Influence of exogenous phytase supplementation on phytate degradation, plasma inositol, alkaline phosphatase, and glucose concentrations of broilers at 28 days of age

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ABSTRACT Inositol is the final product of phytate degradation, which has the potential to serve as an indicator of phytase efficacy. An experiment was conducted to evaluate effects of supplementing broiler diets with phytase on phytate degradation and plasma inositol concentrations at 28 d of age. Twenty-four Ross × Ross 708 male chicks were placed in battery cages (4 birds per cage) from 1 to 21 d of age and individually from 22 to 28 d of age. At 27 d of age, a catheter was placed in the brachial vein of broilers to avoid repeated puncture of the vein during blood collection. At 28 d of age, broilers received 1 of 3 experimental diets formulated to contain 0, 400, or 1,200 phytase units (FTU)/kg, respectively, in diet 1, 2, and 3. Blood was collected 1 h before feeding experimental diets and from 20 to 240 min after feeding experimental diets at 20-min intervals with a final blood collection at 480 min to determine plasma inositol concentrations. Inositol phosphate (IP) ester degradation was determined in gizzard contents and ileal digesta. Broilers provided the 1,200 FTU/kg phytase diet had 60% less (P < 0.01) IP6 concentration in gizzard content (1,264 vs. 4,176 nmol/g) and ileal digesta (13,472 vs. 33,244 nmol/g) than birds fed the 400 FTU/kg diet. Adding phytase at 1,200 FTU/kg increased (P < 0.01) inositol concentrations in gizzard content and ileal digesta of broilers by 2.5 (2,703 vs. 1,071 nmol/g) and 3.5 (16,485 vs. 4,667 nmol/g) fold, respectively, compared with adding 400 FTU/kg. Plasma inositol concentration of broilers was not different (P = 0.94) among the dietary treatments at each collection time. Inositol liberation in the digesta of broilers fed diets with 1,200 FTU/kg phytase did not translate to increased plasma inositol concentrations, which warrants further investigation.

Key words: phytase, phytate, inositol, broiler

INTRODUCTION Phytase is supplemented in broiler diets to enhance phosphorus utilization, which concomitantly decreases feed cost by reducing the inclusion of inorganic phosphate (Selle and Ravindran, 2007). Recently, increasing phytase supplementation beyond 1,000 phytase units (FTU)/kg in broiler diets led to increased amino acid digestibility, apparent metabolizable energy, and growth performance of broilers beyond phosphorus liberation (Gehring et al., 2013). In addition to these extraphosphoric effects of phytase, the liberation of inositol may play a key role in altering growth rate of broilers (Walk et al., 2014). Inositol is the final product of complete phytate degradation through a stepwise liberation of all its phosphate groups. However, information regarding the rate of phytate degradation and inositol liberation in broilers fed a phytase supplemented diet is sparse (Cowieson et al., 2017). In order to determine the rate of phytate degradation in broilers fed phytase beyond 1,000 FTU/kg, digesta inositol phosphate ester and plasma inositol concentration may be used as an indicator of phytase efficacy.

After its liberation, inositol has been reported to be highly absorbed (99.8%) in the small intestine (Croze and Soulage, 2013). Considering its rapid liberation by phytase and efficiency of absorption, the inclusion of phytase beyond 1,000 FTU/kg in a broiler diet may result in a rapid increase of inositol concentration in
the plasma (Cowieson et al., 2015). Plasma inositol concentration may be used as a biomarker for phytase efficacy, which may allow phytase users to identify problems related to phytase destruction during feed manufacturing as well as to unfold alternatives to enhance phytate degradation (Cowieson et al., 2017). Hence, identifying the optimum time to measure plasma inositol concentration is paramount in using inositol as a biomarker for phytase efficacy. An experiment was conducted to determine phytate degradation and an optimum plasma inositol concentration in broilers fed diets supplemented with phytase within 4 h of feeding experimental diets.

**MATERIALS AND METHODS**

All procedures involving care and use of live birds in this experiment were approved by Auburn University Institutional Animal Care and Use Committee (PRN 2017-3067).

**Bird Husbandry**

Twenty-four Ross × Ross 708 male chicks were obtained from a commercial hatchery (Aviagen North America, Huntsville, AL) and received vaccinations for Marek’s disease, Newcastle disease, and infectious bronchitis at 1 d of age. Broilers were placed into 6 battery cages (4 birds per cage; 0.12 m² per bird; Petersime, Gettysburg, OH) with each cage having dimensions of 68 × 68 × 38 cm from 1 to 21 d of age. From 22 to 28 d of age, broilers were placed individually in battery cages (0.46 m²/bird) to prevent other birds from pecking at catheters. Each cage contained a linear feeder and a water trough. Cages were placed in a solid-sided room equipped with forced-air heaters and cooling pads to adjust the temperature. Room temperature at bird placement was set at 33°C and gradually decreased to 24°C until 28 d of age. A 23L:1D photoperiod was provided from 1 to 7 d of age, thereafter a 20L:4D lighting schedule was used. Broilers received feed and water ad libitum throughout the 28-d period. Broilers were provided common starter and grower diets (corn-soybean meal-based) without phytase addition from 1 to 14 and 15 to 27 d of age, respectively. These diets were formulated to contain apparent metabolizable energy, CP, and digestible Lys at 3,185 kcal/kg, 18.1, and 1.08%, respectively. Experimental diet samples were 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).

**Dietary Treatments**

At 28 d of age, the common grower diet was removed from each cage, and broilers were immediately provided experimental diets. Each broiler received 1 of 3 dietary treatments in mash form (Table 1). Dietary treatment 1 was the positive control (PC) diet formulated to contain adequate calcium (0.76%) and nonphytate phosphorus (0.36%) concentrations without phytase addition according to the primary breeder recommendations (Aviagen, 2016). A negative control basal diet was formulated to contain 0.165 and 0.150% lower calcium and nonphytate phosphorus concentrations, respectively, than the PC diet. Diets 2 and 3 were established by supplementing the negative control basal diet with phytase at the expense of sand to contain 400 and 1,200 FTU/kg of diet, respectively. These concentrations were selected to obtain a 3-fold increase of phytase activity to promote rapid phytate degradation and inositol liberation. One FTU is defined as the quantity of phytase required to release 1 μmol of inorganic phosphate from 0.0051 mol/L sodium phytate in 1 min at pH 5.5 and 37°C (Simons et al., 1990). All experimental diets were formulated with corn and soybean meal as primary ingredients to contain apparent metabolizable energy, CP, and digestible Lys at 3,185 kcal/kg, 18.1, and 1.08%, respectively. Experimental diet samples were 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).

**Blood Collection**

A baseline blood collection was performed approximately 1 h before feeding experimental diets. Then, blood samples were collected at 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, and 480 min after feeding of experimental diets to birds. The 20-min interval was selected, as this was the minimum time required to collect blood from the number of replicates chosen. These blood collections allowed for determining the rate of phytate degradation and change in plasma inositol concentration of broilers fed phytase-added diets. To avoid blood sample contamination with saline, 0.3 mL of residual blood was collected using a 1-mL
syringe (25-gauge needle). Then, 1 mL of blood sample was collected using a 1-mL syringe (25-gauge needle), transferred to a 1-mL heparinized tube (BD Microtainer; BD Vacutainer Systems, Franklin Lakes, NJ), and placed on ice until centrifugation. The catheter was flushed with 0.3 mL of heparinized saline (0.9% sodium chloride and 100 unit/mL heparin) after each blood collection to prevent blood clotting. Blood samples were centrifuged at 1,643 × g for 10 min to separate plasma from the whole blood and were stored at −20°C until further analysis.

**Digesta and Tissue Collection**

At the conclusion of blood collections, broilers were euthanized using carbon dioxide asphyxiation followed by cervical dislocation. Gizzard contents were collected by carefully scraping feed contents into a Whirl-Pak bag (Nasco, Fort Atkinson, WI), and ileal digesta samples were collected by gently flushing out the content of the terminal ileum using deionized-distilled water into Whirl-Pak bags (Nasco, Fort Atkinson, WI). The terminal ileum section is defined as the last one-third of the section between the Meckel’s diverticulum and approximately 4 cm anterior from the ileo-cecal junction (Kluth et al., 2005; Rodehutschar et al., 2012). Both gizzard content and ileal digesta samples were immediately frozen by submersion in liquid nitrogen for approximately 5 min to terminate phytase activity. Samples were kept on ice and stored at −20°C until later analysis.

Segments of the small intestine were separated into duodenum (pancreatic loop), jejunum (from the distal duodenal loop to the Meckel’s diverticulum), and ileum (from the Meckel’s diverticulum to the ileo-cecal junction) for analysis of intestinal alkaline phosphatase (ALP) activity. Each segment was longitudinally cut and gently rinsed with a cold phosphate-buffered saline to remove any digesta material. Tissue samples were wrapped in aluminum foil and placed in Whirl-Pak bags (Nasco, Fort Atkinson, WI). Immediately, samples were frozen by submersion in liquid nitrogen for approximately 5 min to terminate ALP activity. Tissue samples were stored at −20°C until further analysis.

**Chemical Analyses**

Plasma samples were separated into 2 aliquots for analyses. One aliquot was sent to the College of Veterinary Medicine at Auburn University for the determination of plasma ALP using colorimetric assay according to the method by Schumann et al. (2011). Each sample was analyzed in duplicate using Hitachi Cobas C331 (Roche Diagnostic, Indianapolis, IN). The other aliquot was mixed with 1 mol perchloric acid in a 1:2 ratio (plasma:HClO₄) to precipitate all protein. The solution was centrifuged at 14,000 × g for 10 min to collect the supernatant. Samples were analyzed in duplicate for inositol and glucose concentrations using HPLC with pulsed amperometric detection at the University of East Anglia School of Biological Sciences in Norwich, England. 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-nmol 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 and glucose peaks were integrated with Chromeleon (ThermoFisher Scientific, Waltham, MA) and DataApex Clarity (DataApex, Prague, Czech Republic) software packages. Concentrations of inositol and glucose were determined by comparing results with standards using a linear least-squares regression.

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

Frozen segments of small intestine tissue samples were thawed in ice for 4 h. The mucosa was gently scraped using a glass slide and placed into a 1.5-mL tube. Mucosa samples were homogenized in a PBS solution with a 20:1 ratio (PBS/mucosa, v/w) using a glass homogenizer. The suspension was subjected for ultrasonication for 15 min to further lyse the cell membrane. Homogenates were centrifuged for 15 min at 1,500 × g, and the supernatant was collected for analysis. Intestinal ALP was analyzed using chicken ALP ELISA kit according to the manufacturer’s procedure (ABclonal, Woburn, MA). Absorbance was measured on a spectrophotometer at 450 nm. (SpectraMax Plus 384; Molecular Devices LLC., San Jose, CA).

**Statistical Analyses**

Dietary treatments were randomly placed into a randomized complete block design with cage location as the blocking factor. Individual bird served as the experimental unit with 8 replications. Concentrations of IP₃, IP₄, IP₅, IP₆, and inositol in gizzard content and ileal digesta were subjected to a 1-way analysis of variance using the MIXED procedure of SAS (2011) by the following mixed-effects model:

\[ Y_{ij} = \mu + \tau_i + \beta_j + e_{ij} \]

where \( \mu \) is the overall mean; the \( \tau_i \) are fixed factor level effects of \( i^{th} \) dietary treatment (diets 1, 2, and 3) such that \( \sum \tau_i = 0 \); the \( \beta_j \) are identically and independently normally distributed random block effects with mean 0 and variance \( \sigma^2_\beta \) such that \( \sum \beta_j = 0 \); and the \( e_{ij} \) are identical...
and independent random errors that follow a normal distribution with mean 0 and variance $\sigma^2$. Statistical significance was considered at $P \leq 0.05$.

Plasma inositol, ALP, and glucose concentrations were analyzed using a 2-way repeated measure analysis of variance with dietary treatment and time as factors. The analysis was conducted using the GLM procedure of SAS (2011) by the following model:

$$Y_{ijk} = \mu + \tau_i + \beta_j + (\tau \beta)_{ij} + \pi_{k(i)} + (\beta \pi)_{jk(i)} + \epsilon_{ijk}$$

where $\mu$ is the overall mean; the $\tau_i$ are fixed factor level effects corresponding to $i$th dietary treatment (diets 1, 2, and 3) such that $\sum \tau_i = 0$; the $\beta_j$ are effects of $j$th collection time (0 to 480 min) such that $\sum \beta_j = 0$; the $(\tau \beta)_{ij}$ are the interaction of the $i$th dietary treatment and $j$th collection time with $\sum (\tau \beta)_{ij} = 0$; the $\pi_{k(i)}$ represent the random effects of bird $k$ nested within dietary treatment $i$; the $(\beta \pi)_{jk(i)}$ are the random interaction effects of bird $k$ and collection time $j$ nested within dietary treatment $i$; and the $\epsilon_{ijk}$ are random errors that follow a normal distribution with mean 0 and variance $\sigma^2$. The baseline plasma concentration was used as a covariate because blood collection was performed before feeding experimental diets. Furthermore, orthogonal polynomial contrasts were performed to determine the effects of dietary treatments over time from 20 to 240 min after feeding experimental diets. A preplanned orthogonal contrast was used to determine differences in plasma concentrations between 240- and 480-min collections. Statistical significance was considered at $P \leq 0.05$.

**RESULTS AND DISCUSSION**

Experimental diet analyses determined that phytase activity in diets 2 and 3 was slightly lower than calculated values (Table 1). However, a 3-fold increase in phytase activity from diets 2 to 3 was maintained. Analyzed concentrations of total IP6 to IP3 and inositol in experimental diets were in agreement among dietary treatments (Table 2). Broilers grew optimally with an average body weight of 1.77 kg before catheter placement from 1 to 27 d of age. During a 15-h recovery period after catheter placement, feed intake was 96 g per bird.

### Table 1. Ingredient and nutrient compositions of experimental diets formulated to contain 0, 400, or 1,200 phytase unit (FTU)/kg of phytase fed to broilers at 28 d of age1

| Item                          | PC     | NC + 400 FTU/kg2 | NC + 1,200 FTU/kg2 |
|------------------------------|--------|------------------|-------------------|
| Ingredient, % as-fed         |        |                  |                   |
| Corn                         | 64.12  | 65.75            | 65.75             |
| Soybean meal                 | 27.85  | 27.60            | 27.60             |
| Vegetable oil                | 4.51   | 3.90             | 3.90              |
| Dicalcium phosphate          | 1.48   | 0.66             | 0.66              |
| Calcium carbonate            | 0.91   | 0.94             | 0.94              |
| Sodium chloride              | 0.40   | 0.40             | 0.40              |
| DL-Methionine                | 0.24   | 0.24             | 0.24              |
| L-Lysine•HCl                 | 0.14   | 0.14             | 0.14              |
| L-Threonine                  | 0.07   | 0.07             | 0.07              |
| Vitamin premix3              | 0.05   | 0.05             | 0.05              |
| Trace mineral premix3        | 0.10   | 0.10             | 0.10              |
| Choline chloride             | 0.07   | 0.06             | 0.06              |
| TBCO4                         | 0.02   | 0.02             | 0.02              |
| Phytase5                      | —      | 0.01             | 0.03              |
| Sand                         | 0.04   | 0.04             | 0.02              |
| Calculated composition, %    |        |                  |                   |
| AME<sub>p</sub>, kcal/kg     | 3.185  | 3.185            | 3.185             |
| Crude protein                | 18.13  | 18.13            | 18.13             |
| Digestible lysine            | 0.98   | 0.98             | 0.98              |
| Digestible sulfur amino acids| 0.74   | 0.74             | 0.74              |
| Digestible threonine         | 0.65   | 0.65             | 0.65              |
| Total phosphorus             | 0.60   | 0.45             | 0.45              |
| Nonphytate phosphorus        | 0.36   | 0.36             | 0.36              |
| Calcium                      | 0.76   | 0.76             | 0.76              |
| Sodium                       | 0.18   | 0.18             | 0.18              |
| Analyzed composition, %      |        |                  |                   |
| Phytase activity, FTU/kg<sup>1</sup> | <50 | 328             | 902              |

Abbreviations: AME<sub>p</sub>, apparent metabolizable energy; NC, negative control; PC, positive control; TBCO (Tri-basic copper chloride).  
<sup>1</sup>One unit of phytase activity (FTU) is defined as the quantity of enzyme required to release 1 µmol of monocalcium phosphate from 0.0051 mol/l sodium phytate in 1 min at pH 5.5 and 37°C.  
<sup>2</sup>Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 18,739 IU; Vitamin D3 (cytocoluprolactate), 66 IU; menadione (menadione sodium bisulfate complex), 4 mg; Vitamin B<sub>12</sub> (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.  
<sup>3</sup>Trace mineral premix include per kg of diet: Mn (manganese sulfate), 120 mg; Zn (manganese sulfate), 100 mg; Fe (iron sulfate monohydrate), 30 mg; Cu (Tri-basic copper chloride), 8 mg; I (ethylene diamine dihydroiodide), 1.4 mg; and Se (sodium selenite), 0.3 mg.  
<sup>4</sup>TBCO (Intellibond C, Micronutrients, Indianapolis, IN).  
<sup>5</sup>Quantum Blue, AB Vista, Marlborough, UK (Analyzed as 4,000 FTU/g).
The consumption of experimental diets during the 8-h experimental period was 66, 72, and 70 g for treatments 1, 2, and 3, respectively (P value = 0.82, pooled standard error of the mean = 13). According to the primary breeder recommendations, daily feed intake of broilers at 28 d of age is 150 g per bird (Aviagen, 2016). Therefore, feed intakes of broilers during the 15-h recovery period and 8-h experimental period were adequate.

In the gizzard contents, concentrations of IP6 to IP3 and inositol of broilers were not different (P > 0.05) between broilers fed diets with phytase addition at 400 FTU/kg compared with feeding the PC diet (Table 2). However, increasing phytase supplementation to 1,200 FTU/kg reduced (P < 0.001) IP6 concentration by 63% compared with that with the PC diet. Total IP3 to IP6 concentrations by 63% compared with that with the PC diet. No differences (P = 0.11) were observed among dietary treatments on IP5 concentration in the ileal digesta of broilers. Effects of adding 400 FTU/kg phytase on the concentrations of IP3, IP4, and inositol in the ileal digesta of broilers were not different (P > 0.05) from feeding the PC diet. However, supplementing phytase at 1,200 FTU/kg increased (P < 0.05) IP3, IP4, and inositol concentrations by 2.5-, 2.0-, and 3.5-fold, respectively, compared with the PC-fed broilers. Similarly, concentrations of IP4 and inositol in ileal digesta of broilers fed diets supplemented with 1,200 FTU/kg phytase were 2.0- and 2.9-fold higher (P < 0.01), respectively, than those in broilers provided 400 FTU/kg supplemented diet. Phytase supplementations at 400 and 1,200 FTU/kg decreased (P < 0.001) total IP3 to IP6 concentrations by 37 and 64%, respectively, compared with the PC diet.

Phytate degradation in the gizzard is critical as the gizzard is the major site of phytase activity and phytate solubility because of its low pH (Selle et al., 2000; Tamim et al., 2004; Selle and Ravindran, 2007). The solubility of phytate at a low pH allows the interaction of phytase and phytate to cleave the carbon-phosphate bond, thereby reducing antinutritive effects of phytate (Zeller et al., 2015). In the present study, there were no differences in IP3 to IP6 and inositol concentrations between broilers fed the control diets and the diet with 400 FTU/kg addition. Despite no statistical differences, the numerical reduction of total IP3 to IP6 concentrations because of 400-FTU/kg phytase supplementation was 27% of the effect from 1,200 FTU/kg phytase supplementation in the gizzard digesta. In contrast, the

### Table 2. Inositol and inositol phosphate (IP) ester concentrations in experimental diets, gizzard content, and ileal digesta of broilers fed diets formulated to contain 0, 400, and 1,200 phytase unit (FTU)/kg of phytase at 28 d of age1,2

| Experimental diets3 | IP6 | IP5 | IP4 | IP3 | Inositol | ∑IP6-IP35 |
|---------------------|-----|-----|-----|-----|----------|----------|
| Total dietary concentrations, nmol/g7 |     |     |     |     |          |          |
| Positive control    | 15,762 | 2,895 | 1,200 | 829 | 1,407 | 20,686 |
| Negative control + 400 FTU/kg phytase | 16,769 | 1,408 | 432 | 553 | 1,261 | 19,162 |
| Negative control + 1,200 FTU/kg phytase | 18,444 | 1,850 | 814 | 697 | 1,337 | 21,805 |
| Gizzard content, nmol/g |     |     |     |     |          |          |
| Positive control    | 6,144ab | 741 | 327 | 280bc | 1,071b | 7,493a |
| Negative control + 400 FTU/kg phytase | 4,176b | 876 | 762 | 407bc | 1,360b | 6,221a |
| Negative control + 1,200 FTU/kg phytase | 1,264bc | 293 | 700 | 580bc | 2,709b | 2,837b |
| Pooled standard error | 826 | 188 | 164 | 77 | 285 | 1,020 |
| Ileal digesta, nmol/g |     |     |     |     |          |          |
| Positive control    | 56,456ab | 6,866 | 1,613b | 713b | 4,667b | 65,698a |
| Negative control + 400 FTU/kg phytase | 33,244b | 4,907 | 2,257b | 958b | 5,617b | 41,301b |
| Negative control + 1,200 FTU/kg phytase | 13,472b | 3,864 | 4,518b | 1,590b | 16,485b | 23,444b |
| Pooled standard error | 5,237 | 1,034 | 626 | 206 | 1,789 | 6,395 |

a,bMeans not sharing a common superscript within column in each section differ significantly (P < 0.05).

1Values represent least-square means of 8 replicate cages with 1 bird per replicate at placement.

2One unit of phytase activity (FTU) is defined as the quantity of enzyme required to release 1 μmol of monocalcium phosphate from 0.0051 mol/l sodium phytate in 1 min at pH 5.5 and 37°C.

3The positive control diet was formulated to contain adequate calcium and phosphorus concentrations. The negative control diet was formulated to contain 0.165 and 0.150% lower calcium and phosphorus concentrations, respectively, than the positive control diet.

4Total amount of IP3 to IP6 concentrations.

5Analyzed concentrations of inositol and inositol phosphate esters in experimental diets.
reduction of total IP3 to IP6 and the increase of inositol when broilers were fed diets with 1,200 FTU/kg phytase indicated that the 3-fold increase in phytase concentration enhanced phytate hydrolysis. In the ileal digesta of broilers, both concentrations of phytase effectively increased phytate degradation, which led to increased inositol liberation. These responses have also been reported in several previous publications, which indicated that the addition of phytase at 500, 1,000, and 1,500 FTU/kg from 1 to 21 d of age decreased IP6 and IP5 in the gizzard contents and ileal digesta of broilers (Walk et al., 2014; Beeson et al., 2017).

Despite the reduction of IP6 due to phytase supplementation, there were accumulations of IP3 and IP4 in the gizzard content and the ileal digesta of broilers. Beeson et al. (2017) also noted that feeding broilers with diets increasing in phytase concentrations from 0, 500, and 1,500 FTU/kg resulted in quadratic reductions of IP5 and IP6, but not on IP3 and IP4 in the ileal digesta of 21-day-old broilers. Similarly, Zeller et al. (2015) measured the proportion of IP3, IP4, and IP5 to the total IP3 to IP5 concentrations in the ileum of broilers fed diets containing added monocalcium phosphate with 0, 500, and 12,500 FTU/kg phytase. These authors observed that when broilers were fed diets with 500 FTU/kg phytase, the greatest accumulation of inositol phosphate ester was IP5, which accounted for 62% of the total IP3 to IP5 concentrations. In contrast, the proportion of IP4 in the ileal digesta was the greatest (39% of the total IP3 to IP5 concentrations) among IP3, IP4, and IP5 when broilers were supplemented with 12,500 FTU/kg phytase. In the present study, the supplementation of 400 FTU/kg phytase resulted in IP5 having the greatest proportion (60%) among the total IP3 to IP5 concentrations. Conversely, providing broilers with 1,200 FTU/kg phytase led to the greatest accumulation of IP4 (45%) among the total IP3 to IP5 concentrations. The accumulation of IP4 even with the high phytase dose may indicate that phytase has higher affinity to phytate esters with higher number of phosphate groups. Wyss et al. (1999) suggested that lower phytate esters are less-effective substrates for phytases derived from Aspergillus niger, A. terreus, and E. coli. It is possible that these phytases may have a higher $K_m$ which indicates a lower affinity to lower inositol phosphate esters. As the phytase in the present study was an E. coli–derived phytase, the accumulation of IP4 from phytate hydrolysis may be expected.

The supplementation of 1,200 FTU/kg phytase reduced total IP3 to IP6 concentrations to approximately one-third of the concentrations in the gizzard content and the ileal digesta of broilers fed the PC diet. These data indicated that antinutritive effects of phytate may still be present and that it may require a higher dose of phytase supplementation to further breakdown phytate. Persson et al. (1998) reported that lower inositol phosphate esters also have the capacity to bind to metal ions, although it may be reduced compared with those having a higher number of phosphate groups. For example, an individual IP6 molecule could bind 5.8 and 4.9 copper and zinc ions, respectively, while an individual IP3 molecule could bind 3.1 and 3.0 copper and zinc ions, respectively. Regardless of the number of ions, the ability of these phytate molecules to bind mineral ions may decrease their availability to broilers leading to poor growth performance. Therefore, it may be necessary to further increase phytase doses to decrease antinutritive effects of phytate. Walk et al. (2018) demonstrated that increasing phytase supplementation from 0, 500, 1,500, and 4,500 FTU/kg linearly decreased total IP2 to IP6 concentrations in the ileal digesta of broilers resulting in a linear increase in body weight gain demonstrating the benefits of higher doses of phytase to further degrade phytate.

The addition of phytase at either 400 or 1,200 FTU/kg in broiler diets did not influence intestinal ALP concentration in the duodenum, jejunum, and ileum (Table 3). In the present study, ALP was measured in the small intestine as it may also contribute to phytate degradation. Despite the increase in phytate degradation and inositol liberation due to increasing phytase concentrations, intestinal ALP in broilers was not altered. It appears that intestinal ALP had high variability, which may be associated with its broad roles in hydrolyzing phosphate esters. In addition to being present in phytate, phosphate esters can also be found in the lipopolysaccharide of gram-negative bacteria. Melo et al. (2016) indicated that ALP might also involve in dephosphorylating lipopolysaccharide of bacteria, which suppresses inflammation caused by bacteria. In addition, ALP may contribute to hydrolyzing ATP along the small intestine (Zhou et al., 2017). These broad functions of intestinal ALP may be a contributing factor causing the high variability with intestinal ALP concentrations. Hence, intestinal ALP may not be a suitable indicator of phytate degradation in broilers.

In addition, ALP concentration was also evaluated in the plasma of broilers. The baseline plasma ALP concentration of broilers before feeding experimental diets was highly variable ($P < 0.001$) (Figure 1). After the introduction of experimental diets, plasma ALP concentration did not differ ($P = 0.45$) among dietary treatments at any of the collection periods. However, effects of time from 20 to 240 min displayed a cubic reduction ($P = 0.007$) of plasma ALP concentration of broilers. In addition, plasma ALP concentration of broilers was not altered ($P = 0.94$) among dietary treatments between 240 and 480 min. ALP is an enzyme primarily responsible for bone mineralization. Owing to its function, ALP concentration in blood circulation can be altered by dietary calcium and phosphorus. For example, plasma ALP concentration in broilers decreased by 5% when increasing dietary nonphytate phosphorus from 4.5 to 5.5 g/kg in a 21-d experimental period (Baradaran et al., 2017). Furthermore, the inclusion of 500 FTU/kg phytase in broiler diet decreased plasma ALP concentration by 24% compared with broilers fed the control diet, indicating a reduction of ALP as mineral availability increases (Huff et al., 1998). In contrast, the measurement of plasma ALP
concentration over time in the present study did not differ among dietary treatments. Cowieson et al. (2017) did not observe effects of feeding diets supplemented with various phytase concentrations on plasma ALP concentration in pigs over 6 h. These researchers attributed the lack of differences in plasma phosphorus and calcium, which may take longer than 6 h.

Plasma inositol concentration of broilers did not vary ($P = 0.94$) at each collection time despite receiving different dietary treatments (Figure 2). Similarly, no dietary treatment effects ($P = 0.37$) were observed on the concentration of plasma inositol in broilers. However, a cubic increase ($P = 0.003$) of plasma inositol concentration from 20 to 240 min was noted regardless of dietary treatments. From 240 to 480 min, there was a 12% reduction from 251 to 222 nmol/mL ($P = 0.019$) of plasma inositol concentration regardless of dietary treatment ($P = 0.30$).

Interestingly, despite the increase of inositol concentration in the gizzard content and the ileal digesta of broilers due to increasing phytase supplementation, no differences of inositol concentrations among dietary treatments were observed in the plasma of broilers over the time course of this study. In contrast, Cowieson et al. (2015) noted an increase in plasma inositol concentration of broilers with increasing doses of phytase from 0, 1,000, 2,000, and 3,000 FTU/kg. In addition, Sommerfeld et al. (2018) observed an increase of inositol concentration in the ileal digesta of broilers with increasing phytase supplementation from 0, 500, 1,500, and 3,000 FTU/kg. However, the only difference noted in the plasma inositol concentration was a 39% increase when adding 500 FTU/kg phytase compared with the control diet, while no further increase of plasma inositol was noted when increasing phytase to 1,500 and 3,000 FTU/kg.

In both studies (Cowieson et al., 2015; Sommerfeld et al., 2018), plasma inositol concentration was measured after feeding diets with phytase addition for 22 d. In the present study, plasma inositol was measured within 8 h after feeding phytase supplemented diets; hence, it is possible that changes in the plasma inositol concentration may require feeding phytase beyond 8 h. Walk et al. (2018) demonstrated that inositol liberated from phytate degradation may accumulate in the terminal ileum of ileum. This accumulation may be an

### Table 3. Intestinal alkaline phosphatase concentrations of broilers fed diets formulated to contain 0, 400, and 1,200 phytase unit (FTU)/kg of phytase at 28 d of age (ng/mL)$^{1,2}$

| Experimental diets$^3$ | Duodenum | Jejunum | Ileum |
|------------------------|-----------|---------|-------|
| Positive control       | 8.42      | 33.15   | 87.35 |
| Negative control + 400 FTU/kg | 7.53   | 35.39   | 107.39 |
| Negative control + 1,200 FTU/kg | 12.74 | 36.52   | 102.37 |
| Pooled standard error  | 2.40      | 8.13    | 16.84 |
| $P$ value              | 0.18      | 0.88    | 0.41  |

$^{1}$Values represent least-square means of 8 replicate cages with 1 bird per replicate at placement.

$^{2}$One unit of phytase activity (FTU) is defined as the quantity of enzyme required to release 1 nmol of monocalcium phosphate from 0.0051 mol/l sodium phytate in 1 min at pH 5.5 and 37°C.

$^{3}$The positive control diet was formulated to contain adequate calcium and phosphorus concentrations. The negative control diet was formulated to contain 0.165 and 0.150% lower calcium and phosphorus concentrations, respectively, than the positive control diet.

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**Figure 1.** Effects of dietary treatments on plasma alkaline phosphatase concentration of broilers at 28 d of age (8 birds per treatment). The positive control diet (---) was formulated to contain adequate calcium and nonphytate phosphorus. A negative control diet was formulated to contain 0.165 and 0.150% lower calcium and nonphytate phosphorus concentrations, respectively. Phytase was added to the negative control diet at 400 (•••) and 1,200 (---) FTU/kg to establish 2 other dietary treatments. Baseline concentration of plasma alkaline phosphatase concentration at 0 min was used as a covariate. Linear, quadratic, and cubic effects over time from 20 to 240 min were analyzed using 2-way repeated measure analysis of variance with dietary treatment and time as factors.
indication of limited absorption in the small intestine depending on the availability of sodium or proton. In contrast, when phytase is provided for 21 d, broilers exhibited higher expressions of sodium/glucose cotransporter 11 and H^+ /myo-inositol transporter than birds consuming diets without phytase supplementation (Walk et al., 2018). The increase of gene expression of inositol transporters may be the factor that

Figure 2. Effects of dietary treatments on plasma inositol concentration of broilers at 28 d of age (8 birds per treatment). The positive control diet (---) was formulated to contain adequate calcium and nonphytate phosphorus. A negative control diet was formulated to contain 0.165 and 0.150% lower calcium and nonphytate phosphorus concentrations, respectively. Phytase was added to the negative control diet at 400 (----) and 1,200 (-----) FTU/kg to establish 2 other dietary treatments. Baseline concentration of plasma inositol concentration at 0 min was used as a covariate. Linear, quadratic, and cubic effects over time from 20 to 240 min were analyzed using 2-way repeated measure analysis of variance with dietary treatment and time as factors.

Figure 3. Effects of dietary treatments on plasma glucose concentration of broilers at 28 d of age (8 birds per treatment). The positive control diet (---) was formulated to contain adequate calcium and nonphytate phosphorus. A negative control diet was formulated to contain 0.165 and 0.150% lower calcium and nonphytate phosphorus concentrations, respectively. Phytase was added to the negative control diet at 400 (----) and 1,200 (-----) FTU/kg to establish 2 other dietary treatments. Baseline concentration of plasma glucose concentration at 0 min was used as a covariate. Linear, quadratic, and cubic effects over time from 20 to 240 min were analyzed using 2-way repeated measure analysis of variance with dietary treatment and time as factors.
caused an increase in plasma inositol concentration when birds were fed increasing phytase doses beyond an 8-h period.

The lack of response in plasma inositol concentrations among dietary treatments is also in contrast with a previous time-series study in pigs. Cowieson et al. (2017) fed pigs (average body weight of 28.7 kg) with diets containing 0, 1,000, and 3,000 FTU/kg phytase as well as 0.2% inositol. In this study, plasma inositol of pigs was measured at 30 min before feeding experimental diets, then at 0, 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, and 360 min after feeding experimental diets. These researchers indicated that plasma inositol concentration varies over time because of experimental diets with maximum concentration reached at 120 min after pigs were fed diets supplemented with inositol. However, at 360 min after feeding experimental diets, the greatest plasma inositol concentration was obtained by feeding diets with 3,000 FTU/kg phytase. The differences between the study by Cowieson et al. (2017) and the present study may likely be associated with species differences in the utilization of inositol after absorption. Avian hemoglobin has been reported to use inositol as a precursor of IP5 for modulating the oxygen affinity of hemoglobin in the red blood cells, while in mammals, this process in facilitated by 2,3-diphosphoglycerate (Lutz, 1980). An in vitro study using 5-day-old chicken plasma incubated with inositol for 6 h demonstrated the increase of inositol uptake by the erythrocyte and inositol incorporation to IP5 with increasing incubation time (Isaacks et al., 1982). These mechanisms led to a reduction of plasma-free inositol from 14.6 to 10.7 μg/mL within 6 h of incubation period. In the present study, it is possible that inositol may be rapidly rephosphorylated after absorption to synthesize IP5, which may explain the reason for the lack of differences in plasma inositol concentration of broilers.

Another possible reason for the lack of response to dietary treatments on plasma inositol concentrations in the present study may be related to the rapid metabolism of inositol. After absorption, inositol may be used for the formation of phosphatidylinositol. The 2 major phosphatidylinositols in the cell membrane are phosphatidylinositol 4,5-bisphosphate and phosphatidylinositol 3,4,5-triphosphate, which have function in cell growth, differentiation, proliferation, and calcium mobilization (Cooper and Hausman, 2013). In intestinal epithelial cells, inositol may be important to synthesize phosphatidylinositol because of the rapid turnover of intestinal epithelial cells (Moran, Jr., 2016). A previous study indicated that the mucosa of the jejunum is completely replaced within approximately 48 h in 2-day-old chicks (Imondi and Bird, 1966) indicating a rapid turnover of intestinal epithelial cells and the need for phosphatidylinositol formation. Moreover, inositol may be metabolized in the liver after absorption in the small intestine. An in vitro study using rat liver cells demonstrated that when liver cells were administered with increasing concentrations of inositol, both inositol uptake and phosphatidylinositol synthesis increased (Prpic et al., 1982). Therefore, in the present study, the increase of inositol liberation due to higher phytase concentrations may lead to an increase of hepatic inositol uptake and phosphatidylinositol formation, which results in the lack of response of dietary treatments on plasma inositol concentrations, especially when inositol was measured after hepatic metabolism in the brachial vein of birds.

No interactive ($P = 0.74$) effects between dietary treatments and collection time were noted on plasma glucose concentration of broilers (Figure 3). In contrast, adding 400 FTU/kg (15,045 nmol/mL) resulted in a higher ($P = 0.038$) plasma glucose concentration of broilers than that without phytase (14,422 nmol/mL) and with 1,200 FTU/kg (14,337 nmol/mL) addition regardless of the collection time. Furthermore, plasma glucose concentration increased ($P = 0.004$) in a cubic manner from 20 to 240 min after feeding experimental diets. There was a 6.8% reduction ($P < 0.001$) in plasma glucose from 240 (15,528 nmol/mL) to 480 min (14,478 nmol/mL) collection time regardless of the dietary treatments. Phytase addition in broiler diets may affect glucose concentration in the plasma through the reduction of endogenous sodium loss. A previous study indicated that an increase in dietary phytate concentration causes an increase in endogenous sodium losses, while the addition of phytase decreases endogenous sodium loss (Cowieson et al., 2004). Because the majority of glucose absorption in the small intestine is facilitated with the sodium-dependent active transport system, the increase in the availability of sodium by phytase addition may also increase glucose concentration in the plasma (Chen et al., 2016). Recent research indicated that the supplementation of phytase at 500 FTU/kg in broiler diets increased blood glucose concentration by 6.7% compared with diets without phytase addition (Cowieson et al., 2013). In the present study, the addition of 400 FTU/kg also increased plasma glucose concentration of broilers by 4.3% regardless of the collection time compared with those fed diets without phytase addition. Interestingly, when phytase supplementation was increased to 1,200 FTU/kg, no difference in plasma glucose concentration was observed compared with the control-fed birds, and this response is not readily explained. However, it is possible that the greater release of inositol by phytase addition at 1,200 FTU/kg inhibited intestinal glucose uptake more than feeding phytase at 400 FTU/kg. This condition occurred as both inositol and glucose may compete for the same transporter (Bevilacqua and Bizzarri, 2018). A previous study in mice demonstrated that increasing supplementation of dietary inositol inhibited intestinal glucose uptake and decreased blood glucose concentration (Chukwuma et al., 2016). Unfortunately, because plasma inositol concentration was not influenced by dietary treatments, it is difficult to determine the exact mechanisms underlying the influence of phytase on plasma glucose concentration at 400 but not at 1,200 FTU. Therefore, the effects of phytase on blood glucose concentration may require further investigation because
of the variable results reported in the literature and the current research. These data demonstrated benefits of adding phytase at 1,200 FTU/kg as it effectively increased phytate degradation and inositol liberation in the gizzard and small intestine of broilers compared with the addition of 400 FTU/kg. Despite the reduction of phytate concentrations, one-third of the total IP3 to IP6 concentrations remained in the small intestine even with the addition of 1,200 FTU/kg suggesting the need for supplementing higher doses of phytase to further degrade phytate. Although increasing phytase concentrations increased inositol concentrations in the gizzard and ileum of broilers, phytase doses did not alter plasma inositol concentrations over time. Future research is warranted to investigate plasma inositol as a biomarker for phytase efficacy possibly by increasing phytase concentrations beyond 1,200 FTU/kg and measuring plasma inositol concentration of broilers beyond an 8-h period after feeding phytase supplemented diets.

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DISCLOSURES

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

REFERENCES

Aviagen. 2016. Ross 708 Broiler: Nutrient Specifications. Aviagen, Huntsville, AL.
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. 96:2234–2253.
Baradaran, N., M. H. Shahir, and Z. A. Kermani. 2017. Subsequent bone and metabolic responses of broilers to high non-phytase phosphorus diets in the starter period. Br. Poult. Sci. 58:435–441.
Bevilacqua, A., and M. Bizzarri. 2018. Inositol in insulin signaling and glucose metabolism. Int. J. Endocrinol. 2018:1968450.
Chen, L., B. Tuo, and H. Dong. 2016. Regulation of intestinal glucose absorption by ion channels and transporters. Nutrients 8:43–53.
Chukwuma, C. I., M. A. Ibrahim, and S. Islam. 2016. Myo-inositol inhibits intestinal glucose absorption and promotes muscle glucose uptake: a dual approach study. J. Physiol. Biochem. 72:791–801.
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., A. Ptak, P. Mackowiak, M. Sassek, E. Pruszynska-Osmalek, K. Zyla, S. Swiatkiewicz, S. Kaczmarek, and D. Jozef. 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.
Cowieson, A. J., F. F. Roos, J. Ruckebusch, J. W. Wilson, P. Guggenbuhl, H. Lu, K. M. Ajunwaon, and O. Adeola. 2017. Time-series responses of swine plasma metabolites to ingestion of diets containing myo-inositol or phytase. Br. J. Nutr. 118:897–905.
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., T. Acamovic, and M. R. Bedford. 2004. The effects of phytase and phytic acid on the loss of endogenous amino acids and minerals from broiler chickens. Br. Poult. Sci. 45:101–108.
Croze, M. L., and C. O. Soulage. 2013. Potential role and therapeutic interests of myo-inositol in metabolic diseases. Biochimie 95:1811–1827.
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.
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.
Huff, W. E., P.A. Moore, Jr, P. W. Waldroup, A. L. Waldroup, J. M. Balog, G. R. Huff, M. C. Rath, T. C. Daniel, and V. Raboy. 1998. Effect of dietary phytase and high available phosphorus corn on broiler chicken performance. Poult. Sci. 77:1899–1904.
Imondi, A. R., and F. H. Bird. 1966. The turnover of intestinal epithelium in the chick. Poult. Sci. 45:142–147.
Isaacks, R. E., C. Y. Kim, A.E. Johnson, Jr, P. H. Goldman, and D. R. Harkness. 1982. Studies on avian erythrocyte metabolism. XII. The synthesis and degradation of inositol pentakis (dihydrophosphate). Poult. Sci. 61:2271–2281.
Kluth, H., K. Mahlhorn, and M. Rodehutscord. 2005. Studies on the intestine section to be sampled in broiler studies on precaecal amino acid digestibility. Arch. Anim. Nutr. 59:271–279.
Laird, S., L. Kuhn, P. Wilcock, and H. M. Miller. 2016. The effects of phytase on grower pig growth performance and ileal inositol phosphate degradation. J. Anim. Sci. 94:142–145.
Lutz, P. L. 1980. On the oxygen affinity of bird blood. Amer. Zool. 20:187–198.
Melo, A. D. B., H. Silveira, F. B. Luciano, C. Andrade, L. B. Costa, and M. H. Rostagno. 2016. Intestinal alkaline phosphatase: potential roles in promoting gut health in weanling piglets and its modulation by feed additives – a review. Asian-australas. J. Anim. Sci. 29:16–22.
Moran, Jr, E.T. 2016. Gastric digestion of protein through pancreozyme action optimizes intestinal forms for absorption, mucin formation, and villus integrity. Anim. Feed Sci. Technol. 221:284–303.
Persson, H., M. Turk, M. Nyman, and A. S. Sandberg. 1998. Binding of Cu2+, Zn2+, and Cd2+ to inositol tri-, teta-, penta-, and hexaphosphates. J. Agric. Food Chem. 46:3194–3200.
Prpic, V., P. F. Blackmore, and J. H. Exton. 1982. Myo-inositol uptake and metabolism in isolated rat liver cells. J. Biol. Chem. 257:11315–11322.
Rodehutscord, M., A. Dieckmann, M. Witzig, and Y. Shastak. 2012. A note on sampling digesta from the ileum of broilers in phosphorus digestibility studies. Poult. Sci. 91:965–971.
SAS Institute Inc 2011. SAS User’s Guide: Statistics. Version 9, 4 ed. SAS Inst. Inc., Cary, NC.
Schumann, G., R. Kluwe, F. Canalias, S. Bossert-Renther, P. F. H. Franck, F. J. Gella, P. J. Jorgensen, D. Kang, J.M. Lessinger, M. Panteghini, and F. Ceriotti. 2011. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37°C. Part 9: Reference procedure for the measurement of catalytic concentration of alkaline phosphatase. Clin. Chem. Lab. Med. 49:1439–1446.
Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Technol. 135:1–41.
Selle, P. H., V. Ravindran, R. A. Caldwell, and W. L. Bryden. 2000. Phytate and phytase: Consequence for protein utilization. Nutr. Res. Rev. 13:255–278.
Simonov, 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. 2018. Influence of phytase or myo-inositol supplements on performance and phytate degradation products in the crop, ileum, and blood of broiler chickens. Poult. Sci. 97:920–929.
Tamim, N. M., R. Angel, and M. Christman. 2004. Influence of dietary calcium and phytase on phytate phosphorus hydrolysis in broiler chickens. Poult. Sci. 83:1358–1367.

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., 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.

Wyss, M., R. Brugger, A. Kronenberger, R. Remy, R. Fimbel, G. Oesterhelt, M. Lehmann, and A. P. G. M. van Loon. 1999. Biochemical characterization of fungal phytases (myo-inositol hexakisphosphate phosphohydrolases): catalytic properties. Appl. Environ. Microbiol. 65:367–373.

Zeller, E., M. Schollenberger, M. Witzig, Y. Shastak, I. Kuhn, L. E. Hoelzle, and M. Rodehutscord. 2015. Interactions between supplemented mineral phosphorus and phytase on phytate hydrolysis and inositol phosphates in the small intestine of broilers. Poult. Sci. 94:1018–1029.

Zhou, K., N. Burello, W. Wang, T. Archbold, H. Leung, E. Kiarie, and M. Z. Fan. 2017. Broiler chickens express differential alkaline phosphatase activity and enzyme affinity in hydrolyzing ATP along the small intestinal longitudinal axis. J. Anim. Sci. 95(Suppl. 4):227 (Abstr.).