ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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International Journal of Poultry Science 8 (10): 919-928, 2009
ISSN 1682-8356

Effect of Adding Phytase to Broiler Diets Containing Low and High Phytate Phosphorus: 1. Performance, Phytate P Hydrolysis, Tibia Ash, Litter Phosphorus and Ca and P Digestion and Retention

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Abstract: A 42 d broiler experiment was conducted to determine the effect of added *Escherichia coli* phytase (Phyzyme-XP 5000G) to low and high phytate P (PP) diets on performance and nutrient digestibility. The experiment consisted of 12 treatments with 2 levels of PP: Low-Phytate (LP) group-0.24% and High-Phytate (HP) group-0.32%. Both LP and HP groups had respective Positive Controls (PCs) with 0.39% in the starter and 0.29% NPP in the grower, respectively and 5 basal diets with graded levels (0, 250, 500, 750 and 1000 FTU's/kg diet) of added phytase. The HP diets contained additional PP because canola and rice bran were substituted for part of the corn and soybean meal. A significant ($p<0.001$) phytase effect was found for Body Weight Gain (BWG) Feed Intake (FI) and Feed:Gain (F:G) ratio on d 42 and ileal P digestibility and % tibia ash for both LP and HP groups on d 42. Supplementation of 250 FTU's phytase/kg diet for both LP and HP basal diets produced equivalent body weights ($p>0.05$) to comparable respective PCs. Supplementing 500 FTU's phytase/kg diet in both LP and HP groups resulted in a comparable ($p>0.05$) % tibia ash to respective PC groups. The litter Total Dissolved P (TDP) and Water-Soluble P (WSP) of pens from broilers fed either the LP or HP basal diets with increasing concentrations of phytase were not significantly different ($p>0.05$) compared to the respective PCs. The research shows that adding feed phytase does not necessarily mean that TDP and WSP will be reduced. The P equivalency determined from 42d ileal digesta for added phtase with the LP and HP diets showed that broilers fed the HP diet with 1000 FTU phytase provided 0.17% digestible P compared to 0.13% digestible P from the LP diet. In order to decrease P excretion in broilers, added feed phytase should be considered equal to feed phosphates for providing available P in the gastrointestinal tract and the combination needs to be low enough for optimum performance and retention.

Key words: Phytase, phytate P, phytase P equivalency value, litter total dissolved P, litter water soluble P

INTRODUCTION

The application of poultry waste with increased P to land can become an environmental concern if P is leached from the soil. This environmental problem is posing a threat to intensive poultry farming on a global basis. The low biological availability of Phytate P (PP) from most feedstuffs for poultry means that meeting the nutritional requirement of P will depend upon feeding feed phosphates with higher availability or feeding a combination of a commercial feed phytase plus feed phosphates. The feeding of different types of P such as Non-Phytate P (NPP) from ingredients, feed phosphates, exogenous phytase and PP to supply optimum P levels for poultry requires specific P digestibility and retention data for all ingredients to minimize P loss in the excreta. Feeding poultry more P than can be retained regardless of P type (exogenous phytase should be considered as a P source) will lead to increased losses of P in poultry waste (Manangi and Coon, 2006). Although research has consistently showed that feed phytase increases PP hydrolysis, there still are a limited number of published reports describing how dietary phytase can effectively be used to reduce litter P based on the P consumed vs. soluble P in the litter. The amount of PP that is present and the extent of bioavailability of P from PP hydrolysis with dietary phytase supplementation may vary depending on the type of ingredients (Leske and Coon, 1999; Ravindran *et al*., 1995). Bio-chemically, the hydrolysis of the PP complex depends on the concentration of enzyme, i.e. phytase and the substrate, i.e. PP. Can more PP in the diet supply increased available P with added feed phytase? The PP contents of European broiler diets are usually higher compared to regular corn-soy diets used in the United States but little research has been conducted showing available P differences from feed phytase with different ingredients and PP levels in the diets.

The objective of this study was to assess the effects of graded levels of *E. coli* phytase (Phyzyme XP 5000G) with low and high dietary PP levels in comparison to their respective Positive Controls (PCs) on a) performance, b) P and Ca digestibility determined from ileal digesta, c) P and Ca retention determined from excreta, d) ileal PP hydrolysis, e) excreta PP hydrolysis and f) % tibia ash in broilers during 6 wk feeding trial. The effects of dietary PP levels and graded levels of phytase on Total Dissolved P (TDP) and Water-Soluble P (WSP) were also determined.

MATERIALS AND METHODS

1-42 d performance: One thousand five hundred and thirty six day-old male Cobb 500 broiler chicks were weighed and randomly assorted into 48 floor pens with wood shavings for litter. The pens were assigned one of 12 diets. The average initial weights of chicks per pen were similar across dietary treatments with 32 chicks per pen with a pen size of 2.4 x 2.1 m and 4 pens per diet. A 23:1 h light to dark schedule was provided. Environmental housing temperatures from d 1-7, 8-14, and 15-42 were 35, 32 and 27°C, respectively.

The chicks were fed experimental starter diets (Table 1) for three weeks. The twelve experimental diets consisted of: 1) Low-Phytate Negative Control (LP NC) basal diet (0.25 NPP, 0.24 PP), 2) LP basal plus phytase at 250 U/kg diet, 3) LP basal plus phytase at 500 U/kg diet, 4) LP basal plus phytase at 750 U/kg diet, 5) LP basal plus phytase at 1000 U/kg diet, 6) Low-Phytate Positive Control (LP PC) diet (0.39 NPP, 0.24 PP) 7) High-Phytate Negative Control (HP NC) basal diet (0.25 NPP, 0.32 PP) 8) HP basal plus phytase at 250 U/kg diet, 9) HP basal plus phytase at 500 U/kg diet, 10) HP basal plus phytase at 750 U/kg diet, 11) HP basal plus phytase at 1000 U/kg diet and 12) High-Phytate Positive Control (HP PC) diet (0.39 NPP, 0.32 PP). The experimental diets were prepared by adding the phytase after the complete basal was mixed.

Chicks were switched to experimental grower diets (Table 1) at the end of three wk and maintained on the grower diets until six wk of age. Broilers were fed experimental grower diets with the respective NPP and phytase concentrations as the starter diets. Body weights and feed intake were obtained at the end of the 3rd and 6th wk. Animal use protocol No. 06012. for the present experiment was approved by IACUC from the University of Arkansas.

Digestibility and retention: On d 21, 288 chicks (6 chicks from each of the 48 pens represented a replication) were selected at random and assigned to metabolic cages. The chicks selected from each of the 12 treatment groups and individually housed in metabolic cages were provided feed and water *ad libitum*. The broiler chicks were fed the same feed from the respective treatments. The broiler chicks utilized for retention and digestibility studies were provided a 23:1 h light to dark lighting schedule and the environmental-temperature maintained at 27°C. Excreta samples were collected from each of the broiler chicks for a 24 h period on d 23. The excreta collections from each of the six broilers (from each floor pen replicate) were pooled, freeze-dried and ground to pass through a 0.5 mm screen in preparation for nutrient and marker analysis. On d 23, all broiler chicks were euthanized by CO_2 inhalation. Digesta samples were collected from the ileum from each bird, pooled for each of the six chicks representing one floor pen, freeze-dried and ground for analysis using the same preparation as described for the excreta samples. The ileum was defined as that portion of the small intestine extending from the Meckel's diverticulum to a point approximately 4-5 cm proximal to the ileo-cecal junction. Tibiae (right and left) were taken from each bird, cleaned and frozen for later analysis. The tibiae from six broiler chicks representing each floor pen were pooled for ash analysis. The ileal digesta, excreta samples and tibiae from 288 broilers, 44 d of age, representing the grower period were also collected utilizing the same environmental conditions, replications and euthanasia procedures as for the 21-23 d broilers. The 42-44 d broilers in the metabolic cages were fed the respective grower diets as their floor pen counterparts.

Chemical analysis: Diets, ileal digesta and excreta samples were analyzed for Total P (TP) and Ca by an inductively coupled plasma emission spectroscopic method as described by Leske and Coon (2002). Acid Insoluble Ash (AIA) was determined in experimental diets, ileal digesta and excreta samples using the dry ash and hydrochloric acid digestion technique of Scott and Balnave (1991). The moisture and N in feed, ileal digesta and excreta were determined by standard AOAC procedures 934.01 (1990) and 990.03 (1995) respectively. PP in the diets was measured as inositol hexa-phosphate by using ion-exchange chromatography as described by Bos *et al*. (1991). The total Retainable P (RP) for each basal diet as reported by Leske and Coon (2002) was determined by measuring total P and acid insoluble ash marker in both feed and excreta and using the equation of Scott and Balnave (1991). The total tract % retention of Ca and TP for broilers fed test diets was determined from excreta samples and % digestibility of Ca and TP was determined from ileum digesta samples. The % ileal digestibility and total tract retention were determined by analyzing the test parmaters in the diet, ileal digesta, or excreta and then using the AIA concentrations with a marker digestibility/retention equation reported by Scott and Balnave (1991). The digestible P equivalency values for phytase were calculated based on the differences between the amount (%) of dietary PP that was hydrolyzed by 42 d broilers fed diets with and without inclusion of phytase using the following equation: [(% ileal PP hydrolysis with dietary phytase inclusion x % PP in the diet)/100]-[(% ileal PP hydrolysis with no dietary phytase inclusion x % PP in the diet)/100]. The digestible P equivalency values obtained for different levels of dietary phytase inclusion were expressed on the DM content of the experimental diets.

The determination of tibia ash consisted of cutting tibiae lengthwise and then removing the fat from the bones in refluxing petroleum ether in a Soxhlet apparatus for 48 h.

Table 1: Composition of experimental diets[1]

	Starter				Grower			
	Low Phytate (LP)		High Phytate (HP)		LP		HP	
Ingredients	Basal	Control	Basal	Control	Basal	Control	Basal	Control
Corn, 9.2% CP	63.29	61.23	54.98	52.92	69.1	66.88	60.72	58.44
Soy meal, 49.5%CP	30.15	30.58	26.67	27.1	25.03	25.49	21.57	22.04
Canola meal	-	-	5	5	-	-	5	5
Rice bran	-	-	5	5	-	-	5	5
Celite	2	2	2	2	2	2	2	2
Corn Oil	1.12	1.73	2.76	3.37	1	1.66	2.66	3.33
Dicalcium Phosphate	0.67	1.44	0.61	1.38	0.18	0.96	0.12	0.9
Limestone	1.37	1.63	1.62	1.85	1.39	1.73	1.66	2.02
Salt	0.47	0.47	0.45	0.47	0.47	0.47	0.46	0.46
Broiler vit premix[2]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Broiler trace minerals[3]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Choline Cl-60%	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Lysine HCl	0.09	0.08	0.09	0.08	0.09	0.08	0.09	0.08
DL-Methionine	0.19	0.19	0.19	0.19	0.08	0.09	0.09	0.09
Selenium Premix-06%	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Ethoxyquin[4]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Mold Curb[5]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Sacox 60[6]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Threonine	0.011	0.01	0.003	-	0.015	0.006	0.006	0.003
Calculated								
Total P (%)	0.49	0.63	0.57	0.71	0.38	0.53	0.46	0.61
Phytate P (%)	0.24	0.24	0.32	0.32	0.24	0.24	0.32	0.32
NPP (%)	0.25	0.39	0.25	0.39	0.14	0.29	0.14	0.29
Ca (%)	0.76	1.03	0.87	1.13	0.65	0.95	0.77	1.07
Protein (%)	21.50	21.50	21.50	21.50	19.50	19.50	19.50	19.50
AME, kcal/kg	2982	2982	2982	2982	3034	3034	3034	3034
Analyzed								
DM (%)	90.82	90.40	91.74	90.91	90.83	90.96	91.50	91.63
Total P (%)	0.50	0.57	0.54	0.70	0.39	0.54	0.41	0.53
Phytate P (%)	0.26	0.26	0.30	0.29	0.25	0.23	0.30	0.30
NPP (%)	0.24	0.31	0.24	0.41	0.14	0.31	0.11	0.23
RP[7]	0.33	0.33	0.36	0.37	0.21	0.27	0.25	0.30
Ca (%)	0.81	0.78	0.99	1.29	0.70	1.11	0.83	1.00
Protein (%)	21.66	20.70	21.10	21.30	19.26	19.20	19.02	19.30
Gross energy, kcal/kg	3998	3939	3996	3994	3925	3930	4057	3999

[1]The starter basal LP (Diet 1) was supplemented with 250 (Diet 2) 500 (Diet 3) 750 (Diet 4) and 1000 (Diet 5) FTU of phytase/kg diet. The Positive Control (PC) LP (Diet 6) was not supplemented with phytase. Similarly, Basal HP was supplemented with 0 (Diet 7) 250 (Diet 8) 500 (Diet 9) 750 (Diet 10) and 1000 (Diet 11) FTU/kg diet. The PC HP (Diet 12) was not supplemented with phytase. The grower diets were also supplemented with phytase similar to starter diets. The phytase enzyme preparation used was Phyzyme XP (5000 FTU phytase/g) from Danisco Animal Nutrition, Marlborough, UK. The analyzed Phytase FTU/kg diet for starter diets 1 through 12 were <50, 277, 422, 976, 1226, <50, <50, 206, 649, 934, 1075, <50, respectively. The analyzed Phytase FTU/kg diet for grower diets 1 through 12 were <50,360, 551, 918, 1246, <50, 75, 300, 730, 900, 1073, <50, respectively.

[2]Vitamin mix provided per kg of diet: vitamin A (vitamin acetate) 7709 IU; Vitamin D3, 3304 ICU; vitamin E, 16.5 IU; niacin, 38.6 mg; D-pantothenic acid, 9.9 mg; riboflavin, 6.6 mg; pyridoxine (pyridoxine HCl) 2.75 mg; thiamine (thiamine mononitrate) 1.54 mg; menadione (menadione nicotinamide bisulfite) 1.5 mg; folic acid, 0.88 mg; biotin, 0.066 mg; vitamin B12, 0.013 mg; ethoxyquin, 132 mg; selenium, 0.1 mg.

[3]Trace mineral mix provided per kg of diet: manganese ($MnSO_4.H_2O$) 100 mg; zinc ($ZnSO_4.7H_2O$) 100 mg; iron ($FeSO_4.7H_2O$) 50 mg; copper $CuSO_4.5H_2O$) 10 mg; iodine ($Ca(IO_3)_2.H_2O$, 1 mg; magnesium (magnesium oxide) 26.5 mg.

[4]Monsanto-sanoquin 6, Monsanto Company, St. Louis, MO

[5]Myco Curb, 65% propionic acid blended with sorbic and benzoic acids, Kemin Industries, 2100 Maury St. P.O. Box 70, Des Moines, IA 50306.

[6] Salinomycin sodium (60g activity/lb) Huvepharma, Sofia, Bulgaria

[7]% Retainable P (RP) was determined by measuring the total P retained from feed by using an acid insoluble ash marker in excreta

The defatted tibiae were oven dried and ashed in ceramic crucibles for 16 h at 600°C. Ash content was expressed as percent tibia ash on a defatted dried basis. Experimental diets were assayed for phytase by Danisco Animal Nutrition, Marlborough, United Kingdom using the method described by Engelen *et al.* (2001). One FTU of phytase is the amount of enzyme that liberates 1 micromole of inorganic phosphate per minute from sodium phytate at pH 5.5 and 37°C.

The collection of litter and analysis of litter WSP was based on the procedure of Self-Davis and Moore (2000). Litter samples were analyzed for TDP by inductively

coupled plasma emission spectroscopic method as described by Leske and Coon (2002).

Statistical analysis: The data, except PCs, were analyzed by 2- way analysis of variance (SAS Institute, 1999) with means compared by least significance difference tests. Data in each group (LP and HP) were compared to the respective PC groups using Dunnet's method. The means of two PCs for each parameter tested were compared using two-group t-test. The significance was tested at $p \leq 0.05$.

RESULTS

Body weight gain, feed intake and feed:gain ratio: The effects of varying levels of phytate and phytase on chick performance during the feeding study are presented in Table 2. The main effect of phytase indicates an increase ($p<0.0001$) in Body Weight Gain (BWG) Feed Intake (FI) and improved Feed:Gain Ratio (F:G) during the 1-42 d period in response to dietary phytase supplementation. A similar response was found during the 22-42 d period. The dietary phytase supplementation had a significant effect on BWG and FI, but not on F:G ($p = 0.1343$) during the 1-21 d period. With the exception of F:G ($p<0.0003$) during the 1-21 d period, the dietary PP levels had no effect ($p>0.05$) on BWG, FI and F:G during the 1-21 d, 22-42 d and 1-42 d periods.

In both LP NC and HP NC fed groups, chicks fed the basal diets with no phytase supplementation showed a reduction ($p<0.05$) in BWG and FI compared to their respective PC groups during 1-42 d feeding period (Table 2). The supplementation of phytase to NCs for all the phytase supplemented groups were comparable to the respective PCs in both the LP and HP fed groups. In the present study, the lowest supplementary levels of 250 units of phytase/kg LP and HP basal (NCs) was adequate to show an equal response ($p<0.05$) in terms of BWG, FI and F:G compared to the broilers fed the LP and HP PC diets. The interactions between PP levels and phytase were non-significant, except for FI during the 22-42 d and 1-42 d periods. Mortality was not affected by dietary treatment in the 42 d floor pen study.

% Tibia ash: There was a significant influence of feeding phytase on % tibia ash during the 1-21 d period and 22-42 d period (Table 3). PP had no influence on % tibia ash. There was a significant interaction of PP and phytase on % tibia ash at 21 d and 42 d. The broilers fed the NC, 250 and 500 units phytase/kg LP diets from 1-21 d had a significantly lower % tibia ash compared to the % tibia ash of broilers fed the PC LP diet. Phytase supplementation with 750 and 1,000 units/kg LP diet increased broiler tibia % ash to an ash value comparable to the broilers fed the PC. The broilers fed the NC HP diet and 250 units phytase/kg HP diet had a lower % tibia ash compared to the broilers fed PC HP diet on d 21, but additions of 500, 750 and 1,000 units of phytase/kg HP diet produced % tibia ash values that

Table 2: Performance of male broilers fed diets containing varying levels of Phytate P (PP) and phytase, 1-42 d[1]

PP	Phytase units/kg	BWG (1-21d) kg	FI (1-21d) kg	F:G (1-21d)	BWG (22-42d) kg	FI (22-42d) kg	F:G (22-42d)	BWG (1-42d) kg	FI (1-42d) kg	F:G (1-42d)
LP	0	0.651	0.900*	1.380*	1.170	2.463^{d}	2.085*	1.820	3.365^{e}	1.780*
LP	250	0.719*	0.952*	1.323*	1.468*	2.928^{*a}	1.993*	2.185*	3.878^{*abc}	1.723*
LP	500	0.726*	0.973*	1.345*	1.49*	2.903^{*ab}	1.950*	2.223*	3.875^{*abc}	1.710*
LP	750	0.748*	0.997*	1.333*	1.510*	2.930^{*a}	1.918*	2.260*	3.925^{*ab}	1.690*
LP	1000	0.727*	0.968*	1.330*	1.333*	2.663^{*c}	2.005*	2.060*	3.630^{*d}	1.718*
LP	(+) control	0.736^{A}	0.978^{A}	1.333^{B}	1.378^{A}	2.763^{A}	2.018^{A}	2.115^{A}	3.743^{A}	1.723^{A}
HP	0	0.614	0.848	1.388*	1.068	2.215^{e}	2.155	1.685	3.058^{f}	1.798*
HP	250	0.700*	0.964*	1.383*	1.460*	2.835^{*ab}	1.945*	2.163*	3.795^{*bcd}	1.720*
HP	500	0.699*	0.950*	1.368*	1.553*	2.988^{*a}	1.955*	2.253*	3.935^{*ab}	1.723*
HP	750	0.761	1.038	1.368*	1.498*	2.985^{*a}	1.995*	2.255*	4.020^{*a}	1.733*
HP	1000	0.718*	0.986*	1.385*	1.360*	2.745^{*c}	1.985*	2.080*	3.730^{*cd}	1.728*
HP	(+) control	0.691^{B}	0.951^{A}	1.385^{A}	1.460^{A}	2.933^{A}	2.003^{A}	2.153^{A}	3.885^{A}	1.755^{A}
Pooled SEM	0.017	0.022	0.014	0.030	0.056	0.032	0.034	0.064	0.016	
Main effects										
PP	LP	0.714	0.958	1.342^{b}	1.395	2.777	1.990	2.110	3.734	1.724
	HP	0.699	0.957	1.378^{a}	1.388	2.754	2.007	2.087	3.708	1.740
Phytase	0	0.633^{c}	0.874^{c}	1.384	1.119^{c}	2.339	2.12^{a}	1.753^{d}	3.211	1.789^{a}
	250	0.710^{b}	0.958^{b}	1.353	1.464^{a}	2.881	1.969^{b}	2.174^{b}	3.836	1.721^{b}
	500	0.712^{b}	0.961^{b}	1.356	1.524^{a}	2.945	1.953^{b}	2.238^{ab}	3.905	1.716^{b}
	750	0.755^{a}	1.017^{a}	1.350	1.504^{a}	2.958	1.956^{b}	2.258^{a}	3.973	1.711^{b}
	1000	0.723^{ab}	0.977^{ab}	1.358	1.346^{b}	2.704	1.995^{b}	2.070^{c}	3.680	1.722^{b}
Source of variation										
PP		0.1447	0.9481	0.0003	0.6976	0.5110	0.4018	0.3014	0.5097	0.1168
Phytase		<0.0001	<0.0001	0.1343	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0001
PP x Phytase		0.6267	0.2449	0.3125	0.1225	0.0216	0.2216	0.1338	0.0150	0.6950

[1]Values are the means of 4 replicate pens (32 birds/pen). Means within columns with no common superscripts differ significantly ($p<0.05$).

*Means with no asterisk sign within the columns are significantly different ($p<0.05$) from Positive Control (PC) within each PP group (LP and HP). PC means are separated by upper case letters (A and B) for significance ($p<0.05$). FI = Feed Intake; BWG = Body Weight Gain; F:G = Feed-to-Gain Ratio; LP = Low Phytate (0.24%) HP = High Phytate (0.32%)

Table 3: The % Phytate P (PP) hydrolysis and tibia ash for 21 and 42 d old male broilers fed diets containing two levels of PP with added phytase[1]

Phytate	Phytase units/kg	21d Ileal PP	42d Ileal PP	21d Excreta PP	42d Excreta PP	21d Tibia ash	42d Tibia ash
		(%)					
LP	0	44.51^{*cd}	26.77	52.99^{bc}	27.12^{f}	47.52^{ef}	46.44^{d}
LP	250	29.74^{*e}	54.42	51.04^{bcd}	52.80^{bcd}	49.68^{d}	50.73^{c}
LP	500	47.50^{*abc}	55.94	50.81^{bcde}	54.36^{abc}	50.03^{cd}	52.84^{*b}
LP	750	55.61^{ab}	67.40	60.41^{ab}	59.51^{ab}	51.76^{*bc}	54.86^{*a}
LP	1000	56.09^{ab}	74.97	67.00^{a}	64.19^{a}	52.42^{*ab}	54.37^{*ab}
LP	(+) control	34.79^{A}	7.69^{B}	24.51^{A}	11.97^{A}	53.35^{A}	54.17^{B}
HP	0	38.16^{cde}	24.93*	38.30^{f}	47.78^{cde}	45.73^{f}	45.05^{d}
HP	250	43.04^{cd}	36.58	50.14^{cde}	42.96^{de}	49.09^{de}	49.51^{c}
HP	500	33.66^{de}	49.86	40.86^{ef}	42.65^{de}	50.86^{*bcd}	55.72^{*a}
HP	750	46.13^{bc}	55.27	44.95^{cdef}	40.59^{e}	53.73^{*a}	55.17^{*a}
HP	1000	58.28^{a}	76.49	42.13^{def}	60.05^{ab}	54.31^{a}	55.84^{*a}
HP	(+) control	5.68^{B}	17.34^{A}	3.25^{B}	12.96^{A}	51.76^{B}	56.70^{A}
Pooled SEM		3.81	4.08	3.48	3.87	0.677	0.685
Main effects							
PP	LP	46.69	55.90^{a}	56.92	51.60	50.28	51.85
	HP	43.85	48.63^{b}	43.28	46.81	50.75	52.26
Phytase	0	41.34	25.85^{d}	44.60	37.45	46.63	45.75
	250	36.39	45.50^{c}	50.53	47.88	49.39	50.12
	500	40.58	52.90^{c}	45.83	48.5	50.44	54.28
	750	50.87	61.33^{b}	52.68	50.05	52.75	55.01
	1000	57.18	75.73^{a}	54.51	62.12	53.37	55.11
Source of variation							
PP		0.2480	0.0084	<0.0001	0.0595	0.2878	0.3517
Phytase		<0.0001	<0.0001	0.0529	<0.0001	<0.0001	<0.0001
PP x Phytase	0.0103	0.1492	0.0332	0.0002	0.0383	0.0187	

[1]Values are the means of 4 replicate pens (6 birds/pen). Means within columns with no common superscripts differ significantly ($p<0.05$). *Means with no asterisk sign within the columns are significantly different ($p<0.05$) from PC within each PP group (LP and HP).
LP = Low Phytate (0.24%) HP = High Phytate (0.32%). PC means are separated by upper case letters (A and B) for significance ($p<0.05$)

were comparable ($p>0.05$) to the % tibia ash from the broilers fed the PC HP diet. The % tibia ash from broilers fed 1,000 units of phytase/kg HP diet surpassed the PC broilers by an additional ($p<0.05$) 2.55 percentage units. The broilers fed the NC and 250 units phytase/kg diet from 1-42 d had significantly lower % tibia ash compared to the % tibia ash of broilers fed their respective PCs for both the LP and HP diets. Broilers fed either the LP or HP diets with 500, 750 and 1000 units phytase/kg diet had the same % tibia ash ($p>0.05$) compared to the % tibia ash from the respective PCs.

% PP hydrolysis: Phytase concentration also had a significant effect on % PP hydrolysis based on disappearance of PP in the ileal digesta from both 21 and 42 d old broilers (Table 3). The interaction of PP and phytase was significant on d 21 but not on d 42 with respect to ileal % PP hydrolysis. The PP levels in the LP fed groups increased ileal PP hydrolysis by 2.84 percentage units on d 21 and 7.27 percentage units on d 42 compared to the HP fed groups. The % PP hydrolysis was lower in the HP PC group on d 21 compared to that of the LP PC group. The ileal % PP hydrolysis was significantly higher in the HP PC group compared to that of LP PC group on d 42.

The phytase level had a significant effect on % PP hydrolysis based on disappearance of PP in excreta from 21 d and 42 d old broilers (Table 3). The data indicate a significant PP and phytase interaction on d 21 and d 42 for PP hydrolysis. The PP in the LP fed groups produced an increase in excreta PP hydrolysis by 13.64 percentage units on d 21 and 4.79 percentage units on d 42 compared to that of HP fed groups. The % PP hydrolysis from excreta was lower in the broiler group fed the HP PC diet compared to that of the LP PC on d 21. There was no difference ($p>0.05$) in % PP hydrolysis in broilers fed the LP PC and HP PC diets on d 42.

% Total P digestibility and retention: Phytase had a significant influence on ileal % TP digestibility on d 21 and d 42 (Table 4). PP levels produced a significant influence ($p<0.05$) on ileal % TP digestibility for 42 d old broilers. The ileal % TP digestibility for both the NC LP and PC LP fed broilers on d 42 was the same but phytase supplemented LP groups had a higher ileal % TP digestibility compared to the PC LP broilers. Broilers fed the HP diet supplemented with 1,000 units of phytase/kg had a significantly higher ileal % TP digestibility compared to the PC HP group.

Phytase also produced a significant effect on % TP retention determined from excreta of both 21 and 42 d old broilers (Table 4). The % TP retention was significantly higher for NC LP broilers compared to PC LP broilers on d 21 but the % TP retentions for broilers

Table 4: Total tract % retention and ileal % digestibility of Total P (TP) for 21 and 42 d old male broilers[1]

Phytate	Phytase units/kg	21 d EPret	42 d EPret	21 d IPdig	42 d IPdig
		---------- (%) ----------			
LP	0	60.76	49.90*	62.91*	48.03*
LP	250	55.11*	57.76*	58.30*	57.86
LP	500	52.57*	52.14*	57.87*	57.15
LP	750	48.70*	38.49*	62.72*	56.20
LP	1000	45.39*	46.21*	66.79*	63.79
LP	(+) control	47.78^{A}	46.43^{A}	61.35^{A}	50.30^{A}
HP	0	58.53	49.75*	60.18	43.19*
HP	250	61.19	50.76*	60.57	50.40*
HP	500	48.05*	37.73*	52.65*	53.12*
HP	750	50.77*	28.94	60.07	54.10*
HP	1000	48.61*	36.81*	64.74	63.81
HP	(+) control	47.01^{A}	45.40^{A}	50.99^{B}	45.67^{A}
Pooled SEM		2.144	3.073	1.821	2.024
Main effects					
PP	LP	52.50	48.90^{a}	61.72	56.60^{a}
	HP	53.43	40.80^{b}	59.65	52.92^{b}
Phytase	0	59.64^{a}	49.83^{ab}	61.55^{b}	45.60^{a}
	250	58.15^{a}	54.26^{a}	59.43^{b}	54.13^{b}
	500	50.31^{b}	44.94^{c}	55.26^{c}	55.42^{b}
	750	49.73^{b}	33.72^{d}	61.40^{b}	55.15^{b}
	1000	47.00^{b}	41.51^{c}	65.77^{a}	63.80^{a}
Source of variation					
PP		0.4996	0.0002	0.0816	0.0076
Phytase		<0.0001	<0.0001	<0.0001	<0.0001
PP x Phytase		0.1231	0.2485	0.3683	0.4210

[1]Values are the means of 4 replicate pens (6 birds/pen). Means within columns with no common superscripts differ significantly ($p<0.05$). *Means with no asterisk sign within the columns are significantly different ($p<0.05$) from PC within each PP group (LP and HP). EPret = Excreta P retention; Ipdig = Ileal P digestibility; LP = Low Phytate (0.24%) HP = High Phytate (0.32%). PC means are separated by upper case letters (A and B) for significance ($p<0.05$)

fed LP diets with phytase were comparable to the PC LP group. Broilers fed the NC HP diet and 250 units phytase/kg HP diet showed significantly higher excreta % TP retention compared to the PC on d 21 but broilers fed HP basal plus 500, 750 and 1,000 units of phytase/kg diet showed a comparable % P retention ($p>0.05$) to the PC HP broilers.

% Ca digestibility and retention: Phytase produced a significant effect on % Ca digestibility determined in the ileal digesta from 21 and 42 d broilers (Table 5). PP levels had no influence ($p>0.05$) on ileal % Ca digestibility for d 21 and d 42 broilers. The interaction of PP and phytase was found only for ileal % Ca digestibility on d 42.

Phytase produced a significant effect on % Ca retention from the excreta at d 21 and d 42 (Table 5). A significant interaction of PP and phytase for excreta % Ca retention was found on both d 21 and d 42.

Litter P: Phytase added to both LP and HP basal diets increased litter TDP and WSP (Table 6). There was no main effect of PP or a significant interaction of PP and phytase for litter TDP and WSP. The litter TDP and WSP from broilers fed the LP and HP diets supplemented with different levels of phytase were not significantly different ($p>0.05$) to the litter TDP and WSP from broilers fed respective LP and HP PC diets.

P equivalency values: The percent dietary digestible P increase or P equivalency values for phytase (250, 500, 750 and 1000 units/kg diet) ranged from 0.08-0.13 for LP and 0.04-0.17 for HP diets (Table 7).

DISCUSSION

There is a limited amount of published research measuring the performance and nutrient digestibility for broilers fed for a period of 6 wk with diets containing higher PP (substrate) levels with and without the addition of phytase. The present 42 d feeding study demonstrated that dietary phytase supplementation improved BWG and F:G irrespective of dietary PP concentration when fed low-P diets. The improvements in BWG and F:G are in agreement with previous reports where broilers were fed diets with added phytase (Dilger *et al*., 2004; Ravindran *et al*., 2006). The FI of chicks was influenced by both dietary PP concentration and the level of phytase. The poor performance in terms of BWG and F:G in broilers fed both NC LP and NC HP diets could be attributed to the reduction in FI. The reduction of FI in NC HP fed birds compared to NC LP fed birds was mainly attributed to the HP basal containing higher PP and potentially lower nutrient and P availability because of the added rice bran and canola substituted for corn and soybean meal. At the low levels of incorporation of rice bran and canola these ingredients caused no deleterious effects as reflected by similar BWG, FI and

Table 5: Total tract % retention and ileal % digestibility of Ca for 21 and 42 d old male broilers[1]

Phytate	Phytase units/kg	21 d ECaret	42 d ECaret	21 d ICadig	42 d ICadig
		(%)			
LP	0	73.68^{a}	47.03^{*cd}	71.46*	51.11^{*bc}
LP	250	70.73^{*ab}	51.60^{*bcd}	70.90*	61.06^{*ab}
LP	500	61.27^{*de}	58.57^{*ab}	64.39*	59.21^{*ab}
LP	750	67.37^{*abcd}	53.33^{*abc}	66.28*	60.14^{*ab}
LP	1000	62.74^{*cde}	48.47^{*cd}	65.07*	54.89^{*abc}
LP	(+) control	61.05^{A}	53.96^{A}	65.27^{A}	47.48^{A}
HP	0	59.08^{*e}	45.17^{*d}	71.07	62.24^{a}
HP	250	67.77^{*abc}	60.01^{a}	68.28	61.68^{a}
HP	500	63.78^{*cde}	49.54^{*cd}	64.34	58.48^{ab}
HP	750	67.10^{*bcd}	53.59^{*abc}	68.65	47.81^{*c}
HP	1000	65.66^{*bcd}	53.65^{*abc}	67.89	45.82^{*c}
HP	(+) control	63.54^{A}	46.80^{A}	53.97^{B}	37.40^{A}
Pooled SEM		2.241	2.498	1.762	3.624
Main effects					
PP	LP	67.16	52.80	67.62	57.28
	HP	64.68	52.39	68.05	55.21
Phytase	0	66.38	46.10	71.27^{a}	56.67
	250	69.25	55.81	69.59^{ab}	61.37
	500	62.53	54.06	64.37^{c}	58.84
	750	67.23	53.45	67.46^{bc}	53.97
	1000	64.20	51.06	66.48^{bc}	50.36
Source of variation					
PP		0.0906	0.7112	0.7036	0.3731
Phytase		0.0470	0.0056	0.0048	0.0446
PP x Phytase		0.0028	0.0159	0.5401	0.0262

[1]Values are the means of 4 replicate pens (6 birds/pen). Means within columns with no common superscripts differ significantly ($p<0.05$). *Means with no asterisk sign within the columns are significantly different ($p<0.05$) from PC within each PP group (LP and HP). Ecaret = Excreta Ca retention; Icadig = Ileal Ca digestibility; LP = Low Phytate (0.24%) HP = High Phytate (0.32%). PC means are separated by upper case letters (A and B) for significance ($p<0.05$)

F:G for broilers fed the PC LP and PC HP diets. Therefore, it can be surmised that the anti-nutritive effects of fiber from rice bran and glucocinolates from canola meal were negligible in the present study. The equal performance of broilers fed PC HP and PC LP diets shows that the RP level (from PP hydrolysis and feed NPP) was too low for broilers fed the NC HP basal thus causing a depressed FI. The FI of broilers fed the HP basal with 250 FTU phytase/kg diet was similar to broilers fed the LP basal with 250 FTU phytase/kg diet. The evidence above suggests, the low available P in the NC HP basal diet and not the potential anti-nutritional factors in rice bran and canola meal was the most important contributing factor in the measured depression in broiler performance. Ravindran *et al.* (2006) have shown that broilers can be fed up to 9% rice bran without causing any deleterious effects on nutrient availability. Cabahug *et al.* (1999) also showed comparable results for broilers fed different levels of rice pollard to manipulate PP in wheat-sorghum-soybean meal basal diets. These researchers reported a phytase and NPP interaction due to larger improvements in BWG and F:G for broilers fed test diets containing the lowest level of NPP plus added phytase. The improvement in broiler performance caused by the addition of phytase in feed may be attributed to an increase in biologically available P as a result of phytate hydrolysis or a release of trace elements or minerals from complexes with phytate. The improvement in the biologically available P with the dietary addition of phytase is reflected in improvements in % tibia ash in broilers fed both LP and HP diets. The present findings indicate a PP and phytase interaction on % tibia ash at both d 21 and d 42. The addition of 250 units of phytase caused an improvement in % tibia ash but a comparable (to PCs) response was obtained with 500 units of phytase at d 42. The present findings are in agreement with Cabahug *et al.* (1999) that reported a significant PP and phytase interaction for % toe ash.

Based on the main effect of phytate with respect to % ileal digestibility and % excreta retention of TP, it can be concluded that the HP diets would cause a decrease in % TP digestibility in the ileum and % TP retention from the excreta compared to the LP diets. The larger reduction in % Ca digestibility in the ileum for 42 d broilers fed 750 and 1,000 units of phytase/kg HP diets compared to broilers fed lower levels of phytase added to the HP diets could be due to an imbalance in the Ca:P ratio caused by the increase in phytase concentration. The higher phytase units in the HP diets increased PP hydrolysis which may have provided an excess of both Ca and available P lowering the % Ca and P retention. Ravindran *et al.* (2006) reported a significant interaction of PP and phytase for ileal P and Ca digestibility, where

Table 6: The concentration of Total Dissolved Phosphorus (TDP) and Water-Soluble Phosphorus (WSP) in the litter for 42 d old broilers[1]

Phytate	Phytase units/kg	TDP mg/kg DM	WSP mg/kg DM
LP	0	315*	278*
LP	250	443*	419*
LP	500	432*	407*
LP	750	581*	564*
LP	1000	461*	433*
LP	(+) control	456[A]	422[A]
HP	0	258*	243*
HP	250	476*	467*
HP	500	434*	411*
HP	750	492*	468*
HP	1000	425*	412*
HP	(+) control	411[A]	387[A]
Pooled SEM		61	60
Main effects			
PP	LP	446	420
	HP	414	397
Phytase	0	282[b]	258[b]
	250	460[a]	443[a]
	500	433[a]	409[a]
	750	530[a]	509[a]
	1000	441[a]	421[a]
Source of variation			
PP		0.4175	0.5631
Phytase		0.0031	0.0016
PP x Phytase		0.8390	0.7641

[1]Values are the means of 4 replicate pens (6 birds/pen). Means within columns with no common superscripts differ significantly ($p<0.05$). *Means with no asterisk sign within the columns are significantly different ($p<0.05$) from Positive Control (PC) within each PP group (LP and HP). DM = Dry Matter Basis. LP = Low Phytate (0.24%) HP = High Phytate (0.32%). PC means are separated by upper case letters (A and B) for significance ($p<0.05$)

Table 7: Digestible phosphorus equivalents produced from low phytate and high phytate diets with added phytase from phytate P hydrolysis and disappearance

Phytase units/kg diet	Low phytate %	High phytate %
250	0.08	0.04
500	0.08	0.08
750	0.11	0.10
1000	0.13	0.17

the improvement in both was higher in the HP diet with added phytase.

The litter soluble P from broilers fed NC diet with 250-1,000 units of phytase/kg was higher compared to broilers fed the NC diets. This increase in WSP with the phytase addition does not mean that dietary phytase supplementation in broiler diets will consistently increase the estimated TP excretion. Firstly, the NC broilers fed LP and HP diets had a lower BWG, lower FI, and poor F:G ratio compared to broilers fed PC LP and PC HP diets or broilers fed LP or HP diets with added phytase. Secondly, there was no difference in TDP/WSP between broilers fed graded levels of phytase and their respective PCs in both LP and HP groups. A key observation from the present study is that broilers fed diets containing 500, 750 and 1000 units of added phytase/kg diet had a lower % retention of TP at both 21 and 42 d. The lower retention of % TP was caused by a diminishing utilization of available P from increased PP hydrolysis with the increased concentration of phytase. A diminishing response to the additional P caused by increased dietary phytase concentration was also found for BWG and F:G ratio as reflected by lack of improvement for broilers fed above 250 units of phytase/kg diet. The lower retention of % TP with increased phytate hydrolysis due to supplemental phytase may be directly related to the released P exceeding the P threshold for broilers. Manangi and Coon (2006) recently reported a P threshold in broilers by conducting research with colostomized broilers. The researchers reported broilers fed increasing levels of dietary P show an increase in plasma P, which eventually plateaus and with additional P intake the broiler will increase P excretion in urine and feces. The researchers showed the biological P threshold was substantially below the suggested NRC (1994) requirement for available P. The present research utilized 0.14% less NPP in basal diets compared to PCs, to allow for increased P release from phytate hydrolysis due to phytase supplementation. If the dietary available P level in the experimental dies had been higher (close to NRC) and supplemented with phytase, the WSP would have been anticipated to be significantly higher than the PCs. The present study shows feeding broilers with graded levels (250-1,000 units/kg) of dietary phytase in either the LP or HP diet with 0.14% less NPP compared to PC does not increase litter soluble P compared to the PC. The present research findings are in agreement with other reports (Moore *et al*., 1998; Applegate *et al*., 2003) that indicate no difference ($p>0.05$) in litter soluble P with and without dietary phytase supplementation when the diets are formulated to have reduced NPP with phytase addition. The exact comparison of the present study with other published reports is difficult since those reports were based either on low or one level of PP and one level of dietary phytase supplementation. The present study shows that the inclusion level of dietary phytase, irrespective of dietary PP, will need to be optimized with added NPP levels in order to minimize litter soluble P in broiler houses.

The dietary digestible P equivalency value of phytase for LP group with 250 units of phytase supplementation was 0.08% where as it was 0.04% for HP group (Table 7). The lower P equivalency value of 250 units of phytase for HP group could be attributed to an overall lower dry matter digestibility of the canola and rice bran ingredients thus additional enzyme concentration/ PP/diet may be needed. With the inclusion of 1000 units of phytase/kg diet, the digestible P equivalency value for LP group was 0.13% whereas for HP group it was 0.17%. The increase in digestible P equivalency value with 1000 units of enzyme could be attributed to higher substrate concentration and enzyme concentration. The

digestible P equivalency values of the phytase in the present research are in agreement with P equivalent values of microbial phytases reported by other researchers. Supplementation of 600 units of phytase/kg feed has been shown to replace 0.1% NPP for broiler chicks (Mitchell and Edwards, 1996). Schoner *et al.* (1991) showed that 700 U microbial phytase was equivalent to 1 g P in the form of Monocalcium Phosphate (MCP) based on P retention in broilers fed corn-soybean meal diet for 2 weeks. Kornegay *et al.* (1996) reported that 939 U microbial phytase was equivalent to 1 g P from defluorinated phosphate in broilers fed corn-soybean meal diets with graded levels of microbial phytase. The P equivalency values reported by Kornegay *et al.* (1996) were with a dietary fixed level of PP (0.24%) in combination with 0.2, 0.27 and 0.34% NPP and 0.88, 1.02 and 1.16% Ca, respectively. The small discrepancy or variation in the phosphorus equivalency values of phytase among various studies in comparison to the present work could be attributed to: a) source of microbial phytase and its efficacy in the biological system, b) source and amount of dietary phytate, c) amount of dietary Ca and available P, d) age of birds or period of study and e) method of accessing P equivalency.

In summary, the anti-nutritive effects on broiler performance of dietary PP (either low or high) and lower NPP levels (0.14% less NPP than PC) were overcome by adding 250 units of phytase/kg basal diet in the current study. The HP diet with 1000 FTU/kg of diet provided 0.17% digestible P compared to 0.13% digestible P from the LP diet showing that additional PP substrate for feed phytase may provide more digestible P for poultry. The increasing concentration of added dietary phytase did not increase the amount of soluble P in the litter compared to PC. However, the addition of phytase levels with the dietary NPP and PP levels utilized in the broiler diets did not decrease soluble P in the litter. The objective of decreasing soluble P in broiler litter can only be achieved by taking into consideration the type and inclusion level of phytase in conjunction with the dietary levels of NPP and PP.

ACKNOWLEDGEMENTS

The authors are indebted to Danisco Animal Nutrition, Marlborough, United Kingdom, for financially supporting this research related to the phytase effect on total P retention and phytate P hydrolysis in broilers. The authors also acknowledge Cobb-Vantress for providing Cobb 500 male broiler chicks.

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