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The Effects of Phytase Supplementation on Performance and Phosphorus Excretion from Broiler Chickens Fed Low Phosphorus-Containing Diets Based on Normal or Low-phytic Acid Barley

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ABSTRACT : A total of 240 day-old broiler chicks were used to study the effects of phytase on performance and phosphorus (P) excretion from birds fed diets containing low phytate barleys formulated without inorganic P. A positive control based on Harrington barley (HB) was formulated to meet requirements for total P. Three experimental diets, based on either HB (0.39% total P with 0.28% phytate P) or the low phytate varieties LP 422 (0.36% total P with 0.14% phytate P) and LP 955 (0.40% total P with 0.01% phytate P), were formulated to be below requirements for total P by removing all the inorganic P from the diet. The four diets were fed with and without 1,000 FTU/kg phytase. Apparent P digestibility was significantly higher ($p<0.01$) for birds fed the low phytate barleys than for birds fed HB either supplemented or un-supplemented with inorganic P. P excretion was significantly lower ($p<0.01$) for birds fed HB without inorganic P than with inorganic P. P excretion was further reduced by the use of the low phytate barleys LP 422 and LP 455 ($p<0.01$). Phytase supplementation did not affect P excretion ($p = 0.39$). Body weight gain and feed intake were highest for birds fed the HB diet with inorganic P and lowest for birds fed the HB diet without inorganic P ($p<0.01$). Among the three low P diets, body weight gain and feed intake of broilers increased as the level of phytate in the barley declined ($p<0.01$). Phytase modestly increased body weight gain ($p = 0.08$) and feed intake ($p = 0.04$). The overall results of this study indicate that it may be possible to reduce the amount of inorganic P used when formulating diets with low phytate barley compared with the levels needed when formulating diets with normal phytate barley. However, it is not possible to completely replace the inorganic P in diets containing low phytate barley without impairing poultry performance. Feeding diets devoid of supplementary inorganic P in combination with low phytate barley resulted in a significant reduction in P excretion by poultry. (**Key Words :** Poultry, Low-phytate Barley, Performance, Phosphorus Excretion)

INTRODUCTION

Considerable research emphasis has been placed on reducing the phosphorus (P) content of poultry manure (Paik, 2003; Maguire et al., 2004; Smith et al., 2004). The reason for this is that long term application of poultry manure to agricultural land often leads to soil P accumulation which has the potential to accelerate P transfer and runoff into water bodies (Maguire et al., 2004). This process can contribute to eutrophication in freshwater ecosystems, and numerous examples of water quality impairment associated with P pollution from livestock operations now exist (Burkholder and Glasgow, 1997; Boesch et al., 2001).

Approximately 50 to 70% of the P found in barley is bound as phytate (myo-inositol 1,2,3,4,5,6-hexakis-dihydrogen phosphate; Ravindran et al., 1994; Li et al., 2001a; Jang et al., 2003). Poultry are very inefficient in degrading phytate P because they do not possess the enzyme phytase that is required to hydrolyze the phytate molecule (Sebastian et al., 1998). The poor digestibility of phytate phosphorus means that additional sources of inorganic P must be used in ration formulation in order to meet the nutritional requirements of poultry, thereby increasing diet cost. Furthermore, low P digestibility leads to excessive fecal excretion of P that can potentially pollute the environment (Paik, 2003; Onyango et al., 2005).

To help address this issue, low-phytate barleys have been developed by USDA scientists (Larson et al., 1998; Raboy et al., 2001). In these barleys, phytic acid accumulation is blocked by a single gene mutation,

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Table 1. Diet composition of experimental diets formulated to determine the effects of phytase on the performance of broilers fed low phosphorus diets based on normal or low-phytate barley

	Harrington Barley		Harrington Barley		LP 422 Barley		LP 955 Barley	
	-Phytase +Dical	+Phytase +Dical	-Phytase -Dical	+Phytase -Dical	-Phytase -Dical	+Phytase -Dical	-Phytase -Dical	+Phytase -Dical
Barley	58.49	58.47	59.63	59.61	59.63	59.61	59.63	59.61
Soybean meal	31.21	31.21	30.98	30.98	30.98	30.98	30.98	30.98
Canola oil	5.62	5.62	5.34	5.34	5.34	5.34	5.34	5.34
Dicalcium phosphate	1.51	1.51	0.00	0.00	0.00	0.00	0.00	0.00
Limestone	1.48	1.48	2.35	2.35	2.35	2.35	2.35	2.35
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin-mineral premix[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Chromic oxide	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Methionine	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Choline	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Endofeed	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Natuphos	0.00	0.02	0.00	0.02	0.00	0.02	0.00	0.02

[1] Supplied per kilogram of diet: 11,000 IU vitamin A (retinyl acetate+retinyl palmitate), 2,200 IU vitamin D_3, 30 IU vitamin E (dl-α-tocopherol acetate), 2.0 mg menadione, 1.5 mg thiamine, 6.0 mg riboflavin, 60 mg niacin, 4 mg pyridoxine, 0.02 mg vitamin B_{12}, 10.0 mg pantothenic acid, 6.0 mg folic acid, 0.15 mg biotin, 0.625 mg ethoxyquin, 500 mg $CaCO_3$, 80 mg Fe, 80 mg Zn, 80 mg Mn, 10 mg Cu, 0.8 mg I, 0.3 mg Se.

resulting in corresponding increases in the free, organic P content of the grain. We and others have reported improvements in P digestibility for broilers as the phytate content of barley declined (Li et al., 2001a; Leytem et al., 2007). Improvements in P digestibility and reductions in P excretion have also been reported as a result of inclusion of the enzyme phytase into poultry diets (Bhanja et al., 2005; Sacakli et al., 2006; Selle et al., 2007; Leytem et al., 2008ab).

Since the development of low-phytate genotypes and phytase supplementation have both been shown to improve the digestibility of P in barley, it may be possible to dramatically reduce the inclusion levels of inorganic P sources when formulating diets for poultry. Jang et al. (2003) evaluated the performance of broilers fed low-phytate barley and found similar performance between birds fed normal barley supplemented with inorganic P and birds fed low P containing diets based on low phytate barley. However, the effect of feeding a combination of low-phytate barley and phytase has not been tested. Therefore, the objective of this experiment was to determine the effects of phytase supplementation on performance and P excretion from young broilers fed diets containing low-phytate barleys formulated without a source of inorganic P.

MATERIALS AND METHODS

Acquisition of barley samples

The low phytate materials were isolated and selected from M2 populations of chemically mutagenized Harrington barley (Larson et al., 1998). Seed stocks of the low phytate barley genotypes LP422 (0.36% total P with 0.14% phytate P), and LP 935 (0.40% total P with 0.01% phytate P) were increased at the Crop Development Centre (University of Saskatchewan). CDC Harrington (0.39% total P with 0.28% phytate P) was included for comparison purposes. Additional details regarding the nutrient content of the barleys can be obtained from Thacker et al. (2006).

Growth trial

A total of 240 one-day old, male broiler chicks (Ross-308 line; Lilydale Hatchery, Wynyard, Saskatchewan) were randomly assigned to one of eight dietary treatments. A positive control, based on Harrington barley (HB) was formulated to meet the National Research Council's (NRC, 1994) recommendations for total phosphorus (Table 1). Three experimental diets were formulated based on either HB, or the low phytate varieties LP 422 and LP 955. The experimental diets were deliberately formulated to be below requirements (NRC, 1994) for total P by removing all of the inorganic P from the diet. All four diets were fed with and without 1,000 FTU/kg phytase (Natuphos 5000, BASF, Ludwigshafen, Germany). The enzyme was obtained from *Aspergillus niger* fermentation and the final product contained 5,000 FTU/g of phytase activity.

Approximately 60% of the diet was comprised of barley. Soybean meal was used as the source of supplementary protein and all diets were supplemented with sufficient vitamins and minerals (other than phosphorus) to meet or exceed the levels recommended by the NRC (1994). Diets were formulated to contain 2,800 kcal ME/kg. 23.0% CP, 1.00% calcium, 0.70 or 0.40% total P, 1.15% lysine, 0.90% methionine and cystine, 0.80% threonine, and 0.31% tryptophan. Actual analyses of the diets are presented in Table 2. The experiment diets were provided in mash form (3 mm screen) and the experiment was conducted over a 14-day period.

The chicks were housed in raised-floor battery cages

Table 2. Chemical analysis of experimental diets formulated to determine the effects of phytase on the performance of broilers fed low phosphorus diets based on normal or low-phytate barley[1]

	Harrington Barley		Harrington Barley		LP 422 Barley		LP 955 Barley	
	-Phytase +Dical	+Phytase +Dical	-Phytase -Dical	+Phytase -Dical	-Phytase -Dical	+Phytase -Dical	-Phytase -Dical	+Phytase -Dical
Moisture	9.39	9.57	9.35	9.35	9.50	9.39	9.42	9.49
Crude protein	23.11	23.27	23.58	23.78	23.71	23.98	23.89	24.51
Lysine	1.10	1.14	1.17	1.11	1.16	1.20	1.13	1.16
Methionine and cystine	0.82	0.84	0.85	0.81	0.79	0.89	0.75	0.84
Threonine	0.77	0.78	0.80	0.76	0.79	0.83	0.78	0.79
Neutral detergent fiber	10.69	11.35	11.65	12.23	14.59	13.01	12.69	11.67
Ether extract	6.72	7.23	7.41	7.61	7.38	7.39	7.27	7.04
Ash	5.95	6.00	5.27	5.27	5.39	5.16	5.20	5.54
Calcium	0.95	0.92	1.00	0.94	1.01	0.93	0.90	1.06
Total phosphorus	0.72	0.72	0.43	0.43	0.40	0.39	0.40	0.42

[1] Chemical analysis conducted in duplicate.

(Jamesway Manufacturing Co., Ft. Atkinson, Wisconsin) with five birds per pen and six replicate pens per treatment. The battery brooder was maintained at a temperature of 35°C for the first week with the temperature gradually reduced to 29°C by the end of second week. Incandescent lighting (23 h light, 1 h dark) was provided with a lighting intensity of 10 lux. Feed and water were available *ad libitum* throughout the experiment. Broilers were weighed individually at the start and weekly thereafter. Weighed amounts of feed were added as required with a weigh back at the conclusion of the experiment to allow for the calculation of feed consumption and feed conversion on a pen basis.

Digestibility determination

Chromic oxide (0.35%) was added to all diets as a digestibility marker and was fed throughout the experimental period. On days 13 and 14, clean excreta (free from feathers and feed) were collected twice a day (morning and afternoon) from plastic liners placed in the excreta collection trays underneath each pen. The excreta samples from the four collections were pooled by placing the samples into an aluminum pan and stirring with a rubber spatula. The pooled samples were then frozen. Prior to analysis, the samples were dried in a forced oven dryer at 55°C for 72 h, followed by fine grinding. Digestibility coefficients for dry matter, calcium and phosphorus were determined using the equations for the indicator method described by Schneider and Flatt (1975). Phosphorus output was calculated using the equations of Dilger and Adeola (2006).

Plasma parameters

At the conclusion of the experiment, the birds were bled via the wing vein using 5 ml EDTA coated vacutainer tubes (Monoject, Sherwood Medical, St Louis, MO). The samples were centrifuged (800×g for 15 min) to collect plasma, which was then stored at -20°C until needed for analysis. Plasma samples were analyzed for phosphorus at the Department of Laboratory Medicine at Royal University Hospital (Saskatoon, Saskatchewan). The samples were analyzed on a Beckman Synchron LX Clinical System following the procedures recommended by the manufacturer (Beckman, 2001).

Chemical analysis

Samples of the barley and the experimental diets were analyzed according to the methods of the Association of Official Analytical Chemists (2007). Analyses were conducted for moisture (AOAC 930.15), crude protein (AOAC 984.13), ash (AOAC method 942.05) calcium (AOAC method 927.02) neutral detergent fiber (AOAC method 2002.04) and ether extract (AOAC method 920.39). Amino acid analysis of the feed was determined by High Performance Liquid Chromatography (Hitachi L-8800 Amino Acid Analyzer, Tokyo, Japan). All samples were hydrolyzed for 24 h at 110°C with 6 N HCl prior to analysis. Sulphur-containing amino acids were analyzed after cold formic acid oxidation for 16 h before acid hydrolysis. The phytate content of the barley was determined following the procedures outlined by Newkirk and Classen (1998). For diets and excreta, the chromic oxide content was determined by the method of Fenton and Fenton (1979), while calcium and phosphorus were determined by inductively-coupled plasma optical-emission spectrometry (Perkin Elmer Optima 4300 DV, Wellesly, MA) as described by Leytem et al. (2007).

Statistical analysis

The pen was the experimental unit for all measurements. Data were analyzed as a 2×4 factorial using the General Linear Models procedure of the Statistical Analysis System Institute, Inc. (SAS, 2004). The factors in the model were phytase supplementation, barley type and their interaction

Table 3. Phosphorus excretion and retention coefficients for dry matter, calcium, and phosphorus from broiler chicks fed low phytate varieties of barley with and without phytase

		Harrington + Dical	Harrington - Dical	LP 422 - Dical	LP 955 - Dical	Average	SEM	p values		
								Barley	Phytase	Interaction
Dry matter (%)	-Phytase	67.2	66.3	63.3	67.1	65.9	0.78	<0.01	0.12	0.01
	+Phytase	68.0	64.1	66.5	68.9	66.9				
	Average	67.6[a]	65.2[b]	64.9[b]	67.9[a]					
Calcium (%)	-Phytase	45.1	39.1	27.5	18.3	32.5	6.61	<0.01	0.33	0.27
	+Phytase	46.1	43.6	21.3	37.6	37.2				
	Average	45.6[a]	41.4[ab]	24.4[c]	28.0[bc]					
Phosphorus (%)	-Phytase	53.8	52.8	63.0	64.9	58.6	1.56	<0.01	0.07	0.74
	+Phytase	56.0	53.9	64.0	68.9	60.7				
	Average	54.9[c]	53.4[c]	63.5[b]	66.9[a]					
Phosphorus excretion (g/kg DMI)	-Phytase	3.41	2.10	1.50	1.45	2.11	0.09	<0.01	0.39	0.92
	+Phytase	3.39	2.08	1.46	1.32	2.06				
	Average	3.40[a]	2.09[b]	1.48[c]	1.39[c]					

[1] Means followed by different letters are significantly different ($p<0.05$).

as fixed effects. Differences were considered significant when $p<0.05$ and $p<0.10$ was considered indicative of a trend.

RESULTS AND DSCUSSION

Dry matter digestibility averaged 66.5% (Table 3). Dry mater digestibility was slightly lower for birds fed the LP 422 diets than for birds fed the other barleys. The lower apparent dry matter digestibility for birds fed the LP 422 diet can be attributed to the higher neutral detergent fiber content of this diet. Fiber is not very digestible by poultry (Janssen and Carre, 1985) and its presence impairs the digestibility of energy and other nutrients contained in the grain (Jozefiak et al., 2004). It is thought that dietary fibre reduces nutrient digestibility due to its physiochemical properties, leading to a more rapid rate of passage and this limits the amount of time available for nutrient breakdown (Burkitt et al., 1972). Phytase had no effect on dry matter digestibility ($p = 0.12$) while there was a significant interaction between type of barley and phytase ($p<0.01$).

Calcium digestibility was slightly higher for the HB diet supplemented with inorganic P than for the HB diet formulated without inorganic P (Table 3). This likely reflects the fact that calcium phosphate is more available than calcium carbonate (Catala-Gregori et al., 2007). The finding of lower ($p<0.01$) calcium digestibility for the low phytate barleys LP 422 and LP 955 compared with HB was somewhat surprising as phytate is known as a strong chelating agent that interacts with divalent cations such as calcium rendering them unavailable for absorption (Li et al., 2001a). An explanation for this apparent anomaly is not readily available. Phytase had no significant effect on calcium digestibility ($p = 0.33$) and there was no significant interaction between barley and phytase ($p = 0.27$).

Apparent P digestibility was significantly ($p<0.01$) higher for birds fed the low phytate barleys LP 422 and LP 955 than for birds fed HB barley either supplemented or unsupplemented with inorganic P (Table 3). This finding supports our previous work (Leytem et al., 2007, 2008ab) and those of others (Li et al., 2001ab; Jang et al., 2003) indicating improved P availability in low phytate barley. Although many previous reports have indicated improvements in P digestibility as a result of phytase supplementation (Peng et al., 2003; Singh et al., 2003; Onyango et al., 2005; Catala-Gregori et al., 2007; Leytem et al., 2008ab), in the present study, there was only a trend ($p = 0.07$) towards improved P availability with phytase addition. The use of the low phytate-containing barleys may have left less room for improvement by phytase addition than occurred with previous studies involving cereal grains containing a normal phytate level.

P excretion was significantly lower for birds fed the low P HB diet than for birds fed the HB diet supplemented with inorganic P (Table 3). P excretion was further reduced by the use of the low phytate barleys LP 422 and LP 455 ($p<0.01$). Phytase supplementation did not significantly ($p = 0.39$) affect P excretion. Reductions in dietary P levels (Maguire et al., 2004; Smith et al., 2004) and inclusion of low-phytate barley (Jang et al., 2003) have both been utilized in the past to reduce P excretion by poultry. The failure of phytase to reduce P excretion conflicts with previous research (Paik et al., 2003; Maguire et al., 2004; Smith et al., 2004) and likely indicates that there is a limit to which P excretion by poultry can be reduced.

Body weight gain was highest ($p<0.01$) for birds fed the HB diet supplemented with inorganic P and lowest for birds fed the HB diet without inorganic P. Among the three low P-containing diets, the body weight gain of broilers increased as the level of phytate in the barley declined ($p<0.01$). Phytase modestly increased body weight gain ($p = 0.08$) and feed intake ($p = 0.04$) and there was a trend for an interaction between barley and phytase for gain ($p = 0.07$) and intake ($p = 0.03$). Feed conversion was unaffected by

Table 4. Performance and plasma parameters for broiler chicks fed low phytate barley varieties with and without phytase (0-14 days)

		Harrington + Dical	Harrington - Dical	LP 422 -Dical	LP 955 -Dical	Average	SEM	p values		
								Barley	Phytase	Interaction
Body weight gain (g)	-Phytase	325.4	200.4	268.7	272.6	266.8	12.9	<0.01	0.08	0.07
	+Phytase	311.6	256.0	274.8	289.2	282.9				
	Average	318.5[a]	228.2[c]	271.7[b]	280.9[b]					
Feed intake (g)	-Phytase	463.8	308.2	395.1	378.6	386.4[x]	17.2	<0.01	0.04	0.03
	+Phytase	444.0	386.3	394.5	424.4	412.3[y]				
	Average	453.9[a]	347.2[c]	394.8[b]	401.5[b]					
Feed conversion	-Phytase	1.43	1.55	1.47	1.40	1.46	0.04	0.10	0.98	0.57
	+Phytase	1.43	1.51	1.44	1.47	1.45				
	Average	1.43	1.53	1.46	1.43					
Mortality (%)	-Phytase	6.7	36.7	13.3	0.0	14.1[x]	4.77	<0.01	<0.01	0.05
	+Phytase	0.0	13.3	0.0	3.3	4.1[y]				
	Average	3.3[a]	25.0[b]	6.7[a]	1.6[a]					
Plasma phosphorus (mmol/L)	-Phytase	2.56	1.07	1.42	1.24	1.57	0.15	<0.01	0.53	0.08
	+Phytase	2.85	1.21	1.05	1.42	1.63				
	Average	2.70[a]	1.14[b]	1.24[b]	1.33[b]					

[1] Means followed by different letters are significantly different ($p<0.05$).

barley type ($p = 0.10$) or phytase supplementation ($p = 0.96$).

The results of the present experiment indicate that although the performance of broilers fed low P-containing diets formulated with low phytate barley was improved compared with birds fed low P diets formulated with normal barley, their performance was not equal to that of birds fed the high P diet. This finding therefore conflicts with that of Jang et al. (2003) who reported equal performance between birds fed normal barley supplemented with P and birds fed low P-containing diets formulated with low-phytate barley. However, the control diet in the Jang et al. (2003) experiment only contained 0.45% total P compared with 0.72% total P in the present study.

Mortality averaged 25.0% for birds fed the HB diet without inorganic P compared with 3.3% for birds fed HB diet with inorganic P ($p<0.01$). Mortality averaged 6.7% and 1.6% for birds fed the LP 422 and LP 955 diets respectively. Phytase addition significantly reduced mortality ($p<0.01$) and there was a significant interaction between barley and phytase addition ($p = 0.05$). The mortality for birds fed the low P, normal phytate barley diet was exceptionally high and would be unacceptable in commercial practice. These birds experienced leg weakness which affected their mobility and the birds became progressively weaker and eventually had difficulty getting to the feeder and had to be euthanized. In contrast, birds on the low-phytate barleys did not experience an appreciable leg weakness and mortality was similar to that of birds fed the high phosphorus diets.

Plasma P averaged 2.7 mmol/L for birds fed the HB diet supplemented with inorganic P and 1.14 mmol/L for birds fed the HB diet deficient in total P ($p<0.01$). Plasma P levels averaged 1.24 and 1.33 mmol/L for birds fed diets based on LP 422 and LP 955 barley. Phytase had no significant effects on plasma P ($p = 0.53$). The increase in plasma P concentration as the phytate content of the diet decreased is consistent with the P digestibility data, where a decrease in phytate concentration of the diet resulted in an increase in P digestibility. This finding provides evidence that the P in low-phytate barley is not only more digestible but is actually absorbed and potentially available for bone mineralization and other metabolic uses.

The overall results of this study indicate that as a consequence of the increased availability of P in low-phytate barley, it may be possible to reduce the amount of inorganic P used when formulating diets with low phytate barley compared with the levels needed when formulating diets with normal phytate barley. However, it is not possible to completely replace the inorganic P in diets containing low phytate barley without impairing poultry performance. The addition of phytase to low P containing diets formulated with low-phytate barley modestly improved body weight gain and feed intake while reducing broiler mortality.

Feeding lower levels of supplementary inorganic P in combination with low phytate barley resulted in a significant reduction in P excretion by poultry. This will allow poultry producers who feed low phytate barley to produce poultry meat in a more environmentally friendly manner than occurs with normal barley thereby helping to minimize the environmental impact of intensive poultry operations. Feeding low-phytate barley would appear to offer poultry producers both economic and environmental benefits in the same package.

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