Phosphorus Composition of Manure from Swine Fed Low-Phytate Grains: Evidence for Hydrolysis in the Animal

April B. Leytem,* Benjamin L. Turner, and P. A. Thacker

Abstract

Including low-phytic-acid grains in swine diets can reduce P concentrations in manure, but the influence on manure P composition is relatively unknown. To address this we analyzed manure from swine fed one of four barley (*Hordeum vulgare* L.) varieties. The barley types consisted of wild-type barley (CDC bold, normal barley diet) and three low-phytic-acid mutant barleys that contained similar amounts of total P but less phytic acid. The phytic acid concentrations in the mutant barleys were reduced by 32% (M422), 59% (M635), and 97% (M955) compared with that in the wild-type barley, respectively. Phosphorus concentrations were approximately one-third less in manures from animals fed low-phytic-acid barleys compared with those fed the wild-type variety. Phytic acid constituted up to 55% of the P in feed, but only trace concentrations were detected in NaOH–EDTA extracts of all manures by solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy. Phosphate was the major P fraction in the manures (86–94% extracted P), with small concentrations of pyrophosphate and simple phosphate monoesters also present. The latter originated mainly from the hydrolysis of phospholipids during extraction and analysis. These results suggest that phytic acid is hydrolyzed in swine, possibly in the hind gut by intestinal microflora before being excreted in feces, even though the animals have little phytase activity in the gut and derive little nutritional benefit from phytate P. We conclude that feeding low-phytic-acid grains reduces total manure P concentrations and the manure P is no more soluble than P generated from normal barley diets.

IN MOST CEREAL GRAINS, P is present primarily as phytic acid (*myo*-inositol hexakisphosphate). Swine are inefficient in utilizing phytic acid P because they do not possess the digestive enzyme phytase that is required to hydrolyze the phytic acid molecule (Pointillart et al., 1984). Mineral phosphate supplements are commonly added to swine diets to prevent P deficiency, although this can lead to high P concentrations in manure; the P accumulates in soil and can contribute to the pollution of water bodies (Sims et al., 2000). To address this, there is considerable current interest in dietary manipulations that decrease P concentrations in manures. Corn (*Zea mays* L.) and barley low-phytic-acid mutants have been isolated (Raboy et al., 2000; Dorsch et al., 2003), which contain the same total P concentration as the wild-type equivalent, but substantially less phytic acid. Feeding these grains to swine improves P utilization and decreases

the P concentration in manure (e.g., Spencer et al., 2000; Veum et al., 2002; Thacker et al., 2003).

In addition to reducing the concentrations of P in manure, dietary modification is expected to influence manure P composition, which may have implications for the environmental fate of manure P (Turner et al., 2002). Compared with phosphate, which is relatively soluble, phytic acid reacts strongly in soils (Anderson et al., 1974; Leytem et al., 2002) to form insoluble complexes that are unlikely to be lost in runoff. Attempts to modify the P composition in manure through dietary manipulation could therefore increase the risk of P transfer in runoff to water bodies. However, little information exists, in part due to a lack of suitable analytical procedures for characterizing the P composition of animal manures. Our aim was to quantify changes in the P composition of manures from swine fed a variety of low-phytic-acid barley diets.

Materials and Methods

Manure was obtained from a feeding trial designed to investigate the digestibility of low-phytic-acid barley fed to finishing pigs (Thacker et al., 2003). Sixteen crossbred barrows weighing an average of 51 ± 5 kg were used in a randomized block design experiment. The pigs were randomly assigned to dietary treatments, which consisted of 99.5% barley and 0.5% Cr_2O_3 (as a marker). Pigs were individually fed for a 7-d acclimation period, followed by 3 d of fecal collection. The barley types consisted of a wild-type barley (CDC bold, normal barley diet) and three low-phytic-acid mutant barleys having phytic acid reductions of 32% (M422), 59% (M635), and 97% (M955). Chemical analysis of the barley feed can be found in Table 1, and further details of the barleys can be found in Dorsch et al. (2003).

Manure was collected from individual animals in a clean room and immediately frozen for storage. Before analysis the manures were dried in a forced air oven dryer at 66°C for 60 h and ground (0.5-mm screen). Manure samples were analyzed for total P by microwave-assisted digestion in concentrated HNO_3 and 30% H_2O_2, with detection by inductively coupled plasma–optical emission spectrometry (ICP–OES).

The P composition of the manures was determined by solution ^{31}P NMR spectroscopy as described by Turner (2004). Briefly, P was extracted in triplicate by shaking 2.00 ± 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 × 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 (Bruker Bio-

A.B. Leytem, USDA-ARS, Northwest Irrigation and Soils Research Laboratory, 3793 N 3600 E, Kimberly, ID 83341. B.L. Turner, Soil and Water Science Department, University of Florida, 106 Newell Hall, P.O. Box 110510, Gainesville, FL 32611. P.A. Thacker, Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK, Canada S7N 5A8. Received 28 Apr. 2004. *Corresponding author (leytem@nwisrl.ars.usda.gov).

Abbreviations: NMR, nuclear magnetic resonance.

Spin, Rheinstetten, Germany). We used 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. 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 parts per million (ppm) relative to 85% H_3PO_4 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 was shown to be able to detect concentrations of P compounds of approximately 0.1 mg P kg^{-1} of dry manure (Turner, 2004).

Results and Discussion

Total manure P concentrations ranged between 8.8 and 13.8 g P kg^{-1} (Table 2). For mutant barley diets, total manure P concentrations were approximately 36% less than for the normal barley diet. Thacker et al. (2003) determined that this reduction in P excretion was a result of greater digestibility of P in the mutant barleys, which was linearly related to phytic acid concentration in the feed.

Extraction with NaOH–EDTA recovered >91% of the total P from all manures. Solution ^{31}P NMR spectra of extracts are shown in Fig. 1. Most of the extracted P was phosphate (86–94%), giving a strong signal at approximately 6.1 ppm (Fig. 1). Signals between 3.4 and 6.0 ppm were assigned to phosphate monoesters, which constituted between 5 and 12% of the extracted P. Phytic acid, which typically has signals at approximately 5.95, 5.06, 4.70, and 4.56 ppm in the ratio 1:2:2:1, was either undetectable or in such small quantities as to prevent accurate quantification. Signals at 4.89 and 5.23 ppm were assigned to β-glycerophosphate and phosphatidic acid, respectively. These compounds are breakdown products of phosphatidyl choline in alkaline solution (Turner et al., 2003). Other signals in the phosphate monoester region (e.g., 4.82, 4.50, 4.41, 4.36 ppm) probably represented mononucleotides originating from the hydrolysis of RNA in alkaline solution. There was also a small proportion of pyrophosphate in all manures (1–2% extracted P), which gave a signal close to −4.4 ppm.

The decreases in total manure P found in this study are similar to those reported in other feeding trials with low-phytic-acid grains. Veum et al. (2002) reported a 40%

Table 1. Chemical analysis of barley samples selected for reduced phytate content (on as-fed basis).†

Barley type	Barley feed properties				
	Moisture	Crude protein	Total P	Phytate P	Digestible energy
	%	%	%	%	kcal kg^{-1}
CDC bold	11.91	9.45	0.40	0.22	3113
M 422	12.11	11.22	0.30	0.15	3026
M 635	12.17	10.87	0.35	0.09	3075
M 955	11.89	12.18	0.40	0.01	3077

† Data taken from Thacker et al. (2003).

decrease in total P excreted from swine fed M422 barley compared with a wild-type, while Spencer et al. (2000) measured a 25% decrease when swine were fed low-phytic-acid corn compared with normal corn. No supplemental P was added in either trial. As the source of grain was the only difference between diets, the decreases in total manure P was attributed to the higher available P and lower phytic acid concentration in the low-phytic-acid grains compared with normal grains.

Since swine cannot digest phytic acid in the gut, it is commonly assumed that swine manure contains undigested phytic acid (Poulsen, 2000; Golovan et al., 2001; Gollany et al., 2003). We therefore expected to find large concentrations of phytic acid in the manure generated from the wild-type (CDC bold) barley diet, and lower phytic acid concentrations in the manures generated from the mutant barley diets. However, our results clearly demonstrate that little phytic acid is excreted in the feces. Phytic acid is extremely stable in the alkaline conditions of the analytical procedure used here (Turner et al., 2003), so a methodological artifact is ruled out. It is therefore likely that degradation of phytic acid is facilitated by microfloral phytase activity in the hind gut (Seynaeve et al., 1999; Skoglund et al., 1997; Jongbloed et al., 1992). Absorption of P in swine occurs in the upper parts of the small intestine and therefore the phosphate generated from hydrolysis of phytic acid in the hind gut is not utilized by the animals and is passed through to the manure (Crenshaw, 2001).

Early work by Rather (1918) concluded that some phytic acid may be absorbed and utilized by swine. Rather (1918) also determined that the majority of phytic acid in feeds can be reduced to inorganic P by catabolic and other changes and eliminated in the feces and urine, as these processes occur in the hind gut where the

Table 2. Phosphorus concentrations in swine manure from animals fed one of four barley varieties containing different concentrations of phytic acid. Phosphorus concentrations were determined by NaOH–EDTA extraction and solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy.

Barley type	Total manure P	NaOH–EDTA-extractable P				
		Total P†	Phosphate‡	Phosphate monoesters‡	Pyrophosphate‡	Phytic acid‡
		g P kg^{-1} dry wt.				
CDC bold	13.82 ± 0.83	13.46 ± 0.83 (97)	13.02 ± 0.81 (94)	0.67 ± 0.16 (5)	0.13 ± 0.03 (1)	trace
M 422	9.11 ± 1.25	8.55 ± 0.80 (95)	7.77 ± 1.11 (86)	1.08 ± 0.33 (12)	0.16 ± 0.05 (2)	trace
M 635	8.82 ± 1.80	8.05 ± 1.60 (91)	7.59 ± 1.41 (86)	1.08 ± 0.37 (12)	0.16 ± 0.09 (2)	trace
M 955	8.81 ± 2.18	8.36 ± 1.94 (95)	7.78 ± 2.13 (88)	0.91 ± 0.21 (11)	0.11 ± 0.04 (1)	ND§

† Values are means ± standard deviations of four animals per feeding treatment, and values in parentheses are the proportion (%) of the total manure P determined by microwave digestion.
‡ Values in parentheses are the proportion (%) of the NaOH–EDTA-extracted P.
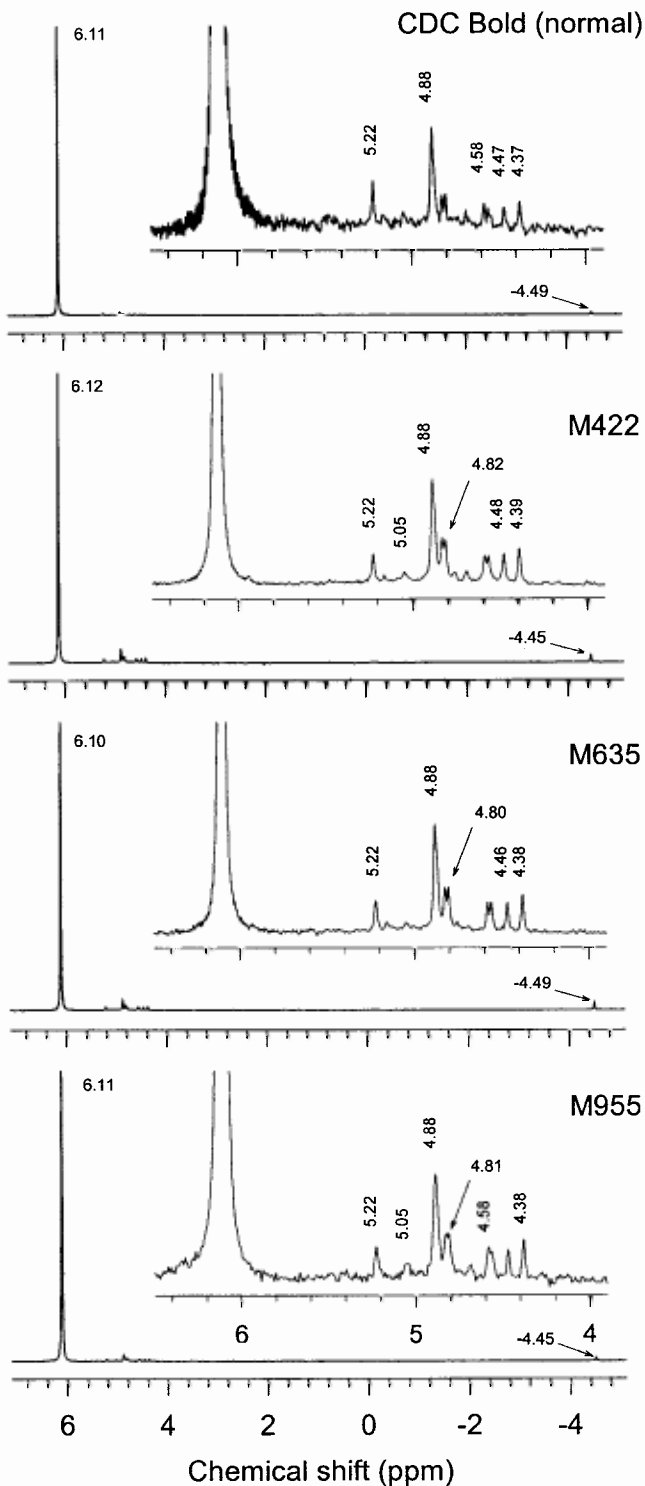§ Not detected.

Fig. 1. Solution ^{31}P nuclear magnetic resonance (NMR) spectra of NaOH–EDTA extracts of manures from swine fed low-phytate barleys.

As dietary manipulation does not influence the P composition of swine manure, the benefits of a reduction in total P as a result of feeding low-phytate grains are unlikely to be compromised by greater P solubility in manure. Manures from swine fed both normal and low-phytate grains are composed primarily of inorganic P, but there is less P excreted from animals fed a diet containing low-phytate grains and therefore less risk of P losses from these manures. Further, it is clearly inappropriate to assume that a reduction in total manure P from swine fed low-phytic-acid grains is due to a reduction in organic P excretion.

Acknowledgment

References

Anderson, G., E.G. Williams, and J.O. Moir. 1974. A comparison of the sorption of inorganic orthophosphate and inositol hexaphosphate by six acid soils. J. Soil Sci. 25:51–62.

Crenshaw, T.D. 2001. Calcium, phosphorus, vitamin D, and vitamin K in swine nutrition. p. 49–66. In A.J. Lewis and L.L. Southern (ed.) Swine nutrition. CRC Press, Boca Raton, FL.

Dorsch, J.A., A. Cook, K.A. Young, J.M. Anderson, A.T. Bauman, C.J. Volkmann, P.P.N. Murthy, and V. Raboy. 2003. Seed phosphorus and inositol phosphate phenotype of barley low phytic acid genotypes. Phytochemistry 62:691–706.

Gollany, H.T., M.A. Schmitt, P.R. Bloom, G.W. Randall, and P.R. Carter. 2003. Extractable phosphorus following soil amendment with manure from swine fed low-phytate corn. Soil Sci. 168:606–616.

Golovan, S.P., R.G. Meidinger, A. Ajakaiye, M. Cottrill, M.Z. Wiederkehr, D.J. Barney, C. Plante, J.W. Pollard, M.Z. Fan, M.A. Hayes, J. Laursen, J.P. Hjorth, R.R. Hacker, J.P. Phillips, and C.W. Forsberg. 2001. Pigs expressing salivary phytase produce low-phosphorus manure. Nat. Biotechnol. 19:741–745.

Jongbloed, A.W., Z. Mroz, and P.A. Kemme. 1992. The effect of supplementary Aspergillus niger phytase in diets for pigs on concentration and apparent digestibility of dry matter, total phosphorus, and phytic acid in different sections of the alimentary tract. J. Anim. Sci. 70:1159–1168.

Leytem, A.B., R.L. Mikkelsen, and J.W. Gilliam. 2002. Sorption of organic phosphorus compounds in Atlantic Coastal Plain soils. Soil Sci. 167:652–658.

Pointillart, A., N. Fontaine, and M. Thomasset. 1984. Phytate phosphorus utilization and intestinal phosphatases in pigs fed low phosphorus wheat or corn diets. Nutr. Rep. Int. 29:473–483.

Poulsen, H.D. 2000. Phosphorus utilization and excretion in pig production. J. Environ. Qual. 29:24–27.

Raboy, V., P.F. Gerbasi, K.A. Young, S.D. Stoneberg, S.G. Pickett, A.T. Bauman, P.P.N. Murthy, W.F. Sheridan, and D.S. Ertl. 2000. Origin and seed phenotype of maize low phytic acid 1-1 and low phytic acid 2-1. Plant Physiol. 124:355–368.

Rather, J.B. 1918. The utilization of phytin phosphorus by the pig. Bull. 147. Univ. of Arkansas Agric. Exp. Stn., Fayetteville.

Seynaeve, M., G. Janssens, M. Hesta, C. van Nevel, and R.O. de Wilde. 1999. Effects of dietary Ca/P ratio, P level and microbial phytase supplementation on nutrient digestibilities in growing pigs: Breakdown of phytic acid, partition of P and phytase activity along the intestinal tract. J. Anim. Physiol. Anim. Nutr. 83:193–204.

Sims, J.T., A.C. Edwards, O.F. Schoumans, and R.R. Simard. 2000. Integrating soil phosphorus testing into environmentally based agricultural management practices. J. Environ. Qual. 29:60–71.

Skoglund, E., T. Larsen, and A.S. Sandberg. 1997. Comparison between steeping and pelleting a mixed diet at different calcium levels on phytate degradation in pigs. Can. J. Anim. Sci. 77:471–477.

Spencer, J.D., G.L. Allee, and T.E. Sauber. 2000. Phosphorus bioavailability and digestibility of normal and genetically modified low-phytate corn for pigs. J. Anim. Sci. 78:675–681.

Thacker, P.A., B.G. Rossnagel, and V. Raboy. 2003. Phosphorus di-

P is not utilized by the animals. The use of phytase in swine diets to increase the utilization of P is still a valid method of increasing P digestibility in swine (Thacker et al., 2004), as the phytase hydrolyzes phytic acid in the portion of the digestive tract where absorption of P can take place and benefit the animal.

gestibility in low phytate barley fed to finishing pigs. Can. J. Anim. Sci. 83:101–104.

Thacker, P.A., B.G. Rossnagel, and V. Raboy. 2004. Effect of phytase supplementation on phosphorus digestibility in low-phytate barley fed to finishing pigs. Arch. Anim. Nutr. 58:61–68.

Turner, B.L. 2004. Optimizing phosphorus characterization in animal manures by phosphorus-31 nuclear magnetic resonance spectroscopy. J. Environ. Qual. 33:757–766.

Turner, B.L., N. Mahieu, and L.M. Condron. 2003. Phosphorus-31 nuclear magnetic resonance spectral assignments of phosphorus compounds in soil NaOH–EDTA extracts. Soil Sci. Soc. Am. J. 67:497–510.

Turner, B.L., M. Papházy, P.M. Haygarth, and I.D. McKelvie. 2002. Inositol phosphates in the environment. Philos. Trans. R. Soc. London Ser. B 357:449–469.

Veum, T.L., D.R. Ledoux, D.W. Bollinger, V. Raboy, and A. Cook. 2002. Low-phytic acid barley improves calcium and phosphorus utilization and growth performance in growing pigs. J. Anim. Sci. 80:2663–2670.