retention (%) of dry matter (DM), energy, and nutrients in broiler chickens fed experimental diets at starter phase (d 1−11 post hatching).

Diet[1]	Phytate P, g/kg	Phytase, FTU/kg	DM	AME, kcal/DMI	AMEn, kcal/DMI	Nitrogen	P	Ca	Zn	No. of replicates
PC[2]	2.8	0	73.2	3,432	3,224	67.4	56.2	48.0	27.4	6
NC2	2.8	0	71.4	3,292	3,091	65.3	36.0	26.3	19.5	6
	Main effect phytate									
	2.3		71.7	3,303	3,101	66.1	56.9	50.2	28.4	30
	2.8		72.4	3,321	3,113	67.7	54.8	43.2	27.4	30
	3.3		72.7	3,345	3,136	68.1	49.4	33.7	26.7	30
	Main effect phytase									
		0	71.3	3,289	3,088	65.4	38.2	27.4	18.9	18
		500	71.8	3,324	3,119	66.8	50.9	39.9	26.0	18
		1,000	72.8	3,339	3,131	67.6	57.0	45.2	28.5	18
		2,000	72.6	3,358	3,148	68.3	60.4	48.2	31.5	18
		4,000	72.9	3,306	3,096	68.3	61.7	51.2	32.6	18
SEM[3]			1.01	4.09	3.74	1.44	3.20	3.20	5.29	
P values										
PC vs. NC2 (0 FTU/kg)			0.22	0.02	0.01	0.30	<0.01	<0.01	0.29	
Phytate × Phytase			0.96	1.00	0.99	0.99	0.80	0.71	1.00	
Phytate linear			0.14	0.11	0.14	0.03	<0.01	<0.01	0.61	
Phytate quadratic			0.65	0.88	0.80	0.43	0.36	0.47	0.95	
Phytase linear			0.03	0.37	0.51	0.01	<0.01	<0.01	<0.01	
Phytase quadratic			0.49	0.06	0.05	0.33	<0.01	<0.01	0.32	

[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 g/kg available P, 1.8 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 is for interaction effects.

improved BW gain and feed efficiency of broiler chicks comparably with birds fed the PC. This indicated that, PhyG was able to efficiently hydrolyze the phytate complex in rice bran, release most of the nutrients required by birds for growth and development (compensated nutrients down spec applied), and recover the performance of birds compared with a nutrient adequate diet. The mitigating effect of phytase is one of the reasons for its increased use in commercial broiler productions as its inclusion is immediately observed in the growth performance of birds.

The adverse effect of P deficiency on bone mineralization has been well documented in previous studies (Dersjant-Li et al., 2018; Babatunde et al., 2020a). There was a clear difference in the tibia ash weight and percentage between birds fed the PC and NC diets without phytase. Because the bones are mostly comprised of P and Ca, a deficiency of these minerals in the diet causes a resorption from the bones to support the P or Ca requirement of birds. Thus, birds fed the deficient NC diets had lower bone mineralization as compared with birds fed the PC diet, except for those NC diets supplemented with 4,000 FTU/kg. There was no effect of PP concentration on tibia ash. However, birds may have utilized the available P from the diet for bone mineralization; and besides the obvious impact of low available P, there was no further negative effect of additional increase in the dietary PP level. The inclusion of PhyG at a low dose of 500 FTU/kg improved the tibia ash of birds by 20% as compared with birds fed the NC diets without phytase. A high dose of PhyG at 4,000 FTU/kg improved the tibia ash percentage of birds by up to 28% as compared with the PC. This observation agrees with previous studies where inclusion of phytase at either traditional or high doses improved bone mineralization considerably and supported the skeletal structure of broiler chickens (Olukosi et al., 2013; Gautier et al., 2018; Babatunde et al., 2020a). Moreover, the high dose of phytase may have improved the ratio of Ca to P from the diet thus increasing the absorption and utilization of both minerals for skeletal development. Thus, under the current experimental conditions and considering bone mineralization, it may be implied that PhyG at highest inclusion compares favorably with phytase from other microbial sources as previously reported in Babatunde et al. (2020a).

As observed previously, birds fed NC diets (without phytase) deficient in minerals, energy and protein had lower AID of energy and nutrients as compared with birds fed the nutrient dense PC. Previous studies have shown that feeding nutrient deficient diets negatively impacted the utilization of these nutrients and the overall production of birds (Ravindran et al., 2001; Dilger et al., 2004). In particular, P deficiency has been known to negatively impact the utilization of energy and other nutrients as P is required in various biochemical reactions that support the metabolism of energy, protein, minerals, and even vitamins (Ravindran et al., 2006; Babatunde et al., 2020a). This impact is however of varying degrees as an increase in the PP of diets did not influence the AID of DM, energy, nitrogen, and zinc but negatively impacted the AID of P and Ca. In contrast to the current study, Ravindran et al. (2000, 2006) observed negative effects of increasing PP from rice pollards and rice bran, respectively, on the AID of nitrogen. However, in both studies, samples were collected from birds at d 21 and 25 post hatching, respectively, as compared with d 11 post hatching in the current study. Although, studies have shown that phytate is able to bind and hinder the utilization of energy, nitrogen, and minerals (Sebastian et al., 1996; Ravindran et al., 2006), it is possible that increased PP concentrations does not

significantly impact their utilization in young broiler chickens. In the current study, P deficiency reduced the AID of nutrients, however an increase in the PP content only impacted the AID of P and Ca which is directly bound by phytate in the complex. Other nutrients may have been indirectly affected by the phytate content in the diets but not in a sufficient manner as to be impacted by a further increase in the PP content.

Previous work from our lab has shown that birds at d 13 or 14 are sensitive to low available P and high phytate content as observed with the AID of P and Ca (Babatunde and Adeola, 2021; Babatunde et al., 2020b). Hypothesis was that when the PP content is increased and nPP kept constant, the amount of P, Ca, as well as other minerals like Zn available to birds reduces significantly in the absence of phytase thereby impacting the AID of this minerals. Surprisingly, the increasing PP content in diets did not affect the AID of Zn. The early age of birds, and consequently their sensitivity to the Zn content in the diet, absorption capacity, or requirement may have impacted the effect of increasing PP on the AID of Zn. Moreover, Zn is usually required in smaller amounts as compared with P or Ca, thus the impact of increasing PP on its utilization may be reduced. Inclusion of phytase improved the AID of DM, energy, nitrogen, P, and Ca as have been observed in previous studies (Ravindran et al., 2000, 2006; Bello et al., 2019).

The AID of P was increased up to 88% when PhyG was included at 4,000 FTU/kg as compared with 0 FTU/kg and matched the AID of P in birds fed the PC. Although there was no interaction between PP and phytase, the percentage difference in the AID of P within each NC diet increased with elevated phytase levels. In the NC3 diets, which had the highest PP content, inclusion of phytase at 4,000 FTU/kg improved the AID of P in birds by up to 137% when compared with birds fed the NC3 without phytase. This observation supported the assumption that with the increase in the phytate substrate pool, the efficacy of phytase was improved in relation to P release. It also indicates that the novel PhyG was very efficacious in releasing most of the P in the phytate rich, rice bran based-diets such that it compares favorably with the PC diet containing inorganic P. Therefore, including PhyG in diets at sufficient doses, could effectively replace all the inorganic P sources including monocalcium phosphate in the diet without compromising on the digestibility of P in birds (Marchal et al., 2021). In contrast with Babatunde et al. (2020a), phytase had no effect on the AID of Zn in the current study.

Feeding diets low in available P has not shown consistent effects on the AID of AA in broiler chickens (Dilger et al., 2004). However, the reduction in the nPP and Lys content of the NC2 diet, negatively impacted the AID of some dispensable and indispensable AA such as His, Met, Trp, and Tyr in broiler chickens. Similarly, increasing the PP content of diets did not affect all AA although, the AID of some AA was low when diets contained 2.8 g PP/kg as compared with 2.3 or 3.3 g PP/kg. This was different from observations in Ravindran et al. (2006) where increasing PP content hindered the AID of most AA. In the current study, it seemed reasonable to note the decrease in the AID of AA when PP content was increased from 2.3 to 2.8 g PP/kg as a higher phytic acid content may bind AA in the gut of chickens and prevent their utilization. However, we could not readily explain the increase in the AID of AA when the PP was further increased from 2.8 to 3.3 g PP/kg. It could be that observations were as a result of changes in the feed composition, fiber content, analyzed P and Ca levels or other analyzed nutrients between the NC diets. The beneficial effects of PhyG in broiler chickens was evident considering the improvements with the AID of all AA and in agreement with previous studies (Amerah et al., 2014; Babatunde et al., 2020a). It suggests that phytase is able to degrade PP before it forms protein-phytate complexes in the gut, reduce the negative impact of phytate on protease function, or improve the Na-K pump function thus, increasing the utilization of AA in diets, and preventing the loss of nitrogen into the environment.

The utilization of energy from diets is important because all biochemical reactions in the body of broiler chickens require energy to function. In the current study, the slight deficiency in the energy content of NC diets as compared with the PC negatively impacted the AME and AMEn of broiler chickens. The deficiency of P would have also played a role in the low energy utilization of the NC diets. In agreement with Ravindran et al. (2000), increasing the PP content had no impact on the utilization of energy. Phytase inclusion improved the AID of energy in the NC diets of the current study and in agreement with previous studies (Olukosi et al., 2008; Woyengo and Wilson, 2019). Observations with the TTR of nutrients followed a similar pattern with the AID of nutrients discussed previously except that the response of TTR of Ca to increasing phytase supplementation was greater than with AID of Ca. Birds fed NC diets without phytase were able to digest Ca to a reasonable extent however, due to the low TTR of P, the excessive Ca released was not retained but excreted. Phytase inclusion improved the retention of Zn which is required for immune health and as a cofactor for many essential biological reactions in broiler chickens (Yi et al., 1996). The disparity in the effect of phytase on Zn digestibility and retention in the current study may have been due to the differences in the site of sample collection.

In conclusion, results from the current trial showed that increasing the PP content of diets negatively impacted the utilization of nutrients by broiler chickens. However, regardless of the PP content of diets in this trial, PhyG improved the growth performance, bone mineralization, and utilization of energy, AA, and minerals in broiler chickens. From this trial, phytase supplementation especially at high doses elicited extraphosphoric effects that alleviated the negative impact of high phytate and the dietary nutrient

deficiency while supporting the productivity of broiler chickens. Therefore, the use of high phytate ingredients in addition to PhyG may be considered when formulating diets for broiler chicks to potentially reduce feed costs as well as P and N emissions, with consequences on sustainability. Lastly, results in this trial reveal that broiler chickens in the starter phase respond to the PP content of diets. Similarly, broiler chickens are able to increase their productivity when fed nutrient deficient diets supplemented with a novel consensus bacterial phytase variant.

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DISCLOSURES

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

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