Characterizing Fecal and Manure Phosphorus from Pigs Fed Phytase Supplemented Diets

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Abstract

We conducted this study to characterize phosphorus (P) forms in feces and manure from pigs fed phytase supplemented diets and to determine if higher phytase levels can result in greater reduction in manure P without increased P solubility. Twenty-eight growing pigs were fed diets containing varying levels of supplemental P and phytase. Phosphorus concentrations in feces, urine and manure were determined and fecal and manure P were fractionated. Phytase addition reduced P concentration in feces and manure but increased urine P concentration. The greatest significant reduction in fecal and manure P was in pigs fed diet containing 2000 U phytase kg^{-1} without supplemental P, with 33% reduction in manure P. Inorganic P constituted more than 85% of fecal and manure P and the percentage decreased with phytase addition. Our study showed that higher phytase levels up to 2000 U phytase kg^{-1} could offer additional advantage of reducing manure P concentration and solubility.

Keywords: Phosphorus, Manure, Phytase, Phosphorus fractionation, Manure phosphorus, Fecal phosphorus, Pigs

1. Introduction

Phosphorus exists in grains mostly as phytate (*myo*-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate). This form of P is not available to non-ruminant animals such as pigs because they lack sufficient digestive phytase enzyme required to break the molecule and release P. To ensure that animals get adequate supply of P, farmers often supplement pig feed with inorganic P for optimum production. The consequence is that manure produced by these animals is high in P. Under intensive animal production, manure P is generated in excess of crop needs (Kellogg et al., 2000). Continuous application of manure causes a build-up of soil P increasing the potential for P loss and possible water quality degradation. Consequently, concerted efforts have been taken to reduce manure P content as a means of decreasing soil P loading and stem the increasing trend of eutrophication.

One of the ways of reducing the P content of manure is through dietary supplementation of pig feed with exogenous phytase. Phytase addition to pig diet increases the availability of grain P and reduces the amount of inorganic P added to the feed and consequently reduces manure P concentration. Phytase addition to pig diets with concomitant reduction in supplemental inorganic P has been reported to reduce fecal P excretion (Omogbenigun et al., 2003). Li et al. (1998) concluded that the use of phytase in pig diets can reduce the use of inorganic P and decrease potential environmental pollution. While phytase inclusion in diets of animals has been reported to decrease total P excretion (Maguire et al., 2005), uncertainty exists regarding its effect on the soluble P portion of the manure. Phytase in broiler diets has been reported to increase the water soluble P portion in poultry manure (Miles et al., 2003); however, in most pig studies, phytase inclusion either decreased or had no effect on water soluble P (Hill et al., 2003; Smith et al., 2004).

Phytase supplementation of about 500 U phytase kg^{-1} with 0.1% unit reduction in available P has been reported to decrease P excretion (Omogbenigun et al., 2003). Results from the 0.1% unit reduction in available P have not been consistent. Harper et al. (1997) and Oryschak et al. (2002) observed a 27 to 28% reduction in P excretion when phytase was supplemented to the diets of growing-finishing pigs while Lei et al. (1993) reported a larger reduction of 35 to 45% for weanling pigs. However, Angel et al. (2005) found no statistical difference in total P excretion in pigs fed diets that were supplemented with 515 U phytase kg^{-1} accompanied by 0.1% unit reduction in available P. Further reduction of 0.2% units in available P at the same level of phytase inclusion reduced poultry litter P concentration (Angel et al., 2005). Thus, there is a need to investigate the effect of further reduction in dietary available P on the forms of P in the manure.

Performance of growing pigs was improved with 750 U phytase kg^{-1} and was close to that achieved with 2 g kg^{-1} of inorganic P (Li et al. 1998). Supplementation of diet with higher levels of phytase could be a good replacement for inorganic P addition in pig diets. Rosen (2002) reported that microbial phytase at 2,500 U phytase kg^{-1} of low-P diet could triple the improvement in feed efficiency of broiler chicks compared to industry level of 634 U phytase kg^{-1} of diet. Veum et al. (2006) supplemented low P diet fed to growing pigs by up to 12,500 U phytase kg^{-1} and observed improved apparent absorption of P, Ca and Mg. These researchers concluded that the maximum effective concentration of phytase is yet unknown.

The sequential fractionation procedure has been used to assess the potential environmental impact of manure P (Sharpley and Moyer, 2000; Ajiboye et al., 2004). The procedure separates manure P into different forms based on their solubility. Efforts have also been made to further investigate each manure P form in a fractionation scheme through chemical speciation. Toor et al. (2005) observed a strong correlation between H_2O-extractable P and dicalcium P in broiler litter while Turner and Leytem (2004) reported that H_2O and $NaHCO_3$ extracted total inorganic phosphate and a small amount of soluble organic P in pig manure.

Most studies with pigs have revealed decreased P content in the feces through phytase addition without taking into consideration the P content of urine (Angel et al., 2005; Ige et al., 2006). As such, more work is needed on manure (feces + urine) samples in order to gain a better understanding of the contribution of urine P. Although urine P has been reported to represent less than 0.5% of the total P excreted (Baxter et al., 2003), phytase supplementation in pig diets has been shown to increase urinary P (Zhang et al., 2003). Therefore, the objective of this study was to characterize the forms of fecal and manure P from pigs fed phytase supplemented diets with concomitant reduction in diet available P. Also we wanted to determine if higher levels of phytase enzyme will lead to greater reductions in manure P without increased solubility of manure P.

2. Materials and Methods

2.1 Housing of Pigs and Dietary Treatments

A total of 28 growing Cotswold pigs obtained from the Glenlea Swine Research Farm, University of Manitoba (Winnipeg, Manitoba, Canada), and seven dietary treatments were used for this study. Each diet was assigned at

random to four pigs for a period of two weeks. The pigs were housed in stainless steel metabolism crates with smooth transparent walls and tenderfoot flooring. The crates were equipped with wire mesh screens and drain trays for separate collection of feces and urine. The dietary treatments used were: positive control (PC) diet that contained NRC (1998) recommended P; a negative control (NC) containing 38 % less available P; NC plus 500 U phytase kg^{-1} (NC + P1); NC plus 1000 U phytase kg^{-1} (NC + P2); double negative control with no added inorganic P (DNC); DNC plus 2000 U phytase kg^{-1} (DNC + P3) and DNC plus 4000 U phytase kg^{-1} (DNC + P4). Microbial phytase (derived from *Aspergillus niger)* was provided by Canadian Biosystems Inc., Calgary, Alberta, Canada. The P and Ca compositions of the 7 diets are presented in Table 1.

2.2 Collection and Pretreatment of Manure Samples

At the end of 2 weeks, each pig was moved into a metabolic crate for separate and quantitative collection of feces and urine. The pigs were allowed to adjust to the diets in the crates for about 3 d before urine and feces collection commenced. Urine and feces were collected during a 48-h period. The urine was collected through an opening on the drain tray into plastic jars containing 10 mL of 6*N* HCl to reduce NH_3 volatilization. Aliquots were taken from daily volume and kept frozen until they were required for sub-sampling and analysis. The feces were weighed and stored at -20^{o}C until sub-sampled. Manure sample was generated from the feces and urine by mixing one-half of the urine and one-half of the feces together in a blender to obtain a homogenous paste. These mixtures were designated as "manure" samples (feces + urine). This resulted in three types of samples: Original feces, derived manure and original urine. The feces and manure samples were then freeze-dried using a *Modulyod-115* Freeze Dryer (Thermo Electron Corporation, Milford, MA. USA.); ground to pass through a 1mm screen and thoroughly mixed before samples were taken for total P analysis. All analyses were performed in quadruplicates. The feces and manure samples were analyzed for total P according to the method of Akinremi et al. (2003). A 4.4 mL portion of sulfuric acid-hydrogen peroxide digestion mixture was added to 0.4 g of feces / manure or diet in a Kjeldahl digestion tube and the mixture was digested in a digestion block for 3 h at 350^{o}C. Phosphorus in the sample digests was determined by the molybdate blue method (Murphy and Riley, 1962). The urine P content was determined directly using the inductively coupled plasma-optical emission spectroscopy (ICP-OES).

2.3 Fecal and Manure P Characterization

The modified Hedley fractionation procedure (Ajiboye et al. 2004) was used to separate P into H_2O-, $NaHCO_3$-, NaOH-, HCl-extractable P, and residual P. A 0.3-g portion of freeze-dried feces or manure was sequentially extracted with 30 mL of deionized H_2O, 0.5M $NaHCO_3$ (pH 8.5), 0.1M NaOH, and 1M HCl solutions in a 50 mL centrifuge tube. The solutions were shaken for 16 h on an end-to-end shaker at 150 excursions per minute at room temperature, centrifuged at 7,000 x *g* for 15 min and vacuum-filtered through 0.45 μm cellulose membrane filter. The total P in each extract was determined as described by Akinremi et al. (2003) through the addition of 1.1 mL of sulfuric acid-hydrogen peroxide acid digestion mixture to an aliquot of the extract and digesting the mixture in a digestion block at 350^{o}C for 1 h. The residue remaining after the sequential fractionation was also digested using the wet oxidation method of Akinremi et al. (2003) and the pH of all digests was adjusted to ~6.5 before P analysis. The P in all extracts and digested samples was measured by the molybdate blue method (Murphy and Riley, 1962), on an Ultrospec 3100 *pro* UV/Visible Spectrophotometer (Bichrom Ltd Cambridge, England) at a wavelength of 882 nm.

2.4 Statistical Analyses

The experimental design was set up as a completely randomized design with 4 replicates per treatment (PC, NC, NC+P1, NC+P2, DNC, DNC+P3, DNC+P4). The Least Significant Difference (LSD) test was used to compare treatment means. Statistical analysis was carried out using the General Linear Models (GLM) procedure of SAS software for Windows, version 9.1 (SAS Institute, Inc., Cary, NC).

3. Results and Discussion

The mean pH of fresh fecal sample was 6.4 while that of fresh derived manure was 7.9. Upon freeze drying, the pH were 5.9 and 6.2 for the fecal sample and manure, respectively. Smith et al. (2001) reported a pH of 8.0 for swine manure from a commercial farm. As such, the addition of small quantity of HCl to urine did not reduce the pH of manure and had no impact on the forms of P reported for manure in this study.

Analysis of variance showed that dietary treatment did not have significant effect on the quantities of feces (P = 0.22) and urine (P = 0.62) produced by the experimental animals. Hence, fecal and urine P excreted by the animals are presented and discussed in terms of their concentrations rather than the quantity produced. Animal performance data for this study has been reported elsewhere (Emiola et al. 2009).

3.1 Effect of supplemental phytase on fecal and manure phosphorus excretion

Low rates (500 and 1000 U phytase kg^{-1}) of phytase enzyme supplementation reduced P concentration in feces and manure (Table 2). The concentration of P in the feces of pigs fed NC diet supplemented with 500 U phytase kg^{-1} (NC+P1) decreased by 21% relative to the NC diets. Addition of 1000 U phytase kg^{-1} to NC diets (i.e. NC+P2) reduced fecal P concentration significantly ($P \leq 0.05$) by 22% (Table 2). Manure P was reduced by 11 and 15% relative to NC diet with the addition of 500 and 1000 U phytase kg^{-1}, respectively. Our results agreed with those of Harper et al. (1997) who observed a 22% decrease in fecal P excretion of growing pigs fed low-P diets with 500 U phytase kg^{-1} diet. Omogbenigun et al. (2003) reported a non-significant 13% reduction in fecal P when 500 U phytase kg^{-1} supplemented diets were fed to piglets.

Addition of 2000 U phytase kg^{-1} significantly reduced the fecal P concentration (Table 2). The concentration of P in feces decreased from 18.4 g kg^{-1} in animals fed DNC diet to 13.5 g kg^{-1} in animals fed with DNC+P3 diet. Similar significant decrease was observed in manure P concentration following supplementation of DNC diet with 2000 U phytase kg^{-1}. Fecal and manure P concentrations were reduced by 27 and 32%, respectively, with 2000 U phytase kg^{-1} supplementation of DNC diet. This magnitude of manure P reduction has potential environmental benefit in reducing P load to soil when manure is applied to soil.

The concentration of P in feces from animals fed the DNC+P4 (diet supplemented with 4000 U phytase kg^{-1}) was not significantly different from that of animals fed DNC diet (18.4 vs. 18.0 g kg^{-1}; Table 2). Similar observation was made in the manure P. Increasing supplemental phytase from 2000 U phytase kg^{-1} to 4000 U phytase kg^{-1} rather than decrease fecal and manure P actually increased it.

The use of supplemental phytase greater than 500 U phytase kg^{-1} was adopted in our dietary treatments to explore the possibility of eliminating supplemental inorganic P in pig diet through the addition of phytase. The highest significant reduction in manure P concentration relative to the control diets was achieved in DNC diet supplemented with 2000 U phytase kg^{-1}. Increasing the phytase supplement to 4000 U phytase kg^{-1} did not produce further decrease in manure P concentration. A plot of manure P concentration against increasing phytase addition to DNC diet showed that the relationship between the two parameters is quadratic (plot not shown) with the effectiveness of phytase in reducing manure P reaching a peak at 2000 U phytase kg^{-1}. Emiola et al. (2009) reported that the performances of animals fed the DNC diet supplemented with 2000 or 4000 U phytase kg^{-1} were comparable to those of animals fed the PC diet. Compared to the PC diet, supplementing the DNC diet with 2000 U phytase kg^{-1} offered the advantages of improving diet P utilization, reducing P excretion, and reducing manure P load to soil upon land application without negatively impacting animal performance. Li et al. (1998) reported that the improvement in the performance of pigs fed low P diet supplemented with 750 U phytase kg^{-1} diet was equivalent to the effect achieved by adding 2 g kg^{-1} of inorganic P (positive control).

3.2 Sequential fractionation of fecal and manure phosphorus

In this study, about 54 to 75% of fecal and 57 to 71% of manure P were extracted by water (H_2O-P; Figs. 1 and 2). These values were similar to the values reported in an earlier study for hog manure (Ige et al., 2006). While phytase addition significantly reduced the concentration of H_2O-P in feces, it did not significantly influence H_2O-P in manure (Table 3 and 4). It is possible that the increase in urine P concentration (which is part of H_2O-P in manure) with phytase addition negated the reduction of H_2O-P in feces. The rate of phytase addition did not have a significant effect on H_2O-P fraction in feces and manure. The concentration of H_2O-P in the manure from pigs fed DNC+P3 diet was statistically similar to that from DNC+P4 diet (Table 4).

The percent inorganic P extracted by water (H_2O-Pi) was greater than 100% in all treatments suggesting that inorganic P was overestimated resulting in negative values of organic P in the extract. Similar overestimation of inorganic P in H_2O-P has been reported by other authors (Ajiboye et al., 2004, 2007). However, while Ajiboye et al. (2004, 2007) determined organic P as the difference between P measured by the inductively couple plasma atomic emission spectroscopy (ICP-AES) and molybdate blue method, organic P in this study was determined as the difference between molybdate blue measured P in digested and undigested P extract. Acid digestion of extract and the subsequent neutralization of the digest for molybdate blue P determination could also interfere with P measurement in the digested sample especially if the concentration of organic P is very low. Turner and Leytem (2004) reported that inorganic P accounted for more than 96% of H_2O-P from swine manure. Ajiboye et al. (2007) complemented sequential extraction with nuclear magnetic resonance (NMR) spectroscopy to confirm that H_2O-P from sequential extraction of hog manure was mostly in the inorganic form.

Sodium bicarbonate solution extracted between 13 and 18% of fecal P and between 15 and 25% of manure P ($NaHCO_3$-P; Figs. 1 and 2). A 500 and 1000 U phytase kg^{-1} addition to NC diet reduced $NaHCO_3$-P by 24 and 42%, respectively. Addition of 2000 U phytase kg^{-1} to DNC diet reduced $NaHCO_3$-P concentration by 45%

while 4000 U phytase kg^{-1} produced only a 13% reduction. As was observed with H_2O-P, the percent inorganic P in some $NaHCO_3$ extracts of feces was greater than 100% resulting in negative values of the organic P. About 95 to 99% of manure $NaHCO_3$-P was in inorganic P form. Ajiboye et al. (2007) reported that P extracted by $NaHCO_3$ was mainly inorganic P.

The sum of H_2O-P and $NaHCO_3$-P fractions constitutes the labile P fraction that is vulnerable to loss through runoff and leaching (Sharpley and Moyer, 2000). The labile P fraction (H_2O-P + $NaHCO_3$-P) accounted for approximately 71 to 89% of P in feces which was similar to the value of 84% reported by Ige et al. (2006). The percentage of labile P in manure samples ranged between 76 and 89% (Fig. 2). This labile P was mainly inorganic P (Table 3). Except with the NC+P1 treatment where the manure labile P was slightly higher than that of NC, addition of phytase to pigs' diet generally reduced the labile P concentration of feces and manure (Tables 3) probably because of greater absorption of phytate hydrolyzed P. Addition of 500 U phytase kg^{-1} to NC diet resulted in a 19% decrease in fecal labile P while addition of 1000 U phytase kg^{-1} resulted in 30% decrease compared to the NC diet. A 27 and 17% decreases in fecal labile P were also observed with the addition of 2000 and 4000 U phytase kg^{-1} to DNC diet, respectively.

While the relationship between phytase levels in DNC diet and labile P concentration in feces was quadratic (plot not shown) with the lowest level of labile P achieved at 2000 U phytase kg^{-1}, phytase levels in DNC diet was inversely and linearly related to manure labile P concentration. The reason for differences in the response of manure and feces labile P to phytase addition to DNC diet was due to the influence of phytase addition on urine P concentration. Several studies have reported on the effect of phytase on swine fecal P, but this study is unique in studying both feces and manure (Omogbenigun et al., 2003; Angel et al., 2005; Power et al., 2006). This study has shown that swine manure behaves differently from feces and this is important as farmers apply manure to their soils and not feces. The quadratic nature of the relationship between fecal labile P concentration and phytase addition to DNC diet suggested that the effectiveness of phytase in reducing fecal P lability probably has a peak beyond which additional increase in phytase will result in greater fecal P lability.

A reduction in labile P concentration in manure with the addition of phytase to diet showed that phytase did not only reduce total P excretion, it has the potential to reduce manure P lability and thus, reduced the environmental impact of land application of manure. Smith et al. (2004) also reported that phytase addition to pig diet significantly reduced P solubility. This, thus, allayed the fear that phytase addition to pig diet could increase manure P solubility (Delaune and Moore, 2001; Smith et al., 2003).

Sodium hydroxide solution extracted 6 to 16% of fecal and 7 to 14% of manure P (NaOH-P). The concentration of NaOH-P extracted from manure and feces was not significantly affected by phytase addition. Inorganic P (NaOH-Pi) constituted between 16 and 61% of P extracted by NaOH in the feces, and between 26 and 69% of P extracted in the manure. Turner and Leytem (2004) reported 59% of hog manure NaOH-P as inorganic while Ajiboye et al. (2007) reported 79% as inorganic P. The wide variation in percent NaOH-Pi among the various dietary treatments was due to the presence or absence of supplemental inorganic P with NaOH-Pi decreasing with decreasing supplemental inorganic P (Table 4). It is possible that inadequate supply of P prevented the reaction of phosphate with cations such Al, Ca, Mg and Fe. This is more so as reduction in supplemental inorganic P translated to reduction in dietary Ca. Turner and Leytem (2004) showed that NaOH-Pi was associated with Ca, Al, Fe and Mg.

Ajiboye et al. (2007) and Turner and Leytem (2004) identified phytic acid as the main organic component of NaOH-P (NaOH-Po) in hog manure. Thus, in this study, phytase addition would be expected to reduce NaOH-Po (i.e. phytic acid) excretion since phytase enhances its hydrolysis. This was, however, not the case. Phytase addition had no significant effect on NaOH-Po. This may be due to the hydrolysis of phytic acid in the hind gut of animals as suggested by Seynaeve et al. (2000) and Leytem and Thacker (2008).

The effect of levels of supplemental inorganic P on HCl-P was more significant than the effect phytase addition to diet. Fecal HCl-P was significantly reduced from 2.9 g kg^{-1} in PC treatment to 1.6 g kg^{-1} in NC treatment and 0.5 g kg^{-1} in DNC treatment (Table 3). There was no significant effect of phytase addition to either NC or DNC diet on fecal and manure HCl-P. The reason for this observation was probably the same as that for NaOH-P. Hydrochloric acid extracted phosphate associated with Ca, Mg, Fe and Al together with phytic acid as the main organic P (Turner and Leytem, 2007). Reduction in supplemental inorganic P resulted in reduction in dietary Ca and consequently reduction in the formation of calcium phosphates.

The effect of phytase addition on residual-P was also not significant. Sodium hydroxide-, HCl- and residual- P are regarded as recalcitrant, and may not be of great environmental risk.

4. Conclusions

Addition of phytase to swine diet resulted in the reduction of excretion of P in feces and manure. The lability of fecal and manure P was also reduced with phytase addition to diet. The greatest percentage of fecal and manure P was in inorganic form. Fecal and manure organic P was not significantly affected by phytase addition probably due to the hydrolysis of phytate P in the hind gut of the animals. The greatest significant reduction in fecal and manure P was observed when 2000 U phytase kg^{-1} was added to DNC diet. This treatment produced 26% less labile manure P than the positive control diet. Although, 500 U phytase kg^{-1} was often used in pig diet formulation, this study showed that higher levels up to 2000 U phytase kg^{-1} with elimination of supplemental inorganic P could offer additional advantage of reducing manure P concentration and manure P solubility. This study showed that phytase enzyme can wholly substitute for inorganic P in pig diet when added at high levels.

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Table 1. Phosphorus and calcium content of the diets used in the study

Diet	Description	Available P (g kg^{-1})	Total P (g kg^{-1})	Calcium (g kg^{-1})
PC	Positive control containing NRC (1998) recommended available P	2.6	5.9	6.3
NC	Negative control with 38 % less supplemental available P	1.6	4.9	5.2
DNC	Double negative control containing no supplemental available P	0.9	4.4	4.8

Table 2. Amount of feces and urine produced and phosphorus concentrations in feces, urine and manure from pigs fed with phytase supplemented diets

	Dietary Treatment							
	PC	NC	NC+P1	NC+P2	DNC	DNC+P3	DNC+P4	SEM
	Quantity (g)							
Feces	0.31	0.23	0.23	0.20	0.35	0.34	0.18	0.02
Urine	1.46	1.50	0.90	1.26	1.37	1.94	1.58	0.13
	---------- P concentration (g kg^{-1})----------							
Feces	22.2^{a}	21.0^{ab}	16.5^{bcd}	16.4^{cd}	18.4^{abc}	13.5^{d}	18.0^{abcd}	0.74
Urine	0.64^{a}	0.04^{d}	0.15^{b}	0.14^{bc}	0.02^{d}	0.05^{cd}	0.03^{d}	0.04
Manure	24.4^{a}	20.7^{ab}	18.4^{bc}	17.5^{bc}	20.6^{ab}	14.1^{c}	17.3^{bc}	0.81

Means in the same row with the same letter are not significantly different at the 0.05 probability level. SEM represents standard error of the mean.

Table 3. Concentrations of P fractions (g kg^{-1}) from sequential fractionation of fecal P as influenced by phytase addition

P forms	PC	NC	NC+P1	NC+P2	DNC	DNC+P3	DNC+P4
H_2O-Pi	11.8^{abc}	12.8^{a}	10.4^{abcd}	9.23^{d}	12.3^{ab}	9.62^{cd}	10.3^{bcd}
H_2O-Po	-0.22^{b}	-0.19^{b}	0.05^{ab}	0.06^{ab}	0.28^{a}	0.13^{a}	0.03^{ab}
H_2O-P	11.6^{ab}	12.6^{a}	10.5^{abc}	9.29^{c}	12.6^{a}	9.75^{bc}	10.3^{bc}
$NaHCO_3$-Pi	3.92^{ab}	4.13^{a}	3.26^{ab}	2.55^{bc}	3.03^{ab}	1.59^{c}	2.64^{bc}
$NaHCO_3$-Po	-0.35^{b}	-0.48^{b}	-0.49^{b}	-0.42^{b}	0.08^{a}	0.13^{a}	0.05^{a}
$NaHCO_3$-P	3.57^{ab}	3.65^{a}	2.77^{abc}	2.13^{bc}	3.11^{abc}	1.72^{c}	2.69^{abc}
Labile-Pi	15.7^{ab}	16.9^{a}	13.7^{abcd}	11.8^{cd}	15.4^{abc}	11.2^{d}	12.9^{bcd}
Labile-Po	-0.57^{b}	-0.67^{b}	-0.44^{b}	-0.36^{b}	0.35^{a}	0.26^{a}	0.08^{a}
Labile-P	15.1^{a}	16.3^{a}	13.2^{ab}	11.4^{b}	15.7^{a}	11.5^{b}	13.0^{ab}
NaOH-Pi	1.66^{a}	1.37^{ab}	0.88^{bc}	0.76^{cd}	0.26^{de}	0.15^{e}	0.47^{cde}
NaOH-Po	1.06^{b}	0.89^{b}	0.86^{b}	1.59^{a}	0.86^{b}	0.80^{b}	1.00^{b}
NaOH-P	2.72^{a}	2.26^{ab}	1.73^{bc}	2.35^{ab}	1.13^{cd}	0.94^{d}	1.47^{cd}
HCl-Pi	1.22^{a}	0.69^{ab}	0.48^{ab}	0.34^{ab}	0.20^{b}	0.10^{b}	0.53^{ab}
HCl-Po	1.70^{a}	0.88^{b}	0.54^{bc}	0.64^{bc}	0.28^{c}	0.29^{c}	0.43^{bc}
HCl-P	2.92^{a}	1.57^{b}	1.03^{bc}	0.98^{bc}	0.48^{c}	0.39^{c}	0.95^{bc}
Residual P	0.50^{a}	0.40^{ab}	0.33^{b}	0.35^{b}	0.29^{b}	0.27^{b}	0.39^{ab}

Means in the same row with the same letter are not significantly different at the 0.05 probability level.

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Table 4. Concentrations of P fractions (g kg^{-1}) from sequential fractionation of manure P as influenced by phytase addition

P forms	PC	NC	NC+P1	NC+P2	DNC	DNC+P3	DNC+P4
H_2O-Pi	12.8^{a}	9.76^{bc}	10.2^{b}	8.30^{bc}	9.16^{bc}	9.08^{bc}	8.15^{c}
H_2O-Po	-1.49^{a}	-1.92^{a}	-0.6^{2a}	-0.58^{a}	-0.43^{a}	-0.52^{a}	-1.01^{a}
H_2O-P	11.3^{a}	7.84^{bc}	9.56^{ab}	7.72^{bc}	8.73^{bc}	8.56^{bc}	7.14^{c}
$NaHCO_3$-Pi	2.84^{ab}	2.88^{ab}	2.14^{abc}	1.86^{bc}	3.27^{a}	1.98^{abc}	1.44^{c}
$NaHCO_3$-Po	0.14^{a}	0.02^{a}	0.09^{a}	0.08^{a}	0.10^{a}	0.10^{a}	0.03^{a}
$NaHCO_3$-P	2.98^{ab}	2.89^{ab}	2.24^{abc}	1.94^{bc}	3.38^{a}	2.08^{abc}	1.47^{c}
Labile-Pi	15.7^{a}	12.6^{b}	12.3^{bc}	10.2^{bc}	12.4^{bc}	11.1^{bc}	9.59^{c}
Labile-Po	-1.35^{a}	-1.90^{a}	-0.52^{a}	-0.50^{a}	-0.32^{a}	-0.42^{a}	-0.98^{a}
Labile-P	14.3^{a}	10.7^{bc}	11.8^{ab}	9.66^{bc}	12.1^{ab}	10.6^{bc}	8.60^{c}
NaOH-Pi	1.51^{a}	1.04^{b}	0.75^{bc}	0.78^{b}	0.37^{cd}	0.24^{d}	0.33^{d}
NaOH-Po	0.69^{b}	0.49^{b}	0.65^{b}	1.08^{a}	0.56^{b}	0.67^{b}	0.56^{b}
NaOH-P	2.19^{a}	1.53^{b}	1.40^{bc}	1.86^{ab}	0.93^{cd}	0.91^{cd}	0.89^{d}
HCl-Pi	1.08^{a}	0.66^{ab}	0.45^{bc}	0.42^{bc}	0.25^{bc}	0.15^{c}	0.25^{bc}
HCl-Po	0.72^{a}	0.41^{bc}	0.33^{bcd}	0.55^{ab}	0.21^{cd}	0.18^{d}	0.30^{cd}
HCl-P	1.80^{a}	1.07^{b}	0.77^{bc}	0.97^{bc}	0.46^{bc}	0.33^{c}	0.55^{bc}
Residual P	0.37^{a}	0.25^{bc}	0.28^{ab}	0.29^{ab}	0.19^{c}	0.22^{bc}	0.26^{bc}

Means in the same row with the same letter are not significantly different at the 0.05 probability level.

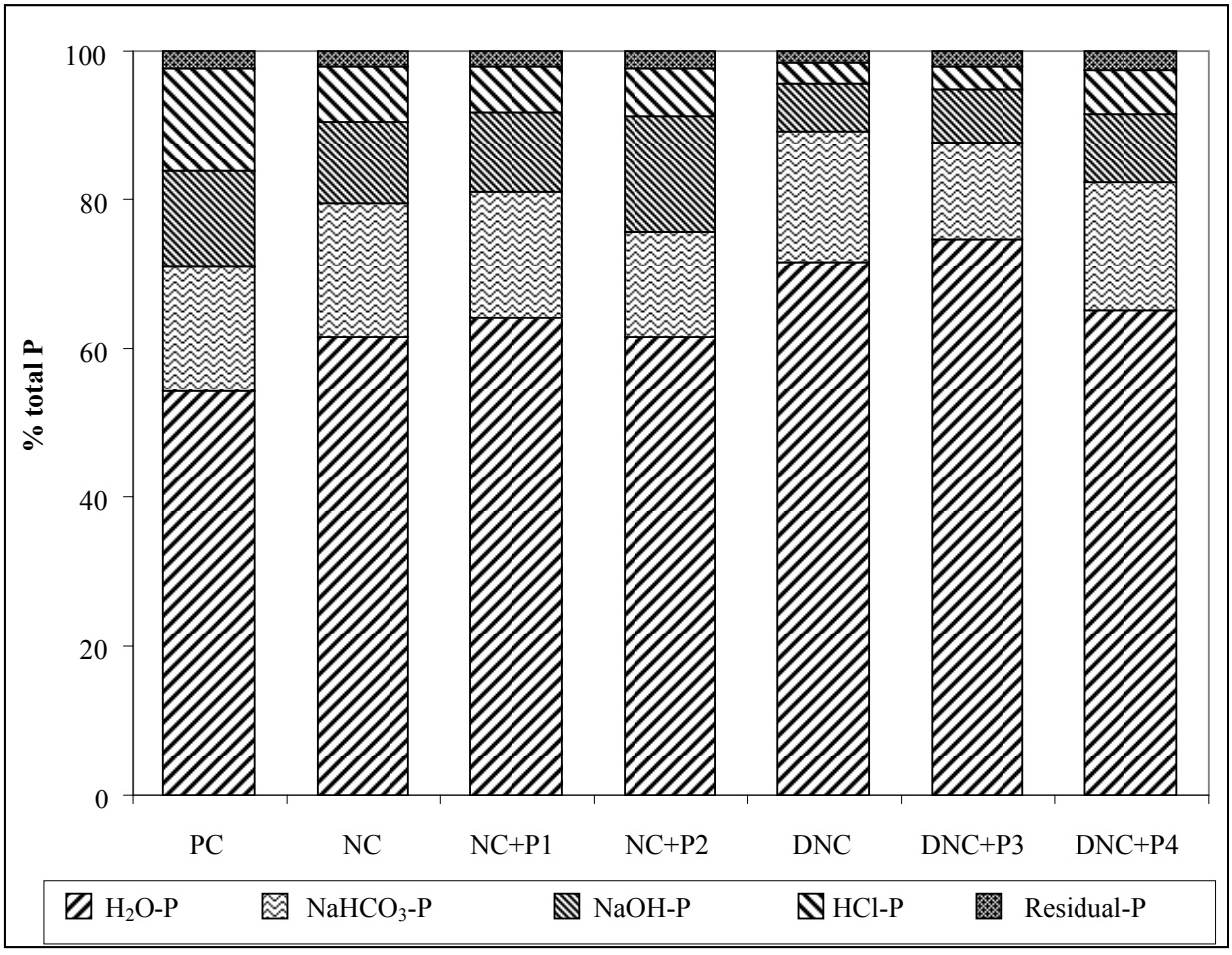

Figure 1. Percentage of fecal total P extracted by deionized water (H_2O-P), 0.5 *M* $NaHCO_3$ (pH 8.5, $NaHCO_3$-P), 0.1 *M* NaOH (NaOH-P), and 1 *M* HCl (HCl-P) and residual P fraction as affected by diet manipulation

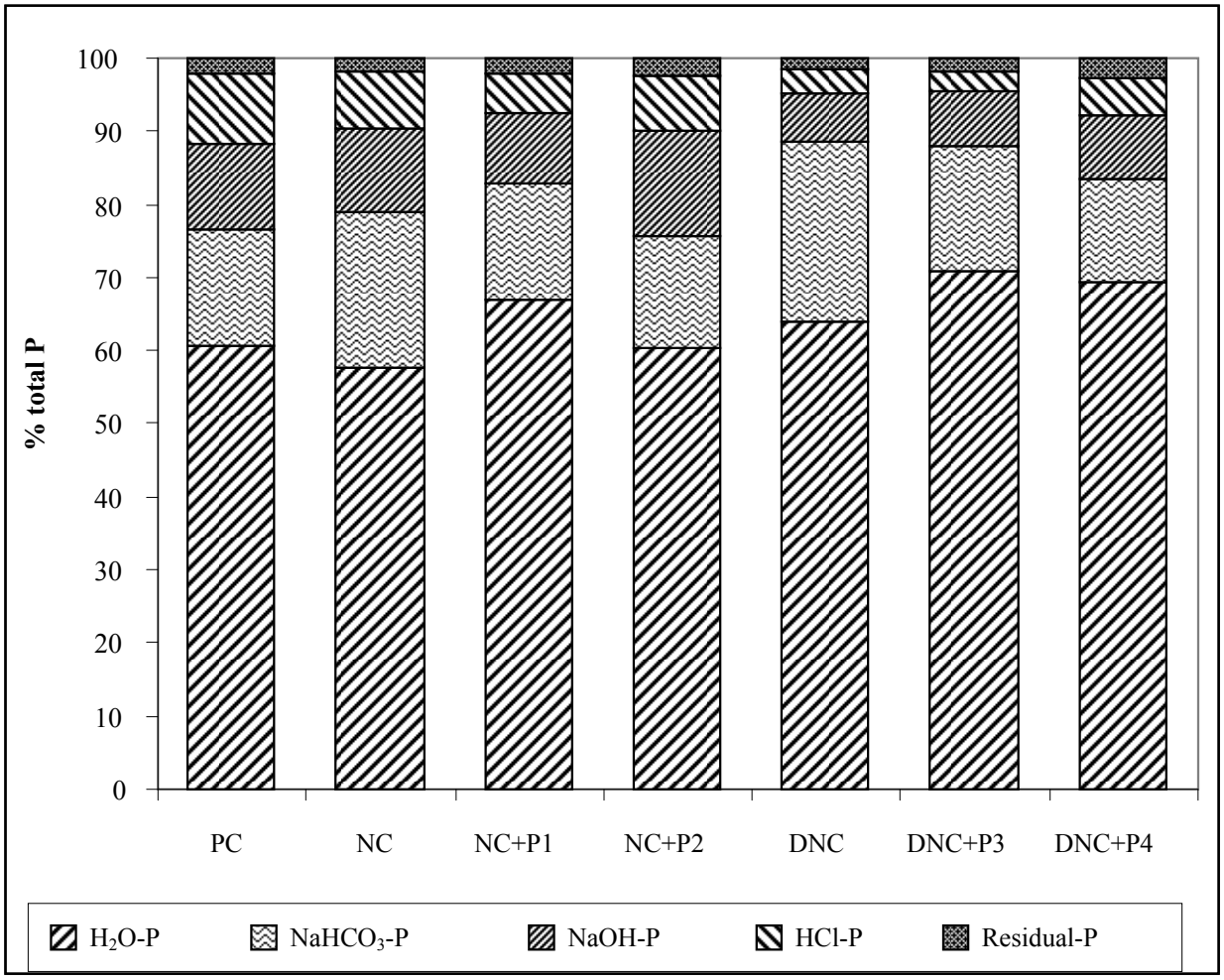

Figure 2. Percentage of manure total P extracted by deionized water (H_2O-P), 0.5 M $NaHCO_3$ (pH 8.5, $NaHCO_3$-P), 0.1 M NaOH (NaOH-P), and 1 M HCl (HCl-P) and residual P fraction as affected by diet manipulation

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