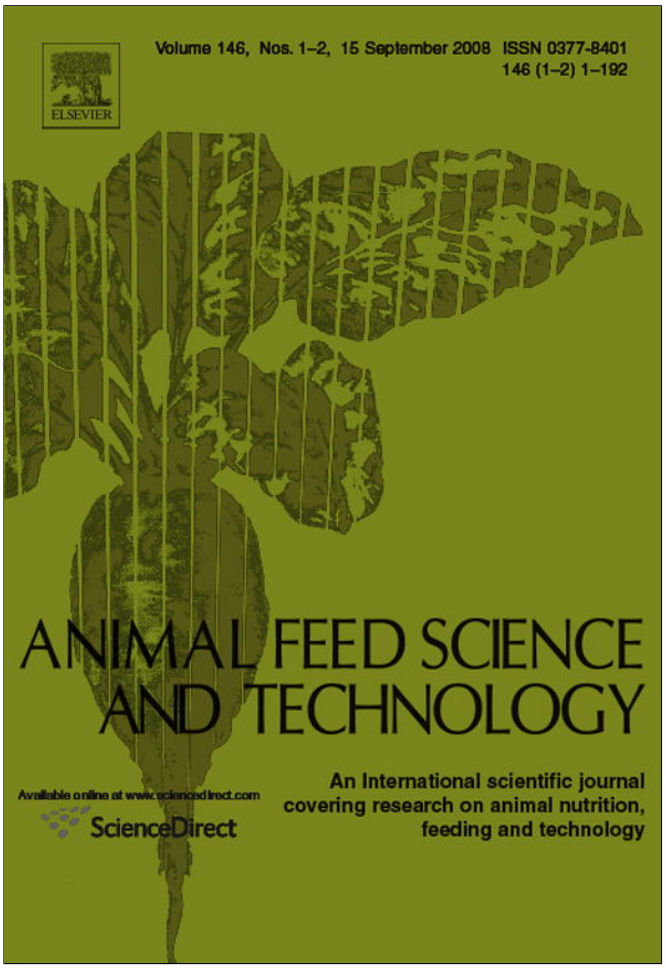

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Short communication

Phytate utilization and phosphorus excretion by broiler chickens fed diets containing cereal grains varying in phytate and phytase content

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Abstract

Eighty, 12-day-old, male broiler chicks, were fed one of four diets to determine the effects of feeding grains varying in phytate phosphorus (P) and intrinsic phytase activity on ileal and excreta P digestibility and composition. The diets contained approximately 970.7 g grain kg^{-1} (maize, high fat–low lignin oat, normal barley or low-phytate barley) with the cereal supplying the sole source of dietary P. The diets were fed for a 7-day acclimation period followed by a 2 day excreta collection while ileal digesta was collected at slaughter on day 21. The coefficients of ileal apparent digestibility (CIAD) for P and phytate P ranged from 0.79 (normal barley) to 0.86 (maize and low-phytate barley) and 0.76 (low-phytate barley) to 0.89 (maize), respectively. The CIAD for phytate P was significantly greater in the maize and high fat–low lignin oat diets, while the low-phytate barley diet had the lowest coefficient ($P>0.002$). The coefficients of total tract apparent digestibility (CTTAD) for P and phytate P ranged from 0.25 (maize) to 0.35 (low-phytate barley) and 0.90 (maize and low-phytate barley) to 0.96 (high fat–low lignin oat), respectively, with no significant differences between diets. There was

Abbreviations: AOAC, Association of Official Analytical Chemists; Ca, calcium; CIAD, coefficient of ileal apparent digestibility; Cr, chromium; CTTAD, coefficient of total tract apparent digestibility; P, phosphorus; ^{31}P NMR, 31-phosphorus nuclear magnetic resonance spectroscopy.

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very little phytate P in excreta regardless of the type of grain fed (<0.13 of total P) with no significant differences between diets. Phytate P degradation was not related to the level of intrinsic phytase in the diet. In summary, current results indicate that, regardless of the type of grain fed, dietary phytate P is highly digestible when large amounts of calcium and P are not added into poultry diets and little phytate P is excreted.

Keywords: Endogenous phytase; Phytate degradation; Poultry; Phosphorus; NMR

1. Introduction

The environmental fate of phosphorus (P) in poultry manure is determined in part by its chemical composition (Leytem et al., 2006). Soil sorption capacity differs among the various forms of P with phytate P being very tightly bound to soil while other P forms such as phosphates are more mobile (Celi and Barberis, 2005). Therefore, dietary factors that influence the amount of phytate P excreted by poultry could alter the solubility of the resultant excreta and impact their potential for P loss once applied to agricultural soils. One factor that could affect the solubility of the P in poultry excreta is the type of grain that they are fed. There is little published data examining phytate P degradation and P characterization of ileal digesta and excreta from birds fed various grains. Therefore, the current experiment was conducted to test the hypothesis that feeding broiler chickens grains, varying in phytate P and intrinsic phytase activities, would influence the utilization of dietary phytate P and the form in which P is excreted.

2. Material and methods

2.1. Experimental diets

Eighty, 12-day-old, male broiler chicks (Ross-308 line, Lilydale Hatchery, Wynyard, Saskatchewan), weighing 1578 ± 161 g, were fed one of four diets in a completely randomized block design (Table 1). The grains used as the principle ingredient in the four experimental diets were commercial grade maize, a newly developed high fat–low lignin oat (CDC SO-1), Harrington barley and a low-phytate, mutant barley (LP 635) containing approximately 0.50 of the phytate P found in normal barley. No supplemental protein, calcium (Ca) or P was included in the diet as their inclusion might have masked differences in phytate P utilization and P excretion resulting from the grain. However, sufficient other vitamins and minerals were added to meet or exceed the levels recommended by the National Research Council (1994). Diets were supplemented with 1 g enzyme (Endofeed, GNC Bioferm Inc., Saskatoon, Sask) kg^{-1} to avoid potential digestibility problems arising from the presence of β-glucans and pentosans in the grains. The experimental diets were provided in mash form (3 mm screen) and contained 3.5 g chromic oxide kg^{-1} as an indigestible marker.

2.2. Bird housing and management

The chicks were housed in raised-floor battery cages (83.8 cm × 45.7 cm × 25.4 cm; Jamesway Manufacturing Co., Ft. Atkinson, WI, USA) with mesh grate floors above excreta collection trays.

Table 1
Ingredient composition and chemical analysis of diets used to determine phytate utilization and ileal and excreta phosphorus characterization of broiler chicks fed diets based on various cereal grains

	Maize	High fat–low lignin oat	Normal barley	Low-phytate LP 635 barley
Ingredients (g kg^{-1} as fed)				
Cereal grain	970.7	969.7	969.7	969.7
Canola oil	15.0	15.0	15.0	15.0
Vitamin–mineral premix[a]	5.0	5.0	5.0	5.0
Salt	5.0	5.0	5.0	5.0
Chromic oxide	3.5	3.5	3.5	3.5
Choline	0.8	0.8	0.8	0.8
Endofeed[b]	0.0	1.0	1.0	1.0
Chemical analysis (g kg^{-1} as fed)				
Moisture	124.2	106.7	105.9	110.3
Crude protein	78.3	118.7	125.7	123.5
Ether extract	44.8	81.2	35.5	36.3
Neutral detergent fibre	109.7	188.4	139.5	156.1
Ash	20.4	39.6	30.2	27.9
Calcium	1.1	1.5	0.7	0.6
Total phosphorus	2.7	3.7	3.6	3.3
Phytate phosphorus	2.1	2.0	1.9	0.9
Endogenous phytase activity (FTU kg^{-1})	16	37	99	83

[a] Supplied per kilogram of diet: 11,000 IU vitamin A (retinyl acetate + retinyl palmitate), 2200 IU vitamin D_3, 30 U vitamin E (dl-α-topheryl 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 calcium carbonate, 80 mg iron, 80 mg manganese, 10 mg copper, 0.8 mg iodine, 0.3 mg selenium.
[b] GNC Bioferm, Saskatoon, Saskatchewan. Provided 750 units g^{-1} of β-glucanase and 650 units g^{-1} of pentosanase.

There were five birds per pen and four replicate pens per treatment. Feed and water were available *ad libitum* throughout the 9-day experiment. The battery brooder temperature was maintained at 22 °C. Incandescent lighting (23 h light, 1 h dark) was provided with a lighting intensity of 10 lux.

The broilers were given a 7-day period to adapt to the experimental diets. Following adaptation, clean excreta (free from feathers and feed) were collected twice a day (morning and afternoon) for two consecutive days from plastic liners placed in the collection trays underneath each pen. The pooled samples were then frozen. Prior to analysis, the samples were dried in a forced air oven at 55 °C for 72 h, followed by fine grinding (<1 mm). Following the final excreta collection on day 21, the birds were killed by cervical dislocation and the entire intestinal length from the proventriculus to the cloaca was excised. The entire content of the ileum from two birds was placed into a single 15 mL conical tube and snap frozen in liquid nitrogen to arrest microbial activity. The samples were lyophilized and ground (<2 mm) prior to analysis.

2.3. Chemical analysis of feed, ileal digesta, and excreta

Samples of the diets were analyzed according to the methods of the Association of Official Analytical Chemists (2005). 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 fibre (AOAC method 2002.04) and ether extract (AOAC method 920.39). Phytase activity in feed (expressed

as "phytase units" or "FTU" per unit of feed; 1 FTU is the amount of phytase that liberates 1 μmol of inorganic P per minute from an excess of sodium phytate at pH 5.5 and 37 °C) was determined according to the method of Engelen et al. (2001).

Samples of feed and excreta were wet-ashed and total P was determined colorimetrically (Pharmacia LKB Ultraspec III, Cambridge, England) using a molybdovanadate reagent (AOAC method 965.17). The ferric precipitation method was used to extract and precipitate the phytate P and the resulting extracts were analyzed for phytate by a colorimetric assay following the procedures described by Leytem et al. (2007). Chromic oxide was determined by the method of Fenton and Fenton (1979). The P composition of the ileal digesta and excreta was determined by solution 31-P nuclear magnetic resonance spectroscopy (^{31}P NMR) as described by Leytem et al. (2007). This method was used as it allows simultaneous identification and quantification of all P compounds with one extraction method and analytical procedure.

2.4. Calculations and statistical analyses

The coefficients of ileal (CIAD) and total tract apparent digestibility (CTTAD) (i.e. overall retention) were calculated using the indicator method based on the following equation:

$$\text{CIAD or CTTAD} = 1 - \left[\frac{\text{Cr}_{\text{diet}}}{\text{Cr}_{\text{out}}} \times \frac{\text{Nut}_{\text{out}}}{\text{Nut}_{\text{diet}}}\right] \quad (1)$$

where Cr_{diet} was the initial chromic oxide concentration in the diet; Nut_{diet} was the initial dietary concentration of the nutrient or dietary component (i.e. dry matter, P and phytate P) being assessed; and Cr_{out} and Nut_{out} were the respective concentrations of either chromic oxide or nutrient/dietary component in the ileal digesta or excreta, respectively. Data were analyzed as a one-way Analysis of Variance using the Proc Mixed procedure of the Statistical Analysis Software Institute (2004). Where appropriate, means for treatments were compared using Tukey's procedure. Differences were considered significant when $P<0.05$.

3. Results

3.1. Chemical analysis of feed

The total P concentrations ranged from 2.7 g P kg^{-1} for the maize diet to 3.7 g P kg^{-1} for the high fat–low lignin oat diet (Table 1). Phytate P concentration ranged from 0.90 g P kg^{-1} for the low-phytate barley to 2.1 g P kg^{-1} for maize. Endogenous phytase activity ranged from 16 FTU phytase kg^{-1} for maize to 99 FTU phytase kg^{-1} for the normal barley.

3.2. Apparent digestibility

Dry matter CIAD ranged from 0.52 for normal barley to 0.72 for maize ($P>0.05$; Table 2). CIAD for P ranged from 0.79 for normal barley to 0.86 for maize and low-phytate barley ($P>0.05$) while phytate P CIAD ranged from 0.76 for low-phytate barley to 0.89 for high fat–low lignin oat ($P>0.002$). The only significant ($P>0.04$) difference in dry matter CTTAD was between maize with the highest coefficient (0.72) and normal barley which had the lowest (0.51). There were no significant differences in CTTAD for P or phytate P which ranged from 0.25 for maize to 0.35 for low-phytate barley, and 0.90 for maize and low-phytate barley to 0.96 for high fat–low lignin oat, respectively.

Table 2
Coefficients of ileal and total tract apparent digestibility for dry matter, phosphorus and phytate phosphorus from diets comprised of maize, normal barley, low-phytate barley and high fat–low lignin oat fed to broilers

Diet	Coefficient of apparent digestibility		
	Dry matter	Phosphorus	Phytate
Ileal			
Maize	0.72 a	0.86 a	0.89 a
Normal barley	0.52 a	0.79 a	0.81 bc
Low-phytate barley	0.65 a	0.86 a	0.76 c
High fat–low lignin oat	0.53 a	0.81 a	0.87 ab
SEM	0.068	0.026	0.020
P>F	0.160	0.200	0.002
Total Tract			
Maize	0.72 a	0.25 a	0.90 a
Normal barley	0.51 b	0.29 a	0.92 a
Low-phytate barley	0.64 ab	0.35 a	0.90 a
High fat–low lignin oat	0.61 ab	0.31 a	0.96 a
SEM	4.27	5.84	3.09
P>F	0.04	0.68	0.49

Within each location, means in the same column followed by the same letter (a, b) do not differ (P>0.05).

3.3. Ileal and excreta phosphorus characterization

Total P in the ileal digesta ranged from 1.32 g P kg^{-1} for maize to 1.67 g P kg^{-1} for normal barley (P>0.05; Table 3). The P in the ileal digesta was dominated by phytate P which ranged from 0.67 g P kg^{-1} for high fat–low lignin oat to 0.85 g P kg^{-1} for normal barley and comprised between 0.50 and 0.63 of the total P in the digesta. There was also a substantial amount of phosphate monoester P (lower inositol phosphate esters), which ranged from 0.37 g P kg^{-1} for maize to 0.63 g P kg^{-1} for normal barley, comprising 0.28–0.38 of the total P in the digesta. The remaining P in the ileal digesta was present as phosphate P and ranged from 0.11 g P kg^{-1} for maize to 0.24 g P kg^{-1} for high fat–low lignin oat. The only significant difference in ileal P composition was for ileal phosphate P (P>0.03) where the high fat–low lignin oat treatment had the greatest concentration and the low-phytate barley and maize treatments had the lowest.

Total P in the excreta ranged from 4.89 g P kg^{-1} for normal barley to 6.92 g P kg^{-1} for maize (Table 3). Phosphate was the dominant form of P found in the excreta ranging from 3.66 g P kg^{-1} for normal barley to 5.54 g P kg^{-1} for maize and comprised 0.75–0.85 of the total excreta P. Excreta phytate P concentrations ranged from 0.27 g P kg^{-1} for low-phytate barley to 0.90 g P kg^{-1} for maize and comprised only 0.05–0.13 of total excreta P. Phosphate monoester P ranged from 0.80 g P kg^{-1} for high fat–low lignin oat to 1.22 g P kg^{-1} for low-phytate barley and comprised 0.14–0.23 of total excreta P. The only significant difference in excreta P composition was for phosphate monoester P (P>0.006) where the high fat–low lignin oat treatment was lower than the other treatments.

4. Discussion

The CTTAD for the P contained in maize of 0.25 is in agreement with the value of 0.29 reported by van der Klis and Versteegh (1996). Leytem et al. (2007) reported that the availability of the P in barley ranged from 0.13 to 0.25 which is somewhat lower than

Table 3
Phosphorus characterization of poultry ileal digesta and excreta determined by sodium hydroxide ethylenediamine tetraacetic acid (NaOH–EDTA) extraction and solution ^{31}P NMR spectroscopy

	NaOH–EDTA extractable P (g P kg dry wt.$^{-1}$)				
	Total P	Phosphate[a]	Phosphate monoesters[a,b]	Phytate[a]	Pyrophosphate[a]
Ileal					
Normal barley	1.67 a	0.19 (0.12) ab	0.63 (0.38) a	0.85 (0.50) a	ND[c]
Low-phytate barley	1.40 a	0.13 (0.09) b	0.52 (0.38) a	0.75 (0.53) a	ND
Maize	1.32 a	0.11 (0.08) b	0.37 (0.28) a	0.84 (0.63) a	ND
High fat–low lignin oat	1.34 a	0.24 (0.18) a	0.42 (0.31) a	0.67 (0.51) a	ND
SEM	0.094	0.029	0.068	0.093	
P>F	0.070	0.026	0.093	0.515	
Excreta					
Normal barley	4.89 a	3.66 (0.75) a	1.14 (0.23) a	0.39 (0.08) a	ND
Low-phytate barley	5.36 a	4.07 (0.76) a	1.22 (0.23) a	0.27 (0.05) a	ND
Maize	6.92 a	5.54 (0.80) a	1.21 (0.18) a	0.90 (0.13) a	ND
High fat–low lignin oat	5.87 a	5.00 (0.85) a	0.80 (0.14) b	0.29 (0.05) a	0.06 (1)
SEM	0.565	0.573	0.075	0.181	
P>F	0.119	0.136	0.006	0.103	

Within each location, means in the same column followed by the same letter (a–c) do not differ (P>0.05).
[a] Values in parenthesis are the proportion of the NaOH–EDTA extracted P.
[b] Values for phosphate monoesters include all monoesters other than phytate.
[c] ND = not detected.

that reported in the present study. The numerically higher P digestibility of the low-phytate barley compared with normal barley agrees with previous work with low-phytate barley variants in broilers (Leytem et al., 2007) and turkey poults (Li et al., 2001).

There is little published data reporting CIAD of cereal grains included in diets with low P and Ca levels. Dilger and Adeola (2006) reported ileal P digestibility coefficients of 0.83–0.85 when broiler chicks were fed low-phytate or conventional soybean meal diets without Ca additions, which was similar to the apparent ileal P digestibilities obtained for all of the cereal grains evaluated in the present study. Tamim and Angel (2003) reported an apparent P absorption of 0.65 when broilers were fed a maize–soybean meal diet. While this is lower than the values obtained in the present study, the diets used by Tamim and Angel (2003) contained higher levels of P from added dicalcium phosphate, which would have reduced the apparent absorption of P at the distal ileum.

Phytate P CIAD ranged from 0.76 to 0.89, which is somewhat comparable to values obtained by Tamim and Angel (2003) and Tamim et al. (2004) who reported that broilers were able to hydrolyze up to 0.69 of phytate P in maize–soybean meal diets. The high CIAD for phytate P in the present study indicated that the majority of phytate P hydrolysis occurred prior to the terminal ileum, with only a 1–18% increase in phytate P hydrolysis in the hind gut. The ability of broilers to hydrolyze phytate P has been reported previously (Ballam et al., 1985; Mohammed et al., 1991) and endogenous brush-border phytase activity has been found in all segments of the small intestine, with the highest levels found in the duodenum (Maenz and Classen, 1998).

The grains used to prepare the diets fed in the present study had varying levels of intrinsic phytase activity. In spite of this, there were few significant differences in phytate P CIAD or CTTAD between treatments. This is particularly evident when comparing the phytate P CIAD of maize and normal barley which were similar (0.89 vs. 0.81, respectively), in spite of a sixfold difference in dietary phytase activity. As there was little difference in phytate P CIAD, even though the intrinsic phytase activity of the grains were different, it appears that the contribution of the endogenous phytase activity of the birds (including microflora phytase activity) had a greater influence on phytate hydrolysis than the intrinsic phytase activity of the grain. Our findings are supported by those of Nelson (1976) who fed birds either maize or maize–wheat-based diets and found no significant differences in phytate P hydrolysis, although *in vitro* tests had previously indicated a higher phytase activity in the maize–wheat diet.

In the present study, the inclusion of Ca or P from inorganic sources such as limestone or dicalcium phosphate was avoided in order to create a more sensitive model to detect differences in phytate P hydrolysis between grains with different endogenous phytase activities. However, while there was no consistent correlation between intrinsic phytase activity of the grains and phytate P hydrolysis, the high-phytate P CIAD (0.76–0.89) reflect the substantial ability of broilers to hydrolyze phytate P in the absence of large quantities of added Ca and P.

The effect of dietary concentrations of Ca and P on the extent of intestinal phytate P hydrolysis has been previously reported. Mohammed et al. (1991) showed that phytate P degradation in broilers up to 4 weeks of age was stimulated in maize–soybean meal diets when the dietary Ca level was decreased. Ballam et al. (1985) demonstrated that high inorganic P levels in broiler diets significantly decreased phytate P hydrolysis. van der Klis and Versteegh (1996) demonstrated a decrease in phytate P hydrolysis of 26–48% in broilers fed diets with higher available P (AvP) than comparable diets with lower AvP.

Although the P CIAD values were high (0.79–0.86), P CTTAD (i.e. overall retention) values were much lower (0.25–0.35). This can be explained by the fact that much of the P absorbed prior to the terminal ileum was not retained, presumably due to insufficient Ca available for bone mineralization. Absorbed P that is not retained is excreted in the urine, since poultry excrete urine and excreta together, this resulted in P CTTAD values that were 58–70% lower than the P CIAD values. This is consistent with previous observations by van der Klis and Versteegh (1996), which showed that increases in dietary Ca concentration reduced P absorption, but the overall retention of P increased as dietary Ca was increased.

There is very little published data on the P composition of excreta obtained from broilers fed different grains. Maguire et al. (2004) reported that litter from birds fed maize-based diets contained 0.26–0.56 of P as phytate P, while concentrations in manure from layers fed maize-based diets ranged from 0.35 to 0.80 of total P (Leytem et al., 2006). Based on these findings, it was expected that there would be reasonable concentrations of phytate P in the excreta in the present study. However, the data clearly show that very little phytate P is excreted when diets are not supplemented with Ca and P, regardless of the type of grain fed. This finding supports previous work where analysis of excreta from broilers fed diets containing various cultivars of barley revealed that only trace amounts of phytate P were excreted, irrespective of the phytate P concentration of the initial feed (Leytem et al., 2007). However, as the diets in the studies by Maguire et al. (2004) and Leytem et al. (2006) also

contained appreciable amounts of inorganic Ca and P, it could be expected that differences in the proportion of phytate P in the excreta were due to the detrimental effects of these minerals on intestinal phytate P hydrolysis.

5. Conclusions

The current study demonstrates that phytate P content and the intrinsic phytase activity of grains has very little influence on overall phytate P utilization in poultry when diets do not contain large amounts of added P or Ca. The broilers were able to hydrolyze over 0.75 of the phytate P present in the cereal-based diets irrespective of the intrinsic phytase activity of the feed. The phytate P hydrolyzed by the birds was presumably absorbed, as P CIAD values were $\geq$0.79. Phytate P degradation was almost complete in poultry excreta with less than 0.13 P in the excreta being in the form of phytate P. Further research should be conducted with diets more typical of those used in commercial poultry production to confirm these findings, as the importance of differences in endogenous phytase activity from cereal grains and its effect on overall availability of P from the diet may previously have been overestimated.

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