Inositol and gradient phytase supplementation in broiler diets during a 6-week production period: 1. effects on growth performance and meat yield[1]

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ABSTRACT An experiment was conducted to evaluate effects of inositol and gradient phytase supplementation on growth performance and meat yield of broilers from 1 to 41 d of age. A total of 1,920 Yield Plus × Ross 708 male chicks were placed in 64 floor pens (30 birds per pen). Each pen received one of the 8 dietary treatments (8 replicate pens) from 1 to 15, 16 to 29, and 30 to 40 d of age. Treatment 1 was formulated to contain 0.165 and 0.150% lower calcium and phosphorus, respectively, than treatment 7 (positive control). Phytase was added to treatment 1 at concentration of 500, 1,500, 4,500, 13,500, and 40,500 phytase units (FTU)/kg to establish treatments 2 to 6, respectively. Treatment 8 was formulated by adding inositol to treatment 7 based on the expected inositol liberation in treatment 6. Feed and birds were weighed at 1, 15, 29, and 40 d of age to determine BW gain, feed intake, and feed conversion. Twelve birds per pen were processed at 41 d of age to determine carcass characteristics. From 1 to 40 d of age, log-quadratic effects of phytase (treatments 1–6) were observed for BW gain ($P = 0.002$) and feed conversion in broilers ($P = 0.018$), whereas feed intake increased log-linearly ($P = 0.045$). The addition of 40,500 FTU/kg of phytase increased cumulative BW gain ($P = 0.001$) and decreased cumulative feed conversion ($P = 0.005$) by 4.7 and 2.6%, respectively, compared with birds subjected to treatment 8. Log-quadratic effects of phytase additions were observed for carcass ($P < 0.001$) and breast meat weights ($P = 0.004$). Growth performance and carcass characteristics of broilers subjected to treatment 7 were similar ($P > 0.05$) to those of birds subjected to treatment 8. These data demonstrate that the extraphosphoric effects of phytase may be associated with increased feed intake of broilers. Inositol supplementation did not provide additional benefits to broilers in this study.

Key words: phytase, phytate, inositol, broiler

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INTRODUCTION

Benefits of adding exogenous phytase in broiler diets to liberate phosphorus and other minerals have been previously reviewed in the literature (Selle and Ravindran, 2007; Bedford et al., 2016). However, recent advancement in phytase research has initiated the addition of phytase in higher concentrations to target extraphosphoric effects of phytase. These effects include increases in amino acid digestibility and AME (Ravindran et al., 2001; Gehring et al., 2013), restoration of digestive enzyme activity (Liu et al., 2008), reduction of antinutritive effects of phytate (Beeson et al., 2017), and release of inositol (Walk et al., 2014). This practice allows integrators to generate more profit through the reduction of dietary inorganic phosphorus and increased growth and meat yield.

Broiler diets are commonly supplemented with 500 to 1,500 phytase units (FTU)/kg to provide phosphorus from the hydrolysis of inositol phosphate (**IP**) 6. As a result, the destruction of IP6 is viewed as the end goal of phytase addition (Bedford and Walk, 2016). However, when focusing on the hydrolysis of IP6 to provide phosphorus, benefits of phytase beyond phosphorus liberation on growth performance and carcass characteristics may be overlooked. Therefore, optimum growth performance

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and meat accretion of broilers should also be considered when determining phytase doses to be used. Unfortunately, previous research evaluating extraphosphoric effects of phytase has been mainly conducted up to 3 wk of age (Walk et al., 2014; Zeller et al., 2015; Beeson et al., 2017). In contrast, the response of extraphosphoric effects of phytase on growth performance of broilers rose to market age, and their carcass characteristics are sparse (Campasino et al., 2014).

Benefits of extraphosphoric effects of phytase may also arise from the liberation of inositol (Walk et al., 2014; Beeson et al., 2017). Owing to its involvement in formation of phosphatidylinositol, inositol may play roles in skeletal muscle development (Lee and Bedford, 2016). However, inconsistency in the response of broilers to inositol supplementation has been observed (Cowieson et al., 2013; Zyla et al., 2013; Farhadi et al., 2017; Sommerfeld et al., 2018b; Pirgozliev et al., 2019), which may require further investigation. Therefore, this experiment was conducted to determine effects of supplementing inositol and elevated phytase doses on growth performance and meat yield of broilers during a 41-day production period.

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).

Husbandry Practices and Measurements

One thousand nine hundred 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. The birds were randomly distributed into 64 floor pens (30 birds per pen; 0.07 m^2 per bird) in a solid-sided house 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 the 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, whereas the grower and finisher diets were provided in pellet form. Feed and water were provided ad libitum throughout the experimental period.

Broilers and feed were weighed at 1, 15, 29, and 40 d of age to determine BW gain, feed intake, and feed conversion ratio (**FCR**) on a pen basis. The incidence of mortality was recorded daily. At 41 d of age, 12 birds per pen were selected for processing. Feed was withdrawn from each pen 10 h before processing. Selected birds were placed in coops and transported to the Auburn University Pilot Processing Plant. The broilers were hung on shackles, electrically stunned, slaughtered, scalded, picked, and manually eviscerated. After 3 h of chilling in an ice bath, carcass (without abdominal fat) and abdominal fat weights were recorded. The whole carcass was cut into the front and back half portions and was packed in ice for 18 h. Pectoralis major (boneless breast) and minor (tenders) muscles were deboned from the front half portion of the carcass and were weighed to determine total breast meat yield. Carcass yield, total breast meat yield, and abdominal fat percentage were calculated relative to the 40-day live BW.

Dietary Treatments

Broilers received one of the 8 dietary treatments during starter (1–15 d), grower (16–29 d), and finisher (30–41 d, Table 1) periods. Corn and soybean meal were used as primary ingredients in each dietary treatment. 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 the 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). In treatment 8, inositol was added to the PC diet (treatment 7) at a concentration of 0.26, 0.25, and 0.23% in the starter, grower, and finisher periods, respectively. 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. AME in starter, grower, and finisher diets was formulated at 3,000, 3,110, and 3,185 kcal/kg, respectively. In addition, amino acid concentrations were

Table 1. Ingredient and nutrient composition of negative control (NC) and positive control (PC) diets fed to broilers during the 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	1.22	2.04	1.03	1.84	0.84	1.65
Calcium carbonate	1.07	1.05	0.99	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	0.17	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
CP	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 TSAA[5]	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. Inositol supplementation was calculated based on the maximum inositol liberation from treatment 6 at 0.26, 0.25, and 0.23% in the starter, grower, and finisher periods, respectively. One unit of phytase activity (FTU) is defined as the quantity of enzyme to liberate 1 μmol of inorganic phosphate per minute from 0.0051 mol/L sodium phytate at pH 5.5 and 37°C.[5]

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

[3]Vitamin premix includes the following per kilogram 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]Total sulfur amino acids.

formulated at 93% as per Aviagen Ross 708 Broiler Nutrition Specifications recommendation (Aviagen, 2016) to mimic commercial practice. Experimental diet samples were collected from each period and were analyzed for phytase activity by ELISA specific for Quantum Blue (ESC, Standard Analytical Method, SAM099; AB Vista) similar to the method by Engelen et al. (2001).

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 1–6) using PROC REG (SAS Institute Inc., 2011). Because phytase doses were not evenly spaced among treatments (0, 500, 1,500, 4,500, 13,500, and 40,500 FTU/kg), these doses 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) as per the following mixed-effects model:

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

where $\mu..$ is the overall mean, ρ_i is identically and independently normally distributed random block effects with a mean of 0 and variance of σ^2_ρ, τ_j is fixed factor level effects corresponding to the j^{th} dietary treatment (treatments 1–8) such that $\sum \tau_j = 0$, and ε_{ij} is identically and independently normally distributed random errors with a mean of 0 and a variance of σ^2. Preplanned orthogonal contrasts were used to detect differences between NC vs. PC, treatment 6 vs. 8,

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
1) Negative control (NC)[2]	65	<50	<50
2) NC + 500 FTU/kg	417	519	641
3) NC + 1,500 FTU/kg	1,250	1,250	1,490
4) NC + 4,500 FTU/kg	3,313	3,350	3,960
5) NC + 13,500 FTU/kg	12,480	13,710	12,690
6) NC + 40,500 FTU/kg	36,640	41,290	42,070
7) Positive control (PC)[2]	695	238	267
8) 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 per minute from 0.0051 mol/L sodium phytate at pH 5.5 and 37°C.

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

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

and treatment 7 vs. 8. Correlation analysis was conducted using PROC CORR by SAS (2011). All statistical significance was considered at $P \leq 0.05$.

RESULTS

Analyzed phytase activity values in experimental diets were in good agreement with the calculated values (Table 2). In treatments 2 to 6, analyzed phytase activity was approximately 93.4% of the calculated values. The activity of phytase in the NC diets and PC diets with inositol addition was less than 65 FTU/kg. However, although the PC diets should contain no phytase inclusion, diet analysis indicated that PC diets in the starter, grower, and finisher periods contained 695, 238, and 267 FTU/kg of phytase. The presence of phytase in the PC diets may be related to diets' residue contamination from treatment 6 as the PC diets were formulated after the completion of treatment 6.

Growth Performance

From 1 to 15 d of age, log-quadratic effects of phytase ($P < 0.01$) in treatments 1 to 6 were observed in all growth performance objectives, wherein the increase of phytase doses increased BW gain and feed intake as well as decreased FCR of broilers (Table 3). Broilers fed with the PC diet had 5.0% higher ($P < 0.001$) BW gain and 3.3% lower ($P = 0.005$) FCR than those provided with the NC diets, but these responses were not reflected ($P = 0.19$) in the feed intake of broilers. Both BW gain and feed intake of broilers fed with diets with inositol supplementation were 5.1 and 3.8% lower ($P < 0.001$), respectively, than birds consuming diets with phytase supplementation at a concentration of 40,500 FTU/kg. Broilers provided with diets with inositol addition (treatment 8) also had 3.6 and 2.5% lower ($P < 0.05$) BW gain and feed intake, respectively, than birds fed with the PC diets. Mortality was not affected by dietary treatments.

From 1 to 29 d of age, increasing phytase doses from 0 to 40,500 FTU/kg in broiler diets led to log-quadratic increases ($P < 0.01$) of BW gain and feed intake and log-quadratic reduction ($P < 0.001$) of FCR (Table 4). Growth performance responses between broilers provided with PC and NC diets did not differ ($P > 0.05$), except the incidence of mortality of broilers fed with the NC diets was 2.9% points higher ($P = 0.028$) than birds provided with the PC diets. The reason for the higher incidence of mortality of broilers fed with the NC diets is unclear; however, these mortalities may be related to culls from leg problems. Inositol addition in broiler diets did not improve ($P > 0.05$) BW gain, feed intake, and FCR compared with those provided with the PC diets. Similarly, broilers consuming PC diets with inositol supplementation had 8.3% lower ($P < 0.001$) BW gain and consumed 3.8% less ($P = 0.005$) feed, while having 6 points higher ($P < 0.001$) FCR than broilers fed with diets enhanced with phytase at a concentration of 40,500 FTU/kg.

From 1 to 40 d of age, BW gain and FCR of broilers increased ($P = 0.002$) and decreased ($P = 0.018$), respectively, in a log-quadratic manner as phytase doses were increased from 0 to 40,500 FTU/kg in treatments 1 to 6 (Table 5). In contrast, a log-linear effect of phytase ($P = 0.045$) from 0 to 40,500 FTU/kg was observed on the feed intake of broilers. These responses demonstrated that the improvement in BW gain of broilers could be attributed to the effect of gradual increase of phytase content in enhancing FCR and feed intake of broilers. BW gain of broilers fed with diets supplemented with phytase at a concentration of 40,500 FTU/kg increased ($P = 0.001$) from 2.784 to 2.914 g, whereas FCR decreased ($P = 0.005$) by 4 points compared with those fed with the PC diets. The NC-fed broilers had a higher ($P = 0.011$) incidence of mortality than birds fed with the PC diets. No differences ($P > 0.005$) were observed on all growth performance objectives when comparing birds provided with the PC diets and PC diets with inositol addition.

Processing Characteristics

Both carcass and breast meat weights displayed increasing log-quadratic responses ($P < 0.01$) as phytase doses were increased from 0 to 40,500 FTU/kg in treatments 1 to 6 (Table 6). In addition, the increase in phytase doses from 0 to 40,500 FTU/kg resulted in increase ($P = 0.042$) in abdominal fat weight of broilers, which may be related to the increased BW of broilers as phytase supplementation was increased. The increased carcass yield due to log-quadratic increase of phytase was near significant ($P = 0.053$). However, no responses ($P > 0.05$) on breast meat yield and abdominal fat percentage were observed when varying dietary treatments. Similarly, broilers fed with the PC and NC diets did not differ ($P > 0.05$) among carcass characteristic responses. Carcass and breast meat weights of broilers fed with diets supplemented with 40,500 FTU/kg of phytase were 104 and 26 g heavier ($P < 0.05$), respectively, than birds consuming the PC diets with inositol addition. Similarly, inositol addition to the PC diets did not provide further benefits ($P > 0.05$) on carcass and breast meat weights when compared with birds provided with the PC diets.

Table 3. Growth performance of broilers fed with diets with gradient phytase supplementation from 1 to 15 d of age.[1]

Item	BW, kg/bird	BW gain, kg/bird	Feed intake, kg/bird	FCR, kg:kg[2]	Mortality, %[3]
Dietary treatment					
1) Negative control (NC)[4]	0.436	0.397	0.511	1.286	2.9
2) NC + 500 FTU/kg[5]	0.445	0.406	0.511	1.258	2.1
3) NC + 1,500 FTU/kg	0.452	0.413	0.515	1.245	1.6
4) NC + 4,500 FTU/kg	0.470	0.431	0.527	1.223	2.9
5) NC + 13,500 FTU/kg	0.462	0.423	0.525	1.240	2.5
6) NC + 40,500 FTU/kg	0.462	0.424	0.525	1.240	2.5
7) Positive control (PC)	0.456	0.417	0.518	1.243	1.3
8) PC + inositol[6]	0.441	0.402	0.505	1.256	1.7
Pooled standard error	0.004	0.004	0.004	0.011	0.9
Source of variation	Probabilities				
Log-linear effect of phytase[7]	<0.001	<0.001	0.005	<0.001	0.55
Log-quadratic effect of phytase[7]	<0.001	<0.001	0.006	0.001	0.61
Treatment 1 vs. 7	0.001	<0.001	0.19	0.005	0.08
Treatment 6 vs. 8	<0.001	<0.001	<0.001	0.28	0.36
Treatment 7 vs. 8	0.012	0.001	0.018	0.39	0.88

Abbreviation: FCR, feed conversion ratio.
[1]Values are least square means of 8 replicate pens, with each pen having 30 birds at placement.
[2]Feed conversion ratio was corrected for mortality.
[3]Mortality was arcsine transformed.
[4]The negative control diet was formulated to contain 0.165 and 0.150% lower calcium and phosphate, respectively, than the positive control diet.
[5]One unit of phytase activity (FTU) is defined as the quantity of enzyme to liberate 1 μmol of inorganic phosphate per minute 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 the diet containing the highest phytase concentration (treatment 6).
[7]Phytase levels were $\log_{10}$ transformed before analysis. Log-linear and log-quadratic effects were analyzed for treatments 1 to 6.

DISCUSSION

The benefits of supplementing phytase beyond 1,000 FTU/kg have been attributed to effects beyond phosphorus liberation, such as further phytate degradation (Zeller et al., 2015), increased amino acid digestibility (Gehring et al., 2013) and AME_n (Ravindran et al., 2001), restoration of enzyme functions (Liu et al., 2008),

Table 4. Growth performance of broilers fed with diets with gradient phytase supplementation from 1 to 29 d of age.[1]

Item	BW, kg/bird	BW gain, kg/bird	Feed intake, kg/bird	FCR, kg:kg[2]	Mortality, %[3]
Dietary treatment					
1) Negative control (NC)[4]	1.617	1.577	2.165	1.372	5.1
2) NC + 500 FTU/kg[5]	1.645	1.604	2.174	1.356	2.6
3) NC + 1,500 FTU/kg	1.670	1.629	2.210	1.356	1.6
4) NC + 4,500 FTU/kg	1.756	1.715	2.272	1.325	2.9
5) NC + 13,500 FTU/kg	1.737	1.699	2.249	1.325	3.4
6) NC + 40,500 FTU/kg	1.725	1.688	2.234	1.324	2.5
7) Positive control (PC)	1.621	1.583	2.168	1.370	2.2
8) PC + inositol[6]	1.587	1.548	2.148	1.388	2.1
Pooled standard error	0.019	0.018	0.024	0.010	1.1
Source of variation	Probabilities				
Log-linear effect of phytase[7]	<0.001	<0.001	0.003	<0.001	0.06
Log-quadratic effect of phytase[7]	<0.001	<0.001	0.003	<0.001	0.08
Treatment 1 vs. 7	0.85	0.83	0.92	0.86	0.028
Treatment 6 vs. 8	<0.001	<0.001	0.005	<0.001	0.65
Treatment 7 vs. 8	0.16	0.15	0.50	0.14	0.74

Abbreviation: FCR, feed conversion ratio.
[1]Values are least square means of 8 replicate pens, with each pen having 30 birds at placement.
[2]Feed conversion ratio was corrected for mortality.
[3]Mortality was arcsine transformed.
[4]The negative control diet was formulated to contain 0.165 and 0.150% lower calcium and phosphorus, respectively, than the positive control diet.
[5]One unit of phytase activity (FTU) is defined as the quantity of enzyme to liberate 1 μmol of inorganic phosphate per minute 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 the diet containing the highest phytase concentration (treatment 6).
[7]Phytase levels were $\log_{10}$ transformed before analysis. Log-linear and log-quadratic effects were analyzed for treatments 1 to 6.

Table 5. Growth performance of broilers fed with diets with gradient phytase supplementation from 1 to 40 d of age.[1]

Item	BW, kg/bird	BW gain, kg/bird	Feed intake, kg/bird	FCR, kg:kg[2]	Mortality, %[3]
Dietary treatment					
1) Negative control (NC)[4]	2.848	2.805	4.166	1.486	7.0
2) NC + 500 FTU/kg[5]	2.881	2.837	4.232	1.492	4.2
3) NC + 1,500 FTU/kg	2.868	2.829	4.238	1.498	2.2
4) NC + 4,500 FTU/kg	2.966	2.924	4.318	1.478	4.0
5) NC + 13,500 FTU/kg	2.973	2.927	4.254	1.454	4.7
6) NC + 40,500 FTU/kg	2.956	2.914	4.239	1.455	3.1
7) Positive control (PC)	2.835	2.790	4.187	1.501	2.2
8) PC + inositol[6]	2.823	2.784	4.158	1.494	3.2
Pooled standard error	0.034	0.033	0.043	0.011	1.3
Source of variation	Probabilities				
Log-linear effect of phytase[7]	0.003	0.002	0.045	0.050	0.040
Log-quadratic effect of phytase[7]	0.005	0.002	0.14	0.018	0.11
Treatment 1 vs. 7	0.75	0.71	0.69	0.27	0.011
Treatment 6 vs. 8	0.001	0.001	0.11	0.005	0.94
Treatment 7 vs. 8	0.76	0.86	0.56	0.60	0.58

Abbreviation: FCR, feed conversion ratio.
[1]Values are least square means of 8 replicate pens, with each pen having 30 birds at placement.
[2]Feed conversion ratio was corrected for mortality.
[3]Mortality was arcsine transformed.
[4]The negative control diet was formulated to contain 0.165 and 0.150% lower calcium and phosphorus, respectively, than the positive control diet.
[5]One unit of phytase activity (FTU) is defined as the quantity of enzyme to liberate 1 μmol of inorganic phosphate per minute 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 the diet containing the highest phytase concentration (treatment 6).
[7]Phytase levels were $\log_{10}$ transformed before analysis. Log-linear and log-quadratic effects were analyzed for treatments 1 to 6.

and inositol liberation (Walk et al., 2014). Effects of phytase on the improvement of nutrient utilization were evident as FCR of broilers in the present research study was decreased with gradual increase in phytase supplementation. These effects translated to improvements in growth performance and meat yield as demonstrated in the present study, wherein phytase doses were increased from 0 to 40,500 FTU/kg.

Table 6. Carcass characteristics of broilers fed with diets with gradient phytase supplementation from 1 to 41 d of age.[1]

		Carcass		Breast meat		Abdominal fat	
Item	Live weight, kg	Weight, kg	Yield, %	Weight, kg	Yield, %	Weight, g	Percentage, %
Dietary treatment							
1) Negative control (NC)[2]	2.917	2.159	74.29	0.780	26.80	26.05	0.89
2) NC + 500 FTU/kg[3]	2.984	2.203	74.17	0.790	26.62	28.17	0.95
3) NC + 1,500 FTU/kg	2.953	2.211	74.91	0.795	26.90	26.51	0.89
4) NC + 4,500 FTU/kg	3.024	2.273	75.11	0.815	26.94	28.17	0.93
5) NC + 13,500 FTU/kg	3.034	2.267	74.68	0.816	26.89	27.97	0.92
6) NC + 40,500 FTU/kg	3.050	2.278	74.73	0.810	26.51	28.48	0.93
7) Positive control (PC)	2.956	2.200	74.32	0.780	26.34	27.11	0.92
8) PC + inositol[4]	2.923	2.174	74.32	0.784	26.79	27.57	0.94
Pooled standard error	0.030	0.024	0.33	0.011	0.23	1.02	0.03
Source of variation	Probabilities						
Log-linear effect of phytase[5]	<0.001	<0.001	0.054	0.002	0.89	0.042	0.27
Log-quadratic effect of phytase[5]	<0.001	<0.001	0.053	0.004	0.83	0.11	0.36
Treatment 1 vs. 7	0.21	0.12	0.94	0.98	0.14	0.39	0.52
Treatment 6 vs. 8	<0.001	<0.001	0.23	0.037	0.35	0.44	0.79
Treatment 7 vs. 8	0.26	0.29	0.99	0.74	0.13	0.70	0.49

[1]Values are least square means of 8 replicate pens, with 12 birds selected from each pen for processing.
[2]The negative control diet was formulated to contain 0.165 and 0.150% lower calcium and phosphorus, 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 per minute from 0.0051 mol/L 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 levels were $\log_{10}$ transformed before analysis. Log-linear and log-quadratic effects were analyzed for treatments 1 to 6.

Shirley and Edwards (2003) also observed a quadratic increase in BW gain of broilers from 1 to 16 d of age when increasing phytase doses from 93.75 to 12,000 FTU/kg. Similarly, Walk et al. (2018) noted a linear increase in weight gain of broilers when feeding 0, 500, 1,500, and 4,500 FTU/kg of bacterial phytase during a 21-d production period. These studies were also in agreement with Augspurger and Baker (2004), who suggested that broilers had an increase in BW gain of 39 g from 302 to 341 g when receiving supplementation of either bacterial or fungal phytase from 500 to 5,000 FTU/kg from 8 to 21 d of age. However, when phytase concentration was increased to 10,000 FTU/kg, BW gain of broilers did not differ with those fed with the diets containing 5,000 FTU/kg of phytase, indicating no further benefits of increasing phytase dose.

The present study also demonstrated the increased response of feed intake due to elevated phytase doses in treatments 1 to 6 in addition to the responses of BW gain and FCR. These data indicated that the extraphosphoric effects of phytase may not only improve nutrient utilization (Ravindran et al., 2001; Shirley and Edwards, 2003; Gehring et al., 2013) but also stimulate feed intake response. In the present study, correlation coefficients between feed intake and BW gain were 0.73 ($P < 0.001$), 0.80 ($P < 0.001$), and 0.73 ($P < 0.001$) from 1 to 15, 1 to 29, and 1 to 40 d of age, respectively. As feed intake increases, digestible nutrient intake also increases leading to the improvement of BW gain. Previously, Walk and Olukosi (2019) conducted a study evaluating the efficacy *E. coli* phytase from *T. reesei* in broilers fed with diets supplemented with 0, 2,000 and 4,000 FTU/kg of phytase. These authors noted that both apparent ileal amino acid digestibility and digestible amino acid intake increased linearly as phytase doses increased. However, BW gain of broilers had a nonsignificant correlation ($P > 0.10$, r = −0.14 to −0.29) with apparent ileal amino acid digestibility. In contrast, the increase in digestible amino acid intake was highly correlated ($P < 0.10$, r = 0.33–0.72) with BW gain of broilers, which indicated that digestible nutrient intake may be a more suitable indicator of phytase efficacy than nutrient digestibility. Therefore, these data indicated that the benefits of extraphosphoric effects of phytase may be due to the digestible nutrient intake.

The effects of phytase in stimulating digestible nutrient intake may be associated with its impact on phytate degradation. Phytate has been reported to reduce feed intake in broilers. Dos Santos et al. (2014) reported a 20.1% reduction in feed intake of broilers from 1 to 21 d of age when increasing dietary phytate phosphorus concentration from 0.18 to 0.29%. However, when 500 FTU/kg of *E. coli* phytase from *T. reesei* was added to the diet containing high phytate phosphorus content, there was no difference in feed intake between the broilers fed with diets containing high and low phytate phosphorus content. In addition, a study by Zeller et al. (2015) provided evidence of phytate degradation by *E. coli* phytase expressed in *T. reesei*. The supplementation of phytase at a concentration of 500 and 12,500 FTU/kg in broiler diets led to 78 and 92% IP6 hydrolysis compared with 67% hydrolysis in diets without phytase addition. As a result, broilers receiving diets with 500 and 12,500 FTU/kg of phytase consumed 9 and 11 g more feed daily than those fed with diets without phytase addition from 1 to 21 d of age. These studies and the present study demonstrated that the extraphosphoric effects of phytase may stimulate nutrient intake in addition to improving nutrient digestibility, which resulted in growth performance enhancement.

The positive influence of phytase on carcass characteristics in the present research study has also been noted in a previous study (Schmeisser et al., 2017), which indicated that bacterial phytase supplementation from *Citrobacter braakii* at a concentration of 1,000 FTU/kg increased breast meat weight of broilers from 90 to 98 g and 220 to 272 g at 21 and 36 d of age, respectively, compared with birds fed with reduced calcium and nonphytate phosphorus diets. These researchers indicated that the benefits of phytase on muscle growth may be attributed to several interconnected factors, such as inositol release and upregulation of myogenesis gene expression (Schmeisser et al., 2017). This study indicated that phytase may increase phospholipase C beta gene expression, which stimulates the release of inositol (1,4,5)-triphosphate from phosphatidylinositol 4,5-bisphosphate. Subsequently, calcium can be released from the endoplasmic reticulum, activating calmodulin and calcineurin A pathways, which promotes muscle growth (Tokomitsu et al., 1999).

Furthermore, Schmeisser et al. (2017) reported the upregulation of genes involved with the insulin growth factor/phosphoinositide 3 kinase pathway, which may be initiated by the release of inositol that mimics the action of glucose (Cowieson et al., 2015). This pathway can activate the mTOR pathway, promoting skeletal myogenesis (Cooper and Hausman, 2013). In addition, measurements of gene expression in the breast muscle of broilers fed with phytase-added diets displayed an increased myocyte enhancer factor 2 gene expression compared with broilers receiving diets without phytase addition (Schmeisser et al., 2017). Previous research indicated that myocyte enhancer factor 2 is responsible for the regulation of myoblast determination protein, which regulates muscle differentiation (McKinsey et al., 2002).

It is interesting that the reduction of calcium and nonphytate phosphorus content in the current research study suppressed BW gain and FCR from 1 to 15 d of age, but not from 1 to 29 and 1 to 40 d of age, and carcass characteristics at 41 d. These responses are similar to those in studies by Walk et al. (2013) and dos Santos et al. (2013). Presumably, younger birds may be more susceptible to a marginal reduction of calcium and nonphytate phosphorus concentrations than older birds (Walk et al., 2013). A previous study reported that the fastest rate of bone formation and mineralization in broilers occurred from 4 to 18 and 4 to 11 d of age, respectively (Williams et al., 2000). Thus, the reduction in calcium and nonphytate phosphorus concentration in the present study may have a more pronounced effect during the starter period than during the later period.

The lack of differences in growth performance from 1 to 29 and 1 to 40 d of age between broilers provided with the NC and PC diets in the present study may also be associated with the concentrations of dietary nonphytate phosphorus. Previous research recommended nonphytate phosphorus concentration of 0.45, 0.29, and 0.23% from 1 to 18, 19 to 32, and 33 to 42 d of age, respectively, for optimum growth performance of broilers (Angel et al., 2005). In the present research study, the PC diets were formulated to contain 0.48, 0.44, and 0.39% of nonphytate phosphorus from 1 to 14 (starter), 15 to 29 (grower), and 30 to 40 d of age (finisher), respectively. When nonphytate phosphorus content was decreased by 0.15% to establish the NC diets, the only concentration lower than that recommended by Angel et al. (2005) was that of nonphytate phosphorus in the NC starter diet. Hence, the lack of differences in growth performance from 1 to 29 and 1 to 40 d of age between broilers fed with the PC and NC diets may be associated with the high concentration of nonphytate phosphorus even after 0.15% reduction. This indicates that recommendation of nonphytate phosphorus content by the primary breeder guidelines may be in excess of the requirements (Aviagen, 2016). This concept is supported by Pieniazek et al. (2017), who reported a reduction in BW and an increase in FCR of broilers from 1 to 14, 1 to 28, and 1 to 42 d of age when fed with NC vs. PC diets. These researchers formulated NC diets to contain 0.28, 0.24, and 0.20% and PC diet to contain 0.45, 0.41, and 0.38% of nonphytate phosphorus from 1 to 14, 15 to 28, and 29 to 42 d of age, respectively.

Inositol has been reported to play roles in various physiological mechanisms, such as signaling mechanisms (Cooper and Hausman, 2013), nutrient transport (Jiang et al., 2013), and hepatic lipid metabolism (Pirgozliev et al., 2019). However, the effects of dietary inositol supplementation on growth performance of broilers have been inconsistent. Cowieson et al. (2013), Zyla et al. (2013), and Sommerfeld et al. (2018b) reported improvements in growth performance of broilers when supplementing inositol in the diets. Conversely, Farhadi et al. (2017) and Pirgozliev et al. (2019) did not observe benefits in growth performance of broilers when provided with diets with inositol inclusion, which are in agreement with the present research study. The reason for this discrepancy of inositol benefits in growth performance of broilers is unclear. Further work should include inositol measurements in the digesta and blood to ascertain whether differences among studies are simply due to differences in basal phytate digestibility, which has been shown to range from 23 to 74% (Rodehutscord et al., 2017), depending on dietary ingredients and specifications. As a result, effects of adding either phytase or inositol to a diet with higher IP6 digestibility may be limited compared with another diet with lower IP6 digestibility (Sommerfeld et al., 2018a; Ingelmann et al., 2019). In addition, inositol concentration for optimizing growth performance of broilers is also not known.

In conclusion, increasing phytase doses up to 4,500 FTU/kg was beneficial for optimizing BW gain and carcass characteristics of broilers from 1 to 40 d. These improvements may be associated with the extraphosphoric effects of phytase on reducing FCR and stimulating feed intake of broilers, which translated to increased digestible amino acid intake. However, this study indicates that the addition of inositol did not provide further benefits in growth performance and meat yield of broilers.

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DISCLOSURES

CLW and MRB are affiliated with the product evaluated in this manuscript.

REFERENCES

Aviagen. 2016. Ross 708 Broiler: Nutrient Specifications. Aviagen, Huntsville, AL.

AminoDat 5.0. 2016. Evonik Industries. Evonik Degussa GmbH, Hanau-Wolfgang, Germany.

Angel, R., W. W. Saylor, A. S. Dhandu, W. Powers, and T. J. Applegate. 2005. Effects of dietary phosphorus, phytase, and 25-hydroxycholecalciferol on performance of broiler chickens grown in floor pens. Poult. Sci. 84:1031–1044.

Augspurger, N. R., and D. H. Baker. 2004. High dietary phytase levels maximize phytate-phosphorus utilization but do not affect protein utilization in chicks fed phosphorus- or amino acid-deficient diets. J. Anim. Sci. 82:1100–1107.

Bedford, M. R., and C. L. Walk. 2016. Reduction of phytate to tetrakisphosphate (IP_4) to triphosphate (IP_3), or perhaps even lower, does not remove its antinutritive properties. Pages 45–51 in Phytate Destruction – Consequences for Precision Animal Nutrition. C. L. Walk, I. Kuhn, H. H. Stein, M. T. Kidd, and M. Rodehutscord, eds. Wageningen Academic Publishers, Wageningen, The Netherlands.

Bedford, M. R., C. L. Walk, and H. V. Masey O'Neill. 2016. Assessing measurements in feed enzyme research: phytase evaluations in broilers. J. Appl. Poult. Res. 25:305–314.

Beeson, L. A., C. L. Walk, M. R. Bedford, and O. A. Olukosi. 2017. Hydrolysis of phytate to its lower esters can influence the growth performance and nutrient utilization of broilers with regular or super doses of phytase. Poult. Sci. 96:2243–2253.

Campasino, A., T. York, C. Wyatt, M. R. Bedford, and W. A. Dozier, III. 2014. Effect of increasing supplemental phytase concentration in diets fed to Hubbard × Cobb 500 male broilers from 1 to 42 days of age. J. Appl. Poult. Res. 23:705–714.

Cooper, G. M., and R. E. Hausman. 2013. Cell signaling. Pages 589–640 in The Cell: A Molecular Approach. 6th ed. Sinauer Associates, Inc., Sunderland, MA.

Cowieson, A. J., R. Aureli, P. Guggenbuhl, and F. Fru-Nji. 2015. Possible involvement of myo-inositol in the physiological response of broilers to high doses of microbial phytase. Anim. Prod. Sci. 55:710–719.

Cowieson, A. J., A. Ptak, P. Mackowiak, M. Sassek, E. Pruszynska-Oszmalek, K. Zyla, S. Swiatkiewicz, S. Kaczmarek, and D. Jozefiak. 2013. The effect of microbial phytase and myo-inositol on performance and blood biochemistry of broiler chickens fed wheat/corn-based diets. Poult. Sci. 92:2124–2134.

Dos Santos, T. T., S. Srinongkote, M. R. Bedford, and C. L. Walk. 2013. Effect of high phytase inclusion rates on performance of broilers fed diets not severely limited in available phosphorus. Asian-aust. J. Anim. Sci. 26:227–232.

Dos Santos, T. T., C. L. Walk, and S. Srinongkote. 2014. Influence of phytate level on broiler performance and the efficacy of 2 microbial phytases from 0 to 21 days of age. J. Appl. Poult. Res. 23:181–187.
Engelen, A. J., F. C. van der Heeft, P. H. G. Randsdorp, and W. A. C. Somers. 2001. Determination of phytase activity in feed by a colorimetric enzymatic method: Collaborative interlaboratory study. J. AOAC Int. 84:629–633.
Farhadi, D., A. Karimi, Gh. Sadeghi, J. Rostamzadeh, and M. R. Bedford. 2017. Effects of a high dose of microbial phytase and myo-inositol supplementation on growth performance, tibia mineralization, nutrient digestibility, litter moisture content, and foot problems in broiler chickens fed phosphorus-deficient diets. Poult. Sci. 96:3664–3675.
Gehring, C. K., M. R. Bedford, and W. A. Dozier, III. 2013. Extra-phosphoric effects of phytase with and without xylanase in corn-soybean meal-based diets fed to broilers. Poult. Sci. 92:979–991.
Ingelmann, C.-J., M. Witzig, J. Mohring, m. Chollenberger, I. Kuhn, and M. Rodehutscord. 2019. Phytate degradation and phosphorus digestibility in broilers and turkeys fed different corn sources with or without added phytase. Poult. Sci. 98:912–922.
Jiang, W. –D., S. –Y. Kuang, y. Liu, J. Jiang, K. Hu, S. –H. Li, L. Tang, L. Feng, and X. –Q. Zhou. 2013. Effects of myo-inositol on proliferation, differentiation, oxidative status and antioxidant capacity of carp enterocytes in primary culture. Aquacult. Nutr. 19:45–53.
Lee, S. A., and M. R. Bedford. 2016. Inositol – An effective growth promotor? World's Poult. Sci. J. 72:743–759.
Liu, N., Y. J. Ru, F. D. Li, and A. J. Cowieson. 2008. Effect of diet containing phytate and phytase on the activity and messenger ribonucleic acid expression of carbohydrase and transporter in chickens. J. Anim. Sci. 86:3432–3439.
McKinsey, T. A., C. L. Zhang, and E. N. Olson. 2002. MEF2: a calcium-dependent regulator of cell division, differentiation and death. Trends Biochem. Sci. 27:40–47.
Ravindran, V., P. H. Selle, G. Ravindran, P. C. H. Morel, A. K. Kies, and W. L. Bryden. 2001. Microbial phytase improves performance, apparent metabolizable energy, and ileal amino acid digestibility of broilers fed a lysine-deficient diet. Poult. Sci. 80:338–344.
SAS Institute Inc. 2011. SAS User's Guide. Statistics. Version 9. 4 ed. SAS Inst. Inc., Cary, NC.
Schmeisser, J., A. –A. Seon, R. Aureli, A. Fridel, P. Guggenbuhl, S. Duval, A. J. Cowieson, and F. Fru-Nji. 2017. Exploratory transcriptomic analysis in muscle tissue of broilers fed a phytase-supplemented diet. J. Anim. Physiol. Anim. Nutr. 101:563–575.
Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Technol. 135:1–41.
Shirley, R. B., and H. M. Edwards, Jr. 2003. Graded levels of phytase past industry standards improves broiler performance. Poult. Sci. 82:671–680.
Simons, P. C. M., H. A. J. Versteegh, A. W. Jongbloed, P. A. Kemme, P. Slump, K. D. Bos, M. G. E. Wolters, R. F. Beudeker, and G. J. Verschoor. 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Br. J. Nutr. 64:525–540.
Sommerfeld, V., S. Kunzel, M. Schollenberger, I. Kuhn, and M. Rodehutscord. 2018b. Influence of phytase or myo-inositol supplements on performance and phytate degradation products in the crop, ileum, and blood of broiler chickens. Poult. Sci. 97: 920–929.
Sommerfeld, V., M. Schollenberger, I. Kuhn, and M. Rodehutscord. 2018a. Interactive effects of phosphorus, calcium, and phytase supplements on products of phytate degradation in the digestive tract of broiler chickens. Poult. Sci. 97:1177–1188.
Tokumitsu, H., N. Takahashi, K. Eto, S. Yano, T. R. Soderling, and M. Muramatsu. 1999. Substrate recognition by Ca^{2+}/calmodulin-dependent protein kinase kinase. Role of the arg-pro-rich inset domain. J. Biol. Chem. 274:15803–15810.
Pieniazek, J., K. A. Smith, M. P. Williams, M. K. Manangi, M. Vazquez-Anon, A. Solbak, M. miller, and J. T. Lee. 2017. Evaluation of increasing levels of microbial phytase in phosphorus deficient broiler diets via live broiler performance, tibia bone ash, apparent metabolizable energy, and amino acid digestibility. Poult. Sci. 96:370–382.
Pirgozliev, V., C. A. Brearley, S. P. Rose, and S. C. Mansbridge. 2019. Manipulation of plasma myo-inositol in broiler chickens: effect on growth performance, dietary energy, nutrient availability, and hepatic function. Poult. Sci. 98:260–268.
Rodehutscord, M., O. Adeola, R. Angel, P. Bikker, E. Delezie, W. A. Dozier, III, M. Umar Faruk, M. Francesch, C. Kwakernaak, A. Narcy, C. M. Nyachoti, O. A. Olukosi, A. Preynat, B. Renouf, A. Saiz del Barrio, K. Schedle, W. Siegert, S. Steenfeldt, M. M. van Krimpen, S. M. Waititu, and M. Witzig. 2017. Results of an international phosphorus digestibility ring test with broiler chickens. Poult. Sci. 96:1679–1687.
Walk, C. L., M. R. Bedford, and O. A. Olukosi. 2018. Effect of phytase on growth performance, phytate degradation and gene expression of myo-inositol transporters in the small intestine, liver and kidney of 21 day old broilers. Poult. Sci. 97:1155–1162.
Walk, C. L., M. R. Bedford, T. S. Santos, D. Pavia, J. R. Bradley, H. Wladecki, C. Honaker, and A. P. McElroy. 2013. Extra-phosphoric effects of superdoses of a novel microbial phytase. Poult. Sci. 92:719–725.
Walk, C. L., T. T. Santos, and M. R. Bedford. 2014. Influence of superdoses of a novel microbial phytase on growth performance, tibia ash, and gizzard phytate and inositol in young broilers. Poult. Sci. 93:1172–1177.
Walk, C. L., and O. A. Olukosi. 2019. Influence of graded concentrations of phytase in high-phytate diets on growth performance, apparent ileal amino acid digestibility, and phytate concentration in broilers from hatch to 28 D post-hatch. Poult. Sci. 98:3884–3893.
Williams, B., S. Solomon, D. Waddington, B. Thorp, and C. Farquharson. 2000. Skeletal development in the meat-type chicken. Br. Poult. Sci. 41:141–149.
Zeller, E., M. Schollenberger, M. Witzig, Y. Shastak, I. Kuhn, L. E. Hoelzle, and M. Rodehutscord. 2015. Interaction between supplemented mineral phosphorus and phytase on phytate hydrolysis and inositol phosphates in the small intestine of broilers. Poult. Sci. 94:1018–1029.
Zyla, K., R. Dulinski, M. Pierzchalaska, M. Grabacka, D. Jozefiak, and S. Swiatkiewicz. 2013. Phytases and myo-inositol modulate performance, bone mineralization and alter lipid fractions in the serum of broilers. J. Anim. Feed Sci. 22:56–62.