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Efficacy of New 6-Phytase from *Buttiauxella* spp. on Growth Performance and Nutrient Retention in Broiler Chickens Fed Corn Soybean Meal-based Diets

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ABSTRACT: A total of 420 day-old male Ross chicks were weighed at d 1 of life and assigned to test diets to assess the efficacy of a new *Buttiauxella* spp. phytase expressed in *Trichoderma reesei*. Diets were: positive control (PC) adequate in nutrients and negative control (NC) diet (40% and 17% less available phosphorous (P) and calcium (Ca), respectively) supplemented with 6 levels of phytase 0, 250, 500, 750, 1,000, and 2,000 phytase units (FTU)/kg of diet. All diets had titanium dioxide as digestibility marker and each diet was allocated to ten cages (6 birds/cage). Diets were fed for 3 wk to measure growth performance, apparent retention (AR) on d 17 to 21 and bone ash and ileal digestibility (AID) on d 22. Growth performance and nutrient utilization was lower ($p<0.05$) for NC vs PC birds. Phytase response in NC birds was linear ($p<0.05$) with 2,000 FTU showing the greatest improvement on body weight gain (20%), feed conversion (7.4%), tibia ash (18%), AR of Ca (38%), AR of P (51%) and apparent metabolizable energy corrected for nitrogen (5.1%) relative to NC. Furthermore, phytase at ≥750 FTU resulted in AID of total AA commensurate to that of PC fed birds and at ≥1,000 FTU improved ($p<0.05$) AR of P, dry matter, and N beyond that of the lower doses of phytase and PC diet. In conclusion, the result from this study showed that in addition to increased P and Ca utilization, the new *Buttiauxella* phytase enhanced growth performance and utilization of other nutrients in broiler chickens in a dose-dependent manner. (**Key Words:** Amino Acid, Broiler, Growth Performance, Phosphorus, Phytase)

INTRODUCTION

Amongst the biotechnological feed enzyme additives, microbial phytases (*myo*-inositol [1,2,3,4,5,6] hexakisphosphate phosphohydrolases) have made the most progress and impact in feed industry. Rapid penetration of phytase in poultry nutrition has been associated with the acceptance as a replacement for inorganic phosphates and concomitant considerable investment in application research, leading to strategic development of highly efficacious microbial phytases (Selle and Ravindran 2007; Adeola and Cowieson, 2011; Kiarie et al., 2013). There is also increasing scientific evidence of anti-nutritive properties of the phytate molecule (Selle and Ravindran 2007; Selle et al., 2012). However, data demonstrating consistent effects of microbial phytases on utilization of other nutrients than release of phytate-bound P has been very variable. For example, Ravindran et al. (2008) and Santos et al. (2008) reported improvement in Ca and other minerals digestibility, whereas Um et al. (2000) reported no improvement on Ca digestibility. For amino acids (AA), Ravindran et al. (1999) and Rutherfurd et al. (2002) reported that exogenous phytases increased the ileal digestibility of AA, whereas Rutherfurd et al. (2004) and Zhang et al. (1999) observed no such effect. Moreover, a review of several independent studies showed that the improvement of phytase supplementation on energy utilization in broiler chickens ranged from –0.7% to 5.2% (Selle and Ravindran, 2007).

The reasons for the aforementioned inconsistencies of phytase effects on digestibility of nutrients other than P are not well understood. However, the effectiveness of feed enzymes in the digestive tract of a bird has been suggested

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to be dependent to enzymes characteristics (source, specific catalytic activity, resistance to pepsin's proteolytic action), substrate characteristics (concentration and accessibility), and digestive tract conditions (moisture content, pH, temperature and the time digesta spends in the tract) (Ravindran, 2013). It is believed that phytate is capable of forming insoluble/indigestible complexes with dietary chemical constituents in the gut, increasing endogenous N losses and eliciting luminal Na excretion to compromise Na-dependent co-transport mechanisms involved in the intestinal uptake of nutrients (Selle et al., 2009; Selle et al., 2012). For example, Onyango et al. (2009) reported that phytic acid increased mucin and endogenous AA losses from the gut of chickens. A gastric simulation report demonstrated that degradation of phytate to lower-level esters dramatically increased the solubility of soya and casein proteins by attenuating phytic acid-protein complexes (Yu et al., 2012). Although commercial *E. coli* and fungal phytases are histidine acid phytases and are active in the acidic pH range, they encompass a range of sizes, structures and catalytic mechanisms (Greiner and Konietzn, 2010; Yu et al., 2014). It has been suggested that perhaps development of novel phytases showing high activity at low pH and exhibiting high stability to pepsin may offer efficient degradation of phytate earlier on in the gastrointestinal tract and thus reduce its anti-nutritional effects (Selle et al., 2012; Ravindran, 2013). *Buttiauxella* phytase has been developed with improved thermostability, pepsin resistance and high activity toward phytic acid and phytic acid-protein complex at low gut pH in comparison to commercial *E. coli* and fungal phytases (Yu et al., 2014). Recent poultry and swine trials showed *Buttiauxella* phytase improved growth performance and nutrient utilization (Adedokun et al., 2013; Amerah et al., 2014). However, its dose response on nutrient utilization in broilers has not been evaluated. The objective of the study was to assess the efficacy of *Buttiauxella* phytase expressed in *Trichoderma reesei* over a range of doses on growth performance, nutrient retention and bone mineralisation in young broiler chicken.

MATERIALS AND METHODS

All birds and experimentation procedures were approved by the University of Manitoba Animal Care Committee and followed the guidelines described by the Canadian Council on Animal Care (CCAC, 2009).

Experimental diets

A corn-soybean meal-based diet formulated to meet or exceed the NRC (1994) nutrient specifications for young broilers served as a positive control (PC) whereas a similarly formulated diet but with 17%, 25%, and 40% less Ca, total and available P contents, respectively, served as the negative control (NC) (Table 1). The NC diet was fed without or with supplemental phytase (250, 500, 750, 1,000, and 2,000 phytase units [FTU]/kg) to give a total of 7 dietary treatments. The phytase (Axtra PHY, a *Buttiauxella* spp. phytase expressed in *Trichoderma reesei*) along with the assay procedures were supplied by Danisco Animal Nutrition (Danisco UK Ltd., Marlborough, Wiltshire, UK). One FTU is the amount of enzyme that liberates one micromole of inorganic phosphate per minute from a sodium phytate substrate at pH 5.5 and 37°C. Diets were prepared in mash form and contained TiO_2 (0.3%) as an indigestible marker.

Table 1. Composition of the control diets, as fed

	Positive control	Negative control
Ingredients (%)		
Corn	60.1	62.7
Soybean meal	33.1	32.5
Soybean oil	2.72	1.50
Sodium bicarbonate	0.20	0.20
Salt	0.14	0.14
Monocalcium phosphate	1.33	0.56
Limestone	1.36	1.27
Lysine-HCl	0.03	0.05
DL-methionine	0.23	0.23
L-threonine	0.04	0.01
Titanium dioxide	0.30	0.30
Vitamin-mineral premix[1]	0.50	0.50
Calculated provisions		
Crude protein (%)	22.5	22.5
ME (kcal/kg)	3,055	3,017
Digestible lys (%)	1.00	1.00
Digestible met+cys (%)	0.77	0.77
Digestible Thr (%)	0.67	0.63
Ca (%)	0.95	0.79
Total P (%)	0.64	0.48
Non-phytate P (%)	0.40	0.24
Ca: available P	2.38	2.63

[1] Supplied per kilogram of diet: vitamin A, 8,255 IU; vitamin D_3, 3,000 IU; vitamin E, 30 IU; vitamin K, 2 mg; Thiamine (vitamin B_1), 4 mg; riboflavin (vitamin B_2), 6 mg; niacin, 41.2 mg; folic acid, 1 mg; biotin, 0.25 mg; pyridoxine, 4 mg; choline, 1,301; Pantothenic acid, 11 mg; vitamin B_{12}, 0.013 mg; Mn, 70 mg; Zn, 80 mg; Fe, 80 mg; Cu, 10 mg; and Na, 1.7 g.

Birds, management, feeding, performance measurements and sample collection

The study used a total of 420, 1-d-old male Ross 308 broiler chicks, which were obtained from a local commercial hatchery (Carlton Hatchery, Grunthal, MB, Canada). The chicks were individually weighed and divided into 70 groups (6 chicks per group) so that the average bird weight per group was similar. The chicks were housed in 70

cages (1 group per cage) in an electrically heated Petersime battery brooder (Incubator Company, Gettysburg, OH, USA). The brooder and room temperatures were set at 32°C and 29°C, respectively, during the first week. Thereafter, heat supply in the brooder was switched off and room temperature was maintained at 29°C throughout the experiment. Light was provided for 24 h throughout the experiment. There were 10 replicate cages per diet, and the diets were fed from d 1 of life for a period of 22 d. Water and diets were provided for *ad libitum* consumption. Feed disappearance on a cage basis was measured on days 7, 14, and 21. The body weight (BW) on a cage basis was determined weekly on days 7, 14, and 21 after withdrawing feed for 4 h. On days 17 to 21, samples of excreta were collected, pooled within a cage, sub-sampled and stored at –20°C for the determination of apparent nutrient retention. On day 22, three birds were randomly selected from each pen and killed by cervical dislocation. The right tibia and middle toe (between the second and third tarsal bones) were obtained for determination of the bone ash content. The digesta (in all birds in a cage) was obtained from the Meckel's diverticulum to 1 cm before ileal-cecal junction. All samples were stored at –20°C until the analyses.

Sample preparation and chemical analysis

The toe samples were oven dried at 45°C for 24 h, weighed, and dry ashed at 550°C for 8 h for determination of toe ash. The tibia samples were defleshed after autoclaving at 121°C for 1 min and dried in an oven at 45°C for 2 d. They were then fat extracted using hexane for 2 d, dried in a fume hood for 2 d to allow the hexane to evaporate and ashed at 550°C in a muffle furnace for 8 h for the determination of tibia ash.

Excreta and ileal samples were lyophilized and, along with diet samples, finely ground in a CBG5 Smart Grind coffee grinder (Applica Consumer Products, Inc., Shelton, CT, USA) and thoroughly mixed before analyses. The diets, digesta and excreta samples were analyzed for dry matter (DM), crude protein (CP), minerals (Ca, Mg, Na, and P) and Ti. Further analysis included AA in diets and ileal digesta samples and gross energy in diets and excreta samples. Dry matter was determined (method 925.09; AOAC, 1990) and N was determined by the combustion method (method 990.03; AOAC, 1990) using combustion analyzer (Model CNS-2000; LECO Corp., St. Joseph, MI, USA) and ethylenediaminetetraacetic acid as a calibration standard. The CP values were derived from multiplying the assayed N values by a factor of 6.25. The samples were wet acid digested with nitric and perchloric acid mixture (AOAC International, 2005; method 990.08) and concentrations of minerals read on an inductively coupled plasma mass spectrometer (Varian Inc, Palo Alto, CA, USA). Samples for Ti analysis were ashed and digested according to the procedures described by Lomer et al. (2000) and read on an inductively coupled plasma mass spectrometer (Varian Inc, USA). Samples for AA analysis were prepared by acid hydrolysis (method 982.30; AOAC, 1984) as modified by Mills et al. (1989). Briefly, about 100 mg of each sample was digested in 4 mL of 6 N HCl for 24 h at 110°C followed by neutralization with 4 mL of 25% (wt/vol) NaOH and cooled to room temperature. The mixture was then equalized to 50 mL volume with sodium citrate buffer (pH 2.2) and analyzed using an AA analyzer (Sykam GmbH, Fürstenfeldbruck, Germany). Samples for analysis of sulfur containing AA (Met and Cys) were subjected to performic acid oxidation before acid hydrolysis. Tryptophan was not determined. Gross energy was determined using an adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL, USA) using benzoic acid as the calibration standard. Phytate in PC and NC diet was determined by the method described by Latta and Eskin (1980).

Calculations and statistical analysis

The content of ash (in g/kg) was calculated as the proportion of the dry, pre-ashed tibia or toe multiplied by 1,000. Apparent ileal digestibility (AID) and apparent retention (AR) of nutrients and apparent metabolizable energy corrected for nitrogen (AMEn) were calculated as described by Woyengo et al. (2010).

Data were analyzed using general linear model procedures (SAS Inst. Inc., Cary, NC, USA). Planned contrasts were used for comparison: PC vs NC; and linear, quadratic and cubic response to supplemental phytase. Contrast coefficients from unequally spaced supplemental phytase were generated using the interactive matrix language procedure of SAS. An α level of $p \leq 0.05$ was used as the criterion for statistical significance.

RESULTS

The analyzed mineral content showed that NC diet had 35.7% and 20.9% lower P and Ca, respectively (Table 2). The other analyzed chemical components were comparable between PC and NC diets. The assayed phytase concentration in the phytase treated diets was 384, 666, 943, 1,102, and 2,312 FTU/kg for NC+250, NC+500, NC+750, NC+1,000 and NC+2,000 FTU/kg, respectively. Assayed phytase in PC and NC basal diets was 82 FTU/kg.

Birds fed the NC diet had poor ($p<0.05$) BW gain (537 vs 639 g), feed intake (793 vs 895 g), feed conversion ratio (FCR) (1.48 vs 1.40), tibia ash content (423 vs 484 g/kg) and toe ash content (45.5 vs 54.3 g/kg) than birds fed PC diet (Table 3). Phytase linearly improved ($p<0.05$) growth performance such that the birds receiving NC+2,000 FTU showed the greatest improvement on BW gain (20%) and

Table 2. Analyzed chemical composition of the basal diets, as fed

Item	Positive control	Negative control
Dry matter (%)	88.9	89.6
Gross energy (kcal/kg)	4,074	3,996
Minerals (%)		
Ca	1.34	1.06
P	0.70	0.45
Mg	0.23	0.27
Na	0.19	0.19
Phytate P (%)	0.28	0.27
Crude protein (%)	19.6	19.6
Amino acids (%)		
Arg	1.33	1.25
His	0.54	0.54
Ile	0.91	0.91
Leu	1.80	1.78
Lys	1.14	1.10
Met	0.51	0.52
Phe	1.05	1.03
Thr	0.79	0.90
Val	1.01	1.03
Ala	1.04	1.03
Asp	2.11	2.06
Cys	0.31	0.31
Glu	3.96	3.91
Gly	0.86	0.85
Pro	1.20	1.18
Ser	0.97	0.90
Tyr	0.63	0.63

FCR (7.4%) relative to birds fed NC diet without phytase. Phytase response on tibia ash content was both linear ($p< 0.01$) and quadratic ($p = 0.02$) as a result of birds receiving NC+2,000 FTU showing higher tibia ash content (8%) relative to birds fed NC+250 FTU. The phytase response was linear ($p = 0.01$) for toe ash. Supplementing NC fed birds with phytase resulted in growth performance and bone ash content commensurate ($p>0.10$) to PC fed birds.

The AID of DM (67.6 vs 64.2%), Ca (42.0 vs 34.0%), Mg (14.0 vs –4.79%), Na (10.4 vs –2.3%) and P (53.1 vs 39.6%) was higher ($p\leq0.01$) in PC fed birds than in birds fed NC without phytase (Table 4). Phytase linearly ($p<0.01$) improved the AID of DM, Mg, and Na in NC diets. Addition of phytase in NC diets improved ($p<0.05$) AID of Ca and P in linear ($p<0.01$) and quadratic ($p<0.01$) fashions. For AID of Ca, phytase addition to NC diet showed a rather strong cubical response ($p = 0.009$) with 500 and 2,000 FTU showing improvement vs NC whereas the other doses had similar AID of Ca to NC. The AID of P in NC diets was restored to that of PC at NC+250 FTU, further phytase dosing improved the AID of P such that birds fed NC+ ≥500 FTU had higher AID of P ($p<0.01$) compared to PC fed birds. The AID of K was not influenced ($p>0.05$) by the dietary treatments.

Reduction of dietary Ca, Na and P content to create NC diets affected the AID of indispensable AA (Table 4). Specifically, the AID of Arg (89.9% vs 88.1%, $p = 0.020$), His (83.3% vs 80.6%, $p = 0.008$), Iso (84.0% vs 81.0%, $p = 0.003$), Leu (84.6% vs 82.0%, $p = 0.006$) and Val (81.8% vs 78.5%, $p = 0.001$) were higher in PC fed birds relative to NC fed birds. This resulted to a lower ($p = 0.042$) AID of mean indispensable AA in NC fed birds compared to PC fed birds. Phytase addition to NC diets exhibited ($p<0.05$) linear and non-linear responses in terms of the AID of indispensable AA. In birds fed NC diets, phytase restored the AID of His, Iso, Leu, Val, and mean of indispensable AA to that of PC fed birds at 750 FTU. However, the AID of Phe and Thr was restored to that of PC at 1,000 FTU. Although the AID of Met did not differ ($p = 0.209$) between PC and NC fed birds, a linear response was observed due phytase such that birds fed NC+2,000 FTU had higher AID of Met relative to NC fed birds. The AID of Lys was not affected ($p>0.05$) by the dietary treatments. Among the dispensable AA, birds fed PC diets showed higher ($p<0.05$)

Table 3. Influence of phytase dose on 21-d body weight gain, feed intake (FI), feed conversion ratio (FCR) and bone ash content of broiler chickens fed corn/soybean diets

Items	PC	Phytase (FTU/kg)						SEM	Contrasts[2]			
		0[1]	250	500	750	1,000	2,000		1	2	3	4
Performance												
Body weight gain (g)	639^{ab}	537^{c}	587^{bc}	598^{ab}	623^{ab}	618a^{b}	642^{a}	18.51	<0.01	<0.01	0.058	0.354
FI (g)	895^{a}	793^{b}	850^{ab}	868^{a}	884^{a}	861^{a}	878^{a}	22.6	0.002	0.042	0.064	0.154
FCR (g/g)	1.40^{bc}	1.48^{a}	1.45^{ab}	1.46^{ab}	1.42^{abc}	1.39^{bc}	1.37^{c}	0.024	0.028	0.002	0.419	0.580
Bone ash (g/kg)												
Tibia	484^{ab}	423^{c}	462^{b}	481^{ab}	485^{ab}	485^{ab}	499^{a}	11.9	0.007	<0.01	0.002	0.145
Toe	54.3^{a}	45.5^{b}	50.1^{ab}	54.8^{a}	53.7^{a}	52.7^{ab}	56.6^{a}	2.669	0.023	0.012	0.188	0.166

PC, positive control; SEM, standard error of the mean.
[1] Negative control, similar to PC except available P and Ca which were less than in PC by 40.0% and 16.8%, respectively.
[2] Contrasts: 1, PC vs negative control; 2, 3, and 4 linear, quadratic and cubic response to supplemental phytase.
Within a row means without common letters are significantly different ($p<0.05$).
N = 10.

Table 4. Influence of phytase dose on ileal apparent digestibility of minerals and amino acids in 21-d old broilers fed corn/soybean meal diets

Items	PC	Phytase (FTU/kg)						SEM	Contrasts[2]			
		0[1]	250	500	750	1,000	2,000		1	2	3	4
Dry matter	67.6^{ab}	64.2^{d}	65.1^{cd}	67.4^{ab}	66.1^{bc}	66.5^{abc}	68.1^{a}	0.62	<0.01	<0.01	0.246	0.117
Minerals												
Ca	42.0^{a}	34.0^{b}	35.3^{b}	44.3^{a}	35.2^{b}	35.9^{b}	42.9^{a}	2.05	0.007	0.011	0.814	0.009
Na	10.4^{ab}	–2.3^{c}	–1.2^{c}	7.8^{ab}	4.1b^{c}	10.8^{ab}	13.1^{a}	2.40	<0.01	<0.01	0.097	0.829
Mg	14.0^{a}	–4.79^{c}	–4.77^{c}	–4.28^{c}	–1.64^{bc}	–1.64^{bc}	5.06^{b}	2.88	<0.01	0.027	0.866	0.196
P	53.1^{b}	39.5^{c}	51.1^{b}	64.9^{a}	67.4^{a}	69.1^{a}	68.2^{a}	1.82	<0.01	<0.01	<0.01	0.035
Amino acids												
Indispensable AA												
Arg	89.9^{a}	88.1^{bc}	87.7^{c}	87.6^{c}	88.5^{abc}	89.4^{ab}	88.8^{abc}	0.51	0.020	0.062	0.375	0.039
His	83.3^{a}	80.6^{b}	80.7^{b}	80.8^{b}	82.0^{ab}	82.9^{a}	82.5^{ab}	0.66	0.008	0.021	0.203	0.184
Ile	84.0^{a}	81.0^{b}	80.7^{b}	81.1^{b}	83.5^{a}	84.9^{a}	84.3^{a}	0.65	0.003	<0.01	0.024	0.008
Leu	84.6^{a}	82.0^{bc}	81.7^{bc}	81.4^{c}	83.4^{ab}	84.5^{a}	83.8^{a}	0.61	0.006	0.003	0.149	0.015
Lys	86.7	84.3	86.9	83.5	87.4	89.1	87.8	2.20	0.452	0.249	0.495	0.500
Met	89.7^{ab}	88.6^{b}	89.1^{ab}	89.2^{ab}	90.4^{ab}	90.3^{ab}	90.6^{a}	0.65	0.209	0.028	0.274	0.796
Phe	81.2^{a}	80.9^{a}	78.6^{ab}	75.3^{b}	75.3^{b}	77.8^{ab}	75.0^{b}	1.31	0.889	0.008	0.090	0.031
Thr	76.9^{bc}	76.9^{bc}	76.6^{bc}	75.9^{c}	77.3^{bc}	79.1^{a}	77.9^{ab}	0.61	0.998	0.031	0.253	0.006
Val	81.8^{a}	78.5^{b}	78.7^{b}	79.2^{b}	81.7^{a}	83.3^{a}	82.6^{a}	0.63	0.001	<0.01	0.005	0.009
Mean IAA	84.2^{a}	82.3^{bc}	82.3^{bc}	81.6^{c}	83.3^{abc}	84.6^{a}	83.7^{ab}	0.63	0.042	0.022	0.249	0.025
Dispensable AA												
Ala	83.2^{a}	80.4^{bc}	79.9^{c}	79.9^{c}	81.8^{ab}	83.2^{a}	82.6^{a}	0.62	0.004	0.001	0.156	0.011
Asp	82.7^{a}	80.0^{bc}	78.7^{c}	78.4^{c}	79.9^{bc}	81.4^{ab}	80.3^{bc}	0.73	0.014	0.150	0.670	0.010
Cys	66.4	64.0	65.9	68.0	60.9	64.6	62.4	3.06	0.597	0.469	0.916	0.551
Glu	88.4^{a}	86.6^{bc}	86.2^{c}	85.8^{c}	86.9^{abc}	87.8^{ab}	87.3^{abc}	0.53	0.026	0.091	0.627	0.041
Gly	78.2^{a}	75.1^{bc}	74.7^{c}	74.7^{c}	76.1^{abc}	78.2^{a}	77.0^{ab}	0.76	0.007	0.011	0.208	0.021
Pro	81.8^{a}	78.8^{b}	79.4^{b}	79.0^{b}	79.7^{ab}	80.6^{ab}	79.9^{ab}	0.81	0.013	0.280	0.390	0.550
Ser	80.8^{a}	78.1^{b}	76.5^{bc}	74.7^{c}	75.6^{bc}	77.6^{b}	76.1^{bc}	0.92	0.048	0.520	0.330	0.020
Tyr	83.4^{a}	81.6^{abc}	81.1^{bc}	80.1^{c}	82.0^{abc}	83.6^{a}	82.5^{ab}	0.75	0.102	0.058	0.471	0.011
Mean DAA	80.6^{a}	78.1^{b}	77.8^{b}	77.6^{b}	77.9^{b}	79.6^{ab}	78.5^{ab}	0.84	0.041	0.393	0.643	0.164
Mean total	82.4^{a}	80.2^{bc}	80.0^{c}	79.6^{c}	80.6^{abc}	82.1^{ab}	81.1^{abc}	0.71	0.036	0.126	0.431	0.068

PC, positive control; SEM, standard error of the mean; AA, amino acids; IAA, indispensable amino acids; DAA, dispensable amino acids.
[1] Negative control, similar to PC except available P and Ca which were less than in PC by 40.0% and 16.8%, respectively.
[2] Contrasts: 1, PC vs negative control; 2, 3, and 4 linear, quadratic and cubic response to supplemental phytase.
Within a row means without common letters are significantly different ($p<0.05$).
N = 10.

AID of all AA except for Cys and Tyr relative to NC fed birds. Phytase addition in NC diets resulted in linear ($p = 0.01$) and non-linear ($p<0.04$) responses on the AID of dispensable AA. Specifically, phytase restored the AID of Ala, Asp, Glu, Gly, and mean dispensable AA to that of PC fed birds at between 750 to 1,000 FTU/kg. Overall, the AID of mean total AA was lower ($p = 0.036$) for NC vs PC , however, phyase at 750 FTU and above resulted in AID of mean total AA commensurate to that of PC fed birds.

The AR of DM and N in birds fed PC and NC diets was similar ($p = 0.382$) (Table 5). Phytase addition in NC diets resulted in linear improvement of AR of DM ($p<0.01$) and N ($p = 0.034$). Birds fed PC diet retained more ($p\leq0.01$) Ca, Mg and P compared to birds fed NC diet without supplemental phytase. Retention of Ca and P exhibited linear ($p<0.01$) and non-linear ($p\leq0.04$) responses to phytase dosing. Adding 250 FTU to NC resulted to AR of Ca commensurate to that of PC; however, the birds fed NC with top phytase dose (2,000 FTU) had greater AR of Ca relative to the PC fed birds. The AR of P in NC diets was restored to that of PC at 250 FTU, further phytase dosing improved the AID of P such that birds receiving 500 and ≥1,000 FTU had higher AID of P ($p<0.01$) compared to PC fed birds. There was no dietary effects ($p> 0.05$) on AR of Na. Birds fed PC diet showed higher AMEn (3.35 vs 3.24 mcal/kg DM, $p<0.01$) compared to birds fed NC without phytase (Table 5). Phytase addition in NC diet improved AMEn in a linear ($p<0.01$) fashion; in this context the

Table 5. Influence of phytase dose on apparent retention (%) and AMEn (kcal/kg DM) in 21-d old broilers fed corn/soybean meal diets

Item	PC	Phytase (FTU/kg)						SEM	Contrasts[2]			
		0[1]	250	500	750	1,000	2,000		1	2	3	4
DM	68.4^{c}	69.1^{c}	70.9^{b}	71.5^{ab}	69.2^{c}	71.9^{ab}	72.8^{a}	0.58	0.382	<0.01	0.870	0.348
Nitrogen	58.5^{c}	59.2^{bc}	59.7^{bc}	60.3^{bc}	58.1^{c}	64.5^{a}	62.9^{ab}	1.46	0.713	0.034	0.662	0.185
Ca	57.7^{b}	49.1^{c}	61.3^{b}	61.0^{b}	60.7^{b}	61.9^{ab}	67.8^{a}	2.25	0.009	<0.01	0.090	0.015
Mg	21.8^{a}	8.60^{d}	13.9^{bcd}	16.2^{abc}	11.1^{cd}	19.9a^{b}	16.7^{abc}	2.18	<0.01	0.018	0.068	0.981
Na	74.9	69.3	75.7	74.1	72.0	73.4	73.3	2.34	0.101	0.666	0.484	0.202
P	51.0^{d}	42.6^{e}	54.8^{cd}	57.9^{bc}	55.6^{cd}	60.9^{ab}	64.1^{a}	1.66	<0.01	<0.01	<0.01	0.010
AMEn	3.35^{abc}	3.24^{d}	3.33^{bc}	3.36^{ab}	3.28^{cd}	3.37^{ab}	3.40^{a}	0.02	<0.01	<0.01	0.285	0.129

AMEn, apparent metabolizable energy corrected for nitrogen; DM, dry matter; PC, positive control; SEM, standard error of the mean.
[1] Negative control, similar to PC except available P and Ca which were less than in PC by 40.0% and 16.8%, respectively.
[2] Contrasts: 1, PC vs negative control; 2, 3, and 4 linear, quadratic and cubic response to supplemental phytase.
Within a row means without common letters are significantly different ($p<0.05$).
N = 10.

AMEn of birds receiving 2,000 FTU was greater by 160 kcal/kg DM than for birds fed the NC diet without phytase.

DISCUSSION

Low levels of Ca and P were chosen for NC diet so that the possibility of restoring normal growth and performance by adding phytase to the diet could be investigated. The 16% decline in BW gain in birds fed NC diet relative to birds fed PC was of similar magnitude to bone ash content reduction in tibia (13%) and toe (16%) and demonstrated essentiality of Ca and P for skeletal integrity and growth performance (Waldroup, 1999). In the body, 99% of Ca and 80% of P reserves are located in the skeleton. The remaining 1% of Ca and 20% of P reserves are extremely important for the biochemical processes and as constituents of the organic components (Kiarie and Nyachoti, 2009). Supplementation of NC diets with phytase significantly improved growth performance and bone mineralization. The efficiency of the phytase in restoring the bird's growth performance and bone mineralization to that of the PC fed birds could be seen at the lowest (250 FTU) inclusion. However, the bird response to phytase dosing was linear with birds receiving NC+2,000 FTU showing the greatest improvement on BW gain (20%), FCR (7.4%), tibia ash (18.0%) and toe ash (24.4%) relative to birds fed NC diet without phytase. Our data agree with a recent study that reported improvement of 24.9%, 8.0%, and 15.8% for BW gain, FCR and tibia ash, respectively in broiler birds fed this phytase at 1,000 FTU over a range of dietary Ca concentrations (0.40% to 1.0%) at a constant 0.28% available P (Amerah et al., 2014).

The foregut of monogastric has only a very limited ability to hydrolyse phytate P (inositol hexaphosphate) in feedstuffs of plant origin due to the lack of significant endogenous phytase activity and low microbial population (Selle and Ravindran, 2007). Phosphorus is absorbed as orthophosphate, and thus utilization of phytate P by monogastrics will largely depend on their capability to hydrolyse phytate. Relative to NC birds, PC birds had most of the available P supplied by monocalcium phosphate but both diets were presented with similar dietary phytate P concentrations (~0.27%). Birds fed NC without phytase had 26% lower AID of P compared to PC fed birds. Addition of phytase at the lowest dose (250 FTU) restored AID of P to that of PC and further dosing resulted to higher AID of P relative to PC. The efficiency of the test phytase on ileal phytate P degradation was recently shown to be between 76 to 88% in broilers fed corn-soybean meal based diets with differing levels of Ca (Amerah et al., 2014). Such a degradation range was not surprising as the level of Ca is known to affect phytase ability to degrade phytate P in the gut (Tamim et al., 2004; Selle et al., 2009). Dephosphorylation of phytate by phytase takes place in a stepwise manner (Selle and Ravindran, 2007). Within the time and environmental constraints in the gut of the chicken, this dephosphorylation is rate limited to product inhibition (perhaps related to pH changes as phosphoric acid is produced) and an inherently lower rate of hydrolysis of the lower molecular weight inositol polyphosphate esters (Greiner et al., 2000; Selle and Ravindran, 2007). However, the observed higher digestibility and retention of P with the addition of higher relative to lower dose of phytase seems to suggest that addition of higher concentrations of phytase allows dephosphorylation to occur more effectively in the relatively restricted conditions within the gastrointestinal tract of the chicken. Similarly, Cowieson et al. (2006) demonstrated that high doses of phytase (>1,200 FTU/kg) improved apparent phytate P digestibility and total P digestibility compared with lower doses of phytase.

It has been suggested that dietary microbial phytases can also improve digestibility of minerals other than P, particularly divalent cations such as Ca and Mg that can complex phytate. The efficacy of phytase in releasing complexed minerals most likely relates to the relative solubility of the phytate-cation complexes in the gastrointestinal tract, which is in turn a function of the pH

and the molar ratio of minerals to phytate present (Wise, 1983; Selle et al., 2009). The site of insoluble phytate-cation complexes formation is relevant to the efficacy of exogenous phytases where the conventional view is that complex formation occurs in the small intestine (Wise, 1983). The site of phytase action in the gastrointestinal tract of poultry has received little attention. However, the crop was reported to be very probably the primary site of phytate dephosphorylation by supplementary phytase (Selle and Ravindran, 2007). To guarantee an efficient phytate dephosphorylation in the crop, stability in an acid environment and resistance to pepsin are properties that are reported to be highly desirable for feed phytases. It is therefore plausible that a phytase capable of rapid degradation of phytate in the acidic region of the crop would attenuate the formation of cations-complexes in the lower gut and therefore increased cations digestion and absorption. The phytase evaluated in the present study has been shown to exhibits a 1.5 to 2.5 fold higher activity than the *E. coli* phytase in the pH range of pH 3 to 5 (Yu et al., 2014). This may explain why phytase improved utilization of Ca and Mg in the present studies and agree with several other studies reporting phytase mediate increase in Ca and Mg retention (Ravindran et al., 2008; Santos et al., 2008; Rutherfurd et al., 2012). Phytate has been shown to reduce the ileal Na digestibility in broilers and piglets (Ravindran et al., 2008; Woyengo et al., 2009). This may explain the linear improvement of the AID of Na due to supplemental phytase.

At present, the mechanisms underlying the AA-associated responses to added phytase are largely speculative. It has been suggested that the *de novo* formation of binary protein-phytate complexes in the acidic regions of the gastrointestinal tract, which are refractory to pepsin activity, may be the key mechanism whereby phytate depresses the digestibility of dietary AA (Cowieson et al., 2004; Onyango et al., 2009; Selle et al., 2012). The other possible mode of action is that phytate may induce increased endogenous AA flows (Cowieson et al., 2004; Selle et al., 2012). Moreover, the capacity of phytate to drag Na into the small intestinal lumen, which is ameliorated by phytase, may mean that phytate could compromise intestinal uptakes of dietary and endogenous AA by impeding Na+-dependent transport systems and Na+, K+-ATPase activity (Glynn, 1993; Woyengo et al., 2012). These phytate mediated mechanisms would presumably singly or collectively depress digestibility and absorption of AA, which should be countered, at least in part, by phytase supplementation (Selle et al., 2012). It is interesting that although the AID of most indispensable AA were improved by the addition of phytase there was a large variation in response that was dependent on the AA type and phytase dose. The AID of mean indispensable AA was 2.8% higher for birds fed NC with 1,000 FTU relative to birds fed NC without phytase. This value was mainly due to higher digestibility of His, Ile, Leu, Thr, and Val whose AID values were respectively 2.9%, 4.8%, 3.0%, 2.9%, and 6.1% higher in birds fed 1,000 FTU than for birds fed NC diet. This is in agreement with findings by Ravindran et al. (1999), and Coweison et al. (2006), who found that the digestibility of Val, Thr, and Ile in particular was improved by phytase. Furthermore, Rutherfurd et al. (2002) found that phytase improved the digestibility of Asp, Val and Thr compared with unsupplemented control. Although published results on the effect of phytase on AA digestibilities vary (Selle et al., 2000; Adeola and Sands, 2003; Selle et al., 2012), it is clear that, when phytase influences AA digestibility coefficients, it does not do so to the same extent for all AA. This may be linked to differential interactions between amino groups and phytate or it may be associated with the ability of phytate to increase the loss of endogenous compounds, such as mucins, that are rich in certain AA (Cowieson et al., 2004).

Consistent with the effect of phytase on AA digestibility, the effect on AME can be variable (Selle and Ravindran, 2009). Cowieson et al. (2006) reported that phytase improved AMEn by 50 to 150 kcal/kg in broiler chickens fed corn-soybean meal based diets depending on the dose of phytase that ranged from 150 to 24,000 FTU/kg. In the current study, phytase improved AMEn by 40 to 160 kcal/kg DM dose dependently. Whether phytase can improve metabolizable energy consistently is still open for debate (Selle and Ravindran, 2007; Selle et al., 2012). However, it is noteworthy that although phytase improved the energy metabolizability to that of the PC diet in the current study, additional beneficial effects of higher doses of phytase was apparent for DM and nitrogen metabolizability. This has been interpreted as the reduced capacity of lower molecular weight isopropyl esters to form insoluble/indigestible complexes with fat and starch, attenuate protein digestibility as previously discussed and increase luminal Na excretion to compromise Na-dependent co-transport mechanisms involved in the intestinal uptake of glucose and certain AA in broilers (Selle et al., 2012). This is supported by recent data that showed that commercial phytase preparation improved gene expression of the peptides, fatty acids, calcium and phosphorous transporters in swine intestines correlating well with growth performance and nutrients utilization (Vigors et al., 2014). The increase in the gene expression of these nutrient transporters would indicate that intestinal nutrient transporter gene expression is a mechanism involved in the uptake of nutrients following the degradation of the phytate molecule. It is important to note that the improvements seen in the current study were obtained despite the fact that the diets were designed to be nutritionally identical except for

Ca and P. Thus, if the NC diet was designed to account for the improvements in nutritional status caused by phytase supplementation, then it is reasonable to have anticipated greater phytase mediated improvements on growth performance. An interpretation has also been put forward involving the role of P as an important element in the active transportation of nutrients in the gut (Selle and Ravindran, 2009; Selle et al., 2012). Thus, Martinez-Amezcua et al. (2006) observed that adding either phytase or inorganic P source to a P deficient diet resulted to comparable AA digestibility in broilers. The comparable increases in AA digestibility generated by the phytase inclusion and inorganic P (Akin to the PC diet in the current study) was interpreted to stem from the provision of additional P because P is essential for transport systems involved in the intestinal uptake of AA (Martinez-Amezcua et al., 2006; Vigors et al., 2014). This may provide additional explanation as to the differences seen in PC vs NC diets in terms of some AA digestibility and AMEn.

It can be concluded that supplemental phytase was effective in improving the nutritional value of corn- and soy-based diet formulated to be suboptimal in terms of available Ca and P. Furthermore, phytase can improve AA digestibility, metabolizability of energy, DM, nitrogen and minerals. The benefits on AA and energy were maximized at relatively higher doses in accord with the increased loss of phytate P as phytase activity increased.

REFERENCES

Adedokun, S. A., A. Owusu-Asiedu, P. Plumstead, and O. Adeola. 2013. The efficacy of graded levels of a new 6-phytase from *Buttiauxella spp*. expressed in *Trichoderma reesei* on ileal amino acid digestibility in pigs fed a corn-soybean meal-wheat midds corn DDGs-based diet. J. Anim. Sci. 91(E-Suppl. 2):411.

Adeola, O. and J. S. Sands. 2003. Does supplemental dietary microbial phytase improve amino acid utilization? A perspective that it does not. J. Anim. Sci. 81:E78-85E.

Adeola, O. and A. J. Cowieson. 2011. Opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. J. Anim. Sci. 89:3189-3218.

Association of Official Analytical Chemists (AOAC). 1984. Official Methods of Analysis. 14th ed. AOAC, Washington, DC, USA.

Association of Official Analytical Chemists (AOAC). 1990. Official Methods of Analysis. 15th ed. AOAC, Washington, DC, USA.

Association of Official Analytical Chemists (AOAC). 2005. Official Methods of Analysis of AOAC International. 18th ed. AOAC Int., Gaithersburg, MD, USA.

Amerah, A. M., P. W. Plumstead, L. P. Barnard, and A. Kumar. 2014. Effect of calcium level and phytase addition on ileal phytate degradation and amino acid digestibility of broilers fed corn-based diets. Poult. Sci. 93:906-915.

Canadian Council on Animal Care. 2009. Guide to Care and Use of Experimental Animals. VI. Canadian Council on Animal Care. Ottawa, ON, Canada.

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.

Cowieson, A. J. and O. Adeola. 2005. Carbohydrases, protease, and phytase have an additive beneficial effect in nutritionally marginal diets for broiler chicks. Poult. Sci. 84:1860-1867.

Cowieson, A. J., T. Acamovic, and M. R. Bedford. 2006. Supplementation of corn–soy-based diets with an *Escherichia coli*-derived phytase: effects on broiler chick performance and the digestibility of amino acids and metabolizability of minerals and energy. Poult. Sci. 85:1389-1397.

Glynn, I. M. 1993. All hands to the sodium pump. J. Physiol. 462: 1-30.

Greiner, R. and U. Konietzny. 2010. Phytases: Biochemistry, Enzymology and Characteristics Relevant to Animal Feed Use. In: Enzymes in Farm Animal Nutrition, 2nd ed., (Eds. M. R. Bedford and G. G. Partridge). CAB International, Wallingford, UK. pp. 96-128.

Greiner, R., N.-G. Carlsson, and M. L. Alminger. 2000. Stereospecificity of myo-inositol hexakisphosphate dephosphorylation by a phytate-degrading enzyme of *Escherichia coli.* J. Biotechnol. 84:53-62.

Kiarie E. and C. M. Nyachoti. 2009. Bioavailability of calcium and phosphorous in feedstuffs for farm animals. In: Phosphorous and Calcium Utilization and Requirements in Farm Animals (Eds. DMSS Vitti and E Kebreab). CAB International, Wallingford, UK. pp. 76-83.

Kiarie, E., L. F. Romero, and C. M. Nyachoti. 2013. The role of added feed enzymes in promoting gut health in swine and poultry. Nutr. Res. Rev. 26:71-88.

Latta, M. and M. A. Eskin. 1980. A simple and rapid colorimetric method for phytate determination. J. Agric. Food Chem. 28:1313-1315.

Lomer, M. C. E., R. P. H. Thompson, J. Commisso, C. L. Keen, and J. J. Powell. 2000. Determination of titanium dioxide in foods using inductively coupled plasma optical emission spectrometry. Analyst 125:2339-2343.

Martinez-Amezcua, C., C. M. Parsons, and D. H. Baker. 2006. Effect of microbial phytase and citric acid on phosphorus bioavailability, apparent metabolizable energy, and amino acid digestibility in distillers dried grains with solubles in chicks. Poult. Sci. 85:470-475.

Mills, P. A., R. G. Rotter, and R. R. Marquardt. 1989. Modification of the glucosamine method for the quantification of fungal contamination. Can J. Anim. Sci. 69:1105-1106.

NRC. 1994. Nutrient Requirements of Poultry. 19th rev. ed. Natl. Acad. Press, Washington, DC, USA.

Onyango, E. M., E. K. Asem, and O. Adeola. 2009. Phytic acid increases mucin and endogenous amino acid losses from the gastrointestinal tract of chickens. Br. J. Nutr. 101:836-842.

Ravindran, V., S. Cabahug, G. Ravindran, and W. L. Bryden. 1999. Influence of microbial phytase on apparent ileal amino acid digestibility of feedstuffs for broilers. Poult. Sci. 78:699-706.

Ravindran, V. 2013. Feed enzymes: The science, practice, and metabolic realities. J. Appl. Poult. Res. 22:628-636.

Ravindran, V., A. J. Cowieson, and P. H. Selle. 2008. Influence of dietary electrolyte balance and microbial phytase on growth

performance, nutrient utilization, and excreta quality of broiler chickens. Poult. Sci. 87:677-688.

Rutherfurd, S. M., T. K. Chung, and P. J. Moughan. 2002. The effect of microbial phytase on ileal phosphorus and amino acid digestibility in the broiler chicken. Br. Poult. Sci. 43:598-606.

Rutherfurd, S. M., T. K. Chung, P. C. H. Morel, and P. J. Moughan. 2004. Effect of microbial phytase on ileal digestibility of phytate phosphorus, total phosphorus, and amino acids in a low-phosphorus diet for broilers. Poult. Sci. 83:61-68.

Rutherfurd, S. M., T. K. Chung, D. V. Thomas, M. L. Zou, and P. J. Moughan. 2012. Effect of a novel phytase on growth performance, apparent metabolizable energy, and the availability of minerals and amino acids in a low-phosphorus corn-soybean meal diet for broilers. Poult. Sci. 91:1118-1127.

Santos, F. R., M. Hruby, E. E. M. Pierson, J. C. Remus, and N. K. Sakomura. 2008. Effect of phytase supplementation in diets on nutrient digestibility and performance in broiler chicks. J. Appl. Poult. Res. 17:191-201.

Selle, P. H., V. Ravindran, R. A. Caldwell, and W. L. Bryden. 2000. Phytate and phytase: Consequences for protein utilisation. Nutr. Res. Rev. 13:255-278.

Selle, P. H. and V. Ravindran. 2007. Microbial phytase in poultry nutrition. Anim. Feed. Sci. Technol. 135:1-41.

Selle, P. H., A. J. Cowieson, and V. Ravindran. 2009. Consequences of calcium interactions with phytate and phytase for poultry and pigs. Livest. Sci. 124:126-141.

Selle, P. H., A. J. Cowieson, N. P. Cowieson, and V. Ravindran. 2012. Protein-phytate interactions in pig and poultry nutrition: A reappraisal. Nutr. Res. Rev. 25:1-17.

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.

Um, J. S., H. S. Lim, S. H. Ahn, and I. K. Paik. 2000. Effects of microbial phytase supplementation to low phosphorus diets on the performance and utilization of nutrients in broiler chickens. Asian Australas. J. Anim. Sci. 13:824-829.

Vigors, S., T. Sweeney, C. J. O'Shea, J. A. Browne, and J. V. O'Doherty. 2014. Improvements in growth performance, bone mineral status and nutrient digestibility in pigs following the dietary inclusion of phytase are accompanied by modifications in intestinal nutrient transporter gene expression. Br. J. Nutr. 112:688-697.

Waldroup, P. W. 1999. Nutritional approaches to reducing phosphorus excretion by poultry. Poult. Sci. 78:683-691.

Wise, A. 1983. Dietary factors determining the biological activity of phytates. Nutr. Abstr. Rev. Clin. Nutr. 53:791-806.

Woyengo T. A., A. J. Cowieson, O. Adeola, and C. M. Nyachoti. 2009. Ileal digestibility and endogenous flow of minerals and amino acids: responses to dietary phytic acid in piglets. Br. J. Nutr. 102:428-433

Woyengo, T. A., E. Kiarie, and C. M. Nyachoti. 2010. Metabolizable energy and standardized ileal digestible amino acid contents of expeller-extracted canola meal fed to broiler chicks. Poult. Sci. 89:1182-1189.

Woyengo, T. A., D. Weihrauch, and C. M. Nyachoti. 2012. Effect of dietary phytic acid on performance and nutrient uptake in the small intestine of piglets. J. Anim. Sci. 90:543-549.

Yu, S., A. J. Cowieson, C. Gilbert, P. Plumstead, and S. Dalsgaard. 2012. Interactions of phytate and myo-inositol phosphate esters (IP1-5) including IP5 isomers with dietary protein and iron and inhibition of pepsin. J. Anim. Sci. 90:1824-1832.

Yu, S., M. F. Kvidtgaard, M. F. Isaksen, and S. Dalsgaard. 2014. Characterization of a mutant Buttiauxella phytase using phytic Acid and phytic acid-protein complex as substrates. Anim. Sci. Lett. 1:18-32.

Zhang X., D. A. Roland, G. R. McDaniel, and S. K. Rao. 1999. Effect of Natuphos phytase supplementation to feed on performance and ileal digestibility of protein and amino acids of broilers. Poult. Sci. 78:1567-1572.