What Aspect of Dietary Modification in Broilers Controls Litter Water-Soluble Phosphorus: Dietary Phosphorus, Phytase, or Calcium?

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ABSTRACT

Environmental concerns about phosphorus (P) losses from animal agriculture have led to interest in dietary strategies to reduce the concentration and solubility of P in manures and litters. To address the effects of dietary available phosphorus (AvP), calcium (Ca), and phytase on P excretion in broilers, 18 dietary treatments were applied in a randomized complete block design to each of four replicate pens of 28 broilers from 18 to 42 d of age. Treatments consisted of three levels of AvP (3.5, 3.0, and 2.5 g kg^{-1}) combined with three levels of Ca (8.0, 6.9, and 5.7 g kg^{-1}) and two levels of phytase (0 and 600 phytase units [FTU]). Phytase was added at the expense of 1.0 g kg^{-1} P from dicalcium phosphate. Fresh litter was collected from pens when the broilers were 41 d of age and analyzed for total P, soluble P, and phytate P as well as P composition by ^{31}P nuclear magnetic resonance (NMR) spectroscopy. Results indicated that the inclusion of phytase at the expense of inorganic P or reductions in AvP decreased litter total P by 28 to 43%. Litter water-soluble P (WSP) decreased by up to 73% with an increasing dietary Ca/AvP ratio, irrespective of phytase addition. The ratio of WSP/total P in litter decreased as the dietary Ca/AvP ratio increased and was greater in the phytase-amended diets. This study indicated that while feeding reduced AvP diets with phytase decreased litter total P, the ratio of Ca/AvP in the diet was primarily responsible for effects on WSP. This is important from an environmental perspective as the amount of WSP in litter could be related to potential for off-site P losses following land application of litter.

Water-soluble P (WSP) release to runoff from manure-amended soils following rainfall has been found to vary considerably primarily due to differences in the concentrations of total P and WSP in the manure (Sharpley and Moyer, 2000; Kleinman et al., 2002a,b; Vadas et al., 2004). Sharpley and Moyer (2000) found a correlation of 98% between WSP in the manure and the amount of P leached from a soil following five simulated rainfall events, which suggested that WSP would be a good indicator to estimate the potential of manure to contribute to P runoff after surface application. Runoff dissolved reactive P (DRP) concentrations from simulated rainfall experiments were also found to be closely related to WSP concentrations in surface-applied manures (Kleinman et al., 2002a). Similar increases in DRP in runoff from fields following application of manures with high WSP were also observed by McGrath et al. (2005). Maguire et al. (2005) reported that feeding reduced P diets to turkeys could decrease DRP losses in runoff relative to a normal diet, when the turkey litters generated from the reduced P diets were land-applied.

In response to research that has demonstrated a strong relationship between manure and litter WSP and P losses in runoff, many states with areas of concentrated poultry production have incorporated a measurement of WSP in manures/litters into their P loss assessment tools. The Maryland Phosphorus Site Index uses a P source coefficient (PSC) to assess the impact of manure/litter source on the potential for off-site P loss, and is calculated using the water-extractable P (WEP) fraction of the manure/litter when available ($PSC = 0.1 + 0.14 \times WEP^{0.86}$; Maryland Cooperative Extension, 2005). The Eucha/Spavinaw Phosphorus Index developed for use in Arkansas uses the soluble reactive P (SRP) fraction of manure/litter as an indication of potential P loss following land application (DeLaune et al., 2006). The Phosphorus Loss Assessment Tool, developed to assess potential P losses in North Carolina, uses a combination of the soluble and nonsoluble P fraction of manures/litters to calculate potential P loss from land application of manure/litters (The N.C. PLAT Committee, 2005). Since the WSP fraction in litters is being used in many areas to assess the potential for off-site P loss following land application, it has become very important to understand what controls the WSP fraction in poultry litters.

One of the fundamental methods for altering both total and WSP in poultry litters has been through diet modification. There has been considerable interest in developing dietary manipulations that decrease the P concentration of poultry litter (Maguire et al., 2004; Smith et al., 2004). For monogastric animals, such as poultry that cannot fully digest phytic acid (*myo*-inositol hexa*kis* dihydrogen phosphate; also commonly known as phytate), dietary strategies have included supplementation of feeds with microbial phytase to increase phytate hydrolysis in the gut, thereby enhancing the availability of dietary P (Cromwell et al., 1993; Coelho and Kornegay, 1996). Phytase is an enzyme that breaks down the undigestable phytate portion in grains by cleaving the phosphate off of the inositol ring, thereby

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Abbreviations: AvP, dietary available P; FTU, phytase activity is expressed as "phytase units" or "FTU" per unit of feed (One phytase unit is the amount of phytase that liberates 1 micromole of inorganic phosphorus per minute from an excess of sodium phytate at pH 5.5 and 37 degrees Celsius); HPLC, high performance liquid chromatography; IP, inorganic phosphorus; NMR, nuclear magnetic resonance spectroscopy; NPP, non-phytate phosphorus; PSC, phosphorus source coefficient; DRP, dissolved reactive P; SRP, soluble reactive P; WSP, water-soluble P.

releasing digestible phosphorus that can be utilized by monogastric animals.

Numerous studies have shown substantial reductions in litter total P when dietary non-phytate P (NPP) was reduced in combination with added phytase enzyme. Several studies have concluded that reduced dietary NPP regimens, when combined with phytase, could reduce litter total P from 29 to 45% (Applegate et al., 2003; Smith et al., 2004; Vadas et al., 2004; McGrath et al., 2005; Angel et al., 2006). While phytase supplementation to feeds has consistently produced a decrease in litter total P, the effects on litter WSP have been inconclusive. Phytase addition to poultry diets was shown to decrease the litter WSP concentration 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 litter WSP concentration (Saylor et al., 2001; Maguire et al., 2004; McGrath et al., 2005), while in two studies, amendment of diets with phytase increased the concentration of WSP in the litter (DeLaune et al., 2001; Vadas et al., 2004).

In addition to diet modification, the P composition of litters themselves has been shown to influence the amount of WSP. An increased proportion of phytate P in poultry litters and manures can have a substantial impact on P solubility, as the proportion of WSP was shown to decrease when phytate P of the manures and litters increased (Leytem and Maguire, 2006). Therefore, dietary factors that influence the amount of phytate excreted by the birds could potentially alter the WSP fraction of the resultant manures and litters.

Minerals such as Ca and other di- and trivalent cations supplemented to diets can form stable complexes with phytate and result in reduced hydrolysis of the phytate P. The formation of stable Ca-phytate complexes that are resistant to hydrolysis by phytase enzyme has been thought to be the mechanism whereby Ca reduces the disappearance of phytate from the small intestine of broilers (Maenz et al., 1999; Angel et al., 2002). Such larger complexes may not be available for hydrolysis by phytase enzymes (both endogenous and supplemented) either as a result of changes to the phytate P structure that preclude it from binding to the substrate-binding site of the enzyme; or due to reduced solubility of the Ca-phytate complex that causes it to precipitate out of solution.

In addition to inhibiting phytate hydrolysis in poultry, the addition of Ca to diets can cause the precipitation of insoluble CaP complexes in litter therefore making the P less soluble. Toor et al. (2005) showed that as dietary Ca increased there was an increase in insoluble CaP precipitates in manures and litters. Cooperband and Ward Good (2002) found that poultry manure contained sparingly soluble Ca and Mg phosphate minerals that controlled soil solution P concentrations following incorporation into a silt loam soil.

The variable effect of diet modification on WSP in litters remains a concern from a litter management standpoint, as litter WSP has been shown to be highly correlated with P losses from land-applied litter. It was evident from the disparity in the literature data that there may be factors other than dietary P and phytase supplementation that influence WSP in poultry litters. To address this, we investigated the effects of dietary available P (AvP), phytase, and Ca levels on total P, WSP, and litter P composition in broilers.

MATERIALS AND METHODS

Broiler Feeding Trial

Broiler chicks were produced from eggs incubated from a 33-wk-old Ross 344 male × Ross 508 female broiler breeder flock that produced feather-sexable chicks housed at the North Carolina State University Chicken Educational Unit. All chicks were sexed after hatching and permanently identified with neck tags. Fourteen male and 14 female chicks were randomly allocated to each of 72 floor pens (3.5 m^2) that contained fresh pine shavings. The chicks were given ad libitum access to water and a standard broiler starter diet that met or exceeded National Research Council (1994) requirements (Table 1). The quantity of starter feed provided per pen was adjusted to provide 907 g per bird alive at 7 d of age.

To evaluate effects of dietary Ca level, AvP level, and phytase enzyme on broiler performance and litter P concentrations, 18 grower treatments were assigned to four replicate

Table 1. Formulation and calculated analyses of the starter diet and basal grower diets.

Ingredients	Starter diet†	Basal grower diet†‡
Corn, g kg^{-1}	556.0	604.5
Soybean meal, g kg^{-1}	328.9	302.0
Poultry meal, g kg^{-1}	35.0	–
Poultry fat, g kg^{-1}	41.5	3.8
Limeston, g kg^{-1}‡	10.8	–
Dicalcium phosphate, g kg^{-1}‡	12.7	–
Sodium chloride, g kg^{-1}	5.0	5.0
Premixes, g kg^{-1}§	6.2	6.2
Lysine HCl, g kg^{-1}	0.7	0.5
L-Threonine, g kg^{-1}	0.8	0.4
DL-Methionine, g kg^{-1}	2.5	1.6
Phytase premix, g kg^{-1}‡¶	–	–
Calculated nutrients#		
Moisture, g kg^{-1}	110.8	110.7
ME, kcal kg^{-1}	3107	3200
Crude protein, g kg^{-1}	230.0	200.0
Lysine, g kg^{-1}	13.3	11.3
Methionine + cysteine, g kg^{-1}	10.1	8.4
Threonine, g kg^{-1}	8.9	7.5
Calcium, g kg^{-1}‡	10.0	–
Available phosphorus, g kg^{-1}‡	4.5	–
Non phytate phosphorus, g kg^{-1}¶	4.5	–
Sodium, g kg^{-1}	2.2	2.0

† Quantity of starter diet fed was adjusted to 907 g per bird alive at 7 d of age after which birds were fed the grower diet to 42 d of age.

‡ The inclusion rate of limestone, dicalcium phosphate, and a bacterial phytase premix (Syngenta Animal Nutrition Inc., Research Triangle Park, NC 27709) was adjusted in the basal grower diet and varied from 7.1 to 16.2, 3.4 to 14.6, and 0.00 or 0.2 g kg^{-1}, respectively, to make 18 diets that had combinations of three levels of available phosphorus of 2.5, 3.0, or 3.5 g kg^{-1}, three levels of calcium of 5.7, 6.9, or 8.0 g kg^{-1}, and either 0.00 or 600 phytase units (FTU) kg^{-1} added phytase enzyme.

§ Premixes provided the following per kg diet: vitamin A, 13 200 IU; vitamin D_3, 4000 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 1200 mg; coccidiostat, 700 mg.

¶ Phytase premix was included at the expense of 1.0 g kg^{-1} phosphorus from dicalcium phosphate and resulted in non-phytate phosphorus levels of 3.5, 3.0, or 2.5 g kg^{-1} in treatments with no added phytase enzyme and 2.5, 2.0, or 1.5 g kg^{-1} in treatments with 600 FTU kg^{-1} added phytase enzyme.

Nutrient compositions calculated from proximate analyses of all ingredients. Final diet composition confirmed by proximate analyses (Table 2).

pens at 14 d of age and fed to 42 d of age. The treatment structure was a 3 × 3 × 2 factorial with three levels of AvP (3.5, 3.0, and 2.5 g kg^{-1}), three levels of Ca (8.0, 6.9, and 5.7 g kg^{-1}), and two levels of a bacterial phytase enzyme (0, or 600 phytase units [FTU] kg^{-1}) (Syngenta Animal Nutrition, Research Triangle Park, NC). Differences in AvP and Ca in diets were achieved by varying the concentration of dicalcium phosphate and limestone in a standard basal grower diet that was formulated to meet or exceed National Research Council (1994) recommendations, except for NPP and Ca (Table 2). Phytase enzyme inclusion in diets mimicked the industry practice of removing 1.0 mg g^{-1} NPP from added dicalcium phosphate and replacing this with 600 FTU of added phytase (Angel et al., 2006). Fine washed river sand was included as an inert filler during diet formulation to allow variable inclusion rates of dicalcium phosphate, limestone, and phytase premix.

Litter Collection and Analysis

Litter samples were collected at 41 d of age from three areas within each pen, mixed thoroughly, and a subsample taken. Fresh litter samples were homogenized in a blender and then immediately analyzed for WSP by shaking the equivalent of 1 g dry litter with 100 mL deionized water for 1 h, filtering through a 0.45-μm membrane, and analyzing total WSP by inductively coupled plasma-atomic emission spectrometry (ICP–AES). The remaining samples were immediately frozen (−80°C), lyophilized, and ground (2 mm) for analysis. Analysis of the litters were as follows: (i) total elements (Ca and P) were determined by microwave-assisted digestion of a 0.5 g dried sample with 8 mL of concentrated HNO_3 and 2 mL of 30% H_2O_2 (v/v) with P and Ca quantified using inductively coupled plasma-optical emission spectrometry (ICP–OES) detection and (ii) phytate P was determined by acid extraction followed by high performance liquid chromatography (HPLC) analysis (Kwanyuen and Burton, 2005). All of the P values reported in the text are as elemental P.

The P composition of the litters was determined by solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy as described by Turner (2004). Samples from two of the four replicate pens per treatment were selected for analysis, due to the expense of ^{31}P NMR analysis. Briefly, P was extracted in triplicate by shaking 2.00 ± 0.01 g of dried litter with 40 mL of a solution containing 0.5 *M* NaOH and 0.05 *M* EDTA for 4 h at 20°C. Extracts were centrifuged at 10000 × *g* for 30 min and aliquots were analyzed for total P by ICP–OES. The remaining solutions from the triplicate extracts were combined, frozen rapidly at −80°C, lyophilized, and ground to a fine powder.

Freeze-dried extracts were redissolved in 0.1 mL of D_2O (for signal lock) and 0.9 mL of a solution containing 1 *M* NaOH and 0.1 *M* 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 operating at 202.456 MHz for ^{31}P. A 5 μs pulse (45°), a delay time of 5.0 s, an acquisition time of 0.8 s, and broadband proton decoupling for all samples was used. The number of scans varied between 3797 and 16091, and spectra were plotted with a line broadening of 1 Hz. Chemical shifts of signals were determined in ppm (ppm) relative to 85% H_3PO_4 and assigned to individual P compounds or functional groups based on literature values (Turner et al., 2003). Signal areas were calculated by integration and P concentrations 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 litters (Turner, 2004).

Table 2. The composition of grower diets fed in the study. Values are presented on an as fed basis and all diets were standardized to contain 88.5% dry matter. All formulated diet concentrations were confirmed with analysis.

	Grower treatment						
Diet	Phytase	Total P	AvP‡	NPP§	Phytate P	Ca	Ca/AvP
	FTU kg^{-1}†	g kg^{-1}					
1	0	4.8	2.5	2.5	2.3	5.7	2.28
2	0	4.8	2.5	2.5	2.3	6.9	2.76
3	0	4.8	2.5	2.5	2.3	8.0	3.20
4	0	5.3	3.0	3.0	2.3	5.7	1.90
5	0	5.3	3.0	3.0	2.3	6.9	2.30
6	0	5.3	3.0	3.0	2.3	8.0	2.67
7	0	5.8	3.5	3.5	2.3	5.7	1.62
8	0	5.8	3.5	3.5	2.3	6.9	1.97
9	0	5.8	3.5	3.5	2.3	8.0	2.29
10	600	3.8	2.5	1.5	2.3	5.7	2.28
11	600	3.8	2.5	1.5	2.3	6.9	2.76
12	600	3.8	2.5	1.5	2.3	8.0	3.20
13	600	4.3	3.0	2.0	2.3	5.7	1.90
14	600	4.3	3.0	2.0	2.3	6.9	2.30
15	600	4.3	3.0	2.0	2.3	8.0	2.67
16	600	4.8	3.5	2.5	2.3	5.7	1.63
17	600	4.8	3.5	2.5	2.3	6.9	1.97
18	600	4.8	3.5	2.5	2.3	8.0	2.29

† FTU, phytase units.
‡ Available P (AvP) calculated using the slope ratio method (Soares, 1995).
§ Non-phytate phosphorus (NPP) defined as the calculated difference between total P and phytate P.

Statistical Analyses

There were four replicate pens per treatment arranged in a randomized complete block design with four blocks. The ratio of litter WSP to total P (WSP/TP) was calculated by taking the WSP and dividing by total P for each pen; the ratio of litter phytate P to total P (phytate P/TP) was calculated in the same manner. Statistical analysis was performed using the Statistical Analysis System (SAS Institute, 1996). All variables were tested for normality using the Shapiro-Wilk test with the PROC CAPABILITY procedure. Where results suggested non-normality, variables were log-transformed before statistical analyses, with untransformed numbers presented in the text.

All data were analyzed using the general linear models (GLM) procedure. A cage of birds served as the experimental unit with 18 cages randomized to all combinations of dietary AvP (three levels), dietary Ca (three levels), and phytase (two levels) within each block. Data were analyzed using a full factorial effects model that included block effects and all possible main effects and interaction effects among treatment factors. Further inspection of the effects of Ca and AvP revealed that the ratio of Ca/AvP appeared to be a good predictor of the response in WSP and the WSP/TP ratio. A best fit model was selected by using a forward stepwise selection procedure that allowed selection of independent variables AvP, Ca, phytase, Ca/AvP, and Ca/AvP^2 (quadratic term) in a model when $P < 0.05$. The term Ca/AvP^2 was included as a potential variable as the relationship between dependent variables and Ca/AvP was nonlinear. Model fit was evaluated using Mallows' Cp statistic, Akaike information criteria, and the Schwarz Bayesian criteria.

RESULTS

The litter P chemical characteristics are shown in Table 3. Total P concentrations in litters from the 18 treatments ranged from 6111 to 10646 mg P kg^{-1}. On

Table 3. Select phosphorus analysis of broiler litters. Data presented are the average of four pens with the standard deviation. Phytate P data in this table was determined with high performance liquid chromatography (HPLC) analysis.†

	Feed formulation			Litter P characteristics				
Diet	Phytase	AvP	Ca	Total P	WSP	WSP/TP	Phytate	Phytate/TP
	FTU kg^{-1}	g kg^{-1}		mg kg^{-1}			mg kg^{-1}	
1	0	2.5	5.7	7632 ± 733	1002 ± 137	0.13	5629 ± 812	0.74
2			6.9	8156 ± 466	934 ± 150	0.11	5603 ± 455	0.69
3			8.0	8232 ± 1163	631 ± 132	0.08	5971 ± 486	0.73
4	0	3.0	5.7	8509 ± 435	1416 ± 332	0.17	5824 ± 768	0.68
5			6.9	8657 ± 968	1230 ± 529	0.14	5616 ± 1044	0.64
6			8.0	8699 ± 923	984 ± 519	0.11	5722 ± 1465	0.65
7	0	3.5	5.7	10 646 ± 606	2363 ± 359	0.22	6129 ± 298	0.68
8			6.9	10 415 ± 1247	1760 ± 655	0.17	6304 ± 507	0.61
9			8.0	9902 ± 1175	1165 ± 251	0.12	6664 ± 614	0.68
10	600	2.5	5.7	6111 ± 705	1043 ± 296	0.17	2760 ± 236	0.45
11			6.9	6343 ± 1083	923 ± 191	0.15	3148 ± 282	0.50
12			8.0	6920 ± 632	807 ± 113	0.12	3436 ± 265	0.50
13	600	3.0	5.7	7267 ± 798	1598 ± 259	0.22	3166 ± 444	0.44
14			6.9	7274 ± 781	1225 ± 217	0.17	3576 ± 358	0.49
15			8.0	6714 ± 955	900 ± 224	0.13	3393 ± 476	0.51
16	600	3.5	5.7	8304 ± 855	2290 ± 632	0.28	3337 ± 523	0.40
17			6.9	7553 ± 549	1353 ± 271	0.18	3515 ± 196	0.47
18			8.0	8141 ± 724	1209 ± 322	0.15	3889 ± 1131	0.49

† FTU, phytase unit; AvP, dietary available P; WSP, water-soluble P; TP, total P.

average, total litter P from broilers fed the phytase-amended diets were approximately 20% lower than broilers fed diets without phytase supplementation. The litter WSP concentrations ranged from 631 to 2363 mg P kg^{-1}, while the ratio of litter WSP/TP ranged from 0.08 to 0.28. There were no differences between the average litter WSP concentrations for the non-phytase and phytase-amended diets, which were 1276 and 1261 mg P kg^{-1}, respectively. The average litter WSP/TP ratio for the phytase-amended diets was 18% higher than diets without phytase supplementation. Litter phytate concentrations (determined by HPLC analysis) ranged from 3148 to 6664 mg P kg^{-1}, while the ratio of phytate P/TP ranged from 0.40 to 0.74. On average, the phytate P content of the litters from diets receiving phytase supplementation were 43% lower than diets without phytase supplementation. In addition, the average phytate P/TP ratio in the litters from diets with phytase supplementation were 30% lower than that of the litters from phytase-amended diets.

The P composition of litters as determined by solution ^{31}P NMR indicated that litters were composed mainly of orthophosphate (inorganic P [IP]), phytate P, lower esters of inositol P (breakdown products of phytate), and small amounts of pyrophosphate (Table 4). Total P recovery by NaOH-EDTA extraction ranged from 94 to 100%. The orthophosphate concentrations ranged from 1876 to 4199 mg P kg^{-1} and comprised between 24 and 47% of the total P in the litters. Phytate P ranged from 2818 to 6317 mg P kg^{-1}, comprising between 37 and 64% of total P in the litters. The concentration of lower inositol P esters ranged from 847 to 1382 mg P kg^{-1} and comprised between 8 and 18% of total P in the litters. There were small concentrations of pyrophosphate that

Table 4. Phosphorus concentrations and standard deviations in poultry litter as determined by NaOH-EDTA extraction and solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy.†

	Feed formulation			Litter P characteristics‡			
Diet	Phytase	AvP	Ca	Orthophosphate	Phytate	Other monoesters	Pyrophosphate
	FTU kg^{-1}	g kg^{-1}		mg kg^{-1}			
1	0	2.5	5.7	1876 ± 345 (26)	4230 ± 310 (59)	998 ± 64 (14)	51 ± 10 (0.7)
2			6.9	2190 ± 287 (25)	5450 ± 223 (61)	1194 ± 160 (13)	57 ± 17 (0.6)
3			8.0	1915 ± 37 (24)	5172 ± 903 (64)	998 ± 339 (12)	54 ± 18 (0.7)
4	0	3.0	5.7	2605 ± 285 (29)	5214 ± 876 (58)	1076 ± 9 (12)	49 ± 40 (0.6)
5			6.9	2696 ± 176 (30)	5011 ± 2072 (56)	1058 ± 121 (12)	76 ± 20 (0.9)
6			8.0	3500 ± 1871 (37)	4608 ± 961 (48)	1328 ± 51 (14)	76 ± 41 (0.8)
7	0	3.5	5.7	4199 ± 613 (37)	6033 ± 467 (53)	1063 ± 367 (9)	69 ± 17 (0.6)
8			6.9	4100 ± 191 (35)	6223 ± 882 (53)	1382 ± 328 (12)	90 ± 13 (0.8)
9			8.0	2795 ± 256 (28)	6317 ± 786 (63)	847 ± 195 (8)	82 ± 13 (0.8)
10	600	2.5	5.7	2502 ± 388 (39)	2818 ± 493 (44)	1123 ± 229 (17)	22 ± 34 (0.3)
11			6.9	2172 ± 5 (34)	2979 ± 247 (47)	1157 ± 436 (18)	35 ± 17 (0.6)
12			8.0	2343 ± 298 (33)	3460 ± 107 (49)	1171 ± 16 (17)	87 ± 12 (1.2)
13	600	3.0	5.7	3645 ± 24 (46)	2930 ± 304 (37)	1305 ± 57 (16)	62 ± 24 (0.8)
14			6.9	2492 ± 316 (36)	3247 ± 63 (47)	1036 ± 54 (15)	72 ± 17 (1.1)
15			8.0	2368 ± 229 (36)	3130 ± 253 (48)	1003 ± 428 (15)	42 ± 54 (0.6)
16	600	3.5	5.7	4057 ± 191 (46)	3423 ± 371 (39)	1283 ± 28 (14)	57 ± 0 (0.6)
17			6.9	3888 ± 98 (47)	3129 ± 391 (38)	1195 ± 306 (14)	67 ± 4 (0.8)
18			8.0	2735 ± 1315 (36)	3582 ± 1195 (47)	1285 ± 378 (17)	49 ± 6 (0.7)

† AvP, dietary available P; FTU, phytase units.
‡ Values in parenthesis are the proportion (%) of the total NaOH–EDTA extracted P.

ranged from 22 to 90 mg P kg^{-1} and comprised approximately 1% of total litter P. The P litter composition between non-phytase and phytase-supplemented diets was similar with the average litter phytate P concentration in phytase-amended diets being 41% lower than litters from non-phytase-amended diets.

We compared the phytate analysis performed by HPLC and ^{31}P NMR to determine how consistent the methods were as well as to determine the effects of reduced sample replication for ^{31}P NMR analysis vs. HPLC analysis. There was a strong positive correlation between the average proportion of phytate in the litter between the two methods (r^2 = 0.84), but the HPLC method gave slightly higher values than the ^{31}P NMR method (^{31}P NMR = 0.88 HPLC; Fig. 1).

Statistical analysis of the litter total P indicated significant differences in the response in total P due to both dietary AvP and phytase ($P < 0.0001$), while dietary Ca and all interactions were not significant (Table 5). As dietary AvP increased, the total P in the litter also increased in a curvilinear manner (Fig. 2). The inclusion of phytase in the diets decreased litter total P at all levels of dietary AvP, and litter total P concentrations were positively correlated with the litter phytate P concentrations (r^2 = 0.78; Fig. 3). Statistical analysis of litter WSP indicated significant differences in the response in WSP due to both dietary Ca and AvP ($P < 0.0001$), while phytase and all interactions were not significant (Table 5). Statistical analysis of litter WSP/TP ratio indicated significant differences in the response in WSP/TP due to dietary Ca, AvP, and phytase ($P < 0.0001$), while all interactions were not significant (Table 5).

Further inspection of the effects of dietary factors revealed that the ratio of dietary Ca/AvP was also a good predictor of the response obtained in litter WSP and WSP/TP ratio. A best fit model including Ca/AvP, Ca/AvP^2, and phytase was selected to describe both litter WSP and WSP/TP based on stepwise regression analysis (Table 5). The more parsimonious model that described the response in litter WSP and WSP/TP in terms of the ratio of dietary Ca/AvP (both linear and quadratic terms) and phytase resulted in an improved fit with less unexplained variance, as indicated by the smaller mean square error of this model, compared with the full factorial effects model that had more parameters. This suggested that the prediction of the response in litter WSP and WSP/TP ratio in future observations would be more accurate using this more parsimonious model in which the dependence of the response in litter WSP and WSP/TP ratio is captured through the dietary Ca/AvP ratio. As the Ca/AvP ratio increased, the litter WSP and WSP/TP ratio decreased in a curvilinear manner (Fig. 4 and 5). After accounting for the effects of Ca/AvP, the litter WSP/TP ratio was higher for those

Table 5. Effects of dietary variables on litter total phosphorus (Total P), water-soluble phosphorus (WSP), and the ratio of water-soluble to total phosphorus (WSP/TP) for both the full factorial model and a reduced model containing dietary calcium to available phosphorus ratios (Ca/AvP) for both litter WSP and WSP/TP.

	Litter P characteristic (Pr > F)		
Diet variable†	Total P	WSP	WSP/TP
	mg kg^{-1}		
Factorial model			
Ca	0.988	<0.0001	<0.0001
AvP	<0.0001	<0.0001	<0.0001
Phytase	<0.0001	0.757	<0.0001
Ca × AvP	0.291	0.368	0.698
Ca × phytase	0.732	0.609	0.743
AvP × phytase	0.175	0.513	0.646
Ca × AvP × phytase	0.652	0.819	0.967
Reduced model			
Ca/AvP		<0.0001	0.009
Ca/AvP^2		0.012	0.095
Phytase		0.745	<0.0001

† AvP, dietary available P.

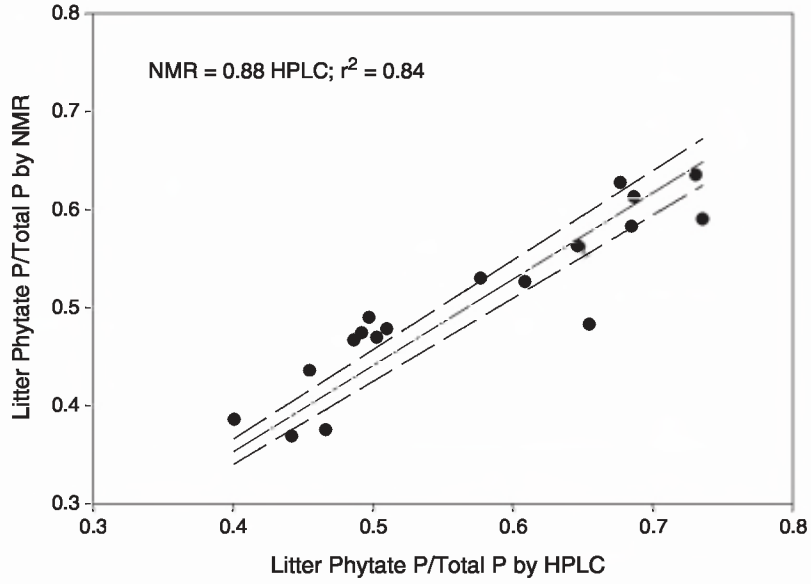

Fig. 1. The comparison of proportion of phytate P to total phosphorus in litters analyzed by high performance liquid chromatography (HPLC) and ^{31}P nuclear magnetic resonance (NMR) spectroscopy. Dashed lines represent the 95% confidence intervals.

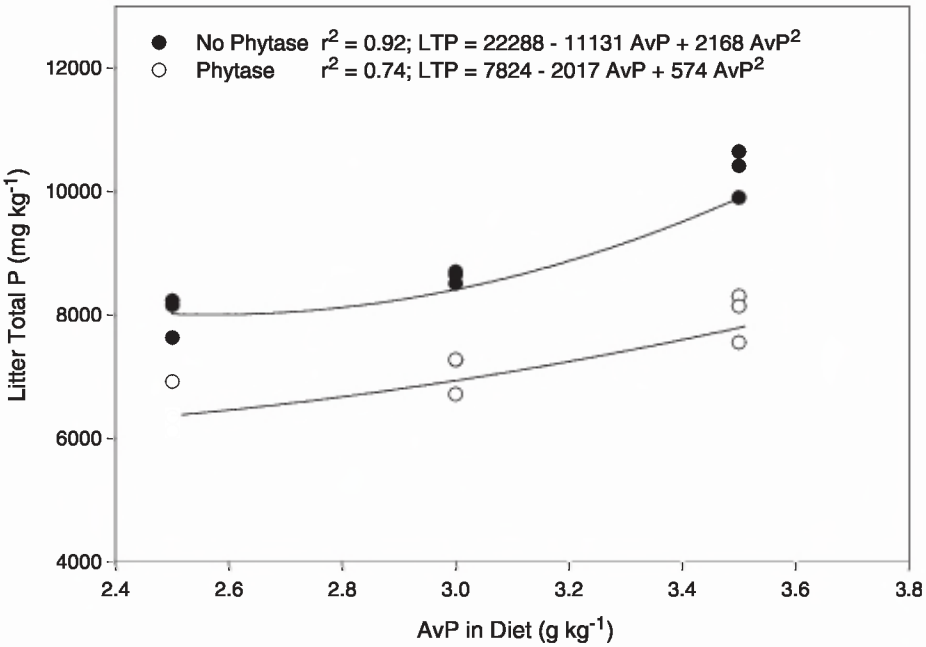

Fig. 2. The effect of dietary available phosphorus on litter total phosphorus concentrations for both non-phytase and phytase-amended diets.

dietary treatments containing phytase vs. those without phytase supplementation.

The proportion of phytate/TP excreted in the litter was negatively correlated with the litter WSP for both the non-phytase and phytase-amended diets (Fig. 6). As the proportion of phytate P in the litter increased, litter WSP decreased. There was also a negative correlation between the dietary Ca/AvP ratio and the fraction of inorganic P in the litter that was water-soluble (Fig. 7). As Ca concentrations relative to AvP in the diet increased, there appeared to be an increase in insoluble CaP precipitates formed in the litter.

DISCUSSION

The positive correlation between dietary AvP levels and total litter P found in this experiment were consistent with other studies that examined the effects of dietary P concentrations on litter total P. Maguire et al. (2004) demonstrated that reducing NPP concentrations in broiler feed (11–40% reduction in NPP over four feeding phases) decreased the total P in the resulting litter by 17%. McGrath et al. (2005) also found that reducing total dietary P levels (6–19% reduction in NPP over four feeding phases) reduced broiler litter total P concentration by 18%. This reduction in litter total P

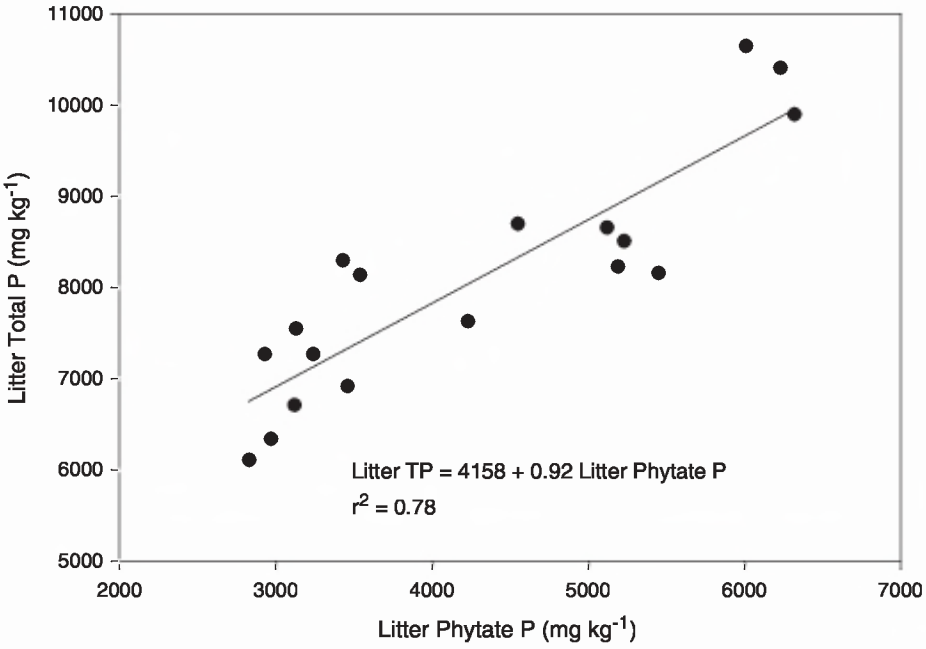

Fig. 3. The relationship between litter phytate phosphorus concentration and litter total phosphorus concentration for all diets.

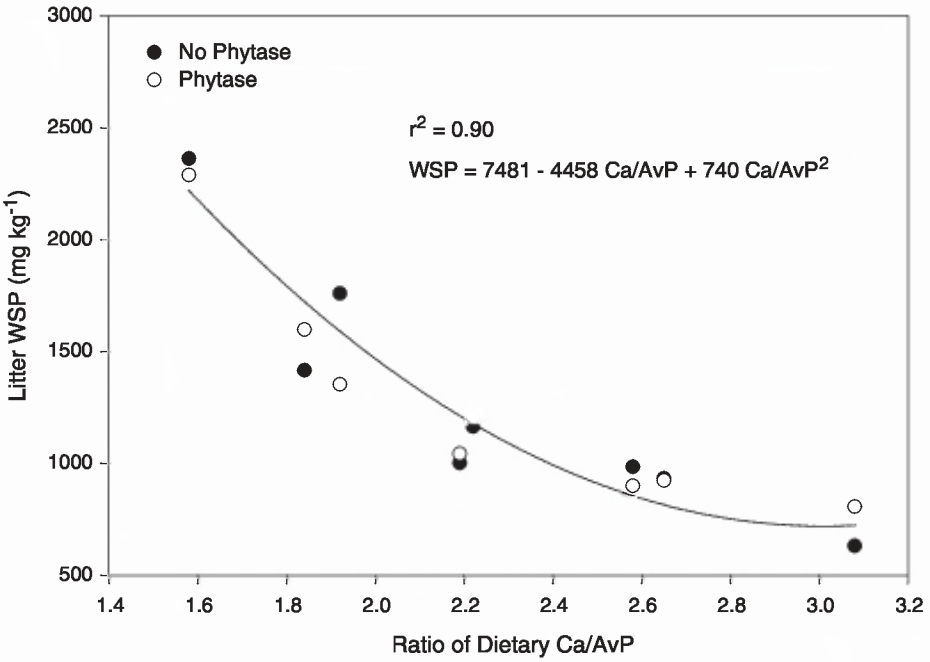

Fig. 4. The influence of dietary calcium to available phosphorus ratio on the litter water-soluble phosphorus concentration of both non-phytase and phytase-amended diets.

was enhanced by the addition of phytase. In the present experiment, phytase addition at the expense of 1.0 mg g^{-1} NPP decreased litter total P by 20% and was comparable to other studies that reported 13 to 33% reductions in total P excretion due to phytase addition (Applegate et al., 2003; Miles et al., 2003; Maguire et al., 2004; Vadas et al., 2004; Angel et al., 2005).

The positive correlation between litter phytate P and litter total P was caused by a combination of dietary effects. Data points with the lowest phytate P and total P concentrations represent treatments with added phytase enzyme that hydrolyzed phytate P and presumably also facilitated increased P absorption by the bird (Fig. 3). Data points with the highest phytate P and total P concentrations were from diets that had high dietary AvP concentrations and were without phytase supplementation.

A decrease in litter phytate P from broilers fed supplemental phytase has been demonstrated in several

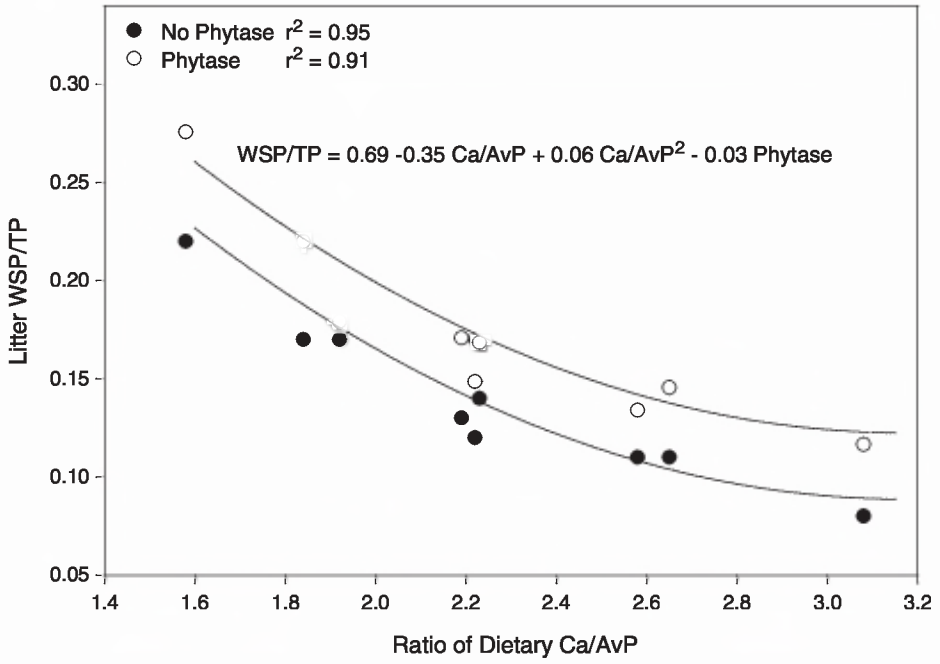

Fig. 5. The influence of dietary calcium to available phosphorus ratio on the litter water-soluble phosphorus to total phosphorus ratio of both non-phytase and phytase-amended diets.

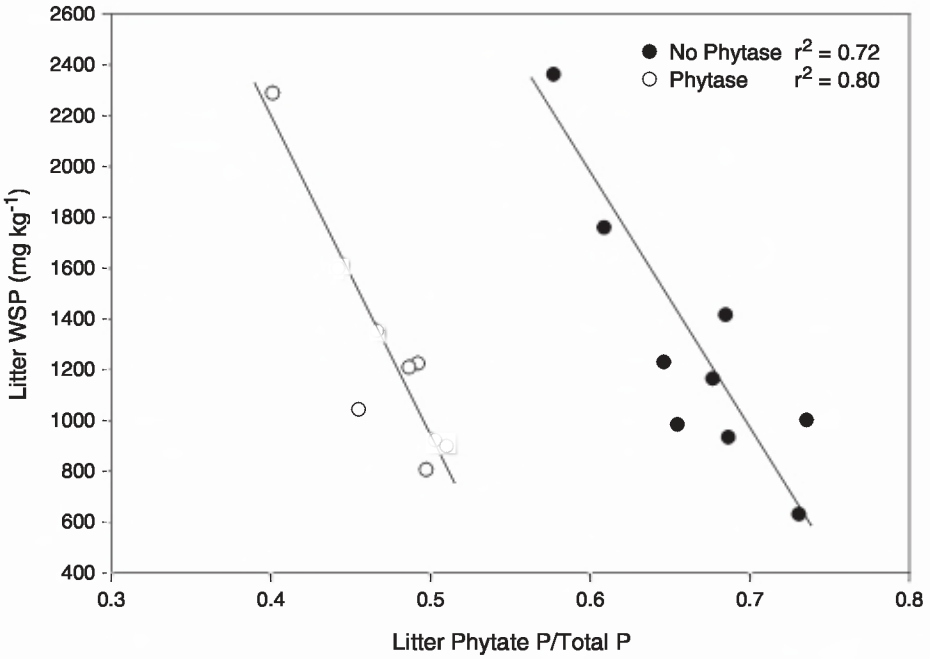

Fig. 6. The relationship between litter phytate phosphorus to total phosphorus ratio and litter water-soluble phosphorus for both non-phytase and phytase-amended diets.

studies. Maguire et al. (2004) demonstrated that the addition of phytase to broiler diets decreased phytate P concentrations in the litter compared with equivalent non-phytase-amended diets. McGrath et al. (2005) analyzed the phytate P content of litters from broilers fed a variety of diets with and without phytase addition, and found that diets containing phytase had lower phytate P content than diets without phytase. Toor et al. (2005) analyzed litter samples generated from broilers fed diets with and without phytase and concluded that dietary phytase addition decreased phytate P concentrations in litters.

The negative effects of increased dietary AvP on phytate degradation have been demonstrated in several studies. Ravindran et al. (2000) demonstrated that increasing dietary AvP concentrations lowered the digestibility of P by the bird, presumably due to a decrease in phytate hydrolysis. Ballam et al. (1985) demonstrated

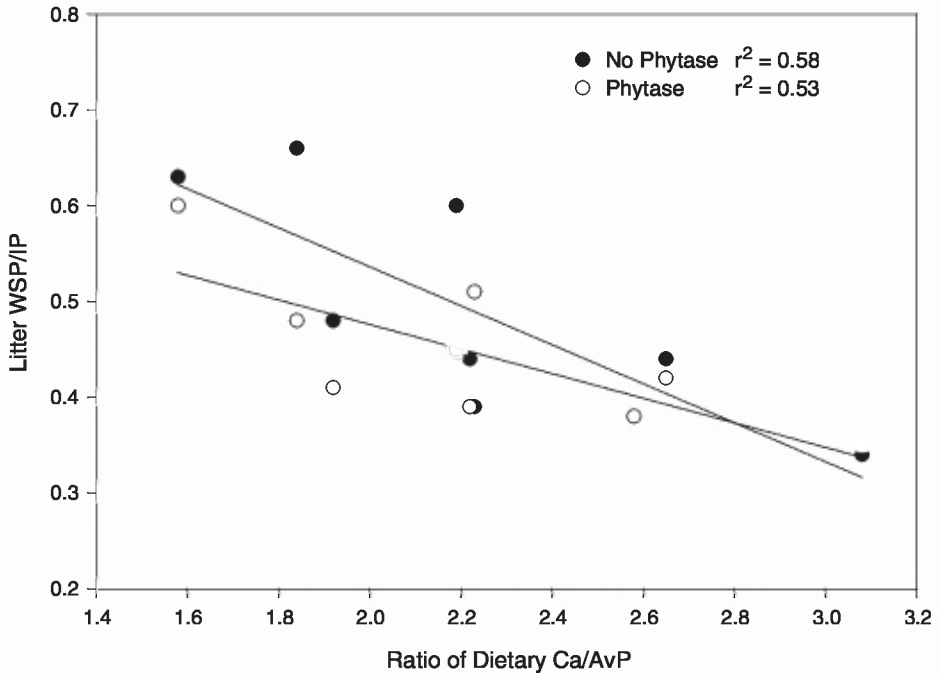

Fig. 7. The relationship between dietary calcium to available phosphorus ratio and the fraction of inorganic phosphorus (IP) in litter (determined by ^{31}P nuclear magnetic resonance [NMR] spectroscopy) that was water-soluble.

that an increase in inorganic P in broiler diets significantly decreased phytate P hydrolysis in their birds. van der Klis and Versteegh (1996) demonstrated a decrease in phytate P hydrolysis of between 26 and 48% in broilers fed diets with higher AvP than comparable diets with lower AvP, while maintaining a constant Ca/AvP ratio in the diet.

In the present study, the WSP in the litters decreased as the phytate P content in the litters increased (Fig. 6). Sequential extraction of broiler litter has shown that P compounds extracted in deionized water were predominantly inorganic P and that the majority of phytate P was only extracted with stronger extractions such as HCl or NaOH (Turner and Leytem, 2004). Therefore, litters that have a greater proportion of phytate P will have lower WSP concentrations. Examination of available literature has shown this same trend in broiler litter (Maguire et al., 2004; Toor et al., 2005) and manure from laying hens (Leytem et al., 2006).

The main dietary component affecting litter WSP in the present study was the Ca/AvP ratio. As the Ca/AvP ratio increased, litter WSP decreased irrespective of phytase treatment. These effects of increasing Ca/AvP on litter WSP were due to a combination of several factors. The calculated optimal Ca/AvP ratio that maximized both absorption and retention of P in broilers was found to be approximately 2.2 (van der Klis and Versteegh, 1996). At a high dietary Ca/AvP, phytate P hydrolysis and P absorption were reduced due to Ca/phytate and CaP precipitates in the gut. At a low dietary Ca/AvP, more P appeared to be absorbed but was not retained by the bird and was presumably excreted in the urine.

In the present study, an increase in dietary Ca generally decreased phytate P hydrolysis leading to greater excretion of phytate P (Table 3 and 4). In addition, increasing the dietary levels of Ca reduced the proportion of inorganic P that was soluble, presumably due to formation of stable CaP complexes (Fig. 7). The formation of stable CaP complexes in litter and manure with increasing dietary Ca have been reported previously (Cooperband and Ward Good, 2002; Toor et al., 2005). Therefore, as the dietary Ca/AvP ratio increased, WSP decreased due to an increase in both phytate excretion and formation of insoluble CaP complexes. At lower dietary Ca/AvP ratios, phytate was hydrolyzed and increased P absorption, but due to the imbalance of serum Ca and P, the absorbed P was presumably excreted in the urine, which further increased P solubility (urinary P has been shown to be primarily soluble inorganic P).

When the WSP/TP ratio in the litters was determined, the Ca/AvP was still the dominant factor controlling this relationship, but there was also a significant difference between diets with and without phytase. After accounting for effects of dietary Ca/AvP, there was an increase in litter WSP/TP with phytase additions (Fig. 5). This should be expected, as additions of phytase were shown to significantly decrease the amount of phytate P in litter, which would in turn increase the proportion of total P that was water-soluble. Vadas et al. (2004) also demonstrated that broiler manure from diets equivalent in Ca/AvP with and without phytase supplementation had a greater proportion of WSP in litters from phytase-amended diets, although they did not reduce the AvP in the diets with phytase addition.

Previous studies that have examined the influence of changes in dietary NPP (or AvP) and phytase additions on WSP have frequently not taken the influence of the Ca/NPP ratio into account. Typically, test diets were formulated to have constant Ca levels while the NPP in the diet was decreased, with and without phytase addition. By designing feeding trials in this manner, a decrease in dietary P, which may be accompanied with phytase supplementation, and a constant Ca concentration resulted in feeds that had higher Ca/NPP ratios. Therefore, by increasing the Ca/NPP ratio, WSP values in the resulting litters and manures would generally decrease, but this decrease could not strictly be attributed to decreasing NPP or the inclusion of phytase in the diets. In other words, there has been a hidden confounding factor in many published studies.

As an example, Applegate et al. (2003) demonstrated that decreasing dietary NPP and adding phytase to an industry standard diet decreased litter WSP, which was attributed to the decrease in dietary NPP and inclusion of dietary phytase. When we examine the formulated diets in this study, we found that there was an increase in Ca/NPP ratio from 2.2 in the industry diet to 3.0 in the industry diet with phytase supplementation and 3.5 in the lower NPP diet with phytase supplementation (Ca/NPP calculated as a weighted average of values across the four diets fed). When the Ca/NPP ratio was plotted vs. litter WSP there was a linear decrease in WSP with increasing Ca/NPP that possessed an $r^2 = 0.99$ for the three treatments. Therefore, the effects on WSP in this study were more likely a result of increasing the Ca/NPP ratio rather than the reduction of dietary NPP or the supplementation of diets with phytase.

Further, Vadas et al. (2004) demonstrated that WSP decreased in manures from three diets with decreasing dietary AvP that had phytase supplementation. The decrease in WSP was attributed to the decrease in dietary AvP, but when we examined the Ca/AvP ratio in the grower diets, we found that as dietary AvP was decreased from 4.0 to 2.0 g kg^{-1}, the Ca/AvP ratio increased from 2.1 to 3.1 (Ca/AvP calculated as a weighted average of the diets fed). Further, when we plotted the Ca/AvP vs. litter WSP concentrations there was a strong inverse correlation of WSP to Ca/AvP ($r^2 = 0.82$). In another study, Maguire et al. (2003) examined the influence of reducing dietary NPP with and without addition of both phytase and 25-0H-D_3 + phytase. They found a similar reduction in the proportion of WSP with all treatments relative to the control, which was attributed to reduced dietary NPP concentrations and phytase additions. However, the Ca/NPP ratio for the control diet was 1.7 while the treatment diets all had a ratio of 3.3, therefore the difference in litter WSP in the control may have been due to a much lower Ca/NPP ratio rather than other diet modifications.

These three examples were presented to demonstrate that including the potential effects of Ca/NPP or Ca/AvP

ratio in the interpretation of the data can provide greater insight into the processes controlling WSP in litter. Research involving dietary manipulation of poultry to alter P excretion into the environment is very complex and properly accounting for the effects of diet on P excretion is difficult, as there are many different factors reported to affect the ultimate outcome. Therefore, accounting for all of them simultaneously can be difficult.

CONCLUSIONS

In this study, the dietary Ca/AvP ratio was a better indicator of changes in litter WSP than either dietary AvP or phytase additions. Earlier research that has examined the influence of dietary modification on WSP excretion did not take into account the effects of dietary Ca and the resulting Ca/AvP or Ca/NPP ratio on WSP excretion which, in part, may explain some of the variation in the reported results. Since many poultry producing regions are using a measurement of litter/manure WSP to assess the potential impacts of land application on P losses, a better understanding of what is really controlling WSP levels in poultry litters and manures must be developed to better manage these with respect to environmental protection.

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