Interaction of Calcium and Phytate in Broiler Diets. 2. Effects on Total and Soluble Phosphorus Excretion[1]

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ABSTRACT Dietary Ca has been reported to influence the amount of phytate excreted from broilers and affect the solubility of P in excreta. To address the effects of dietary Ca and phytate on P excretion, 12 dietary treatments were fed to broilers from 16 to 21 d of age. Treatments consisted of 3 levels of phytate P (0.10, 0.24, and 0.28%) and 4 levels of Ca (0.47, 0.70, 0.93, and 1.16%) in a randomized complete block design. Feed phytate concentrations were varied by formulating diets with 3 different soybean meals (SBM): a low-phytate SBM, a commercial SBM, and a high phytate Prolina SBM having phytate P concentrations of 0.15 to 0.51%. Fresh excreta was collected from cages during 2 separate 24-h periods; collection I commenced after the start of dietary treatments (16 to 17 d) and collection II followed a 3-d adaptation period (19 to 20 d). Ileal samples were also collected at 21 d. Excreta samples were analyzed for total P, water soluble P (WSP), and phytate P, whereas ileal samples were analyzed for total P and phytate P. Results indicated that excreta total P could be reduced by up to 63% and WSP by up to 66% with dietary inclusion of low-phytate SBM. There was a significant effect of dietary Ca on both the excreta WSP and the ratio of WSP:total P. As dietary Ca increased, the excreta WSP and WSP:total P decreased, with the effects being more pronounced following a dietary adaptation period. There was a linear relationship between the slope of the response in WSP to dietary Ca and feed phytate content for excreta from collection II ($r^2 = 0.99$). There was also a negative correlation between excreta phytate concentration and excreta WSP during both excreta collections. The response in WSP to dietary manipulation was important from an environmental perspective because WSP in excreta has been related to potential for off-site P losses following land application.

Key words: broiler, soluble phosphorus, excreta, phytate, environment

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INTRODUCTION

Water soluble P (**WSP**) release to runoff from manure-amended soils has varied considerably due to concentration differences in manure total P and WSP (Sharpley and Moyer, 2000; Kleinman et al., 2002a,b; Vadas et al., 2004). Sharpley and Moyer (2000) found a 98% correlation between WSP in manure and the amount of P leached from soils following simulated rainfall events. This suggested that WSP was a good indicator for estimating the potential of manure to contribute to P runoff after surface application.

Diet modification has been one of the fundamental methods for altering both total P and WSP in manure. There has been interest in manipulating diets to decrease P concentration in poultry manure (Maguire et al., 2004; Smith et al., 2004). For monogastric animals that cannot fully digest phytate P, dietary strategies have included genetic development of mutant grains that contain substantially less phytate P than the wild-type equivalents (Raboy et al., 2000; Dorsch et al., 2003). Feeding such low phytate (**LP**) grains has been shown to improve P utilization in poultry (Li et al., 2001a,b) and reduce P excretion (Jang et al., 2003).

Manure P composition has been found to influence the concentration of manure WSP. An increased proportion of phytate P in poultry litters and manures can impact P solubility; the proportion of WSP decreased when phytate P concentrations of manures and litters increased (Leytem and Maguire, 2006). Therefore, dietary factors that influence the amount of phytate P excreted could potentially alter the WSP fraction of the resultant manures.

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Table 1. Proximate composition of 3 soybean meals that varied in phytate concentration used to determine the effects of dietary phytate and calcium on total and soluble P excretion in broiler chicks[1,2]

Soybean meal source	CP	Lys	Total P	IP3[3]	IP4[3]	IP5[3]	IP6[3]	Total IP[3]	IP6 P[3,4]	IP6 P[3]
	(g/kg)									(% of) total P
HP Prolina	489.6	3.09	7.80	0.99	0.73	4.71	18.17	24.60	5.12	66
Commercial	503.3	3.09	6.10	1.12	0.21	2.24	16.09	19.66	4.53	74
Low phytate	502.5	3.10	6.10	1.52	0.07	0.85	5.35	7.78	1.51	25

[1]Abbreviations: high phytate (HP); inositol phosphate esters (IP).

[2]Nutrient composition based on analyzed nutrient values and standardized to 88% dry matter.

[3]IP esters analyzed by HPLC (Kwanyuen and Burton, 2005).

[4]Phytate P represented the P content as IP6, calculated as 0.2818 × IP6 (Angel et al., 2002).

Supplemental minerals, such as Ca and other divalent and trivalent cations, can form stable complexes with phytate and result in reduced hydrolysis of phytate P. Increased dietary Ca has been shown to reduce phytate P hydrolysis in poultry, although manure phytate P concentrations were not measured (Ballam et al., 1985; Scheideler and Sell, 1987; van der Klis and Versteegh, 1996; Tamim et al., 2004). In addition to inhibiting phytate P hydrolysis, addition of Ca to diets caused precipitation of insoluble CaP complexes in poultry manure, which made manure P less soluble (Toor et al., 2005).

Leytem et al. (2007) recently reported that increasing the dietary Ca to available P ratio (**Ca:AvP**) in broiler diets decreased litter WSP due to decreased phytate P hydrolysis and increasing CaP precipitation in the gut. However, the extent that dietary Ca can influence WSP in manure generated from broiler diets containing variable levels of phytate P has not yet been examined. To address this, we investigated the effects of dietary phytate P and Ca levels on manure total P, WSP, and phytate P excretion from broilers fed diets containing soybean meal (**SBM**) with varying phytate P levels. In addition, we examined the impact of the dietary adaptation period on properties of these excreta.

MATERIALS AND METHODS

Broiler Feeding Trial

All animal research was approved by the North Carolina State University Institutional Animal Care and Use Committee. Broiler chicks were hatched from eggs obtained from Ross 344 × 508 broiler breeders housed at the institution. Chicks were feather-sexed at hatching, and 816 male chicks were permanently identified with neck tags. Thereafter, 17 chicks were randomly allocated to each of 48 electrically heated battery brooders located within 2 environmentally controlled brooding rooms. To reduce vertical temperature and lighting differences between tiers in the battery brooders, only the middle 4 tiers of cages in each 6-tier battery brooder were utilized. Brooding temperatures within cages were initially set at 33°C and reduced gradually to 25°C by 21 d of age. From 1 to 15 d, all birds had ad libitum access to feed and water and received a standard corn-soy broiler starter diet containing 3,150 kcal ME/kg, 23.0% CP, 0.9% Ca, and 0.45% nonphytate P (**NPP**). To reduce variation in BW between pens, all chicks were weighed at 12 d and chicks with extreme BW excluded so that 13 chicks per pen remained with a BW of 342 ± 12 g.

To obtain practical broiler diets that differed in phytate P concentration, SBM produced from 3 different cultivars of soybeans that differed in their natural phytate P content were utilized (Table 1). The high phytate (**HP**) Prolina SBM (0.51% phytate P; Burton et al., 1999) and the LP SBM (0.15% phytate P; Wilcox et al., 2000) were generated from soybeans selected for improved protein or reduced phytate P content, respectively. A commercial SBM (0.45% phytate P) with similar protein and amino acid content and which was intermediate in its phytate P concentration was selected as a control. Further, to increase the range of phytate P in the final diets, degermed dehulled (**DGDH**) corn was used in diet formulation (~0.09% phytate P).

Three basal diets that differed in the source of SBM were formulated to contain similar nutrient profiles with the exception of dietary Ca levels (Table 2). Finely ground limestone was added to each of the basal diets at the expense of washed sand to obtain graded levels of dietary Ca of 0.47, 0.70, 0.93, and 1.16% in the final diets. Further, to reduce differences between diets, the formulated moisture content was fixed at 11.5% by varying the inclusion of water, whereas levels of added soy oil in diets were held constant at 6.2%. Using the analyzed phytate P concentration of the DGDH corn and SBM, final diets contained phytate P levels of 0.10, 0.24, and 0.28% (Table 2). Further information regarding diets and dietary analysis can be found in the companion paper (Plumstead et al., 2008). After birds were fasted for 16 h at 15 d of age, the 12 experimental diets were assigned to 4 pens of birds each and fed from 16 to 21 d of age.

Excreta and Ileal Collection

Metal trays under battery cages were lined with clean plastic prior to the beginning of each excreta collection. Excreta samples were collected at 2 separate times during the experiment, once when birds were first given access to the test diets and a second sample following a dietary adaptation period. Collection I commenced for 24 h at 16

Table 2. Formulation and calculated analyses of the basal diets used to determine the effects of dietary phytate and calcium on total and soluble P excretion in broiler chicks

	Basal diet		
	HP Prolina soy	Commercial soy	Low phytate soy
Ingredient	(%)		
DGDH corn[1]	45.80	46.74	46.65
Soybean meal[2]	40.06	39.32	36.44
Monocalcium phosphate	1.21	1.09	0.59
Limestone[3]	0.34 to 2.15	0.44 to 2.25	0.64 to 2.45
Lysine HCl	0.00	0.04	0.04
DL-Methionine	0.27	0.28	0.25
L-Threonine	0.01	0.05	0.09
Premixes[4]	0.55	0.55	0.55
Sodium chloride	0.50	0.50	0.50
TiO_2 marker	0.40	0.40	0.40
Celite marker	2.00	2.00	2.00
Soybean oil	6.20	6.20	6.20
Filler (sand)	0.10 to 1.91	0.43 to 2.24	0.76 to 2.56
Water[5]	0.74	0.16	3.09
Calculated nutrient[6]			
Moisture,[5] %	11.50	11.50	11.50
ME, kcal/kg	3,059	3,059	3,059
Crude protein, %	23.0	23.0	23.0
Lysine, %	1.33	1.33	1.33
Methionine + Cysteine, %	0.97	0.97	0.97
Threonine, %	0.88	0.88	0.88
Arginine, %	1.63	1.55	1.50
Calcium,[3] %	0.47 to 1.16	0.47 to 1.16	0.47 to 1.16
Total P, %	0.65	0.54	0.42
NPP,[7] %	0.35	0.35	0.35
Calcium: NPP ratio	1.35 to 3.31	1.35 to 3.31	1.35 to 3.31
Phytate P, %	0.28	0.24	0.10
Sodium, %	0.20	0.20	0.20
DEB,[8] (mEq)	27.47	26.84	24.98

[1]Degermed dehulled (DGDH) corn (Moeser et al., 2002).

[2]Three different sources of soybean meal described in Table 1 were used in formulation.

[3]The inclusion rate of limestone added to the basal diets was varied to provide 4 different levels of dietary calcium of 0.47, 0.70, 0.93, and 1.16%. Washed sand was used as an inert filler to adjust the volume of each diet formulation to 100%.

[4]Premixes provided the following per kilogram of diet: vitamin A, 13,200 IU; vitamin D_3, 4,000 IU; vitamin E, 66 IU; vitamin B_{12}, 39.6 μg; riboflavin, 13.2 mg; niacin, 110 mg; D-pantothenate, 22 mg; menadione (K_3), 4 mg; folic acid, 2.2 mg; thiamine, 4 mg; pyridoxine, 8 mg; D-biotin, 252 μg; selenium (as Na_2SeO_3), 0.30 mg; manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; cobalt, 1.0 mg; choline chloride 1,200 mg.

[5]The moisture percentage of diets was adjusted to 11.5% with water.

[6]Nutrient compositions calculated from proximate analyses of all ingredients. Final diet composition was confirmed by proximate analyses of all diets.

[7]Nonphytate P (NPP) calculated as analyzed P – P as phytic acid (IP6).

[8]Dietary electrolyte balance (DEB) calculated as mEq (Na + K – Cl).

d of age after birds had first been fasted for 16 h after which dietary treatments were initiated. The experimental period (24 h) was chosen to minimize any physiological adaptations of the gastrointestinal tract to the diets (Tamim et al., 2004). Collection II was composed of 24 h from 19 to 20 d after the birds had been adapted to the diets for 72 h. All of the excreta that had accumulated during each 24-h period was collected from each tray, thoroughly homogenized, and subsampled with subsamples immediately refrigerated. At 21 d, 10 chicks per pen were weighed and killed by cervical dislocation and the terminal 13 cm of ileum removed 3 cm anterior to the ileo-cecal junction. Ileal contents were gently expressed, pooled by cage, frozen, lyophilized, and ground (<2 mm) prior to analysis.

Sample Analysis

A portion of the excreta subsample (prior to refrigeration) was dried for 24 h in an oven at 100°C to determine dry weight. Fresh excreta samples were immediately analyzed for WSP by shaking the equivalent of 1 g of dry excreta with 100 mL of deionized water for 1 h, filtering through a 0.45-μm membrane, and analyzing total WSP by inductively-coupled plasma atomic-emission spectrometry (Optima 2000, Perkin Elmer, Wellesley, MA). The remaining excreta samples were frozen, lyophilized, and ground (<2 mm) for analysis. Analysis of the excreta and ileal samples for total elements and phytate were as follows: (i) total elements (Ca and P) were determined by microwave-assisted digestion of a 0.5-g dried sample

Table 3. Select P analysis of broiler excreta from collection I (16 to 17 d, no dietary adaptation) from chicks fed soybean meal (SBM)-based diets containing high phytate (HP) Prolina, commercial, and low phytate SBM and 4 levels of Ca (0.47 to 1.16%)

	Treatment nutrient value		Excreta P characteristic				
Diet	SBM	Ca[1] (%)	Total P (g/kg)	WSP[2] (g/kg)	WSP:TP[3] (ratio)	Phytate P (g/kg)	Phytate P:TP[4] (ratio)
1	HP Prolina	0.47	14.87	13.29	0.89	5.94	0.40
2	HP Prolina	0.70	14.42	12.94	0.90	6.69	0.46
3	HP Prolina	0.93	14.50	11.57	0.80	5.67	0.39
4	HP Prolina	1.16	13.79	10.45	0.76	7.27	0.53
5	Commercial	0.47	11.54	9.98	0.87	5.33	0.46
6	Commercial	0.70	10.54	8.60	0.81	5.57	0.53
7	Commercial	0.93	10.79	7.78	0.72	6.26	0.58
8	Commercial	1.16	10.76	6.50	0.60	6.37	0.59
9	Low phytate	0.47	6.47	5.29	0.82	1.18	0.18
10	Low phytate	0.70	6.25	4.59	0.73	1.67	0.27
11	Low phytate	0.93	6.32	4.32	0.68	1.84	0.29
12	Low phytate	1.16	6.11	3.66	0.60	2.16	0.35
SEM			0.16	0.15	0.01	0.23	0.01
Source of variation			(Probability > *F*)				
SBM			<0.0001	<0.0001	0.16	<0.0001	<0.0001
Ca			0.0002	<0.0001	<0.0001	0.002	<0.0001
SBM × Ca[5]			0.22	0.004	0.03	0.93	0.44

[1]Ca = the calcium content of the formulated diets.
[2]WSP = water soluble P.
[3]WSP:TP = the ratio of water soluble P to total P.
[4]Phytate:TP = the ratio of phytate P to total P.
[5]Interaction means depicted in Figure 1.

with 8 mL of concentrated HNO_3 and 2 mL of 30% H_2O_2 (vol/vol) with all elements quantified using inductively-coupled plasma optical-emission spectrometry (4300DV, Perkin-Elmer) detection; and (ii) phytate P was determined by acid extraction followed by HPLC analysis (Agilent HPLC 1100 series, Agilent Technologies, Wilmington, DE; Kwanyuen and Burton, 2005).

Statistical Analyses

All data were analyzed using the Mixed Models procedure of SAS (SAS Institute, 2004). There were 4 replicate cages per treatment arranged in a randomized complete block design with 4 blocks. Two blocks were located in each of 2 rooms. A cage of birds served as the experimental unit. Data were analyzed using a factorial model that included SBM, dietary Ca level, and the interaction of SBM × Ca level as fixed effects with block as a random effect. Orthogonal polynomial contrasts were used to assess the significance of the ability of linear or quadratic models to describe the response in the dependent variable to increasing Ca level. Where appropriate, means separation was carried out using Tukey's HSD with an alpha level of 0.05. Statements of statistical significance were based upon $P < 0.05$ unless otherwise stated.

RESULTS

The analyzed nutrient content of diets was presented in the companion paper (Plumstead et al., 2008). Although all diets had been formulated to contain the same calculated NPP of 0.35%, a lower than expected phytate P content of the HP Prolina SBM resulted in the analyzed percentage NPP of these diets being slightly higher (0.40%) than that of the commercial and LP SBM diets (0.31 and 0.32% NPP, respectively).

From collection I, the total excreta P ranged from 6.11 to 14.87 g of P/kg, while the WSP ranged from 3.66 to 13.29 g of P/kg (Table 3). The ratios of excreta WSP:total P ranged from 0.60 to 0.90. The average total P, WSP, and WSP:total P of the excreta generated from the various diets followed the trend HP Prolina > commercial > LP SBM. Excreta phytate P ranged from 1.18 to 7.27 g of P/kg, with the lowest average phytate P concentration observed in the LP SBM treatment with no significant differences between the remaining diets. The ratios of excreta phytate P:total P ranged from 0.18 to 0.59, with the average ratio following the trend commercial > HP Prolina > LP SBM. The ANOVA indicated that SBM had a significant effect on total P, WSP, phytate P, and phytate P:total P, whereas Ca significantly affected all variables measured. The interaction of SBM and Ca was only significant for the WSP and the WSP:total P variables and is discussed below.

In collection II, following a 72-h dietary adaptation period, the excreta total P, WSP, and phytate P ranged from 4.87 to 14.01 g of P/kg, 2.44 to 13.00 g of P/kg, and 0.92 to 7.36 g of P/kg, respectively, following the trend HP Prolina > commercial > LP SBM (Table 4). The excreta WSP:total P ratio ranged from 0.34 to 0.94 with the HP Prolina SBM having the greatest average ratio whereas there were no significant differences between the other SBM. The excreta phytate P:total P ratio ranged from 0.17 to 0.62, with the LP SBM having the lowest average ratio and no significant differences between the remaining SBM. As with collection I, both SBM and Ca had signifi-

Table 4. Select P analysis of broiler excreta from collection II (19 to 20 d) from chicks fed soybean meal (SBM)-based diets containing high phytate (HP) Prolina, commercial, and low phytate SBM and 4 levels of Ca (0.47 to 1.16%)

	Treatment nutrient value		Excreta P characteristic				
Diet	SBM	Ca[1] (%)	Total P (g/kg)	WSP[2] (g/kg)	WSP:TP[3] (ratio)	Phytate P (g/kg)	Phytate P:TP[4] (ratio)
1	HP Prolina	0.47	13.90	13.00	0.94	5.07	0.36
2	HP Prolina	0.70	14.01	10.73	0.77	6.88	0.49
3	HP Prolina	0.93	13.40	7.21	0.54	7.34	0.55
4	HP Prolina	1.16	13.10	5.73	0.44	7.36	0.56
5	Commercial	0.47	10.59	9.44	0.89	4.42	0.42
6	Commercial	0.70	9.72	6.68	0.69	5.33	0.54
7	Commercial	0.93	9.74	4.32	0.44	6.05	0.62
8	Commercial	1.16	9.72	3.28	0.34	5.59	0.57
9	Low phytate	0.47	5.38	4.18	0.78	0.92	0.17
10	Low phytate	0.70	4.87	3.06	0.63	1.62	0.33
11	Low phytate	0.93	4.96	2.64	0.53	2.28	0.47
12	Low phytate	1.16	5.08	2.44	0.48	1.94	0.38
SEM			0.10	0.23	0.02	0.21	0.03
Source of variation			(Probability > *F*)				
SBM			0.0002	<0.0001	0.0003	<0.0001	0.04
Ca			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SBM × Ca[5]			0.19	<0.0001	0.0003	0.19	0.77

[1]Ca = the calcium content of the formulated diets.

[2]WSP = water soluble P.

[3]WSP:TP = the ratio of water soluble P to total P.

[4]Phytate:TP = the ratio of phytate P to total P.

[5]Interaction means depicted in Figures 2 and 3.

cant effects on all variables measured, whereas the interaction of SBM and Ca was only significant for the WSP and WSP:total P variables as discussed below.

The regression of excreta WSP vs. dietary Ca indicated that there was a significant negative relationship between these variables for both sets of excreta (Figure 1, Table 5). As dietary Ca increased, the excreta WSP decreased, with the effects being more pronounced for both the HP Prolina and commercial SBM in collection II (resulting in a significant Ca × SBM interaction). The slope of the response in excreta WSP with increasing dietary Ca was lowest for the LP SBM with no significant differences between the remaining SBM for both collection I and II. The slope of the response of excreta WSP to increasing dietary Ca concentrations for collection II was linearly related to the phytate P content of the diets with the equation being: WSP = –47.47 × %phytate P + 2.3 (r^2 = 0.99).

Table 5. Regression parameters for the relationship between excreta water soluble P (WSP; y, g/kg) vs. dietary Ca (x, %) and the excreta water soluble P to total P ratio (WSP:TP; y) vs. dietary Ca (x, %) for excreta from collection I (16 to 17 d) and II (19 to 20 d) from chicks fed soybean meal (SBM)-based diets containing high phytate (HP) Prolina, commercial, and low phytate SBM and 4 levels of Ca (0.47 to 1.16%)[1]

SBM	Intercept	Slope	r^2
Collection I			
WSP			
HP Prolina	15.56 ± 0.61[a]	–4.29 ± 0.72[a]	0.70***
Commercial	12.21 ± 0.50[b]	–4.90 ± 0.58[a]	0.82***
Low phytate	6.29 ± 0.22[c]	–2.25 ± 0.26[b]	0.84***
WSP:TP			
HP Prolina	1.02 ± 0.05[a]	–0.22 ± 0.05[b]	0.52**
Commercial	1.06 ± 0.03[a]	–0.38 ± 0.038[a]	0.87***
Low phytate	0.96 ± 0.03[a]	–0.31 ± 0.04[ab]	0.84***
Collection II			
WSP			
HP Prolina	18.14 ± 1.02[a]	–11.01 ± 1.19[a]	0.85***
Commercial	13.32 ± 0.57[b]	–9.06 ± 0.67[a]	0.92***
Low phytate	5.08 ± 0.36[c]	–2.45 ± 0.42[b]	0.69***
WSP:TP			
HP Prolina	1.28 ± 0.08[a]	–0.74 ± 0.09[a]	0.83***
Commercial	1.26 ± 0.83[a]	–0.82 ± 0.05[a]	0.94***
Low phytate	0.95 ± 0.06[b]	–0.42 ± 0.07[b]	0.74***

[a–c]Means within a treatment and column lacking a common superscript differ significantly ($P \leq 0.05$).

[1]Regression lines are depicted in Figures 1, 2, and 3.

**, ***Denotes model significance at the $P \leq 0.01$ and $P \leq 0.001$ levels, respectively.

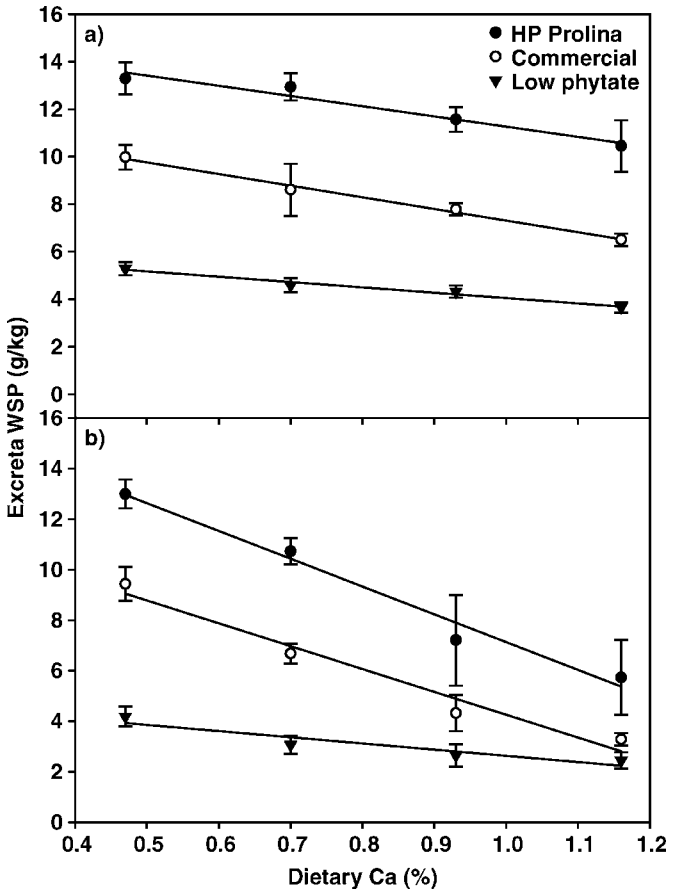

Figure 1. The relationship between dietary calcium (%) and excreta water soluble P (WSP; g/kg) for a) excreta collection I (16 to 17 d) and b) collection II (19 to 20 d) from chicks fed soybean meal (SBM)-based diets containing high phytate (HP) Prolina, commercial, and low phytate SBM and 4 levels of Ca (0.47 to 1.16%). Statistics for interaction effects can be found in Tables 3 (collection I) and 4 (collection II). Statistics for regression analysis can be found in Table 5.

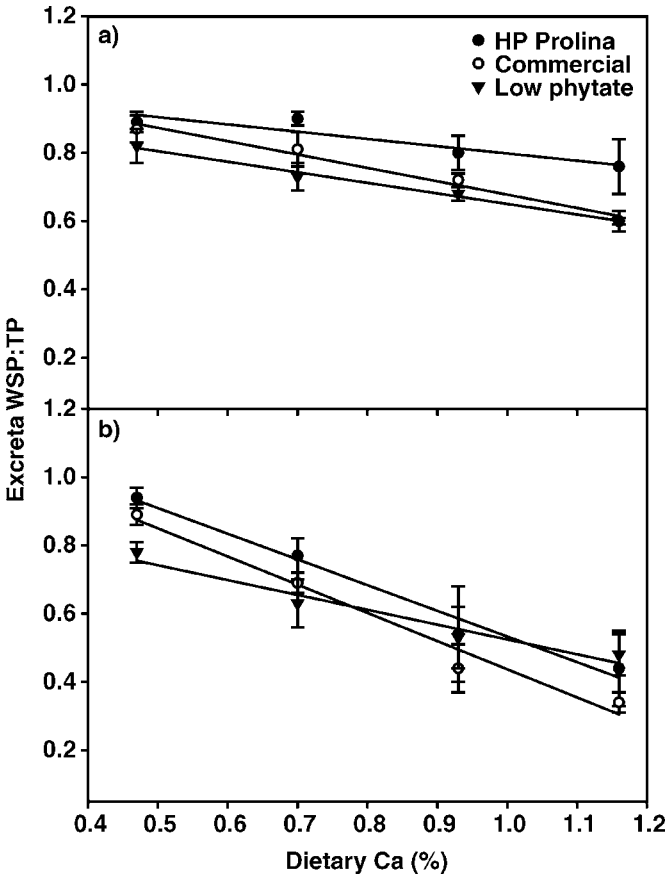

Figure 2. The relationship between dietary calcium (%) and excreta water soluble P to total P ratio (WSP:TP) for a) excreta collection I (16 to 17 d) and b) collection II (19 to 20 d) from chicks fed soybean meal (SBM)-based diets containing high phytate (HP) Prolina, commercial, and low phytate SBM and 4 levels of Ca (0.47 to 1.16%). Statistics for interaction effects can be found in Tables 3 (collection I) and 4 (collection II). Statistics for regression analysis can be found in Table 5.

The regression of excreta WSP:total P vs. dietary Ca also indicated a significant negative correlation between the 2 variables for both collection I and II (Figure 2, Table 5). Again, as with WSP, as dietary Ca increased the excreta WSP:total P decreased. The response in excreta WSP with increasing dietary Ca for collection I was greatest in the commercial SBM with no significant differences between the remaining SBM, whereas for collection II the LP SBM was significantly different having the least response with no differences between the remaining SBM (resulting in a significant Ca × SBM interaction).

Following a period of adaptation to the test diets, there was a significant effect of dietary Ca on the excreta phytate P:total P that was best fit with a quadratic function (r^2 = 0.36 to 0.81, Figure 3). As dietary Ca increased there was an increase in the proportion of phytate P excreted from the birds. Excreta phytate P:total P was negatively correlated with WSP within SBM with correlation coefficients of –0.36 to –0.94 for collection I and –0.70 to –0.90 for collection II. A comparison of the phytate P content of the ileal digesta vs. the excreta indicated that there was a substantial amount of phytate P hydrolysis that occurred as a result of microbial activity in the ceca, which ultimately affected excreta WSP (Figure 4).

DISCUSSION

When the dietary phytate P was decreased from 0.24 to 0.10% by replacing commercial SBM with LP SBM in diets there was a decrease in excreta total P from collection I and II of 42 and 49%, respectively (Tables 3 and 4). This was similar to other studies that have examined the impact of reducing dietary phytate P on total P excretion in broilers. Jang et al. (2003) reported that broiler chicks consuming LP corn-based diets excreted 33% less P than chicks fed normal diets. Penn et al. (2004) reported a decrease of 41% in total P excretion from turkeys fed diets containing normal and LP corn, whereas Li et al. (2001b) reported that poults fed LP barley diets excreted 41% less total P than poults fed normal barley diets. Although the difference between HP Prolina and LP SBM in excreta total P of 56 and 63% in collection I and II in the present study was even higher, this could not be attributed to differences in phytate P content of the diets alone. The HP Prolina diets had a higher determined NPP compared

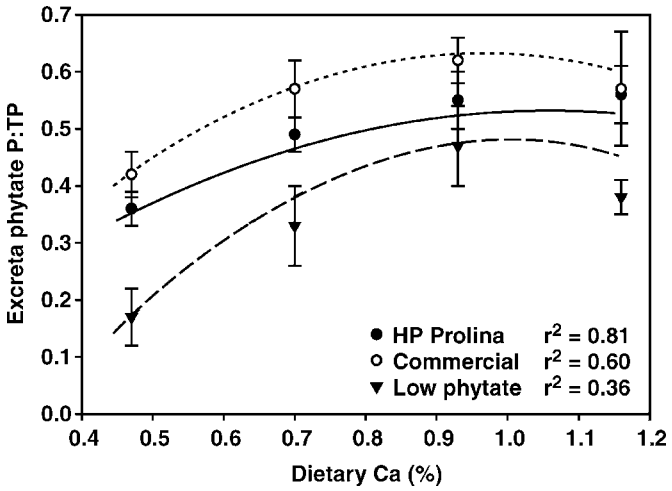

Figure 3. The relationship between dietary calcium (%) and the excreta phytate P to total P ratio (phytate P:TP) for excreta collection II (19 to 20 d) from chicks fed soybean meal (SBM)-based diets containing high phytate (HP) Prolina, commercial, and low phytate SBM and 4 levels of Ca (0.47 to 1.16%). Statistics for interaction effects can be found in Table 4.

with the commercial and LP SBM diets, which would have contributed to a higher total P in excreta.

The inclusion of LP SBM in diets in lieu of commercial SBM also resulted in a 46 and 48% reduction in WSP in excreta from collections I and II, respectively. Penn et al. (2004) reported a WSP decrease of 48% in litter from turkeys fed a LP corn vs. a normal corn diet, whereas Smith et al. (2005) reported a WSP decrease of 36% in broiler litters from diets containing LP vs. normal corn.

In the present study, there was a decrease of 73 and 75% in phytate P excreted between the HP Prolina and LP SBM diets for collection I and II, respectively (Tables 3 and 4). Toor et al. (2005) reported a decrease of 47% in phytate P excretion from broilers fed diets containing normal corn vs. LP corn. The large decreases in excreta phytate P found in the present study were likely due to the fact that the main source of phytate P in the diet was from the SBM, as DGDH corn was used in the diets, whereas Toor et al. (2005) fed normal corn based diets. van der Klis and Versteegh (1996) reported that the phytate P degradation in corn was only 16% whereas that of SBM was 61%. Therefore, the phytate P in SBM may be more susceptible to hydrolysis than that found in corn, which may account for the much higher percentage of phytate P degradation in the present study.

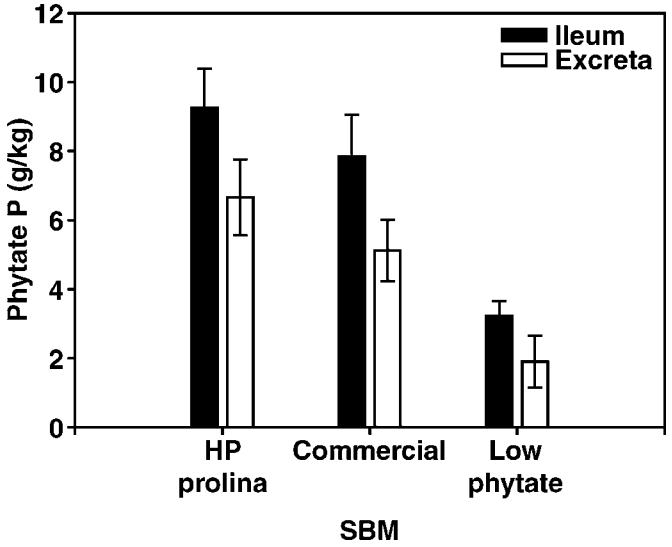

Figure 4. Average (means ± SE) phytate P (g/kg) measured across calcium level for the ileal and excreta samples generated from broiler chicks fed soybean meal (SBM)-based diets containing high phytate (HP) Prolina, commercial, and low phytate SBM and 4 levels of Ca (0.47 to 1.16%).

The influence of increasing dietary Ca on both excreta WSP and the WSP:total P ratio was highly significant for collections I and II (Tables 3 and 4). As the dietary Ca increased within each diet, the WSP decreased from 31 to 35% for collection I and from 42 to 65% for collection II. However, the significant Ca × SBM source interaction in the present study suggested that the relationship between dietary Ca and either excreta WSP or the WSP:total P ratio was dependent upon the dietary phytate P concentration. At higher dietary phytate P concentrations (HP Prolina or commercial SBM), the slope of the response in both excreta WSP and WSP:total P to increasing dietary Ca (higher Ca:NPP ratio) was greater than when diets contained reduced phytate P concentrations from inclusion of LP SBM (Table 5).

The dietary adaptation period significantly influenced the response in excreta WSP and WSP:total P to increasing dietary Ca. The slope of the response of excreta WSP and WSP:total P to increasing Ca was greater for the HP Prolina and the commercial SBM treatments following a 3-d dietary adaptation period, whereas there was no difference in the LP SBM treatment (Table 5). This suggested that when phytate P was present in broiler diets an adaptation period was necessary to determine true effects of diet on P excretion.

The effects of increased dietary Ca on excreta WSP were attributed to a combination of several factors. The calculated optimal Ca:AvP ratio that maximized absorption and retention of P in broilers was reported to be approximately 2.2 (van der Klis and Versteegh, 1996), whereas the Ca:NPP ratio in the present study ranged from 1.34 to 3.63. At a high dietary Ca, phytate P hydrolysis, and therefore P absorption, was reduced due to Ca-phytate precipitates in the gut and excretion of phytate P (Figure 3). At a low dietary Ca, P appeared to be absorbed but was later excreted in the urine, presumably due to insufficient Ca for P retention to occur (Plumstead et al., 2008). Therefore, it would be important to balance the dietary Ca:NPP ratio to maximize P absorption by the bird, thereby decreasing total P excretion.

Plumstead et al. (2008) reported that the optimum ratio of Ca:NPP that resulted in the highest P retention and lowest P excretion was 2.53:1, 2.40:1, and 2.34:1 for the HP Prolina, commercial SBM, and LP SBM treatments, respectively. These ratios also resulted in lower excreta WSP concentrations and were near the point where the

responses to Ca addition that changed excreta WSP concentrations were insignificant (Figure 1b). Further addition of Ca beyond these ratios would not be beneficial in terms of reducing total P or WSP excretion and would only result in further potential interference in phytate P hydrolysis and P absorption by the birds.

Leytem et al. (2007) found that an increased ratio of dietary Ca:AvP significantly decreased WSP excretion in broilers fed corn-soy-based diets with and without phytase addition. Leytem et al. (2007) postulated that this was due to the combined effect of Ca interference with phytate P hydrolysis in the birds, as well as a decrease in the proportion of inorganic P that was soluble due to CaP precipitation in the gut. It has been demonstrated in several studies that increasing dietary Ca reduced phytate P hydrolysis in poultry (Ballam et al., 1985; Scheideler and Sell, 1987; van der Klis and Versteegh, 1996; Tamim et al., 2004). Toor et al. (2005) also reported that as dietary Ca increased there was an increase of insoluble CaP precipitates in the resulting excreta.

In the present study, there was a strong negative correlation between the proportion of phytate P in the excreta and excreta WSP, particularly when birds were allowed a dietary adaptation period, as with collection II ($r = -0.7$ to -0.9, $P < 0.002$). As the proportion of phytate P in the excreta increased, the excreta WSP decreased due to the decreased solubility of phytate P relative to inorganic P. Sequential extraction of broiler litter has shown that P compounds extracted in water were predominantly inorganic P and that the majority of phytate P was only extracted in stronger extractions such as HCl or NaOH (Turner and Leytem, 2004). Therefore, litters or excreta that have a greater proportion of phytate P will have lower WSP concentrations. Examination of available literature revealed this same trend in broiler litter (Maguire et al., 2004; Toor et al., 2005: Leytem et al., 2007) and excreta from laying hens (Leytem et al., 2006).

The WSP content of both broiler litters and excreta can be influenced by microbial activity in the ceca. In the present study, the amount of phytate P decreased by 28 to 41% from the ileal digesta to the excreta (Figure 4). As phytate P concentrations decreased, a resulting increase in WSP could be expected. Kerr et al. (1999) reported that the levels of phytate P were very low in the ceca of broiler chicks and that the disappearance of phytate P from the ileum to the ceca indicated that microbial activity in the ceca had a large impact on phytate P hydrolysis.

In the present study, dietary Ca had a significant influence on both excreta WSP and WSP:total P across several DGDH corn-SBM-based diets. The excreta WSP was influenced by the dietary phytate P levels and dietary adaptation period. Earlier research that examined the influence of dietary modification on WSP excretion did not take into account the effects of dietary Ca and the resulting Ca:NPP ratio on WSP excretion, which, in part, may explain some of the variation in previously reported results. Many poultry producing regions have begun using a measurement of litter/excreta WSP to assess the potential impacts of land application on P losses. A better understanding of what may be controlling WSP levels in poultry litters and excreta must be developed to improve their management with respect to environmental protection.

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