

Enzymatic Characterization of Organic Phosphorus in Animal Manure

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ABSTRACT

Information on the forms of P present in animal manure may improve our ability to manage manure P. In most investigations of manure P composition, only inorganic and total P are determined, and the difference between them is assigned as organic P. In this study, we explored the possibility of identifying and quantifying more specific organic P forms in animal manure with orthophosphate-releasing enzymes. Pig (*Sus scrofa*) manure and cattle (*Bos taurus*) manure were first sequentially fractionated into water-soluble P, NaHCO₃-soluble P, NaOH-soluble P, HCl-soluble P, and residual P. The fractions were separately incubated with wheat phytase, alkaline phosphatase, nuclease P1, nucleotide pyrophosphatase, or their combinations. The released orthophosphate was determined by a molybdate blue method. Part of the organic P in those fractions could be identified by the enzymatic treatments as phytate (i.e., 39% for pig manure and 17% for cattle manure in water-soluble organic P), simple phosphomonoesters (i.e., 43% for pig manure and 15% for cattle manure in NaOH-soluble organic P), nucleotide-like phosphodiester (2–12%), and nucleotide pyrophosphate (0–4%). Our data indicate that the enzymatic treatment is an effective approach to identify and quantify the organic P forms present in animal manures.

PHOSPHORUS from animal manure is sometimes associated with significant degradation of surface water quality. Management practices are needed to optimize recycling of manure P while minimizing adverse environmental consequences of manure application to cropland. The chemical composition of phosphorus in animal manure is one of the factors to determine the mechanisms of its transport and potential bioavailability. Therefore, identification and quantification of the various P forms in animal manure may significantly contribute to effective manure P management. At present, however, little research has been reported that addresses P forms in animal manure. Studies on this topic have often adapted methods used for soil analysis (Barnett, 1994a,b; Dao, 1999; Leinweber et al., 1997; Peperzak et al., 1959). Sequential fractionation procedures have been used to characterize P forms in soil and manure (Barnett, 1994a,b; Bowman and Cole, 1978; Hedley et al., 1982; Leinweber et al., 1997; Sharpley and Moyer, 2000; Sui et al., 1999). Classes of P compounds are functionally defined by the extractants removing them from soil or manure, such as resin P or H₂O-P, NaHCO₃-P, NaOH-P, and H₂SO₄-P or HCl-P. Organic P is estimated by the difference of total P and inorganic P. The sequential fractionation procedures are based on the assumption that chemical extractants selectively dissolve discrete groups of P compounds; therefore, such operationally defined P fractions are subject to broad interpretation (Sui et al., 1999).

Phosphorus-31 nuclear magnetic resonance (NMR)

offers another way to identify and quantify the relative amount of organic P. The procedure was first applied to 0.5 M NaOH soil extracts in 1980 (Newman and Tate, 1980). This method has identified structural features of alkali-soluble P mainly as orthophosphate, monoester P, diester P, and pyrophosphate (Condron et al., 1985; Hawkes et al., 1984; Leinweber et al., 1997; Newman and Tate, 1980; Rubæk et al., 1999). Identification of more specific P forms by ³¹P NMR, such as glucose-6-phosphate and teichoic acid in NaOH extract, has been reported recently (Guggenberger et al., 1996; Pant et al., 1999). To our knowledge, no study has been reported that correlates the P data obtained from ³¹P NMR to that taken from sequential fractionation.

In nature, phosphatases catalyze chemical reactions that release orthophosphate from various types of organic phosphorus compounds. Those enzymes provide a possibility to enzymatically identify and quantify organic P forms in manure. That is, if a manure sample is incubated with a specific phosphatase, the resulting inorganic phosphate concentration will represent the corresponding type of organic P and its amount in the sample. Phosphatases (acid and alkaline) and phytase have been used to release organic phosphorus compounds in soil solutions (Hayes et al., 2000; Pant et al., 1994; Shand and Smith, 1997). Bishop et al. (1994) reported enzymatic mineralization of organic phosphorus in a volcanic soil in Chile in which soil samples were incubated with four phosphate-releasing enzymes, and then ³¹P NMR analysis of both untreated and treated 1 M NaOH soil extracts was used to show the change in soil organic phosphorus. To our knowledge, no such biological or enzymatic approaches have been used in the characterization of organic P in animal manure.

The objective of this study was to evaluate an enzymatic approach for quantifying manure-derived organic P compounds. As a first step in exploring this novel enzymatic approach, we examined the release of orthophosphate from pig and cattle manure by several commercially available phosphatases.

MATERIALS AND METHODS

Manures

Two fresh animal manures with straw litter, a pig manure and a dairy cattle slurry manure, were collected from local farms. The manures were freeze-dried and ground. Those manure particles passing through a 0.991-mm sieve were stored at -20°C and used for the experiments reported here.

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Abbreviations: NMR, nuclear magnetic resonance; P_{-a} and P_{+a}, orthophosphate determined after samples were incubated in the absence and presence of alkaline phosphatase; P_{-w} and P_{+w}, orthophosphate determined after samples were incubated in the absence and presence of wheat phytase.

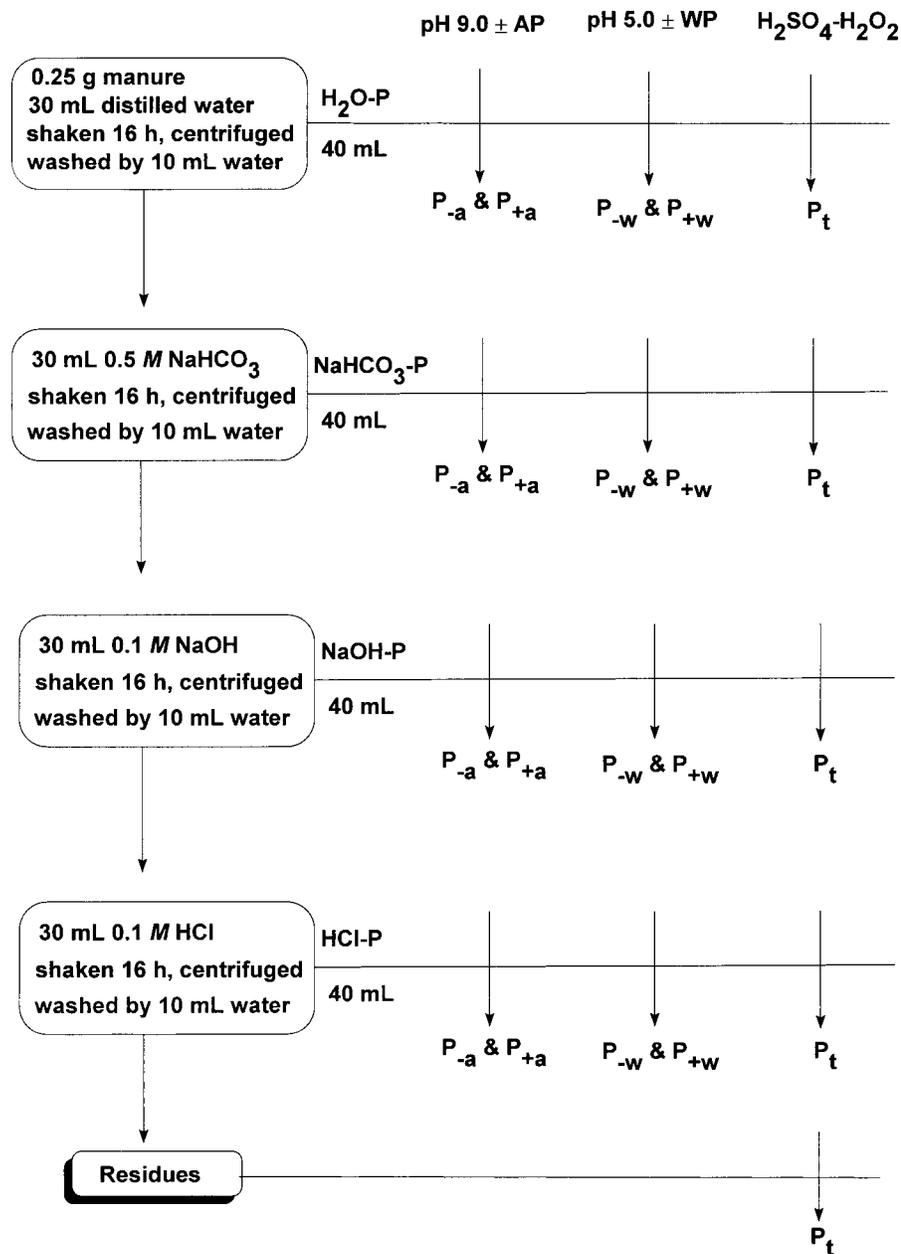


Fig. 1. Sequential P fraction and methods of releasing orthophosphate. P_{-a} and P_{+a}, orthophosphate determined after samples were incubated in the absence and presence of alkaline phosphatase (AP); P_t, total phosphorus; P_{-w} and P_{+w}, orthophosphate determined after samples were incubated in the absence and presence of wheat phytase (WP).

Sequential Fractionation of Manure Phosphorus

A modification of the method presented by Sui et al. (1999) was used in this study (Fig. 1). Duplicate samples were fractionated. Each manure sample (0.25 g) was placed in a centrifuge tube with 30 mL of distilled water. The tubes were set horizontally on the platform of an orbital shaker (250 rpm) for 16 h at room temperature (22°C). The samples were then centrifuged at 2800 × g for 30 min at 4°C, and the supernatant was decanted and saved. The residues were then suspended in 10 mL of water. The suspension was shaken for 1 h (250 rpm on the orbital shaker) and the residues were spun down in the same way as above. The two supernatants were pooled and used for the P assay. The same procedure was repeated for fractionation by 0.5 M NaHCO₃, 0.1 M NaOH, and 0.1 M

HCl (Fig. 1). Those fractions were neutralized to pH 7.0 by appropriate amounts of 2 M HCl or NaOH.

Enzymes and their Activities

All enzymes used were purchased from Sigma¹ (St. Louis, MO) (Table 1). The purchased crude wheat phytase contained phosphate. Consequently, it was necessary to dialyze by taking 0.4 g of the enzyme in 2.5 mL of 10 mM potassium acetate buffer (pH 5.0) in a Slide-A-Lyzer dialysis cassette (Pierce,

¹ Trade or manufacturer's names mentioned in the paper are for information only and do not constitute endorsement, recommendation, or exclusion by the USDA Agricultural Research Service.

Table 1. Enzymes used in the study.

	Enzyme					
	Acid phosphatase	Alkaline phosphatase	Fungal phytase	Wheat phytase	Nucleotide pyrophosphatase	Nuclease P1
Source	Type 1, wheat germ	Type VII-S, bovine intestinal mucosa	<i>Aspergillus ficuum</i>	wheat	Type II, <i>Crotalus adamanteus</i> venom	<i>Penicillium citrinum</i>
Enzyme Commission number	3.1.3.2	3.1.3.1	3.1.3.8	3.1.3.26	3.6.1.9	3.1.30.1
Function	Release of orthophosphate (P_i) from R- PO_4 (monoester)	Release of P_i from R- PO_4 (monoester)	Release of P_i from phytate	Release of P_i from phytate	Cleavage of a dinucleotide bond	Endonucleolytic cleavage of RNA and DNA
Optimal conditions:†						
pH	4.8	9.8	2.5	5.15	7.4	5.3
Temp., °C	37	37	37	55	37	37
Actual assay conditions:						
pH	5.0	5.0	2.5	5.0	5.0	5.0
Temp., °C	22	22	22	22 or 55‡	37–55§	37–55

† Data from the supplier.

‡ 22°C used in substrate specificity experiment, 55°C used in other experiments.

§ 37°C for 15 min, and then 55°C for 15 min in the presence or absence of wheat phytase. Refer to Materials and Methods for detail.

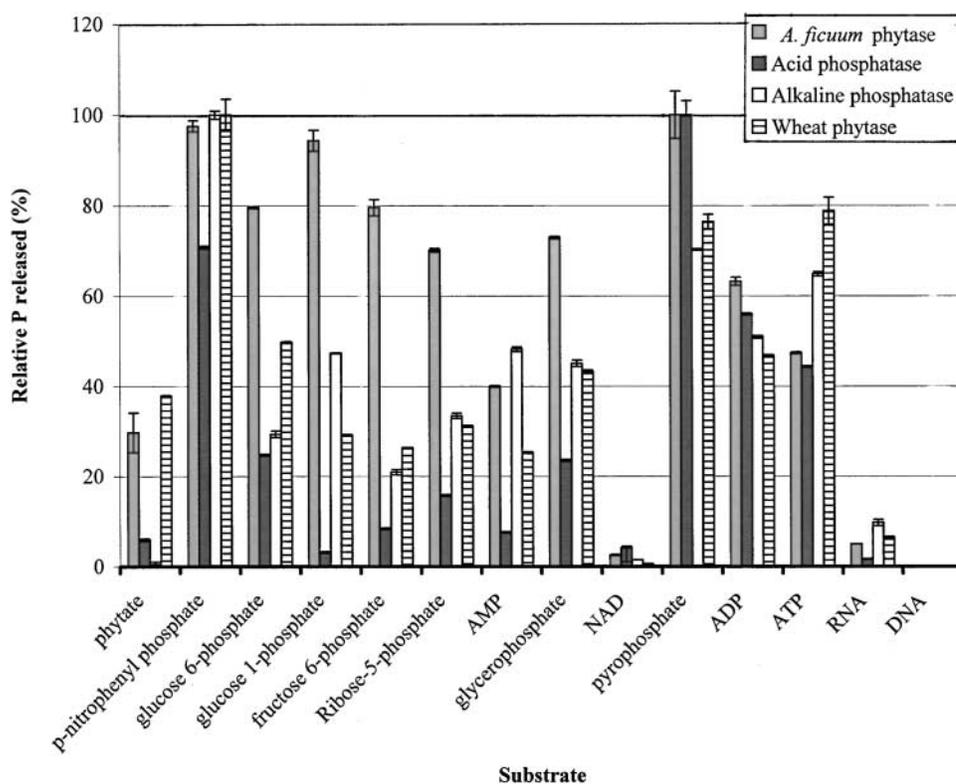


Fig. 2. Substrate of orthophosphate-releasing enzymes.

Rockford, IL) for 8 h (600-mL buffer by three times) to remove orthophosphate. The enzyme solution was then centrifuged for 5 min by a microfuge at $15\ 800 \times g$. Other enzymes were used without pretreatments. One unit (U) of enzyme activity was defined as liberation of 1.0 μ mole of orthophosphate (or appropriate products in case of non-phosphate-releasing enzymes) from appropriate substrates.

Enzymatic release of orthophosphate (P_i) from organic P compounds was carried out under or near the optimal conditions for the enzyme as stated by the supplier. During the substrate specificity experiment for the orthophosphate-releasing enzymes, all incubations were carried out at room temperature (22°C) in order to keep the results more comparable (Fig. 2). All substrates but RNA and DNA contained 10 mM total P.

The concentrations of RNA from baker's yeast and DNA from salmon testes were 3 mg substrate per 1 mL reaction mixture. All enzymes were used in 1 unit per mL of reaction mixture. Reaction mixtures were incubated for 30 min at room temperature prior to orthophosphate determination. The 100% of relative activity was 7.35 mM P released during the incubation for *Aspergillus ficuum* phytase in 100 mM glycine-HCl buffer (pH 2.5), 4.65 mM P released for wheat germ acid phosphatase in 100 mM acetate buffer (pH 5.0), 2.22 mM P released for Bovine intestinal mucosa Type VII-S alkaline phosphatase in 100 mM Tris-HCl buffer (pH 9.0; Sigma, St. Louis, MO) with 1 mM $MgCl_2$ and 0.1 mM $ZnCl_2$, and 4.63 mM P released for wheat phytase in 100 mM acetate buffer (pH 5.0). The assay solutions of DNA were cloudy, but no blue color developed.

Nucleotide pyrophosphatase and nuclease hydrolyze relevant di- or polynucleotides to orthophosphate-containing monomers. The orthophosphate in these monomers was then released by wheat phytase. In 0.5 mL of 100 mM acetate buffer (pH 5.0), 0.5 mM nucleotide pyrophosphate (NAD) or 0.5 mg mL⁻¹ DNA were first incubated for 15 min at 37°C in the presence of 0.15 unit of nucleotide pyrophosphatase plus 1 mM MgCl₂, or in the presence of 1.7 units of nuclease P1, or in the absence of both. Half of the reaction mixtures (0.25 mL) were mixed with 0.013 units of wheat phytase. Then, all reaction mixtures were incubated for another 30 min at 55°C.

Release of Organic Phosphorus from Manure Extraction Fractions

A portion of each fraction (250 µL in a total 500-µL reaction mixture) was incubated for 30 min in the presence or absence of wheat phytase (0.1 U mL⁻¹) at 55°C or alkaline phosphatase (1 U mL⁻¹) at room temperature. The reaction mixture for the phytase reaction was 100 mM potassium acetate (pH 5.0). The reaction mixture for the alkaline phosphatase reaction was 100 mM Tris-HCl buffer (pH 9.0) with 1 mM MgCl₂ and 0.1 mM ZnCl₂. Total P was released by H₂SO₄-H₂O₂ digestion

(Thomas et al., 1967). Orthophosphate in these mixtures was then assayed by a molybdate blue method modified by the inclusion of citrate-arsenate reagent to minimize the interference of labile organic and inorganic phosphorus (Dick and Tabatabai, 1977).

Designation of Phosphorus Forms

Simple monoester P in a given sample was calculated from the difference in orthophosphate contents determined after incubation in the presence and absence of alkaline phosphatase (P_{+a} - P_{-a} in Fig. 3). Phytate-like P was calculated from the difference in orthophosphate released with phytase and alkaline phosphatase (P_{+w} - P_{+a} in Fig. 3). DNA-like P was the difference of orthophosphate content in a given sample preincubated with or without nuclease P1 (P_{nu} - P_{+w} in Fig. 4). Organic pyrophosphates were calculated from the difference in orthophosphate content in a given sample preincubated with or without nucleotide pyrophosphatase (P_{py} - P_{+w} in Fig. 4). Total organic P was determined by the difference in P from an H₂SO₄-H₂O₂ digested sample and inorganic P (undigested sample). Inorganic P was calculated as the average P contents measured at pH 5.0 and at pH 9.0 without enzymatic incubation (P_{-a} and P_{-w}).

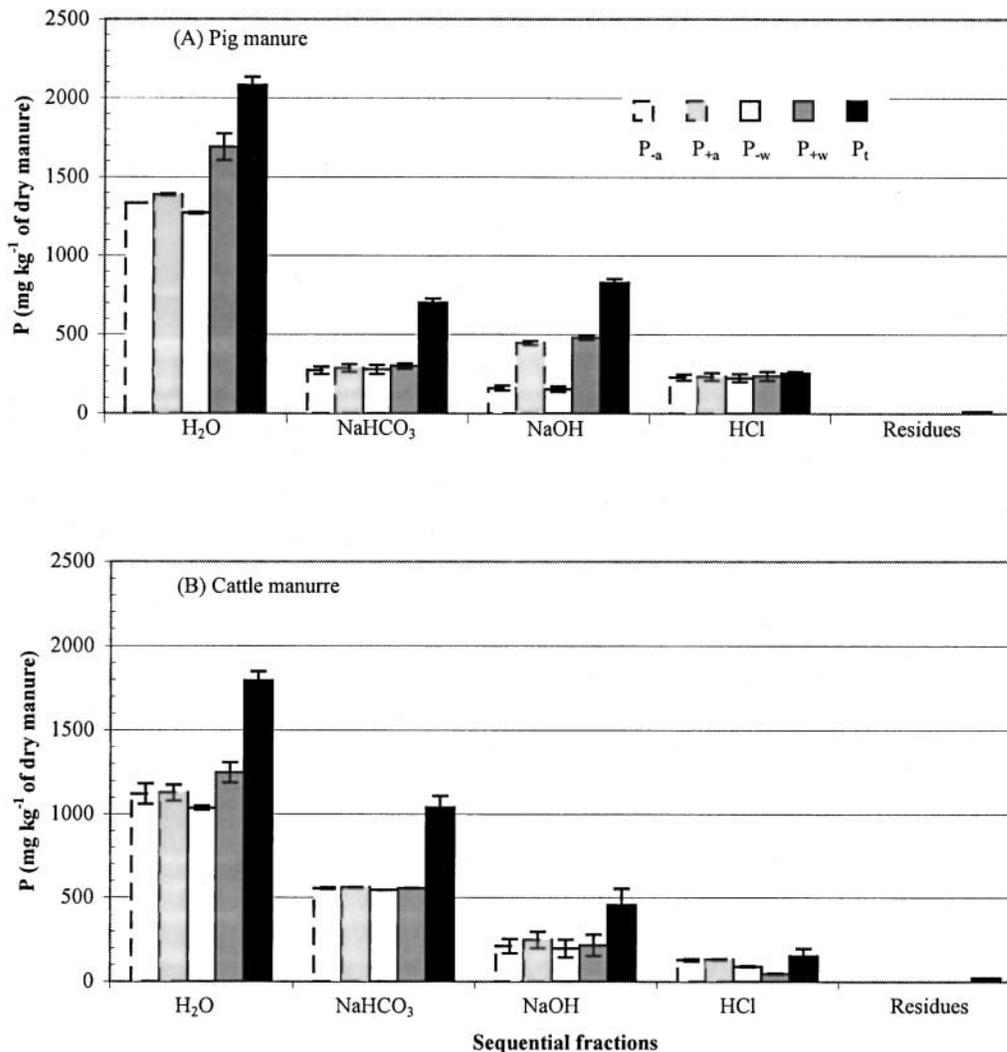


Fig. 3. Orthophosphate content of sequentially extracted P fractions in (A) pig and (B) cattle manure. P_{-a} and P_{+a}, orthophosphate determined after samples were incubated in the absence and presence of alkaline phosphatase; P_t, total phosphorus; P_{-w} and P_{+w}, orthophosphate determined after samples were incubated in the absence and presence of wheat phytase.

RESULTS AND DISCUSSION

Substrate Specificity of Phosphate-Releasing Enzymes

Acid phosphatase, alkaline phosphatase, fungal phytase, and wheat phytase were tested for their substrate specificity on 14 phosphorus compounds (Fig. 2). All four enzymes showed rather high activities on various phosphomonoesters, but little or no activity on NAD and phosphodiester (RNA and DNA). This observation may reflect the intrinsic properties of these enzymes, but may also be due to the possibility that more than one phosphate-releasing enzyme is present in these enzyme preparations. One useful observation from Fig. 2 is that the relative activities of alkaline phosphatase and wheat phytase on the tested compounds were similar, with a difference being seen in phytate, from which alkaline phosphatase was unable to release orthophosphate. To verify this observation, the incubation time and enzyme concentration were increased. Phosphorus released by wheat phytase (1.5 units per mL) from phytate (10 mM P) was 3.28 mM at 15 min and 8.94 mM at 105 min. On

the other hand, P released by alkaline phosphatase (2 units per mL) from phytate was still not significant with 0.078 mM at 15 min and 0.095 mM at 105 min. These results suggest that P released by alkaline phosphatase may reflect the content of most simple phosphomonoesters, and the difference between P released by alkaline phosphatase and wheat phytase may reflect the content of phytate.

Phosphorus Distribution in Fractions of Sequential Treatments

Two types of animal manure, one from monogastric pigs and one from polygastric cattle, were used in this study. The specific activity of crude wheat phytase from the supplier was very low (0.03 unit per mg solid), compared with 2745 units per mg of protein for the alkaline phosphatase reagent. In other words, the purchased phytase preparation contained many impurities. In addition, precipitates were observed during orthophosphate determination by the modified molybdate-blue method when the enzyme was used at a concentration of 1 unit

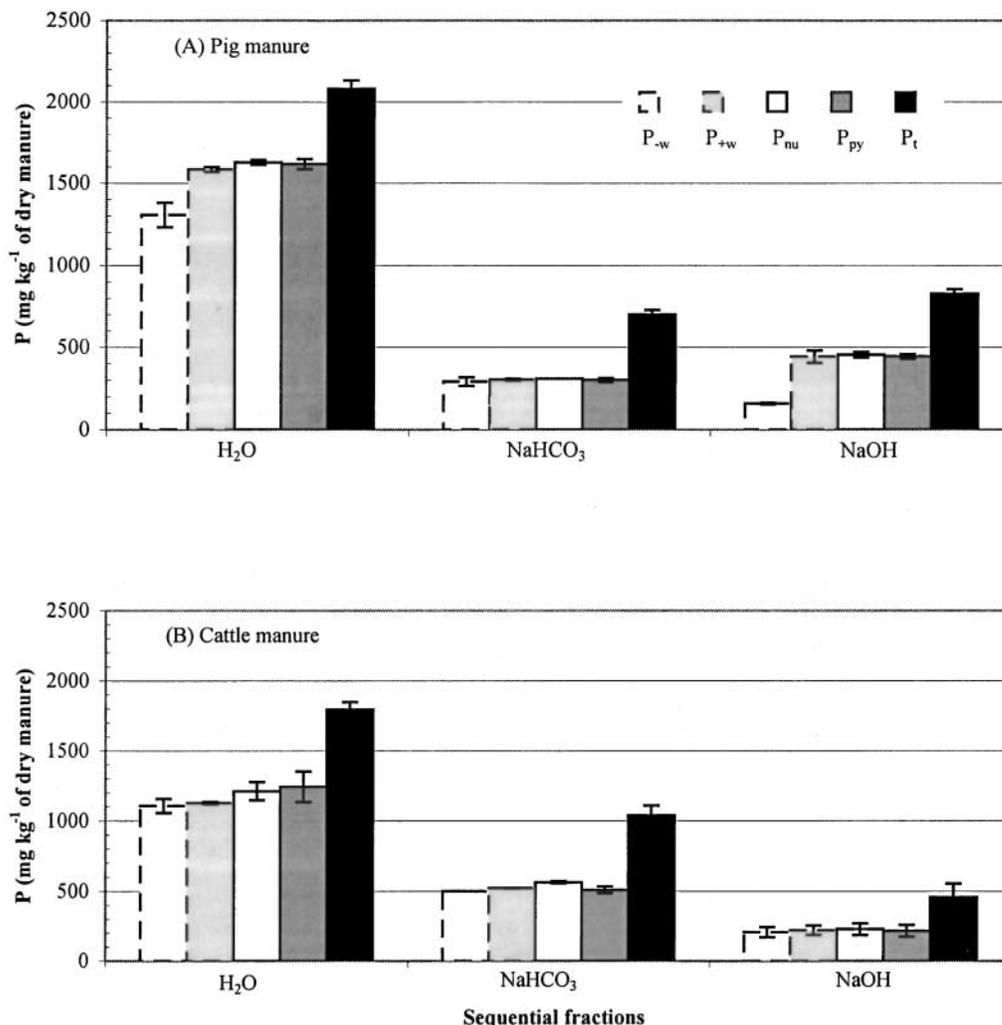


Fig. 4. Orthophosphate released by nuclease P1 and nucleotide pyrophosphatase in pig (A) and cattle (B) manure. P_{nu} and P_{py}, orthophosphate determined in the presence of nuclease P1 or nucleotide pyrophosphatase and wheat phytase; P_t, total phosphorus; P_{-w} and P_{+w}, orthophosphate determined after samples were incubated in the absence and presence of wheat phytase.

per mL in the P-releasing incubation. To reduce interference, the concentration of wheat phytase was changed to 0.1 unit per mL of reaction mixture, and the incubation temperature was increased from room temperature to 55°C (at which the activity was increased by about five- or sixfold). The activity of wheat phytase under such conditions was still far more than the amount required to release relevant organic P from the samples.

Total P and inorganic P were 3875 ± 9 and 1959 ± 60 mg per kg of dry pig manure and 3450 ± 187 and 1946 ± 78 mg per mg of dry cattle manure, respectively (Fig. 3). The data indicated that about 49% of P in pig manure was in organic forms, and about 44% of P in the cattle manure was present as organic P.

Organic phosphorus mainly distributed in H₂O, NaHCO₃, and NaOH fractions. Treatment of these fractions with the two enzymes did provide information about P forms found in the sequential fractions, in addition to the conventionally determined values for inorganic P and total P (Fig. 3).

In H₂O fractions of both manures, P_{-a} was about 5 to 8% greater than P_{-w} (Fig. 3). This might be caused by measurement interference or by decomposition of some organic P at pH 9.0. In the pig manure fraction, 4% more orthophosphate was observed after treatment with alkaline phosphatase. Treatment with wheat phytase released 33% more P than observed for untreated controls in pig manure, and 20% more in cattle manure. The substrate specificity in Fig. 2 suggested that the inability of alkaline phosphatase acting on phytate is the cause of the difference. Pant et al. (1994) reported that phytase released nearly twice as much P as acid and alkaline phosphatases did in water extracts of soil. The authors suggested that a significant part of the inositol-type material was unavailable, either "hidden" or protected from the two more general phosphatases. In fact, we believe that characterizing the difference as phytate seems more convenient and easier to understand.

The P forms in NaHCO₃ fractions seem to be similar in both manure samples (Fig. 3). In the NaHCO₃ fraction, enzymatic treatments increased orthophosphate by only 6.3 and 1.9% for pig and cattle manure, respectively, still leaving 96 and 98% of the organic P forms unknown. Hayes et al. (2000) reported that only a small proportion (1–9%) of NaHCO₃-extracted organic P in three soil samples was hydrolyzable by wheat phytase. Otani and Ae (1999) also reported a negligible amount of organic P in NaHCO₃ extracts from a range of soils. Our data indicated that most NaHCO₃-extractable organic P from animal manure was also unhydrolyzable by phytase and alkaline phosphatase. This supports the claim by Hayes et al. (2000) that the common supposition that considers NaHCO₃-extracted organic P to be labile may not be true.

Unlike the H₂O fractions, wheat phytase in NaOH fractions did not release more organic P than did alkaline phosphatase. Both enzymes were unable to release 44% of the total P in pig manure, and 49% of the total P in cattle manure. This observation suggests that phytate is not a major component of the NaOH fractions.

It is difficult to compare the results found in this

study with the few data reported in other animal manure organic P investigations due to the different experimental approaches. The most similar investigation is the report by Leinweber et al. (1997). The investigators sequentially fractionated the P in liquid pig manure and chicken (*Gallus gallus*) manure to resin P (11–29%), NaHCO₃-P (10–13%), NaOH-P (3–10%), H₂SO₄-P (17–27%), and residual P (39–41%). Leinweber et al. (1997) considered at least a portion of residual P to represent insoluble mineral phases.

In contrast, concentrations of HCl-P (4–7%) and residual P (less than 1%) were very low in our study. In soils, HCl- or H₂SO₄-P was designated apatite P (Ca-associated), and residual P was considered occluded P (Hedley et al., 1982; Tiessen et al., 1983). There are two possible causes for the low P portion of the two fractions in our study. First, theoretically, most P in animal manure should be present as the products or residues of the biological processes of the animal itself or of microbes in its intestinal system. In other words, mineral apatite P or occluded P should not be major components of animal manure. Second, we discarded the manure materials too large to pass through a 0.991-mm sieve. This practice might have reduced the residual P content because these large feedstuff and litter materials may be expected to contain indigestible P that can only be released through H₂O₂-acid treatment. Recently, Sharpley and Moyer (2000) reported that HCl-P varies from 1 to 33% in six types of animal manure, and residual P was less than 3% in five of six manures. Therefore, it is not uncommon that P forms present in animal manure vary widely depending on factors such as animal species, diet composition, and manure management. It may be speculated that optimal manure management may vary to the same extent as does manure P composition.

Further Exploration of the Unknown Phosphorus Forms

It was already shown in Fig. 2 that both alkaline phosphatase and wheat phytase were not active on NAD that contains nucleotide pyrophosphate bonds, nor on RNA and DNA that contain phosphodiester bonds. Two enzymes, a nucleotide pyrophosphatase that hydrolyzes NAD to nicotinamide mononucleotide (NMN) and adenosine monophosphate (AMP), and a nuclease P1 that cleaves RNA and DNA to produce 5'-phosphomonoesters, were used to test the presence of such P forms in both animal manures. As shown in Table 2, both enzymes acted on their own substrate only. Wheat phytase then released orthophosphate from their cleaved products. These results indicate that the two enzymes can be used to release the relevant P form when present in manure.

The two enzymes were then added to the sequential fractions. The fractions treated with nuclease P1 prior to addition of wheat phytase released slightly more orthophosphate (1.6 to 7.5%) than untreated samples for both manures (Fig. 4). Nucleotide pyrophosphatase did not increase orthophosphate released by wheat phytase except for the H₂O fraction of cattle manure. The two

Table 2. Orthophosphate released from NAD and DNA by different enzymatic actions.

Enzyme	P released	
	NAD	DNA
	μM	
Nucleotide pyrophosphatase		
- wheat phytase	0.3 ± 0.3†	-‡
+ wheat phytase	159 ± 12	-‡
Nuclease P1		
- wheat phytase	0.3 ± 0.3	5.9 ± 0.6
+ wheat phytase	14 ± 1	268 ± 21
Control		
- wheat phytase	-2.0 ± 1.4	-‡
+ wheat phytase	31 ± 5	-‡

† Mean ± standard error.

‡ The P assay solution was cloudy, but no blue color was observed.

enzymes were not able to release most of the unknown organic phosphorus in the three fractions. The differences in P_{+w} between the data in Fig. 3 and 4 were 6, 1, and 8% in the three sequential fractions for pig manure, and 10, 6, and 2.3% for cattle manure. Precipitation caused by high protein concentrations during the P_{+w} assay may have caused the disagreement observed for the two sets of measurements. In each set of data, however, the interference was minimized because the measurements were obtained under the same conditions.

Designation of Organic Phosphorus Forms in Manure Fractions

Although release of organic phosphorus in soil solutions has been investigated previously (Hayes et al., 2000; Pant et al., 1994; Shand and Smith, 1997), identification of specific phosphorus types in those soil solutions was not the purpose of these investigations. These investigators referred to phosphorus released by phytase, acid phosphatase, and alkaline phosphatase, generally as enzyme-labile (hydrolysable) P. We believe that it is reasonable to designate more specific P forms released by these enzymes based on the substrate specificity of the phosphate-releasing enzymes (Fig. 2 and Table 2). The difference in P in the presence and absence of alkaline phosphatase represents the content of simple or general phosphomonoesters (Table 3). The difference in P between phytase and alkaline phosphatase reflects the amount of phytate in a sample. The difference in P for manure incubated with phytase in the presence and absence of nuclease P1 was contributed by nucleotide-like phosphodiester. The action of nucleotide pyrophosphatase revealed the content of organic pyrophosphate. Some negative figures in Table 3 were due to the result of the protein-molybdate blue precipitation during P_{+w} assay. We designated the percentage of the corresponding phosphorus form as zero.

The five designated types of organic P forms were distributed unequally in the sequential extraction fractions (Table 3). Phytate was most prevalent in the H₂O fraction and accounted for 39% of the organic P in pig manure, and 17% of the organic P in cattle manure. This finding is consistent with the lack of phytase enzymes in pig digestion systems (Wodzinski and Ulla, 1996). Simple phosphomonoesters comprised the major or-

Table 3. Designation of organic phosphorus forms in pig and cattle manure released by enzymatic treatment.

P form	Pig manure fraction					Cattle manure fraction					
	H ₂ O	NaHCO ₃	NaOH	H ₂ O	NaHCO ₃	H ₂ O	NaHCO ₃	NaOH	H ₂ O	NaHCO ₃	NaOH
Organic†	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹					
Simple P	779 ± 49‡	426 ± 53	671 ± 41	715 ± 18	488 ± 67	6 ± 6	4.5 ± 1.5	250 ± 51	37 ± 6	32 ± 14	8 ± 8
monoesters†	55 ± 6	14 ± 1.5	286 ± 27	6 ± 6	4.5 ± 1.5	43 ± 4	1.5 ± 0.8	37 ± 6	15 ± 2.4	15 ± 2.4	15 ± 2.4
Phytate-like†	302 ± 50	14 ± 6	34 ± 1	121 ± 12	-4.5 ± 1.5	5.1 ± 0.1	17 ± 1.7	-32 ± 14	0	0	0
DNA-like§	43 ± 29	5 ± 5	13 ± 20	84 ± 54	43 ± 8.8	1.9 ± 3.0	12 ± 7.6	8 ± 8	3.2 ± 3.2	3.2 ± 3.2	3.2 ± 3.2
Organic pyrophosphates§	33 ± 15	-2 ± 3	2 ± 27	116 ± 79	-11 ± 20	0.3 ± 4.0	116 ± 11	-8.3 ± 4.3	0	0	0
Organic P unreleased	377 ± 41	368 ± 33	337 ± 35	395 ± 162	435 ± 35	50 ± 5.3	55 ± 23	210 ± 40	89 ± 7.3	84 ± 16	84 ± 16
	%	%	%	%	%	%	%	%	%	%	%
	100 ± 6.3	100 ± 12	100 ± 6	100 ± 2.5	100 ± 14	0.8 ± 0.8	1 ± 0.3	100 ± 20	1 ± 0.3	1 ± 0.3	1 ± 0.3

† Calculated from results shown in Fig. 3.

‡ Mean ± standard error.

§ Calculated from results shown in Fig. 4.

ganic P form identified in the NaOH fractions. There are some phosphodiester (DNA and RNA) in each fraction (1.1–12% with error range of 1.1–8%), but no fractions contained remarkably high concentrations of this type of organic P. The relatively low concentration of organic pyrophosphate was fractionated into H₂O soluble parts (less than 5%). It is worth pointing out that although the specific amounts of each P form between pig and cattle manure were different, properties of the P in each fraction were similar, thereby indicating validity of the method used.

CONCLUSIONS

The organic P in the sequential fractions of two manure samples, one from monogastric pig and one from polygastric cattle, were distributed in H₂O, NaHCO₃, and NaOH fractions. Enzymatic treatment revealed the different properties of the organic P in these fractions. About half of the organic P in H₂O fractions was enzymatic hydrolysable, mainly in the form of phytate for pig manure, or more complicated forms in cattle manure. Simple monoester P was the major hydrolysable organic P in the NaOH fractions, with 43% of organic P in pig manure, and 15% of organic P in cattle manure. Nearly 90% of organic P in the NaHCO₃ fractions was not hydrolysable with the tested enzymes, which does not support the supposition that NaHCO₃-extracted organic P is labile. This study may provide a baseline for a more detailed enzymatic investigation of organic P in animal manure, and probably in soils as well.

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