Inositol and gradient phytase supplementation in broiler diets during a 6-week production period: 2. Effects on phytate degradation and inositol liberation in gizzard and ileal digesta contents[1]

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ABSTRACT An experiment was conducted to evaluate effects of dietary phytase and inositol supplementation on phytate degradation in gizzard and ileal digesta contents of broilers from 1 to 43 d of age. One thousand nine hundred and twenty Yield Plus × Ross 708 male chicks were placed in 64 floor pens (30 birds/pen). Each pen received 1 of 8 dietary treatments from 1 to 43 d of age. Treatment 1 was formulated to contain 0.165 and 0.150% lower calcium and phosphorus, respectively, than treatment 7. Treatments 2 to 6 were formulated by adding phytase at 500, 1,500, 4,500, 13,500, and 40,500 phytase units (**FTU**)/kg, respectively, to treatment 1. Treatment 8 was established by adding inositol to treatment 7 based on the maximum inositol liberation in treatment 6. At 15, 29, and 43 d of age, gizzard contents, ileal digesta, and blood were collected for analysis of inositol and inositol phosphate (**IP**) 2, 3, 4, 5, and 6 concentrations. Increasing phytase from 0 to 40,500 FTU/kg resulted in log-quadratic reductions ($P < 0.01$) of inositol pentaphosphate and inositol hexakisphosphate concentrations in the gizzard and ileal digesta contents of broilers at 15, 29, and 43 d of age. The increase in phytase doses in treatments 1 to 6 reduced IP3 and IP4 concentrations in a log-quadratic manner ($P < 0.05$) at each collection period in gizzard contents but only at 43 d of age in the ileal digesta of broilers ($P < 0.01$). Log-quadratic increases ($P < 0.05$) of inositol concentrations were observed in gizzard and ileal digesta contents when increasing phytase activity from 0 to 40,500 FTU/kg at each collection period, which translated to a log-quadratic increase ($P < 0.01$) in plasma inositol concentration of broilers at 15, 29, and 43 d of age. Phytase supplementation up to 40,500 FTU/kg may benefit broilers by reducing phytate concentrations in the gizzard and ileal digesta contents. Moreover, inositol release in the ileal digesta may translate to increased plasma inositol concentration.

Key words: broiler, phytase, phytate, inositol

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INTRODUCTION

Exogenous phytase is supplemented in broiler diets to liberate additional phosphorus (Selle and Ravindran, 2007). Moreover, extra-phosphoric effects of phytase have been reported to also enhance growth performance of broilers beyond those fed diets with adequate phosphorus concentration (dos santos et al., 2013; Campasino et al., 2014). These extra-phosphoric effects have been associated with enhancements in nutrient availability through the degradation of phytate to inositol (Beeson et al., 2017). Previous research demonstrated that an *Escherichia coli* phytase addition in broiler diets of up to 3,000 phytase units (**FTU**)/kg increased phytate degradation, inositol liberation, phosphorus, calcium, and amino acid digestibility (Sommerfeld et al., 2018).

The degradation of phytate in broiler diets occurs through a stepwise removal of phosphate groups of phytate from inositol hexakisphosphate (**IP6**) to inositol (Selle and Ravindran, 2007). Many previous studies have demonstrated the efficacy of phytase in degrading IP6 (Tamim et al., 2004). However, it appeared that

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broilers may have difficulties in degrading lower phytate esters when provided phytase at 500 FTU/kg, such as inositol triphosphate (**IP3**) and inositol tetraphosphate (**IP4**) (Zeller et al., 2015a,b; Bedford and Walk, 2016). A previous study indicated the accumulation of IP3 and IP4 even when supplementing *E. coli* phytase up to 1,500 FTU/kg (Walk et al., 2014; Beeson et al., 2017). In addition, these lower phytate esters may have similar antinutritive effects with IP6 to chelate nutrients and compromise digestive enzyme efficacy leading to poor growth performance of broilers (Persson et al., 1998; Yu et al., 2012). Therefore, it is important to consider phytase efficacy on the extent of phytate degradation not only for IP6 but also for IP3 and IP4 (Bedford and Walk, 2016). Unfortunately, research evaluating the efficacy of phytase to minimize IP3 and IP4 accumulations in the digesta of broilers is sparse (Zeller et al., 2015a,b; Sommerfeld et al., 2018).

The result of further degradation of lower phytate esters is inositol liberation. After its liberation, inositol can be effectively absorbed in the small intestine (Croze and Soulage, 2013), which has been reported to provide growth-promoting effects (Cowieson et al., 2013; Zyla et al., 2013). Previous research observed an increase in plasma inositol concentrations when feeding broilers with elevated phytase concentrations (Cowieson et al., 2015). These attributes of inositol may allow for its use as an indicator of phytase efficacy to degrade phytate (Cowieson et al., 2017). However, additional data are needed to evaluate the extent of inositol concentration in the plasma of broilers provided phytase supplementation beyond 1,500 FTU/kg. Therefore, the current

Table 1. Ingredient and nutrient composition of negative (NC) and positive control (PC) diets fed to broilers during starter (1–15 d of age), grower (16–29 d of age), and finisher (30–41 d of age) periods.

	Starter		Grower		Finisher	
Item	NC	PC	NC	PC	NC	PC
Ingredient, %						
Corn	57.47	55.82	61.63	59.98	66.70	65.05
Soybean meal	36.61	36.86	31.69	31.94	26.63	26.88
Vegetable oil	1.18	1.80	2.29	2.91	2.62	3.24
Dicalcium phosphate	1.23	2.04	1.03	1.84	0.84	1.65
Calcium carbonate	1.07	1.05	0.98	0.96	0.90	0.87
Sodium chloride	0.40	0.39	0.40	0.40	0.40	0.40
Builder sand[1]	0.70	0.70	0.70	0.70	0.70	0.70
DL-Methionine	0.30	0.30	0.27	0.27	0.25	0.25
L-Lysine•HCl	0.17	0.17	0.17	0.16	0.16	0.16
L-Threonine	0.09	0.09	0.08	0.08	0.07	0.07
Mineral premix[2]	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix[3]	0.10	0.10	0.08	0.08	0.05	0.05
Choline chloride	0.07	0.07	0.07	0.07	0.07	0.07
Titanium dioxide	0.50	0.50	0.50	0.50	0.50	0.50
Xylanase[4]	0.01	0.01	0.01	0.01	0.01	0.01
Calculated nutrient composition, % (unless otherwise noted)						
AME_n, kcal/kg	3,000	3,000	3,110	3,110	3,185	3,185
Crude protein	21.83	21.82	19.80	19.79	17.77	17.77
Digestible lysine	1.19	1.19	1.07	1.07	0.95	0.95
Digestible sulfur amino acids	0.88	0.88	0.81	0.81	0.74	0.74
Digestible threonine	0.80	0.80	0.72	0.72	0.63	0.63
Digestible valine	0.89	0.89	0.81	0.81	0.73	0.73
Digestible isoleucine	0.84	0.84	0.75	0.75	0.67	0.67
Calcium	0.80	0.96	0.71	0.87	0.62	0.78
Nonphytate phosphorus	0.33	0.48	0.29	0.44	0.24	0.39
Sodium	0.18	0.18	0.18	0.18	0.18	0.18

[1]The NC basal diet (treatment 1) was supplemented with *E. coli* phytase expressed in *Trichoderma reesei* at the expense of sand (Quantum Blue 5G, AB Vista Feed Ingredients, Marlborough, UK; analyzed as 7,700 FTU/g) at 500, 1,500, 4,500, 13,500, and 40,500 FTU/kg of diet to create treatments 2 to 6, respectively. The PC basal diet (treatment 7) was supplemented with inositol at the expense of sand to generate treatment 8.[5,6]

[2]Trace mineral premix include per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100 mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (ethylenediamine dihydriodide), 1.4 mg; and Se (sodium selenite), 0.3 mg.

[3]Vitamin premix includes per kg of diet: vitamin A (vitamin A acetate), 18,739 IU; vitamin D_3 (cholecalciferol), 6,614 IU; vitamin E (DL-alpha tocopherol acetate), 66 IU; menadione (menadione sodium bisulfate complex), 4 mg; vitamin B_{12} (cyanocobalamin), 0.03 mg; folacin (folic acid), 2.6 mg: D-pantothenic acid (calcium pantothenate), 31 mg; riboflavin (riboflavin), 22 mg; niacin (niacinamide), 88 mg; thiamin (thiamin mononitrate), 5.5 mg; biotin (biotin), 0.18 mg; and pyridoxine (pyridoxine hydrochloride), 7.7 mg.

[4]Econase XT, AB Vista Feed Ingredients, Marlborough, UK.

[5]One unit of phytase activity (FTU) is defined as the quantity of enzyme to liberate 1 μmol of inorganic phosphate per min from 0.0051 mol/L sodium phytate at pH 5.5 and 37°C.

[6]Inositol supplementation was calculated based on the maximum inositol liberation from Treatment 6 at 0.26, 0.25, and 0.23%, respectively, in the starter, grower, and finisher periods.

experiment was designed to determine effects of elevated phytase supplementation and inositol addition on the extent of phytate degradation and plasma inositol concentrations of broilers.

MATERIALS AND METHODS

All experimental procedures regarding live birds were approved by the Institutional Animal Care and Use Committee at Auburn University (PRN 2018-3254).

Bird Husbandry

The present study used the same set of birds, husbandry practices, and dietary treatments with the companion manuscript (Tables 1 and 2). One thousand nine hundred and twenty Yield Plus × Ross 708 male broilers were obtained from a commercial hatchery (Aviagen North America, Huntsville, AL). All birds were vaccinated for Marek's disease, Newcastle disease, and infectious bronchitis. Birds were randomly distributed into 64 floor-pens with 30 birds per pen (0.07 m^2 per bird). Broilers were housed in a solid-sided building equipped with vent boards, exhaust fans, evaporative cooling pads, forced-air heaters, and an electric controller to maintain optimum ventilation and temperature. Each pen was provided with a tube feeder, a nipple drinker line, and litter from 2 previous flocks. At placement, the house temperature was adjusted to 33°C and gradually decreased to 20°C at 41 d of age. Photoperiod was provided at 23L:1D from 1 to 7 d of age and 20L:4D for the remainder of the experimental period. In addition, light intensity was set at 30 lux at chick placement and was subsequently dimmed to 10 and 5 lux at 7 and 14 d after hatch, respectively. The intensity was verified at bird level (30 cm) using a photometric sensor (Extech 407026 Heavy Duty Light Meter; Extech Instruments, Nashua, NH). Three feeding programs were provided from 1 to 15, 16 to 29, and 30 to 41 d of age as starter, grower, and finisher diets, respectively. The starter diet was provided in a crumble form, while the grower and finisher diets were provided in pellet form. Feed and water were provided ad libitum throughout the experimental period.

Table 2. Analyzed activity of phytase (FTU/kg) in the starter (1–15 d of age), grower (16–29 d of age), and finisher (30–41 d of age) diets.[1]

Dietary treatments	Starter	Grower	Finisher
Negative control (NC)[2]	65	<50	<50
NC + 500 FTU/kg	417	519	641
NC + 1,500 FTU/kg	1,250	1,250	1,490
NC + 4,500 FTU/kg	3,313	3,350	3,960
NC + 13,500 FTU/kg	12,480	13,710	12,690
NC + 40,500 FTU/kg	36,640	41,290	42,070
Positive control (PC)[2]	695	238	267
PC + inositol[3]	65	<50	<50

[1]One unit of phytase activity (FTU) is defined as the quantity of enzyme to liberate 1 μmol of inorganic phosphate from 0.0051 mol/L sodium phytate per minute from sodium phytate at pH 5.5 and 37°C. Phytase activity was analyzed by ELISA specific for Quantum Blue (ESC, Standard Analytical Method, SAM099; AB Vista) according to method by Engelen et al. (2001).

[2]The negative control diet was formulated to contain 0.165 and 0.150% lower calcium and phosphorus, respectively, than the the positive control diet.

[3]Inositol supplementation was calculated based on the maximum inositol liberation from the diet containing the highest phytase concentration (treatment 6).

Dietary Treatments

Broilers received 1 of 8 dietary treatments during starter (1–15 d), grower (16–29 d), and finisher (30–43 d; Table 1) periods. A negative control (**NC**) diet (treatment 1) was formulated to contain 0.165 and 0.150% lower calcium and nonphytate phosphorus concentrations, respectively, than the positive control (**PC**) diet (treatment 7). Dietary concentrations of calcium and nonphytate phosphorus in the starter, grower, and finisher periods were formulated based on Aviagen Ross 708 Broiler Nutrition Specification recommendation (Aviagen, 2016). Treatments 2 to 6 were formulated similar to treatment 1 with additions of *E. coli* phytase from *Trichoderma reesei* at 500, 1,500, 4,500, 13,500, and 40,500 FTU/kg, respectively, at the expense of sand (Quantum Blue 5G; AB Vista, Marlborough, UK). One FTU is defined as the quantity of phytase required to release 1 μmol of inorganic phosphate from 0.0051 mol/L solution of sodium phytate in 1 min at pH 5.5 and 37°C (Simons et al., 1990). The concentration of phytase was selected to determine the extent of phytate degradation in the gizzard and ileum of broilers. In treatment 8, inositol was added to the PC diet (treatment 7) at 0.26, 0.25, and 0.23%, respectively, in the starter, grower, and finisher periods. Inositol was added to evaluate effects of inositol supplementation compared with phytase addition. The amount of inositol addition was calculated based on the expected inositol liberation in treatment 6 with the assumption that all phytate content in treatment 6 can be completely degraded, which consequently liberates inositol. Phytate phosphorus concentration was calculated by the amount of feed ingredients multiplied by 0.17 and 0.48% for corn and soybean meal, respectively (AminoDat 5.0, 2016). Then, inositol concentration was calculated by multiplying the concentration of phytate phosphorus in the diet by 0.968, which is the ratio of inositol to phosphorus in phytate. Amino acid concentrations were formulated at 93% of Aviagen Ross 708 Broiler Nutrition Specifications recommendation (Aviagen, 2016) to mimic commercial practice. Experimental diet samples were collected from each period and analyzed for phytase activity by ELISA specific for Quantum Blue (ESC, Standard Analytical Method, SAM099; AB Vista) similar to the method described by Engelen et al. (2001).

Sample Collections

At 14 d of age, 4 birds per pen were sacrificed using carbon dioxide asphyxiation followed by cervical dislocation for necropsy, while at 28 and 43 d of age, 2 birds per pen were sacrificed using carbon dioxide asphyxiation

followed by cervical dislocation. At least 3 mL of blood was collected from each bird via heart puncture for determination of plasma inositol concentrations. Blood samples were collected into a 4.5-mL heparinized tube (S-Monovette 4.5-mL LH; Sarstedt, Nümbrecht, Germany) and placed on ice until centrifugation. Blood samples were centrifuged at 1,643 × *g* for 10 min to separate plasma from the whole blood and stored at −20°C until further analysis. Gizzard and ileal digesta contents were collected for analyses of phytate breakdown, inositol phosphate ester disappearance, and inositol concentration. Gizzard contents were collected by carefully scraping feed contents into a Whirl-Pak bag (Nasco, Fort Atkinson, WI), and ileal digesta was collected by gently flushing out the content of the terminal ileum using deionized-distilled water into Whirl-Pak bags (Nasco, Fort Atkinson, WI). This section is defined as the terminal one-third of the section between the Meckel's diverticulum and approximately 4 cm anterior from the ileo-cecal junction (Rodehutscord et al., 2012). Both gizzard and ileal digesta samples were immediately frozen by submersion in liquid nitrogen for approximately 5 min to terminate any remaining phytase activity. Samples were kept on ice and stored at −20°C until later analysis.

Chemical Analyses

Plasma samples were prepared by mixing with 1 M perchloric acid in a 1:2 ratio (plasma:$HClO_4$) to precipitate all protein. Samples were centrifuged at 14,000 × *g* for 10 min to collect the supernatant and were sent to the University of East Anglia School of Biological Sciences in Norwich, England, for analysis of inositol concentration using HPLC with pulsed amperometric detection. Samples were diluted 50-fold in 18.2 mΩ × cm water. An aliquot (20 μL) was injected into a 4 × 250-mm MetroSep Carb 2 (Metrohm, Runcorn, UK) HPLC column. The column was eluted at a flow rate of 0.5 mL/min with 150-mmol NaOH. Another aliquot (5 μL) was injected onto a 2 × 100-mm Metrosep Carb 2 (Metrohm, Runcorn, UK) column with guard column eluted at a flow rate of 0.2 mL/min with the same solvent. Inositol peaks were integrated with Chromeleon (ThermoFisher Scientific, Waltham, MA) and DataApex Clarity (DataApex, Prague, Czech Republic) software packages. Inositol concentration was determined by comparing results with standards using a linear least squares regression.

Samples of gizzard and ileal digesta contents were lyophilized (VirTis Genesis 25 ES; SP Industries Inc., Warminster, PA) and ground by using an electric coffee grinder. Samples were analyzed at the University of East Anglia School of Biological Science in Norwich, England, for IP6, inositol pentaphosphate (**IP5**), IP4, IP3, and inositol biphosphate (**IP2**) concentrations using high-performance ion chromatography with postcolumn derivatization and UV detection at 290 nm. Inositol was quantified using HPLC with pulsed amperometric detection (Laird et al., 2016).

Diet and ileal digesta samples were analyzed for titanium dioxide concentration to determine IP6 and total IP2 to IP6 disappearances according to the method described by Short et al. (1996). Titanium dioxide content in diet samples was analyzed in quadruplicate, while duplicate analyses were conducted for the ileal digesta. Samples of diet (600 mg) and ileal digesta (200 mg) were placed into porcelain crucibles and ashed for 12 h at 580°C. After ashing, samples were transferred to a 50-mL beaker by rinsing porcelain crucibles using 10 mL of sulfuric acid (7.4 M). The solutions were heated (250°C) on a hot plate to dissolve solid particles for approximately 60 min. After cooling, the solutions were rinsed using 10 mL of distilled water into a glass beaker containing 25 mL of distilled water. Twenty milliliters of hydrogen peroxide (30%) was added to each beaker, and the solution was diluted to 100 mL with distilled water. Solutions were kept at room temperature for at least 48 h before absorbance measurement at 410 nm using a spectrophotometer (SpectraMax Plus 384; Molecular Devices LLC., San Jose, CA). Titanium concentration was determined by comparing absorbance results with known standards using a linear least-squares regression.

Calculations

Concentrations of IP6, total IP2 to IP6, and titanium dioxide from feed and ileal digesta analyses were used to calculate percent IP6 and total IP2 to IP6 disappearances using the following equation:

$$IP6\ disappearance = \left[1 - \left(\frac{TiO_{2\ Diet}}{TiO_{2\ Digesta}}\right) \times \left(\frac{IP_{Digesta}}{IP_{Diet}}\right)\right] \times 100$$

where $TiO_{2\ Digesta}$ and $TiO_{2\ Diet}$ represent the analyzed concentrations of titanium dioxide in the ileal digesta and diets, respectively, and $IP_{Digesta}$ and IP_{Diet} indicate the analyzed IP6 or total IP2 to IP6 concentrations in ileal digesta and diets, respectively.

Statistical Analyses

This study was designed as a randomized complete block with pen location as the blocking factor. Individual pen represents the experimental unit with 8 replications. Regression analysis was conducted to determine linear and quadratic effects of phytase supplementations (treatments 1 to 6) using PROC REG (SAS Institute Inc., 2011). Because phytase concentrations were not evenly spaced among treatments (0, 500, 1,500, 4,500, 13,500, and 40,500 FTU/kg), these concentrations were log transformed [$\log_{10}$(FTU+1)] before regression analysis to obtain normally distributed data (Shirley and Edwards, 2003; Gehring et al., 2013). In addition, analysis of variance was performed using PROC MIXED

(SAS Institute Inc., 2011) with the following mixed-effect model:

$$Y_{ij} = \mu.. + \rho_i + \tau_j + \varepsilon_{ij}$$

where $\mu..$ is the overall mean; the ρ_i are identically and independently normally distributed random block effects with mean 0 and variance $\sigma^2{}_\rho$; the τ_j are fixed factor level effects corresponding to the j^{th} dietary treatment (treatments 1–8) such that $\sum \tau_j = 0$; and the ε_{ij} are identically and independently normally distributed random errors with mean 0 and a variance σ^2. Preplanned orthogonal contrasts were used to detect differences between NC vs. PC, treatment 6 (NC with 40,500 FTU/kg addition) vs. 8 (PC with inositol addition), and treatment 7 (PC) vs. 8 (PC with inositol addition). Correlation analysis was conducted using PROC CORR (SAS Institute Inc., 2011). Statistical significance was considered at $P \leq 0.05$.

RESULTS

Analyses of phytase activity in treatments 2 to 6 were approximately 93.4% (CV = 14%) of the calculated values (Table 2). However, analysis of the PC diets in the starter, grower, and finisher periods had phytase activity of 695, 238, and 267 FTU/kg, respectively. Phytase activity in the PC diets may have originated from dietary treatment 6 phytase residue, which were manufactured before the PC diets.

In the gizzard contents of broilers, an increase of phytase concentrations in treatments 1 to 6 resulted in log-quadratic reductions ($P < 0.05$) of IP3, IP4, IP5, IP6, and total IP3 to IP6 at 15, 29, and 43 d of age (Tables 3–5). These responses were accompanied by the log-quadratic increase ($P < 0.05$) of inositol concentration at 15, 29, and 43 d of age. Broilers consuming the PC diets had lower ($P < 0.001$) IP6 and total IP3 to IP6 but higher ($P < 0.001$) IP4 and IP3 concentrations than birds provided the NC diets at 15, 29, and 43 d of age. In contrast, inositol concentrations were similar ($P > 0.05$) between broilers fed the PC or NC diets at all collection periods. Phytase supplementation at 40,500 FTU/kg decreased ($P < 0.05$) IP3, IP4, IP5, IP6, and total IP3 to IP6 of broilers compared with birds receiving the PC diets with inositol addition at 15, 29, and 43 d of age. Moreover, higher ($P < 0.05$) IP ester concentrations were also observed in broilers fed the PC diets with inositol than in birds provided the PC diets at 15, 29, and 43 d of age. Inositol concentration of broilers fed PC diets supplemented with inositol was similar ($P > 0.05$) to that of birds fed diets with 40,500 FTU/kg phytase at 15, 20, and 43 d of age. However, higher ($P < 0.001$) inositol concentration in broilers fed diets with inositol supplementation than in birds fed the PC diets was only observed at 15 d of age.

Table 3. Concentrations of inositol phosphate (IP) esters and inositol (nmol/g) in the gizzard digesta of broilers at 15 d of age fed diets with gradient phytase supplementation.[1]

Item	IP6	IP5	IP4	IP3	∑IP	Inositol
Dietary treatments						
Negative control (NC)[2]	4,953	520	264	102	5,839	457
NC + 500 FTU/kg[3]	3,197	606	625	209	4,636	604
NC + 1,500 FTU/kg	639	238	1,042	333	2,252	794
NC + 4,500 FTU/kg	116	39	291	153	599	1,486
NC + 13,500 FTU/kg	23	9	46	93	171	1,831
NC + 40,500 FTU/kg	11	2	12	79	104	2,536
Positive control (PC)	569	193	984	346	2,092	802
PC + inositol[4]	3,429	532	643	180	4,784	2,434
Pooled standard error	232	33	104	30	247	147
Source of variation	Probabilities					
Log-linear effect of phytase[5]	<0.001	<0.001	0.32	0.99	<0.001	<0.001
Log-quadratic effect of phytase[5]	<0.001	<0.001	0.002	0.019	<0.001	<0.001
Treatment 1 vs. 7	<0.001	<0.001	<0.001	<0.001	<0.001	0.07
Treatment 6 vs. 8	<0.001	<0.001	<0.001	0.017	<0.001	0.64
Treatment 7 vs. 8	<0.001	<0.001	0.016	<0.001	<0.001	<0.001

Abbreviations: ∑IP, total IP6 to IP3; IP6, inositol hexakisphosphate; IP5, inositol pentaphosphate; IP4, inositol tetraphosphate; IP3, inositol triphosphate.

[1]Values are least-square means of 8 replicate pens, with 4 birds selected per pen for necropsy at 15 d of age. Concentrations of inositol biphosphate (IP2) were below limit of detection.

[2]The negative control diet was formulated to contain 0.165 and 0.150% lower calcium and phosphorus concentrations, respectively, compared with the positive control diet.

[3]One unit of phytase activity (FTU) is defined as the quantity of enzyme to liberate 1 μmol of inorganic phosphate from 0.0051 mol/L sodium phytate per minute from sodium phytate at pH 5.5 and 37°C.

[4]Inositol supplementation was calculated based on the maximum inositol liberation from the treatment containing the highest phytase concentration (Treatment 6).

[5]Phytase concentrations were $\log_{10}$ transformed before analysis. Log-linear and log-quadratic effects were analyzed for treatments 1 to 6.

Table 4. Concentrations of inositol phosphate (IP) esters and inositol (nmol/g) in the gizzard digesta of broilers at 29 d of age fed diets with gradient phytase supplementation.[1]

Item	IP6	IP5	IP4	IP3	∑IP	Inositol
Dietary treatments						
Negative control (NC)[2]	5,516	620	352	163	6,651	322
NC + 500 FTU/kg[3]	2,273	520	1,439	451	4,682	297
NC + 1,500 FTU/kg	873	287	1,729	608	3,498	286
NC + 4,500 FTU/kg	27	16	126	146	316	673
NC + 13,500 FTU/kg	20	22	57	121	220	805
NC + 40,500 FTU/kg	0	7	11	87	105	841
Positive control (PC)	1,897	484	1,772	468	4,620	803
PC + inositol[4]	4,526	627	455	161	5,768	1,098
Pooled standard error	276	63	163	48	318	297
Source of variation	Probabilities					
Log-linear effect of phytase[5]	<0.001	<0.001	0.24	0.45	<0.001	0.014
Log-quadratic effect of phytase[5]	<0.001	<0.001	<0.001	<0.001	<0.001	0.011
Treatment 1 vs. 7	<0.001	0.11	<0.001	<0.001	<0.001	0.37
Treatment 6 vs. 8	<0.001	<0.001	0.045	0.25	<0.001	0.29
Treatment 7 vs. 8	<0.001	0.10	<0.001	<0.001	0.007	0.31

Abbreviations: ∑IP, total IP6 to IP3; IP6, inositol hexakisphosphate; IP5, inositol pentaphosphate; IP4, inositol tetraphosphate; IP3, inositol triphosphate.

[1]Values are least-square means of 8 replicate pens, with 2 birds selected per pen for necropsy at 29 d of age. Concentrations of inositol biphosphate (IP2) were below limit of detection.

[2]The negative control diet was formulated to contain 0.165 and 0.150% lower calcium and phosphorus concentrations, respectively, than the positive control diet.

[3]One unit of phytase activity (FTU) is defined as the quantity of enzyme to liberate 1 μmol of inorganic phosphate from 0.0051 mol/L sodium phytate per minute from sodium phytate at pH 5.5 and 37°C.

[4]Inositol supplementation was calculated based on the maximum inositol liberation from the treatment containing the highest phytase concentration (treatment 6).

[5]Phytase concentrations were $\log_{10}$ transformed before analysis. Log-linear and log-quadratic effects were analyzed for treatments 1 to 6.

In the ileal digesta, increasing supplementation of phytase from 0 to 40,500 FTU/kg in broiler diets decreased ($P < 0.001$) IP5, IP6, and total IP2 to IP6 concentrations in a log-quadratic manner at 15, 29, and 43 d of age (Tables 6–8). However, a log-quadratic reduction ($P < 0.001$) of IP4 due to increasing phytase concentrations was obtained only at 29 and 43 d of age, while a log-quadratic IP3 reduction ($P = 0.011$) was observed only at 43 d of age. No log-quadratic reduction ($P > 0.05$) of IP2 was observed at any of the collection periods with increasing dietary phytase doses. Increasing phytase doses in treatments 1 to 6 resulted in log-quadratic increase ($P < 0.001$) of inositol concentrations at each collection period. At 15, 29, and 43 d of age, broilers provided the PC diets had lower ($P < 0.05$) IP6 and total IP2 to IP6 concentrations than birds provided the NC diets. Conversely, IP3 and IP4 concentrations were higher ($P < 0.001$) in broilers provided the PC diets than in those consuming the NC diets. Broilers fed the PC diets had higher ($P = 0.048$) inositol concentration only at 15 d of age than broilers provided the NC diets. Feeding broilers diets formulated with phytase at 40,500 FTU/kg decreased ($P < 0.001$) IP6 and total IP2 to IP6 compared with birds consuming the PC diets with inositol addition at every collection period. However, inositol concentration of broilers fed diets supplemented with phytase (40,500 FTU/kg) was 25, 39, and 41% higher ($P < 0.01$) than that in broilers fed the PC diets with inositol addition at 15, 29, and 43 d of age, respectively. Furthermore, broilers receiving PC diets with inositol addition had lower ($P < 0.001$) IP3 and IP4 concentrations but higher ($P < 0.001$) inositol concentrations than birds provided the PC diets only at 15, 29, and 43 d of age.

Effects of phytase additions in treatments 1 to 6 also led to quadratic increase ($P < 0.001$) of apparent ileal disappearance of IP6 and total IP2 to IP6 concentrations at 15, 29, and 43 d of age (Table 9). Providing broilers the PC diets resulted in lower ($P < 0.001$) ileal disappearances of IP6 and total IP2 to IP6 concentrations than feeding the NC diets or the PC diets with inositol addition. Broilers receiving diets with phytase addition at 40,500 FTU/kg had higher ($P < 0.001$) ileal IP6 and total IP2 to IP6 disappearances than broilers consuming the PC diets with inositol addition.

Moreover, increasing phytase addition from 0 to 40,500 FTU/kg resulted in log-quadratic increase of plasma inositol concentrations of broilers at 15, 29, and 43 d of age (Table 10). Broilers receiving the PC diets had higher ($P < 0.01$) plasma inositol concentrations at 15 and 43 d of age than broilers provided the NC diets. The addition of dietary inositol to the PC diets resulted in increased ($P < 0.01$) plasma inositol of broilers compared with that in those consuming the PC diets only at 15, 29, and 43 d of age. However, plasma inositol concentrations of broilers fed the PC diets with inositol

Table 5. Concentrations of inositol phosphate (IP) esters and inositol (nmol/g) in the gizzard digesta of broilers at 43 d of age fed diets with gradient phytase supplementation.[1]

Item	IP6	IP5	IP4	IP3	∑IP	Inositol
Dietary treatments						
Negative control (NC)[2]	5,255	513	291	178	6,236	352
NC + 500 FTU/kg[3]	1,480	323	1,530	536	3,869	443
NC + 1,500 FTU/kg	341	50	767	551	1,708	576
NC + 4,500 FTU/kg	286	53	362	254	954	827
NC + 13,500 FTU/kg	135	18	58	117	327	1,044
NC + 40,500 FTU/kg	145	6	25	114	290	1,414
Positive control (PC)	1,837	411	1,549	593	4,390	302
PC + inositol[4]	4,911	727	891	371	6,901	1,026
Pooled standard error	380	70	185	56	504	326
Source of variation	Probabilities					
Log-linear effect of phytase[5]	<0.001	<0.001	0.25	0.52	<0.001	0.025
Log-quadratic effect of phytase[5]	<0.001	<0.001	<0.001	<0.001	<0.001	0.043
Treatment 1 vs. 7	<0.001	0.28	<0.001	<0.001	0.010	0.90
Treatment 6 vs. 8	<0.001	<0.001	0.002	0.002	<0.001	0.27
Treatment 7 vs. 8	<0.001	0.002	0.015	0.007	0.001	0.07

Abbreviations: ∑IP, total IP6 to IP3; IP6, inositol hexakisphosphate; IP5, inositol pentaphosphate; IP4, inositol tetraphosphate; IP3, inositol triphosphate.

[1]Values are least-square means of 8 replicate pens, with 2 birds selected per pen for necropsy at 43 d of age. Concentrations of inositol biphosphate (IP2) were below limit of detection.

[2]The negative control diet was formulated to contain 0.165 and 0.150% lower calcium and phosphorus concentrations, respectively, than the positive control diet.

[3]One unit of phytase activity (FTU) is defined as the quantity of enzyme to liberate 1 μmol of inorganic phosphate from 0.0051 mol/L sodium phytate per minute from sodium phytate at pH 5.5 and 37°C.

[4]Inositol supplementation was calculated based on the maximum inositol liberation from the treatment containing the highest phytase concentration (treatment 6).

[5]Phytase concentrations were $\log_{10}$ transformed before analysis. Log-linear and log-quadratic effects were analyzed for treatments 1 to 6.

Table 6. Concentrations of inositol phosphate (IP) esters and inositol (nmol/g) in the ileal digesta of broilers at 15 d of age fed diets with gradient phytase supplementation.[1]

Item	IP6	IP5	IP4	IP3	IP2	∑IP	Inositol
Dietary treatments							
Negative control (NC)[2]	42,960	3,779	1,536	440	234	48,949	7,485
NC + 500 FTU/kg[3]	39,832	5,482	3,092	697	364	49,467	8,350
NC + 1,500 FTU/kg	23,084	5,408	4,631	1,207	326	34,656	12,490
NC + 4,500 FTU/kg	3,884	1,318	4,036	1,720	145	11,103	21,143
NC + 13,500 FTU/kg	405	122	1,517	951	271	3,266	21,070
NC + 40,500 FTU/kg	190	49	202	96	212	749	26,314
Positive control (PC)	17,082	5,634	8,812	2,973	336	34,837	10,642
PC + Inositol[5]	47,661	5,637	2,256	612	458	56,623	21,014
Pooled standard error	2,867	690	757	273	104	3,485	1,278
Source of variation	Probabilities						
Log-linear effect of phytase[5]	<0.001	0.002	0.96	0.34	0.89	<0.001	<0.001
Log-quadratic effect of phytase[5]	<0.001	<0.001	0.18	0.63	0.27	<0.001	<0.001
Treatment 1 vs. 7	<0.001	0.032	<0.001	<0.001	0.46	0.001	0.048
Treatment 6 vs. 8	<0.001	<0.001	0.039	0.16	0.10	<0.001	0.002
Treatment 7 vs. 8	<0.001	0.91	<0.001	<0.001	0.40	<0.001	<0.001

Abbreviations: ∑IP, total IP6 to IP2; IP6, inositol hexakisphosphate; IP5, inositol pentaphosphate; IP4, inositol tetraphosphate; IP3, inositol triphosphate; IP2, inositol bisphosphate.

[1]Values are least-square means of 8 replicate pens, with 4 birds selected per pen for necropsy at 15 d of age.

[2]The negative control diet was formulated to contain 0.165 and 0.150% lower calcium and phosphorus concentrations, respectively, than the positive control diet.

[3]One unit of phytase activity (FTU) is defined as the quantity of enzyme to liberate 1 μmol of inorganic phosphate from 0.0051 mol/L sodium phytate per minute from sodium phytate at pH 5.5 and 37°C.

[4]Inositol supplementation was calculated based on the maximum inositol liberation from the treatment containing the highest phytase concentration (treatment 6).

[5]Phytase concentrations were $\log_{10}$ transformed before analysis. Log-linear and log-quadratic effects were analyzed for treatments 1 to 6.

Table 7. Concentrations of inositol phosphate (IP) esters and inositol (nmol/g) in the ileal digesta of broilers at 29 d of age fed diets with gradient phytase supplementation.[1]

Item	IP6	IP5	IP4	IP3	IP2	∑IP	Inositol
Dietary treatments							
Negative control (NC)[2]	56,615	5,821	1,393	246	613	64,688	3,189
NC + 500 FTU/kg[3]	33,354	7,817	5,758	1,279	536	48,744	4,708
NC + 1,500 FTU/kg	13,689	4,451	6,631	1,523	764	27,059	10,294
NC + 4,500 FTU/kg	2,145	1,085	5,117	2,827	1,034	11,171	17,243
NC + 13,500 FTU/kg	391	109	1,024	546	784	2,854	20,716
NC + 40,500 FTU/kg	314	52	283	105	594	1,348	22,879
Positive control (PC)	31,783	9,332	9,468	2,537	1,290	54,409	4,449
PC + inositol[4]	47,115	6,243	2,100	440	773	56,673	16,469
Pooled standard error	3,000	559	803	255	137	3,434	1,180
Source of variation	Probabilities						
Log-linear effect of phytase[5]	<0.001	<0.001	0.79	0.34	0.37	<0.001	<0.001
Log-quadratic effect of phytase[5]	<0.001	<0.001	<0.001	0.16	0.37	<0.001	<0.001
Treatment 1 vs. 7	<0.001	<0.001	<0.001	<0.001	<0.001	0.023	0.42
Treatment 6 vs. 8	<0.001	<0.001	0.07	0.37	0.31	<0.001	<0.001
Treatment 7 vs. 8	<0.001	<0.001	<0.001	<0.001	0.005	0.61	<0.001

Abbreviations: ∑IP, total IP6 to IP2; IP6, inositol hexakisphosphate; IP5, inositol pentaphosphate; IP4, inositol tetraphosphate; IP3, inositol triphosphate; IP2, inositol bisphosphate.

[1]Values are least-square means of 8 replicate pens, with 2 birds selected per pen for necropsy at 29 d of age.

[2]The negative control diet was formulated to contain 0.165 and 0.150% lower calcium and phosphorus concentrations, respectively, than the positive control diet.

[3]One unit of phytase activity (FTU) is defined as the quantity of enzyme to liberate 1 μmol of inorganic phosphate from 0.0051 mol/L sodium phytate per minute from sodium phytate at pH 5.5 and 37°C.

[4]Inositol supplementation was calculated based on the maximum inositol liberation from the treatment containing the highest phytase concentration (treatment 6).

[5]Phytase concentrations were $\log_{10}$ transformed before analysis. Log-linear and log-quadratic effects were analyzed for treatments 1 to 6.

Table 8. Concentrations of inositol phosphate (IP) esters and inositol (nmol/g) in the ileal digesta of broilers at 43 d of age fed diets with gradient phytase supplementation.[1]

Item	IP6	IP5	IP4	IP3	IP2	∑IP	Inositol
Dietary treatments							
Negative control (NC)[2]	60,746	6,204	1,902	175	609	69,637	1,728
NC + 500 FTU/kg[3]	27,367	7,101	7,648	1,733	783	44,632	4,120
NC + 1,500 FTU/kg	9,185	2,901	8,357	2,761	795	24,000	8,894
NC + 4,500 FTU/kg	2,868	604	3,229	1,425	510	8,637	13,339
NC + 13,500 FTU/kg	1,885	296	1,387	311	596	4,475	14,893
NC + 40,500 FTU/kg	419	32	126	41	474	1,092	18,548
Positive control (PC)	27,515	6,487	7,324	1,884	1,301	44,512	3,512
PC + inositol[4]	47,628	6,360	2,793	476	977	58,234	13,118
Pooled standard error	3,078	590	1,016	398	178	3,966	1,225
Source of variation	Probabilities						
Log-linear effect of phytase[5]	<0.001	<0.001	0.58	0.71	0.51	<0.001	<0.001
Log-quadratic effect of phytase[5]	<0.001	<0.001	<0.001	0.011	0.14	<0.001	<0.001
Treatment 1 vs. 7	<0.001	0.68	<0.001	0.001	0.004	<0.001	0.26
Treatment 6 vs. 8	<0.001	<0.001	0.043	0.39	0.031	<0.001	0.001
Treatment 7 vs. 8	<0.001	0.84	<0.001	0.007	0.16	0.008	<0.001

Abbreviations: ∑IP, total IP6 to IP2; IP6, inositol hexakisphosphate; IP5, inositol pentaphosphate; IP4, inositol tetraphosphate; IP3, inositol triphosphate; IP2, inositol bisphosphate.

[1]Values are least-square means of 8 replicate pens, with 2 birds selected per pen for necropsy at 43 d of age.

[2]The negative control diet was formulated to contain 0.165 and 0.150% lower calcium and phosphorus concentrations, respectively, than the positive control diet.

[3]One unit of phytase activity (FTU) is defined as the quantity of enzyme to liberate 1 μmol of inorganic phosphate from 0.0051 mol/L sodium phytate per minute from sodium phytate at pH 5.5 and 37°C.

[4]Inositol supplementation was calculated based on the maximum inositol liberation from the treatment containing the highest phytase concentration (treatment 6).

[5]Phytase concentrations were $\log_{10}$ transformed before analysis. Log-linear and log-quadratic effects were analyzed for treatments 1 to 6.

Table 9. Ileal disappearance (%) of inositol phosphate 6 (IP6) and total inositol phosphate 6 to 2 (∑IP) of broilers at 15, 29, and 43 d of age fed diets with gradient phytase supplementation.[1]

	15 d		29 d		43 d	
Item	IP6	∑IP	IP6	∑IP	IP6	∑IP
Dietary treatments						
Negative control (NC)[2]	32.97	30.49	−26.34	−9.84	−9.29	−10.22
NC + 500 FTU/kg[3]	35.72	26.28	49.90	33.00	41.77	16.46
NC + 1,500 FTU/kg	60.24	46.04	76.15	56.74	80.52	56.13
NC + 4,500 FTU/kg	92.36	81.43	96.15	82.49	92.46	83.35
NC + 13,500 FTU/kg	99.38	95.60	99.12	94.95	96.82	93.48
NC + 40,500 FTU/kg	99.69	98.49	99.40	98.05	98.31	97.49
Positive control (PC)	73.75	52.11	48.04	20.53	50.52	29.49
PC + inositol[4]	29.31	24.64	0.61	−2.17	−8.58	−15.22
Pooled standard error	4.54	5.40	3.78	4.93	7.17	6.87
Source of variation	Probabilities					
Log-linear effect of phytase[5]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Log-quadratic effect of phytase[5]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Treatment 1 vs. 7	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Treatment 6 vs. 8	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Treatment 7 vs. 8	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Abbreviations: ∑IP, total IP6 to IP2; IP6, inositol hexakisphosphate; IP5, inositol pentaphosphate; IP4, inositol tetraphosphate; IP3, inositol triphosphate; IP2, inositol bisphosphate.

[1]Values are least-square means of 8 replicate pens, with 4, 2, and 2 birds selected per pen for necropsy at 15, 29, and 43 d of age, respectively.

[2]The negative control diet was formulated to contain 0.165 and 0.150% lower calcium and phosphorus concentrations, respectively, than the positive control diet.

[3]One unit of phytase activity (FTU) is defined as the quantity of enzyme to liberate 1 μmol of inorganic phosphate from 0.0051 mol/L sodium phytate per minute from sodium phytate at pH 5.5 and 37°C.

[4]Inositol supplementation was calculated based on the maximum inositol liberation from the treatment containing the highest phytase concentration (treatment 6).

[5]Phytase concentrations were $\log_{10}$ transformed before analysis. Log-linear and log-quadratic effects were analyzed for treatments 1 to 6.

addition were similar ($P > 0.05$) to birds provided diets with 40,500 FTU/kg phytase at all collection periods.

DISCUSSION

The efficacy of phytase in hydrolyzing phytate in the present study was evident as supplementing phytase at 40,500 FTU/kg decreased concentrations of total IP2 to IP6 in both gizzard and ileal digesta contents to less than 10% of the total IP2 to IP6 concentrations in broilers fed diets with 500 FTU/kg phytase addition. Similarly, the disappearance of total IP esters increased up to 98% when phytase was supplemented at 40,500 FTU/kg indicating the near-complete destruction of all phytate esters. The reduction of phytate content in the gastrointestinal tract of broilers may likely be the reason for increased feed intake of broilers, which was presented in the companion article. Previous research demonstrated that supplementing dietary phytate to grass carp resulted in higher cholecystokinin and cocaine- and amphetamine-regulated transcript, which promote feed intake reduction (Liu et al., 2014). In contrast, the efficacy of phytase in hydrolyzing phytate was observed to increase digesta passage rate and feed intake in broilers (Watson et al., 2006). The increase of feed intake may likely be associated with enhanced BW gain and meat accretion of broilers.

In the present study, increasing IP3 and IP4 concentrations in the gizzard and ileal digesta were observed with the addition of phytase at 1,500 and 4,500 FTU/kg even after reductions of IP5 and IP6 concentrations. As a result, both IP3 and IP4 were the most dominant phytate degradation products among total IP3 to IP6 concentrations. Similarly, Walk and Olukosi (2019) observed that IP4 accounted for the majority (46%) of total IP3 to IP6 in the gizzard digesta of broilers fed diets with 2,000 FTU/kg of *E. coli* phytase from *T. reesei* at 18 d of age. Interestingly, increasing phytase dose to 4,000 FTU/kg also resulted in the accumulation of IP4 (47% among total IP3 to IP6 concentrations) in the ileal digesta of broilers (Walk and Olukosi, 2019). The accumulation of IP3 and IP4 may occur because of the fact that these lower IP esters are poor substrates for commercial phytases (Wyss et al., 1999; Bedford and Walk, 2016). Previous research indicated that as more phosphate groups are released from a phytate molecule, the binding capacity of phytase to phytate esters decreases, resulting in the accumulation of lower phytate esters (Menezes-Blackburn et al., 2015). Alternative strategy to reduce the accumulation of IP4 may be implemented by increasing the concentration of phytase in broiler diets (Bedford and Walk, 2016). In the present research, the efficacy of phytase in degrading IP3 and IP4 in the gizzard and ileal digesta contents was evident

Table 10. Plasma inositol concentration (μmol) of broilers fed diets with gradient phytase supplementation from 1 to 43 d of age.[1]

Item	15 d	29 d	43 d
Dietary treatment			
Negative control (NC)[2]	247	234	234
NC + 500 FTU/kg[3]	305	299	260
NC + 1,500 FTU/kg	392	396	374
NC + 4,500 FTU/kg	517	472	456
NC + 13,500 FTU/kg	544	480	450
NC + 40,500 FTU/kg	473	458	469
Positive control (PC)	332	276	354
PC + inositol[4]	474	402	526
Pooled standard error	34	29	30
Source of variation	Probabilities		
Log-linear effect of phytase[5]	<0.001	<0.001	<0.001
Log-quadratic effect of phytase[5]	<0.001	<0.001	<0.001
Treatment 1 vs. 7	0.022	0.29	0.005
Treatment 6 vs. 8	0.98	0.15	0.16
Treatment 7 vs. 8	<0.001	0.002	<0.001

[1]Values are least-square means of 8 replicate pens with 4, 2, and 2 birds selected per pen for blood collections at 15, 29, and 43 d of age, respectively.

[2]The negative control diet was formulated to contain 0.165 and 0.150% lower calcium and phosphorus concentrations, respectively, than the positive control diet.

[3]One unit of phytase activity (FTU) is defined as the quantity of enzyme to liberate 1 μmol of inorganic phosphate from 0.0051 mol/L sodium phytate per minute from sodium phytate at pH 5.5 and 37°C.

[4]Inositol supplementation was calculated based on the maximum inositol liberation from the diet containing the highest phytase concentration (treatment 6).

[5]Phytase concentrations were $\log_{10}$ transformed before analysis. Log-linear and log-quadratic effects were analyzed for treatments 1 to 6.

when the concentrations of these phytate esters decreased and inositol concentration increased after the supplementation of 13,500 and 40,500 FTU/kg phytase.

The present research also demonstrated varying magnitudes of phytate degradation between the gizzard and ileal digesta. Log-quadratic reductions of IP3 and IP4 in gizzard digesta of broilers fed treatments 1 to 6 were observed at all collection periods, whereas log-quadratic reduction of IP4 in the ileal digesta was observed at 29 and 43 d of age and IP3 only at 43 d of age. The inconsistency of phytate degradation in the ileal digesta compared with gizzard contents may be attributed to the change of pH from the gizzard to the small intestine (Schlemmer et al., 2001). The gizzard is the primary site of phytate degradation because of its low pH (Selle and Ravindran, 2007). However, as pH increases in the small intestine, phytate solubility decreases and dietary phytase activity is reduced. In a study using pigs, Schlemmer et al. (2001) indicated that when feeding nonextruded diets, 57% of all phytate hydrolysis products in the gastric chyme are soluble in the liquid phase. Conversely, up to 87% of these IP esters precipitate in the small intestine, presumably with minerals or proteins, resulting in low phytate degradation and unavailability of nutrients. Xu et al. (1992) demonstrated that IP4 reduced the solubility of calcium and zinc by approximately 40 and 90%, respectively, when pH rises from 5 to 6.

The difficulties in degrading lower phytate esters may also be influenced by an interactive effect of phytase dose and age of broilers (Olukosi et al., 2020). In research presented herein, concentrations of IP3 and IP4 in the ileal digesta increased as phytase dose is increased from 0 to 1,500 FTU/kg but decreased when phytase is further increased to 40,500 FTU/kg. However, as the age of broilers increased, the accumulation of IP3 and IP4 concentrations also increased in broilers supplemented with phytase at 1,500 FTU/kg. Data evaluating the efficacy of phytase in degrading lower IP esters in broilers up to 6 wks of age are lacking. However, Olukosi et al. (2020) demonstrated that broilers at 28 d of age had higher concentrations of IP3 and IP4 than birds at 7 d of age when fed diets with phytase supplementation at either 1,500 or 3,000 FTU/kg. The higher accumulation of IP3 and IP4 concentrations with the increase of age may be attributed to older birds having greater feed intake and digestive capacity than younger birds. This was evident as the proportion of IP4 to total IP esters in the present study increased from 14, 26, to 34% at 15, 29, and 43 d of age, respectively, in the ileal digesta of broilers fed diets with 1,500 FTU/kg. In addition, the influence of age on phytase efficacy may vary because of young birds having less developed gastrointestinal tract than older birds. From 6 to 10 d of age, the small intestine of broilers undergoes a rapid increase in size relative to body weight (Sklan, 2001). Presumably, this may have led to changes in endogenous phytase activity in the small intestine. Morgan et al. (2015) reported that ileal phytase activity and phytate hydrolysis of broilers fed diets without phytase addition increased from 4 to 14 d of age. As dietary phytase may be inactive in the small intestine of broilers, phytate degradation may occur because of the activity of intestinal brush border phytase (Maenz and Classen, 1998).

In the present study, broilers fed the PC diets appeared to have greater phytate degradation and ileal IP disappearance than those provided the NC diets and the PC diets with inositol addition. The unexpected presence of phytase in the PC diets may explain the greater occurrence of phytate degradation in broilers provided the PC diets. However, despite unexpectedly having phytase activity, there seems to be accumulation of IP3 and IP4 in the gizzard and ileal digesta contents of broilers fed the PC diets indicating the inability of degrading lower phytate esters with small amount of dietary phytase (695, 238, and 267 FTU/kg in the starter, grower, and finisher diets, respectively). This condition was evident as ileal inositol concentration was similar between broilers fed the NC and PC diets except in the ileal digesta at 15 d of age. Previous research has also reported that adding dietary *E. coli* phytase from *T. reesei* at 500 FTU/kg resulted in the accumulation of IP4 in the gizzard digesta of broilers (Zeller et al., 2015a).

In addition, negative IP disappearance values were observed in broilers fed the NC diets and PC diets supplemented with inositol at 29 and 43 d of age, but not in broilers at 15 d of age. Negative values of IP disappearance have been observed in previous studies when broilers were fed diets with low or no phytase addition. Perryman et al. (2017) noted a 9.3% ileal IP6

disappearance in broilers, while Zeller et al. (2016) reported a 7.0% IP6 disappearance in the crop of broilers fed diets without phytase supplementation. In addition, Olukosi et al. (2020) observed a 19.1% IP6 disappearance in turkeys receiving diets with 500 FTU/kg phytase addition at 28 d of age, but not at 7 d of age.

Negative IP6 disappearance values may occur when there is an elevated ratio of marker in the diet to digesta or a decreased ratio of IP6 in the diet to digesta. The ratio of marker in the diet to digesta may increase because of a slower passage rate, which slows down marker flow to the ileal digesta. A previous study indicated that broilers receiving diets without phytase addition had 20% slower rate of passage than broilers fed phytase-supplemented diets (Watson et al., 2006). In addition, the reduction of IP6 ratio in the diet to digesta may also lead to a negative IP6 disappearance value. This condition may occur either because of reverse peristalsis of the liquid fraction containing IP6 (Sacranie et al., 2007) or more complete digestion of starch and protein with increasing age (Batal and Parsons, 2002) resulting in higher concentration of IP6 in the digesta. In the research reported herein, the increase in ileal IP6 concentration in birds fed the NC diets from 42,960 to 56,615 and 60,746 nmol/g at 15, 29, and 43 d of age decreased the ratio of IP6 in the diet to digesta from 0.43 to 0.32 and 0.26, respectively. As the ratio of IP6 in the diet to digesta decreases, the ratio of marker to IP6 may rise to above 1, which produced negative IP disappearance values.

The current research also added free inositol in treatment 8 to further evaluate its role when phytase is supplemented in broiler diets. The amount of dietary inositol was calculated based on the assumption that all phytate in treatment 6 can be degraded to inositol. Hence, the amount of free inositol in treatment 8 should be similar to complete liberation of inositol in treatment 6 (40,500 FTU/kg). However, inositol concentration in the ileal digesta of broilers fed diets supplemented with phytase (40,500 FTU/kg) was higher than that in birds fed the PC diets with inositol addition at all phases. Analysis of dietary treatments in the starter, grower, and finisher phases indicated that phytate phosphorus concentrations in treatment 6 were 0.34, 0.33, and 0.30%, which translate to 0.33, 0.32, and 0.29% inositol (0.968 ratio of inositol to phytate phosphorus), respectively. However, the addition of inositol in treatment 8 was 0.26, 0.25, and 0.23% in the starter, grower, and finisher diets, respectively, which were lower than the expected inositol liberation in treatment 6. This underestimation may likely be linked to the variation in phytate phosphorus concentration in feed ingredients. Previous research reported phosphorus concentration in corn and soybean meal, which ranges from 0.16 to 0.26% and 0.28 to 0.45%, respectively (Eeckhout and De Paepe, 1994; Leske and Coon, 1994; Ravindran et al., 1994; Selle and Ravindran, 2007; Tahir et al., 2012). In contrast, phytate phosphorus concentrations used in the present study were 0.17 and 0.48% for corn and soybean meal, respectively (AminoDat 5.0, 2016).

Effects of increasing phytase concentrations on the liberation of inositol in both gizzard and ileal digesta contents resulted in increased plasma inositol concentrations of broilers. After the liberation, inositol can be absorbed in the jejunum and upper ileum using sodium-dependent and proton-dependent myo-inositol cotransporters (Walk et al., 2018). Previously, the supplementation of phytase has also been reported to increase plasma inositol concentrations of broilers (Cowieson et al., 2015; Sommerfeld et al., 2018). In pigs, plasma inositol concentration was observed to peak 360 min after the introduction of feed containing 3,000 FTU/kg of bacterial phytase from *Aspergillus oryzae* (Cowieson et al., 2017) indicating the rapid degradation of phytate by a high-phytase dose.

The response of plasma inositol due to phytase supplementation enabled the use of plasma inositol as a biomarker for phytase efficacy of phytate degradation. In the current research, at 15, 29, and 43 d of age, correlations between plasma inositol and total IP esters in the gizzard contents of broilers provided treatments 1 to 6 were r = −0.70, −0.73, and −0.64 ($P < 0.001$) and correlations with total IP esters in the ileal digesta were r = −0.63, −0.77, and −0.68 ($P < 0.001$), respectively. These data demonstrated that the increase of plasma inositol may be attributed to the reduction of total IP esters in the gizzard and ileal digesta contents; thus, allowing plasma inositol to be used as a biomarker for phytase efficacy.

In conclusion, supplementation of phytase up to 40,500 FTU/kg promoted phytate degradation, especially IP3 and IP4. The degradation of IP3 and IP4 is critical, as antinutritive effects of these lower IP esters may still be present to chelate nutrients. Moreover, further degradation of IP3 and IP4 was shown to liberate inositol. The reduction of antinutritive effects of phytate along with inositol liberation may contribute to the enhancement of growth performance and meat accretion of broilers. In addition, elevated plasma inositol concentration after its liberation indicates the possibility of its use as a biomarker for phytase efficacy on phytate degradation.

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DISCLOSURES

C. L. W. and M. R. B. are affiliated with the product evaluated in this article.

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