Phosphorus characterization in feces from broiler chicks fed low-phytate barley diets

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Abstract: The inclusion of low phytate grains in poultry diets can reduce the phosphorus (P) content of poultry feces, but their influence on fecal P composition is not well established. To assess this, 100 male broiler chicks (21 days old) were fed dietary treatments based on either a wild-type barley or one of three low phytate mutant barleys with 59, 62 and 99% reductions in phytate P, compared with the normal barley diet. The birds were housed in raised-floor battery cages with mesh grate floors above fecal collection trays with five birds per pen and five pens per treatment. The birds were fed for 9 days and feces were collected twice a day during the last 2 days of the experiment. Total P concentrations were 14–24% lower in feces from birds fed low phytate barley diets compared with those fed the normal barley diet. Phosphorus digestibility increased ($P < 0.05$) as phytate in the barley diet decreased. Phosphate was the major P fraction in the feces (69–75% extracted P) regardless of the type of barley fed. Phytate constituted only 3–12% of the P in the feces, indicating its hydrolysis in the bird. Overall, these results suggest that feeding low-phytate barley diets can reduce P concentrations in poultry feces without causing significant changes in P composition.

Keywords: low-phytate barley; phosphorus; broilers; feces

INTRODUCTION

Phytate (salts of *myo*-inositol hexakisphosphate) is the primary storage form of phosphorus (P) in cereal grains.[1] Poultry are inefficient in utilizing phytate P because they do not produce intestinal phytase, a digestive enzyme required to release the P from phytate.[2] As a result, inorganic P is commonly added to poultry diets to prevent P deficiency, although over-supplementation can lead to high concentrations of P in poultry feces. The P subsequently accumulates in soil and can contribute to the eutrophication of water bodies.[3] Consequently, there is considerable interest in developing dietary manipulations that will decrease the P concentration of poultry feces.[4,5] One approach is the development of mutant grains that contain substantially less phytate than the wild-type equivalent.[6,7] Feeding these grains has been shown to improve P utilization in poultry.[8–11]

In addition to reducing the P concentration of feces, dietary modification has the potential to alter the forms in which P is excreted. This may have important implications for the fate of fecal P in the environment following land application.[12] Phosphate is relatively soluble in soil, whereas phytate is retained strongly and is unlikely to be lost in run-off.[13–15] As a result the phytate content of manures can exert a strong influence on phosphate solubility following application to soil. Alteration of the P composition of feces through dietary manipulation could influence the transport of P to water bodies following land application.[16]

Poultry litter (feces mixed with bedding material) and manure can contain high concentrations of phytate. For example, Maguire *et al.*[4] reported that litter from broilers and turkeys fed corn-based diets contained between 26 and 56% of total P as phytate, while Leytem *et al.*[17] reported that phytate concentrations in manures from layers fed corn based diets ranged from 35 to 80% of total P. However, recent analysis of pigs fed diets containing various cultivars of barley revealed that only trace amounts of phytate were excreted, irrespective of the phytate concentration of the initial feed.[18] Clearly, our understanding of the metabolism of phytate in monogastric animals is limited and information is needed on the P composition of feces obtained from a wide range of animals and diets. The objective of this experiment was to quantify changes in fecal P composition from poultry fed diets containing different varieties of low-phytate barley.

MATERIALS AND METHODS

All birds in this experiment were cared for under the guidelines of the Canadian Council on Animal Care.[19]

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Table 1. Ingredient composition and chemical analysis of diets using normal and low-phytate barley

	Barley used			
Composition/analysis	Copeland	M422	M635	M955
Diet formulation (g kg^{-1} as fed)				
Barley	970.7	970.7	970.7	970.7
Canola oil	15	15	15	15
Table salt	5.0	5.0	5.0	5.0
Vitamin/mineral mix[a]	5.0	5.0	5.0	5.0
Chromic oxide	3.5	3.5	3.5	3.5
Choline chloride	0.8	0.8	0.8	0.8
Chemical composition (g kg^{-1} as fed)				
Moisture	91	93	92	90
Crude protein	124	129	124	126
Ash	31	30	31	31
Ether extract	32	34	33	37
Neutral detergent fiber	130	167	150	157
Total phosphorus[b]	3.5	3.0	3.0	3.0
Phytate[c]	3.2	1.2	1.1	trace

[a] Supplied per kilogram of diet: 11 000 IU vitamin A (retinyl acetate + retinyl palmitate), 2200 IU vitamin D_3, 30 IU 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 and 0.3 mg selenium.

[b] No supplemental calcium or P (i.e. limestone or dicalcium phosphate) was used in formulating the diets but sufficient other vitamins and minerals were added to meet or exceed the levels recommended by the NRC.[20]

[c] Phytate determined with an HPLC method.

Digestibility trial

One hundred, 21-day-old male broiler chicks (Ross-308 line, Lilydale Hatchery, Wynyard, Saskatchewan), weighing an average of 1098 ± 35 g, were used in a completely randomized block design and fed one of four dietary treatments formulated using different varieties of barley. 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 fecal collection trays. There were five birds per pen and five replicate pens per treatment. Feed and water were available *ad libitum* throughout the 9-day experiment. The battery brooder was maintained at 22 °C. Incandescent lighting (23 h light, 1 h dark) was provided with a lighting intensity of 10 lux.

The barleys used in the diets were a wild-type barley (cv. Copeland) and three low-phytate mutant barleys (M422, M635 and M955) with 59, 62, and 99% reductions in phyate P, respectively, compared with the normal barley diet (Table 1). Further details regarding the barley varieties can be found in Dorsch *et al.*[7] The experimental diets are shown in Table 1. No supplemental calcium or P (i.e. limestone or dicalcium phosphate) were used in formulating the diets but sufficient other vitamins and minerals were added to meet or exceed the levels recommended by the NRC.[20] The experimental diets were provided in mash form (3 mm screen).

The broilers were given a 7-day period to adapt to the experimental diets. Following the adaptation period, clean feces (free from feathers and feed) were collected twice a day (morning and afternoon) for two consecutive days from plastic liners placed in the fecal collection trays underneath each pen. The fecal 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 air oven at 55 °C for 72 h, followed by fine grinding (<1 mm). Apparent digestibility coefficients for dry matter and P were calculated using the equations for the indicator method described by Schneider and Flatt.[21]

$$\%DM = 100 - \left(100 \times \frac{c_{feed}}{c_{feces}}\right)$$

and

$$\%P = 100 - \left(100 \times \left[\frac{c_{feed}}{c_{feces}} \times \frac{P_{feces}}{P_{feed}}\right]\right)$$

where DM and P are dry matter and phosphorus digestibility, respectively; and c_{feed} and c_{feces} are the amounts of chromic oxide in feeds and feces, respectively, in g kg^{-1}. P_{feed} and P_{feces} are the amounts of phosphorus in the feeds and feces, respectively, in g kg^{-1}.

Chemical analysis

Samples of the experimental diets and feces were analyzed according to the methods of the Association of Official Analytical Chemists.[22] Analyses were conducted for moisture (AOAC 930.15), crude protein (AOAC 984.13), ash (AOAC method 942.05) and ether extract (AOAC method 920.39). Neutral detergent fiber was analyzed using the method of Van Soest *et al.*[23] Total P was determined using the wet-ash nitric–perchloric acid method with phosphate detected colorimetrically (Pharmacia LKB Ultraspec III, Cambridge, UK) using a molybdovanadate reagent (AOAC method 965.17).[24] The ferric precipitation method was used to extract and precipitate the phytate P and the resulting extracts were analyzed for phytate by the colorimetric assays of Raboy *et al.*[25] and Chen *et al.*[26] Chromic oxide was determined by the method of Fenton and Fenton.[27]

The P composition of the feed and feces was determined by NaOH–EDTA extraction and solution ^{31}P NMR spectroscopy as described by Turner.[28] Three of the five replicates per treatment were randomly selected for analysis. Briefly, P was extracted in triplicate by shaking 2.00 ± 0.01 g of dried feed or feces with 40 mL of a solution containing 0.5 mol L^{-1} NaOH and 0.05 mol L^{-1} EDTA for 4 h at 20 °C. Extracts were centrifuged at $10\,000 \times g$ for 30 min and aliquots were analyzed for total P by inductively coupled plasma optical emission spectrometry (Perkin Elmer Optima 4300 DV, Wellesly, MA, USA). The

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remaining solutions from the triplicate extracts were combined, frozen rapidly at −80 °C, lyophilized, and ground to a fine powder.

Freeze-dried extracts were re-dissolved in 0.1 mL of D_2O (for signal lock) and 0.9 mL of a solution containing 1 mol L^{-1} NaOH and 0.1 mol L^{-1} EDTA, and then transferred to a 5-mm NMR tube. Solution ^{31}P NMR spectra were obtained using a Bruker Avance DRX 500 MHz spectrometer (Rhinestetten, Germany) operating at 202.456 MHz for ^{31}P. Samples were analyzed using a 5 μs pulse (45°), a delay time of 5.0 s, an acquisition time of 0.8 s, and broadband proton decoupling. The number of scans varied between 4000 and 16 000, and spectra were plotted with a line broadening of 1 Hz. Chemical shifts of signals were determined in parts per million (ppm) relative to 85% H_3PO_4 and assigned to individual P compounds or functional groups based on literature values.[29] Signal areas were calculated by integration and P concentrations were calculated by multiplying the proportion of the total spectral area assigned to a specific signal by the total P concentration (g P kg^{-1} dry feces) in the original extract. This NMR procedure detects concentrations of P compounds of approximately 0.1 mg P kg^{-1} of dry feces.[28]

Statistics

Statistical analysis of the data was performed using PROC ANOVA procedures of the Statistical Analysis System Institute.[30] Student–Newman–Keul's multiple range tests were used to determine significant differences between treatment means. A simple regression analysis was used to relate P digestibility to the phytate content of the feed. The slope of the line and intercept were generated using Microsoft® Excel.

RESULTS

The chemical analysis of the barley diets confirmed that there was a reduction in the phytate content of the mutant barley varieties (Table 1). The wild-type barley diet (Copeland) contained 3.2 g kg^{-1} phytate P, while the mutant barley diets M422 and M635 contained phytate concentrations of 1.2 and 1.1 g kg^{-1}, respectively. The M955 barley diet contained only trace amounts of phytate. Total P concentration ranged from 3.0 g kg^{-1} in the mutant barley diets to 3.5 g kg^{-1} in the Copeland diet.

Solution ^{31}P NMR spectra of feed extracts are shown in Fig. 1. Extraction with NaOH–EDTA recovered >67% of the total feed P. Phytate, which gives signals at approximately 5.9, 5.0, 4.7 and 4.5 ppm in the ratio 1:2:2:1 in alkaline solution, accounted for the main differences in P composition of the feeds. As the phytate content in the feeds decreased, the phosphate content of the feed increased as shown by a relative increase in the signal at 6.1 ppm.

The selection for reduced phytate content did not appear to appreciably alter the chemical composition of the barley diets as crude protein, moisture, ash,

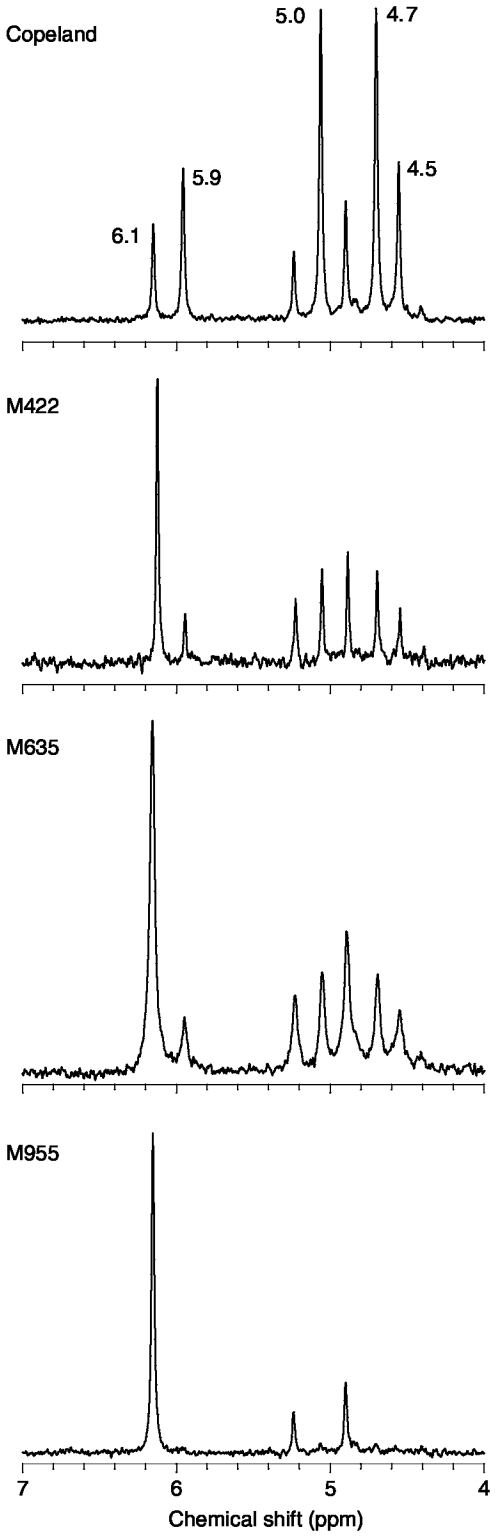

Figure 1. Solution ^{31}P NMR spectra of NaOH–EDTA extracts of feed fed to broiler chicks, including a wild-type (Copeland) and three low-phytate barley varieties (M422, M635 and M955).

and ether extract contents were not dramatically different between the mutant and wild-type barley diets (Table 1). There was an increase in neutral detergent fiber in the mutant barley diets.

The only significant difference ($P < 0.05$) in apparent digestibility of dry matter was between the highest (M955, 58.0%) and lowest (M422, 48.9%)

values (Table 2). There was no significant increase ($P < 0.05$) in apparent P digestibility as the level of phytate in the barley diets decreased with the exception of M955 whose P digestibility value was significantly greater than the other three barleys (Table 2). The relationship between the phytate content of the diet and apparent P digestibility was described by a linear regression model: % apparent P digestibility = 21.65–32.8 (% phytate); $r^2 = 0.34$; $P = 0.007$; $n = 20$.

Total fecal P concentrations ranged between 4.84 and 6.41 g P kg^{-1} (Table 3). For mutant barley diets, total fecal P concentrations were 13–24% lower than those from birds fed the normal barley diet. The greatest reduction in fecal P concentration was for birds fed the M422 barley diet. Solution ^{31}P NMR spectra of fecal extracts are shown in Fig. 2. Extraction with NaOH–EDTA recovered >92% of the total fecal P (Table 3). Most of the extracted P was phosphate (69–75%; Table 3), as indicated by the strong signal at approximately 6.1 ppm (Fig. 2). Signals between 3.4 and 6.0 ppm were assigned to phytate and phosphate monoesters, which constituted between 24 and 33% of the extracted P. Of these, phytate (signals at 5.9, 5.0, 4.7 and 4.5 ppm in the ratio 1:2:2:1) accounted for only a small proportion of the total P (3–12%). Signals at 4.9 and 5.2 ppm were assigned to β-glycerophosphate and phosphatidic acid, respectively. These compounds are breakdown products of phosphatidyl choline in alkaline solution.[29] There were significant differences between diets for NaOH–EDTA extractable P, phosphate, and phytate P ($P < 0.05$).

Table 2. Apparent digestibility coefficients for dry matter and phosphorus from diets comprised of normal and low-phytate varieties of barley

	Digestibility	
Barley used	Dry matter (%)	Phosphorus (%)
Copeland	53.7 ± 2.5^{ab}	13.0 ± 4.0^{b}
M422	48.9 ± 4.2^{b}	14.5 ± 5.4^{b}
M635	53.5 ± 2.6^{ab}	16.4 ± 4.8^{b}
M955	58.0 ± 6.3^{a}	24.8 ± 6.2^{a}

Values are means ± standard error of five pens per treatment.
Means followed by same letter in the same column do not differ statistically ($P < 0.05$).

DISCUSSION

The experimental diets used in the present experiment were formulated to quantify changes in fecal P composition from poultry diets containing different varieties of low phytate barley, free from the influence of other confounding P sources. As a result, they were deliberately formulated without additional P sources such as soybean meal and dicalcium phosphate as inclusion of these additional P sources would have hindered our ability to detect differences in P excretion for broilers fed the different barley varieties. Our diets contained over 97% barley and are therefore atypical of diets that would be fed commercially to broiler chicks.

The dry matter apparent digestibility of the barley-based diets was slightly lower than other reported values for barley, but not out of line based on diet formulation. Marquardt *et al.*[31] reported a dry matter digestibility of 67.5% for broilers fed barley-based diets, but their diets only included 69% barley, while the remainder of the diet was soybean meal, soybean concentrate, corn starch and tallow which are all highly digestible thereby increasing the overall digestibility of their diets. The lower apparent dry matter digestibility for birds fed the M422 diet can be attributed to the higher neutral detergent fiber content of this diet. Fiber is not very digestible by poultry and its presence impairs the digestibility of energy and other nutrients contained in the grain.[32,33] It is thought that dietary fiber reduces nutrient digestibility due to its physico-chemical properties, leading to a more rapid rate of passage, thus limiting the amount of time available for nutrient breakdown.[34]

Apparent P digestibility was somewhat low but was in line with data from other studies. Li *et al.* reported a P digestibility of 28% from broilers fed a normal barley diet.[9] However, this diet contained

Table 3. Phosphorus composition in the feces of poultry fed a variety of normal and low-phytate barley diets

		NaOH–EDTA extractable P				
Barley used	Total manure P	Total NaOH–EDTA P*	Phosphate†	Phosphomonoesters†‡	Phospholipids†	Phytate†
Copeland	6.41 ± 0.23	6.36 ± 0.11 (99)^{a}	4.46 ± 0.14 (70)^{a}	1.19 ± 0.13 (19)^{a}	ND[4]	0.74 ± 0.28 (12)^{a}
M422	4.84 ± 0.17	4.48 ± 0.35 (93)^{c}	3.20 ± 0.05 (72)^{c}	0.95 ± 0.06 (19)^{a}	ND	0.34 ± 0.03 (8)^{ab}
M635	5.39 ± 0.27	4.93 ± 0.31 (92)^{bc}	3.42 ± 0.25 (69)^{c}	1.18 ± 0.05 (26)^{a}	ND	0.34 ± 0.03 (7)^{ab}
M 955	5.59 ± 0.44	5.15 ± 0.44 (92)^{b}	3.88 ± 0.27 (75)^{b}	1.10 ± 0.13 (21)^{a}	0.04 ± 0.07 (0.7)	0.14 ± 0.25 (3)^{b}

Phosphorus concentrations were determined by NaOH–EDTA extraction and solution ^{31}P NMR. Results are given as g P kg dry wt^{-1}.
* Values are means ± standard deviation of three pens per feeding treatment, and values in parenthesis are the proportion (%) of the total manure P determined by microwave digestion.
† Values in parenthesis are the proportion (%) of the NaOH–EDTA extracted P.
‡ Values for phosphate monoesters include all monoesters other than phytate.
ND, not detected.
$^{a-c}$ Means in the same column followed by the same letter do not differ ($P > 0.05$).

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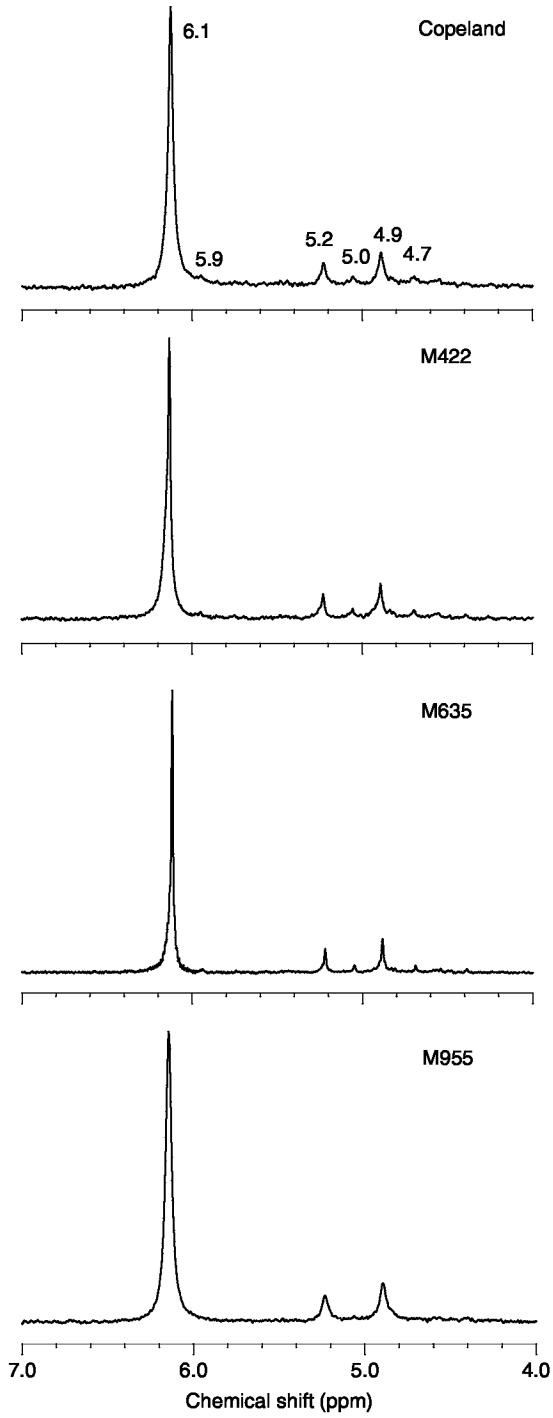

Figure 2. Solution ^{31}P NMR spectra of NaOH–EDTA extracts of feces from broiler chicks fed a variety of barley feeds, including a wild-type (Copeland) and three low-phytate barley varieties (M422, M635 and M955).

dicalcium phosphate and β-glucanase which would greatly improve P digestibility. Fan and Sauer[35] found that the P digestibility of barley fed to growing-finishing pigs was only 28%, which would be expected to be much higher than for poultry. The improvement in P utilization from feeding low-phytate barley diets agrees with previous studies in which low-phytate barley improved P digestibility in poultry.[8,9] Feeding low-phytate corn also improved P utilization for poultry.[11,36–38]

Since poultry do not fully digest phytate in the gut, it is commonly assumed that poultry feces contain undigested phytate. Indeed, characterization of broiler litters and manures by solution ^{31}P NMR spectroscopy showed as much as 80% phytate in manures from birds fed corn-based diets.[4,28,39] Therefore, we expected to find large concentrations of phytate in the feces of broiler chicks fed the wild-type (Copeland) barley diet, and lower phytate concentrations in the feces generated from the mutant barley diets. However, our results clearly demonstrate that little phytate was excreted in the feces of birds fed barley-based diets and that the majority of P was excreted as phosphate, irrespective of the phytate content of the diet. Phytate is extremely stable in the alkaline conditions of the analytical procedure used here, so the results are not a methodological artifact.[29]

There are several possible explanations for the marked difference between this and previous studies that reported high phytate contents in poultry litter and manures.[4,39,40] There are large differences in grain phytase activity between corn and barley, which could influence phytate hydrolysis in the bird: corn has an average of 20 units phytase kg^{-1} while barley has an average of 540 units phytase kg^{-1}.[41] There may have been an influence of bird age, since most previous studies examined litter and manure from mature birds and the present study was performed with younger chicks. However, there is little evidence that age alters specific phytase activity in the digestive tract of poultry.[42–44]

Another possibility is that the present study had no supplemental calcium added to the diets, which can inhibit phytate hydrolysis in broiler chicks. Tamin *et al.*[45] found that addition of 0.5% calcium to a broiler diet resulted in a 63% reduction in ileal phytate hydrolysis. Maenz *et al.*[46] found that mineral–phytate complexes reduced the hydrolysis of phytate by microbial phytase and that the potency of inhibitors was in the order of $Zn^{2+} \gg Fe^{2+} > Mn^{2+} > Fe^{3+} > Ca^{2+} > Mg^{2+}$ at neutral pH. In addition, ternary complexes of phytate, calcium and protein may be formed.[47] The lack of added calcium in these diets may have allowed the broilers to hydrolyze the phytate in the grains and therefore there was little phytate excreted in the manures.

Assays of phytase activity in different sections of the gastrointestinal tract of chickens have shown that hindgut micro-organisms have an important impact on phytate hydrolysis.[42–44] Therefore, it is likely that the phytate degradation observed in the present experiment was also facilitated by microfloral phytase activity in the hindgut of broilers in a similar manner to that reported previously for swine fed barley.[18] In monogastric animals, there are two primary mechanisms involved in phosphate absorption: namely, an active transport system and a passive transport system. Active transport occurs primarily in the proximal small intestine while passive transport occurs primarily in the jejunum and

ileum.[48,49] Therefore, any P liberated as a result of phytate hydrolysis in the hindgut cannot be utilized and, as indicated by our digestibility data, is simply excreted. This finding does not negate the potential benefits from including phytase in the diet of poultry, as dietary phytase hydrolyzes phytate in that portion of the digestive tract (crop and proventriculus) where absorption of P can still take place and benefit the bird.[50]

CONCLUSION

This study indicates that there is little phytate excreted from broilers fed both normal and low phytate barley diets. Broilers fed low phytate barley versus a normal barley diet excrete less total P, but the overall composition of P is the feces is similar. Our results highlight the importance of obtaining information on the P composition of feces when assessing the impact of an animal's diet on the solubility and environmental fate of P in feces. Additional information is now required for other monogastric animals fed a range of diets.

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