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Phosphorus Fractionation in Manure from Swine Fed Traditional and Low-Phytate Corn Diets

Brian J. Wienhold* and Phillip S. Miller

ABSTRACT

Traditional corn (*Zea mays* L.) (TC), the primary grain used in swine (*Sus scrofa*) diets, stores a majority of its P as phytate, which is largely unavailable for digestion by nonruminant animals. Low-phytate corn (LPC) contains similar amounts of total P but a smaller percentage of P as phytate. When fed to swine, LPC increases P utilization and reduces P content of manure. While differences in P content between manure from animals fed TC and LPC diets have been documented, solubility and lability of manure P have not been compared. Manure P was characterized in manure from swine fed either LPC or TC diets in 2000 and 2001. Total P was lower (20 vs. 34 g kg^{-1}) and N to P ratio was higher (4.5 vs. 3.3) in LPC manure than in TC manure. Manures were sequentially extracted with deionized water, 0.5 *M* $NaHCO_3$, 0.1 *M* NaOH, and 1.0 *M* HCl. Extracts were analyzed for inorganic and total P. Most P (approximately 80%) in the extracts was in the inorganic form. Concentration of P in the water-extractable fraction was lower for LPC manure (10.2 g kg^{-1} in 2000 and 9.7 g kg^{-1} in 2001) than for TC manure (13.6 g kg^{-1} in 2000 and 17.0 g kg^{-1} in 2001). Percentage of total P in each extract was in the order of: H_2O (60%), HCl (22%), $NaHCO_3$ (12%), NaOH (8%), and residue (<1%). Total P and distribution of P in extracts indicates swine are able to utilize more P contained in LPC feed but the composition of P excreted in LPC manure is similar to TC manure. Solubility, crop availability, and lability of P in LPC manure should be similar to that of TC manure.

MANURE can be an excellent fertilizer source containing essential plant nutrients such as N, P, and K. Swine excrete 0.42 Mg of P each year (Sweeten, 1992). However, swine manure has an N to P ratio (e.g., 3:1) lower than that needed by the crop (e.g., corn grain has a N to P ratio of 6:1) resulting in excess P being applied to the soil when manure is applied at rates to meet the N needs of the crop. Excess P in surface soil has the potential for leaching and runoff losses that contribute to eutrophication of surface water (Sharpley et al., 1998). Applying manure at rates to meet the P needs of the crop results in the need for greater land areas, increased manure hauling costs, and the need for additional N application to meet crop needs.

Corn is the primary feed grain used in swine diets. Traditional corn (TC) stores P in an organic molecule called phytate or phytic acid (*myo*-inositol 1,2,3,4,5,6-hexa-*kis*phosphate). Phosphate is hydrolized from the inositol of the phytate molecule by the enzyme phytase. Nonruminant animals such as swine and poultry lack the ability to produce phytase and phytate P use in these animals is low (Wodzinski and Ulla, 1996). Low utilization of phytate P requires that inorganic P be added to feed to ensure that P needs of the animal are met. Supplemental P represents an additional cost in animal feeding operations and inefficient use of phytate P results in high P content of manure.

One strategy for reducing the P content of manure is to increase the bioavailability of P contained in the grain thereby reducing the need for P supplementation. Low-phytate corn (LPC) contains a mutant gene that results in grain containing similar amounts of total P as TC but a larger proportion of that P as phosphate rather than phytate (Ertl et al., 1998). Utilization of P in LPC is greater than in TC for nonruminant animals such as swine (Spencer et al., 2000; Sands et al., 2001) and poultry (Ertl et al., 1998). Increased utilization of feed P reduces the need for supplemental P and results in less P being excreted by the animal (Spencer et al., 2000; Sands et al., 2001). Ertl et al. (1998) reported higher bird weights and lower feed to gain ratios in broiler chicks and Sands et al. (2001) reported higher average daily gains for swine fed LPC diets when compared with TC diets.

Phosphorus-containing compounds in manures vary greatly in solubility, availability for plant uptake, and absorption potential in soils. Several fractionation schemes have been developed for analyzing the P composition of manure. Peperzak et al. (1959) used a fractionation scheme developed by McAuliffe and Peech (1949) to analyze the P forms present in a number of manure types. Barnett (1994b) modified the procedure developed by McAuliffe and Peech (1949) to separate manure P into inorganic, acid soluble, and residual forms. The modified procedure was used to analyze the P composition in several types of animal manure (Barnett, 1994a). More recently a number of studies have modified the soil P fractionation procedure developed by Hedley et al. (1982) to analyze P fractions in manure. Dou et al. (2000) determined the effect of shaking time and repeated extractions on manure P release from dairy manure and proposed a sequential extraction procedure that could be used to quickly fractionate P in manure. In their procedure, H_2O- and $NaHCO_3$–extractable P were designated as readily soluble, HCl-extractable P was designated as somewhat soluble, residues were designated as stable, and the availability of NaOH-extractable P was not designated (Dou et al., 2000). Sharpley

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Abbreviations: LPC, low-phytate corn; TC, traditional corn.

and Moyer (2000) used a similar sequential extraction procedure to fractionate manure P. They then determined correlations between P contained in the various fractions and P released from manure during simulated rainfall events. In their study, H_2O- and $NaHCO_3$–extractable P were highly correlated with P released during simulated runoff. These studies have demonstrated that sequential fractionation of manure P has utility for assessing the potential for environmental contamination and availability of nutrients for crop utilization.

While LPC has the potential to reduce the P content of manure, the composition of the excreted P has not been analyzed in detail. The objective of the present study was to use a sequential fractionation procedure to compare P composition of manure from swine fed a LPC diet to that from swine fed a TC diet. Knowledge of the P composition of manure from swine fed LPC diets will contribute to understanding environmental impacts associated with land application and agronomic utilization of nutrients contained in this type of manure.

MATERIALS AND METHODS

Corn exhibiting the low-phytate trait (var. X1127PP; Pioneer Hi-Bred International, Des Moines, IA) and the same variety without the low-phytate trait (var. Alicia; Pioneer) were grown under irrigation near Shelton, NE in 1998. Recommended practices for irrigation, fertilizer application, and pest control were used to optimize yield. The stands were harvested and stored separately until used as feed. Grain N and C concentration was determined using a Model NA 1500 NCS analyzer (Carlo Erba Instruments, Milan, Italy) (Schepers et al., 1989). Grain total P concentration was determined using an H_2SO_4 and H_2O_2 digest (Thomas et al., 1967) and phytate P concentration was determined using the titration method of Wheeler and Ferrel (1971).

The two corn sources were used to prepare feed appropriate for a starter-phase swine diet in the spring of 1999, 2000, and 2001. Each diet was comprised of 74% corn, 22% soybean meal, and 4% salt, vitamins, and supplements. These diets provided 3250 kcal kg^{-1} of metabolizable energy and 180 g kg^{-1} of crude protein and would meet animal needs at a consumption level of 0.95 kg $animal^{-1}$ d^{-1}. The starter-phase diet using TC was supplemented with 14 g kg^{-1} calcium phosphate ($CaHPO_4{\cdot}2H_2O$) and the diet using LPC was supplemented with 7 g kg^{-1} calcium phosphate resulting in P concentrations in the diets of 6.3 g kg^{-1} in the TC diet and 4.9 g kg^{-1} in the LPC diet. Swine fed these two diets exhibited similar average daily gain and daily feed intake (Paschold, 2001).

Each year the two diets were fed to a mixed-sex group of starter-phase swine at a commercial hog farm. The same breeding herd was used throughout the study. After weaning, the piglets were moved to a feeding barn and placed in elevated pens. After the piglets had acclimated to the elevated pens (one week), each of the two diets were fed to all piglets in six randomly assigned elevated pens (10 pigs per pen). Trays were positioned under each pen, avoiding feeder and watering points, so that only manure and urine were collected. Slurry collection was initiated at least 3 d after the diets were presented to the pigs. The piglets remained in the elevated pens until they reached approximately 25 kg and were then transferred to hoop barns for finishing. Five samples from swine fed each feed were collected over a two-week feeding period in 2000 and 2001. Slurry samples were air-dried and stored for P characterization. In addition, five slurry samples from swine fed each of the diets were sent to a commercial laboratory for gravimetric determination of dry matter of subsamples dried at 105°C (USEPA, 1983), colorimetric determination of total P in nitric acid–perchloric acid digests (Johnson and Ulrich, 1959), determination of total N using the Dumas method (Tate, 1994), and colorimetric determination of inorganic N (USEPA, 1983).

Manure P was characterized using the sequential extraction procedure described by Dou et al. (2000). Thirty milliliters of extractant was added to 0.3 g of air-dry manure, placed on a shaker at 150 strokes min^{-1} for 1 h, centrifuged at 3000 rpm for 10 min, and vacuum-filtered through nitrocellulose filter paper. Material retained on the filter was rinsed back into the centrifuge tube and the procedure was repeated two additional times. The three filtrates were combined and inorganic P concentration determined spectrophotometrically at 882 nm using the phosphomolybdate blue method (Murphy and Riley, 1962). Material retained on the filter was returned to the centrifuge tube and extracted with the next extractant. Extractants were used in the order: deionized water, 0.5 *M* $NaHCO_3$, 0.1 *M* NaOH, 1.0 *M* HCl. Material remaining on the filter after the final extraction and an aliquot of each extract was sent to a commercial laboratory and analyzed for total P (Johnson and Ulrich, 1959).

Total and inorganic P in each extract is reported as concentration on a dry-matter basis. Differences in P concentrations among the extracts for each manure type in both years were determined using PROC GLM. Means were separated using the DIFF statement with differences considered significant at $P < 0.05$ (SAS Institute, 1996).

RESULTS AND DISCUSSION

Grain C and total P concentrations did not differ between the two corn types (Table 1). Total N concentration was slightly lower in the LPC hybrid than in the TC hybrid. Phytate P comprised 83% of total P in the TC hybrid and 24% in the LPC hybrid. The percentage of total P comprised as phytate P in TC is similar to the 84.6% reported by Ravindran et al. (1994). The 60% reduction in phytate P with no difference in total P between the two hybrids is similar to that reported by Ertl et al. (1998). The decrease in phytate P in LPC hybrids has been shown to improve utilization of P in monogastric animals such as poultry (Ertl et al., 1998) and swine (Spencer et al., 2000; Sands et al., 2001). The increase in utilization decreases the proportion of P in the feed that is excreted (Spencer et al., 2000; Sands et al., 2001).

Dry-matter content was greater in manure from swine fed the LPC diet (7.2 ± 0.6%) than from swine fed the TC diet (3.6 ± 0.9%). Reasons for differences in manure

Table 1. Chemical characteristics of traditional corn (TC) and low-phytate corn (LPC) used to make swine diets on a dry-matter basis.†

Nutrient	TC	LPC
	g kg^{-1}	
C	455 ± 7a	449 ± 6a
N	17.1 ± 0.4b	13.6 ± 0.4a
Total P	2.89 ± 0.10a	3.11 ± 0.40a
Phytate P	2.40 ± 0.09b	0.75 ± 0.10a

† Means within a row followed by different letters are significantly different at $P < 0.05$.

dry-matter content are not known as there were no apparent differences in average daily gain or daily feed intake for swine fed these two diets (Paschold, 2001). When expressed on a dry-matter basis, total N and organic N concentrations in manure from swine fed the LPC diet were similar to that of manure from swine fed the TC diet (Table 2). Inorganic N and total P concentrations were greater in manure from swine fed the TC diet than from swine fed the LPC diet. Higher inorganic N concentrations in manure from swine fed the TC diet are probably due to slightly higher N contents in those diets. The lower total P concentrations in manure from swine fed the LPC diet probably result from the lower P content in the LPC ration and greater P uptake by swine consuming LPC rations (Spencer et al., 2000; Sands et al., 2001). Phosphorus concentration in the LPC diet was 78% that of the TC diet but the P concentration of LPC manure was only 60% that of TC manure. Total P concentrations in both manure types are within the range of values previously reported for swine manure (Barnett, 1994a; Sharpley and Moyer, 2000).

Manure from swine fed the LPC diet had a higher N to P ratio than did manure from swine fed the TC diet (Table 2). When used as a crop nutrient source, manure with a higher N to P ratio will increase soil P levels at a slower rate when applied at rates to meet the N needs of the crop and will require less additional inorganic fertilizer N when applied at rates to meet the P needs of the crop. When applied at a rate to meet the N need of the crop, the higher N to P ratio in the LPC manure would result in 20 to 30% less P being applied. The reduction in P applied that would result using LPC manure from this study is similar to that reported by Ertl et al. (1998).

Sequential extraction was used to determine if there were differences in P forms between manure from swine fed the two diets. Recovery of P (sum of P in extracts as a percentage of total P in original sample) for the sequential extraction procedure ranged from 98 to 109%. The recovery of P in this study was similar to that reported by Dou et al. (2000). The coefficient of variation in the sum of P in the extracts in this study (8.7%) was higher than the 1.3% reported by Dou et al. (2000) and similar to the 8% reported by Barnett (1994b). The higher coefficient of variation in this study compared with that reported by Dou et al. (2000) is probably due to their use of replicate analyses from a single composite manure sample compared with our use of multiple manure samples.

Table 2. Chemical characteristics of manure (dry-matter basis) from swine fed either a traditional corn (TC) diet or a low-phytate corn (LPC) diet.†

	Diet	
Nutrient	**TC**	**LPC**
N to P ratio‡	**3.3 ± 0.5a**	**4.5 ± 0.4b**
	g kg^{-1}	
Total N	**108.8 ± 13.0a**	**90.1 ± 11.6a**
Organic N	**46.2 ± 10.9a**	**51.1 ± 8.8a**
Inorganic N	**62.6 ± 4.1b**	**39.0 ± 3.6a**
Total P	**33.6 ± 1.1b**	**19.8 ± 1.2a**

† Means within a row followed by different letters are significantly different at $P < 0.05$, except where indicated.

‡ Means within the row followed by different letters are significantly different at $P < 0.10$.

For both manure types in both years, water-soluble total and inorganic P concentrations were higher than P concentrations in other extracts (Fig. 1). Water-soluble total and inorganic P concentrations were greater in TC manure than in LPC manure in both years. The higher concentration of P in TC manure reflects the higher P content in TC feed. Most of the P extracted with water was in inorganic forms (approximately 80%). Others have also reported that water-soluble P comprises the largest fraction of manure P and that most of the extracted P is in inorganic forms (Dou et al., 2000; Sharpley and Moyer, 2000; He and Honeycutt, 2001). A reduction in water-extractable P observed for LPC manure has environmental implications as water-extractable P has been reported to be highly correlated with total and inorganic P leached from manure and thereby susceptible to runoff losses (Sharpley and Moyer, 2000).

Total and inorganic P concentrations in remaining extracts for each manure were in the order HCl > $NaHCO_3$ = NaOH with very little P remaining in the residue (Fig. 1). Inorganic and total P concentrations in

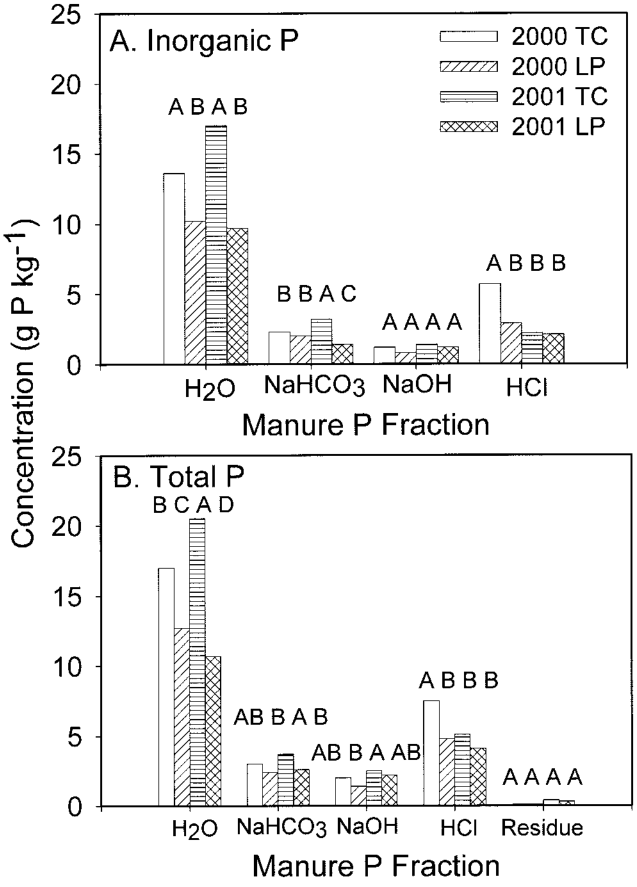

Fig. 1. Concentration of (*A*) inorganic and (*B*) total P expressed on a dry-matter basis in sequential extracts of manure produced by swine fed traditional corn (TC) or low-phytate corn (LPC) diets. Bars within a group with different letters above are significantly different at $P < 0.05$.

$NaHCO_3$ extracts were similar in LPC and TC manure in 2000 and greater in TC manure than in LPC manure in 2001 (Fig. 1). Sharpley and Moyer (2000) found that organic and inorganic P in $NaHCO_3$ extracts were correlated with P leached from manure but the correlation was weaker than that for water-extractable P. Inorganic and total P concentrations in NaOH extracts were similar between the two manure types in both years (Fig. 1). Inorganic and total P concentration in HCl extracts was greater in TC manure than in LPC manure in 2000 but similar between the two manure types in 2001. Concentrations of P in NaOH and HCl represent more recalcitrant forms of P. Most of the P contained in the HCl, $NaHCO_3$, and NaOH extracts (60–80%) was in the inorganic form. Total P in residue was similar among manure types in both years (Fig. 1). While there is much work to be done to identify specific P compounds present in manure, fractionation is an inexpensive way to rapidly estimate P solubilities and labilities (Sharpley and Moyer, 2000).

When expressed as a percentage of total P, the amount of P contained in water extracts in 2001 was greater for TC manure than for TC manure in 2000 or LPC manure in both years (Table 3). The amount of P contained in the $NaHCO_3$ and NaOH extracts was greater in 2001 than in 2000 for both manure types. The amount of P contained in HCl extracts from TC manure was greater in 2000 and less in 2001 than from LPC manure (Table 3). The distribution of P in various extracts in this study was similar to the distribution of P in poultry manure reported by Dou et al. (2000) and in swine manure reported by He and Honeycutt (2001) but differs from that reported for swine manure (23% in water, 15% in $NaHCO_3$, 51% in NaOH, 10% in HCl, and 1% in residue) by Sharpley and Moyer (2000). Differences between the present study and that of Sharpley and Moyer (2000) are probably due to procedure differences (manure extracted three times with each extractant in this study versus once in theirs) and composition differences in the swine diets (starter-phase diet in this study versus finishing-phase diet in their study). Dou et al. (2000) demonstrated that substantial amounts of P are extracted during the second and third extractions with water or $NaHCO_3$ thus explaining the higher amounts of P in those extracts in this study.

Table 3. Percentage of total phosphorus contained in each extract for sequential extraction of manure dry matter produced by swine fed traditional corn (TC) and low-phytate corn (LPC) diets.†

	Diet			
	TC		LPC	
Extract	**2000**	**2001**	**2000**	**2001**
	% of total P			
H_2O	58 ± 1a	66 ± 1b	59 ± 1a	55 ± 2a
$NaHCO_3$	10 ± 0.6a	12 ± 0.6b	11 ± 0.6a	14 ± 0.7b
NaOH	7 ± 0.4a	8 ± 0.4b	7 ± 0.4a	11 ± 0.4c
HCl	26 ± 0.9c	16 ± 1.0a	23 ± 0.9b	21 ± 1.0b
Residue	0.3 ± 0.2a	1.1 ± 0.2b	0.6 ± 0.2a	1.5 ± 0.2b

† Means within a row followed by different letters are significantly different at $P < 0.05$.

Given the high variability often observed in manure nutrient content, the reasonable agreement across studies in P distribution among extracts is evidence that while the P content relative to the N content of manure from swine fed a LPC diet will be lower, the solubility and lability of that P is similar to that of manure from swine fed a TC diet. When used as a crop nutrient source, availability of P in manure from swine fed LPC diets should be similar to that from manure from swine fed a TC diet. Nutrient management recommendations developed for manure from swine fed TC diets should be applicable to manure from swine fed LPC diets.

CONCLUSIONS

Manure produced by swine fed LPC diets during the starter phase had lower P concentrations and higher N to P ratios than manure from swine fed TC diets. The lower concentration of P in LPC manure will reduce P loading rates when this type of manure is land-applied at rates to meet the N needs of a crop. The higher N to P ratio of LPC manure (4.5:1) is more similar to that needed by a corn crop (6:1) than is the N to P ratio of TC manure (3.3:1), making LPC manure a more balanced fertilizer source. In addition, the concentration of water-soluble P was lower in LPC manure than in TC manure. When expressed as a percentage of total P, differences in the distribution of P among the fractions were small and the solubility and lability of P is similar for the two manure types.

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