Phosphorus Utilization and Characterization of Ileal Digesta and Excreta from Broiler Chickens Fed Diets Varying in Cereal Grain, Phosphorus Level, and Phytase Addition

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ABSTRACT Both intrinsic and exogenous phytase in poultry feeds can alter phytate utilization and the solubility of P excreted. This experiment determined the effects of feeding diets varying in cereal grain, P concentration and phytase addition on phytate and P utilization and P characterization of ileal digesta and excreta. Twelve treatments, consisting of diets based on corn, wheat, barley, or high fat-low lignin oat and 3 P treatments (low P with 0.30% nonphytate P; low P + 1,000 phytase units of phytase; high P with 0.45% nonphytate P), were fed to 300 broilers using a factorial design. Fresh excreta were collected at 20 and 21 d and ileal digesta was collected at 21 d. Ileal digesta and excreta were analyzed for total P, phytate P and Ca, with P composition determined by solution ^{31}P nuclear magnetic resonance spectroscopy. Excreta samples were also analyzed for water soluble P (WSP). Apparent ileal digestibility coefficients for phytate P and total P ranged from 0.03 to 0.42 and 0.56 to 0.71, respectively. Diets supplemented with phytase had greater phytate P hydrolysis than unsupplemented diets. Apparent total tract digestibility coefficients for phytate P and total P ranged from 0.10 to 0.73 and 0.43 to 0.61, respectively. Across cereal grains, there was almost a 3-fold increase in total tract phytate P hydrolysis with phytase supplementation. The P composition of ileal digesta was predominantly phytate P (70 to 88% of total P), whereas excreta phytate P ranged from 26 to 76% of total P. Excreta WSP ranged from 3.2 to 7.5 g kg^{-1} and was least for the barley diets. There was a 25% reduction in excreta WSP from the high P to the low P + phytase diets and a 37% reduction from the high P to the low P diets. As cereal grain had little influence on phytate digestibility, it is unlikely that intrinsic phytase in grain has much influence on phytate utilization by poultry. Both total P and WSP in excreta were reduced by the low P diet and the low P + phytase diet, irrespective of cereal grain, which reduces the risk of P transfer to water bodies when excreta are applied to land as fertilizer.

Key words: broiler, phosphorus, phytate, phytase, cereal

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INTRODUCTION

The availability of P from cereal grains is largely determined by the percentage of P in the grain bound as phytate, which can vary between 50 and 85% of the total P (NRC, 1994; Ravindran et al., 1994). Poultry are relatively inefficient in utilizing phytate P because they do not produce significant quantities of the digestive enzyme phytase that is required to hydrolyze the phytate molecule (Sebastian et al., 1998). The poor digestibility of phytate P means that inorganic sources of P must be used in diet formulation to meet the nutritional requirements of birds, thereby increasing the cost of poultry production.

One way to overcome the poor availability of phytate P in poultry diets is by supplementing the diet with exogenous phytase. The addition of phytases to corn-soy diets has been shown to improve P digestibility by 10 to 24% (Camden et al., 2001; Rutherford et al., 2002). In addition to improved P utilization, numerous studies have also shown reductions in total excreta P of 29% to 45% when dietary nonphytate P (**NPP**) was reduced in combination with added phytase (Applegate et al., 2003; Smith et al., 2004; Vadas et al., 2004; McGrath et al., 2005; Angel et al., 2006).

In addition to exogenous phytases, feed ingredients also contain phytase. The presence of these intrinsic phytases in ingredients used in poultry diets may influence phytate degradation and utilization, thereby alter-

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ing the total amount and composition of the P excreted. The intrinsic phytase activities in grains such as wheat and barley can be high [500 to 1,200 phytase units (**FTU**)·kg^{-1}], whereas the activities in grains such as corn and oat tend to be quite low (15 to 40 FTU·kg^{-1}) and could have a varying effect on phytate degradation when fed to animals (Eeckhout and De Paepe, 1994).

Whereas phytase supplementation to corn-soy diets has consistently decreased the total P in excreta, the effects on the water soluble P (**WSP**) in excreta has been inconclusive. Phytase addition to poultry diets was shown to decrease the WSP concentration in excreta by 35.6% (Applegate et al., 2003) and by 29% (McGrath et al., 2005). However, in other studies, phytase supplementation to diets had no effect on excreta WSP concentration (Saylor et al., 2001; Maguire et al., 2004; McGrath et al., 2005), whereas in 2 studies, amendment of diets with phytase increased the concentration of WSP in excreta (DeLaune et al., 2001; Vadas et al., 2004). As many poultry producing states are starting to regulate land application of excreta based on its WSP concentration, the variable effect of diet modification on WSP in excreta remains a concern from a management standpoint, as excreta WSP has been shown to be highly correlated with P losses following land application.

Although there is evidence that phytate can be utilized by poultry fed diets containing grains with high levels of intrinsic phytase (Leytem et al., 2007), there has been no published work that has examined the effects of intrinsic grain phytase activity or the addition of exogenous phytase to grains with high intrinsic phytase activities on the WSP concentrations of the resulting excreta. Therefore, the following experiment was conducted to determine the effects of feeding broiler chickens diets varying in cereal grain, P concentration, and phytase addition on phytate and P utilization as well as P characterization in ileal digesta and excreta and the effects on excreta WSP.

MATERIALS AND METHODS

Experimental Design

A total of 300 one-day-old male broiler chicks (Ross-308 line, Lilydale Hatchery, Wynyard, Saskatchewan, Canada), weighing an average of 44.8 ± 1.5 g, were fed 1 of 12 diets arranged in a 3 × 4 factorial design (Table 1). The experimental diets were based on 1 of 4 cereal grains (diets contained approximately 56.67% grain) consisting of feed-grade corn, wheat, or barley as well as a newly developed high fat-low lignin oat (CDC SO-1; Thacker and Rossnagel, 2005). Within each cereal grain, 2 diets were formulated to be deficient in P (0.30% NPP), whereas the remaining diet was formulated to meet NRC (1994) requirements for P (0.45% NPP). The P-deficient diets were fed with and without phytase (1,000 FTU phytase per kg of diet; Natuphos BASF, Ludwigshafen, Germany). In addition to phytase, all diets were supplemented with 0.1% Endofeed (GNC Bioferm, Saskatoon, Saskatchewan, Canada) and 0.1% Avizyme 1300 (Danisco Animal Nutrition, Scarborough, Ontario, Canada) to provide β-glucanase and xylanase enzymes to avoid potential digestibility problems arising from the presence of β-glucans and pentosans in the cereal grains.

All diets were formulated to contain 2,950 kcal of ME·kg^{-1} by varying the amount of canola oil and corn starch added to the ration. The diets were also formulated to provide a similar pattern of essential amino acids by adding supplemental lysine, threonine, methionine, and tryptophan. All diets contained sufficient vitamins and trace minerals to meet NRC (1994) recommendations. The diets were provided in mash form (3-mm screen) and contained 3.5 g·kg^{-1} of chromic oxide as an indigestible marker.

Bird Housing and Management

The birds used in this experiment were cared for according to the guidelines of the Canadian Council on Animal Care (1993). The chicks were housed in raised-floor battery cages (83.8 cm × 45.7 cm × 25.4 cm; Jamesway Manufacturing Co., Ft. Atkinson, WI) with mesh grate floors above excreta collection trays. There were 5 birds per pen and 5 replicate pens per treatment. Feed and water were available ad libitum throughout the 21-d experiment. The battery brooder temperature was initially set at 29°C and gradually reduced to 22°C by d 21. Incandescent lighting (23 h light, 1 h dark) was provided with a lighting intensity of 10 lx.

Broilers were weighed at the start (d 1) and end of the experiment (d 21) as well as at weekly intervals. Weighed amounts of feed were added as required with a single weigh back at the conclusion of the experiment to allow for the calculation of feed consumption and feed conversion on a pen basis. On d 20 and 21, 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 4 collections were pooled by placing the samples into an aluminum pan and stirring with a rubber spatula. The pooled samples were then frozen. Before analysis, the samples were lyophilized, followed by fine grinding (<2 mm).

Following the final excreta collection on d 21, the birds were killed by cervical dislocation and the entire intestinal length from the proventriculus to the cloaca excised. The ileum was identified as the segment extending distally from Meckel's diverticulum to the ileocecal junction. The content of the ileum from 2 birds was gently expressed and placed into a single 15-mL conical tube and snap frozen in liquid nitrogen to arrest microbial activity. Two pooled samples were collected from each pen. The samples were lyophilized and ground (<2 mm) before analysis.

Table 1. Ingredient composition of diets used to determine the effects of cereal grain (corn, wheat, high fat-low lignin oat, or barley), P concentration (low or high), and phytase supplementation (− or +) on ileal and excreta P digestibility and P characterization for broiler chicks (% as fed)

	Corn			Wheat			High fat-low lignin oat			Barley		
	Low	Low	High	Low	Low	High	Low	Low	High	Low	Low	High
Item	−	+	−	−	+	−	−	+	−	−	+	−
Cereal	56.67	56.67	56.18	56.67	56.67	56.18	56.67	56.67	56.18	56.67	56.67	56.18
Soybean meal	31.64	31.64	31.37	31.64	31.64	31.37	31.64	31.64	31.37	31.64	31.64	31.37
Corn starch	6.98	6.96	6.92	2.79	2.77	2.77	0.00	0.00	0.00	0.00	0.00	0.00
Canola oil	0.00	0.00	0.00	4.72	4.72	4.68	7.43	7.41	7.39	7.51	7.49	7.45
Salt	0.50	0.50	0.50	0.50	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	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	0.35	0.35	0.35	0.35
Enzyme[2]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Choline	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Threonine	0.15	0.15	0.15	0.10	0.10	0.10	0.08	0.08	0.08	0.12	0.12	0.12
Methionine	0.28	0.28	0.28	0.19	0.19	0.19	0.26	0.26	0.26	0.21	0.21	0.21
Tryptophan	0.09	0.09	0.09	0.03	0.03	0.03	0.03	0.03	0.03	0.06	0.06	0.06
Lysine	0.10	0.10	0.10	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Limestone	1.43	1.43	1.41	1.34	1.34	1.33	1.12	1.12	1.10	1.41	1.41	1.39
Dicalcium phosphate	1.03	1.03	1.87	0.88	0.88	1.71	1.14	1.14	1.96	0.75	0.75	1.59
Phytase[3]	0.00	0.02	0.00	0.00	0.02	0.00	0.00	0.02	0.00	0.00	0.02	0.00

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

[2]Combination of 0.1% Endofeed (GNC Bioferm, Saskatoon, Saskatchewan, Canada) and 0.1% Avizyme (Danisco, Scarborough, Ontario, Canada) to provide β-glucanase and xylanase enzymes.

[3]Natuphos (BASF, Ludwigshafen, Germany) provided 1,000 phytase units per kilogram of diet.

Chemical Analysis of Feed, Ileal Digesta, and Excreta

Samples of the experimental diets were analyzed (Table 2) according to the methods of the Association of Official Analytical Chemists (1990). Analyses were conducted for moisture (AOAC 930.15), CP (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. (1991). Amino acid analysis 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 before analysis. Sulfur-containing amino acids were analyzed after cold formic acid oxidation for 16 h before acid hydrolysis.

Phytase activity in feed was determined according to the method of Engelen et al. (2001; phytase activity is expressed as FTU per unit of feed; 1 FTU is the amount of phytase that liberates 1 μmol of inorganic P per min

Table 2. Chemical analysis of diets used to determine the effects of cereal grain (corn, wheat, high fat-low lignin oat, or barley), P concentration (low or high), and phytase supplementation (− or +) on ileal and excreta P digestibility and P characterization in broiler chicks (% as fed)

	Corn			Wheat			High fat-low lignin oat			Barley		
	Low	Low	High	Low	Low	High	Low	Low	High	Low	Low	High
Item	−	+	−	−	+	−	−	+	−	−	+	−
Moisture	7.43	8.42	7.86	7.63	8.10	8.12	6.53	6.58	6.51	6.88	7.19	6.99
Ash	5.76	5.63	5.88	5.71	5.31	6.01	6.31	6.68	6.93	5.76	5.75	6.56
Crude protein	21.86	20.97	20.62	22.43	23.20	23.21	21.93	21.15	22.20	22.07	21.84	22.74
Ether extract	2.09	2.23	2.15	6.02	5.62	5.69	9.98	10.29	10.68	9.23	9.18	9.24
Neutral detergent fiber	8.51	7.31	7.45	10.01	9.90	10.06	15.85	15.22	15.01	11.45	10.08	10.45
Lysine	1.18	1.16	1.11	1.16	1.11	1.10	1.21	1.14	1.19	1.15	1.13	1.13
Methionine + cystine	0.82	0.82	0.78	0.80	0.81	0.83	0.92	0.87	0.84	0.83	0.82	0.81
Threonine	0.90	0.91	0.85	0.80	0.84	0.84	0.87	0.82	0.86	0.90	0.89	0.87
Calcium	0.89	0.84	0.94	0.83	0.81	0.96	0.77	0.92	0.97	0.72	0.72	1.04
Total P	0.59	0.57	0.71	0.60	0.59	0.72	0.63	0.67	0.83	0.53	0.52	0.76
Phytate P	0.26	0.25	0.25	0.31	0.30	0.28	0.28	0.28	0.32	0.26	0.26	0.25
Nonphytate P[1]	0.33	0.32	0.46	0.29	0.29	0.44	0.35	0.39	0.51	0.27	0.26	0.51
Phytase (phytase units/kg)	166	1,352	142	889	1,809	899	50	845	ND[2]	370	1,875	491

[1]Defined as the difference between total P and phytate P.

[2]ND = nondetectable.

from an excess of sodium phytate at pH 5.5 and 37°C). Phytate P was determined by acid extraction followed by HPLC analysis (Agilent HPLC 1100 series, Agilent Technologies, Wilmington, DE; Kwanyuen and Burton, 2005).

Experimental diets, excreta, and ileal samples were analyzed for total elements (Ca, P, Cr_2O_3) by modification of the method of Fenton and Fenton (1979) as follows: 0.5 g of dried sample was ashed and then digested with the digestion mixture and all elements were quantified using inductively coupled plasma optical-emission spectrometry (4300DV, Perkin-Elmer, Wellesley, MA) detection. The P composition of the ileal and excreta samples was determined by solution ^{31}P nuclear magnetic resonance spectroscopy as described by Leytem et al. (2007). Samples from 3 of the 5 replicate pens per treatment were randomly selected for analysis because of the expense of ^{31}P nuclear magnetic resonance analysis.

Calculations and Statistical Analyses

The apparent ileal (**AIDC**) and total tract (i.e., overall retention) nutrient digestibility coefficients (**ATTDC**) were calculated using the indicator method based on the following equation:

$$\text{AIDC or ATTDC} = 1 - [(Cr_{diet}/\ Cr_{out}) \times (Nut_{out}/\ Nut_{diet})], \quad [1]$$

where Cr_{diet} was the initial chromic oxide concentration in the diet; Nut_{diet} was the initial dietary concentration of the nutrient being assessed; and Cr_{out} and Nut_{out} were the respective concentrations of either chromic oxide or nutrient in the ileal digesta or excreta, respectively. Further, the total nutrients excreted (**TNE_g**) per kilogram of DM intake (**DMI**) were calculated for P and WSP using the ratio of chromic oxide intake to chromic oxide output (Dilger and Adeola, 2006):

$$TNE_g\ (g/kg\ of\ DMI) = NcE \times (Cr_{diet}/Cr_{out}), \quad [2]$$

where NcE was the concentration of the respective nutrient in the excreta; Cr_{diet} was the initial chromic oxide concentration in the diet; and Cr_{out} was the concentration of chromic oxide in the excreta.

The pen was the experimental unit for all measurements. Statistical analysis was performed using the Statistical Analysis System (SAS Institute, 2004). All variables were tested for normality using the Shapiro-Wilk test with the PROC CAPABILITY procedure. Where results suggested nonnormality, variables were log-transformed before statistical analyses, with untransformed numbers presented in the text. Data were analyzed as a factorial ANOVA using the GLM procedure of SAS with grain, P treatment, and their interaction as fixed effects. Where appropriate, means separation was carried out using the Ryan-Einot-Gabriel-Welsch multiple range test with an α level of 0.05.

RESULTS

Performance data are presented in Table 3. The 0 to 21 d gain in BW ranged from 439.9 to 760.4 g with a significant main effect of grain and grain × treatment interaction. Average BW gains were 699.7, 677.2, 613.8, and 507.2 for the wheat, barley, corn, and high fat-low lignin oat diets, respectively, following the trend wheat = barley > corn > oat. The high fat-low lignin oat diets had the only significant difference in BW due to P treatment and had a BW gain of 567.7, 514.2, and 439.8 for the low P, low P + phytase, and high P treatments, respectively, with the low P and high P treatments being significantly different. Cumulative feed intake averaged 943.4, 879.2, 868.2, and 671.5 for the wheat, corn, barley, and high fat-low lignin oat diets, respectively, and had a significant main effect of grain with the oat diet having significantly less feed intake than the other diets, which did not differ. The feed conversion ratios ranged from 1.25 to 1.45 with a significant main effect of grain and grain × treatment interaction. Mortality ranged from 0 to 8% and was not significantly different with respect to grain or treatment.

Apparent ileal digestibility coefficients are presented in Table 4. The AIDC for DM ranged from 0.64 to 0.74 with a significant effect of grain and grain × P treatment interaction. The AIDC for phytate P ranged from 0.03 to 0.42 with significant main effects of both grain and P treatment. Average AIDC for phytate P were 0.28, 0.26, 0.26, and 0.10 for high fat-low lignin oat, barley, wheat, and corn treatments, respectively, with the corn diet being significantly less than the other diets which did not differ. Diets having phytase supplementation had significantly greater phytate P digestibility (0.33) than the low (0.18) and high P diets (0.17), which were not significantly different. The AIDC for P ranged from 0.56 to 0.71, with a significant main effect for both grain and P treatment. Average AIDC for P were 0.67, 0.64, 0.61, and 0.57 for high fat-low lignin oat, wheat, barley, and corn treatments, respectively, with the only significant difference between the high fat-low lignin oat and corn diets. High P diets had a greater total P digestibility (0.64) than low P diets (0.60) but were not significantly greater than the low P + phytase diets (0.63). The AIDC for Ca ranged from 0.43 to 0.68 with a significant main effect of grain. Average AIDC for Ca were 0.66, 0.57, 0.56, and 0.46 for high fat-low lignin oat, wheat, barley, and corn treatments, respectively, with the only significant difference being between the oat and corn diets.

Apparent total tract digestibility coefficients are presented in Table 5. The ATTDC for DM ranged from 0.60 to 0.73 with a significant main effect of grain and grain × P treatment interaction. The corn diets had the greatest average DM digestibility coefficients (0.72), whereas the high fat-low lignin oat diets had the least

Table 3. Body weight gain, feed intake, feed conversion ratio (FCR), and mortality in broiler chicks fed diets varying in cereal grain, P concentration, and phytase additions (values are means of 5 replicates of 5 birds per pen)

	Performance			
Item	0 to 21 d of BW, g	Feed intake, g	FCR	Mortality, %
Barley				
Low P	663.4	877.2	1.32	4.0
Low P + phytase	671.5	838.0	1.25	0.0
High P	696.9	894.7	1.28	8.0
Corn				
Low P	624.9	906.4	1.45	0.0
Low P + phytase	621.7	894.8	1.44	4.0
High	594.7	836.2	1.40	4.0
High fat-low lignin oat				
Low P	567.7	720.4	1.27	4.0
Low P + phytase	514.2	674.8	1.31	0.0
High P	439.9	619.4	1.42	4.0
Wheat				
Low P	637.5	877.8	1.38	0.0
Low P + phytase	760.4	1,002.1	1.32	4.0
High P	701.3	950.4	1.36	4.0
SEM	13.04	17.21	0.01	1.04
P-value				
Grain	<0.0001	<0.0001	<0.0001	0.97
Treatment	0.27	0.59	0.17	0.45
Grain × treatment	0.02	0.12	0.01	0.84

(0.61). The ATTDC for phytate P ranged from 0.10 to 0.73 with a significant main effect of P treatment and grain × P treatment interaction. As with ileal phytate P digestibility, diets with phytase amendment had the greatest phytate P digestibility coefficients (0.64), whereas the low (0.24) and high P (0.19) diets were not significantly different, except for the barley diets where the low P treatment (0.36) was greater than the high P treatment (0.11). There was, approximately, a 3-fold increase in phytate P ATTDC when diets were supplemented with phytase.

The ATTDC for P ranged from 0.43 to 0.61 with a significant main effect of both grain and P treatment. Average ATTDC for P were 0.56, 0.56, 0.52, and 0.46 for corn, barley, wheat, and high fat-low lignin oat, respectively, and followed the trend corn = barley > wheat > oat. The ATTDC for P was significantly different between all P treatments and was greatest in the

Table 4. Apparent ileal digestibility coefficients for DM, phytate P, P, and Ca from broiler chicks fed diets varying in cereal grain, P concentration, and phytase additions (values are means of 5 replicates of 4 birds per pen)

	Apparent digestibility coefficient			
Item	DM	Phytate P	Total P	Ca
Corn				
Low P	0.71	0.03	0.56	0.52
Low P + phytase	0.74	0.22	0.59	0.51
High P	0.73	0.06	0.57	0.36
Wheat				
Low P	0.67	0.18	0.57	0.55
Low P + phytase	0.71	0.42	0.62	0.52
High P	0.73	0.19	0.64	0.63
High fat-low lignin oat				
Low P	0.65	0.20	0.64	0.67
Low P + phytase	0.64	0.30	0.67	0.64
High P	0.65	0.29	0.71	0.68
Barley				
Low P	0.71	0.30	0.64	0.65
Low P + phytase	0.70	0.40	0.63	0.43
High P	0.67	0.16	0.65	0.61
SEM	0.005	0.024	0.008	0.020
P-value				
Grain	<0.0001	0.002	<0.0001	0.001
P treatment	0.20	0.0007	0.02	0.21
Grain × P treatment	0.0007	0.30	0.25	0.08

Table 5. Apparent total tract digestibility coefficients for DM, phytate P, P, and calcium from broiler chicks fed diets varying in cereal grain, P concentration, and phytase additions (values are means of 5 replicates of 5 birds per pen)

	Apparent digestibility coefficient			
Item	DM	Phytate P	Total P	Ca
Corn				
Low P	0.71	0.16	0.57	0.57
Low P + phytase	0.73	0.64	0.61	0.58
High P	0.73	0.10	0.51	0.58
Wheat				
Low P	0.66	0.25	0.51	0.57
Low P + phytase	0.70	0.73	0.55	0.58
High P	0.71	0.23	0.49	0.55
High fat-low lignin oat				
Low P	0.62	0.21	0.46	0.47
Low P + phytase	0.60	0.59	0.51	0.60
High P	0.62	0.39	0.43	0.52
Barley				
Low P	0.69	0.36	0.59	0.51
Low P + phytase	0.67	0.62	0.59	0.50
High P	0.62	0.11	0.49	0.49
SEM	0.006	0.040	0.008	0.010
P-value				
Grain	<0.0001	0.08	<0.0001	0.005
P treatment	0.49	<0.0001	<0.0001	0.40
Grain × P treatment	<0.0001	0.006	0.16	0.21

Table 6. Phosphorus characterization of ileal digesta from broiler chicks fed diets varying in cereal grain, P concentration, and phytase additions (determined by NaOH-EDTA extraction and solution ^{31}P nuclear magnetic resonance spectroscopy; values are means of 3 replicates of 4 birds per pen)

		NaOH–EDTA extractable P			
Item	Total P	Phosphate[1]	Phosphate monoesters[1,2]	Phytate[1]	Pyrophosphate[1]
	g·kg of dry wt^{-1} of P				
Corn					
Low P	9.68	0.90 (9)	0.57 (6)	8.21 (85)	0.11 (1)
Low P + phytase	10.80	2.12 (19)	1.19 (11)	7.50 (70)	0.09 (1)
High P	12.10	2.73 (21)	0.96 (8)	8.96 (71)	0.23 (2)
Wheat					
Low P	8.63	0.60 (7)	0.45 (5)	7.63 (88)	0.02 (0.3)
Low P + phytase	8.37	1.08 (14)	1.21 (15)	6.08 (71)	0.02 (0.2)
High P	10.53	1.01 (10)	0.94 (9)	8.58 (82)	0.10 (1)
High fat-low lignin oat					
Low P	7.12	0.63 (9)	0.22 (3)	6.27 (88)	ND[3]
Low P + phytase	6.70	0.80 (12)	0.55 (8)	5.35 (80)	0.03 (0.3)
High P	7.66	0.92 (12)	0.56 (7)	6.18 (81)	0.05 (0.6)
Barley					
Low P	7.28	0.57 (8)	0.51 (7)	6.20 (85)	ND
Low P + phytase	6.89	0.83 (12)	0.81 (12)	5.25 (76)	ND
High P	8.87	1.09 (12)	0.65 (7)	7.12 (80)	0.03 (0.3)
SEM	0.303	0.125	0.070	0.236	0.011
P-value					
Grain	<0.0001	<0.0001	0.04	<0.0001	<0.0001
P treatment	<0.0001	<0.0001	0.007	0.0005	<0.0001
Grain × P treatment	0.37	0.44	0.91	0.78	0.12

[1]Values in parentheses are the proportion (%) of the NaOH–EDTA extracted P.

[2]Values for phosphate monoesters include all monoesters other than phytate.

[3]ND = not detected.

phytase amended diets (0.57), which, on average, was 7% greater than the low P diets (0.53) and 18% greater than the high P diets (0.48).

The total P excreted per kilogram of DMI was also calculated to provide a better indication of the potential effects of P treatment on P loading in the environment and ranged from 2.08 to 5.72 g·kg^{-1} of DMI. There was a significant effect of grain ($P < 0.0001$), P treatment ($P < 0.0001$), and their interaction ($P = 0.002$). The barley, high fat-low lignin oat, and wheat diets followed the trend high $P >$ low $P =$ low P + phytase and had a 46, 26, and 25% reduction in total P output per kilogram of DMI from the high P to the low P and low P + phytase treatments, respectively. The corn diets followed the trend high $P >$ low $P >$ low P + phytase with a 29% decrease in total P output per kilogram of DMI in the low P compared with the high P treatment and a 37% reduction in total P output per kilogram of DMI in the low P + phytase compared with the high P treatment.

The ATTDC for Ca ranged from 0.47 to 0.60 with a significant main effect of grain. Average ATTDC for Ca was 0.58, 0.57, 0.53, and 0.50 for the corn, wheat, high fat-low lignin oat, and barley treatments, respectively, with the corn and wheat treatments being significantly greater than the barley treatment.

The P characterization of the ileal digesta is shown in Table 6. There was a significant main effect of both grain and P treatment for all P measurements. Total P in ileal digesta ranged from 6.70 to 12.10 g·kg^{-1} following this trend: corn (10.86 g·kg^{-1}) > wheat (9.18 g·kg^{-1}) > barley (7.68 g·kg^{-1}) = oat (7.16 g·kg^{-1}) and was, on average, 20% greater in the high P diets (9.79 g·kg^{-1}) vs. the low (8.19 g·kg^{-1}) and low + phytase (8.19 g·kg^{-1}) diets, which were not significantly different. The majority of P in ileal digesta was in the form of phytate P, which ranged from 70 to 88% of the total P in ileal digesta. Diets with phytase amendment had, on average, 19% less phytate P (6.04 g·kg^{-1}) than both the high (7.71 g·kg^{-1}) and low P diets (7.08 g·kg^{-1}), which did not differ significantly.

Phosphate P in ileal digesta ranged from 0.57 to 2.73 g·kg^{-1}, comprising between 7 and 21% of total P, with the phytase amended (1.2 g·kg^{-1}) and high P (1.4 g·kg^{-1}) diets having significantly greater phosphate concentrations than the low P diet, which had only 0.68 g·kg^{-1}. Phosphate monoester P, which includes all inositol esters other than phytate, ranged from 0.22 to 1.21 g·kg^{-1}, comprising between 3 and 15% of the total P, with the phytase amended (0.94 g·kg^{-1}) and high P diets (0.78 g·kg^{-1}) having, on average, twice the concentration of the low P diets (0.44 g·kg^{-1}). There were small amounts of pyrophosphate present, but in most cases this represented less than 1% of total P.

The P characterization of excreta is shown in Table 7. Total P in excreta ranged from 7.03 to 15.44 g·kg^{-1} with significant main effects of grain and P treatment as well as their interaction. Average total P concentrations in the excreta were 11.47, 10.95, 10.11, and 8.34 g·kg^{-1} for corn, wheat, high fat-low lignin oat, and barley, respectively, with the corn and wheat diets having the greatest concentration whereas barley had the

least. Both the low P (9.07 g·kg^{-1}) and low P + phytase treatments (8.58 g·kg^{-1}) were not significantly different for any of the grains and produced significantly less total P in excreta than the high P diets (12.98 g·kg^{-1}) with an average decrease of 32% across diets. The phytate P concentration in excreta ranged from 2.54 to 8.67 g·kg^{-1}, comprising between 26 and 76% of the total excreta P. There was a significant main effect of both grain and P treatment on phytate P in excreta, with birds fed the corn diets having significantly greater phytate P concentrations (6.56 g·kg^{-1} of P) compared with the other grains (~5.0 g·kg^{-1} of P). Birds fed diets having phytase additions had significantly less phytate P in excreta (2.94 g·kg^{-1}, 56% reduction) than birds fed nonphytase amended diets, which did not differ significantly (6.90 and 6.32 g·kg^{-1} for the high and low P diets).

Phosphate concentration in excreta ranged from 1.50 to 6.10 g·kg^{-1}, comprising between 20 and 57% of total excreta P. Average phosphate concentrations were 4.66, 4.43, 4.00, and 2.53 g·kg^{-1} for high fat-low lignin oat, wheat, corn, and barley treatments, respectively, with birds fed barley diets having significantly less phosphate concentration in excreta than the other diets, which did not differ significantly. The average phosphate concentration in excreta were 5.25, 4.23, and 2.24 g·kg^{-1} for the high P, low P + phytase, low P treatments, respectively, with all treatments being significantly different. Phosphate monoester concentrations in excreta ranged from nondetectable (**ND**) to 1.80 g·kg^{-1}, comprising between 0 and 19% of the total P, whereas pyrophosphate concentrations ranged from ND to 0.15 g·kg^{-1} and were less than 2% of total excreta P.

The WSP in excreta ranged from 3.2 to 7.5 g·kg^{-1} with significant main effects of grain and P treatment (Figure 1). The average WSP concentration in excreta were 5.73, 5.47, 5.24, and 3.77 g·kg^{-1} for the wheat, corn, high fat-low lignin oat, and barley treatments, respectively, with the barley treatment having a significantly less WSP concentration compared with the other grains ($P < 0.0001$), which did not differ significantly. The average WSP concentration in excreta was significantly different for all P treatments and was greatest ($P < 0.0001$) for birds fed the high P diets (6.37 g·kg^{-1}) and least for birds fed the low P (4.02 g·kg^{-1}) diets with the low P + phytase (4.75 g·kg^{-1}) amended diets falling in the middle. There was a 25% reduction in excreta WSP from the high P to the low P + phytase diets, and a 37% reduction in excreta WSP from the high P to the low P diets. The WSP output per kilogram of DMI was also calculated and was significantly affected by both the grain ($P < 0.0001$) and P treatment ($P < 0.0001$), whereas the interaction term was insignificant. The average output of WSP was 2.02, 1.76, 1.49, and 1.30 g kg·DMI^{-1} for the high fat-low lignin oat, wheat, corn, and barley diets, respectively, following the trend oat > wheat > corn = barley. The WSP output was significantly different for all P treatments and was reduced 29% by feeding the low P diet (3.00 g of P kg·DMI^{-1}) vs. the high P diet (4.27 g of P

Table 7. Phosphorus characterization of manure from broiler chicks fed diets varying in cereal grain, P concentration, and phytase additions (determined by NaOH-EDTA extraction and solution ^{31}P nuclear magnetic resonance spectroscopy; values are means of 3 replicates of 5 birds per pen)

		NaOH–EDTA extractable P			
Item	Total P	Phosphate[1]	Phosphate monoesters[1,2]	Phytate[1]	Pyrophosphate[1]
	g·kg of dry wt.$^{-1}$ of P				
Corn					
Low P	9.97	1.98 (20)	0.39 (4)	7.60 (76)	0.05 (0.5)
Low P + phytase	9.01	3.95 (44)	1.64 (18)	3.42 (37)	0.06 (0.7)
High P	15.44	6.09 (39)	0.68 (4)	8.67 (56)	0.16 (1)
Wheat					
Low P	9.29	2.59 (28)	ND[3]	6.75 (72)	0.13 (1.5)
Low P + phytase	9.57	5.23 (55)	1.80 (19)	2.54 (26)	0.09 (0.9)
High P	13.98	5.48 (39)	0.93 (7)	7.57 (54)	0.15 (1)
High fat-low lignin oat					
Low P	9.40	2.90 (31)	0.86 (9)	5.64 (60)	0.06 (0.6)
Low P + phytase	8.77	4.99 (57)	0.97 (11)	2.81 (32)	0.11 (1.2)
High P	12.16	6.10 (50)	0.99 (8)	5.08 (42)	0.15 (1.2)
Barley					
Low P	7.61	1.50 (20)	0.80 (10)	5.32 (70)	ND
Low P + phytase	7.03	2.76 (39)	1.29 (18)	2.98 (43)	0.05 (0.7)
High P	10.39	3.33 (32)	0.78 (7)	6.28 (61)	0.10 (1)
SEM	0.412	0.276	0.101	0.356	0.011
P-value					
Grain	<0.0001	<0.0001	0.99	0.0001	0.05
P treatment	<0.0001	<0.0001	0.0001	<0.0001	0.002
Grain × P treatment	0.002	0.06	0.05	0.05	0.53

[1]Values in parentheses are the proportion (%) of the NaOH–EDTA extracted P.

[2]Values for phosphate monoesters include all monoesters other than phytic acid.

[3]ND = not detected.

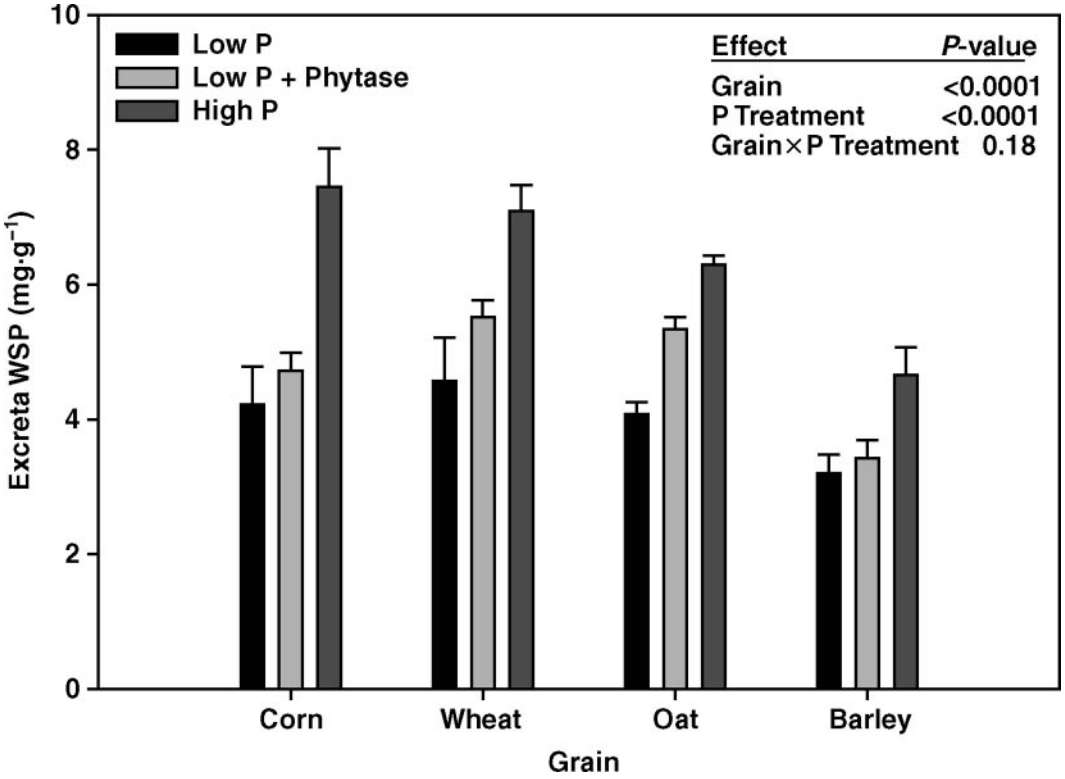

Figure 1. The water soluble P (WSP) concentrations of poultry excreta from broiler chicks fed diets varying in cereal grain, P concentration, and phytase additions. Error bars represent 5 pens of 5 birds per pen.

kg·DMI^{-1}) and was reduced 34% by feeding the low P + phytase diet (2.82 g of P kg·DMI^{-1}) compared with the high P diet.

DISCUSSION

There was no significant main effect of P treatment on performance in the present study, although there was a main effect of grain and a significant interaction term for BW and FCR. The chicks on the high fat-low lignin oat diets had significantly less feed intake than the other treatments, which did not differ significantly, and therefore BW gain over the 21-d period was much less for the oat diets compared with the other grain diets. The only significant effects of P treatment on FCR was in the barley and oat treatments, where the low P + phytase had the best FCR and the low P diet the poorest in the barley treatment, whereas the low P and low P + phytase treatments had the best FCR in the high fat-low lignin oat diets and the high P treatment had the poorest FCR.

The performance data are similar to that reported in other studies utilizing different cereal grains in broiler diets. Svihus and Gullord (2002) fed a variety of cereal-based diets (77% cereal) to broilers up to 21 d and reported 0 to 21 d BW gains of 612, 629 , and 644 g for barley, oat, and wheat diets, respectively, which is similar to values in the present study of 677, 507, and 670 g, respectively, for these same dietary treatments. Svihus and Gullord (2002) reported cumulative feed intakes of 848, 876, and 861 g for barley-, oat-, and wheat-based diets, respectively, compared with the feed intakes in the present study, which averaged 870, 672, and 943 g, respectively, for these same treatments. The main difference between the present study and that by Svihus and Gullord (2002) is the low feed intake of the chicks on the oat-based diets and resulting less BW gain. Differences in the type of cultivar (i.e., high fat-low lignin vs. conventional oat) used may partially explain this apparent discrepancy. Svihus and Gullord (2002) reported FCR of 1.39, 1.40, and 1.34 for barley-, oat-, and wheat-based diets compared with the FCR in the present study, which averaged 1.28, 1.30, and 1.35, respectively, for these same treatments.

Yu et al. (2004) fed corn-based diets (45% corn) to broilers from 0 to 21 d with low P (0.35% NPP), low P + phytase, and high P (0.45% NPP) treatments. They reported 0 to 21 d BW gains, cumulative feed intake, and FCR values of 662 g, 985 g, and 1.58 for the low P diet; 682 g, 990 g, and 1.45 for the low P + phytase diets; and 689 g, 980 g, and 1.42 for the high P diets, respectively. These values were very similar to those found in the present study where 21 d BW gain ranged from 595 to 625 g, feed intake ranged from 836 to 906 g, and FCR ranged from 1.42 to 1.45. The feed intake in the present study was slightly less than that found by Yu et al. (2004), which also resulted in slightly lower 0 to 21 d BW gain.

There was no evidence of any interaction between grain source and P treatment on ileal phytate P hy-

drolysis and P digestibility. Between sources of grains, ileal P digestibility decreased from 67 to 57% with high fat-low lignin oat having the greatest and corn having the least P digestibility. The AIDC for P in the present study are similar to reported values for cereal-based diets. Wu et al. (2004) reported ileal P digestibility coefficients of 0.67 and 0.77 for barley-based diets without and with phytase supplementation, respectively. They also reported ileal P digestibility coefficients for corn-based diets of 0.70 and 0.77 without and with phytase, respectively, and for wheat-based diets of 0.58 and 0.70 with and without phytase, respectively. Ileal phytate hydrolysis in the present study was enhanced 2-fold with phytase amendment. Whereas there was a significant main effect of grain, the only difference in ileal phytate hydrolysis was in the corn-based diets, which were less than the other diets. Large differences in the intrinsic phytase activity of oat, wheat, and barley grains had no effect on the amount of phytate hydrolyzed at the terminal ileum.

The lack of response in phytate hydrolysis due to the intrinsic phytase activity of the grains is likely due to a combination of several factors. Plant phytases have been shown to be most active at a pH of approximately 5 (Angel et al., 2002), whereas the fungal phytase used in the present study (*Aspergillus niger*) has 2 pH optimums at pH 2 and 5 (Irving and Cosgrove, 1972). The solubility of phytate in the presence of Ca at the molar ratios present in most feed (~6) is approximately 90% at a pH <4.5 and decreases to approximately 10% at pH 5 and becomes insoluble above pH 6 (Grynspan and Cheryan, 1983; Pontoppidan et al., 2007). Because plant phytases would be most active at a pH near 5, the decreased solubility of Ca-phytate complexes at this pH would prevent hydrolysis of the phytate by the intrinsic phytase activity of the grain.

In addition, the stability of plant phytases has been shown to be low at pH <3.0 with no activity present when a wheat phytase was subjected to a solution with pH 2.5 for 15 min (Phillippy, 1999). It has also been demonstrated that the activity of wheat phytase decreased with increasing pepsin and pancreatin concentrations in vitro (Phillippy, 1999). Therefore, because of a combination of low pH, pepsin, and pancreatin in the gastrointestinal tract of broilers, much of the intrinsic phytase activity of the grains may have been inactivated. In contrast, *A. niger* was shown to be stable at low pH (pH 2.5 to 3.5) and was not impaired by the presence of pepsin or pancreatin (Phillippy, 1999), and therefore would have had greater efficacy than the intrinsic phytase of the grains.

Although exogenous phytase addition increased the amount of phytate hydrolyzed almost 2-fold, only 22 to 42% of the dietary phytate had been hydrolyzed at the terminal ileum by the fungal phytase source added to diets in the present study. The relatively low proportion of phytate hydrolyzed by fungal phytase is comparable with previous reports of 35% hydrolysis reported by Selle and Ravindran (2007). The addition of phytase had no effect on the AIDC for Ca, which was only affected by grain source, being significantly greater in the high fat-low lignin oat vs. corn diets. The greater hydrolysis of phytate P in the phytase amended diets resulted in these diets having similar P digestibility as the high P diets, even though these diets had proportionately more highly available inorganic P added during the diet formulation.

The total tract apparent phytate P digestibilities reported in the present study ranged from 0.10 to 0.36 in nonphytase amended diets, which is similar to other published data. Jang et al. (2003) reported total tract phytate P digestibility coefficients for barley and corn, which were 0.39 and 0.24, respectively. Grain source had no effect on the amount of phytate hydrolyzed over the total digestive tract. Phytate P digestibility was affected by P treatment, although the significant interaction of grain source and P treatment for total phytate P digestibility indicated that the effects of P treatment were not consistent across all grain sources. Although grain source had no effect on the amount of phytate P hydrolyzed, hydrolysis was increased approximately 3-fold in phytase amended diets. It is also interesting to note that the difference in the percentage of phytate hydrolyzed from phytase amended diets vs. diets with no phytase increased between the terminal ileum and the excreta, which suggests that phytase continued to hydrolyze phytate in the hind gut.

The total tract apparent P digestibilities reported in the present study ranged from 0.43 to 0.61, which is similar to other published data. Juanpere et al. (2004) reported total tract P digestibility coefficients of 0.58, 0.62, and 0.65 for barley-based diets with high P, low P, or low P + phytase treatments, respectively. Hernández et al. (2005) reported total tract P digestibility coefficients for corn and wheat as 0.58 and 0.72. Wu et al. (2004) reported total tract P digestibility coefficients for wheat with and without phytase addition as 0.51 and 0.40, respectively. Total tract P digestibility had significant main effects for both grain and P treatment, with the P digestibility in the grains following the trend corn = barley > wheat > oat. The greater phytate hydrolysis in phytase amended diets also led to a greater ATTDC for P, which was 7% greater than the low P diets and 18% greater than the high P diets. Although phytate hydrolysis was enhanced with phytase amendment, this did not affect the total tract Ca digestibility, which was only affected by type of grain.

To our knowledge, there are no published data reporting the P composition of ileal digesta from broilers fed a variety of cereal grains with and without phytase addition. Additionally, there are few published data on the P composition of excreta obtained from broilers fed different cereal grains and the impact of phytase addition on excreta P composition. Recent work in our laboratory indicated that excreta from broiler chicks fed diets containing cultivars of barley varying in phytate content contained only trace amounts of phytate, irrespective of the phytate concentration of the initial

feed (Leytem et al., 2007). Similarly, broiler chicks fed diets based on cereal grains (corn, oats, barley) varying in phytate content and intrinsic phytase activity excreted less than 13% of the P in their excreta in the form of phytate (Leytem et al., 2008). However, both these experiments involved the feeding of diets in which the cereal grain supplied the sole source of dietary Ca and P and were therefore atypical of diets that would be used in commercial poultry production. Maguire et al. (2004) reported that litter from broilers and turkeys fed corn-based diets contained between 26 and 56% of total P as phytate, whereas phytate P concentrations in excreta from laying hens fed corn-based diets ranged from 35 to 80% of total P (Leytem et al., 2006).

In the present study, the total ileal P was dominated in all cases by phytate P which ranged from 70 to 88% of total P. There was a main effect of grain with corn and wheat having the greatest phytate P concentrations, whereas barley and high fat-low lignin oat had the least. As there was little intrinsic phytase activity in both corn and oat diets but high values in the barley and wheat diets, it is evident that intrinsic phytase activities of the grains had little influence on phytate hydrolysis in the upper gastrointestinal tract. In contrast, exogenous phytase addition increased phytate hydrolysis and resulted in phytase amended diets having 19% less phytate P concentrations than either the low P or high P diets.

Breakdown of phytate continued in the hind gut, and phytate P composed between 26 and 72% of total P in the excreta. As with the ileal digesta, there was a main effect for both grain and P treatment with corn having the greatest phytate P concentration, whereas high fat-low lignin oat had the least, again suggesting that intrinsic phytase activity of the diets had little influence on phytate hydrolysis. There was a significant main effect of P treatment and a significant grain × P treatment interaction for monoester P concentrations, which ranged from ND to 19% of total excreta P. The small amount of monoester P found in both the ileal digesta and excreta suggested that the lower inositol esters (breakdown products of phytate) are not very stable and are rapidly hydrolyzed to release phosphate P.

In nonphytase amended treatments, the excreta monoester P was ≤10% of total P, whereas phosphate P in all cases was at least twice that amount or greater. This indicates that as phytate is being hydrolyzed in the gut, the predominant product is phosphate as opposed to lower inositol phosphate esters. The addition of phytase decreased the phytate P concentration of excreta by 56%. As phytate degradation occurred, there was an increase in phosphate, which ranged from 20 to 57% of total excreta P. There was more phosphate monoester P in the excreta from the phytase amended diets, which indicates that continual action of exogenous phytase in the hind gut is producing intermediate inositol phosphate esters that are not being broken down to phosphate as quickly as diets without phytase addition, most likely because of the larger amount of these lower inostiol phosphates being generated via action of the exogenous phytase.

With the addition of phytase or feeding a low P diet, there was a 32% decrease in the total P excreted by the birds. There was also a decrease in total P output per kilogram of DMI, ranging from 25 to 46% when feeding a low P or low P + phytase diet compared with the high P diet. This same trend was seen in the excreta WSP concentrations with the low P and low P + phytase treatments, respectively, having 37 and 25% less WSP compared with the high P diets. The WSP output per kilogram of DMI was reduced by 29% by feeding a low P diet and 34% by feeding a low P + phytase diet compared with the high P diet. Although the wheat and barley diets had high levels of intrinsic phytase activities, this did not translate into an enhanced WSP concentration in the excreta. In addition, grain source did not affect the amount of phytate P hydrolyzed from phytase in the intestine or the ability of phytase to reduce P excretion. This is important from an environmental perspective because nutritionists will be able to vary the source of cereal grains in diets without affecting the amount of WSP in the excreta.

Even though the addition of phytase to diets decreased the amount of phytate P in the excreta, this did not translate into greater WSP concentrations than those in the high P diets. In previous work, it was demonstrated that decreasing the percentage of phytate in the excreta increases the proportion of WSP in the excreta, leading to greater extractable P in soils amended with these excreta (Leytem et al., 2006). However, these effects on increased soil extractable P were only short-term and as the phytate in the excreta breaks down over time, soil extractable P increases in soils treated with excreta having high phytate P contents. Therefore, the potential negative effects of phytase addition decreasing excreta phytate concentrations is less of a concern from an environmental standpoint than the effects on total P, which will ultimately affect the potential for off-site P transport from soils fertilized with poultry excreta.

In conclusion, the P composition of ileal digesta was dominated by phytate P (70 to 88% of total P), whereas excreta phytate P ranged from 26 to 76% of total P. Excreta WSP ranged from 3.2 to 7.5 $g \cdot kg^{-1}$ and was least for the barley diets. There was a 25% reduction in excreta WSP from the high P to the low P + phytase diets and a 37% reduction from the high P to the low P diets. The WSP output per kilogram of DMI was reduced by 29% by feeding a low P diet and 34% by feeding a low P + phytase diet compared with feeding the high P diet. As cereal grain had little influence on phytate digestibility, it is unlikely that intrinsic grain phytase has much influence on phytate utilization by poultry. Both excreta total P and WSP were reduced by the low P diet and the low P + phytase diet, ir-

respective of cereal grain, which reduces the risk of P transfer to water bodies when excreta are applied to farm land.

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