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Effect of Phytase on Ileal Amino Acid Digestibility, Nitrogen Retention and AMEn for Broilers Fed Diets Containing Low and High Phytate Phosphorus

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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. GE retention was significantly improved 6% percentage points for 42 d broilers fed either LP or HP diets with added phytase however a significant interaction indicated the improvement in energy retention caused by phytase was primarily from the broilers fed the HP diet. Overall there was no significant main effect of phytase on GE retention of 21 d broilers but the energy retention of HP diet was significantly less than for LP fed broilers and broilers fed the LP diet showed a significant improvement an interaction suggested the phytase would only benefit broilers fed the LP diet. The ileal % digestibility of threonine, tryptophan and serine were affected by PP levels, phytase and the interaction of PP and phytase for 21 d broilers, whereas cystine was the only amino acid to show all three main effects for 42 d broilers. In general, the interaction of phytase and PP for ileal digestibility of specific amino acids was caused by a lack of positive response of phytase when added to the HP diet for 21 d broilers, whereas phytase showed an influential trend of improving ileal digestibility of all amino acids ($p = 0.1284$) in either of the HP or LP diets for the older 42 d broiler. The slight improvement of ileal digestible amino acids seemed to correlate with a 6.2 percentage point improvement in % ileal N digestibility for 42 d broilers fed either the LP or HP diets. Broilers fed the LP PC diet had a higher % ileal nitrogen and energy digestion than broilers fed the HP PC diet at 21 d but there was no difference in 42 d broilers fed the two different PP diets. In summary adding phytase to broiler diets improved energy utilization by 2.1-4.9% for both LP and HP fed broilers and an 1.9-6.1% improved ileal % tAA digestibility for LP fed broilers.

Key words: Phytase, phytate P, ileal DE, AME, ileal AA digestibility

INTRODUCTION

Since energy, protein and P are each expensive components of feed, the ability of a feed phytase to improve both ME and amino acid availability in addition to providing available P from PP has become an important economic decision for the poultry industry to use. The addition of an exogenous phytase to the feed has been shown to increase the utilization of P from PP but the literature is mixed regarding the effect of phytase on improving protein and energy utilization in non-ruminant animals. Farrel *et al.* (1993) Yi *et al.* (1996) Namkung and Leeson (1999) Ravindran *et al.* (2000) Selle *et al.* (2003a,b) and Shirley and Edwards (2003) indicate improvements in both Amino Acid (AA) and energy utilization with dietary phytase supplementation whereas the reports of Biehl and Baker (1997) Ravindran *et al.* (2000) and Selle *et al.* (2000) indicate improved protein/AA utilization with no increase in energy utilization. Some reports (Zhang *et al.*, 1999; Peter and Baker, 2001) indicate no improvement in protein and AA utilization when adding phytase.

The objectives of this study were to assess the effects of graded levels of microbial phytase (Phyzyme XP 5000G) with low and high dietary PP levels in comparison to their respective positive controls (PCs) on a) N and Gross Energy (GE) digestibility determined from ileal digesta, b) N and GE retention determined from excreta and c) ileal AA digestibility in broilers during 6 wk feeding trial.

MATERIALS AND METHODS

Poultry husbandry and diets: 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.

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 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 6 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.

The ileal digesta and excreta sample 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 same grower diets as respective treatment.

Chemical analysis: 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. The GE of test feed, ileal digesta and excreta samples were measured with a Parr Bomb Calorimeter. The feed and ileal digesta AA were analyzed using ion-exchange chromatography at the Central Analytical Laboratory (University of Arkansas, Fayetteville, AR) following acid (6N HCl) hydrolysis for 22 h at 110°C. Met and Cys were quantified in samples after oxidizing samples with performic acid before acid hydrolysis. Alkaline hydrolysis was carried out for tryptophan analysis. The total tract % retention of Gross Energy (GE) and N for broilers fed test diets was determined from excreta samples and % ileal digestibility of GE, N and amino acids were determined from ileum digesta samples. The % digestion/retention was determined by analyzing the test components 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 ileal Digestible Energy (DE) and total tract AME for the test feeds was determined by the equation utilized by Hong *et al*. (2002). The DE was determined using ileal digesta samples and AME was determined using excreta samples. The DE/AME of the feed was determined by subtracting [GE of digesta/excreta* (feed AIA/digesta or excreta AIA)] from the GE of the test diet. Experimental diets were assayed for phytase by Danisco Animal Nutrition, Marlborough, United Kingdom. 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.

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

Ileal N digestibility and total tract N retention: Phytase did not have an effect on ileal % N digestibility on d 21 but had a significant influence on the ileal % N digestibility for 42 d broilers (Table 2). PP had no influence ($p>0.05$) on N digestibility for d 21 and d 42 broilers. An interaction bewteen PP and phytase was found only for ileal % N digestibility for d 21 broilers. The ileal % N digestibility for the NC HP fed group was significantly lower compared to the group fed PC HP on d 42 but the addition of phytase from 250-1,000 units/kg HP diet improved the ileal % N digestibility. The ileal % N digestibility values for the broilers fed the HP diets with phytase were comparable ($p>0.05$) to the ileal % N digestibility for the PC HP broilers. An increased PP level in the diets caused a significant reduction in total tract % N retention for both 21d and 42 d broilers (Table 2).

Table 2: The % ileal digestibility and total tract % retention of N for 21 and 42 d old broilers[1]

Phytate	Phytase units/kg	21 d INdig	42 d INdig	21 d ENret	42 d ENret
		(%)			
LP	(+) control	85.79[A]	79.92[A]	41.07[A]	33.64[A]
HP	(+) control	77.36[B]	79.69[A]	34.59[A]	48.54[A]
LP	0	81.19[abc]	76.44*	42.78*	33.82*
LP	250	79.03[d]	76.98*	38.81*	32.32*
LP	500	79.92[abcd]	77.64*	42.40*	34.13*
LP	750	80.70[abcd]	76.73*	39.20*	35.04*
LP	1000	81.88[a]	82.28*	38.99*	36.76*
HP	0	80.29[abcd]	75.36	38.30*	30.62*
HP	250	81.60[ab]	77.77*	24.30*	28.69*
HP	500	79.21*[cd]	78.92*	22.12*	19.54*
HP	750	79.31*[cd]	77.58*	32.18*	22.18*
HP	1000	79.72[bcd]	80.25*	27.60*	33.41*
Pooled SEM		0.720	0.895	4.171	4.852
Main effects					
PP	LP	80.54	78.02	40.44[a]	34.41[a]
	HP	80.03	77.93	29.53[b]	27.27[b]
Phytase	0	80.74	75.90[c]	40.54	32.22
	250	80.32	77.38[bc]	32.59	30.50
	500	79.57	78.19[b]	33.70	27.88
	750	80.01	77.16[bc]	35.69	28.61
	1000	80.80	81.26[a]	33.30	35.08
Source of variation					
PP		0.2663	0.9456	0.0002	0.0224
Phytase		0.4100	<0.0001	0.2433	0.5214
PP x Phytase		0.0270	0.2967	0.4110	0.6316

[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). ENret=excreta N retention; Indig = Ileal N digestibility; LP = Low Phytate (0.24%) HP = High Phytate (0.32%). Positive control means are separated by upper case letters (A and B) for significance ($p<0.05$)

Ileal GE digestibility and total tract GE retention: Phytase produced a significant influence on ileal % GE digestibility on d 42 but not on d 21 (Table 3). PP also produced a significant response in ileal % GE digestibility for d 42 but no effects were found for 21 d broilers. The interaction of phytase and PP on ileal % GE digestibility was significant on d 21, but not on d 42. Among HP groups, phytase supplementation at 250, 500 and 1000 units/kg diet improved ileal % GE digestibility equal to PC ($p>0.05$). The broilers fed the HP basal plus 750 units phytase/kg diet did not improve their ileal % GE digestibility compared to the NC on d 42. The broilers fed the HP basal diet with phytase seemed to show more response on d 42 compared to d 21 and broilers fed the LP basal plus phytase tended to show more response for d 21 compared to d 42.

Phytase produced a significant increase in total tract GE retention for broilers at d 42 but not at d 21 (Table 3). The dietary PP levels produced a reduction in GE retention at both d 21 and d 42. The interaction of phytase vs. PP for GE retention was also significant on both d 21 and d 42.

Ileal Digestible Energy (DE) and total tract AME: Phytase had a significant effect on ileal DE on both d 21 and d 42 but the dietary PP concentrations did not show any significant effect on DE (Table 4). The interaction of PP and phytase had a significant effect on ileal DE on d 21 but not on d 42. The analyzed ileal DE for broiler chicks fed the LP PC was significantly higher than the determined DE of broiler chicks fed the HP PC diet on d 21. There was no difference in the analyzed ileal DE on d 42 for broilers fed LP and HP PC diets.

The level of dietary PP, phytase and interaction of PP and phytase had a significant effect on total tract AME at both d 21 and d 42 (Table 4). The AME determined for the LP PC broilers on d 21 was higher than the AME determined for the broilers fed HP PC. There was no difference in the analyzed AME on d 42 for the LP and HP PCs.

Ileal AA digestibility: Phytase produced a significant effect on % digestibility for some of the Essential Amino Acids (EAA) such as Met, Trp and Thr on d 21 (Table 5) and % digestibility of Cys, Thr and Trp on d 42 (Table 6). PP had a significant influence on % digestibility of Thr and Trp on d 21. There was an interaction effect ($p<0.05$) of PP and phytase on ileal % digestibility for Cys, Met, Thr and Trp on d 21 and for Cys, Thr and Trp on d 42. Phytase increased the ileal % digestibility of the Non-Essential Amino Acid (NEAA) Ser on d 21 and d 42. PP also showed a significant influence on the ileal digestibility of Ser on both d 21 and d 42. The interaction effect of PP and phytase was obtained only for Ser on

Table 3: The % ileal digestibility and total tract % retention of Gross Energy (GE) for male broilers at 21 and 42 d of age[1]

Phytate	Phytase units/kg	21 d IGEdig	42 d IGEdig	21 d EGEret	42 d EGEret
		(%)			
LP	(+) control	78.33^{A}	74.29^{A}	75.75^{A}	76.72^{A}
HP	(+) control	68.86^{B}	74.57^{A}	70.18^{B}	78.24^{A}
LP	0	68.61^{bcd}	72.72*^{b}	71.86*^{bcd}	74.55*^{a}
LP	250	66.74^{d}	75.97*^{a}	71.72^{bcd}	75.58*^{a}
LP	500	68.99^{abcd}	75.49*^{a}	74.97*^{ab}	76.16*^{a}
LP	750	71.55^{ab}	74.84*^{ab}	73.83*^{abc}	77.23*^{a}
LP	1000	71.47^{ab}	76.15*^{a}	76.05*^{a}	76.39*^{a}
HP	0	69.63*^{abcd}	69.64^{c}	71.53*^{cd}	65.77^{c}
HP	250	72.34*^{a}	74.16*^{ab}	69.90*^{de}	76.42*^{a}
HP	500	67.61*^{cd}	74.44*^{ab}	67.62*^{e}	71.22^{b}
HP	750	67.03*^{d}	69.37^{c}	69.23*^{de}	69.55^{b}
HP	1000	71.00*^{abc}	74.51*^{ab}	69.63*^{de}	75.92*^{a}
Pooled SEM		1.20	0.79	1.19	1.09
Main effects					
PP	LP	69.52	75.03^{a}	73.69	75.98
	HP	69.47	72.32^{b}	69.58	71.77
Phytase	0	69.12	71.18^{b}	71.69	70.16
	250	69.54	75.07^{a}	70.81	76.00
	500	68.3	75.04^{a}	71.29	73.69
	750	69.29	72.11^{b}	71.53	73.39
	1000	71.23	75.33^{a}	72.84	76.15
Source of variation					
PP		0.9456	<0.0001	<0.0001	<0.0001
Phytase		0.1943	<0.0001	0.5347	<0.0001
PP x Phytase		0.0041	0.0681	0.0286	0.0002

[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) EGEret=excreta GE retention; IGEdig = Ileal GE digestibility; LP = Low Phytate (0.24%) HP = High Phytate (0.32%). Positive control means are separated by upper case letters (A and B) for significance ($p<0.05$)

d 21. Although phytase supplementation (main effect) did not significantly influence ileal % digestibility of the entire group of EAA, NEAA and Total Amino Acids (TAA) on d 42, there was a numerical improvement in the % digestibility of EAA ($p = 0.1016$) NEAA ($p = 0.1867$) and TAA ($p = 0.1284$) by 5.08, 3.92 and 4.67 percentage units, respectively, for broilers fed the LP and HP basal diets supplemented with 1,000 units of phytase/kg diet compared to the NCs. Among the groups of broilers fed LP diets on d 42, the NC and 250 units phytase/kg diet had a lower ileal % digestibility of EAA, NEAA and TAA compared to the PC whereas the ileal % digestibility of the EAA, NEAA and TAA from broilers fed the basal diets plus 500, 750 and 1,000 units of phytase/kg diet were comparable to the PC group.

DISCUSSION

The change in dietary digestible amino acids, potential anti-nutritional constituents and fiber with the inclusion of 5% canola meal and 5% rice bran in the HP diet may have contributed to the significant reductions in ileal % GE digestion, % N retention, % GE retention and AME in this study. However, these results in addition to previous reports provide strong evidence that the level of PP was a significant contributory factor in the changes in nutrient digestibility and retention observed in this study.

The % N digestibility in the ileum was affected by the interaction of PP levels and phytase in the diets at d 21, whereas at d 42 the % ileal N digestibility was only affected by the level of phytase added to the diets (Table 2). Ravindran *et al.* (2000) measured N digestibility in 25-d-old broilers fed sorghum-wheat-soybean meal based diets, where PP levels were manipulated with rice pollard and concluded that ileal nitrogen and AA digestibilities were negatively influenced by phytate level, but the negative effects were overcame by phytase. Cowieson *et al.* (2004) and Cowieson and Ravindran (2007) demonstrated that the ingestion of Phytic acid as sodium phytate by 21-d-old broiler chicks has anti-nutritive effects including increase in endogenous N and amino acid flow at the terminal ileum. In both studies, the addition of microbial phytase was shown to reduce the anti-nutritive affect of phytic acid.

In the current study, a significant interaction was detected between PP and phytase at d 21. This interaction was due to the higher digestibility coefficients for birds fed the HP diets with 250 FTU/ kg compared to those receiving the LP diets with 250 FTU/ kg of phytase. As nitrogen digestibility coefficients did not follow a consistent trend, the interaction detected does not appear to be the result of dietary treatment. At d 42, ileal N digestibility was linearly improved by phytase, yet no interaction between phytate and phytase was detected over this period. Similarly, Ravindran *et al.* (2000) reported that the addition of graded levels of phytase to

Table 4: Ileal Digestible Energy (DE) and AME for 21 and 42 d broilers[1]

Phytate	Phytase units/kg	21 d ileal DE, kcal/kg	42 d ileal DE, kcal/kg	21 d excreta AME, kcal/kg	42 d excreta AME, kcal/kg
		---------- (DM) ----------			
LP	(+) control	3413^{A}	3210^{A}	3301^{A}	3315^{A}
HP	(+) control	3026^{B}	3255^{A}	3084^{B}	3415^{A}
LP	0	3015^{bcd}	3129*	3158*^{bc}	3208*^{bc}
LP	250	2929^{cd}	3306*	3148*^{bc}	3289*^{ab}
LP	500	3013^{bcd}	3301*	3275*^{ab}	3331*^{ab}
LP	750	3121^{ab}	3213*	3221*^{b}	3315*^{ab}
LP	1000	3212*^{a}	3265*	3418*^{a}	3275*^{abc}
HP	0	3065*^{abc}	3125*	3149*^{bc}	2952^{e}
HP	250	3175*^{a}	3301*	3067*^{cd}	3402*^{a}
HP	500	2921*^{cd}	3176*	2922*^{d}	3145^{cd}
HP	750	2867*^{d}	3040*	2962*^{d}	3047de
HP	1000	3116*^{ab}	3304*	3056*^{cd}	3367*^{a}
Pooled SEM		52	49	52	48
Main effects					
PP	LP	3058	3243	3244	3283
	HP	3029	3189	3031	3182
Phytase	0	3040	3127^{b}	3153	3080
	250	3052	3304^{a}	3107	3346
	500	2967	3239^{a}	3098	3238
	750	2994	3126^{b}	3091	3181
	1000	3164	3284^{a}	3237	3321
Source of variation					
PP		0.3821	0.0932	<0.0001	0.0024
Phytase		0.0084	0.0010	0.0480	<0.0001
PP x Phytase		0.0007	0.1664	0.0042	0.0002

[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 within each PP group (LP and HP). DM = Dry Matter Basis. LP = Low Phytate (0.24%) HP = High Phytate (0.32%). Positive control means are separated by upper case letters (A and B) for significance ($p<0.05$)

broiler diets from d 0-21 linearly increase apparent ileal N digestibility, but no interaction between phytate and phytase was detected. Nitrogen retention at 21 d and at 42 d was affected by PP level, but not by phytase suggesting other factors in the HP PP diets apart from the PP and phytase level may have had an affect on N retention.

The improvement in ileal % N and GE digestion, ileal DE, total tract % GE retention and AME for broilers fed diets with supplemental phytase compared to broilers fed the LP and HP NC diets with no added phytase may have been caused by phytase hydrolyzing potential PP complexes in the gastrointestinal tract. PP has the ability: a) to form metallic complexes, protein complexes, and complexes with lipids (Cosgrove, 1980) and b) to inhibit ∝-amylase digestion of starch (Knuckles and Betschart, 1987). Previous research has shown an approximate 4.5% improvement in AME or AMEn (kcal/kg) for broilers fed diets supplemented with 750-800 units of dietary phytase (Shirley and Edwards, 2003; Ravindran *et al*., 2000) compared to broilers fed diets without added phytase. An alternative or additional mode of action by which phytase may improve energy and nutrient digestibility was describe by Cowieson *et al*. (2004) and Cowieson and Ravindran (2007) who demonstrated that the ingestion of phytic acid by 21-d-old chicks has anti-nutritive effects including increase in endogenous N and amino acid flow at the terminal ileum. In both studies, the addition of microbial phytase was shown to reduce the anti-nutritive affect of phytic acid. Other studies reported by Edwards (1993) and Biehl and Baker (1997) have reported the addition of dietary phytase did not significantly improve energy utilization in their research. Given the role of phytic acid as shown in the studies above, failure to detect an improvement in energy utilization could be due to the level of phytate in the diets used in these studies. In contrast, Ravindran *et al*. (2006) reported a significant PP ($p<0.05$) and phytase trend ($p = 0.07$) on DE with broilers based on ileal digesta samples at d 21. The present study shows a significant ($p<0.05$) interaction of PP and phytase on ileal DE and total tract AME at d 21 and total tract AME at d 42. The significant interaction between PP and phytase at d 21 for ileal DE was due to higher digestibility coefficients for the birds fed the LP diets with 750 FTU/kg phytase compared to the birds fed the LP diets with 250 FTU/kg of added phytase, with the opposite effect in the group fed the HP diets.

The 42 d broilers showed a higher energy utilization than the 21 d broilers, irrespective of dietary phytate content, when the broilers were fed diets supplemented with dietary phytase. The variation between ileal and total

Table 5: Ileal amino acid digestibility (%) for 21 day-old male broilers fed diets containing varying levels of phytate P (PP) and phytase[1]

Phytate	Phytase units/kg	Arg	Cys	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
		(%)										
LP	(+) control	91.18^{A}	76.94^{A}	87.82^{A}	88.05^{A}	88.96^{A}	90.29^{A}	93.45^{A}	88.89^{A}	79.60^{A}	79.72^{A}	86.52^{A}
HP	(+) control	87.02^{B}	68.68^{B}	82.04^{B}	81.28^{B}	82.55^{B}	84.41^{B}	90.14^{B}	82.98^{B}	73.40^{B}	76.69^{A}	78.73^{B}
LP	0	89.18*	66.71^{cde}	83.86	84.33	85.08	88.02*	90.30^{d}	85.27	72.04^{ab}	75.35^{*a}	82.39
LP	250	87.73	65.79^{de}	82.20	82.36	82.79	86.69	90.80^{cd}	83.59	68.34^{c}	70.77^{d}	80.21
LP	500	87.72	68.31^{bcd}	82.42	83.34	83.40	86.64	90.22^{d}	83.96	71.04^{bc}	71.64^{d}	82.03
LP	750	88.36	71.55^{ab}	83.81	84.29	84.77	86.96	91.06^{cd}	84.95	72.28^{ab}	70.72^{d}	82.98
LP	1000	88.96*	73.84^{*a}	84.48	84.69	85.44	87.75*	93.11^{*a}	85.62	74.90^{a}	75.29^{*c}	82.82
HP	0	89.00	69.09^{*bcd}	83.45*	83.94*	84.53*	87.55*	91.91^{*abc}	84.92*	73.56^{*ab}	81.80^{ab}	81.53*
HP	250	88.75	72.81^{*a}	84.30*	84.22*	85.23*	87.23*	92.65^{ab}	85.61	74.61^{*a}	82.47^{a}	82.65
HP	500	87.58*	70.18^{*abc}	82.68*	82.91*	83.73*	86.25*	91.38^{*bcd}	84.05*	70.74^{*bc}	80.10^{*ab}	80.65*
HP	750	88.19*	67.55^{*cde}	84.11*	83.60*	84.64*	86.77*	90.15^{*d}	85.17*	74.05^{*a}	79.08^{*b}	81.98*
HP	1000	88.52*	64.49^{*e}	84.26*	84.57	85.56	86.22*	91.48^{*bcd}	85.86	73.37^{*ab}	78.97^{*b}	83.09
Pooled SEM		0.534	1.271	0.689	0.786	0.777	0.786	0.547	0.727	0.991	1.060	0.847
Main effects												
PP	LP	88.39	69.24	83.36	83.80	84.30	87.21	91.10	84.68	71.72	72.75	82.09
	HP	88.41	68.82	83.76	83.85	84.74	86.80	91.51	85.13	73.27	80.48	81.98
Phytase	0	89.09	67.90	83.66	84.14	84.80	87.79	91.11	85.10	72.80	78.57	81.96
	250	88.24	69.30	83.25	83.29	84.12	86.96	91.72	84.60	71.48	76.62	81.43
	500	87.65	69.24	82.55	83.12	83.56	86.45	90.80	84.01	70.89	75.87	81.34
	750	88.27	69.55	83.96	83.95	84.71	86.86	90.60	85.07	73.16	74.90	82.48
	1000	88.74	69.17	84.37	84.63	85.50	86.99	92.29	85.73	74.14	77.13	82.95
Source of variation												
PP		0.9578	0.6099	0.3639	0.9261	0.3759	0.4159	0.2388	0.3405	0.0196	<0.0001	0.8401
Phytase		0.1068	0.7216	0.1124	0.3127	0.1429	0.5585	0.0248	0.2061	0.0188	0.0213	0.2845
PP x Phytase		0.6846	<0.0001	0.4021	0.4944	0.3640	0.7711	0.0081	0.5450	0.0059	0.0121	0.1823

Phytate	Phytase units/kg	Ala	Asp	Glu	Gly	Ser	Tyr	EAA	NEAA	TAA
		(%)								
LP	(+) control	87.73^{A}	87.07^{A}	92.26^{A}	82.87^{A}	85.00^{A}	87.61^{A}	86.49^{A}	87.09^{A}	86.70^{A}
HP	(+) control	80.86^{B}	81.41^{B}	87.74^{B}	75.84^{B}	81.91^{B}	81.76^{B}	80.72^{B}	81.59^{B}	81.03^{B}
LP	0	83.58	83.45	90.19*	78.26	77.44^{cde}	83.14	82.05	82.68	82.27
LP	250	81.27	81.39	89.43	75.39	73.12^{f}	80.49	80.12	80.18	80.14
LP	500	82.63	83.10	88.56	76.35	76.35^{e}	81.88	80.98	81.48	81.15
LP	750	83.28	83.55	90.04*	77.88	78.05^{bcde}	83.12	81.98	82.65	82.21
LP	1000	84.34	84.32*	90.17*	78.46	81.57^{*a}	84.14	83.35	83.83	83.52
HP	0	82.67*	83.84	89.53*	78.07*	80.01^{*abc}	82.63*	82.84*	82.79*	82.83*
HP	250	83.87*	83.82	89.77	78.95	80.44^{*ab}	83.79*	83.68	83.44*	83.60
HP	500	81.90*	82.53*	88.62*	76.75*	77.32^{de}	82.17*	81.84*	81.55*	81.74*
HP	750	83.41*	83.41*	89.49*	78.45*	80.47^{*ab}	83.92*	82.30*	83.19*	82.61*
HP	1000	84.17	84.03	89.94	79.41	79.39^{*abcd}	83.61*	82.40*	83.42*	82.76*
Pooled SEM		0.837	0.647	0.582	0.831	0.919	0.737	0.726	0.714	0.718
Main effects										
PP	LP	83.02	83.16	89.68	77.27	77.31	82.55	81.69	82.17	81.86
	HP	83.20	83.53	89.47	78.33	79.53	83.22	82.61	82.68	82.71
Phytase	0	83.13	83.64	89.16	78.17	78.73	82.88	82.45	82.74^{ab}	82.55
	250	82.57	82.60	89.60	77.17	76.78	82.14	81.90	81.81^{b}	81.87
	500	82.27	82.82	88.59	76.55	76.84	82.02	81.41	81.52^{b}	81.45
	750	83.35	83.48	89.77	78.17	79.26	83.52	82.14	82.92^{ab}	82.41
	1000	84.26	84.18	90.05	78.93	80.48	83.88	82.88	83.63^{a}	83.14
Source of variation										
PP		0.7327	0.3858	0.5772	0.0523	0.0006	0.1605	0.0545	0.1244	0.0722
Phytase		0.1777	0.1281	0.1291	0.0604	0.0011	0.0662	0.3466	0.0384	0.1879
PP x Phytase		0.2477	0.1678	0.9051	0.2140	0.0004	0.0834	0.0572	0.1059	0.0753

[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). EAA = Total Essential Amino Acids; NEAA = Total Non- Essential Amino Acids; TAA = Total Amino Acids; LP = Low Phytate (0.24%); HP = High Phytate (0.32%). Positive control means are separated by upper case letters (A and B) for significance ($p<0.05$)

tract energy utilization could be attributed to age of the broilers and the sampling (ileal vs. excreta) location (Scott *et al*., 1998) and also the possible action of caecal microflora or hind gut metabolism. An interesting observation is that PC LP broilers showed significantly higher energy utilization compared to PC HP birds at d 21 based on both ileal and total tract AME and GE retention. This finding is supported by Ravindran *et al.* (2006) who reported a similar effect in broilers at 21 d. The difference in energy utilization was not observed at d 42, where broilers fed PC LP and PC HP produced the same DE and AME values. It is unclear why energy utilization in younger birds was so negatively affected by PP levels. The lower energy utilization in PC HP is well manifested in terms of significantly lower performance and phytate hydrolysis compared to PC LP birds

Table 6: Ileal amino acid digestibility (%) for 42 day-old male broilers fed diets containing varying levels of phytate P (PP) and phytase[1]

Phytate	Phytase units/kg	Arg	Cys	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
		(%)										
LP	(+) control	88.03^{A}	73.78^{B}	84.41^{A}	82.88^{A}	84.80^{A}	86.48^{A}	89.87^{A}	84.74^{A}	75.83^{A}	77.64^{B}	81.32^{A}
HP	(+) control	70.56^{B}	79.26^{A}	79.23^{B}	76.94^{B}	77.06^{B}	81.72^{B}	78.25^{B}	76.91^{B}	75.32^{A}	85.87^{A}	74.66^{B}
LP	0	85.07	66.63^{d}	80.40	79.17*	81.05	82.16*	86.99*	81.20	71.18	68.76	76.95
LP	250	85.59*	67.92^{cd}	80.91	80.42*	82.19*	82.97*	85.05	82.06*	70.95	70.35	78.20*
LP	500	86.20*	72.58^{*bc}	81.91*	81.14*	82.70*	83.32*	87.51*	82.48*	72.41*	73.41*	79.37*
LP	750	85.40*	73.99^{*b}	81.60*	80.94*	82.78*	83.62*	87.19*	82.55*	72.26*	71.88	79.46*
LP	1000	89.79*	72.22^{*bcd}	85.73*	85.11*	86.99*	88.34*	90.78*	86.99*	79.21*	78.00*	82.93*
HP	0	85.08	70.00^{bcd}	79.72*	79.31*	81.29*	82.78*	87.50*	81.50*	69.78*	67.57	76.27*
HP	250	86.45	72.43^{*bc}	83.24*	82.06*	83.85*	85.61*	89.17	83.63*	73.63*	71.43	80.56*
HP	500	83.20	72.57^{*bc}	80.37*	78.55*	80.46*	80.10*	84.68*	80.48*	65.89*	72.04	77.59*
HP	750	86.57	69.93^{bcd}	82.68*	81.51*	83.24*	84.70*	87.47*	83.66*	69.20*	75.29*	79.53*
HP	1000	81.01	80.71^{*a}	83.76*	81.67*	82.88*	85.37*	86.94*	82.71*	77.15*	83.68*	80.11*
Pooled SEM		2.063	1.941	1.571	1.914	1.754	2.253	2.098	1.739	2.294	2.431	1.943
Main effects												
PP	LP	86.41	70.67	82.11	81.36	83.14	84.08	87.50	83.06	73.20	72.48	79.38
	HP	84.46	73.13	81.95	80.62	82.35	83.71	87.15	82.40	71.13	74.00	78.81
Phytase	0	85.40	68.31	80.06	79.24	81.17	82.47	87.25	81.35	70.48^{b}	68.17^{c}	76.60
	250	86.02	70.18	82.08	81.24	83.02	84.29	87.11	82.85	72.29^{b}	70.89^{c}	79.38
	500	84.70	72.58	81.14	79.84	81.58	81.71	86.10	81.48	69.15^{b}	72.73^{bc}	78.48
	750	85.99	71.96	82.14	81.23	83.01	84.16	87.33	83.11	70.73^{b}	73.59^{b}	79.50
	1000	85.40	76.46	84.75	83.39	84.94	86.85	88.86	84.85	78.18^{a}	80.84^{a}	81.52
Source of variation												
PP		0.1455	0.0543	0.8747	0.5474	0.4783	0.7977	0.7929	0.5538	0.1634	0.3302	0.6469
Phytase		0.9596	0.0036	0.0681	0.2580	0.2491	0.2117	0.7747	0.2769	0.0042	0.0002	0.1801
PP x Phytase	0.1149	0.0346	0.6198	0.6368	0.4861	0.6215	0.3662	0.4277	0.4029	0.5484	0.7241	

Phytate	Phytase units/kg	Ala	Asp	Glu	Gly	Ser	Tyr	EAA	NEAA	TAA
		(%)								
LP	(+) control	83.61^{A}	82.80^{A}	88.64^{A}	78.38^{A}	82.79^{A}	83.99^{A}	82.71^{A}	83.37^{A}	82.94^{A}
HP	(+) control	75.17^{B}	76.78^{B}	80.57^{B}	70.44^{B}	74.40^{B}	73.24^{B}	77.80^{B}	75.10^{B}	76.85^{B}
LP	0	80.30*	80.26*	85.93	73.30	79.34	79.87	78.14	79.83	78.74
LP	250	80.99*	80.96*	86.55*	74.34*	79.21	80.54	78.78	80.43	79.36
LP	500	81.55*	81.56*	87.06*	75.60*	80.35*	81.01	80.28*	81.19*	80.60*
LP	750	81.44*	81.54*	86.84*	75.49*	79.25	81.22*	80.15*	80.96*	80.44*
LP	1000	85.60*	85.24*	90.47*	80.33*	87.16	86.58*	84.19*	85.90*	84.79*
HP	0	79.37*	79.34*	86.47*	73.42*	78.28*	79.54*	78.26*	79.40*	78.66*
HP	250	83.44*	82.16*	88.19	77.76*	80.54*	82.26*	81.10*	82.39*	81.55*
HP	500	79.13*	78.92*	85.77*	73.77*	72.61*	77.70*	77.81*	77.98*	77.87*
HP	750	81.98*	80.37*	87.97	75.88*	75.58*	80.89*	80.34*	80.45*	80.38*
HP	1000	82.60*	81.22*	86.08*	77.49*	79.71*	80.00*	82.36*	81.18*	81.95*
Pooled SEM		1.818	1.639	1.415	2.064	2.048	1.984	1.867	1.791	1.831
Main effects										
PP	LP	81.97	81.91	87.37	75.81	81.06^{a}	81.85	80.31	81.66	80.79
	HP	81.30	80.40	86.90	75.67	77.35^{b}	80.08	79.98	80.28	80.08
Phytase	0	79.83	79.80	86.20	73.36	78.81^{b}	79.70	78.20	79.62	78.70
	250	82.21	81.56	87.37	76.05	79.88^{ab}	81.40	79.94	81.41	80.46
	500	80.34	80.24	86.41	74.69	76.48^{b}	79.36	79.04	79.59	79.24
	750	81.71	80.95	87.41	75.69	77.41^{b}	81.06	80.25	80.71	80.41
	1000	84.10	83.23	88.28	78.91	83.43^{a}	83.29	83.28	83.54	83.37
Source of variation										
PP		0.5622	0.1561	0.5994	0.9108	0.0075	0.1691	0.7799	0.2320	0.5470
Phytase		0.1723	0.2790	0.5932	0.1223	0.0191	0.3147	0.1016	0.1867	0.1284
PP x Phytase		0.5678	0.5878	0.2265	0.6115	0.1427	0.2823	0.7283	0.3935	0.6212

[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). EAA = Total Essential Amino Acids; NEAA = Total Non- Essential Amino Acids; TAA = Total Amino Acids; LP = Low Phytate (0.24%); HP = High Phytate (0.32%). Positive control means are separated by upper case letters (A and B) for significance ($p<0.05$)

(Manangi *et al*., 2009). The improvement in both PC LP and PC HP at d 42 could be attributed to gut maturity as it is suggested that digestive system maturation increases the AME (March *et al*., 1973; Peterson *et al*., 1976; Shires *et al*., 1980). It is unlikely that, the observed negative effect on AME in the HP PC group was due to fiber from rice bran and canola meal at such low levels of inclusion, furthermore both PC LP and PC HP birds performed similarly at d 42.

With the exception of % ileal digestibility of TAA at d 42, the response to dietary phytase supplementation in both LP and HP groups on % ileal digestibility of TAA was not significant. The % ileal digestibility of TAA on d 42 indicates that the addition of phytase at 500 to 1000 FTU/kg diet produced a comparable response to PC for the LP group. On the other hand, the data shows improvements in % digestibility for some amino acids in both 21 d and 42 d broilers. Ravindran *et al*. (2006)

reported a significant interaction effect of phytate and phytase for most of the amino acids with the exception of Met and Trp. The researchers found an improvement in ileal digestibility due to dietary phytase (main effect) supplementation for all the amino acids. In addition, studies measuring the effect of phytate on endogenous losses demonstrated that the effect on individual amino acids vary depending on their contribution to endogenous proteins (Cowieson and Ravindran, 2007). The differences in the present research compared to previous findings on the effects of phytase on amino acid digestibility could be attributed to the birds age, duration of the experiment, sample collection site and dietary ingredients. The comparable response in amino acid digestibility in birds fed NC LP with 500 and 1000 FTU phytase and PC LP could be attributed to overcoming an effect of P deficiency as suggested by Martnez-Amezcua *et al*. (2006). Martnez-Amezcua *et al*. (2006) suggested that P is an important and necessary mineral for membrane function and active transporters such as the Na/K ATPase pump, which are essential for amino acid absorption. The lack of difference for amino acid digestibility for broilers fed PC HP and NC HP with phytase supplementation could be attributed to phytate characteristics and the chemical or structural properties of phytate-protein complexes in specific feed ingredients as speculated by Ravindran *et al*. (2006). It is also possible that the level of phytate supplied by the addition of rice bran and canola meal in the current study were not high enough to illicit a phytate effect on endogenous protein loss. The fact that the PC LP birds showed a significant improvement in the % ileal digestibility of TAA compared to PC HP birds is an indication that the phytate-protein complex in rice bran and canola meal may have affected the protein digestibility. The possibility exists that the additional fiber component of rice bran and canola meal compared to the LP diets may have also affected the amino acid digestibility. However, higher levels of rice bran and canola/rapeseed meal were used by Ravindran *et al*. (2006) who reported a significant effect of phytate and phytase. This suggests the higher phytate levels in these ingredients may be a major contributing factor to the lower digestibility of AA in the HP diets. The effect of phytase on energy and protein utilization with different dietary ingredients with varied phytate levels is an area that requires further investigation.

In summary, dietary phytase (500-1000 units/kg diet) improved energy utilization 2.1-4.9% for both LP and HP fed broilers and improved ileal % TAA digestibility 1.9-6.1% for LP fed broilers. These data suggest phytate is a likely factor in the energy and amino acid responses to phytase. Further research with different dietary ingredients and varing levels of phytate is required to quantify the relationship between phytate and phytase.

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