Thermo-stable *Escherichia coli* phytase improves growth performance and nutrient utilization in broilers fed mash and pelleted corn–soybean-meal-based diets

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Woyengo, T. A., Emiola, A., Owusu-Asiedu, A., Guenter, W., Simmins, P. H. and Nyachoti, C. M. 2010. **Thermo-stable *Escherichia coli* phytase improves growth performance and nutrient utilization in broilers fed mash and pelleted corn–soybean-meal-based diets**. Can. J. Anim. Sci. **90**: 1–8. The bio-efficacy of *Escherichia coli*-derived phytase produced by thermo-protective coating technology (coated phytase) was evaluated in mash or pelleted broiler diets. Three hundred and twenty-four broilers were divided into 54 groups of six birds and fed nine corn-based diets (six replicates per diet) from 1 to 22 d of age. The nine diets were a positive control (PC) (calcium, 0.9% and non-phytate phosphorus, 0.45%) fed as mash plus 2×4 factorial of a negative control (calcium, 0.78% and non-phytate phosphorus, 0.26%) fed as mash (NC-M) or pellet (NC-P) and with coated phytase at 0, 500, 600 or 700 FTU kg^{-1}. The diets were pelleted at 80°C, and pressure load of 40 psi. Body weight gain (BWG) for the PC diet was higher ($P < 0.001$) than that for the NC-M or NC-P diet. The BWG and tibia ash were linearly increased ($P < 0.001$) by increased level of the phytase in NC-M or NC-P diet. The BWG for NC-M or NC-P diet reached that of the PC diet when phytase was supplemented at 600 or 700 FTU kg^{-1}. In conclusion, the coated phytase improved nutrient utilization in broilers, and its bio-efficacy was unaffected by the pelleting process.

Key words: Broiler, digestibility, pelleting, performance, thermostable-phytase

Woyengo, T. A., Emiola, A., Owusu-Asiedu, A., Guenter, W., Simmins, P. H. et Nyachoti, C. M. 2010. **La phytase thermostable d'*Escherichia coli* améliore la croissance et l'assimilation des aliments chez les poulets de chair nourris de pâtée ou d'agglomérés de maïs et de tourteau de soja**. Can. J. Anim. Sci. **90**: 1–8. Les auteurs ont évalué l'efficacité biologique de la phytase d'*Escherichia coli* préparée avec un enduit thermoprotecteur (phytase enrobée) quand on s'en sert pour enrichir la ration en pâtée ou en agglomérés des poulets de chair. Trois cent vingt-quatre poulets de chair ont été répartis en 54 groupes de six et ont reçu neuf rations à base de maïs (six répétitions par ration) du 1^er au 22^e jour. Les neuf rations étaient les suivantes : ration témoin positive (0,9% de calcium et 0,45% de phosphore non phytique) sous forme de pâtée (PC), plus répartition factorielle 2×4 de rations témoin négatives (0,78% de calcium et 0,26% de phosphore non phytique) sous forme de pâtée (NC-M) ou d'agglomérés (NC-P) enrichies avec 0, 500, 600 ou 700 FTU de phytase enrobée par kg. Les rations ont été agglomérées à une température de 80°C et à une pression de 40 livres au pouce carré. La ration PC accroît plus ($P < 0{,}001$) le gain de poids corporel (GPC) que les rations NC-M ou NC-P. Le GPC et le poids des cendres du tibia augmentent de façon linéaire ($P < 0{,}001$) avec la concentration de phytase dans la ration NC-M ou NC-P. Le GPC des oiseaux nourris avec la ration NC-M ou NC-P atteint un niveau équivalent à celui obtenu avec la ration PC dès que le supplément de phytase atteint 600 ou 700 FTU par kg. On en conclut que la phytase enrobée rehausse l'assimilation des aliments chez le poulet de chair et que le procédé d'agglomération n'en modifie pas l'efficacité biologique.

Mots clés: Poulet de chair, digestibilité, agglomération, rendement, phytase thermostable

About two-thirds of the total phosphorus (P) in feedstuffs of plant origin is present in the phytate form (Eeckhout and De Paepe 1994). Phosphorus in this form is only partially available to poultry, thus necessitating supplementation of their diets with inorganic forms of P, which are expensive, to meet P requirements. In addition, phytate has also been reported to form complexes with divalent cations and amino acids (AA) (Sebastian et al. 1997), and starch (Thompson and Yoon 1984), leading to reduced digestibility and utilization of these nutrients by poultry. Thus, the presence of phytate in diets for poultry can reduce the efficiency of nutrient utilization, which may lead to increased cost of feeding and environmental pollution due to increased discharge

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Abbreviations: **AA**, amino acids; **AID**, apparent ileal digestibility; **AME**, apparent metabolizable energy; **BWG**, body weight gain; **DM**, dry matter; **FCR**, feed conversion ratio; **FI**, feed intake; **PC**, positive control of unabsorbed nutrients, especially nitrogen (N) and P to the environment (Lenis and Jongbloed 1999).

Several studies have demonstrated that exogenous dietary phytase improves nutrient utilization and performance of broilers through phytate hydrolysis (Bedford 2000; Selle and Ravindran 2007). Thus, several phytase products have been developed and are commercially available. Most of the commercially available phytases are from *Aspergillus niger*, *Peniophora lycii* and *Escherichia coli* (Selle and Ravindran 2007), and they have an optimum temperature of 40 to 60°C, beyond which they lose most of their activity (Ullah et al. 2000; Lassen et al. 2001; Seonho et al. 2005). Slominski et al. (2007) reported a reduction in the activity of a commercial phytase in excess of 50% after pelleting a diet at 67°C, whereas Boyce and Walsh (2006) reported reduction in activities of four commercial phytases by a range of 14 to 72% after heating them for 5 min at 80°C. Poultry feeds are, in most cases, steam pelleted at 65 to 80°C before feeding to improve feed efficiency (Lei and Stahl 2001). Thus, the higher temperatures that are encountered during feed pelleting could limit the efficacy of the phytases if they are included in the diets before pelleting.

Phytase may be applied to a diet after pelleting either as granules or sprayed on as a liquid to avoid the loss of activity that is associated with pelleting (Selle and Ravindran 2007). However, this method of phytase application requires heavy investment in the application equipment and may result in non-uniform distribution of phytase in the diet. Thus, the use of phytase that can withstand the heat that diets are subjected to during pelleting would be more advantageous as it would allow thorough mixing of phytase with diet prior to pelleting, leading to uniform distribution of phytase in the diet. Thermostable phytase can be achieved through alteration of genes that encode the phytase (Garrett et al. 2004) or application of thermo-protective coating technology (Barletta 2007). The objective of this study was to determine the effect of an *E. coli* derived phytase that was produced by a thermo-protective coating technology on performance and nutrient utilization of broilers fed corn–soybean-meal-based diets in mash or pelleted form from hatch to 21 d of age.

MATERIALS AND METHODS

Birds and Housing

Three hundred and twenty-four 1-d-old male broiler chicks (Ross 308) were obtained from a commercial hatchery (Carlton Hatchery, Grunthal, MB) and used in this experiment, which lasted for 21 d. The chicks were individually weighed upon arrival and divided into 54 groups (six birds per group) of similar body weights. They were then group-weighed, and each group was housed in a cage in electrically heated Petersime battery brooders (Petersime Incubator Company, Gettysburg, OH). The brooder and room temperatures were set at 32 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 daily throughout the experiment. All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Protocol Management and Review Committee, and birds were handled in accordance with guidelines described by the Canadian Council on Animal Care (CCAC 1993).

Experimental Diets

The nine experimental diets included a positive control (PC) diet (fed as mash) plus 2×4 factorial of a negative control fed as mash (NC-M) or pellet (NC-P) and with coated phytase at 0, 500, 600 or 700 FTU kg^{-1} (Tables 1 and 2). The PC diet was formulated to meet or exceed the National Research Council (1994) nutrient requirements for broiler chicks (Table 1). The negative control diet was similar to the PC diet, except that the calcium (Ca) and non-phytate P levels were reduced by 0.20 and 0.19 percentage units, respectively, so as to maximize response to enzyme supplementation (Table 1), and was mixed as a basal diet, from which all other diets (except the PC diet) were derived.

Table 1. Composition of the basal diets used in the study (as-fed basis)

Item	Positive control	Negative control
Ingredients (%)		
Corn	54.22	53.22
Soybean meal	38.21	38.03
Sodium bicarbonate	0.20	0.20
Canola oil	4.00	4.00
Monocalcium phosphate	1.52	0.62
Limestone	1.39	1.47
Iodized salt	0.30	0.30
L-Lysine (79.0%)	0.12	0.12
DL-Methionine (99.0%)	0.24	0.24
Titanium dioxide	0.30	0.30
Mineral and vitamin premix[z]	0.50	0.50
Calculated nutrient composition		
ME (kcal kg^{-1})	3060	3060
CP (%)	22.9	22.8
Ca (%)	0.90	0.78
Total P (%)	0.70	0.52
Non-phytate P (%)	0.45	0.26
Lysine (%)	1.39	1.38
Methionine (%)	0.59	0.59
Threonine (%)	0.87	0.87
Analyzed nutrient composition		
AME (kcal kg^{-1})	2992	2922
CP (%)	22.6	22.4
Ca (%)	1.14	0.95
Total P (%)	0.72	0.53

[z]Supplied per kilogram of diet: vitamin A, 8255 IU; vitamin D_3, 3000, 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, 1301; pantothenic acid, 11 mg; vitamin B_{12}, 0.013 mg; Mn, 70 mg; Zn, 80 mg; Fe, 80 mg; and Cu, 10 mg.

Table 2. Coated phytase activity in the experimental diets

Diet[z]	Phytase activity (FTU kg^{-1})
1. PC	71
2. NC-M	60
3. NC-P	73
4. NC-M + 500 FTU kg^{-1}	568
5. NC-P + 500 FTU kg^{-1}	558
6. NC-M + 600 FTU kg^{-1}	661
7. NC-P + 600 FTU kg^{-1}	608
8. NC-M + 700 FTU kg^{-1}	786
9. NC-P + 700 FTU kg^{-1}	758

[z]PC, positive control diet in mash form; NC-M, negative control diet in mash form; NC-P, negative control diet in pellet form.

The coated phytase used was a 6-phytase (EC 3.1.3.26; Danisco Animal Nutrition, Marlbourough, UK), which was derived from *E. coli*, and was produced by a thermo-protective coating technology. The diets were pelleted at a temperature of 80°C and pressure load of 40 psi. All the diets contained titanium dioxide (0.3%) as an indigestible marker.

Experimental Procedures

The nine diets were randomly allocated to 54 cages to give six replicates per diet. The body weight and feed consumption for each cage were determined weekly on days 8, 15 and 22 after withdrawing feed for 2 h. On days 19, 20 and 21, samples of excreta were collected, pooled per cage and stored frozen at −20°C for the determination of apparent nutrient retention. Care was taken during the collection of excreta samples to avoid contamination from feathers and other foreign materials. On the last day of the experiment (day 22), all birds were killed by cervical dislocation. The left tibiae and the contents of the ileum (from Meckel's diverticulum to approximately 1 cm above the ileal–cecal junction) were obtained and stored frozen at −20°C for later determination of tibia ash and apparent ileal nutrient digestibilities.

Sample Preparation and Chemical Analyses

The tibiae 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 12 h for the determination of tibia ash. Ileal and excreta samples were freeze-dried and finely ground in a grinder (CBG5 Smart Grind, Applica Consumer Products, Inc., Shelton, CT) to pass through a 1-mm screen and thoroughly mixed before analysis. Basal (PC and negative control) diet samples were similarly ground before analysis.

Dry matter (DM) was determined according to the method of the Association of Official Analytical Chemists (AOAC 1990, method 925.09), and gross energy was determined using the Parr adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL). Nitrogen was determined using a N analyzer (Model NS-2000; LECO Corporation, St. Joseph, MI). Samples for Ca and P analyses were ashed and digested according to AOAC (1990) procedures (method 990.08) and read on a Varian Inductively Coupled Plasma (Varian Inc, Palo Alto, CA). Diet and digesta samples were analyzed for AA according to the AOAC (1990, method 982.30) procedure, using a cation exchange column in an LKB 4151 Alpha plus AA analyzer (LKB Biochrom, Cambridge, UK) equipped with an LKB 4029 programmer and a 3393A Hewlett-Packard Integrator (Hewlett-Packard Co., Avondale, PA). Briefly, 100 mg of each sample was hydrolyzed with 6 mol L^{-1} HCl at 110°C for 24 h. Cysteine and methionine were determined by oxidizing the sample with performic acid prior to hydrolysis. Tryptophan was not determined. Samples for titanium analysis were analyzed according to the procedure of Myers et al. (2004). Phytase activity in the diets was conducted by Danisco Animal Nutrition (Marlborough, UK) as described by Engelen et al. (2001).

Calculations and Statistical Analysis

Apparent ileal digestibility (AID) and apparent retention of nutrients were calculated using titanium as the indigestible marker (Nyachoti et al. 1997). Apparent metabolizable energy (AME; kcal kg^{-1}) was calculated using the following equation:

$$\text{AME (kcal kg}^{-1}) = \{[\text{apparent retention of gross energy (\%)}] \times [\text{dietary gross energy content (kcal kg}^{-1})]\}/100$$

Tibia ash was calculated as follows: Tibia ash (%) = 100 × (weight of tibia ash/weight of fat free tibia).

Data were analyzed using the mixed model procedure (PROC MIXED) of the SAS statistical program (SAS 9.1, SAS Institute, Inc., Cary, NC) in a completely randomized design. Specific contrasts were used to determine the main effects of diet form (Mash vs. pellet) and phytase, and their interaction. Linear and quadratic contrasts for unequally spaced levels (Gill 1978) were performed to assess the effect of increasing the level of the phytase in the NC-M or NC-P diets. Confidence intervals (95%) for the PC diet means were calculated for comparison of the PC diet with the phytase-supplemented diets. That is, means for phytase-supplemented diets that were lower than the lower confidence limits for the PC diet means were considered to be significantly lower than those for the PC diet, whereas means for phytase-supplemented diets that were higher than the upper confidence limits for the PC diet means were considered to be significantly higher than those for the PC diet.

RESULTS

The recovery of the supplemental phytase in the diets ranged from 89 to 104% (Table 2). The loss of phytase activity in the diets due to pelleting ranged from 4.53 to 10.98% of the original activity. The analyzed AME, crude protein and P values in the basal diets were close to the calculated values, whereas the analyzed Ca values were slightly higher than the calculated values (Table 1). Data for body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) and tibia ash are presented in Table 3. The dietary treatment effects on BWG, FI, and FCR at 1, 2 and 3 wk of age were similar and thus only data on the same response criteria at 3 wk of age are presented. The BWG, FI and tibia ash of chicks fed the NC-M or NC-P diet were lower ($P < 0.001$) than those for chicks fed the PC diet. An increase in the level of the phytase supplementation to the NC-M or NC-P diet from 0 to 700 FTU kg^{-1} resulted in a linear increase ($P < 0.001$) in BWG, FI and tibia ash. There was an interaction ($P < 0.05$) between diet form and phytase on BWG, FI and tibia ash, such that the values of these response criteria were lower for the NC-P diet than for NC-M diet when the phytase was supplemented at 0 FTU kg^{-1}, but not when it was supplemented at the higher levels. The BGW and FI values for the NC-M or NC-P diets with phytase at 600 or 700 FTU kg^{-1} reached ($P > 0.05$) those for the PC diet, whereas tibia ash values for the phytase-supplemented NC-M or NC-P diets did not reach ($P < 0.05$) that for the PC diet.

Data for AID of P, Ca and N, indispensable AA, and dispensable AA, are shown in Tables 4, 5 and 6, respectively. The AID of P was higher ($P = 0.034$) for pellet diets than for mash diets, whereas AID of N was lower ($P = 0.013$) for pellet diets than for mash diets. An increase in the level of the supplemental phytase from 0 to 700 FTU kg^{-1} resulted in a linear increase ($P < 0.001$) in the AID of P and N for the NC-M and NC-P diets. Also, the AID of Ca in NC-M and NC-P diets tended to increase linearly ($P = 0.064$) with increased level of the phytase supplementation. There was an interaction ($P = 0.039$) between diet form and phytase on AID of P such that the AID of this nutrient was higher for NC-P diet than for NC-M diet when phytase was supplemented at 0 and 500 FTU kg^{-1}, but not when it was supplemented at the higher levels. Increasing the level of the phytase from 0 to 700 FTU kg^{-1} resulted in a linear increase ($P < 0.05$) in AID of methionine, valine and proline, and tended to linearly increase ($P < 0.1$) the AID of arginine, aspartic acid, glutamic acid, glycine and serine (Tables 5 and 6).

The apparent retention of DM and N, and AME values are shown in Table 7. An increase in the level of phytase from 0 to 700 FTU kg^{-1} resulted in a linear increase in the apparent retention of DM and N, and AME values. There was, however, no effect of diet form and interaction between diet form and phytase on the apparent retention of DM and N, and AME values.

DISCUSSION

The loss of phytase activity in the diets due to pelleting was less than 11% of the original activity, which is lower

Table 3. Effect of dietary treatment on body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) and tibia ash of broilers from 1 to 22 d of age

Diet[z]	BWG ($g\ d^{-1}$)	FI ($g\ d^{-1}$)	FCR ($g\ g^{-1}$)	Tibia ash (%)[y]
PC	41.3	55.7	1.35	52.4
NC-M	36.9	47.6	1.29	45.9
NC-P	33.6	42.5	1.27	43.3
NC-M + 500 FTU kg^{-1}	37.1	47.2	1.28	48.6
NC-P + 500 FTU kg^{-1}	38.7	51.3	1.33	49.6
NC-M + 600 FTU kg^{-1}	39.8	51.3	1.29	49.8
NC-P + 600 FTU kg^{-1}	40.5	52.4	1.30	50.6
NC-M + 700 FTU kg^{-1}	41.3	51.4	1.24	50.5
NC-P + 700 FTU kg^{-1}	41.3	53.2	1.29	50.6
SEM	0.88	1.28	0.03	0.62
Contrasts				
Diet form	0.666	0.577	0.101	0.686
Phytase	<0.001	<0.001	0.047	<0.001
Diet form × phytase	0.037	0.007	0.121	0.023
Phytase linear[x]	<0.001	<0.001	0.976	<0.001
Phytase quadratic[w]	0.052	0.529	0.138	0.579
CL for PC[v]				
Lower limit	39.8	53.2	1.29	51.4
Upper limit	42.8	59.8	1.39	53.4

[z]PC, positive control diet in mash form; NC-M, negative control diet in mash form; NC-P, negative control diet in pellet form.
[y]Percentage of free-fat bone.
[x]Phytase linear = linear effect of phytase.
[w]Phytase quadratic = quadratic effect of phytase.
[v]95% confidence limits for positive control diet mean.

Table 4. Effect of dietary treatment on apparent ileal digestibility (%) of phosphorus, calcium and nitrogen in broilers at 3 wk of age

Diet[z]	Phosphorus	Calcium	Nitrogen
PC	43.5	40.0	79.2
NC-M	41.3	44.6	77.5
NC-P	53.5	57.6	75.0
NC-M + 500 FTU kg^{-1}	59.3	58.4	81.3
NC-P + 500 FTU kg^{-1}	63.9	65.4	79.4
NC-M + 600 FTU kg^{-1}	65.7	60.2	81.7
NC-P + 600 FTU kg^{-1}	60.8	54.6	79.7
NC-M + 700 FTU kg^{-1}	65.8	60.4	81.6
NC-P + 700 FTU kg^{-1}	65.0	58.2	79.8
SEM	2.39	4.67	1.10
Contrasts			
Diet form	0.034	0.370	0.013
Phytase	<0.001	0.158	<0.001
Diet form × phytase	0.039	0.211	0.991
Phytase linear[y]	<0.001	0.064	<0.001
Phytase quadratic[x]	0.691	0.332	0.359
CL for PC[w]			
Lower limit	35.0	23.3	76.2
Upper limit	52.1	56.7	82.3

[z]PC, positive control diet in mash form; NC-M, negative control diet in mash form; NC-P, negative control diet in pellet form.
[y]Phytase linear = linear effect of phytase.
[x]Phytase quadratic = quadratic effect of phytase.
[w]95% confidence limits for positive control diet mean.

Table 5. Effect of dietary treatment on apparent ileal digestibility (%) of indispensable amino acids in broilers at 3 wk of age

	Amino acids[y]								
Diet[z]	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
PC	85.1	78.7	77.0	77.8	81.6	94.5	79.7	65.6	73.0
NC-M	82.9	75.7	74.4	72.7	80.1	91.1	76.2	62.8	68.9
NC-P	81.1	73.6	73.1	71.6	78.4	66.9	74.7	61.3	68.7
NC-M + 500 FTU kg^{-1}	81.6	73.0	71.4	70.5	77.3	91.6	73.8	60.8	67.0
NC-P + 500 FTU kg^{-1}	85.0	77.9	77.8	75.7	82.1	88.4	79.6	65.3	72.9
NC-M + 600 FTU kg^{-1}	85.7	79.3	79.1	77.8	83.5	92.3	80.4	68.9	74.8
NC-P + 600 FTU kg^{-1}	82.9	76.7	76.4	73.9	80.0	90.4	76.3	61.0	72.4
NC-M + 700 FTU kg^{-1}	84.7	76.6	76.5	74.3	80.4	87.6	78.3	65.8	72.2
NC-P + 700 FTU kg^{-1}	85.0	78.1	76.6	75.8	81.5	89.4	78.3	67.9	74.2
SEM									
Contrasts									
Diet form	0.836	0.788	0.718	0.773	0.887	0.073	0.989	0.745	0.411
Phytase	0.308	0.390	0.341	0.312	0.466	0.102	0.474	0.425	0.123
Diet form × phytase	0.194	0.271	0.240	0.188	0.102	0.086	0.129	0.191	0.331
Phytase linear[x]	0.072	0.158	0.160	0.127	0.236	0.025	0.149	0.160	0.046
Phytase quadratic[w]	0.618	0.677	0.723	0.692	0.755	0.327	0.748	0.373	0.462
CL for PC[v]									
Lower limit	80.7	72.7	68.8	71.8	76.4	92.6	73.4	56.5	65.5
Upper limit	89.4	84.6	85.2	83.9	86.9	96.3	86.0	74.8	80.4

[z]PC, positive control diet in mash form; NC-M, negative control diet in mash form; NC-P, negative control diet in pellet form.
[y]Arg, arginine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Thr, threonine; Val, valine.
[x]Phytase linear = linear effect of phytase.
[w]Phytase quadratic = quadratic effect of phytase.
[v]95% confidence limits for positive control diet mean.

than the values reported by Slominski et al. (2007) and Boyce and Walsh (2006) for commercial phytases after steam pelleting and heat treatment of the diets, respectively. The negative control diet from which the NC-M and NC-P diets were derived was formulated to be deficient in Ca and non-phytate P so as to maximize

Table 6. Effect of dietary treatment on apparent ileal digestibility (%) of dispensable amino acids in broilers at 3 wk of age

	Amino acids[y]							
Diet[z]	Ala	Asp	Cys	Glu	Gly	Pro	Ser	Tyr
PC	76.3	70.7	58.8	82.0	70.6	75.5	72.3	74.2
NC-M	73.3	67.6	58.5	79.0	69.5	73.1	66.8	72.4
NC-P	71.4	66.5	51.0	77.5	67.5	70.0	65.3	70.7
NC-M + 500 FTU kg^{-1}	70.8	66.0	61.5	77.7	67.4	71.4	66.1	67.0
NC-P + 500 FTU kg^{-1}	75.1	72.1	60.6	81.1	72.1	74.8	70.3	76.8
NC-M + 600 FTU kg^{-1}	76.6	72.1	62.9	82.4	73.5	77.0	71.9	78.0
NC-P + 600 FTU kg^{-1}	73.6	71.8	60.4	79.7	69.8	74.3	67.3	72.6
NC-M + 700 FTU kg^{-1}	75.4	70.3	67.4	81.5	72.7	77.4	70.0	72.5
NC-P + 700 FTU kg^{-1}	75.7	71.2	62.4	81.2	72.8	74.7	72.6	72.5
SEM								
Contrasts								
Diet form	0.953	0.456	0.367	0.835	0.895	0.418	0.925	0.793
Phytase	0.387	0.291	0.451	0.251	0.326	0.136	0.231	0.680
Diet form × phytase	0.359	0.518	0.955	0.325	0.348	0.376	0.269	0.153
Phytase linear[x]	0.146	0.095	0.114	0.066	0.099	0.034	0.053	0.545
Phytase quadratic[w]	0.446	0.992	0.830	0.546	0.427	0.458	0.474	0.928
CL for PC[v]								
Lower limit	70.6	63.2	45.4	77.7	62.6	69.4	65.3	60.7
Upper limit	81.9	78.2	72.1	86.1	78.6	81.6	79.2	87.8

[z]PC, positive control diet in mash form; NC-M, negative control diet in mash form; NC-P, negative control diet in pellet form.
[y]Ala, alanine; Asp, aspartic acid; Cys, cysteine; Glu, glutamic acid; Gly, glycine; Pro, proline; Ser, serine; Tyr, tyrosine.
[x]Phytase linear = linear effect of phytase.
[w]Phytase quadratic = quadratic effect of phytase.
[v]95% confidence limits for positive control diet mean.

Table 7. Effect of dietary treatment on the apparent retention of dry matter and nitrogen, and apparent metabolizable energy (AME) of broilers at three weeks of age

Diet[z]	Dry matter (%)	Nitrogen (%)	AME (kcal kg^{-1})
PC	81.3	62.4	2992
NC-M	77.2	57.5	2922
NC-P	79.6	58.9	3004
NC-M + 500 FTU kg^{-1}	80.7	63.9	3109
NC-P + 500 FTU kg^{-1}	81.1	64.8	3121
NC-M + 600 FTU kg^{-1}	81.2	64.2	3134
NC-P + 600 FTU kg^{-1}	82.2	66.7	3171
NC-M + 700 FTU kg^{-1}	81.8	66.2	3153
NC-P + 700 FTU kg^{-1}	82.9	64.6	3136
SEM	1.09	1.71	41
Contrasts			
Diet form	0.126	0.534	0.347
Phytase	0.007	<0.001	<0.001
Diet form × phytase	0.826	0.692	0.709
Phytase linear[y]	<0.001	<0.001	<0.001
Phytase quadratic[x]	0.781	0.485	0.479
CL for PC[w]			
Lower limit	78.2	56.5	2847
Upper limit	84.4	66.8	3137

[z]PC = positive control diet in mash form; NC-M = negative control diet in mash form; and NC-P = negative control diet in pellet form.
[y]Phytase linear = linear effect of phytase.
[x]Phytase quadratic = quadratic effect of phytase.
[w]95% confidence limits for positive control diet mean.

the response to phytase supplementation. The BWG, FI and tibia ash of broilers fed the NC-M or NC-P diet were poorer than those of birds fed the PC diet, confirming that Ca and non-phytate P were indeed limiting nutrients in the negative control diet.

Phytase has been reported to improve performance and bone mineralization of broilers by improving nutrient digestibility (Bedford 2000; Selle and Ravindran 2007), which is in agreement with results from the current study. The BWG, FI and tibia ash content of broilers fed NC-M or NC-P diets increased linearly with increasing level of supplemental phytase from 0 to 700 FTU kg^{-1}, implying that the optimum level of the phytase used in the current study with regard to improving growth performance and bone mineralization is around 600 FTU kg^{-1} (mash and pellet) for BWG, >700 FTU kg^{-1} (mash) or 700 FTU kg^{-1} (pellet) for FI, and >700 FTU kg^{-1} (mash and pellet diets) for tibia ash. Therefore, it could be inferred that the optimal level (compared with the PC diet) of the phytase used in the current study for bone mineralization is higher than that 700 FTU kg^{-1}.

The BWG and tibia ash content for the NC-M and NC-P diets did not differ at each level (500, 600 or 700 FTU kg^{-1}) of phytase supplementation, indicating that diet pelleting did not affect the influence of the phytase on growth performance and bone mineralization. Also, the BWG values for the NC-M or NC-P diets with the supplemental phytase at 600 or 700 FTU kg^{-1} reached that for the PC diet, indicating that the phytase supplementation at 600 FTU kg^{-1} or more was able to restore the growth performance of broilers fed the NC-M or NC-P diets. The tibia ash values for the phytase-supplemented diets did not, however, reach that for the PC diet, indicating that supplementation of the phytase up to 700 FTU kg^{-1} was not able to restore bone mineralization in NC-M or NC-P diets for broilers. As previously mentioned, the tibia ash values increased linearly with an increase in level of phytase supplementation from 0 to 700 FTU kg^{-1}, implying that a further increase in the level of phytase (beyond 700 FTU kg^{-1}) might have resulted in a further increase in tibia ash content. Shirley and Edwards (2003) reported that the performance and bone mineralization of broilers fed a low Ca and P diet were restored by phytase supplementation at 1500 and 3000 FTU kg^{-1}, respectively, indicating that higher levels of phytase are required for the restoration of bone mineralization than for the restoration of growth performance of broilers fed low Ca and P diets. Therefore, the bone mineralization of birds fed the NC-M or NC-P diets in the current study might have been restored to the PC diet level by phytase supplementation if the supplementation level was greater than 700 FTU kg^{-1}.

It is interesting to note that AID of P for the NC-P diet was superior to that of the NC-M diet. This could be due to improvement in P availability as a result of subjecting the NC-P diet to heat during pelleting. Others have also reported improved P availability in broilers due to heat treatment (El-Hady and Habiba 2003; Amezcua and Parsons 2007). The mechanisms by which heat treatment improves P availability are, however, not yet known. The AID of P was improved by phytase supplementation of the NC-M or NC-P diets, which is in agreement with results from several previous studies (Shirley and Edwards 2003; Dilger et al. 2004; Onyango et al. 2005; Olukosi et al. 2007), and it is due to the hydrolysis of phytate by phytase (Bedford 2000; Selle and Ravindran 2007). The AID of P responded linearly to increasing levels of supplemental phytase from 0 to 700 FTU kg^{-1} in the NC-M or NC-P diets. This implies that a further increase in the level of phytase (beyond 700 FTU kg^{-1}) may have resulted in a further increase in AID of P, which supports the hypothesis mentioned earlier that a further increase in the level of phytase may have resulted in a further increase in tibia ash content. The improved AID of Ca with increased level of the phytase supplementation to the NC-M or NC-P diets could also have been due to the hydrolysis of phytate by the phytase.

The AID of N was lower for pelleted diets than for mash diets, which could be attributed to the effect of steam pelleting the diet at high temperature, and may help explain the poorer performance of birds fed pelleted basal diet compared with those fed the mash basal diet. Phytate directly binds protein as binary phytate-AA complexes or indirectly via cationic bridges as tertiary phytate-mineral-protein complexes (Reddy and Salunkhe 1981). In theory, addition of microbial phytase to phytate-containing diets could potentially improve AA digestibility by releasing the phytate-bound AA. Various researchers have shown improvements in AA digestibility with phytase supplementation in broilers (Ravindran et al. 1999; Ravindran et al. 2001; Dilger et al. 2004; Ravindran et al. 2006). In the current study, phytase supplementation to NC-M or NC-P diets improved the AID of N and of some AA, which is in agreement with results from the previously mentioned studies. The improved N and AA digestibilities with increased level of phytase supplementation implies that the dietary concentration of these nutrients can be reduced to a certain extent without an adverse effect on performance of broilers if the phytase is utilized as a dietary supplement.

Phytase supplementation improved the retention N for NC-M and NC-P diets, which could have been due to improved AA digestibility by phytase supplementation. Phytase supplementation also improved the AME values for NC-M and NC-P diets. Phytase has previously been shown to improve the AME value of diets for broilers (Namkung and Leeson 1999; Ravindran et al. 2000; Olukosi et al. 2007) and results from the present study are in agreement with this. The improvement in dietary AME value due to phytase supplementation has been attributed to its ability to improve the digestibility of fat, carbohydrate and other nutrients (Selle and Ravindran 2007). Like N and AA digestibilities, the improved AME with increased level of phytase supplementation implies that the dietary AME value can be reduced to a certain extent without any negative effect on the performance of broilers if phytase is utilized as a dietary supplement.

In conclusion, an increase in the level of coated phytase from 0 to 700 FTU kg^{-1} resulted in a linear improvement of performance, bone mineralization and nutrient digestibility of broilers fed the low Ca and P corn–soybean-meal-based diet in either mash or pellet form, indicating that the bio-efficacy of the coated phytase used in the current study was not affected by the pelleting process. Therefore, the coated phytase used in the current study can be supplemented to diets that are to be pelleted. The coated phytase did not restore bone mineralization of broilers fed the low Ca and P corn–soybean-meal-based diet to the PC diet fed birds in either mash or pellet form. However, the linear improvement of tibia ash and P digestibility with increased level of supplementation of coated phytase from 0 to 700 FTU kg^{-1} implies that a further increase in level of phytase supplementation (beyond 700 FTU kg^{-1}) could have resulted in a further increase in bone mineralization. Therefore, there is a need to compare bone mineralization of broilers fed a nutrient-adequate diet with those fed a low Ca and P diet when the latter is supplemented with coated phytase at levels greater than 700 FTU kg^{-1}.

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