The effect of phytase and fructooligosaccharide supplementation on growth performance, bone quality, and phosphorus utilization in broiler chickens

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ABSTRACT An experiment was conducted to investigate the effects of phytase and 2 levels of fructooligosaccharide (FOS) supplementation on growth performance, bone mineralization, and P utilization of broiler chickens. A total of 210 day-old male broiler chickens (Ross) were randomly placed into 7 dietary treatments consisting of 6 replicates with 5 birds per pen. The experiment was designed as an augmented 2 × 3 factorial arrangement with 0 or 500 U/kg of phytase and 0, 0.5% or 1% of FOS added to a reduced Ca (0.8%) and available P (0.25%) negative control diet (NC). A positive control diet (PC) that contained 1% Ca and 0.45% available P was also included. During the entire experimental period, phytase supplementation significantly improved ($P < 0.05$) the feed conversion ratio (FCR), BW gain (BWG), and feed intake. Birds fed the PC diet showed significantly higher bone mineral density (BMD) and bone mineral content (BMC) in both femur and tibia bones ($P < 0.0001$) than those fed the NC diet. Phytase supplementation increased femur BMD ($P < 0.05$), whereas FOS decreased femur BMD and BMC ($P < 0.05$). Phosphorus utilization was significantly higher for the NC diet ($P < 0.0001$). Phytase alone and in combination with 0.5% FOS increased P utilization significantly when compared with other treatments ($P < 0.05$). Fructooligosaccharides, especially at the level of 0.5%, increased P retention. In conclusion, phytase supplementation in low Ca and P diets improved growth performance, bone quality, and P utilization. However, supplementing NC diets with phytase and FOS did not result in bone mineralization values comparable with that of the PC diet. The application of dietary FOS alone had a negative effect on broiler bone quality.

Key words: phytase, fructooligosaccharide, bone quality, phosphorus utilization, broiler chicken

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INTRODUCTION

Broiler diets based on ingredients of plant origin contain large amounts of unavailable P in the form of phytate-P, which is poorly hydrolyzed by the endogenous enzymes of monogastric animals (Ravindran et al., 1995). Dietary supplementation of inorganic phosphate is often necessary to meet available P requirements for poultry. However, the addition of inorganic P increases the cost of the feed and results in high concentrations of P in the manure. As a consequence, animal waste applied to the soil leads to environmental pollution, allowing excreted P to wash into overland water systems, which causes eutrophication (Boling et al., 2000; McGrath et al., 2005). Supplementation of exogenous phytase in broiler chicken rations has been proven to improve the hydrolysis of phytate-P, increase P digestibility, reduce P excretion into the environment, and lower the cost of inorganic phosphate addition (Nahm, 2002; Knowlton et al., 2004; Coppedge et al., 2011; Powell et al., 2011). Although it is generally recognized that a 0.1% reduction of available P content can be attained with phytase supplementation, several studies suggest that only a 0.05% reduction is achieved with phytase supplementation (Slominski, 2011), indicating that developing additional strategies is necessary to further improve utilization of phytate-P provided by phytase supplementation in poultry diets. Promising results have been observed on the growth performance of broiler chickens by supplementing phytase. For example, Simons et al. (1990) reported that the use of phytase increased bird performance and improved bone mineralization, while El-Sherbiny et al. (2010) examined broiler diets containing a reduced level of dicalcium phosphate and concluded that the addition of 500 U/kg phytase improved body weight gain (**BWG**), feed intake (**FI**), and the feed conversion ratio (**FCR**) of the birds from 23 to 40 d of age.

Fructooligosaccharides (**FOS**) are short-chain nondigestible carbohydrates extracted from plant sources (e.g., chicory root, onion, beet and cane sugar) and are considered to have prebiotic properties (Williams et al.,

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2008; Kim et al., 2011a). Several studies have been conducted to evaluate the effect of FOS supplementation on broiler chickens' growth performance. For example, increased BWG and decreased FCR were reported by Ammerman et al. (1988), Bailey et al. (1991), and Xu et al. (2003). Variations in the levels of FOS supplementation may affect the growth rate and performance parameters of the birds (Yang et al., 2009). However, there is no well-defined recommendation for FOS supplementation in poultry diets.

Bone weakness and skeletal disorders such as tibial dyschondroplasia and rickets are existing problems associated with rapid bone growth in broiler chickens that lead to economic losses and animal welfare issues (Fleming, 2008; Kim et al., 2011b). Improved nutrient utilization and mineral absorption have positive influences on bone development and thus reduce the incidence of leg problems in broiler chicken production (Swiatkiewicz and Arczewska-Wlosek, 2012). The addition of phytase has been demonstrated to have positive effects on bone ash content and bone mineralization in broilers fed low available P diets (Angel et al., 2006; Woyengo et al., 2008; Coppedge et al., 2011). El-Sherbiny et al. (2010) reported that phytase increased dietary Ca and P utilization, reduced Ca and P excretion, and improved tibia breaking strength and tibia ash percentage in broiler chickens. FOS also have the potential ability to increase mineral bioavailability due to their effect on bacterial fermentation in the intestine (Gudiel-Urabano and Goni, 2002; Zafar et al., 2004; Ohta, 2006). Xu et al. (2003) indicated that a diet containing 0.4% FOS had positive effects on intestinal morphology in broilers, which may lead to improved mineral absorption. The growth of probiotic-like bacteria (such as *Bifidobacteria* and *Lactobacilli*) stimulated by FOS supplementation produces short-chain fatty acid (**SCFA**), resulting in acidification of the gastrointestinal tract (**GIT**) (Wang et al., 2010; Bogusławska-Tryk et al., 2012). An acidic pH in gastrointestinal segments, such as gizzard, duodenum, jejunum, and ileum, is favorable for mineral solubility as well as for phytase activity (Selle et al. 2009; Walk et al., 2012). Therefore, dietary FOS could potentially increase phytase efficacy by facilitating phytate hydrolysis and thus improve mineral utilization.

To date, no studies have been conducted examining P utilization and bone mineralization in broiler chickens fed diets supplemented with FOS alone or in combination with phytase. The hypothesis of this study was that FOS supplementation would increase phytase efficacy and the combination of the 2 would act additively to improve growth performance, bone quality, and total P utilization in broiler chickens. The objectives of this study were: 1) to determine the interaction between phytase and FOS on P utilization and skeletal integrity in broiler chickens fed low Ca and available P (reduced by 0.2 percentage points) diets and 2) to examine the optimum FOS inclusion level in broiler rations.

MATERIALS AND METHODS

Birds and Housing

A total of 210 day-old male Ross × Ross 308 chicks were obtained from a local commercial hatchery (Carltons Hatchery, Grunthal, Manitoba, Canada). The chicks were housed in electrically heated Jamesway battery brooders (James Mfg. Co., Mount Joy, PA) for the first 4 d pre-experimental period with the temperature maintained at 32°C. On d 5, birds were individually weighed and sorted into 5 weight classes. Groups of 5 birds, 1 from each weight class, were then randomly assigned to 42 battery pens such that the average initial BW was similar across pens. The chickens were raised to 21 d to obtain data for the duration of starter to grower phase. During the experimental period, birds were housed in 3 electrically heated Alternative Design Super Brooders (Alternative Design Manufacturing & Supply, Inc., Siloam Springs, AR) under a controlled environment. The temperature was monitored daily and was gradually reduced until a temperature of 24°C was reached on d 21. Light was provided for 24 h throughout the experimental period. The experimental protocol was approved by the University of Manitoba Animal Care Protocol Management and Review Committee, and birds were handled in accordance with the guidelines established by the Canadian Council on Animal Care (CCAC, 1993).

Dietary Treatments Seven dietary treatments were randomly assigned to 6 replicate cages of 5 birds each. Composition and analyzed nutrient values of the experiment diets are shown in Table 1. The experiment was designed as an augmented 2 × 3 factorial arrangement with 0 or 500 U/kg phytase (Bio-Phytase 5000G, Canadian Bio-Systems Inc., Calgary, Alberta) and 0, 0.5, or 1% FOS (Nutraflora P-95, Ingredion, Etobicoke, Ontario, Canada) in a low Ca and available P diet. A positive control (**PC**) diet contained adequate levels of Ca and available P. The 7 dietary treatments included PC, wheat-, corn-, soybean meal–based diet containing 1% Ca and 0.45% available P; negative control (**NC**), wheat-, corn-, soybean meal–based diet containing 0.8% Ca and 0.25% available P; NC + phytase; NC + 0.5% FOS; NC + phytase + 0.5% FOS; NC + 1% FOS; and NC + phytase + 1% FOS. As an indigestible marker, 0.3% titanium dioxide (Aldrich-248576, Sigma-Aldrich, Oakville, Ontario, Canada) was incorporated into the diets. The PC diet was fed to all the chickens for the first 4 d adaption period, and the experimental diets were provided from d 5 to 21. Water and feed were allowed ad libitum. The basal diet was formulated to meet or exceed the National Research Council nutrient requirements for broiler chickens (NRC, 1994).

Growth Performance and Sample Collection The BWG and FI for each pen were recorded on d 14 and 21. The FCR was calculated as g feed/g gain. Body weight gain, FI, and FCR were corrected for mortality and were calculated for d 5 to 14, d 15 to 21 and

Table 1. Composition and analysis of experimental diets (as-fed basis).

Item	PC[1]	NC[2]	NC + phytase	NC + 0.5% FOS	NC + phytase + 0.5% FOS	NC + 1% FOS FOS	NC + phytase + 1% FOS
Ingredient (% of diet)							
FOS[3]	–	–	–	0.5	0.5	1	1
Phytase (U/kg)[4]	–	–	500	–	500	–	500
Wheat	35.80	36.00	36.00	35.26	35.26	35.10	35.10
Corn	29.78	31.45	31.45	31.74	31.74	31.28	31.28
Soybean meal	20.46	19.36	19.36	19.58	19.58	19.52	19.52
Canola meal	4.25	5.00	5.00	4.72	4.72	4.70	4.70
Canola oil	4.50	4.00	4.00	4.00	4.00	4.20	4.20
Limestone	1.38	1.46	1.46	1.46	1.46	1.46	1.46
Dicalcium phosphate	1.76	0.65	0.65	0.65	0.65	0.65	0.65
DL-metionine	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-lysine HCl	0.12	0.14	0.14	0.14	0.14	0.14	0.14
Threoninie	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Mineral premix[5]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[6]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Titanium dioxide[7]	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Calculated composition[8]							
ME (kcal/kg)	3,111	3,119	3,119	3,105	3,105	3,101	3,101
CP (%)	21.3	21.3	21.3	21.2	21.2	21.1	21.1
Ca (%)	1.00	0.80	0.80	0.80	0.80	0.80	0.80
Available P (%)	0.45	0.25	0.25	0.25	0.25	0.25	0.25
Met + Cys (%)	0.97	0.98	0.98	0.98	0.98	0.97	0.97
Met (%)	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Lys (%)	1.08	1.08	1.08	1.08	1.08	1.08	1.08
Thr (%)	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Analyzed composition							
CP (%)	21.0	20.7	20.7	20.6	20.6	20.8	20.8
Ca (%)	1.24	0.98	0.98	0.95	0.95	0.90	0.90
Total P (%)	0.73	0.54	0.54	0.56	0.56	0.53	0.53
Available P (%)	0.45	0.23	0.23	0.26	0.26	0.23	0.23
Phytase activity (U/kg)	42	65	544	107	545	99	539
DM (%)	90.3	89.3	89.4	89.3	89.3	89.1	89.6

[1]PC: Positive control, wheat-, corn-, and soybean meal–based diet containing adequate Ca and available P (1% Ca and 0.45% available P).

[2]NC: Negative control, wheat-, corn-, and soybean meal–based diet containing low Ca and available P (0.8% Ca and 0.25% available P).

[3]Nutraflora P-95, Short-Chain Fructooligosaccharides (scFOS), contains 4.5% sugar (fructose + glucose + sucrose), 34.2% GF_2 (glucose + 2 molecules fructose), 48.9% GF_3 (glucose + 3 molecules fructose), and 12.4% GF_4 (glucose + 4 molecules fructose) on DM basis (Ingredion, Etobicoke, ON, Canada).

[4]Bio-Phytase 5000G (Canadian Bio-Systems Inc., Calgary, AB, Canada) wheat was used as a carrier for 0 or 500 U/kg phytase in diets to equal 100%.

[5]Supplied per kilogram of diet: Mn, 70 mg; Zn, 80 mg; Fe, 80 mg; Cu, 10 mg; Se, 0.3 mg; I, 0.5 mg; and NaCl, 4.3 g.

[6]Supplied per kilogram of diet: vitamin A, 8,250 IU; vitamin D_3, 3,000 IU; vitamin E, 30 IU; vitamin B_{12}, 0.013 mg; vitamin K, 2 mg; riboflavin, 6 mg; pantothenic acid, 11 mg; niacin, 41.6 mg; choline, 1,300.8 mg; folic acid, 4 mg; biotin, 0.25 mg; pyridoxine, 4 mg; thiamine, 4 mg; endox (antiox), 125 mg; DL-methionine, 500 mg; virginiamycin (Stafac-22), 11mg; and monensin sodium (Coban), 99 mg.

[7]Aldrich- 248576 (Sigma-Aldrich Co. LLC, ON, Canada).

[8]Concentrations were calculated based on NRC (1994) guidelines.

the entire experimental period. The production index (**PI**) was calculated for the total experimental period using the following equation (Swiatkiewicz et al., 2011): PI = [BW (kg) × survival (%)/age × FCR (kg)] × 100.

On d 21, a total of 42 birds (one bird from each pen; 6 birds per treatment) were euthanized by cervical dislocation. Individual BW was recorded from all sacrificed birds. Left femur and tibia bones were collected for the analysis of bone mineralization parameters. The bones were cleaned of the attached tissue, wrapped in 1× phosphate buffered saline–soaked cheesecloth, and stored at –20°C. Excreta samples from each pen were collected for 3 h and immediately stored at –20°C. Care was taken during collection to avoid contamination from feathers, feed, and foreign materials. The excreta samples were then frozen and freeze-dried (VirTis 25LL freeze-dryer, VirTis Co. Inc., Gardiner, NY) for P analysis. Ileal tissue samples were collected and snap freezed at –80°C for the determination of mineral transporters expression, including calcineurin-like EF hand protein (**CHP**); transient receptor potential cation channel, subfamily V 6 (**TRPV6**); and phosphate transporter solute carrier family 20 (**SLC20A**) by quantitative real-time PCR (qRT-PCR).

Dual Energy X-Ray Absorptiometry

Bone mineral density (**BMD**), bone mineral content (**BMC**), and bone area (**BA**) of the femur and tibia bones were measured using a dual energy x-ray absorptiometry (pDEXA, Norland Medical System, Inc. Fort Atkinson, WI). Quality assurance calibration was performed each time before scanning. The femur and tibia bones were placed in a standardized orientation in each scan. The detected BMD was normalized to

a 2-dimensional bone area instead of a true volume, which represented a combination of bone thickness and density, and was expressed as g/cm^2 (Schreiweis et al., 2005; Kim et al., 2012). All scans were obtained at a scout speed of 40 mm/sec and at a measure speed of 20 mm/sec, with the resolution of 1.0 mm × 1.0 mm. The bone mineralization parameters were corrected by total individual BW as a covariance.

Chemical Analysis

Experimental diets and excreta samples were finely ground and thoroughly mixed using a coffee grinder (CBG5 SmartGrind; Applica Consumer Products Inc., Shelton, CT). Dry matter of the diets and excreta samples was determined using the 934.01 method of AOAC (1990). The diet samples' CP (N × 6.25) levels of were determined using a nitrogen analyzer (NS-2000, Leco Corp., St. Joseph, MI). Samples for the analysis of Ca and total P were ashed at 600°C for 12 h in a muffle furnace and digested in 1% HNO_3 and 5 N HCl according to AOAC (1990) method 990.08. Calcium and total P concentrations were measured using an inductively coupled plasma optical emission spectrometer (AES Vista, Varian Inc., Palo Alto, CA). Phytate P in the diet was determined as described by Haug and Lantzsch (1983). Available P was calculated as total P minus phytate P. Phytase activity was determined according to Slominski et al. (2007). The measurements of TiO_2 in the diets and excreta samples were carried out according to the method of Lomer et al. (2000), and titanium dioxide (**TiO_2**) levels were determined using the Varian inductively coupled plasma optical emission spectrometer. Diet and excreta TiO_2 and total P values were used to calculate apparent P digestibility (**APD**) using the following equation: APD (%) = 100 − [(TiO_{2Diet}/$TiO_{2Excreta}$) × (total $P_{Excreta}$/total P_{Diet}) × 100]. The retained and excreted P (% of diet) was calculated based on APD and expressed as actual total P content of the diet. P excretion was also calculated as g/bird of total P consumed and g/kg of P in excreta (DM basis).

Statistical Analysis

All data were subjected to one-way ANOVA as a completely randomized design using the GLM procedure of SAS (SAS software release 9.1, SAS Institute Inc. Cary, NC). A set of preplanned orthogonal contrasts was applied to analyze the difference between PC and NC treatments and to determine the main effect of phytase and FOS as well as their interaction (Marini, 2003). Treatment means were compared using Duncan's multiple-range test. Differences were considered significant at $P < 0.05$. Least square means were separated using SAS macro pdglm800 (Saxton, 1998).

RESULTS

Diets and Growth Performance

The analyzed diet compositions of P, Ca, and phytase activity of the 7 dietary treatments are listed in Table 1. All values were within acceptable ranges and are in agreement with calculated compositions. Small amounts of endogenous phytase activities were observed in all diets, which is because phytase is naturally present in some feedstuffs (e.g., wheat).

During 5 to 14 d of age, no statistically significant effects on BWG or FI were observed for all treatments, which indicated that the 2 dietary supplements and the application of reduced levels of Ca and available P did not significantly impact birds' growth and feed consumption at an early age (Table 2). Although there were no interactions between phytase and FOS, the NC + phytase diet showed improved FCR when compared with the NC, NC + 0.5% FOS, and NC + 1% FOS diets.

From 15 to 21 d of age, there were no interactions between phytase and FOS. However, phytase supplementation significantly increased BWG ($P < 0.05$) and FI ($P < 0.05$) of birds among the NC treatments (Table 3). The results of the entire experimental period (d 5 to 21) showed that phytase supplementation increased BWG ($P < 0.05$) and decreased FCR ($P < 0.05$). In addition, treatments supplemented with a combination of phytase and 1% FOS showed an increase in BWG ($P < 0.05$) and FI ($P < 0.05$) compared to the NC diet containing 0.5% FOS and the NC diet, respectively, during the 15 to 21 d period (Table 3) and the entire experiment period (Table 4). The PI (Table 4) calculated for the entire experimental period, and when taking into account mortality, age, BW, and FCR values, showed no statistical difference among the treatments. Growth performance of broilers fed NC diets did not differ from that of the PC, which in part may have resulted from the application of both FOS and phytase. Similarly, the 2 levels of FOS used in this study showed no significant difference in the birds' growth performance parameters. No interaction was detected between FOS and phytase in the NC diets on growth performance parameters.

Bone Quality

There were no interactions between phytase and FOS on bone quality. The results of the mineralization parameters of femur and tibia bones showed great differences between the PC and NC treatments (Table 5). Birds had significantly higher BMD and BMC ($P < 0.0001$) in the standard Ca and available P diet (PC) when compared with the low Ca and available P NC diets. These results suggest that reducing Ca and available P 0.2 percentage points from the NRC recommended level was sufficient to reduce bone quality, but the supplemented diets failed to bring up bone

Table 2. Effect of phytase and fructooligosaccharides (FOS) supplementation on growth performance of broiler chickens from 5 to 14 d of age[1].

Item	Body weight gain (BWG; g/bird)	Feed intake (FI; g/bird)	Feed conversion ratio (FCR; g feed/g gain)
Diet			
PC[2]	290.9	380.1	1.31[a,b]
NC[3]	272.9	364.7	1.34[a]
NC + phytase[4]	291.5	369.7	1.27[b]
NC + 0.5% FOS	290.0	389.2	1.34[a]
NC + phytase + 0.5% FOS	283.1	374.4	1.33[a,b]
NC + 1% FOS	277.2	370.5	1.34[a]
NC + phytase + 1% FOS	294.3	384.2	1.31[a,b]
SEM	2.90	3.49	0.008
Contrasts			
PC vs. NC	0.472	0.657	0.574
Phytase + FOS	0.511	0.566	0.918
Effects among NC			
Phytase	0.130	0.863	0.029
FOS	0.828	0.280	0.294
Phytase × FOS	0.177	0.309	0.447
FOS 0.5% vs. 1%	0.912	0.641	0.598

[1]Means of 6 replicate pens of 5 birds each.

[2]PC: Positive control, adequate Ca and available P (1% Ca and 0.45% available P).

[3]NC: Negative control, low Ca and available P (0.8% Ca and 0.25% available P).

[4]Provided 500 U/kg of diet.

[a–b]Means with different superscripts within a column differ significantly ($P < 0.05$).

Table 3. Effect of phytase and fructooligosaccharides (FOS) supplementation on growth performance of broiler chickens during 15 to 21 d of age[1].

Item	Body weight gain (BWG; g/bird)	Feed intake (FI; g/bird)	Feed conversion ratio (FCR; g feed/g gain)
Diet			
PC[2]	391.3[a]	570.1[a,b]	1.46
NC[3]	350.1[a,b]	518.3[b]	1.48
NC + phytase[4]	368.4[a,b]	552.0[a,b]	1.50
NC + 0.5% FOS	323.2[b]	526.6[a,b]	1.69
NC + phytase + 0.5% FOS	377.2[a,b]	549.7[a,b]	1.46
NC + 1% FOS	347.1[a,b]	548.2[a,b]	1.62
NC + phytase + 1% FOS	395.2[a]	580.4[a]	1.47
SEM	7.43	6.97	0.033
Contrasts			
PC vs. NC	0.134	0.224	0.400
Phytase + FOS	0.056	0.144	0.224
Effects among NC			
Phytase	0.012	0.048	0.090
FOS	0.530	0.218	0.628
Phytase × FOS	0.575	0.947	0.324
FOS 0.5% vs. 1%	0.265	0.152	0.752

[1]Means of 6 replicate pens of 5 birds each.

[2]PC: Positive control, adequate Ca and available P (1% Ca and 0.45% available P).

[3]NC: Negative control, low Ca and available P (0.8% Ca and 0.25% available P).

[4]Provided 500 U/kg of diet.

[a–b]Means with different superscripts within a column differ significantly ($P < 0.05$).

mineralization to the same values as that found in birds fed the PC diet. However, the addition of phytase improved femur BMD among the NC diets ($P < 0.05$). The NC diet supplemented with phytase alone exhibited higher femur BMD (0.1210 g/cm^2) compared to the other NC diets; higher femur BMC (0.7010 g) and tibia BMD (0.1165 g/cm^2) compared with the NC diet containing 0.5% of FOS (0.6157 g and 0.1030 g/cm^2, respectively). In contrast, FOS supplementation exhibited negative effects among the NC treatments on femur BMD ($P < 0.01$) and BMC ($P < 0.05$), which indicates that FOS may not be a suitable supplement in broiler diets for maintaining or improving bone mineralization. The BA did not show much difference after being adjusted by individual bird BW as a covariate, except the tibia area of birds fed the NC diets were bigger than those of birds fed the PC diet. No significant effects on bone parameters were observed between diets supplemented with 0.5% and 1% FOS or due to phytase × FOS interactions.

Table 4. Effect of phytase and fructooligosaccharides (FOS) supplementation on growth performance of broiler chickens during the entire experimental period[1] (5 to 21 d of age).

Item	Body weight gain (BWG; g bird)	Feed intake (FI; g bird)	Feed conversion ratio (FCR; g feed g gain)	Production index (PI)[2]
Diet				
PC[3]	682.3[a,b]	950.2[a,b]	1.39	477.6
NC[4]	623.1[a,b]	883.0[b]	1.42	481.1
NC + phytase[5]	659.9[a,b]	921.7[a,b]	1.40	498.9
NC + 0.5% FOS	613.2[b]	915.8[a,b]	1.51	484.0
NC + phytase + 0.5% FOS	660.3[a,b]	924.1[a,b]	1.40	500.3
NC + 1% FOS	624.2[a,b]	918.7[a,b]	1.49	484.2
NC + phytase + 1% FOS	689.5[a]	964.6[a]	1.40	484.1
SEM	9.08	9.28	0.016	4.16
Contrasts				
PC vs. NC	0.147	0.288	0.347	0.382
Phytase + FOS	0.078	0.196	0.235	0.461
Effects among NC				
Phytase	0.011	0.126	0.037	0.231
FOS	0.661	0.284	0.490	0.772
Phytase × FOS	0.821	0.712	0.531	0.694
FOS 0.5% vs. 1%	0.385	0.379	0.808	0.488

[1]Means of 6 replicate pens of 5 birds each.

[2]Overall mortality of each treatment at 21 d were 6.7, 0, 0, 3.3, 0, 3.3, and 6.7% (SEM = 1.13), respectively.

[3]PC: Positive control, adequate Ca and available P (1% Ca and 0.45% available P).

[4]NC: Negative control, low Ca and available P (0.8% Ca and 0.25% available P).

[5]Provided 500 U/kg of diet.

[a–b]Means with different superscripts within a column differ significantly ($P < 0.05$).

Table 5. Effect of phytase and fructooligosaccharides (FOS) supplementation on femur and tibia bone mineral density (BMD), bone mineral content (BMC), and bone area (BA) of broiler chickens[1].

	Femur			Tibia		
Item	BMD (g/cm^2)	BMC (g)	BA (cm^2)	BMD (g/cm^2)	BMC (g)	BA (cm^2)
Diet						
PC[2]	0.147[a]	0.862[a]	5.8	0.149[a]	1.190[a]	7.9[b]
NC[3]	0.112[b,c]	0.674[b]	6.0	0.108[b,c]	0.927[b]	8.6[a]
NC + phytase[4]	0.121[b]	0.701[b]	5.8	0.117[b]	0.938 [b]	8.1[a,b]
NC + 0.5% FOS	0.102[d]	0.616[c]	6.0	0.103[c]	0.879[b]	8.5[a,b]
NC + phytase + 0.5% FOS	0.110[c,d]	0.655[b,c]	6.0	0.107[b,c]	0.850[b]	8.0[a,b]
NC + 1% FOS	0.109[c,d]	0.652[b,c]	6.0	0.108[b,c]	0.886[b]	8.2[a,b]
NC + phytase + 1% FOS	0.110[c,d]	0.660[b,c]	6.0	0.109[b,c]	0.911[b]	8.4[a,b]
SEM	0.0023	0.0131	0.04	0.0026	0.0200	0.09
Contrasts						
PC vs. NC	<0.0001	<0.0001	0.386	<0.0001	<0.0001	0.174
Effects among NC						
Phytase	0.021	0.129	0.354	0.201	0.929	0.129
FOS	0.006	0.038	0.685	0.211	0.154	0.909
Phytase × FOS	0.411	0.747	0.536	0.658	0.721	0.210
FOS 0.5% vs. 1%	0.256	0.293	0.903	0.406	0.322	0.857
Phytase + FOS vs. others	0.775	0.860	0.763	0.761	0.389	0.423

[1]Bone parameters were adjusted by total individual BW as a covariance of 6 replicates in each treatment, using least square means ± SEM; average individual BW of each treatment (n = 6): 817.1 g, 779.1 g, 717.3 g, 764.5 g, 780.3 g, 734.1 g, and 838.2 g (SEM = 16.12), respectively.

[2]PC: Positive control, adequate Ca and available P (1% Ca and 0.45% available P).

[3]NC: Negative control, low Ca and available P (0.8% Ca and 0.25% available P).

[4]Provided 500 U/kg of diet.

[a–d]Means with different superscripts within a column differ significantly ($P < 0.05$).

Phosphorus Utilization

The effect of phytase and FOS supplementation on broiler chickens' P utilization is presented in Table 6. No interactions between phytase and FOS related to P utilization were observed. The birds' apparent P digestibility significantly increased with low dietary Ca and available P content ($P < 0.0001$), and P excretion was significantly reduced ($P < 0.0001$). Similar to the growth performance and bone integrity results, phytase supplementation had a positive effect on improving APD and P retention ($P < 0.05$) and significantly reduced the amount of P excretion (% of diet; $P < 0.05$). FOS increased P retention among the NC diets (% of diet; $P < 0.05$), particularly at the 0.5%

Table 6. Effect of phytase and fructooligosaccharides (FOS) supplementation on phosphorus utilization in broiler chickens at 21 d of age[1].

Item	Apparent phosphorus digestibility (%)	P retention (% of diet)	P excretion		
			% of diet	g/bird of total P consumed	g/kg excreta (DM basis)
Diet					
PC[2]	29.2[d]	0.213[c]	0.517[a]	4.72[a]	22.16[a]
NC[3]	40.7[c]	0.220[b,c]	0.320[b]	2.77[b]	13.47[b]
NC + phytase[4]	49.1[a,b]	0.265[a,b]	0.275[b]	2.60[b]	12.58[b]
NC + 0.5% FOS	46.1[a,b,c]	0.258[a,b,c]	0.302[b]	2.74[b]	13.01[b]
NC + phytase + 0.5% FOS	51.2[a]	0.287[a]	0.273[b]	2.54[b]	12.01[b]
NC + 1% FOS	42.9[b,c]	0.228[b,c]	0.302[b]	2.79[b]	12.96[b]
NC + phytase + 1% FOS	46.2[a,b,c]	0.245[a,b,c]	0.285[b]	2.61[b]	11.80[b]
SEM	1.45	0.0066	0.0146	0.133	0.625
Contrasts					
PC vs. NC	<0.0001	0.028	<0.0001	<0.0001	<0.0001
Effects among NC					
Phytase	0.012	0.018	0.018	0.204	0.160
FOS	0.223	0.046	0.794	0.936	0.730
Phytase × FOS	0.601	0.639	0.639	0.996	0.988
FOS 0.5% vs. 1%	0.120	0.021	0.675	0.732	0.885
Phytase + FOS vs. others	0.080	0.080	0.116	0.324	0.165

[1]Means of 6 replicate pens of 5 birds each.

[2]PC: Positive control, adequate Ca and available P (1% Ca and 0.45% available P).

[3]NC: Negative control, low Ca and available P (0.8% Ca and 0.25% available P).

[4]Provided 500 U/kg of diet.

[a–d]Means with different superscripts within a column differ significantly ($P < 0.05$).

inclusion level ($P < 0.05$), while the diet containing a combination of phytase and 0.5% FOS had a significantly greater APD value (52.2%) in comparison with the NC diet (40.7%; $P < 0.05$). No interactions between FOS and phytase supplementation were observed related to P utilization parameters. Expression of genes for CHP, TRPV6, and SLC20A from the ileal tissues was not significantly different across the treatments (data not shown; $P > 0.05$).

DISCUSSION

Effect of Dietary Phytase Supplementation

The results of dietary phytase supplementation in the present study agree with previous findings in that the addition of phytase to low Ca and available P diets is known to enhance growth performance of broilers (Woyengo et al., 2008; Coppedge et al., 2011; El-Sherbiny et al., 2010; Chung et al., 2013). However, Angel et al. (2005) reported that growth performance was not affected when available P content was reduced from 0.45 to 0.35% and then from 0.35 to 0.25% during starter (1 to 18 d) and grower (18 to 32 d) phases, respectively, in phytase-supplemented broiler diets. Similar results were found by Silversides et al. (2004) and Walk et al. (2012) with a 0.1% reduction of available P. However, Woyengo et al. (2010) found that birds fed phytase-supplemented low P (0.2% lower) diets did not have performance values comparable to birds fed an adequate P diet. Our results showed that 500 U/kg phytase supplementation increased BWG and decreased FCR at 15 to 21 and 5 to 14 d of age, respectively, as well as for the entire experimental period. A possible explanation could be that phytase supplementation induced improved P retention and energy utilization. Several studies have shown that phytase improved AME_n values, ileal digestibility of P, and P retention in broilers fed a low Ca and available P diet (Ravindran et al., 2000; Woyengo et al., 2010). Pirgozliev et al. (2011) evaluated the net energy for production in broilers and demonstrated a 15.6% increase with phytase supplementation.

The bone mineralization results from the current study confirm that low Ca and available P diets are indeed P deficient, which in turn impairs bone quality. Previous studies indicated that the tibia ash percentage and bone breaking strength of birds fed low Ca and available P diet were improved by phytase supplementation; however, the values were not equivalent to those of the PC diet (Powell et al., 2008; Woyengo et al., 2008; El-Sherbiny et al., 2010). Few studies have used a DEXA or other bone densitometer to examine the BMD and BMC of broilers fed phytase-supplemented diets. Angel et al. (2006) reported that whole body and tibia BMD and BMC of 49-d-old birds were higher in those fed diets with 0.2% available P and 600 U/kg of phytase, although the values were lower than those fed the PC diet containing 0.3% available P. Chung et al. (2013) found similar results, showing that phytase supplementation improved bird femur and tibia BMD and BMC when compared with birds fed the low P control diet (available P reduced by 0.1%). In the present study, the addition of phytase had a positive effect on femur BMD among NC treatments, indicating that phytase supplementation increased the release of available P for more effective bone mineralization. However, the concentration of dietary available P was still inadequate

for normal bone development. Thus, increased phytase efficacy in low Ca and available P diets would be expected to further improve bone mineralization.

Phytase supplementation as a means of improving P utilization is a common practice in poultry nutrition (Woyengo et al., 2008; El-Sherbiny et al., 2010). As reviewed by Slominski (2011), numerous studies have elucidated the efficacy of phytase in improving total tract P digestibility in broiler chickens, which indicates that the P utilization could be improved with phytase supplementation by liberating P from phytate-P and preventing the formation of insoluble Ca-phytate complexes in poultry diets (Woyengo et al., 2010). Our results showed that APD significantly increased by 8.4, 10.5, and 5.5 percentage points following phytase addition, when comparing the NC + phytase, NC + phytase + 0.5% FOS, and NC + phytase + 1% FOS diet with the NC diet, respectively. These results are in agreement with the findings of Ravindran et al. (2000) and Woyengo et al. (2010). At the same time, our results showed that phytase-supplemented diets also improved P retention and reduced P excretion when expressed as actual amounts of total P present in the diet, which is in agreement with the findings of Ravindran et al. (2000) and Powell et al. (2008), who reported that phytase supplementation reduced P excretion.

Effect of Dietary FOS Supplementation

In the current study, FOS supplementation did not significantly affect broiler chicken growth performance when they were fed the low Ca and available P diets. Research findings on the effect of FOS on growth performance parameters are inconsistent. Decreased FI and BWG, and improved FCR were found by Williams et al. (2008) with 0.6% FOS added to standard broiler rations. Kim et al. (2011a) reported that 0.25% FOS could be used as an alternative to antibiotic growth promoters to improve productivity in broilers up to 28 d of age. Altered gastrointestinal fermentation intensity caused by different levels of FOS supplementation may partially explain the variations observed in broiler performance. Any carbohydrate that passes through the GIT is a potential source for fermentation by gut microbiota. The metabolism of FOS produces SCFAs, hydrogen and carbon dioxide gases, and bacterial cell mass (Cummings et al., 2001). It has been reported that excessive levels of FOS (1%) may cause diarrhea and generate carbon dioxide and hydrogen gases due to intensive fermentation in the GIT, thus decreasing production performance (Cummings et al., 2001; Xu et al., 2003). Human prebiotic feeding studies have well reported that undesired symptoms are associated with gas production and the laxative effect. Daily ingestion of 15 g FOS increased bloating, abdominal discomfort, and stool output (Stone-Dorshow and Levitt, 1987; Gibson et al., 1995). Xu et al. (2003) demonstrated that the addition of 0.4% FOS increased ADG and FCR in broiler chickens, whereas 0.8% FOS had no effect on those parameters. However, in the present study, there was no significant difference in growth performance of birds fed 0.5% or 1% FOS. Moreover, individual birds may react differently to FOS supplementation because the composition of gut microflora induced by FOS supplementation varies in different individuals and with different FOS inclusion rates (Williams et al., 2008). Therefore, this inherent variability could lead to different rates of SCFA production and different levels of intestinal immune system stimulation, which may eventually result in the performance variations observed in the current study. The reason for reduced FCR but not FI in FOS-supplemented diets may be attributed to reduced BWG caused by FOS ingestion. In a study with rats, it was demonstrated that dietary FOS decreased abdominal fat tissue weight and intestinal mesenteric fat mass and improved insulin sensitivity (Shinoki and Hara, 2011). Moreover, SCFA production stimulated by FOS may suppress body fat accumulation, change mesenteric adipocyte properties, and lower energy intake. Therefore, it would be interesting to investigate how FOS affects intestinal fat mass and related gene expression.

Dietary FOS supplementation had a negative effect on femur BMD and BMC in birds fed low Ca and available P diets, which indicates that FOS does not beneficially and does deleteriously affect bone mineralization in broiler chickens. At this time, limited data are available from the literature on the effect of FOS on bone parameters in poultry. Kim et al. (2006) investigated bone breaking strength and mineralization parameters in laying hens fed a FOS-supplemented (0.75%) alfalfa molting diet and concluded that tibia breaking strength was comparable to control hens. However, DEXA results showed that femur and tibia BMD and BMC for the birds fed the FOS-supplemented diet did not reach the values of hens fed the control diet. Similar to our findings, Kim et al. (2011b) reported that FOS did not beneficially affect the bone growth and skeletal integrity of broilers fed diets adequate in Ca and available P and supplemented with 2% or 4% FOS. In a study with prebiotic fructans added at 0.1% to deficient Ca and available P diets, Swiatkiewicz et al. (2011) observed that Ca and available P levels did not affect growth performance but did negatively influence bone biomechanical parameters of broilers at 21 and 42 d of age and that the supplemental fructans did not improve growth performance or bone quality. These findings are contradicted by studies with rats showing that dietary FOS increased mineral absorption and bone mineralization (Lopez et al., 2000; Zafar et al., 2004; Ohta, 2006). Thus, there may be interspecies differences in the beneficial effects of FOS supplementation (Kim et al., 2011b).

Additive Effects of Phytase and FOS The combination of phytase and 1% FOS showed significant improvement in FI and BWG of birds during the entire

trial period, particularly when compared with the NC and NC + 0.5% FOS diets. Increased APD and P retention were observed for the diet containing phytase and 0.5% of FOS when compared to the NC diet and the diet containing 1% FOS. These results suggest that FOS and phytase may have additive effects in promoting growth performance and P utilization in broiler chickens. Adding FOS to the phytase-supplemented diet may further facilitate phytate hydrolysis by prohibiting the formation of Ca-phytate complexes and improving digestive enzyme activities. In the present study, the PC diet had a Ca to available P ratio of 2.8:1, whereas the NC diets had a Ca to available P ratio of 4:1. Wider Ca to available P ratios may lead to the formation of insoluble Ca-phytate complexes in the intestine, which could lower exogenous phytase efficacy and reduce the availability of dietary Ca and P (Selle et al., 2009). The growth performance and APD data for phytase + FOS treatments (NC + phytase + 0.5% FOS and NC + phytase + 1% FOS) reached or exceeded the values for controls, which revealed that FOS and phytase may have a synergistic effect in alleviating the negative impact of diets with a wide Ca to available P ratio. Furthermore, Xu et al., 2003 documented that intestinal bacteria colonization induced by FOS increased amylase, protease, and other digestive enzyme activities in broilers. In addition, phytase has been reported to improve amino acid, fat, protein, and starch digestibility (Selle and Ravindran, 2007; Pirgozliev et al., 2011). These functions may act collectively to improve energy utilization and counteract harmful properties of phytate, thus contributing to the positive effects observed in the current study. In contrast, the combination of FOS and phytase did not result in any beneficial effects on bone quality, and expression of Ca and P transporter genes (CHP1, TRPV6, and SLC20A2) was not different among the treatments, which indicated that supplemental phytase and FOS act mainly by modifying the microenvironment of the gut in ways such as releasing phytate-P from the diet, providing beneficial compounds (e.g., SCFAs) for utilization, and increasing enzyme activities to assist nutrient absorption, rather than directly affecting the host's ability to transport minerals or transform these minerals to bone content. Mechanisms and specific pathways remain unclear, and further research is needed.

In summary, our results confirmed that phytase improved growth performance, bone quality, and P utilization in broiler chickens fed diets with a 0.2% reduction in Ca and available P. Dietary FOS supplementation negatively affected bone mineralization; thus, it may not be a suitable supplement for enhancing bone quality in broilers. Supplementary phytase and FOS in the low Ca and available P diet failed to improve bone mineralization parameters to the same levels as the adequate Ca and available P diet. Further research is needed to explain the mechanisms associated with dietary FOS supplementation and the synergetic effects of phytase and FOS in broiler rations.

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