

The Influence of Manure Phytic Acid on Phosphorus Solubility in Calcareous Soils

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ABSTRACT

Manure characteristics can influence the potential for P transfer in runoff following land application of manures. This research assessed the influence of manure characteristics on P solubility in calcareous soils using manures from poultry (*Gallus Domesticus*) fed a variety of grain-based diets with the manures containing a range of total P (5.6–16.4 g P kg⁻¹), water-extractable P (WEP, 0.9–4.7 g P kg⁻¹), phytic acid P (0.1–7.6), total N/P ratios (2.6–5.1), and total C/P ratios (19.5–75.7). In addition, mono-ammonium phosphate fertilizer and reagent grade inositol hexaphosphate (phytic acid [PA]), were included, as well as a control treatment with no P additions. Treatments were incorporated into two soils (Portneuf [Coarse-silty, mixed, superactive, mesic Durinodic Xeric Haplocalcids] and Millville [Coarse-silty, carbonatic, mesic Typic Haploxerolls]) at three rates (10, 20, and 40 mg P kg⁻¹) and incubated for a total of 18 wk with subsamples taken at 2, 5, 9, and 18 wk. Soil samples were analyzed for inorganic and organic NaHCO₃ (Olsen) extractable P and select soils were analyzed at 0 and 12 wk by ³¹P nuclear magnetic resonance spectroscopy (NMR) for soil P characterization. The percentage of WEP and PA (of total P) in the manures were linearly related ($r^2 = 0.94$). Increases in Olsen P over time were positively related to the percentage of monoester P in the treatments. At 2 wk, there was a strong negative correlation between the amount of PA added in the treatments and increases in Olsen P. However, by 18 wk, Olsen P was more closely related to the amount of C or N added with the treatments. Changes in PA content of manures due to dietary modification may influence P sorption on calcareous soils in the short-term while other characteristics such as C/P ratio may exert a stronger influence over changes in soil test P over longer time periods.

ANIMAL PRODUCTION in the USA is valued at more than \$100 billion and has consolidated significantly over the past 20 yr, with a larger number of animals being produced on an increasingly smaller land base (Kellogg et al., 2000). Such consolidation of animal production can generate regional and farm-scale nutrient surpluses that can potentially contribute to nonpoint source nutrient pollution of water bodies, because nutrient imports in feed and mineral fertilizer can exceed nutrient exports (Sharpley et al., 1994; Sims et al., 1998). Overapplication or mismanagement of manure can increase the risk of degrading surface and ground water quality with excess nutrients (Sharpley, 1996; Sims et al., 1998, 2000).

Phosphorus is a particular problem, because it can accumulate in soil and reach concentrations greater than

those needed for optimum crop production. This is due in part to unfavorable N/P ratios in manures relative to the uptake of these nutrients by most crops, which results in overapplication of P when manures are applied to meet the N requirement of the crop (Mikkelsen, 2000). As a result, long-term manure application to agricultural land often leads to soil P accumulation which has the potential to accelerate P transfer in runoff to water bodies. This process can contribute to eutrophication in freshwater ecosystems, and numerous examples of water quality impairment associated with P pollution from animal operations now exist (Boesch et al., 2001; Burkholder and Glasgow, 1997; U.S. Geological Survey, 1999).

The environmental fate of P in animal manures is partly determined by its chemical composition. However, few studies have fully characterized manure P and determined the effect of various P compounds on P reactivity in soils. It has been shown that the soil sorption capacity differs for various organic P compounds, with inositol hexaphosphate (also called phytate-P, PA) being very tightly bound by soils while other organic P compounds such as nucleotides, DNA, and glucose phosphates are more mobile in soils (Celi and Barberis, 2005). Manure characteristics, other than organic P content, may also affect the manure P retention in soils. For example, the addition of manure can lead to complexation of Fe and Al by organic ligands, which decreases precipitation of P with these metals. These ligands can also compete for P sorption sites, which increase the concentration of soluble P (Iyamuremye et al., 1996).

As new feed technologies aimed at P reduction in manures are adopted, the P composition of manures may be altered, which in turn can potentially influence its reactivity in soils. For example, the addition of phytase enzymes to improve P availability in feeds can result in a decrease in total P in manure by up to 40% (Sims et al., 1999). This reduction in total P may also be accompanied by a change in the forms of P in the manure. For example, research has shown that these manures contain greater amounts of inorganic P than manure generated from conventional feeds (Sims et al., 1999; Maguire et al., 2003, 2004). The use of genetically mutated corn (containing low concentrations of PA) for feed can also reduce the total P excreted by monogastric animals (Spencer et al., 1998) and may change the P composition in the manure. As feed modifications result in manures with less PA, a situation may be created with

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Abbreviations: CCE, calcium carbonate equivalent; ICP-OES, inductively coupled plasma optical emission spectroscopy; NMR, nuclear magnetic resonance; NPP, non-phytate P; NTA, 0.1 M sodium nitrilotriacetate; OC, organic carbon; PA, phytic acid; WEP, water-extractable P.

an enhanced potential for undesirable P losses (Smith et al., 2004; Maguire et al., 2005).

Variability in soluble P in runoff from manure-amended soils is also linked to differences in soil properties. However, most studies have been performed on Eastern USA soils, where rain fed agriculture occurs primarily on acidic soils, and the dynamics of P in soil are primarily controlled by Al and Fe. In contrast, agricultural soils of the Western USA are typically irrigated and contain high CaCO₃ and low organic matter concentrations. Inorganic P reactions in such soils are conventionally considered to be controlled primarily by Ca (Lindsay, 1979), although some studies found that P sorption in calcareous soils was more closely related to the amount of organically complexed Fe and Mn (Leytem and Westermann, 2003), the iron oxide and clay content (Hamad et al., 1992; Ryan et al., 1985; Solis and Torrent, 1989), and aluminum oxides (Castro and Torrent, 1998).

Organic P compounds also have the ability to form complexes or precipitate with metal cations, the extent of which is dependent on the type of organic P (Celi and Barberis, 2005). Inositol phosphates, such as PA, have a high affinity for complexation with metal cations (Cosgrove, 1980; Nolan and Duffin, 1987). In soils, complexation with metals, particularly Fe and Ca, increase the stabilization of organic P compounds (Harrison, 1987; House and Denison, 2002). In calcareous soils, there is great potential for the formation of complexes between organic P compounds and Ca containing minerals. For example, precipitation of PA with calcite occurs at very low concentrations of PA (Celi et al., 2000). This explains why, in some calcareous soils, the organic P content is positively correlated to the calcium content (Harrison, 1987).

It has also recently been shown that the amount of C added with manures can influence changes in extractable P with manure additions to calcareous soils (Leytem et al., 2005; Leytem and Westermann, 2005). The inverse relationship found between the increase in extractable soil P and the addition of C in manure in these studies strongly suggests the importance of microbial processes in regulating P solubility following manure application to calcareous soils. This is potentially important, because few models or indexes account for microbial immobilization. In the Leytem et al. (2005) study, it was shown that this effect overrides the influence of soil chemical properties such as CaCO₃ concentration, clay content, pH, or amorphous metal concentrations on soil P sorption, at least in the short term.

The previous studies examining the influence of manure characteristics on P sorption in calcareous soils used manures with a narrow range of organic P concentrations. One question that arises is; will the effects of added C still be the controlling characteristic for P solubility in manure amended calcareous soils when there is a wide range in organic P concentrations in the manures. Therefore, the objective of this study was to determine the impact of altering the organic P fraction of manures, in particular PA, on P reactivity and solubility in calcareous soils.

MATERIALS AND METHODS

Soil Sampling and Analysis

Two calcareous soils (0- to 30-cm depth), a Portneuf and Millville, were used in the incubation study; both are irrigated agricultural soils in the semiarid Western USA (Table 1). Particle size was determined by the hydrometer method (Gee and Bauder, 1986). Organic C (OC) was determined using the method of Walkley and Black (1934). The pH was measured in a saturated paste using a combination electrode (Robbins and Wiegand, 1990). Calcium carbonate equivalent (CCE) was determined with the titrimetric method of Allison and Moodie (1965). Water-extractable P was determined using inductively coupled plasma optical emission spectroscopy (ICP-OES) after extraction of 1 g of soil with 100 mL of deionized water, shaken for 1 h, and filtered through a 0.45- μ m membrane. Sodium bicarbonate-extractable P was determined using the method of Olsen et al. (1954). Oxalate-extractable Al (Al_{ox}) and Fe (Fe_{ox}) were obtained by extraction with 0.2 M ammonium oxalate at pH 3 to obtain a measure of the amorphous Al and Fe in the soils (Jackson et al., 1986). Organically complexed Fe (Fe_{nta}) and Mn (Mn_{nta}) were extracted with 0.1 M sodium nitrilotriacetate (NTA) at pH 8.3, with a soil/solution ratio 1:200, and filtration through a 0.45- μ m membrane (Yuan et al., 1993). Water-extractable and bicarbonate-extractable P was quantified by the colorimetric method of Murphy and Riley (1962). Oxalate and NTA extractable Fe, Al, and Mn were quantified by ICP-OES detection.

Manure Collection and Analysis

Six manures were obtained from dietary studies using either laying hens or broiler chicks. Four manures were from laying hens fed corn-based diets (C1–C4) with variation in non-phytate P (NPP), calcium addition (Ca), and one diet having phytate addition (C4). Dietary P and Ca content are described in Table 2. The manures from diets C1–C4 were obtained from 41-wk-old Hy-line W36 hens, with 84% hen egg production, and consuming 93.2 g hen⁻¹ d⁻¹. There were two birds per pen and diets were fed for 10 wk before manure collection. Excreta were collected by placing pans under four cages per diet for a 48-h period. After which time, the manure was pooled by diet

Table 1. Select physical and chemical properties of soils used in the incubation study.

Soil Properties	Soils	
	Portneuf	Millville
Classification	coarse-silty, mixed superactive, mesic Durinodic Xeric Haplocalcids	coarse-silty, carbonatic, mesic Typic Haploxeroll
Clay [†] , g kg ⁻¹	180	146
OC [‡] , g kg ⁻¹	7.5	13.5
pH [§]	7.6	7.9
CCE , g kg ⁻¹	170	496
WEP [#] , mg kg ⁻¹	0.03	2.33
Olsen P ^{††} , mg kg ⁻¹	4.0	18.8
Al _{ox} ^{‡‡} , g kg ⁻¹	0.65	0.96
Fe _{ox} ^{‡‡} , g kg ⁻¹	0.45	0.97
Fe _{nta} , g kg ⁻¹	0.50	0.46
Mn _{nta} , g kg ⁻¹	0.27	0.29

[†] Particle size determined by method of Gee and Bauder (1986).

[‡] Organic carbon (Walkley and Black, 1934).

[§] pH determined with 1:1 soil/solution ratio.

^{||} Calcium carbonate equivalent (Allison and Moodie, 1965).

[#] Water extractable P.

^{††} Bicarbonate extractable P.

^{‡‡} Oxalate extractable Al and Fe (Jackson et al., 1986).

^{|||} Nitrilotriacetate extractable Fe and Mn (Yuan et al., 1993).

Table 2. Select properties of the manures used in the incubation studies. (All measurements on a dry weight basis).

Manure property	C1	C2	C3	C4	B1	B2
Diet composition†	0.54% TP, 0.32% NPP, 3.8% Ca	0.38% TP, 0.16% NPP, 3.8% Ca	0.38% TP 0.16% NPP, 3.56% Ca	0.38% TP 0.16% NPP, 3.56% Ca, 400 U phytase kg ⁻¹	0.35% TP 0.11% NPP	0.30% TP 0.30% NPP
Total P, g kg ⁻¹	16.4	9.8	10.1	10.3	6.4	5.6
Water extractable P, g kg ⁻¹	4.7	0.9	1.64	3.8	4.7	4.1
% Water extractable P of Total P	28	10	16	37	74	73
Total Ca, g kg ⁻¹	64.7	70.3	46.5	57.6	1.0	0.8
Total Al, g kg ⁻¹	1.00	0.80	0.69	0.67	0.09	0.05
Total Fe, g kg ⁻¹	0.84	0.56	0.61	0.59	0.43	0.40
Total Mn, g kg ⁻¹	0.35	0.31	0.32	0.30	0.25	0.28
Total N, g kg ⁻¹	42.1	40.8	41.1	42.9	24.9	28.4
Total C, g kg ⁻¹	320	318	325	313	418	423
N/P	2.56	4.14	4.08	4.18	4.67	5.07
C/P	19.5	32.4	32.3	30.5	65.2	75.7

† TP refers to Total P; NPP is non-phytate P.

and freeze dried. The remaining two manures were obtained from 21-d old male broiler chicks fed a barley-based diet containing either wild-type barley (B1) or low-phytate barley (B2). The birds were housed in raised-floor battery cages with mesh grate floors above fecal collection trays with five birds per pen and five pens per treatment. Feed and water were available ad libitum throughout the 9-d experiment. During the final 2 d of the experiment, clean feces (free from feathers and feed) were collected twice a day from plastic liners placed in the fecal collection trays underneath each pen of birds. Before analysis, the samples were dried in a forced oven dryer at 55°C for 72 h, followed by fine grinding.

Analysis of the manures was as follows: (i) total elements (Al, Ca, Fe, Mn, P) were determined by microwave-assisted digestion of a 0.5-g dried sample with 8 mL of concentrated HNO₃ and 2 mL of 30% (v/v) H₂O₂ with ICP-OES detection; (ii) WEP was determined in a 1:100 manure (freeze dried) to deionized water ratio, shaken for 1 h, filtered through a 0.45- μ m membrane, and analyzed by ICP-OES; (iii) total N and C were determined by combustion of a 50-mg sample in a FlashEA1112 CNS analyzer (CE Elantech, Lakewood, NJ).

The P composition of manures was determined by solution ³¹P NMR spectroscopy as described by Turner (2004). Briefly, P was extracted in triplicate by shaking 2.00 \pm 0.01 g of dried manure 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 10 000 \times 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 re-dissolved in 0.1 mL of D₂O (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 ³¹P NMR spectra were obtained using a Bruker Avance DRX 500 MHz spectrometer (Bruker, Germany) operating at 202.456 MHz for ³¹P. We used a 5- μ s pulse (45°), a delay time of 5.0 s, an acquisition time of 0.8 s, and broadband proton decoupling for all samples. The number of scans varied between 9000 and 14 000, and spectra were plotted with a line broadening of 1 Hz. Chemical shifts of signals were determined in ppm relative to 85% (v/v) H₃PO₄ and assigned to individual P compounds or functional groups based on literature reports (Turner et al., 2003). Signal areas were calculated by integration and P concentrations were calculated by multiplying the proportion of the total spectral area assigned to a specific signal by the total P concentration (g P kg⁻¹ dry manure) in the original extract. This NMR procedure detects concentrations of P compounds of approximately 0.1 mg P kg⁻¹ of dry manure (Turner, 2004).

Incubation Study

Each of the six manures was incorporated individually (in triplicate) into 100 g portions of each of the two soils at three P based application rates (10, 20, and 40 mg P kg⁻¹). A fertilizer treatment (mono-ammonium phosphate, MAP) and reagent grade inositol hexaphosphate (PA) were applied at the same P rate for comparison with manure P, and a control (unamended soil) was included for each soil. The rates used were typical of those used for potato (*Solanum spp.*) production in Idaho where recommended P applications range from 0 to 36 mg P kg⁻¹, depending on bicarbonate-extractable P concentration and soil lime content (McDole et al., 1987).

After incorporation, the soils were incubated in 250-mL polyethylene containers in a constant temperature room (22°C) in a completely randomized design. Two holes were made in the tops of the incubation containers to allow gas exchange and prevent anaerobic conditions during the incubation. Soil moisture content (20% by weight) was maintained by adding deionized water at weekly intervals, and moisture varied <1% between adjustments.

Soils were incubated for 18 wk with subsamples (approximately 3 g) taken after 2, 5, 9, and 18 wk. Moist subsamples of the incubation soil mixtures were analyzed for bicarbonate extractable P using the method of Olsen et al. (1954), P was quantified using the colorimetric method of Murphy and Riley (1962). Molybdate-reactive P in soil bicarbonate extracts includes only inorganic orthophosphate (Coventry et al., 2001). Total P in the extracts was determined by the same procedure following acid-persulphate digestion. Briefly, samples (1 mL) were acidified by adding 0.15 mL of 3 M H₂SO₄ and digested (in autoclave) with 3.85 mL of 26 mM K₂S₂O₈ (20 mM final concentration) at 120°C and 100 kPa for 45 min. Organic P was calculated as the difference between total and inorganic P. One set of manure-treated soils (Portneuf, C1-C4 treatments only) was chosen for ³¹P NMR analysis, due to the high cost of this analysis. Extractions for ³¹P NMR analysis were made immediately after manure incorporation and then at 12 wks, using the above method with the following exception: extracts were performed by shaking 5 g of soil with 100 mL of a solution containing 0.25 M NaOH and 0.05 M EDTA for 16 h at 22°C (Cade-Menun and Preston, 1996).

Calculation of Increase in Olsen P

The increase in Olsen P was calculated for the six manure, PA, and fertilizer treatments evaluated in the incubation study. This calculation allows comparison of the increase in Olsen P due to treatment application, thereby normalizing for soil test

Table 3. Phosphorus concentrations in poultry manure. Phosphorus concentrations were determined by NaOH-EDTA extraction and solution ^{31}P NMR spectroscopy.

	Total manure P	NaOH-EDTA extractable P				
		Total NaOH-EDTA P†	Phosphate‡	Phosphate monoesters‡	Phytic acid‡	Pyrophosphate‡
g P kg dry wt. ⁻¹						
C1	16.41	15.61 (95)	7.21 (46)	0.58 (4)	7.61 (49)	0.21 (1)
C2	9.82	9.49 (97)	1.22 (13)	0.55 (6)	7.62 (80)	0.10 (1)
C3	10.07	9.12 (91)	2.02 (22)	0.60 (7)	6.38 (70)	0.13 (1)
C4	10.26	9.61 (94)	5.33 (56)	0.65 (7)	3.39 (35)	0.13 (1)
B1	6.41	6.36 (99)	4.46 (70)	1.18 (19)	0.74 (12)	ND¶
B2	5.59	5.15 (92)	3.88 (75)	1.10 (21)	0.14 (3)	ND

† Values in parenthesis are the proportion (%) of the total manure P determined by microwave digestion.

‡ Values in parenthesis are the proportion (%) of the NaOH-EDTA extracted P.

¶ ND, not detected.

P changes without manure addition. After addition of 10, 20, or 40 mg P kg⁻¹ and incubation, we calculated the increase in Olsen P in treated soils (P_T) as follows:

$$\text{Increase in Olsen P (mg kg}^{-1}\text{)} = \text{Soil P}_T - \text{Soil P}_{\text{Control}}$$

Statistics

All statistical analyses were performed using the Statistical Analysis System (SAS Institute, 1999). We used linear regression analysis (GLM), Pearson correlation, and analysis of variance using Duncan's multiple range test ($P < 0.05$) for mean separation. Slope comparisons were performed using the Mixed procedure and were determined to be different as evidenced by non-overlap of the associated 95% confidence intervals.

RESULTS

Soil Properties

These two soils were selected as they had differences in physical and chemical properties commonly associated with P sorption (Table 1). The Portneuf soil had greater clay content (180 g kg⁻¹) and lower OC content (7.5 g kg⁻¹) than the Millville soil (146 g clay kg⁻¹ and 13.5 g OC kg⁻¹). The pH values were similar with both soils being somewhat alkaline (pH 7.6 and 7.9). The Millville soil had a much greater CCE (496 g kg⁻¹) than the Portneuf soil (170 g kg⁻¹), as well as greater oxalate-extractable Al and Fe concentration (0.96 and 0.97 g kg⁻¹, respectively). The organically complexed Fe and Mn in both soils were similar and were approximately 0.48 and 0.28 g kg⁻¹, respectively. The Millville soil had greater bicarbonate-extractable P (18.8 mg kg⁻¹) and WEP (2.33 mg kg⁻¹) than the Portneuf soil (4.0 and 0.03 mg kg⁻¹, respectively). Crop responses to P additions are not expected in soils with Olsen P concentrations > 20 to 25 mg P kg⁻¹ (McDole et al., 2005).

Manure Characterization

Select chemical properties of the manures are reported in Table 2. There was a range in total P of the six manures (5.6–16.4 g kg⁻¹) with C1 (corn-based industry standard diet) having the greatest total P while the diets from broilers fed barley (B1 and B2) had the least total P. The WEP ranged from 0.9 to 4.7 g kg⁻¹, with B1 and C1 having the greatest WEP. The percentage WEP of TP ranged from 10 to 74%, with the manures from the two barley-based diets having the greatest percentage. Total Ca ranged from 0.8 to 70.3 g

kg⁻¹, with the two barley-based diets having the least Ca concentrations. The ranges in total Al, Fe, and Mn were as follows: 0.05 to 1.00 g Al kg⁻¹, 0.40 to 0.84 g Fe kg⁻¹, and 0.25 to 0.35 g Mn kg⁻¹, with the manures from the barley-based diets having the least concentrations. The average manure total N for the corn-based diets was 41.7 g kg⁻¹, while it was 26.6 g kg⁻¹ for the barley based diets, resulting in N/P ratios between 2.56 and 5.07. The average manure total C for the corn-based diets was 319 g kg⁻¹, while it was 421 g kg⁻¹ for the barley-based diets, resulting in a range of C/P ratios of 19.5 to 75.7.

The NaOH-EDTA extraction for ^{31}P NMR analysis recovered between 91 and 99% of the total P in the manure samples (Table 3). There was a wide range in phosphate (13–75% of total extractable P) and PA (3–80% of total extractable P) concentrations in the manures. The proportion of other monoesters (excluding PA) in the manures varied with the manures from the corn based diets having small proportions of other monoesters (~7% of total extractable P), while the manures from the barley based diets had much greater proportions of other monoesters (~20% of total extractable P). There were also small amounts (<1% of total extractable P) of pyrophosphate detected in the manure samples from the corn based diets. There was no significant relationship between Total P and WEP in the manures. The manure WEP/Total P and PA/Total P were negatively related (WEP/TP = -0.87 PA/TP + 84.6, $r^2 = 0.94$, $P < 0.001$; Fig. 1).

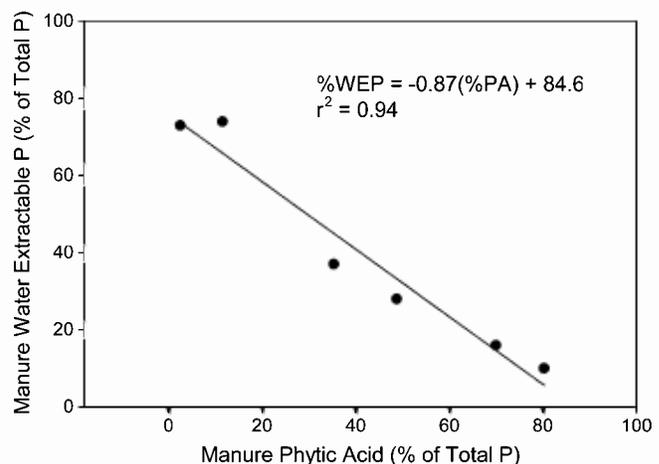


Fig. 1. Relationship between the percentage of phytic acid P in manures and the percentage of water extractable P in the manures.

Incubation Study

In the Portneuf soil, there was an initial increase in Olsen P following treatment application for all but the PA treatment, after which there was a decrease in Olsen P for the mono-ammonium phosphate treatment, an increase for the PA, C2 and C3 treatments, and little change in the remaining treatments over the 18-wk incubation period (Fig. 2a, 40 mg kg⁻¹ rate only). Treatment application on the Millville soil caused an initial increase in Olsen P following treatment application for all but the PA and C2 treatments, after which there was an increase in all treatments from 2 to 5 wk followed by a decrease in Olsen P for all but the PA treatment during the remaining incubation period (Fig. 2b, 40 mg kg⁻¹ rate only).

The Portneuf soil had an initial increase of Olsen organic P for some of the treatments. Generally speaking, the organic P decreased over the first 9 wk then was stable from 9 to 18 wk (Fig. 3a). Treatment effect on Olsen organic P was more pronounced in the Millville soil, where there was an initial increase in Olsen organic P with treatment application followed by a decrease over time, the only exception being the B2 treatment, which had an increase in Olsen organic P from 9 to 18 wk (Fig. 3b).

The change in Olsen P over time was linearly related to the percentage of PA present in the treatments (Fig. 4, $r^2 = 0.64$). As the percentage of PA increased in the treatment, the Olsen P also increased over the 18-wk incubation study, suggesting that the PA was being hydrolyzed over time and releasing soluble P. To investi-

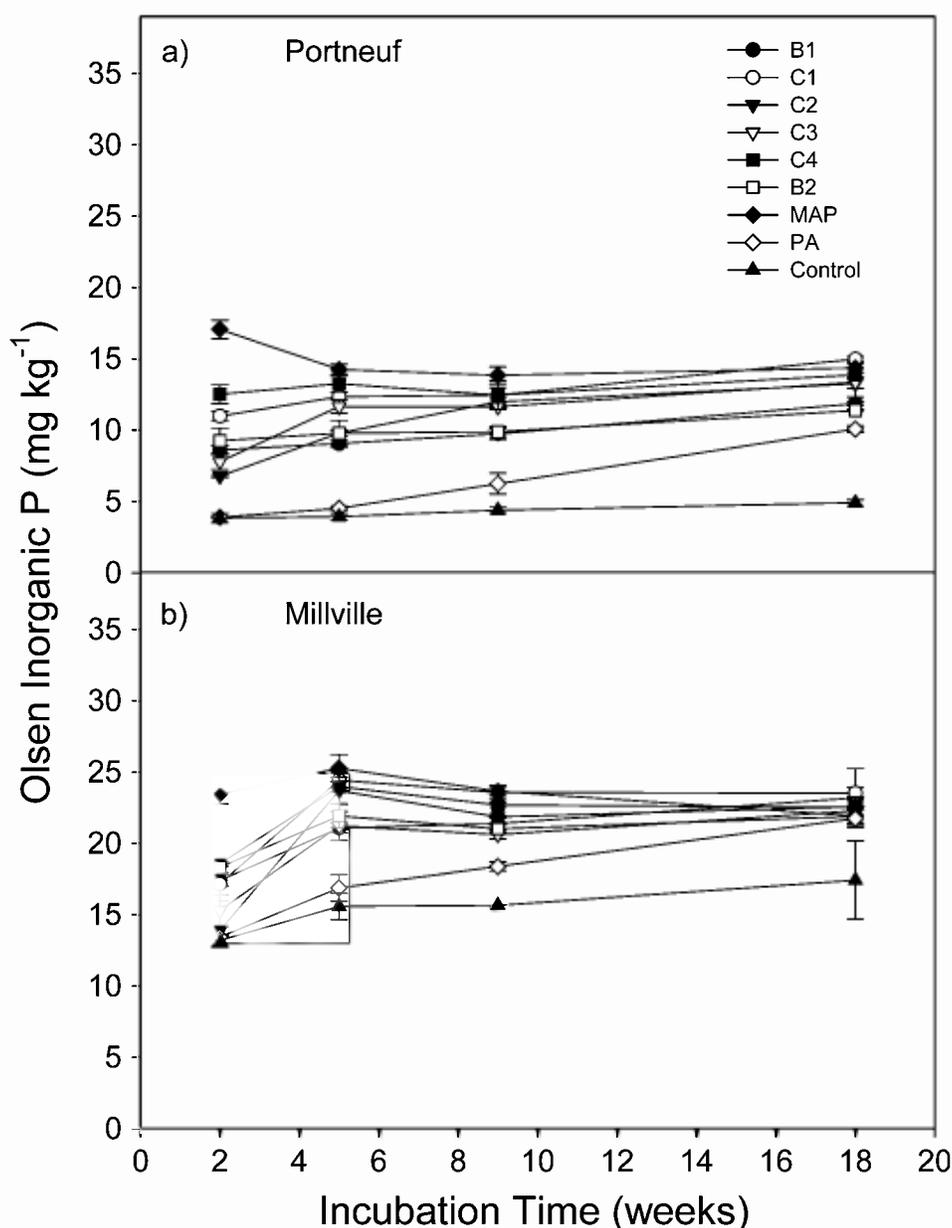


Fig. 2. Change in Olsen inorganic P for all treatments over the 18-wk incubation period at the 40 mg kg⁻¹ application rate on the (a) Portneuf and (b) Millville soils.

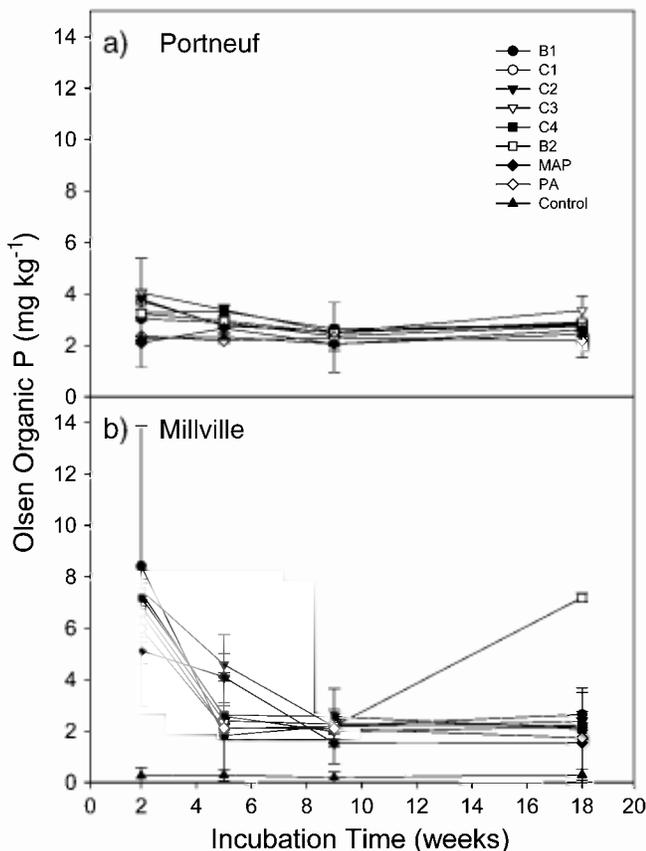


Fig. 3. Change in Olsen organic P for all treatments over the 18-wk incubation period at the 40 mg kg^{-1} application rate on the (a) Portneuf and (b) Millville soils.

gate this, ^{31}P NMR was used to monitor changes in PA over the first 12 wk of incubation. The presence of PA was detected in all samples analyzed immediately following incorporation of the treatments into the soils (as indicated by peaks at 4.5, 4.7, 5.1, and 5.9 ppm); however, after 12 wk of incubation, there were no measurable amounts of PA present, supporting the theory that the increase in Olsen P over this time was due in part to hydrolysis of PA (Fig. 5, only C2 spectra shown).

Treatment was significant at all rates for Olsen P on both the Portneuf and Millville soils ($P < 0.05$). The effect of treatment on increasing Olsen P with application rate was more pronounced at 2 wk than at 18 wk on both soils (Fig. 6; 2- and 18-wk data shown). At 2 wk, the mono-ammonium phosphate treatment had the greatest increase in Olsen P and the PA treatment had the least increase in Olsen P with increasing application rate on both soils (Fig. 6a and 6c, Table 4). There were some differences in increases in Olsen P with application rate between the manure samples at 2 wk, but by the 18 wk sampling date there were few differences between these treatments (Fig. 6b and 6d). Treatment differences were most pronounced at the highest P application rate (40 mg P kg^{-1}) on the Portneuf soil and generally followed the trend: $\text{MAP} > \text{C4} > \text{C1} > \text{B2} = \text{B1} > \text{C3} > \text{C2} > \text{PA}$. The treatment properties most closely correlated with increases in Olsen P on the two soils were the percentage of phosphate ($r = 0.85$, $P > 0.001$) and

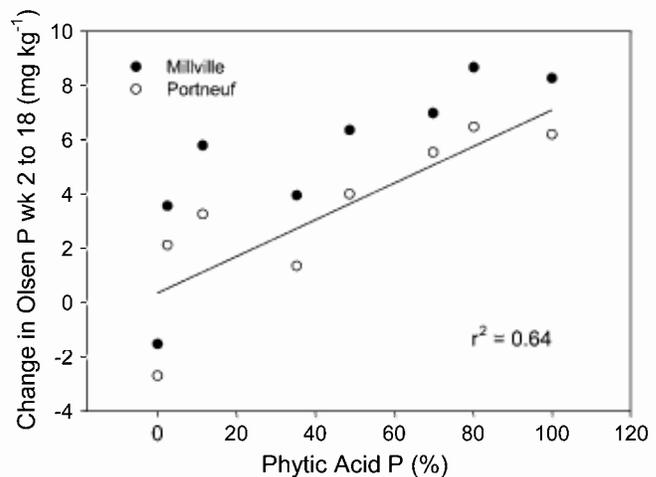


Fig. 4. The relationship between the change in Olsen P from 2 to 18 wk of incubation and the percentage of phytic acid in the treatments for both soils.

percentage of PA ($r = -0.76$, $P > 0.001$). There was a strong linear relationship between manure PA content and increases in Olsen P following 2 wk of incubation for all of the manures (Fig. 7, $r^2 = 0.57$). This relationship improved to $r^2 = 0.90$ when the manures from the barley-based diets were dropped out of the model (Fig. 7).

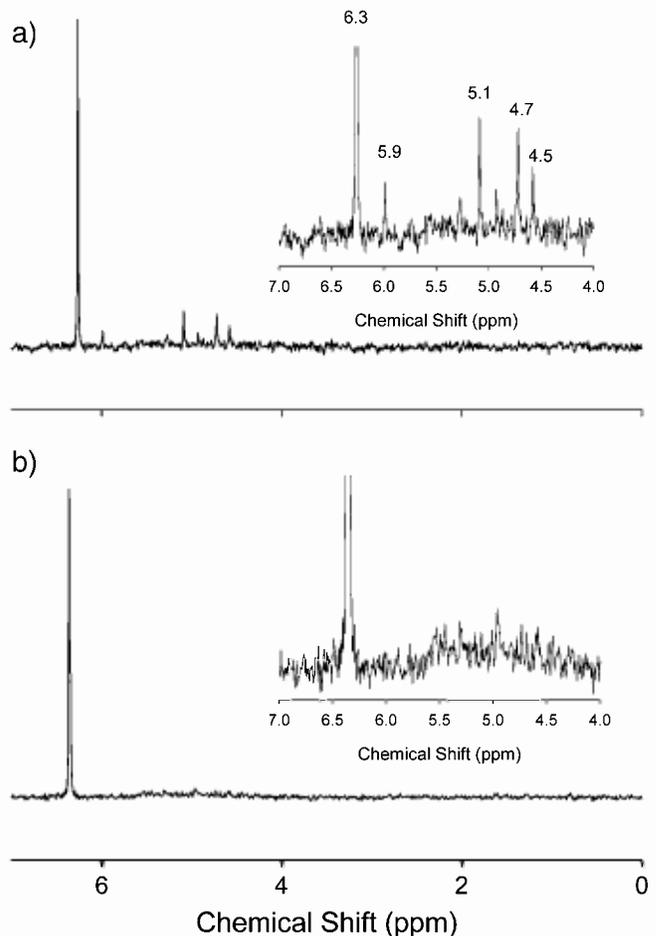


Fig. 5. The ^{31}P nuclear magnetic resonance spectra (^{31}P NMR) for soils receiving manure from C2 and extracted (a) immediately following manure incorporations and (b) after 12 wk of incubation.

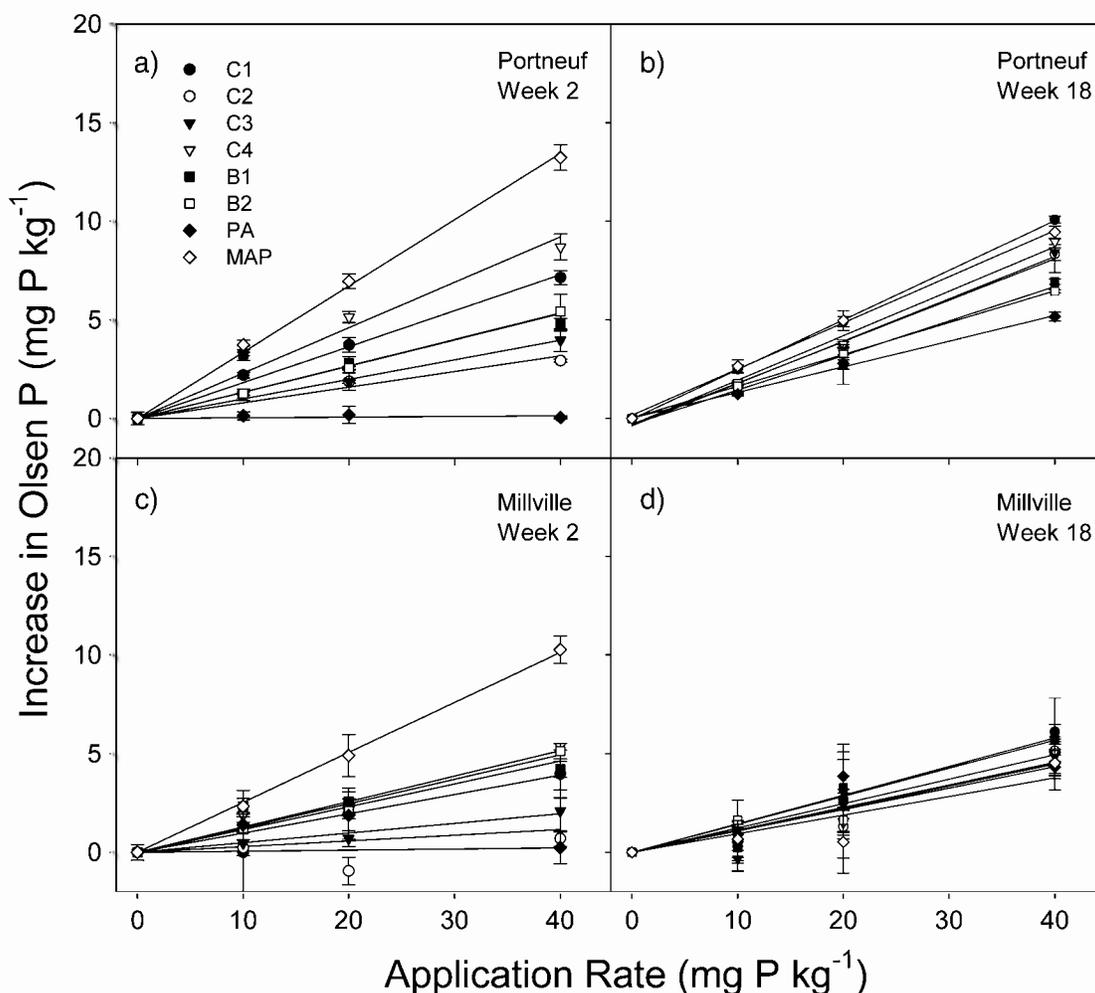


Fig. 6. The increase in Olsen P with treatment application rate for (a) Portneuf soil at 2 wk, (b) Portneuf soil at 18 wk, (c) Millville soil at 2 wk, and (d) Millville soil at 18 wk.

As incubation time increased, the relationship between added PA in the manure and Olsen P became insignificant. As time progressed in the incubation study, the relationship between the amounts of C added with the manure became significantly related to Olsen P on the Portneuf soil. At 18 wk, the manure C/P ratio

was linearly related to Olsen P on the Portneuf soil ($r^2 = 0.91$; Fig. 8a), while there was a poor correlation on the Millville soil ($r^2 = 0.17$). However, a similar trend was seen with the manure N/P ratio on the Millville soil

Table 4. Regression parameters for the relationship between increase in Olsen P (y , mg kg^{-1}) and manure application rate (x , mg P kg^{-1}), after 2 wk of incubation on the Portneuf and Millville soils.

Treatment	Regression Coefficients			
	Portneuf		Millville	
	Slope	r^2	Slope	r^2
C1	0.18 b‡	0.99***	0.11 bc	0.88***
C2	0.07 d	0.94***	0.01 d	NS†
C3	0.10 d	0.99***	0.05 cd	0.95***
C4	0.21 b	0.96***	0.13 b	0.91***
B1	0.10 cd	0.77***	0.10 bc	0.88***
B2	0.14 c	0.99***	0.13 b	0.99***
PA	0.00 e	NS	0.00 d	NS
MAP	0.33 a	0.99***	0.26 a	0.99***

*** Signifies significance at the 0.001 probability level.

† NS is not significant.

‡ Values within columns followed by the same letter are not significantly different at $P > 0.05$ level.

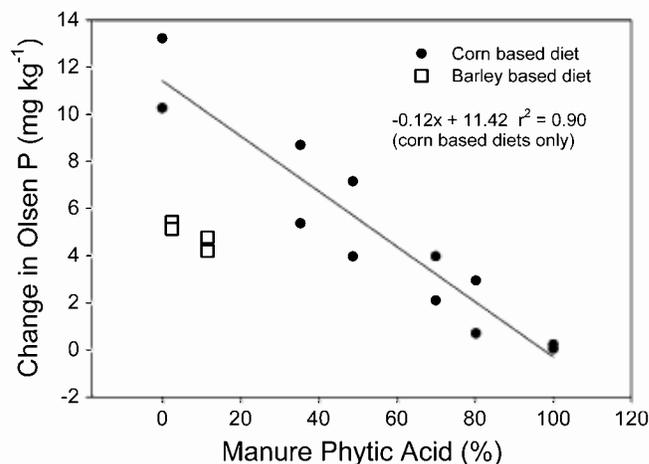


Fig. 7. The relationship between manure phytic acid and change in Olsen P for all treatments on both the Portneuf and Millville soils. Regression equation is for manures from the corn-based diets only.

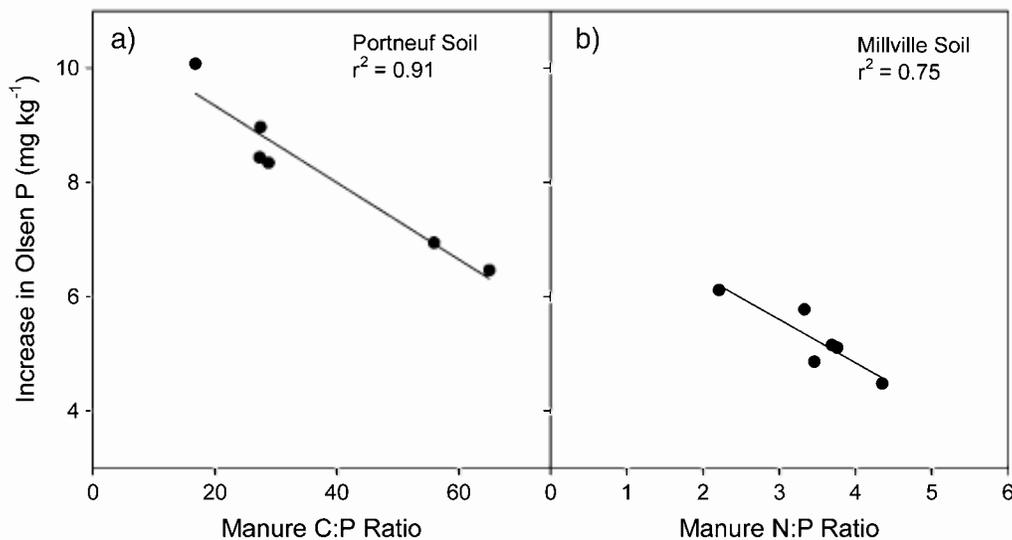


Fig. 8. The relationship between increases in Olsen P resulting from manure application of 40 mg P kg^{-1} following 18 wk incubation and (a) manure C/P ratio on the Portneuf soil and (b) manure N/P ratio on the Millville soil.

and at 18 wk, manure N/P was linearly related to Olsen P ($r^2 = 0.75$; Fig. 8b).

DISCUSSION

Manure total and WEP concentrations were similar to those reported in other studies evaluating diet modification in poultry (Maguire et al., 2003; Penn et al., 2004; Smith et al., 2004; Vadas et al., 2004). The greatest total P concentration was found in C1, which is a standard industry diet for laying hens. This diet had the greatest NPP content and therefore the birds excreted the greatest amount of total P. Birds fed this diet were receiving approximately $298 \text{ mg of NPP bird}^{-1} \text{ d}^{-1}$ which is considerably higher than the reported requirement of $159 \text{ mg NPP hen}^{-1} \text{ d}^{-1}$ (Boling et al., 2000). The broilers fed the barley-based diet excreted on average only 53% of the total P that was excreted by the average corn fed laying hen. The barley based diets had no inorganic P supplementation and it has been demonstrated by Yingran et al. (2005) that the intrinsic phytase content of barley ($582\text{--}1016 \text{ units kg}^{-1}$) is greater than that of corn ($15\text{--}70 \text{ units kg}^{-1}$). The combination of these two factors may explain the lower total P content of the manures from the barley based dietary treatments, along with the differences in bird type and management systems.

The proportion of WEP in the manures was directly related to the proportion of PA, the greater the PA content, the lower the WEP fraction. This trend was also observed in broiler litters generated from diet modification where $\text{WEP/TP} = -1.07 \text{ PA/TP} + 0.88$, $r^2 = 0.85$ (Maguire et al., 2004). Therefore, the manures containing the lowest percentage of PA had the greatest proportion of WEP (barley-based diets and phytase amended corn-based diet).

The change in Olsen P over the 18-wk incubation period varied between treatments and was greatest for those treatments having the greatest proportion of PA. Conversely, Olsen organic P decreased over the first

9 wk of incubation for all treatments on both soils. An increase in WEP with a concomitant decrease in water-extractable organic P was also seen in soils treated with turkey manures from modified diets (Maguire et al., 2003), but unfortunately, no P speciation data was obtained for these manures.

A similar increase in water and Olsen extractable P was observed for up to 8 wk for soil fertilized with poultry litter from a commercial diet, whereas significant increases were not observed from the same soil fertilized with litter from poultry fed a phytase modified diet (Smith et al., 2005). Unfortunately, Smith et al. (2005) did not determine the specific P compounds in the poultry litters used in the study. Maguire et al. (2004) also reported an increase in WEP from 5 to 29 d of incubation of broiler litters from modified diets in a Rumford loamy sand, the greatest increases in this case were from the manure treatments having the greatest PA content. The ^{31}P NMR data obtained in the present study suggests that the increase in Olsen P from 0 to 12 wk of incubation could be due to hydrolysis of PA in the manures. There were measurable amounts of PA present in soil extracts of treated soils (corn-based manure treatments only), which disappeared over the 12-wk incubation period resulting in greater orthophosphate concentrations.

In the present study, the effects of treatment on increases in Olsen P with increasing application rate was more pronounced at 2 wk than at 18 wk, presumably because of the release of P over time from those treatments having the greatest PA content. At 2 wk, the greatest response in Olsen P was from the mono-ammonium phosphate treatment on both soils, while the PA treatment had little effect on increases in Olsen P. This increase in Olsen P was highly correlated to the amount of PA present in the treatments. The greater the PA content the less response was generated in Olsen P. This linear relationship was very strong, especially when the two manures from the barley-based diets were dropped from the model.

The manures resulting from barley fed broilers generated relatively lower increases in Olsen P even though they have very low levels of PA. This could be explained by the large differences in the C/P ratio of these manures (average C/P = 60) compared with the manures from the laying hens fed corn (average C/P = 25). There was more than twice the amount of C added with the manure generated from barley fed broilers. Leytem et al. (2005) showed that the amount of C added with manures to calcareous soils had a large influence on extractable P, the greater the manure C/P ratio, the less Olsen P extracted.

Maguire et al. (2004) also demonstrated that manure PA influenced extractable P in manure-treated soils, and that this influence was more pronounced at 5 vs. 29 d of incubation. In their study, after 5 d of incubation, the water-soluble P was greatest for the manures having the lowest PA content on three out of the four soils studied. As with our study, these treatment effects disappeared over time. Maguire et al. (2004) showed that after a 29-d incubation, the effects of treatment on Mehlich-3 (M3) extractable P were minimal, although on one soil they evaluated, the manure having the greatest PA content had the lowest M3 extractable P, even after 29 d of incubation.

As time progressed throughout the present incubation study, manure properties other than P composition began to have a greater influence on P solubility. On the Portneuf soil, the manure C/P ratio became increasingly linearly related to Olsen P from 2 to 18 wk (Data not shown). Heterotrophic soil microbes are commonly limited by the availability of organic substrate, and can respond rapidly when this becomes available (De Nobili et al., 1996). Microbial processes exert a marked influence on P concentrations in solution, which can alter soluble P levels (Seeling and Zasocki, 1993; Turner and Haygarth, 2001). In many Western irrigated calcareous soils, where soil C levels tend to be low (~1%), C addition has the potential to stimulate microbial activity thereby reducing the soluble P levels via microbial uptake.

In Leytem et al. (2005), application of manures with similar P composition but a wide range of C/P ratios resulted in greater microbial biomass P concentrations. The importance of this was demonstrated by the strong negative correlation between added C and the increase in extractable P for all soils examined, irrespective of soil chemical or physical properties considered likely to regulate P solubility in these soils. This phenomenon is rarely, if ever, considered in studies of manure P dynamics following land application. However, given that microbes in almost all soils are strongly limited by the availability of labile C, it is likely to be highly important wherever manure is applied to soil. For semiarid calcareous soils, which tend to have low OC concentrations, the C/P ratio of added manure can be a key regulator of subsequent soil P solubility.

The same strong trend between manure C/P ratio and Olsen P from 2 to 18 wk was not observed on the Millville soil, although on this soil the manure N/P ratio had a linear relationship to Olsen P at 18 wk. Since the Millville soil had twice the OC content of the Portneuf

soil, it is possible that C was not as limiting in this soil and therefore a microbial response may have been more pronounced with N additions as opposed to C additions. A readily available supply of N from manures has been shown to significantly increase soil microbial biomass following manure incorporation (Gaind et al., 2006). Microbial biomass C, N, and P have also been shown to be positively related to one another (Ghoshal and Sigh, 1995). Therefore, increases in readily available sources of C and N, such as those found in manures, has the potential to stimulate increases in microbial biomass. This increase in microbial biomass increases the incorporation of available soil P into microbial biomass, thereby reducing soil P solubility.

CONCLUSIONS

Modifying poultry diets by the addition of phytase, use of low phytate grains, as well as the use of grains with varying intrinsic phytase levels can alter the amount of PA excreted by poultry. As the amount of PA in the resulting manures is reduced, there is an increase in the WEP fraction of the manures. When these manures are land applied and incorporated into calcareous soils, the PA content of the manures can influence the short term P solubility. Manures having a high PA content may be less soluble initially as PA is strongly bound by the soils. This may be particularly important in calcareous soils, as PA is known to precipitate with Ca minerals at low concentrations. Over time, due to microbial breakdown of PA and other organic forms of P, differences in soil P solubility resulting from application of manures may decrease, thereby ameliorating any influence that diet modification had on manure-treated soils. Dietary treatments that reduce total P in manures will have the greatest benefits in reducing potential environmental impacts associated with land application, as less P will be applied if manures are applied based on N application rates, which is still a common practice.

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