FORMS AND REACTIVITY OF MANURE PHOSPHORUS FROM PHYTASE FED SWINE IN MANITOBA SOILS

BY

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A Thesis Submitted to the Faculty of Graduate Studies in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

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ABSTRACT

Abioye, Olakulehin Stephen. M.Sc., The University of Manitoba, July, 2007. Forms and Reactivity of Manure Phosphorus from Phytase Fed Swine in Manitoba Soils. Major Professor; Dr. O.O. Akinremi

Growing interests in dietary manipulation to reduce P excretion in animal manure and P loss from agricultural soils to the environment have led to strategies such as the use of phytase in monogastric animal diets. The efficacy of phytase has been confirmed by several studies that reported its ability to hydrolyze phytate P present in grain feeds and thus, reduce manure total P. However, the solubility of manure P from phytase supplemented diets in soils is not well known, and as thus, the environmental implications of dietary P manipulation require further investigation.

Two related studies were carried out in the laboratory to investigate the fate of manure phosphorus (P) from pigs fed phytase supplemented diets in Manitoba soils. The first study characterized the forms of manure P from phytase supplemented swine diets to evaluate their potential environmental impact. The seven dietary treatments fed randomly to a total of 28 growing pigs were: a positive control that contained P at the NRC (1998) recommendations (NRC), a negative control (RED) containing 0.1 percentage units reduction (about 33%) in available P from 1998 NRC recommendations, RED with 500 U of phytase kg^{-1} of diet (RED + P1), RED with 1000 U of phytase $kg^{-1}$of diet (RED + P2), a double negative control with no added inorganic P (DNC), DNC plus 2000 U of

phytase kg^{-1} of diet (DNC + P3) and DNC plus 4000 U of phytase kg^{-1} of diet (DNC + P4).

The second study examined the solubility of manure P from the manure collected from the first study. Manure collected from the first study were applied at a rate of 75 kg of total P ha^{-1} of soil to surface samples from four Manitoba soils (0-15 cm); Osborne clay (Rego Humic Gleysol/Gleysolic Humic Vertisol), Red River clay (Gleyed Rego Black Chernozem/Gleyed Humic Vertisol), Ladywood very fine sandy loam (Gleyed Dark Gray Chernozem), and Glenhope loamy fine sand (Gleyed Rego Black Chernozem).

In the first experiment, total P in feces and manure were significantly reduced ($p < 0.05$) with phytase addition to the diets. The labile P concentration (sum of H_2O–P and $NaHCO_3$-P) was about 71 to 89% and 77 to 89% of total P in both feces and manure, respectively. Phytase addition to the diets reduced the labile P in feces.

The solubility of P was greatest in the calcareous soils amended with the manure from the DNC diets and solubility of P varied with time and extracting solutions. Although, a combination of physico-chemical properties (e.g. CEC, Exchangeable Ca^{2+}), texture seems to play a significant role, as P solubility increased in coarse textured soils after longer period of incubation (16wks). However, our results showed that phytase supplementation in the diets of pigs did not affect the solubility of manure P in amended soils.

ACKNOWLEDGEMENT

I wish to acknowledge the opportunity my supervisor, Dr. Wole Akinremi gave in accepting me into a graduate program under his tutelage. His great contribution, constructive and helpful criticism is of invaluable worth to the successful completion of this project. My gratitude also goes to other members of my advisory committee: Dr. Martin Nyachoti and Dr. Don Flaten – for their contribution, support and guidance.

I also want to thank these agencies for providing the financial support for this project: Manitoba Livestock and Manure Management Initiative Inc. (MLMMI), Manitoba Rural Adaptation Council (MRAC), Sustainable Development Innovation Fund (SDIF). My profound gratitude also goes to all the graduate students, faculty members, administrative and technical staff of the Department of Soil Science – many thanks to Rob Ellis for his technical support, instruction and advice. It was a great privilege working with the members of the Soil Chemodynamics Research Group, and more importantly, my warmest appreciation goes to Mrs. Mulikat Bammeke for her technical instruction, efforts and moral advice towards my successful laboratory experience. I would also like to say a big thanks to Gregg Morden of Geological Sciences for his immediate help in some analytical works of my research results and Peter Haluschak for helping with the soil collection from the field. All thanks to Dr. Crow of Animal Science Department for his suggestions and help on the statistical analysis of the second study.

My appreciation also goes to the following fellows: Sola Ajiboye – Thanks for your help and encouragement, Bola-Mola Opapeju, thanks for being there always and to

the ‘friends like brothers’, ‘Lekan Olatuyi and ‘Deji Dunmola – we are born to overcome!!!

My appreciation would be incomplete without acknowledging the *‘Blessing of my life’,* OYEBUKOLA – your patience, prayers and encouragement are peerless!!!

And to the One who ordered my steps this far, the One whose Grace is always sufficient in times of need, The Almighty God, to you alone be all the thanks and all my life, I will keep working with you.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CEC	Cation exchange capacity
DCP	Dicalcium phosphate
d.f	Degree of freedom
DNC	Double negative control
DNC+P3	Double negative control plus 2000U of phytase kg^{-1} diet
DNC+P4	Double negative control plus 4000U of phytase kg^{-1} diet
L	Litre
M	mole L^{-1}
M3-P	Mehlich-3 extractable phosphorus
MK-P	Modified Kelowna extractable phosphorus
NPP	Non-phytate phosphorus
NRC	National Research Council
O-P	Olsen extractable phosphorus
Pi	Inorganic phosphorus
Po	Organic phosphorus
Pt/ TP	Total phosphorus
PTU/ U	Phytase units
rpm	Revolution per minute
W-P	Water extractable phosphorus
WSP	Water soluble phosphorus
vs.	Versus

1. BACKGROUND INFORMATION

Phosphorus (P) is an indispensable nutrient element for the growth of both terrestrial and aquatic plants. It is also essential for animal growth as its input has long been recognized as necessary to maintain profitable crop and animal production. Manure has been identified as a beneficial source of nutrients (nitrogen, phosphorus, and potassium) that can be used to increase crop production. However, the current intensification of animal production in many parts of the world has led to an increase in available manure P than required by the crops (Kellogg et al., 2000). Moreover, manure applications have been in excess of crop P requirements due to the fact that its applications were based on crop nitrogen (N) requirements. Flaten et al. (2003) reasoned that N:P ratio of manure is typically 3:1 or less, whereas the N:P ratio required by most crops is 4:1 or more; therefore, the application of manure to meet crop N requirements usually results in overloading the soil with P. The over-application of P fertilizers and manures beyond optimum levels for crop growth results in the potential increase in the transfer of P from soil to solution and eventually to surface water.

Substantial amounts of P entering surface water (lakes, other surface impoundments, and streams) contribute to accelerated eutrophication. Eutrophication is a major problem affecting the world's freshwaters leading to impaired water quality, restricting its use for fisheries, recreation, industry, and drinking (CEC, 1992; USEPA, 1996, Carpenter et al., 1998). Eutrophication is caused by inputs of phosphorus from urban, industrial and agricultural sources into surface water bodies and as a result, the water quality declines

due to increased algal growth, oxygen depletion (caused by the death and decomposition of algae), fish kills and release of algal toxins (Flaten et al., 2003). This increasing incidence of eutrophication globally is caused in part by nonpoint sources of P, especially from agricultural land (Sharpley et al., 2000). Tunney (2000) reported that the Irish Environmental Protection Agency (IEPA) estimates that half the phosphorus (P) loss to water comes from agriculture and P is generally the element causing eutrophication of freshwaters.

Monogastric animals (poultry and swine) have limited ability to digest the complex grain P (phytate P) in their feeds. As a result, a high amount of P is excreted in their feces. However, to enhance the digestibility of the phytate P present in the feed grain of monogastric animals and consequently reduce the excretion of P in feces, several technologies have been identified through the use of additives in the diets of the animals. One of these is the use of phytase, an enzyme that breaks down the indigestible phytic acid (phytate) portion in grains and oil seeds, thereby releasing digestible phosphorus for the animals. For example, Beers and Jongbloed (1992) showed that phytase addition to corn-soybean meal diet could increase digestible P by as much as 1g kg^{-1}. Shortly after this technological breakthrough, considerable concerns were raised suggesting that the use of phytase to reduce fecal P excretion might contribute to loss of P environmentally. These were the concerns of Barnes (2002) for example, who suggested that the phytase inclusion in broiler diets may increase the solubility of P in the litter and as a result, enhance P loss in runoff. This concern stemmed from the report of DeLaune et al. (2001) who observed a significant increase in the soluble P in the runoff water from plots treated with control + phytase litter compared to the control litter of broilers.

Recent studies (Moore et al. 1998; Gilley et al. 2001; Baxter et al. 2003; Maguire et al. 2003; and Maguire 2004) have showed that phytase supplementation in diets of animals either decreased or did not have a significant effect on the water soluble P and P loss in runoff. While many of these studies have been conducted mostly on poultry, limited work has been done to investigate this reactivity in swine manure P from phytase supplemented diets in soils. Therefore, this study was conducted to characterize the amounts and forms of manure P from phytase supplemented diets of swine as a first step in assessing the fate of manure P. Moreover, this study also investigated the solubility of manure P from phytase amended diets in calcareous and non-calcareous soils.

1.1 Swine Production in Manitoba

Manitoba's hog industry has experienced significant changes in recent years. The industry has undergone greater changes in its overall structure and has become more capital intensive over the years. Pig farms are now generally fewer in number but larger in size as has also occurred in the United States and other parts of the world. In Manitoba, a total number of 1,740 farms and about 1,324 pigs/farm in October 2000 has reduced to as low as 1,280 farms but as much as 2,342 pigs/farm in October 2006 (Statistics Canada, 2006).

Currently, Manitoba is the third largest producer of pork in Canada with approximately 8.6 millions hogs produced in 2005 (Manitoba Conservation, 2006). About 3.6 million of these hogs were locally slaughtered in Manitoba processing plants,

while about 3.6 million weanlings and over a million live-slaughter hogs were exported to the United States in 2005. In terms of job opportunities, about 16,000 to 17,000 people benefit from the industry and the growth of many other businesses and services that relate to the industry in Manitoba has been enhanced, contributing about $2 billion economically (MAFRI, 2006)

Moreover, the involvement of the feed companies has strengthened the hog industry. Among all, the large expanse of land in Manitoba for agricultural production has also made livestock production easier. However, there have been rising concerns about the expansion of the hog industry, especially regarding issues like odour, disposal of large amounts of manure, food safety and environmental degradation. In an attempt to balance between sustainable agricultural practices and environmental protection, the government and industry have developed policies, recommendations, guidelines, regulations and services to protect the environment (Manitoba Agriculture 1998; MAFRI 2006; Manitoba Phosphorus Expert Committee 2005; and Manitoba Conservation, 2006).

The management of the large amount of swine manure and various nutrients it contains is an issue affecting the sustainability of the hog industry. Increasing concentrations of manure nutrients such as nitrogen and P in Manitoba waterways and water bodies has prompted regulations for the land application of animal manure. The current P regulation introduced in 2006 has placed a temporary pause on new or expanded barns in the province (Manitoba Conservation, 2006).

The magnitude of manure P input is not the only factor affecting P movement from soil to water bodies. Several other factors such as soil type and management, amount of P removal, and transport processes could affect P movement. However, the

source factor is an important target in order to mitigate P loss from soil to water as it is impossible to control natural rainfall and difficult to influence soil hydrology. Moreover, Manitoba's regulations focus on reducing excessive P build-up in the soil to protect the rivers, streams and lakes. These control the amount of P that is applied in a localized area, restricts the risk of P loss and minimizes P movement to surface water by limiting the quantity of P in manure that is applied to land.

1.2 Phytate P in Feeds of Monogastric Animals

Oilseed meals, cereal grains and by-products used in the feeds of monogastric animals contain a high proportion of P in a naturally occurring organic complex called phytate or phytic acid. This complex is called myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate. Cosgrove (1966) regarded phytate as the primary storage form of phosphates in plant seeds. Two thirds of the total P in the cereal seeds, grain legumes and oil-bearing plants is present in phytate form (Simons et al., 1990). Phytate consists of an inositol ring

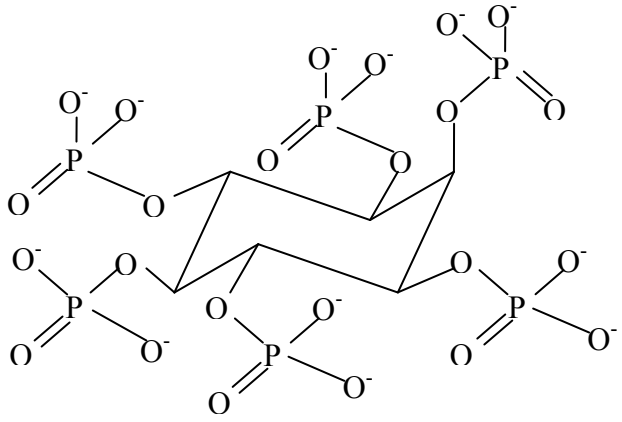

Figure 1. Structure of myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate (phytic acid)

with six phosphate radicals (Fig 1). Phytate becomes very reactive as a result of the phosphate radicals, and can chelate divalent cations such as Ca^{2+} and Zn^{2+}, and thus reduce the availability of nutrients such as proteins, amino acids, starch and other carbohydrates (Ravindran et al. 1995).

Phytate P is almost indigestible by monogastric animals (poultry and swine). Non-ruminants do not produce sufficient amount of extracellular phytase in their intestine to fully digest the complex P form in phytic acid. However, Pointillart et al. (1984) and Cromwell (2005) reported that pigs produce phytase in their hind gut, which might not be of great significance because of poor P absorption in the large intestine.

Phosphorus in the grain feedstuffs may vary in bioavailability to the animals. Cereal phytic acid was reported not to be uniform in kernel composition due to the

morphological components of the seed (Oberleas, 1973). Tonroy et al. (1973) in their results reported extremely low P availability in corn (*Zea mays* L.) and grain sorghum [*Sorghum bicolor* (L.) Moench], but slightly higher availability in barley (*Hordeum vulgare*) and soybean (*Glycine max* L.). Phytate concentration in plants has also been attributed to the stage of maturity, degree of processing, cultivar, climatic conditions, and the geographical location where they are grown (Reddy et al., 1982). McCance and Widdowson, (1944) also attributed the higher P availability in some cereal grain feedstuffs (wheat, rye, triticale and barley) to the presence of naturally occurring endogenous phytase in their seed coat. The presence of endogenous phytase in these grain feedstuffs also affects P availability in their by-products (Pointillart et al. 1984; Jongbloed and Kemme, 1990b; Cromwell and Coffey, 1993,). A combination and use of feedstuffs that are high in endogenous phytase, such as mentioned above and their by-products (wheat bran, wheat middlings) can be sources of phytase needed by pigs and poultry.

1.3 Dietary Manipulation of Feeds to Reduce P Excretion

Maguire et al. (2005) suggested that dietary manipulation strategies offer the most effective and economically viable means of dealing with the environmental problems associated with nutrient excretion in pig manure. Reduction in P content of manure will reduce the on-farm accumulation of P and may alleviate the problem of excess manure application (by increasing the N:P ratio to meet crop requirements). Therefore,

manipulating the dietary P supplement of the animals helps in balancing P input and output in animal production.

1.3.1 Increasing the Efficiency of Animals for Dietary P Uptake

Due to the inability of the monogastric animals (poultry and pigs) to efficiently digest phytate P in grain feeds, the animals become deficient in P. To correct this deficiency, nutritionists supplement feeds with inorganic P sources in form of mono- and di-calcium phosphates based on available P rather than on total P (De Lange et al. 1999) because the P availability in mineral sources are high. This inorganic P supplementation contributes to P enrichment of manures and litters.

Alternatively, some feed ingredients have endogenous phytase activity in them which vary in most cereals. These endogenous plant phytases have been shown to be effective in feeds. Scheuermann and co workers (1988) showed that phytase from wheat can hydrolyze phytates from other ingredients. A combination of these cereals rich in phytase can possibly eliminate the supplementation of inorganic P. This may have combined benefit of increasing the utilization of phytate P by the animals and also reducing P excretion in feces and manure.

For those cereals, such as corn, that have low levels of endogenous phytase activity, varieties of low-phytic acid concentration in corn (high available phosphorus corn), barley (*Hordeum vulgare* L.), soybean [*Glycine max* (L.) Merr.] and wheat (*Triticum aestivum* L.) have been developed which do not affect the amount of total P in the seed (Raboy et al., 1990; Rasmussen and Hatzack, 1998; Wilcox et al. 2000; Guttieri et al. 2004). Several workers have shown that low-phytate corn increases the

bioavailability of P to non-ruminant animals compared to the conventional (traditional) or normal corn (Cromwell et al. 1998; Ertl et al. 1998; Spencer et al. 2000; Veum et al. 2001). Not only do these low-phytate crop varieties enhance available P to animals, they reduce the need for supplemental P, leading to reduced P excretion.

1.3.2 Minimizing Excess Dietary P

Overfeeding of P to animals increases the P content of their manure and subsequently endangers the environment with excessive P loading (Ebeling et al., 2002). Dietary P intake supplied above the optimum for animals' dietary requirements does not increase the yield of milk in dairy animals (Brodison et al., 1989; Morse et al., 1992). Also, this excessive intake of P leads to reduced profitability due to increased feed costs as reported by Knowlton and Kohn (1999). Morse et al. (1992) demonstrated a reduction in P excreted by 23% in feces and 17% in total P when dairy cows' P intake was decreased from 82 to 60 g day^{-1}. However, they demonstrated that there was an increase in excretion of P in feces by 49% and 37% in total P by raising the dietary P intake of the animals from 82 to 112 g day^{-1}. One of the possible ways is to manipulate the dietary P intake of animals by feeding to their optimum requirements without overfeeding. This requires that the optimum requirements are known.

1.3.3 Supplementation of Diets with Phytase

The phytate P is relatively stable but can be hydrolysed to inositol and inorganic phosphates by an enzyme (myo-inositol hexaphosphate phosphohydrolase) (Sebastian *et al.,* 1998). Simons et al. (1990) first reported promising results of using microbial phytase

in pig feeds using a strain of *Aspergillus ficuum*. They reported an increase in digestibility of P in the range of 27 to 51% with the use of phytase. Other workers have also documented that phytase could make P more digestible by non-ruminants thus reducing the amount of P excreted in feces and manure (Harper et al., 1997; Sands et al., 2001; Beaulieu et al., 2004). Phytase could also eliminate the need for supplemental inorganic P in the diets (Grandhi, 2001) and reduce the rate of P accumulation in soil, thus, minimizing the negative effects of manure on the environment. Campbell and Bedford (1992) stated that phytase has a great potential to increase the availability of P and thus, provides a cost effective alternative to inorganic P supplementation especially in areas where livestock production is intensive.

Phytase not only increases the absorption of P, it also enhances the absorption of Ca, Mg, Zn through its breaking down of the phytate (Adeola, 1996). The amount of phytase units that would be sufficient to replace the inorganic P supplementation is, however, a debatable issue. Early studies have shown that about 1000 units of phytase kg^{-1} diet maximized responses in pigs (Jongbloed et al., 1996). Meanwhile, Kornegay (1999) proposed that about 550 phytase units per kg of diet will release 0.10% P and more recently Augspurger et al. (2003) reported the release of about 75% of dietary unavailable P with high levels of dietary of dietary *E. coli* phytase (1,500 FTU/kg). These researchers suggested that more studies should be done with higher level of phytase.

1.4 Solubility of Manure from Phytase Supplemented Diets

While phytase supplementation in diets reduced P content of manure (Smith et al. 2004), diets containing phytase have also been said to increase soluble P component in

manure, and subsequently increase the soluble P in runoff (Delaune and Moore, 2001). Moreover, as reported by Applegate et al. (2003) that phytase can enhance phytate P availability to poultry, there have been concerns that phytase could increase WSP in poultry litter and hence, the potential loss to adjacent water bodies (Vadas et al., 2004). Moore et al. (1998) earlier reported that phytase addition with reduced inorganic P in poultry diets did not significantly decrease total or water-extractable P. Meanwhile Applegate et al. (2003) showed that P levels in broiler litters are associated with the P concentration in the feed and not the use of phytase. Correct formulation of diets with phytase would reduce litter P (Maguire et al., 2004). Most studies involving pigs have shown that phytase, with appropriate reductions in supplemental inorganic P, either decreases or has no effect on P excretion in manure (Baxter et al., 1998; Hill et al., 2003; Angel et al., 2005). More recently, Ige et al. (2006) showed that enzyme addition in swine diets did not significantly affect water-extractable P but reduced the $NaHCO_3$-P fraction in pig feces. They reported a significant reduction in the labile P fraction of manure from enzyme supplemented diets.

1.4.1 Solubility of Manure Phosphorus in Soils

The current application of manure to soil based on the N requirements of crops has lead to overapplication of P partly due to the unfavorable N/P ratios in manure when compared to uptake by crops (Mikkelsen, 2000). The applied P then accumulates in agricultural soils which may increase soluble P concentration in soils, dissolved P in surface runoff waters and downward movement of P to groundwater (Lui et al., 1997;

Haygarth, 1998). The soluble P contains the dissolved organic and inorganic P forms. These are P forms with which runoff and leaching waters can readily transport. The movement of this P into freshwater bodies contributes to increased algal growth, oxygen depletion (caused by algal death and decomposition), fish kills, and release of algal toxins that characterize eutrophication (Schindler, 1977; Sharpley et al., 2000). Due to the potential for a significant loss of the soluble P to the environment from soils that are frequently manured, there is need for a better understanding of the factors contributing to P transfer from agricultural land to adjacent water bodies.

1.5 Factors Affecting P Availability in the Soil

The fate of manure P in soil is determined by several factors including the properties of both soil and manures. Such properties and the forms of P present in manure would determine the key processes involved in P movement within the soil. The key processes that affect the fate of P include precipitation-dissolution, sorption-desorption, mineralization-immobilization, and the mechanisms of P movement are leaching, runoff and erosion. These chemical, biological and physical reactions in soil that control P solubility could be altered by manure application (Chang et al. 1991). Details of the factors influencing soil P solubility are described below.

1.5.1 Amount and Relative Availability of Soil Minerals

Adsorption and desorption of P are the main processes that influence the amount of P in the solid phases. In areas of intense weathering, P is released into the soil solution

by the dissolution and desorption of the precipitates formed by Fe, Al and P on the soil mineral surfaces. In contrast, in soils with less weathering incidence, such as calcareous soils, Ca is conventionally believed to primarily control P reactions (Lindsay, 1979). However, much of the P adsorption to $CaCO_3$ surfaces is being attributed to Fe oxides impurities. Hamad et al. (1992) observed that P sorption is related to iron oxide and clay content while Leytem and Westermann (2003) reported that the adsorption of P is related to organically complexed Fe and Mn.

Phosphorus sorption capacity is low in organic and very sandy soils. In organic soils, humic substances adhere to clay and metal oxides, which lower the P sorption capacity. Duxbury and Peverly (1978) stated that organic soils can show high leaching losses from the mineralization of organic matter. Following manure application to calcareous soils, Leytem et al. (2005) concluded that microbial processes influenced P solubility. This is in agreement with the works by Seeling and Zasocki (1993) and by Turner and Haygarth (2001). The capacity of sandy soils to adsorb P can be easily overwhelmed due to low quantity of reactive mineral phases.

1.5.2 Effect of Soil pH on Phosphorus Solubility

Dissolved ionic P forms in the soil solution are largely affected by soil pH. Optimum pH for most plants is from pH 5.5 to 6.5 because P availability is at its maximum at this pH range (Stevenson, 1986) bringing P adsorption to soil minerals to a minimum (Lindsay, 1979). Soil pH will greatly influence the reactivity of soil constituents for soluble P. Availability of P in acid soils is generally favored by increases in pH values, this is as a result of reduction in the activities of Fe and Al oxides that fix P

at low pH range. Liming of acid soils is proposed primarily to increase phosphate availability to plants (Sanchez and Uehara, 1980). High rates of liming, however, have been reported to have a negative impact on P solubility (Sumner, 1979) by forming insoluble Ca-P minerals.

Significant effects of soil pH on the distribution of phosphate species were reported by Schachtman et al. (1998). The range of pH found in agricultural soils (4.0 to 9.0) differentiates the P species into monovalent ($H_2PO_4^-$) or divalent (HPO_4^{2-}) orthophosphate anions, and both are readily available for plant uptake. Foth and Ellis (1997) showed that below pH 6.0, the monovalent $H_2PO_4^-$ species is prevalent and at neutral pH, the proportions of orthophosphate forms are equal. However, the divalent (HPO_4^{2-}) species is predominates at pH above 7.2.

1.5.3 Effects of Soil Organic Matter on P Solubility

Humic materials from organic matter could adhere to the surfaces of clay, metal oxides and hydroxides forming a protective layer that blocks P adsorption onto these surfaces. Also, decomposing organic matter can produce organic acids (anions) that could compete with phosphate anions for adsorption sites. Moreover, the organic acids could also chelate free Al, Fe and Ca, making them unavailable for precipitation with soluble phosphate ions and as such, increase P availability. Humic acid application to soils, except those with high sodium (Na) content increased the recovery of Olsen P in a study by Delgado et al. (2002). In semiarid calcareous soils with low organic C, Leytem et al. (2005) demonstrated an overriding effect of C/P ratio of added manure in regulating soil

P solubility, over soil chemical properties such as clay content, pH, $CaCO_3$ concentration, etc.

1.5.4. Roles of Types of Soil Amendments on P Solubility

Various organic and inorganic amendments used to provide soluble P and to build the labile pool of soil P vary widely in soil P solubility. Kashem et al. (2004) observed that the labile P levels in amended soils follow an increasing order of: biosolids < cattle manure < hog manure < fertilizer. Differences could be as a result of the varying amount of P in the amendments, resulting from the variations in animal species and diet, effect of handling and storage practices of the amendments, and treatment with P sorbing agents (e.g. alum). Decreases were observed in the water-soluble P fraction and runoff losses of poultry litter treated with alum from the pasture receiving litter (Moore et al., 2000). Shreve et al. (1995) also reported a decrease in dissolved P concentrations from 83 mg L^{-1} in normal broiler litter to 11 and 19 mg L^{-1} in alum- and $FeSO_4$-treated broiler litters, respectively, in surface runoff of fescue pastures. Studies have characterized solubility of various P sources and such studies have been used to assess the fate of P in manured soils (Leinweber et al., 1997; Dou et al., 2000; Ajiboye et al., 2004).

1.5.5. Manure Characteristics and its Effect on Soluble P

The type of manure and the variability in manure properties can considerably affect soluble P release to runoff from manure amended soils (Sharpley and Moyer, 2000; Kleinman et al., 2002). These researchers observed that the variation in soluble P release

to runoff is primarily due to the differences in total and soluble P concentrations of manure. Kleinman et al. (2005) concluded that high water content promotes the dissolution of P compounds in manure. Properties of manure other than organic P content may have a significant influence on the P solubility in soils. The amount of C in manure influenced changes in extractable P in manured soils (Leytem et al. 2005; Leytem and Westermann, 2005). Iyamuremye et al. (1996) reported that organic ligands in manure can complex Fe and Al decreasing P precipitation by these metals; besides, the organic ligands can compete for sorption sites, thus increasing P concentration in solution.

The investigation of the forms of P in swine manure from phytase supplemented diets would enable us to determine the fate of manure P in treated soils. Moreover, the application of these swine manure P to soil will give an insight into the factors that contribute to the solubility of the manure P and how they are affected by dietary modification. Thus, the overall objectives for this study were to investigate the forms of P in swine manure and feces from phytase supplemented diets, to examine the effect of phytase addition on the manure and fecal P content. And lastly, to investigate P solubility of manure from phytase supplemented diets in calcareous and non-calcareous Manitoba soils.

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2. CHARACTERIZING FECAL AND MANURE PHOSPHORUS FROM PIGS FED PHYTASE SUPPLEMENTED DIETS

2.1 Abstract

Phytase supplementation with concomitant reduction in available P has been reported to reduce total P in feces. Whereas the effect of phytase in reducing the P concentration of manure is well known, we do not have adequate knowledge about the influence of phytase on the forms of P in swine manure and their relative solubility. We conducted this study to characterize the amount and forms of P in feces and manure from pigs fed phytase supplemented diets. A total of 28 growing pigs were fed seven different dietary treatments: A positive control that contained P at NRC (1998) recommendations (NRC), a negative control (RED) containing 0.1 percentage units reduction (about 33%) in available P from the 1998 NRC recommendations, RED with 500 U of phytase kg^{-1} of diet (RED + P1), RED with 1000 U of phytase $kg^{-1}$of diet (RED + P2), a double negative control with no added inorganic P (DNC), DNC plus 2000 U of phytase kg^{-1} of diet (DNC + P3) and DNC plus 4000 U of phytase kg^{-1} of diet (DNC + P4). The fecal material and urine were collected separately and a sub-sample of manure was derived by mixing the feces and urine. Total P in feces, urine and manure was determined. Fecal and manure P were fractionated using the modified sequential fractionation procedure. Total P in feces and manure was significantly reduced ($p < 0.05$) by 26% and 32%, respectively, with phytase addition while urine total P increased by 33 to 73%. The labile

P concentration (sum of H_2O –P and $NaHCO_3$-P) was approximately 71 to 89% of total P in both feces and manure. Phytase addition to the diets significantly reduced ($p < 0.10$) the labile P in feces; however, the reduction in labile P was not significant in the manure. Phytase addition beyond the 2000 U kg^{-1} of diet tends to increase both the total and labile P. Overall, the addition of phytase to pig diets reduced the total and labile P in the manure and feces and this may reduce the loss of P to the environment.

2.2 Introduction

Phosphorus is an essential element for both plant and animal growth. Due to intensive animal production, manure P has been generated in excess of crop needs (Kellogg et al., 2000). Consequently, concerted efforts have been taken to reduce manure P content as a means of decreasing soil P loading and averting possible eutrophication. One of the ways to reduce manure P is through dietary supplementation with exogenous phytase, a phosphatase enzyme that catalyzes the hydrolysis of phytic acid / phytate (*myo*-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate) in animal diets (Jongbloed and Lenis, 1998).

Phytase addition in diets of swine with concomitant reduction in supplemental inorganic P has been reported to reduce fecal P excretion (Yi et al., 1996; Kees deLange et al., 1999; Grandhi 2001; Omogbenigun et al., 2003). Li et al. (1998) concluded that the use of phytase in swine 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., 2004: 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 manure (Miles et al., 2003, Vadas et al., 2004) however, in most swine studies, phytase inclusion either decreased or had no effect on water soluble P (Baxter et al., 1998; Baxter et al., 2003; Hill et al., 2003; and Smith et al., 2004a).

Phytase supplementation of about 500 U complemented by a 0.1 percentage unit reduction in available P has been reported to decrease P excretion (Omogbenigun et al., 2003). Results from the 0.1 percentage 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. (1993a) reported a larger reduction of 35 to 42% for weanling pigs. However, Angel et al. (2005) could not observe a statistical difference in total P excretion after 0.1 percentage units reduction in the available P with 515 U of phytase addition to swine diet. Further reduction of 0.2 percentage units in dietary NPP at the same level of phytase inclusion reduced litter P concentration, but not the percentage of H_2O-P relative to the normal diet. Thus, there is a need to investigate the effect of further reduction in available P in the diet on the forms of P in the manure.

Most studies on swine have observed decreased P content in the feces through phytase addition without taking into consideration the P content of urine (Veum et al., 2001; Baxter et al., 2003; Angel et al., 2005; Ige et al., 2006). As such, more work needs to be done on manure (feces + urine) samples in order to gain a better understanding of the contribution of urine P. Although reports had it that the urine P represents < 0.5% of the total P excreted (Baxter et al., 2003), phytase inclusion in swine diets have been reported to increase urinary P (Zhang et al., 2003; Baxter et al., 2003, Ige et al., 2006). The inclusion of urine P will provide more complete understanding of phytase effects on P excretion by swine.

The sequential fractionation procedure has been used to assess the potential environmental impact of manure P (Sharpley and Moyer 2000; Dou et al., 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 inorganic phosphate and a small amount of soluble organic P in swine manure but NaOH and HCl extracted P forms that are less soluble (Turner and Leytem, 2004; Toor et al., 2005). Therefore, the objective of this study was therefore to characterize the forms of fecal and manure P from pigs fed phytase supplemented diets with concomitant reduction in available P in diets. This will serve as a first step in determining the behavior of P in manured soils.

2.3 Materials and Methods

2.3.1 Housing of Pigs and Dietary Treatments

A total of 28 growing Cotswold pigs obtained from the University of Manitoba, Glenlea Swine Research Farm 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: A positive control that

contained P at NRC (1998) recommendations (NRC); a negative control containing 0.1 percentage units reduction (about 33% in mass) in available P (RED); RED plus 500 U of phytase kg^{-1} of diet (RED + P1); RED plus 1000 U of phytase $kg^{-1}$of diet (RED + P2); a double negative control with no added inorganic P (DNC); DNC plus 2000 U of phytase kg^{-1} of diet (DNC + P3) and DNC plus 4000 U of phytase kg^{-1} of diet (DNC + P4).

2.3.2 Collection and Pretreatment of Manure Samples

At the end of the 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 over 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 urine pH and NH_3 volatilization. Aliquots were taken from daily volume and kept frozen till required for sub-sampling and analysis. The feces were weighed and stored at -20^{o}C until sub-sampled. A "manure" sample was generated from the feces and urine by sub-sampling and mixing. The feces and urine were divided into two halves based on their weights and volumes, respectively. One-half of the urine and one-half of the feces were mixed together using a blender to obtain a homogenous paste. This mixture was designated as the "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 fine sizes (about 1mm screen) and thoroughly mixed before samples were taken for total P analysis. All analyses were performed in

quadruplicates. The feces and the 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).

2.3.3 Fecal and Manure P Characterization

The modified sequential fractionation procedure of 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 hrs on an end-to-end shaker at 150 epm (excursions per minute) at room temperature, centrifuged at 10,000 rpm for 15 min and vacuum-filtered through 0.45 μm cellulose membrane filter. The inorganic P (Pi) in these extracts was determined using the molybdate blue method. The total P (Pt) 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. Analysis of P was through the use of the molybdate blue method (Murphy and Riley, 1962) after adjusting the pH to 6.5 to 7.0. After all extractions, the residue left was also digested using the wet oxidation method of Akinremi et al. (2003) and the P in all extracts and residual P were 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. The organic P (Po) in each extract was estimated as the difference between Pt and Pi.

2.3.4 Statistical Analyses

The experimental design was set up as a completely randomized design with 4 replicates per treatment (NRC, RED, RED+P1, RED+P2, DNC, DNC+P3, DNC+P4). A contrast of treatment means through a simple comparison of treatment means, i.e. the Least Significant Difference (LSD) test of two means was used. 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).

2.4 Results and Discussion

Enzyme supplementation to the RED diets reduced the total P in the feces and manure (Table 2.1). The total P in the feces of pigs fed RED + P1 decreased by 21% relative to the RED diets. However, this was not statistically significant ($p > 0.05$) and may be as a result of variability among animals. Similarly, the addition of 1000 U of phytase per kg of diet to the RED diets (i.e. RED + P2) lowered the total P (16.4 g kg^{-1}) excretion in the feces by 22% when compared to the RED. This indicates that enzyme addition is effective in reducing the total P content of swine feces (Table 2.1). 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 of phytase / kg of diet. Omogbenigun et al. (2003) reported 13% reduction when 500 U / kg diet were fed to piglets. Angel et al. (2005) observed a low TP content in feces of pigs fed RED diet that was supplemented with 515 U of phytase kg^{-1} of diet. However, there was no statistically significant difference in the TP content of feces from NRC and RED diets.

Table 2.1 Total P concentrations in feces, urine and manure from phytase supplemented swine diets

	Dietary Treatments†							
	NRC	**RED**	**RED+P1**	**RED+P2**	**DNC**	**DNC+P3**	**DNC+P4**	**SEM‡**
	----------Total P 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}	1.55
Urine	0.64^{a}	0.04^{d}	0.15^{b}	0.14^{bc}	0.02^{d}	0.05^{cd}	0.03^{d}	0.03
Manure	24.4^{a}	20.7^{ab}	18.4^{bc}	17.5^{bc}	20.6^{ab}	14.1^{c}	17.3^{bc}	1.71

† NRC, diet that contain P at the National Research Council (1998) recommendation; RED, diet with 0.1 percentage units reduction in the available P from NRC (1998) recommendation; RED+P1, reduced diet with 500U of phytase per kg of diet; RED+P2, reduced diet with 1000U of phytase units per kg of diets; DNC, double negative control with no inorganic P; DNC+P3, DNC with 2000U of phytase supplement per kg of diet, DNC+P4, DNC with 4000U of phytase per kg of diet.
‡Pooled standard error of the mean
§Means in the same row with the same letter are not significantly different at the 0.05 probability level.

There was about a 26% reduction in the total P of swine feces when 2000 U of phytase / kg diet was added to the DNC. This reduction was statistically significant ($p \leq 0.05$). On the other hand, the total P of the feces when 4000 U of phytase was added to the DNC diet was not significantly different from the DNC (18.0 vs. 18.4 g kg^{-1})

indicating that phytase supplementation of 2000 U /kg of DNC diet is the best level for reducing P excretion in this study (Table 2.1).

While enzyme addition reduced both fecal and manure total P, the urinary total P was markedly higher in enzyme supplemented diets than those without enzyme addition. This increase in the urinary P output suggests that phytase addition to the pig diets enhanced phytate P digestibility and availability (Zhang et al., 2003 and Ige et al., 2006). However, the total P in urine is much smaller than that in feces and manure. Our result is consistent with that of many researchers that, percent P in urine is smaller than that of feces (MacLean et al., 1983; Baxter et al., 2003; Zhang et al., 2003; Ige et al., 2006). Baxter et al. (2003) reported that the diets used in their experiment was lower in available P than diets that met NRC requirements and thus the urine P was considerably lower than would be expected for pigs fed diets that contain P at NRC requirements. Reducing the available P in our diets by 0.1 percentage units and by totally eliminating mineral P, significantly ($p \leq 0.01$) reduced the P in the urine relative to the NRC diets. At the higher levels of phytase addition (2000 U and 4000 U/kg of diet), there was no significant increase in the urine P in these diets compared to the DNC diets. Thus, the elimination of inorganic P from these diets was sufficient in removing any excess P in the urine of animals that were fed the DNC diets with or without phytase.

The effects of phytase supplementation in reducing manure total P are less substantial at lower levels of phytase addition (500 U and 1000 U/kg diet) when compared to feces. These differences in response could be attributed to the contribution of urine P in the manure samples. At higher levels of phytase additions (2000 U and 4000 U/kg of diet), however, the reduction in the P content of manure was comparable to that

of the feces. The total P of manure from RED diet was smaller than manure from pigs that were fed NRC diets by 15%. The addition of phytase (RED+P1) reduced manure P non-significantly by 11% compared to the RED diet. Increasing the level of phytase addition (RED+P2) resulted in a non-significant 15.4% reduction in manure P compared to RED diet (Table 2.1). Higher levels of phytase addition to the DNC diet had pronounced and statistically significant ($p \leq 0.05$) effect on manure P with a 32% reduction when 2000 U of phytase /kg diet was added to DNC diets. In comparison, the addition of 4000 U of phytase /kg to the DNC diet resulted in a 16% reduction in manure P content. These results confirmed that the 2000 U is the optimum level of phytase that could be added to the DNC diet in this study, as was observed for the feces. These higher levels of phytase addition were included in our dietary treatment to examine if the supplementation of inorganic P could be eliminated through the addition of phytase. Li et al. (1998) reported that the improvement in performance of pigs fed low P diet supplemented with 750 phytase units kg^{-1} diet was equivalent to the effect achieved by adding 2g kg^{-1} inorganic phosphorus (positive control). These researchers concluded that phytase use in swine diets can reduce inorganic P. Toor et al. (2005) reported a 36% reduction in poultry litter P content when they fed reduced NPP (0.2% reduction) and 600 U phytase /kg diet to the birds relative to the normal corn diet. Because little research has been conducted with complete swine manure (feces + urine), there is limited results in the literature to compare our work with.

2.4.1 Effect of Dietary Treatment on Fecal and Manure P in each Fraction

In the feces, no statistically significant effect of treatment was evident at the 0.05 probability level when H_2O and $NaHCO_3$ - P in RED diet were compared with those of NRC diet (Table 2.2). These two fractions of P (H_2O- and $NaHCO_3$ – P) are of environmental significance as they constitute the labile P fraction that can be lost to surface runoff. However, in manure, the H_2O-P fractions of RED and DNC diets were significantly different from those of the NRC diet at the 0.01 probability. This suggests that the smaller urine P content in the RED and DNC diets (Table 2.1) relative to the NRC diet resulted in a significant reduction in the manure soluble P fractions in these diets. Poulsen (2000) concluded that excess dietary P would result in high levels of urinary P.

Table 2.2 P concentration of different fractions in feces as influenced by phytase addition

	Dietary Treatments†						
P Fractions	**NRC**	**RED**	**RED+P1**	**RED+P2**	**DNC**	**DNC+P3**	**DNC+P4**
	----------g kg^{-1}----------						
H_2O	$11.8^{abc§}$	12.8^{a}	10.4^{abcd}	9.23^{d}	12.4^{ab}	9.62^{dc}	10.3^{bcd}
$NaHCO_3$	3.92^{ab}	4.13^{a}	3.26^{ab}	2.55^{bc}	3.03^{ab}	1.59^{c}	2.64^{bc}
Labile	15.7^{ab}	16.9^{a}	13.7^{abcd}	11.8^{dc}	15.4^{abc}	11.2^{d}	12.9^{bcd}
NaOH	2.71^{a}	2.26^{ab}	1.73^{bc}	2.35^{ab}	1.12^{dc}	0.94^{d}	1.46^{dc}
HCl	2.92^{a}	1.57^{b}	1.02^{bc}	0.98^{bc}	0.48^{c}	0.39^{c}	0.95^{bc}
Residual	0.50^{a}	0.40^{ab}	0.33^{b}	0.35^{b}	0.28^{b}	0.27^{b}	0.39^{ab}

† NRC, diet that contain P at the national research council (1998) recommendation; RED, diet with 0.1 percentage units reduction in the available P from NRC (1998) recommendation; RED+P1, reduced diet with 500 U of phytase per kg of diet; RED+P2, reduced diet with 1000 U of phytase units per kg of diets; DNC, double negative control with no inorganic P; DNC+P3, DNC with 2000 U of phytase supplement per kg of diet, DNC+P4, DNC with 4000 U of phytase per kg of diet.

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

Phytase addition to both RED and DNC diets significantly reduced ($p \leq 0.05$) both H_2O- and $NaHCO_3$- P fractions in the feces (Table 2.2). For instance, the supplementation of 1000 U of phytase kg^{-1} diet to RED diets resulted in an average of 9231 mg kg^{-1} and 2551 mg kg^{-1} P in both H_2O and $NaHCO_3$- P fractions, respectively. This resulted in H_2O-P reduction of about 28% in the phytase supplemented diets and about 38% percentage reduction in $NaHCO_3$-P of phytase supplemented diet. Similarly, when 2000 U kg^{-1} diet was added to the DNC, H_2O-P of the feces declined from 12331 mg kg^{-1} to 9618 mg kg^{-1} with a 22% reduction in H_2O-P. The corresponding reduction of 48% in the $NaHCO_3$ fraction of the same treatment was from 3034 mg kg^{-1} to 1592 mg kg^{-1} (Table 2.2). However, in the manure, phytase addition of 1000 U kg^{-1} diet to the

RED diet reduced the H_2O-P from a mean of 9756 to 8300 mg kg^{-1} (15% reduction) in Table 2.4. The $NaHCO_3$-P in manure decreased from 2875 mg kg^{-1} to 1856 mg kg^{-1} (35% reduction) at the same phytase addition in the RED diet. When 2000 U kg^{-1} was added to DNC diet, manure H_2O-P decreased by about 1% from 9156 to 9081 mg kg^{-1}. The reduction in manure $NaHCO_3$ fraction of the same dietary treatment was about 40% (Table 2.4). The impact of phytase addition at reducing the labile P fractions in our study was less substantial in manure compared to feces. This result agrees with the works of Xavier et al. (2004) who reported that the high solubility of urine P increased the proportion of soluble P in manure (feces + urine), though not significantly, when phytase and low-phytate feeds were fed to pigs.

Table 2.3 Mean squares of the analysis of variance for the effect of dietary treatment on the fecal and manure P fractions

		Feces				
		----------Mean Squares----------				
Sources of variations	df	Water-P†	$NaHCO_3$-P‡	NaOH-P§	HCl-P	Residual-P
Diet	6	7.61*	2.99*	1.77***	2.94***	0.02*
Error	21	2.60	0.86	0.21	0.44	0.01
		Manure				
		----------Mean Squares ----------				
Sources of variations	df	Water-P†	$NaHCO_3$-P‡	NaOH-P§	HCl-P	Residual-P
Diet	6	10.0**	1.75 ns	1.05***	0.99**	0.01*
Error	21	1.81	0.80	0.11	0.18	0.003

* significant at 5% level, ** significant at 1% level, *** significant at 0.1% level, and ^{ns} not significant
† Water-P = H_2O Pi + H_2O Po,
‡ $NaHCO_3$-P = $NaHCO_3$-Pi + $NaHCO_3$-Po
§ NaOH-P = NaOH-Pi + NaOH-Po

Table 2.4 P concentration of different fractions in manure as influenced by phytase addition

	Dietary Treatments†						
P Fractions	**NRC**	**RED**	**RED+P1**	**RED+P2**	**DNC**	**DNC+P3**	**DNC+P4**
	----------g kg^{-1}----------						
H_2O	$12.8^{a§}$	9.76^{bc}	10.2^{b}	8.30^{bc}	9.16^{bc}	9.08^{bc}	8.15^{c}
$NaHCO_3$	2.84^{ab}	2.87^{ab}	2.14^{abc}	1.86^{bc}	3.27^{a}	1.97^{abc}	1.44^{c}
Labile	15.7^{a}	12.6^{b}	12.3^{bc}	10.2^{bc}	12.4^{bc}	11.1^{bc}	9.59^{c}
NaOH	2.19^{a}	1.52^{b}	1.40^{bc}	1.86^{ab}	0.92^{cd}	0.91^{cd}	0.89^{d}
HCl	1.79^{a}	1.07^{b}	0.77^{bc}	0.96^{b}	0.46^{bc}	0.33^{c}	$0.54b^{c}$
Residual	0.36^{a}	0.25^{bc}	0.28^{ab}	0.29^{ab}	0.19^{c}	0.22^{bc}	0.26^{bc}

† NRC, diet that contain P at the National Research Council (1998) recommendation; RED, diet with 0.1 percentage units reduction in the available P from NRC (1998) recommendation; RED+P1, reduced diet with 500 U of phytase per kg of diet; RED+P2, reduced diet with 1000 U of phytase units per kg of diets; DNC, double negative control with no inorganic P; DNC+P3, DNC with 2000 U of phytase supplement per kg of diet, DNC+P4, DNC with 4000 U of phytase per kg of diet.

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

2.4.2 P Fractions in Feces and Manure

In this study, about 54 to 75% of the total P in feces was extracted by water while $NaHCO_3$ extracted between 12 to 19% of total P depending on the diets (Figure 2.1). Toor et al. (2005) found that most broiler litter P was in H_2O-P (56 to 77%). These two fractions (H_2O- and $NaHCO_3$-P) were slightly higher in manure with the H_2O-P ranging from 62 to 73% and the $NaHCO_3$ fraction was between 12 to 23% (Figure 2.2). Thus, the labile P fraction (% H_2O-P + % $NaHCO_3$-P) accounted for about 72 to 89% in feces and ranged from 76% to 88% in manure. Our results agreed with those of Ige et al. (2006)

who reported that 84% of the total P in feces was present in labile forms. Qian and Schoenau (2000) also reported a 70% labile P fraction in the manure while Ajiboye et al. (2004) reported that P in hog manure was mainly in labile forms.

There was a non-significant 18% reduction in the water extractable P in feces collected from the RED+P1 diet compared to the RED diet (Table 2.2). On the other hand, the water extractable P for RED+P2 diets was significantly reduced by 28% compared to the RED diets in the feces ($p \leq 0.05$). The addition of higher levels of phytase in the DNC+P3 diet also resulted in a significant reduction of 22% in the feces water extractable P relative to the DNC diet. The feces water extractable P in the DNC+P4 diet, however, resulted in a non-significant 17% decrease, which was not significantly different from that of the DNC diet.

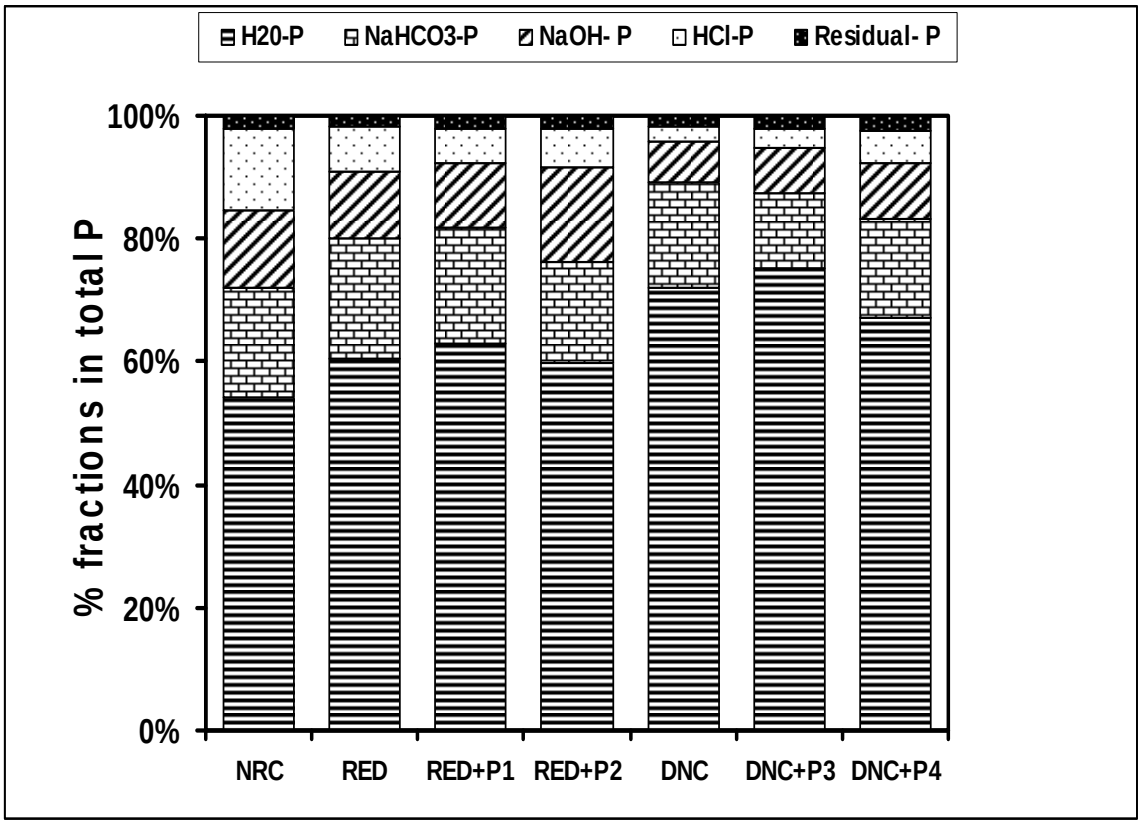

Figure 2.1 Percentage of fecal total P extracted by deionized water (H_2O-P), 0.5 *M* $NaHCO_3$ (pH 8.5, $NaHCO_3$-P), 0.1 *M* NaOH (NaOH-P), and 1 *M* HCl (HCl-P) and residual P fraction as affected by diet manipulation. NRC, National Research Council recommendation (1998); RED, Reduced P (0.1 percentage units) from NRC; RED+P1, RED plus 500 U of phytase kg^{-1} diet; RED+P2, RED plus 1000 U of phytase kg^{-1} of diet; DNC, Double negative control with no inorganic P; DNC+P3, DNC plus 2000 U of phytase kg^{-1} diet, and DNC+P4, DNC plus 4000 U of phytase kg^{-1} diet.

Phytase supplementation decreased the $NaHCO_3$-extractable P by 13.0 to 47.5% depending on the level of addition and diet, resulting in a 16 to 30.4% reduction in the labile P fraction of the phytase supplemented diets. Ige et al. (2006) reported 14 and 18% reduction in labile P resulting from phytase addition to swine diets containing barley-raw pea and barley-micronized peas, respectively. This reduction in the labile P content of

manure following phytase supplementation is desirable for the environment since the labile fraction is prone to runoff and leaching losses (Sharpley and Moyer, 2000). In the manure samples, the effect of phytase addition was less substantial as the water extractable P was reduced by 10 to 14% depending on the diet. This effect resulted in a 2 to 23% reduction of the labile P fraction in the manure. Ajiboye et al. (2004) concluded that P in hog and cattle manures was mainly in labile forms.

No significant differences were observed ($p \leq 0.05$) in the NaOH- , HCl- and residual- P fractions as indicated by the mean squares (Tables 2.3) of both feces and manure samples from the phytase supplemented diets. This showed that phytase addition did not influence these recalcitrant fractions. These fractions were 2 to 15% of the total P in feces and 1 to 14% in manure (Figures 2.1 and 2.2). These results were in agreement with the works of Ajiboye et al., 2004, who reported about 14% of total P in these recalcitrant fractions. The maximum values are also similar to the recalcitrant fraction of 14% that was reported by Ige et al. (2006). These authors concluded that the recalcitrant fractions may not be of great agronomic or environmental impact because of their small proportion of total P.

Moreover, while labile P fractions were decreased by phytase supplementation, the percentages of total P that is labile, especially the water extractable fraction was increased. The addition of 500 U of phytase kg^{-1} diet to RED diet was not significant but slightly increased the percentage of total P extracted by water in the feces from 60 to 63% (Fig 2.1). Similarly, phytase addition of 2000 U kg^{-1} to DNC diet increased the percentage of total fecal P that was extracted by water from 72 to 75%.

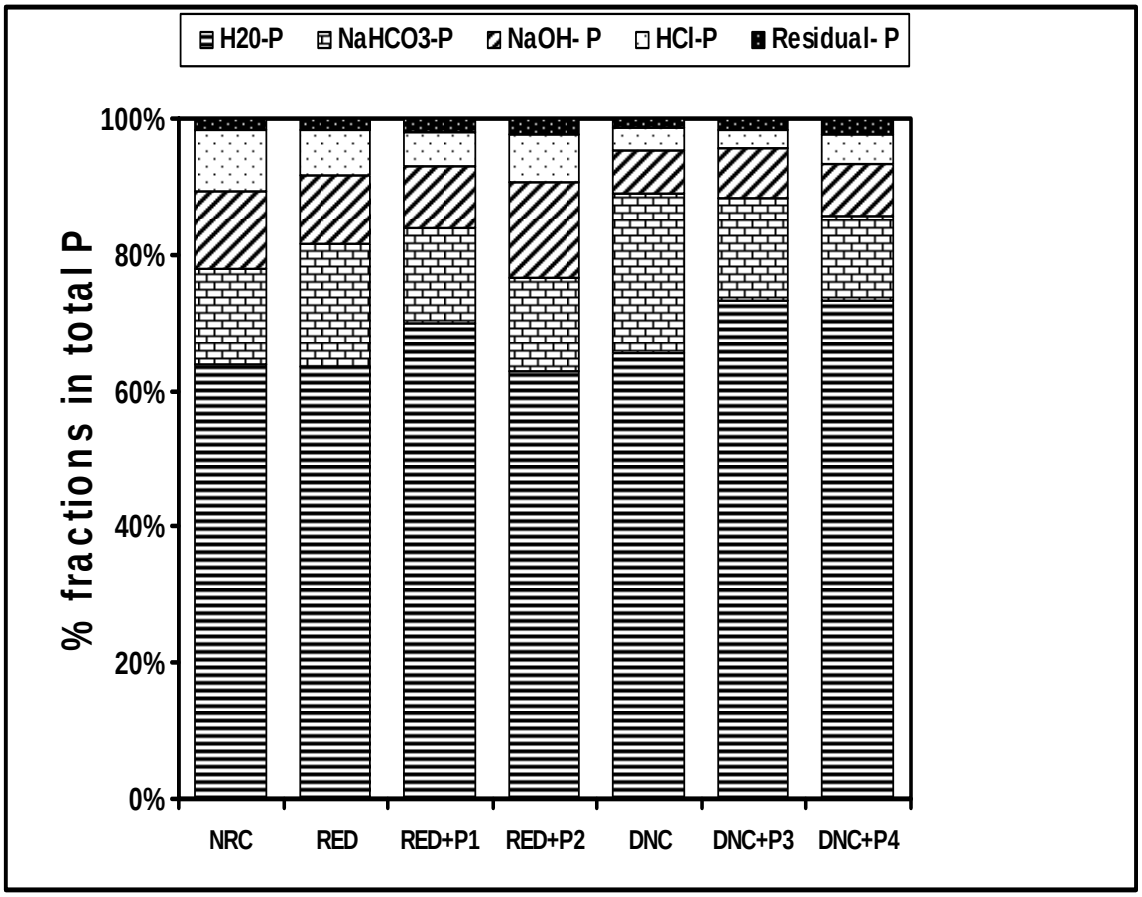

Figure 2.2 Percentage of manure total P extracted by deionized water (H_2O-P), 0.5 *M* $NaHCO_3$ (pH 8.5, $NaHCO_3$-P), 0.1 *M* NaOH (NaOH-P), and 1 *M* HCl (HCl-P) and residual P fraction as affected by diet manipulation. NRC, National Research Council recommendation (1998); RED, Reduced P (0.1 percentage units) from NRC; RED+P1, RED plus 500 U of phytase kg^{-1} diet; RED+P2, RED plus 1000 U of phytase kg^{-1} of diet; DNC, Double negative control with no inorganic P; DNC+P3, DNC plus 2000 U of phytase kg^{-1} diet, and DNC+P4, DNC plus 4000 U of phytase kg^{-1} diet.

A similar non-significant result was obtained for the manure samples (Fig. 2.2) where the percentage of total P extracted by water increased from 63 to 70% with a 500 U phytase addition kg^{-1} to RED diet and from 65 to 73% when 2000 U of phytase was added to the DNC diet. Our results do not agree with those by Maguire et al. (2003) who

showed a decrease in water soluble P (WSP) as a percent of total P (TP) when 600 U of phytase $kg^{-1}$was added to turkey diets. This could be due to the quantity of phytase supplemented or the reduction in NPP requirement (42%). Angel et al. (2005) reported no difference in the WSP content of excreta from birds and feces from pigs fed diets with and without 515 U of phytase addition. The total P recovery by the sequential extraction procedure ranged from 98 to119% in feces and 88 to 139% depending on the diets. Our recovery of P was similar to that reported by Wienhold and Miller (2004) and Turner and Leytem (2004).

2. 5 Summary and Conclusions

Reducing available P (NPP) in diets with phytase addition reduced both total and labile P in feces and manure. Thus, potential detrimental effect on the adjacent water bodies and subsequent eutrophication can be reduced. Our results showed that phytase supplementation of 1000 U and 2000 U kg^{-1} of diet to the RED and DNC diets, respectively, generally decreased total P concentrations in the manure and feces. However, supplementation beyond 2000 U kg^{-1} of diet caused an increase in P excretion which showed that the 2000 U was the best level of phytase from an environmental point of view when no inorganic P was added to the diets in our study. Also the impact of adding phytase in reducing P excretion was greater on the feces than on the manure, indicating the importance of including urine P as was done in this study.

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3. PHOSPHORUS SOLUBILITY IN MANITOBA SOILS TREATED WITH PIG MANURE FROM PHYTASE SUPPLEMENTED DIETS

3.1 Abstract

Dietary manipulation to reduce P losses from manure and manure amended soils has become one of the ways to address environmental concerns arising from intensive animal production. Therefore, we investigated the solubility of manure P, produced from phytase supplemented swine diets, in Manitoba soils. Seven composite manure samples generated from replicates of seven dietary treatments: NRC (a positive control that contains P at NRC (1998) recommendations), RED (a negative control containing 0.1 percentage units reduction, about 33% in available P from 1998 NRC recommendations), RED+P1 (RED plus 500 U of phytase kg^{-1} of diet), RED+P2 (RED plus 1000 U of phytase $kg^{-1}$of diet), DNC (a double negative control with no added inorganic P), DNC+P3 (DNC plus 2000 U of phytase kg^{-1} of diet), DNC+P4 (DNC plus 4000 U of phytase kg^{-1} of diet) were applied at a rate of 75 kg of total P ha^{-1} of soil to four surface Manitoba soils (0-15 cm). The soils used were: Osborne (fine textured and calcareous), Red River (fine textured and non-calcareous), Ladywood (coarse textured and calcareous), and Glenhope (coarse textured and non-calcareous). The treated soils and a control soil (CON) were incubated for 1 wk, 4 wk and 16 wk after which they were extracted using either Mehlich-3 (M3-P), Modified Kelowna (MK-P), Olsen (O-P) or water (W-P). The different methods extracted varying amounts of P depending on the soil and the period of incubation. The order of M3-P solubility was: Osborne>Red river>Ladywood=Glenhope. The absence of inorganic P supplementation in swine diets

increased P solubility in amended calcareous soils. The W-P increased at the end of 4 wk by 2 to 35% and then decreased between 4 wk and 16 wk by 2 to 25% in fine textured soils. In coarse textured soils, W-P decreased at week 4 by 54 to 91% and then increased between week 4 and week 16 of incubation by 52 to 93%. We concluded that addition of phytase to swine diets had no effect on P solubility in manure amended soils and that the absence of inorganic P (reduction of supplemental Ca) in the swine diet increased manure P solubility in amended calcareous soils. Therefore, caution should be exercised in modifying diets to reduce P excretion in manure.

3.2 Introduction

The hog industry in Manitoba has experienced expansion over the years (Statistics Canada, 2006). However, a major challenge facing the industry relates to the nutrient content in manure and its impact on the environment. Poor digestibility of feed grain P by monogastric animals (poultry and swine), results in greater P concentration in their manure. Moreover, current application of manure based on the crop N requirements, leads to more P application than crop removal (Sharpley, 1999) and subsequent long term P accumulation in soils (Sims et al., 2000). This build up of P in soils may potentially lead to increased P losses to water bodies and impairment of water quality (Sharpley et al., 1996; Sims et al., 1998; Maguire and Sims, 2002). The increase in P concentration of many Manitoba streams with time (Jones and Armstrong, 2001) has prompted the establishment of water quality policies and guidelines (Manitoba Conservation 2001).

Nutritional management strategies have been reported to offer the most cost-effective means of reducing the negative impact associated with non-ruminant production (Leneman et al. 1993). Feed additives such as phytase have been known to increase the efficiency of dietary P utilization and thereby reduce the amount and costs of supplemental P in feeds (Jongbloed and Lenis, 1991; Council for Agricultural Science and Technology, 2002; Applegate et al., 2003). However, the evaluation of the environmental implication of phytase supplemented diets can only be obtained by understanding the solubility and reactivity of the manure P in amended soils.

Considerable literatures exists on solubility of manure P in soil for poultry (broiler and turkey) litter and manure generated from phytase supplemented diets (Applegate et al., 2003; Miles et al., 2003; Maguire et al., 2004; Vadas et al., 2004; Smith et al., 2004b; Maguire et al., 2005). In contrast, the solubility in soils of swine manure P from pigs fed phytase supplemented diets has not been thoroughly investigated. Angel et al. (2005) observed that data on the water soluble P (WSP) in manure of pigs fed phytase supplemented diets are scarce. Smith et al. (2004a) did not observe a significant effect of dietary phytase on the dissolved P loss in runoff from their study with swine manure. Phytase inclusion in pigs' diets either decreased or had no effect on the loss of manure dissolved and total P in runoff (Baxter et al., 2003; Gilley et al., 2001).

While reduced supplemental P and phytase addition in diets can lower WSP in manure and manure-amended soils (Maguire et al., 2003; Angel et al., 2005), the effects of total elimination of inorganic P through the addition of phytase in diets of monogastric animals have not been examined in manure-amended soils. Li et al. (1998) reported that the improvement in performance of pigs fed low P diet supplemented with 750 phytase units kg^{-1} diet was equivalent to the effect achieved by adding 2g kg^{-1} inorganic phosphorus (positive control). These researchers concluded that phytase use in swine diets can reduce inorganic P. Toor et al. (2005) fed reduced non phytate P (NPP) (0.2%) and 600 U phytase kg^{-1} diet to birds and found a reduced concentration of H_2O-P relative to the normal corn diet. Similarly, Vadas et al. (2004) added 11 500 PTU (phytase units) kg^{-1} of diet with about 0.2% reduction in available P in poultry diets and reported that phytase addition with or without conservative P reduction (from 0.3 to 0.4%) to poultry rations may not result in a consistent decrease in manure or litter total P. Lack of

statistical difference in total P (TP) excretion convinced Angel et al. (2005) that their reduced diets were not sufficiently low in dietary P.

Moreover, the understanding of swine manure P chemistry in soils over a period of time is important in predicting P solubility and minimizing environmental risks. The behavior of swine manure P in amended soils is dominated by reactions of inorganic P (Peperzak et al., 1959; Gerritse and Vriesema, 1984). Leytem et al. (2004) reported that swine manures are dominated by inorganic phosphate, regardless of the initial diet composition. Solubility and availability of applied soil P is influenced by a number of soil characteristics. For instance, soil available P is influenced by soil texture, because of clay content and soil organic C (Sharpley and Sisak, 1997). Elias-Azar et al. (1980) had earlier reported an increase in relative availability of manure P with increasing pH and sand content.

Determining the relative P solubility of manure from phytase supplemented swine diets will be beneficial in assessing potential P risks of land-applied manure. Therefore, the objectives of this study were to investigate:

- The solubility of manure P from phytase amended diets in soils.
- The effect of incubation time on manure P solubility in these soils.
- The influence of soil type on the solubility of manure P from phytase supplemented swine diets

3.3 Materials and Methods

3.3.1 Characteristics of Soil and Manure Samples

Four surface Manitoba soils (coarse and fine textured calcareous and non-calcareous) were collected from different locations and used for this study. The soils were: Osborne (Rego Humic Gleysol/Gleysolic Humic Vertisol), Red River (Gleyed Rego Black Chernozem/Gleyed Humic Vertisol), Ladywood (Gleyed Dark Gray Chernozem), Glenhope (Gleyed Rego Black Chernozem). A composite sample of each soil was taken from the surface layer (0-15 cm), air dried, crushed and ground to pass through a 2-mm sieve on a roller-grinder and stored in plastic bags prior to incubation. The amendment used in this study was from the freeze-dried manure samples of the first study of the thesis. A composite of manure sample from each dietary treatment was formed by mixing equal amounts of each of the four replicates properly to give a total of seven manure treatments. The air dried soils were mixed with the swine manure generated from the first study at a rate of 75 kg of total P ha^{-1} of soil. The choice of this rate was to have detectable changes in P solubility in the laboratory.

Properties of soils and manure treatments are presented in Tables 3.1 and 3.2. The initial extractable P concentration in the soil was determined using the $NaHCO_3$ method (Olsen et al., 1954). The particle size analysis was carried out using the pipette method following dispersion with sodium hexametaphosphate and removal of organic matter with hydrogen peroxide (Sheldrick and Wang, 1993). Soil pH was measured using a 1:2 soil to water ratio (Hendershot et al., 1993). Total P in manure treatments was determined by the H_2O_2-H_2SO_4 wet oxidation method (Akinremi et al., 2003).

3.3.2 Soil Amendments and Incubation Study

The experiment was carried out using the seven composite manure generated from these seven dietary treatments: NRC (a positive control that contains P at NRC (1998) recommendations), RED (a negative control containing 0.1 percentage units reduction, about 33% in available P from 1998 NRC recommendations), RED+P1 (RED plus 500 U of phytase kg^{-1} of diet), RED+P2 (RED plus 1000 U of phytase $kg^{-1}$of diet), DNC (a double negative control with no added inorganic P), DNC+P3 (DNC plus 2000 U of phytase kg^{-1} of diet), and DNC+P4 (DNC plus 4000CU of phytase kg^{-1} of diet). The air dried soil (100 g) was mixed with the manure at a rate of 75 kg P ha^{-1}. Each treatment was replicated four times. The soil was incubated at field capacity for 1, 4, and 16 wks at 20^{o}C in 500-mL glass jars with perforated lids to allow for gaseous exchange and prevent anaerobic condition. Samples were weighed weekly and water was added to keep the moisture content at field capacity. After each period of incubation, the soils were air dried, ground and stored for subsequent analysis.

3.3.3 Chemical Analysis

The soils were analyzed with four different extracting solutions including Mehlich-3 (M3-P), Modified Kelowna (MK-P, Ashworth and Mrazek, 1995), Olsen (O-P) and Water (W-P). A 2.5-g of soil sample was weighed into a 50-mL centrifuge tube and was extracted with 25 mL of Mehlich-3 solution (0.2 *M* CH_3COOH + 0.25 *M* NH_4NO_3 + 0.015 *M* NH_4F + 0.013 *M* HNO_3 + 0.001 *M* EDTA) and shaken for 5 mins (at 120 excursions per minute) on a reciprocal shaker. The same procedure was used for

Modified Kelowna extraction (0.015 *M* NH_4F + 1.0 *M* NH_4OAc + 0.5 *M* CH_3COOH); however, the solution was shaken for 15 mins on a reciprocating shaker (150 excursions per minute). The extracts were then filtered into plastic vials using medium retention filter paper (Whatman® No. 40). Water extraction was done by adding 20 mL of deionized water to a 2.0-g air dried soil sample in a 50-mL centrifuge tube and shaking on a reciprocal shaker for 1 hr (at 120 excursions per minute) (Self-Davis et al., 2000). The tube was centrifuged at 10,000 rpm for 10 min at room temperature and the supernatant was filtered under suction using a 0.45-μm cellulose membrane. Meanwhile, Olsen P extraction was by adding 20-mL of 0.5 M $NaHCO_3$ (pH = 8.5) plus 0.25 g charcoal and the solution was shaken on a reciprocal shaker for 30 min (at 120 excursions per minute). The extract was filtered using medium retention filter paper (Whatman® No. 40). All extracts were then analyzed for the molybdate reactive P using the molybdate blue method (Murphy and Riley, 1962).

Table 3.1 Selected properties of the soils used for the incubation study

Property	**Osborne**	**Red River**	**Ladywood**	**Glenhope**
Classification	Calcareous	Non- Calcareous	Calcareous	Non- Calcareous
§Soil textural class	HC	HC	VFSL	LFS
$CaCO_3$ (% Eq.)	5.7	0	11.0	1.3
CEC ($cmol_c\ kg^{-1}$)	42.0	39.1	11.2	11.3
Field Capacity (%wt)	25.0	25.0	18.2	17.1
†% sand	9	6	73	83
% silt	41	25	17	9
% clay	50	69	10	8
pH	7.7	6.8	7.7	7.5
¶Exch Ca^{2+} ($cmol_c\ kg^{-1}$)	17.5	11.9	8.44	4.86
Exch Mg^{2+} ($cmol_c\ kg^{-1}$)	3.52	4.42	2.07	1.19
Exch K^+ ($cmol_c\ kg^{-1}$)	2.98	3.06	0.40	0.23
Exch Na^+ ($cmol_c\ kg^{-1}$)	0.44	0.46	0.28	0.28
Exch Al^{3+} ($cmol_c\ kg^{-1}$)	0.01	0.01	0.01	0.01
Exch Fe^{3+} ($cmol_c\ kg^{-1}$)	0.01	0.01	0.01	0.01
Extractable P ($mg\ kg^{-1}$ soil)‡	33.1	23.0	7.7	5.7

§HC: Heavy Clay; VFSL: Very fine sandy loam; LFS: Loamy fine sand.
†Source: Manitoba Soil Survey, MAFRI Winnipeg.
¶Exchangeable Ca^{2+}, Mg^{2+}, K^+, Al^{3+}, and Fe^{3+} in $cmol_c\ kg^{-1}$.
‡Olsen P.

Table 3.2 Analysis of elements present in the manure treatments after composting four replicates

Manure Treatments†	% P¶	% Ca	% Mg	% Al	% Fe
NRC	2.12[a§]	1.04[a]	0.56[d]	0.06	0.12
RED	1.87[b]	1.09[a]	0.62[bc]	0.06	0.11
RED+P1	1.74[b]	0.89[b]	0.64[b]	0.05	0.09
RED+P2	1.46[c]	0.76[c]	0.49[e]	0.04	0.07
DNC	1.77[b]	1.06[a]	0.63[b]	0.02	0.05
DNC+P3	1.30[d]	0.77[c]	0.59[cd]	0.05	0.08
DNC+P4	1.85[b]	1.03[a]	0.76[a]	0.07	0.10

† NRC, diet that contain P at the National Research Council (1998) recommendation; RED, diet with 0.1 percentage units reduction in the available P from NRC (1998) recommendation; RED+P1, RED diet with 500 U of phytase per kg of diet; RED+P2, RED diet with 1000 U of phytase units per kg of diets; DNC, double negative control with no inorganic P; DNC+P3, DNC with 2000 U of phytase supplement per kg of diet, DNC+P4, DNC with 4000 U of phytase per kg of diet.
§Means in the same column with the same letter are not significantly different at the 0.05 probability level.
¶% element analyzed by manure digestion and determination by inductively couple plasma-optical emission spectroscopy.

3.3.4 Statistical Analyses

The incubation experiment was carried out as a completely randomized design (CRD) with eight treatments (comprising of seven manure treatments and a control), four replicates and three factors (Soil, treatments and period of incubation). Extractable P from Mehlich-3, Modified Kelowna, Olsen and water were analyzed statistically by two-

way and three-way analysis of variance (ANOVA) using the Generalized Linear Models (GLM) procedure (SAS Institute, Inc., Cary. NC) at 0.05 level of significance.

3.4 Results and Discussions

3.4.1 Soil Characteristics

The four soils used for this study comprised of two fine textured (Osborne and Red River, calcareous and non-calcareous respectively), and two coarse textured soils (Ladywood and Glenhope with the former being calcareous and the latter, non-calcareous). In the fine textured soils, the cation exchange capacity (CEC) was 42.0 $cmol_c\ kg^{-1}$ in Osborne and 39.1 $cmol_c\ kg^{-1}$ in the Red River soil. Their $CaCO_3$ equivalent was 5.7% in Osborne but 0% in Red River soils (Table 3.1). The CEC measured in the Ladywood and Glenhope soils (11.2 and 11.3 $cmol_c\ kg^{-1}$) were similar, however, differences in their calcareous nature were shown in the $CaCO_3$ equivalents. Ladywood had a $CaCO_3$ percentage equivalence of 11.0 and the Glenhope had 1.3%.

Comparing the physical properties of each of the two categories (fine vs. coarse texture) of soils indicated that the Osborne soil has lower clay content but higher silt percentage than the Red River soil. The Ladywood and Glenhope soils had the same field capacity. The proportion of sand is, however, slightly higher in the Glenhope than in the Ladywood soil. The Olsen extractable P was 33.1 and 23.0 mg kg^{-1} respectively, in the Osborne and Red River soils and 7.7 and 5.7 mg kg^{-1} in Ladywood and Glenhope, respectively. These Olsen extractable P concentrations would be regarded as low P soils

(Ladywood and Glenhope) and very high P soils (Osborne and Red River) according to Manitoba's Soil Fertility Guide (Manitoba Agriculture and Food, 2001).

3.4.2 Manure P Characteristics

Reduction in the available P of the NRC diets produced a significant decrease ($p \leq 0.05$) in the manure P generated from pigs fed RED and DNC diets respectively (Table 3.2). Phytase supplementation of diets had significant effects on the composite manure P produced. Addition of 1000 U of phytase to RED diets resulted in a significant reduction in manure P from 1.87 to 1.46%. Similarly, manure P produced in the DNC diet was significantly decreased from 1.77% to 1.30% with addition of 2000 U of phytase to the DNC diet. However, the manure P increased when 4000 U of phytase was added to the DNC diet, indicating that supplementation to DNC diets is optimum at the 2000 U of phytase for this study. The inclusion of these higher levels of phytase into the DNC diets was to determine if the addition of high levels of phytase could totally eliminate the supplementation of inorganic P in diets. A detailed discussion of these results is presented in Chapter 2 of this thesis.

3.4.3 Solubility of Manure Phosphorus in Soils following Dietary Manipulation

The amount of P extracted from the amended soils differs depending on the extracting solution. Since the soils had varying intrinsic extractable P, there were also differences in the solubility of P in both amended and unamended soils (Tables 3.3 and 3.4). Mehlich-3 extracted the greatest amount of P in all the soils. Mehlich-3 extractable

P ranged from 79 to 105 mg kg^{-1} in Osborne soil, 51 to 68 mg kg^{-1} in Red river soil, 14 to 34 mg kg^{-1} in

Table 3.3 Extractable P concentration (mg kg^{-1}) after incubation of amended calcareous soils

	§H_2O			Olsen			Kelowna			Mehlich-3		
Treatment	1wk	4wk	16wk	1wk	4wk	16wk	1wk	4wk	16wk	1wk	4wk	16wk
						Osborne soil						
¶CON	5.61^{c}†	6.40^{c}	6.28^{e}	28.7^{d}	29.4^{b}	35.4^{c}	57.1^{b}	58.8^{c}	62.2^{c}	88.7^{c}	90.4^{b}	93.1^{b}
NRC	7.36^{b}	7.94^{b}	7.78^{bc}	33.7^{cb}	31.8^{b}	34.7^{cd}	56.2^{b}	55.9^{c}	63.2^{c}	79.4^{d}	80.3^{c}	84.9^{c}
RED	7.56^{b}	8.05^{b}	7.50^{cd}	31.6^{cd}	31.8^{b}	33.6^{de}	56.2^{b}	55.1^{c}	61.6^{cd}	79.9^{d}	78.9^{c}	81.4^{cd}
RED+P1	7.11^{b}	8.14^{b}	7.22^{d}	31.2^{cd}	31.5^{b}	32.5^{e}	56.1^{b}	55.8^{c}	58.4^{e}	79.0^{d}	79.7^{c}	80.0^{d}
RED+P2	7.69^{b}	7.88^{b}	7.49^{cd}	33.4^{c}	29.3^{b}	33.0^{e}	58.0^{b}	56.6^{c}	59.5^{de}	82.1^{d}	78.9^{c}	81.9^{cd}
DNC	8.45^{a}	8.87^{a}	7.96^{ab}	39.9^{a}	36.5^{a}	40.7^{ab}	71.3^{a}	68.6^{ab}	74.1^{a}	105.4^{a}	102.8^{a}	104.9^{a}
DNC+P3	7.83^{ab}	8.32^{b}	7.41^{d}	40.8^{a}	37.2^{a}	39.9^{b}	70.2^{a}	70.4^{a}	69.8^{b}	104.9^{a}	102.6^{a}	102.1^{a}
DNC+P4	7.25^{b}	8.02^{b}	8.15^{a}	36.5^{b}	35.1^{a}	41.7^{a}	67.1^{a}	65.4^{b}	74.0^{a}	99.8^{b}	99.9^{a}	104.5^{a}
						Ladywood soil						
CON	2.54^{e}	0.58^{a}	1.42^{d}	6.51^{e}	5.91^{c}	5.28^{d}	7.7^{d}	9.5^{d}	7.94^{e}	16.3^{d}	14.8^{f}	14.4^{d}
NRC	4.02^{cd}	1.63^{a}	3.40^{b}	17.4^{a}	13.5^{a}	11.3^{bc}	14.7^{bc}	13.2^{c}	18.1^{b}	31.4^{b}	28.5^{cd}	29.8^{b}
RED	3.83^{d}	1.18^{a}	3.37^{b}	15.4^{bc}	13.8^{a}	11.1^{bc}	14.0^{c}	13.9^{bc}	17.2^{cd}	29.7^{c}	27.4^{e}	27.9^{c}
RED+P1	4.59^{b}	1.49^{a}	3.31^{b}	16.6^{ab}	13.1^{ab}	11.3^{bc}	16.9^{ab}	22.2^{a}	16.9^{d}	30.3^{cb}	28.9^{c}	27.7^{c}
RED+P2	4.77^{b}	1.33^{a}	3.48^{b}	15.8^{bc}	14.2^{a}	11.8^{ab}	14.9^{bc}	16.3^{b}	17.6^{cb}	31.6^{b}	29.8^{b}	29.0^{b}
DNC	5.22^{a}	0.73^{a}	3.43^{a}	16.2^{ab}	14.2^{a}	12.2^{a}	17.2^{a}	15.3^{cb}	18.7^{a}	33.5^{a}	31.5^{a}	31.3^{a}
DNC+P3	4.41^{bc}	1.15^{a}	3.39^{b}	14.3^{dc}	13.2^{a}	10.9^{c}	14.8^{bc}	13.1^{c}	16.9^{d}	30.1^{bc}	27.8^{de}	27.6^{c}
DNC+P4	4.01^{cd}	1.01^{a}	3.10^{c}	13.4^{d}	11.9^{b}	11.2^{bc}	14.9^{bc}	14.0^{cb}	17.5^{cd}	29.4^{c}	27.2^{e}	27.4^{c}

§H_2O = Water extractable P; Olsen = $NaHCO_3$-extractable P; Kelowna = Modified Kelowna Extractable P; and Mehlich-3 = Mehlich-3 extractable P.

¶CON = Gross P extracted in control (unamended) soil.

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

Table 3.4 Extractable P concentration (mg kg^{-1}) after incubation of amended non-calcareous soils

	§H_2O			Olsen			Kelowna			Mehlich-3		
Treatment	1wk	4wk	16wk	1wk	4wk	16wk	1wk	4wk	16wk	1wk	4wk	16wk
						Red River soil						
¶CON	2.33^{d}†	3.60^{d}	2.76^{e}	33.2^{d}	31.2^{d}	41.4^{e}	37.3^{ab}	31.8^{c}	38.4^{d}	53.8^{b}	51.3^{d}	59.2^{e}
NRC	3.13^{ab}	4.28^{ab}	4.16^{b}	36.2^{bc}	35.5^{bc}	44.5^{cd}	36.3^{abc}	38.7^{a}	40.7^{c}	61.5^{a}	61.7^{ab}	62.4^{cde}
RED	3.^{34a}	4.54^{a}	4.70^{a}	37.7^{ab}	36.0^{abc}	44.0^{d}	37.0^{ab}	38.6^{a}	42.3^{b}	63.5^{a}	63.1^{a}	63.4^{bcd}
RED+P1	3.14^{ab}	3.75^{dc}	3.56^{c}	37.3^{abc}	31.2^{d}	46.5^{b}	35.2^{bc}	33.9^{bc}	43.6^{ab}	61.8^{a}	55.6^{d}	68.2^{a}
RED+P2	3.34^{a}	4.01^{bc}	3.57^{c}	37.4^{abc}	33.8^{c}	44.4^{cd}	37.3^{a}	40.5^{a}	40.1^{c}	63.8^{a}	59.4^{bc}	61.4^{de}
DNC	2.98^{bc}	4.28^{ab}	3.46^{cd}	35.6^{c}	37.0^{ab}	50.2^{a}	34.7^{c}	38.6^{a}	44.4^{a}	61.3^{a}	62.1^{ab}	66.6^{ab}
DNC+P3	2.82^{c}	3.69^{d}	3.04^{de}	36.9^{abc}	35.1^{bc}	45.4^{cb}	34.8^{c}	34.7^{b}	40.5^{c}	61.7^{a}	56.8^{cd}	63.2^{bc}
DNC+P4	3.22^{ab}	4.45^{a}	3.36^{cd}	38.4^{a}	38.4^{a}	46.6^{b}	37.1^{ab}	40.0^{a}	43.5^{ab}	63.8^{a}	59.3^{bc}	65.2^{abc}
						Glenhope soil						
CON	1.44^{d}	0.34^{b}	3.03^{bc}	5.39^{c}	5.78^{c}	5.62^{e}	6.8^{d}	7.5^{c}	7.25^{f}	12.2^{f}	11.5^{g}	11.6^{f}
NRC	2.50^{a}	0.40^{b}	1.23^{d}	17.5^{a}	15.6^{a}	15.9^{b}	17.4^{a}	18.6^{a}	19.5^{a}	29.7^{ab}	28.3^{b}	28.5^{ab}
RED	2.24^{bc}	0.28^{b}	2.63^{c}	16.0^{a}	15.9^{a}	16.0^{b}	15.9^{bc}	17.5^{ab}	18.4^{bc}	29.6^{ab}	27.2^{cd}	27.2^{c}
RED+P1	2.40^{abc}	0.31^{b}	4.19^{ab}	17.3^{a}	16.0^{a}	15.7^{b}	14.7^{c}	17.7^{ab}	18.1^{cd}	28.6^{c}	26.6^{d}	26.6^{c}
RED+P2	2.43^{ab}	0.51^{b}	3.97^{ab}	17.9^{a}	15.5^{a}	14.8^{c}	15.3^{c}	17.6^{ab}	17.5^{d}	30.1^{a}	28.9^{a}	25.6^{d}
DNC	2.50^{a}	0.53^{b}	4.46^{a}	15.6^{ab}	15.9^{a}	15.9^{b}	16.6^{ab}	17.9^{ab}	18.6^{bc}	29.0^{bc}	27.4^{c}	28.0^{b}
DNC+P3	2.46^{ab}	0.54^{a}	4.56^{a}	15.7^{a}	14.9^{ab}	17.9^{a}	15.5^{bc}	17.0^{ab}	18.8^{b}	26.7^{d}	25.5^{e}	29.0^{a}
DNC+P4	2.18^{c}	1.00^{b}	3.83^{ab}	13.4^{b}	13.5^{b}	13.4^{d}	14.9^{c}	15.9^{b}	15.9^{e}	24.6^{e}	23.2^{f}	23.5^{e}

§H_2O = Water extractable P; Olsen = $NaHCO_3$-extractable P; Kelowna = Modified Kelowna Extractable P; and Mehlich-3 = Mehlich-3 extractable P.

¶CON = Gross P extracted in control (unamended) soil.

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

Table 3.5 Sums of squares for the factorial model, and sum of squares of each model species as a proportion of the total sum of squares (Eta values) in each extraction method.

		§H_2O		**Olsen**		**Kelowna**		**Mehlich-3**	
Source	†df	Type I SS	‡η^2 (%)	Type I SS	η^2 (%)	Type I SS	η^2 (%)	Type I SS	η^2 (%)
Soil	3	1739.49***	77.20	52987.00***	89.08	145501.36***	94.78	273175.07***	93.70
Period	2	74.01***	3.28	644.87***	1.08	824.57***	0.54	283.31***	0.10
Treatment	7	57.66***	2.56	2001.07***	3.36	2270.43***	1.48	6053.73***	2.08
Soil*Period	6	251.24***	11.15	1864.58***	3.13	244.58***	0.16	359.78***	0.12
Soil*Treatment	21	35.39***	1.57	1093.70***	1.84	3145.03***	2.05	10068.82***	3.45
Period*Treatment	14	7.33***	0.32	119.01***	0.20	100.45**	0.07	116.76**	0.04
Soil*Period*Treatment	42	36.37***	1.61	271.20***	0.46	513.68***	0.33	416.97***	0.14
Error	288	51.84	2.30	498.44	0.84	909.69	0.59	1078.48	0.37
Total SS	383	2253.33		59479.88		153509.79		291552.92	

§H_2O = Water extractable P; Olsen = $NaHCO_3$-extractable P; Kelowna = Modified Kelowna Extractable P; and Mehlich-3 = Mehlich-3 extractable P.

**significant at 0.01 level, ***significant at 0.001 level

‡η^2 (Eta values)$_{species}$ = $\frac{SS_{Species}}{SS_{Total}}$ x 100 % (Fern and Monroe, 1996)

†df = degree of freedom

Ladywood and 12 to 30 mg kg^{-1} in Glenhope soils. The amount of P extracted by the Modified Kelowna (Kelowna) and Olsen methods were generally similar with the Kelowna being slightly higher in few cases. Water extractable P was the smallest regardless of the soil. It varied from as low as 0.28 to a high of 7.94 mg kg^{-1} depending on the soil type and manure treatment.

In the Osborne soil, the application of manure decreased the Mehlich-3 soil test P. Table 3.3 showed that the application of swine manure to the soil decreased the soil test P in the NRC, RED, RED+P1 and RED+P2 treatments when compared to the unamended (CON) soil. For instance, at the end of 4 wk incubation, the Mehlich-3 P in the CON treatment was 90.4 mg kg^{-1} compared to the NRC treatment which was 80.3 mg kg^{-1}. Bond et al. (2006) recently reported that soils with greater initial M3-P regardless of fertilization rate and soil type would have an increased WSP following fertilization. They showed the importance of avoiding P applications to soils that are high in M3P in order to reduce the risk of P loss. However, our results showed that in some soils, the application of moderate amounts of swine manures can actually reduce soil test P rather than increase it.

The main treatment effects (Soil, Period and Manure) are highly significant ($p <$ 0.001), as well as their two-way and three-way interactions (Soil*Period*Treatment) were all significant at 0.01 probability (Table 3.5). However, as shown above (Table 3.5), the soil, accounted for the greatest proportion of the total variance in P solubility as measured by the various extractants. The proportion of soil's contribution to the variation in extracted P ranged from 77% to as high as 95% indicating that the soil is the main effect that accounted for the greatest proportion of the total sum of squares.

3.4.4 Effects of Dietary Treatments on the Manure P Solubility in Soils

It is worthwhile to note that manure was applied to these soils based on the same total P rate (75 kg ha^{-1}). This resulted in the addition of manure with different concentrations of water soluble P (WSP) to the soils (Table 3.6). This is consequence of the fact that modifying diets to reduce P excretion in animals affected total and WSP concentrations in different ways (Maguire et al., 2004). The effects of dietary modification on manure P solubility in soils vary with the calcareous nature as well as the textural characteristics of the soils. In the calcareous soils (Osborne and Ladywood), DNC treatment had the highest P solubility while RED+P1 treatment was the least soluble. There was, however, no statistically significant effect of phytase addition on P solubility of manured soils.

Table 3.6 Dietary P formulation and P fractions extracted in manure used for the incubation study.

			P extracted sequentially by			
Diets¶	P formulation	Phytase	H_2O	$NaHCO_3$	NaOH	HCl
		U kg^{-1}‡	----------g kg^{-1}----------			
CON						
NRC	NRC†		12.8^{a}§	2.84^{ab}	2.19^{a}	1.79^{a}
RED	NRC – 0.1% P		9.76^{bc}	2.87^{ab}	1.52^{b}	1.07^{b}
RED+P1	NRC – 0.1% P	500	10.2^{b}	2.14^{abc}	1.40^{bc}	0.77^{bc}
RED+P2	NRC – 0.1% P	1000	8.30^{bc}	1.86^{bc}	1.86^{ab}	0.96^{b}
DNC	NRC – DCP		9.16^{bc}	3.27^{a}	0.92^{cd}	0.46^{bc}
DNC+P3	NRC – DCP	2000	9.08^{bc}	1.97^{abc}	0.91^{cd}	0.33^{c}
DNC+P4	NRC – DCP	4000	8.15^{c}	1.44^{c}	0.89^{d}	0.54^{bc}

¶CON = unamended control soil, NRC = manure from the diet containing P at the National Research Council recommendation (1998); RED = manure from a reduced diet containing P of 0.1 percentage unit reduction of NRC; RED+P1 = manure from diet containing RED plus 500 U kg-1 phytase; RED+P2 = manure from diet containing RED plus 1000 U kg-1 phytase; DNC = manure from diets with no inorganic P; DNC+P3 = manure from DNC diet plus 2000 U kg-1 phytase; DNC+P4 = manure from DNC diets plus 4000 U kg-1 phytase.

†NRC = National Research Council; DCP = Dicalcium phosphate.

‡Units of phytase per kg of diet

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

The solubility of manure phosphorus from the NRC treatments was not different from that of P solubility of the RED treatment in all soils, although the WSP concentration of RED manure was significantly less than that of the NRC. (Tables 3.3, 3.4 and 3.6). Addition of phytase to the RED diets did not have a consistent influence on the solubility of manure P in soils. Depending on the soil type, phytase supplementation generally has no significant effect on the solubility of P in manured soils. Gilley et al. (2001) reported that phytase addition to swine diets did not have a significant effect on the soluble P losses in runoff.

One striking and interesting effect of dietary treatment observed in the calcareous soils was the significant increase in solubility of manure P from the DNC treatments compared to CON, NRC and RED treatments. In Osborne and Ladywood soils, there were significant increases in P solubility ($p < 0.05$) in the DNC diets both at short (1wk) and long term (16wks) incubation periods compared to CON, NRC and RED treatments (Table 3.3). These two soils are calcareous in nature as shown by their high pH (7.7) and their $CaCO_3$ contents, which was 5.7% in Osborne and 11.0% in Ladywood (Table 3.1). It should be noted that the DNC treatment was from the diet that contained no inorganic P (dicalcium phosphate). This indicates that the absence of cations such as Ca in these diets may contribute to the increase in solubility of manure P in soils. Ige et al. (2005) concluded that exchangeable Ca is one of the factors that influenced P retention capacity. Thus, the solubility of manure P increased, as the retention capacities of these soils were reduced. This implies that the appropriate supplementation of inorganic Ca-P in swine diets could serve as a means of reducing manure P solubility in amended soils.

In the Osborne soil, after 1wk incubation, the water extractable P in DNC treatment was 8.45 mg kg^{-1} which was greater than the 7.83 mg kg^{-1} that was measured in the treatment containing 2000 U of phytase (Table 3.3). This difference was not statistically significant at this time, however, after 16 wk incubation, the difference between these two treatments was significant (7.96 vs 7.41 mg kg^{-1}). In the Mehlich-3 extractions, there was no difference as the extractable P was from 105.4 to 104.9 mg kg^{-1} when 2000 U of phytase was added to the DNC dietary treatment. The extractable P then decreased significantly with the addition of 4000 U of phytase in the DNC treatment. Similar and even more significant effects were observed in the Ladywood soil (Table 3.3). Thus, the absence of inorganic P supplementation in diets increased P solubility in calcareous soils (probably due to the absence of supplemental Ca).

3.4.5 Effects of Incubation Time on the Solubility of Manure P

There was a significant effect of incubation time on the solubility of manure P in the four soils used. This effect varied depending on the soil texture and the extraction method used. In some soils, depending on the extraction method used, there was an initial increase at 1 wk and this was reduced at 4 wks, which later increased after 16 wk of incubation. And in other soils, the extraction methods were able to show an increase in P solubility with time. These differences may be as a result of the differences in the physico-chemical properties of the soils (Table 3.1). Osborne soil has a higher CEC than the Red River soil and a greater concentration of exchangeable Ca^{2+} than the Red River soil. The clay content in the Osborne was 50% and that of Red River was 69%.

Ladywood soil has a similar CEC as the Glenhope soil, greater level of exchangeable Ca^{2+}, and greater clay content (10%) than the Glenhope soil (8%).

3.4.5.1 Effect of Incubation Time on Manure P Solubility in Fine Textured Soils as Measured using Water Extraction.

Figure 3.1 illustrates the effects of incubation time on solubility of manure P in the Osborne and Red River soils. In the Osborne soil, water extractable P from the manure amended soil increased between 1wk and 4 wk incubation and then declined between 4 wk and 16 wk (Figure. 3.1a). The increase between 1 wk and 4 wk ranged between 2 to 13%. These changes in the water extractable P with time could be due to the mineralization of organic and release of insoluble P forms in the native soil, as the increase was great in the control soil (12% between 1 and 4 wk). The decline in the extractable P may be due to some forms of retention. Water extracts the labile and dynamic fraction of soil P and changes in water extractable P followed the same pattern with time in manured soils as we observed in the control soil (Fig 3.1a). This shows that manure treatments in the Osborne soil had less influence on the mineralization of P with time. Increases in soil WSP following the addition of poultry litter or inorganic P were significantly correlated to initial soil P (Pot et al., 2003). This shows the soil's ability to buffer moderate input of P.

A similar trend was observed in the water extractable P of the Red River soil (Figure 3.1b), however, with greater percentage increase of 16 to 35% between 1 and 4 wk of incubation in all treatments. This increase was greatest in the control soil (35%) compare to the treated

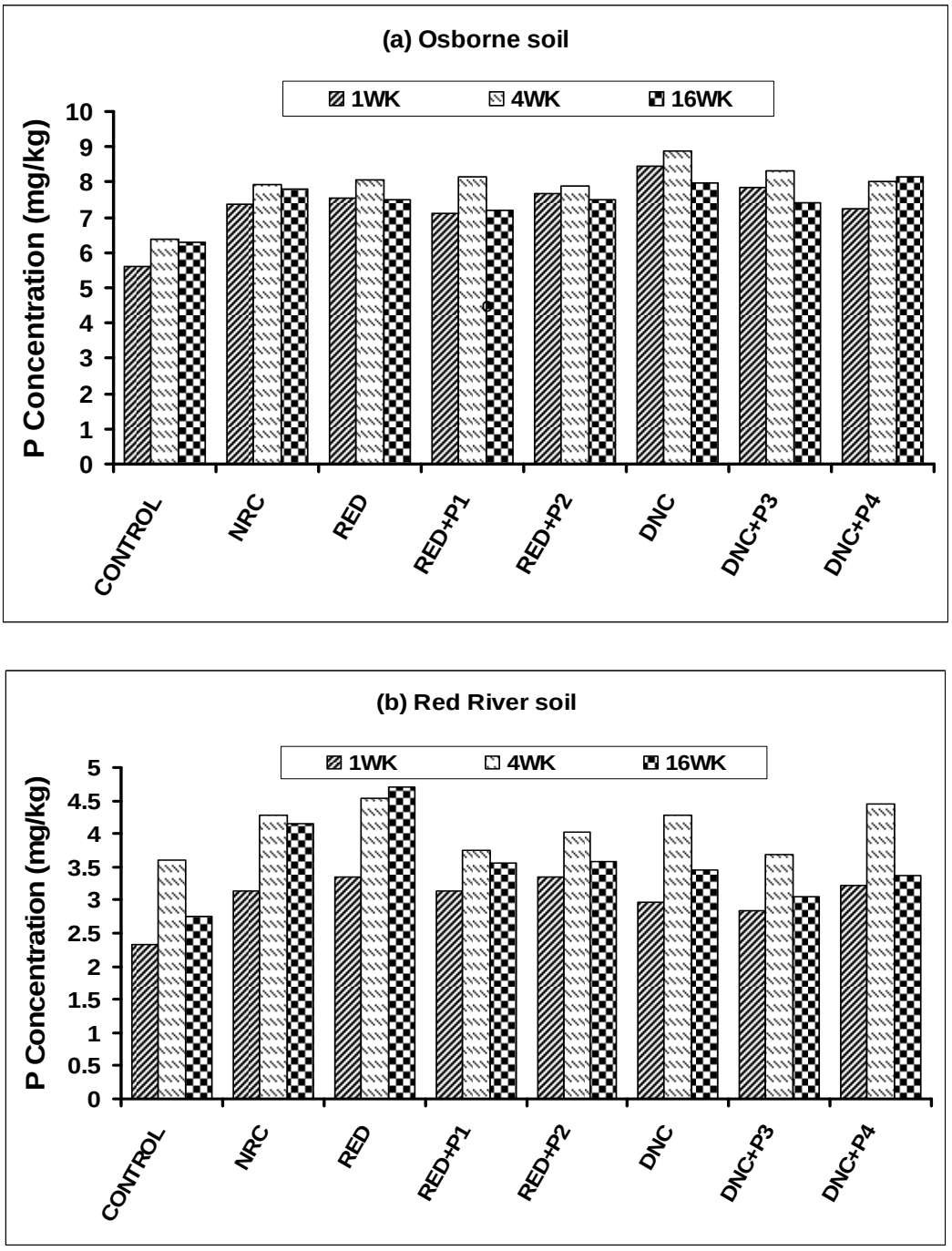

Figure 3.1 Effects of incubation time on Water P solubility in (a) Osborne and (b) Red River soils

soils. The percentage reduction of water soluble P between 4 and 16 wk ranged from 2 to 24% in the Red River clay soil. The greater solubility observed in the Red River soil than the Osborne soil could be as a result of differences in their P saturation indices (not determined). Hooda et al. (2000) found that P saturation index (PSI) was the most significant soil property for predicting water desorbable P from contrasting soils.

3.4.5.2 Effect of Incubation Time on Manure P Solubility in Fine Textured Soils as Measured using Olsen and Kelowna Extraction.

There was no change in the Olsen extractable P between 1 and 4 wk in the Osborne control soil, but an increase of about 17% was observed between the 4 and 16 wk of incubation (Figure 3.2). However, in the treated soil, the Olsen extractable P declined, although not significant (4 – 12%) between the periods of 1 and 4 wks, probably due to the retention of P in the soil (Figure 3.2a). After 16 wk of incubation, there was an increase of about 3 to 15% in the Olsen extractable P of the amended Osborne soil relative to the amount at 4 wk. This indicates mineralization of P in the amended soil after 16 wk.

The same trend of an initial decline and followed by an increase in extractable P was observed in the Olsen extractable P of Red River soil. However, the reductions and increases in the Red River clay were greater than those of the Osborne soil (Figure 3.2b). Between 1 wk and 4

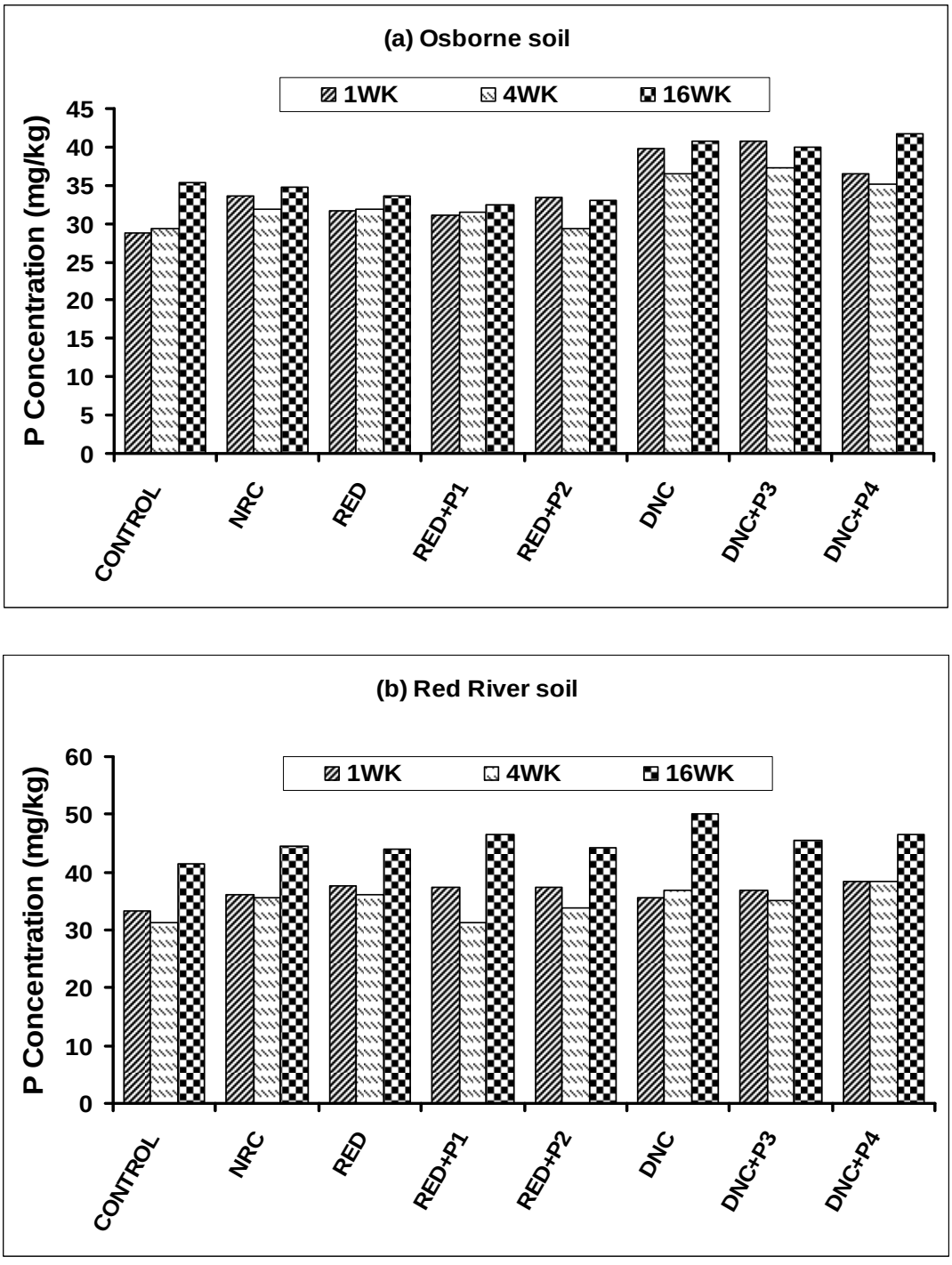

Figure 3.2 Effects of incubation time on Olsen P solubility in (a) Osborne and (b) Red River soils

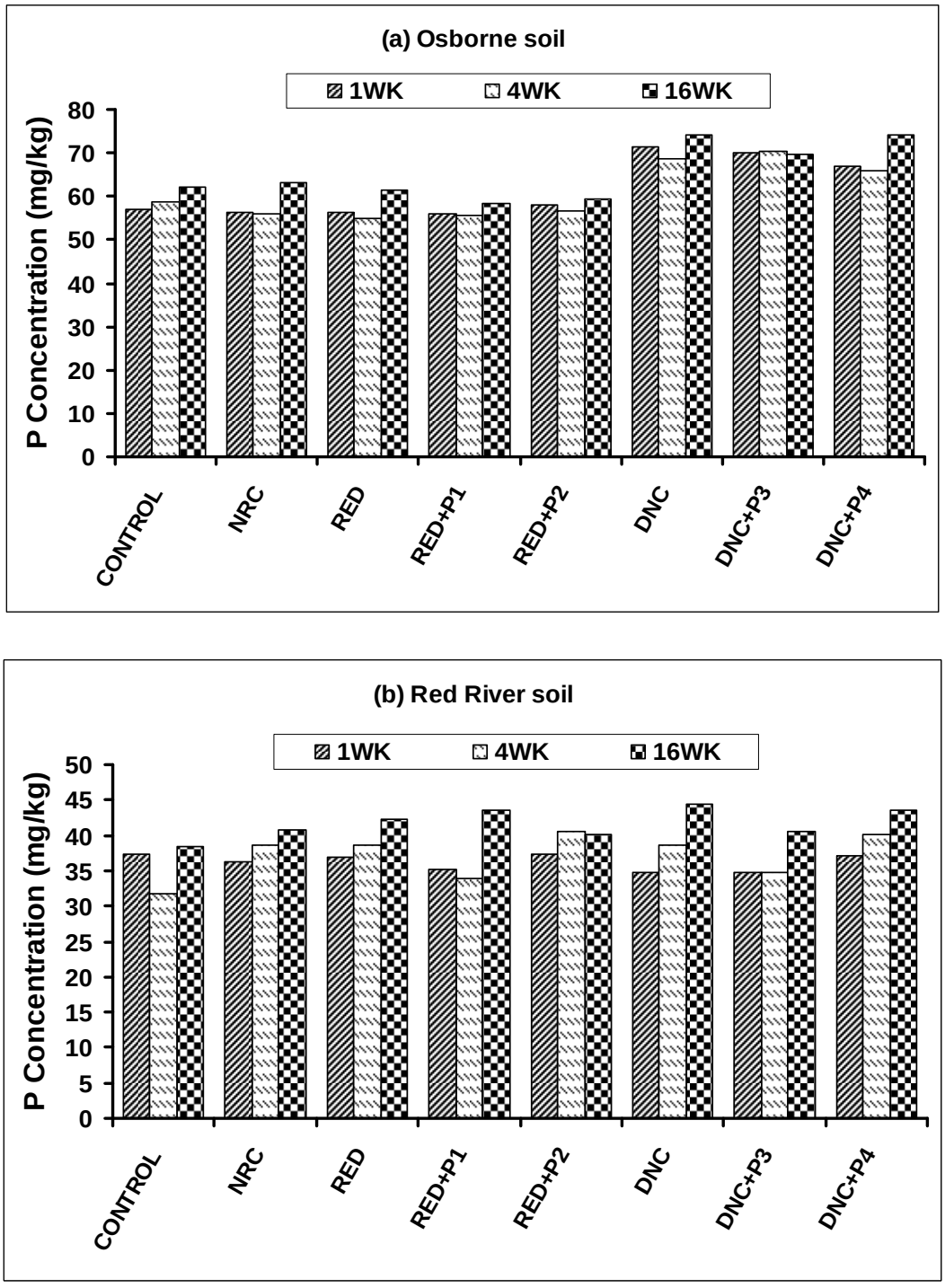

Figure 3.3 Effects of incubation time on Modified Kelowna P solubility in (a) Osborne and (b) Red River soils

wk of incubation, a reduction of 2 to 16% was observed in the Red river soil. This decline was followed by a sharp increase in solubility of the treated soil between 4 wk and 16 wk. This increase in solubility between 4 wk and 16 wk of incubation ranged from 18 to 33% depending on the treatment. Differences in P solubility with time in these heavy clay soils may be as a result of differences in their P saturation indices. High P saturated soils are less able to retain added P (Maguire and Sims, 2002; Sims et al., 2002)

Similar to the pattern observed in water and Olsen P in the two soils, the pattern of Kelowna P in the Osborne soil is not different from that of the Red River clay (Fig. 3.3). While there was little effect of time in the Osborne soil, there were greater changes in the extracted P with incubation time in the Red River clay. These changes were similar to what we observed for other extractants.

3.4.5.3 Effect of Incubation Time on Manure P Solubility in Fine Textured Soils as Measured using Mehlich-3 Extraction.

The Mehlich-3 extractable P behaved similarly in these two soils with virtually no significant change through the periods of incubation, probably because Mehlich-3 extracted a larger pool that is not responsive to time. Although manure treatment influenced the extractable P in the Osborne soil, there was no change in soluble P with incubation time (Figure 3.4a). Mehlich-3 extracted greater amounts of P in the control soil than in the NRC, RED, RED+P1, RED+P2 treated soils. For example, in the RED+P1 amended Osborne soil, the Mehlich-3 extractable P was 79.7 mg kg^{-1} at the end of 4 wk and 80 mg kg^{-1} at the end of 16 wk. Meanwhile, it was 90.4 and 93 mg kg^{-1} respectively, at the end of 4 and 16 wks in the control soil.

The Mehlich-3 extractable P in the manure amended Red River soil at the three periods of incubation were also similar. The large pool of P that Mehlich-3 extracts, compared to water or Olsen, may not allow us to see small changes in P solubility with time in all the treatments. Mehlich-3 extracts the highest amount of P from soil due to the presence of strong acid reagents in the solution which enable it to dissolve some precipitated and organic P.

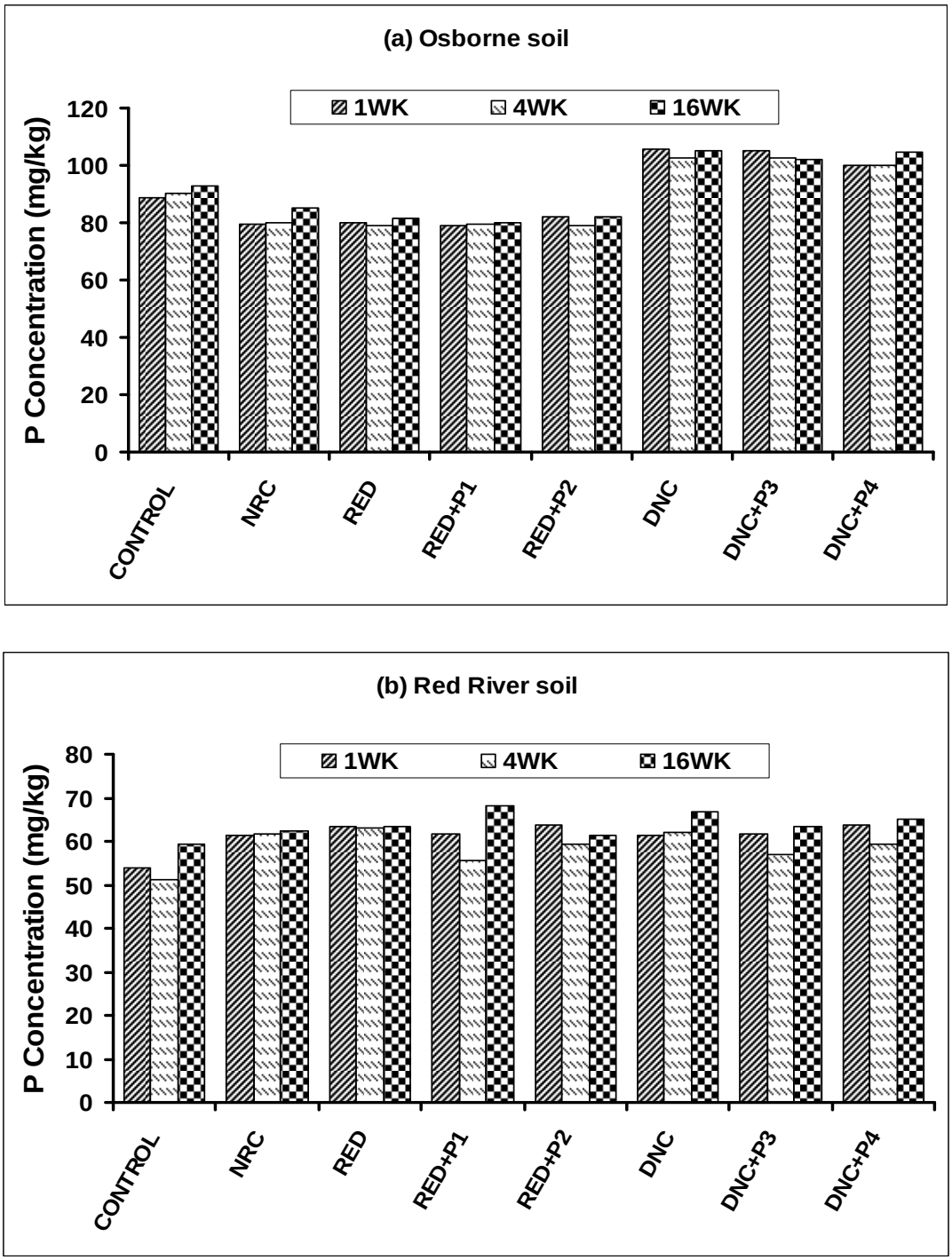

Figure 3.4 Effects of incubation time on Mehlich-3 P solubility in (a) Osborne and (b) Red river soil

3.4.5.4 Effect of Incubation Time on Manure P Solubility in Coarse Textured Soils as Measured using Water Extraction.

The water extractable P in the Ladywood soil declined sharply at 4 wk and thereafter increased in this soil at 16 wk (Figure 3.5a). The sharp decline between 1 wk and 4 wk was observed in all dietary treatments of this soil with a percentage reduction ranging from 59 to 91%. On the other hand, between 4 wk and 16 wk of incubation, there was an increase, ranging from 52 to 85% in the water soluble P of Ladywood soil. However, this increase did not totally compensate for the initial decrease in soluble P between week 1 and 4 of incubation as the water soluble P at 16 wk was still smaller than the amount that was measured after 1 wk. Water extracts a small but labile soil P fraction that can be used to estimate P amount in surface runoff (Hartikainen, 1982; Pote et al., 1996) and the sharp decline between week 1 and 4 could be due to retention processes including immobilization, adsorption and precipitation with soil Ca^{2+} and Mg^{2+}. Following incorporation into a silt loam soil, poultry manure was found to contain Ca and Mg phosphate minerals that controlled soil solution P concentrations. (Cooperband and Ward Good, 2002). Ige et al., (2005) concluded that P retention capacity of Manitoba soils was significantly influenced by exchangeable Ca and Mg. Following this initial disappearance of labile P, the increase between week 4 and 16 could be due to mineralization of organic P from the soil. Kashem et al., (2004) observed an increase in the labile biosolids P with a concomitant decrease in non-labile P with incubation time and they attributed this effect to conversion of organic P to inorganic P in the amended soil. The fact that this trend occurred in the control soil indicates that it was not solely due to the addition of manure to the soil.

The results obtained in the Glenhope soil were similar to that of the Ladywood, suggesting that it was due mainly to the coarse texture nature of these soils in contrast to what was observed for the heavy textured soils.

Also, at 4 wk, there was a very sharp decline in the extractable P of Glenhope soil (figure 3.5b). The decrease in W-P solubility ranged from 54 to 88% at 4 wk of incubation relative to 1 wk. Between 4 wk and 16 wk, there was an increase in extractable P which ranged between 67 and 93%. Unlike in the Ladywood soil, this later increase was greater than the initial decrease between week 1 and 4 with the exception of the NRC treatment. From the results of the Glenhope soil, it appears that the addition of phytase slightly increased the solubility of P in the amended soil after 16 week of incubation. This is because the treatment with phytase (RED+P1 and RED+P2) had higher levels of W-P than their non-phytase counterparts (NRC and RED). Other authors have noted that including phytase in the diet, with reduction in non-phytate P led to more WSP added in poultry litters (Maguire et al., 2004). Due to the small amount of P extracted by water these differences will probably not have an agronomic impact but may be important environmentally.

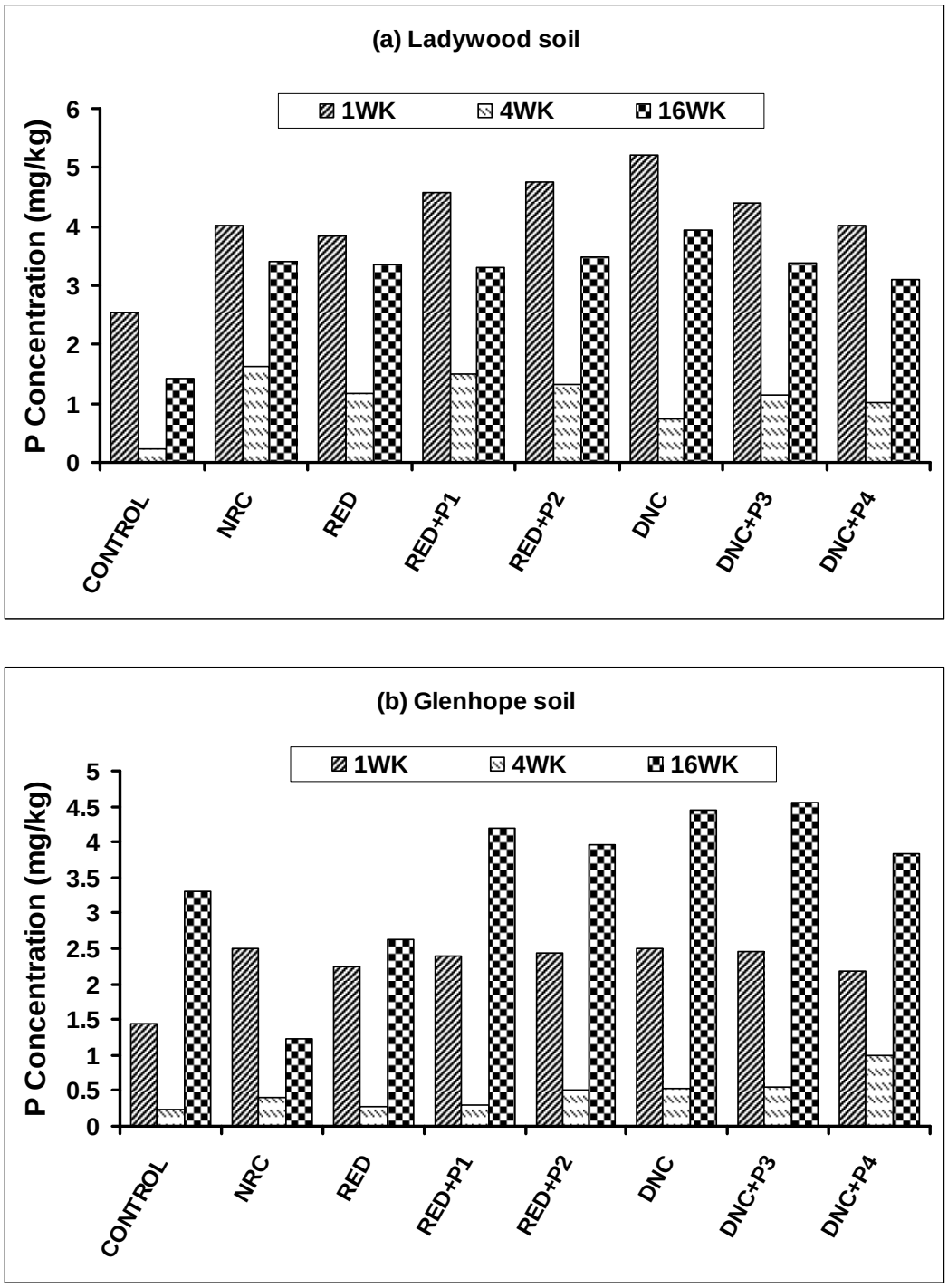

Figure 3.5 Effects of incubation time on Water P solubility in (a) Ladywood and (b) Glenhope soil

3.4.5.5 Effect of Incubation Time on Manure P Solubility in Coarse Textured Soils as Measured using Olsen and Kelowna Extraction.

In the Ladywood soil, there was a decline in Olsen P with incubation time (Figure 3.6a). For example, in the DNC amended soil, the soluble P declined from 16.2 mg kg^{-1} to 14.2 mg kg^{-1} between 1 wk and 4 wk, a 12% reduction and further declined to 12.2 mg kg^{-1} between 4 wk and 16 wk, a 14% reduction. The percentage reduction in the Olsen extractable P in all treatments ranged from 9 to 22% after 4 wk and varied between 5 to 20% after 16 wk.

In Glenhope soil, the Olsen extractable P either showed a small decline or remained unchanged with the incubation time (Figure 3.6b). For example, in the RED+P1 amended soil, Olsen extractable P declined from 17.3 mg kg^{-1} after 1 wk to 16.0 after 4 wk and then 15.7 mg kg^{-1} after 16 wk. This resulted in a percentage decline of 7% after 4 wk and 2% after 16 wk. On the other hand, in the DNC amended Glenhope soil, Olsen extractable P at the end of 1 wk was 15.6 mg kg^{-1} and this remained unchanged at 4 and 16 wk.

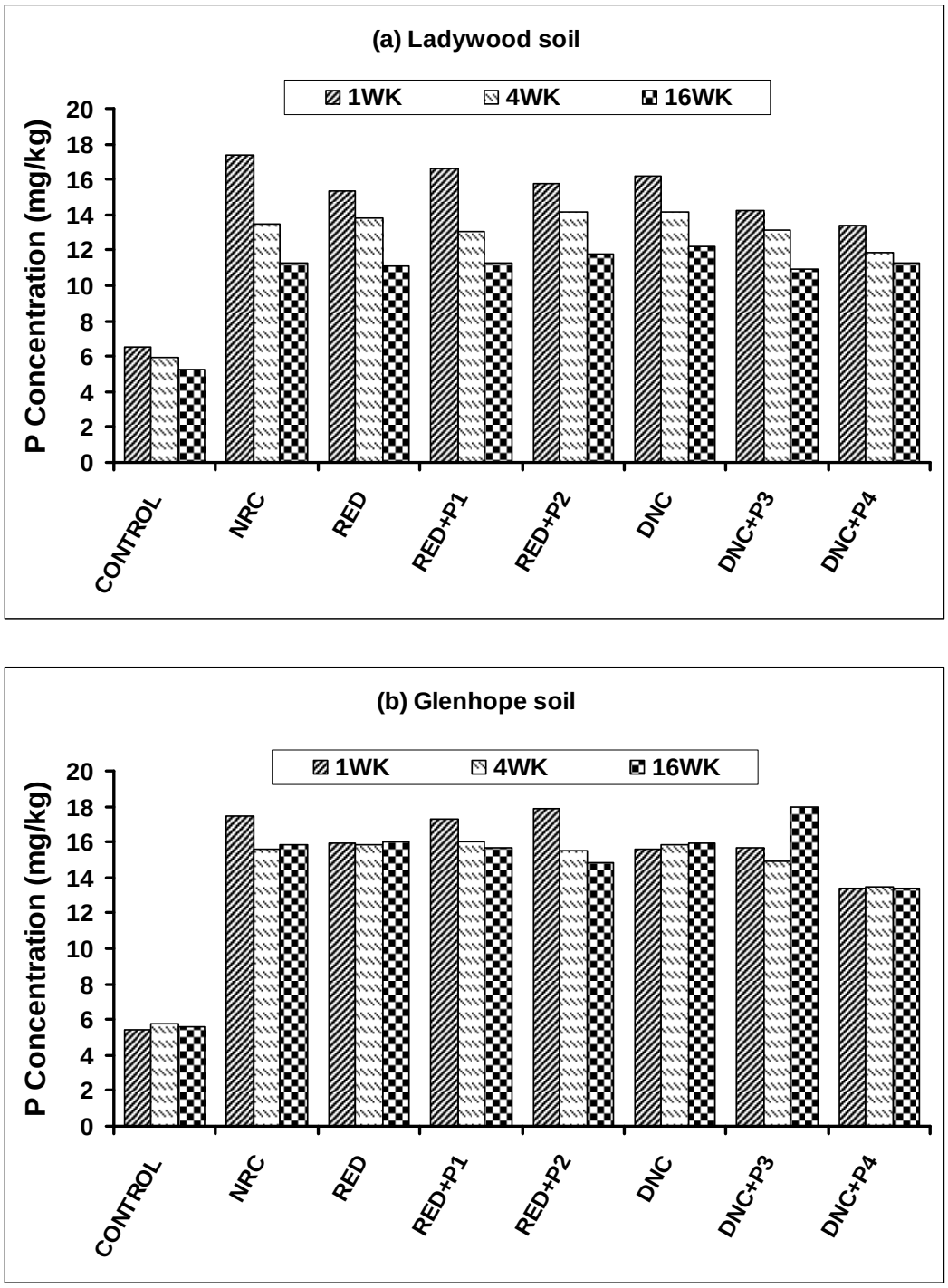

Figure 3.6 Effects of incubation time on Olsen P solubility in (a) Ladywood and (b) Glenhope soil

The two soils behaved in a similar manner with the Kelowna extraction (Figure 3.7). Overall, the results showed small increases in extractable P with incubation time. This is the reverse of the trend in Olsen P. This shows that these extractants behaved differently in manured soil because the control seems to be consistent between the two extractants, Olsen and Kelowna.

The slight increase in the Kelowna extractable P of the Glenhope soil was greater at 4 wk than at the 16 wk incubation. The percentage increase between 1 wk and 4 wk ranged from 6 to 17% and from 2 to 9% between 4 wk and 16 wk in the Glenhope soil. In the DNC amended soil, the extractable P increased from 16.6 mg kg^{-1} at 1 wk to 17.9 mg kg^{-1} after 4 wk showing a 7% increase and this slightly increased to 18.6 mg kg^{-1} after 16 wk incubation, a 4% increase.

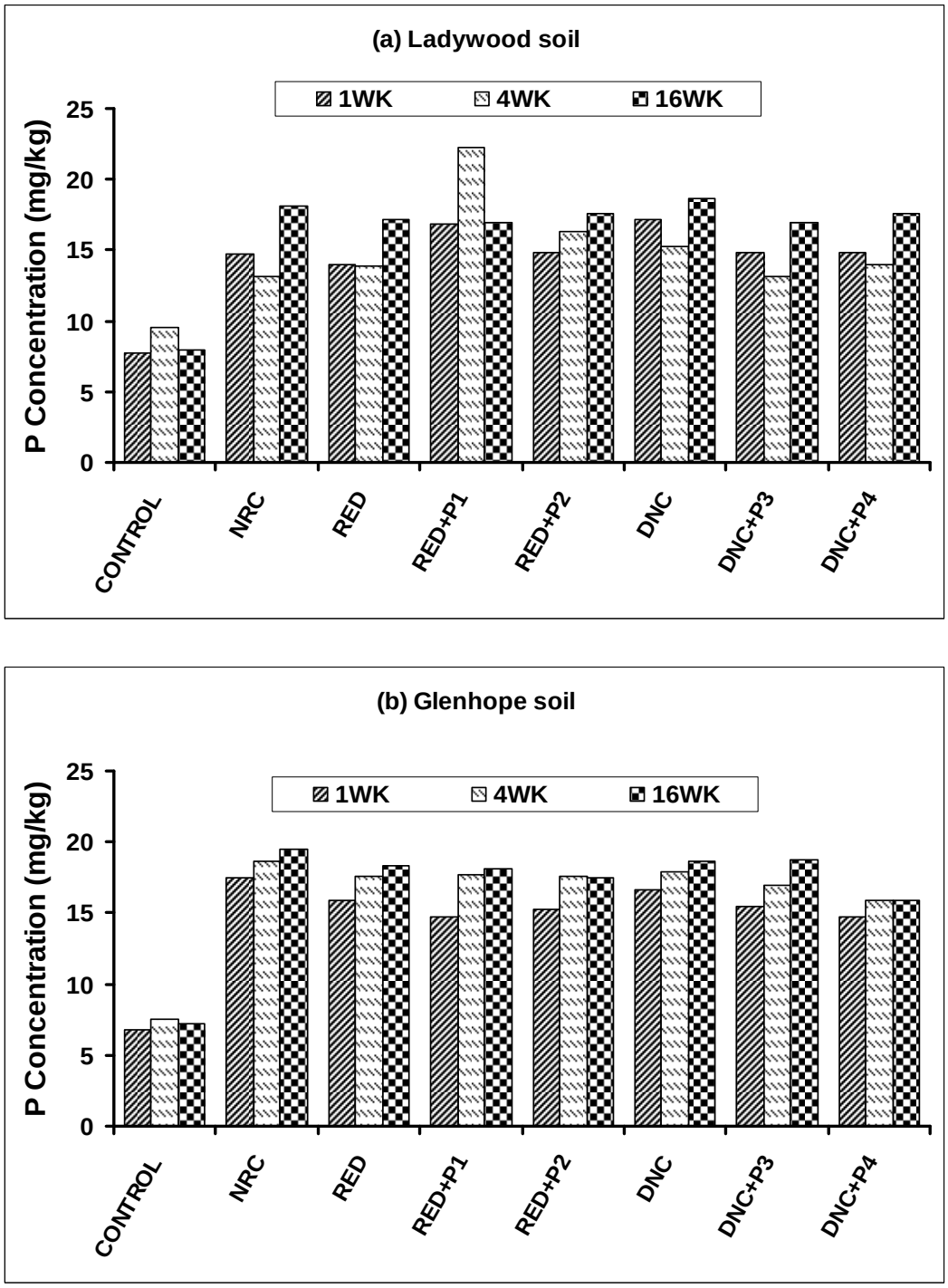

Figure 3.7 Effects of incubation time on Modified Kelowna P solubility in (a) Ladywood and (b) Glenhope soil

3.4.5.6 Effect of Incubation Time on Manure P Solubility in Coarse Textured Soils as Measured using Mehlich-3 Extraction.

Similar to the Kelowna extract, the Mehlich-3 P solubility in the manure treatments decreased slightly with incubation time. In the Ladywood soil, the Mehlich-3 P decreased in the DNC treatment from 33.5 mg kg^{-1} after 1 wk to 31.5 mg kg^{-1} after 4 wk and then 31.3 mg kg^{-1} after 16 wk (Figure 3.8a). In this soil, the percentage decrease after 4 wk was non-significant between 5 to 9% and just between 1 to 4% reduction was observed between 4 wk and 16 wk of incubation. The extractable P after 4 wk could be said to remain constant even till 16 wk. This short time (4 wk) of reaction and the low P concentration in the soil P indicates that sorption may be the main retention mechanisms in this soil. Kashem et al., (2004) demonstrated that the rapid surface sorption mechanisms explained why extractable P of soils incubated with hog and cattle manures remained relatively unchanged after 4 wks. They reported that the rapidity of surface sorption mechanisms may be explained by the observation of Qian and Schoenau (2000) that the addition of hog manure did not increase the soil most labile extractable P after 2 and 16 wk of incubation.

In the Glenhope soil, the Mehlich-3 P declined in the manure treatment after 1 wk and thereafter remained relatively constant after the 16 wk incubation (Figure 3.8b). For example, in the RED+P1 manure amended Glenhope soil, the M-3 P declined from 28.6 mg kg^{-1} after 1 wk to 26.5 mg kg^{-1} after 4 and 16 wks. The decline ranged from 3 to 8% after 4 wk and then remained constant after 16 wk incubation. The retention mechanism

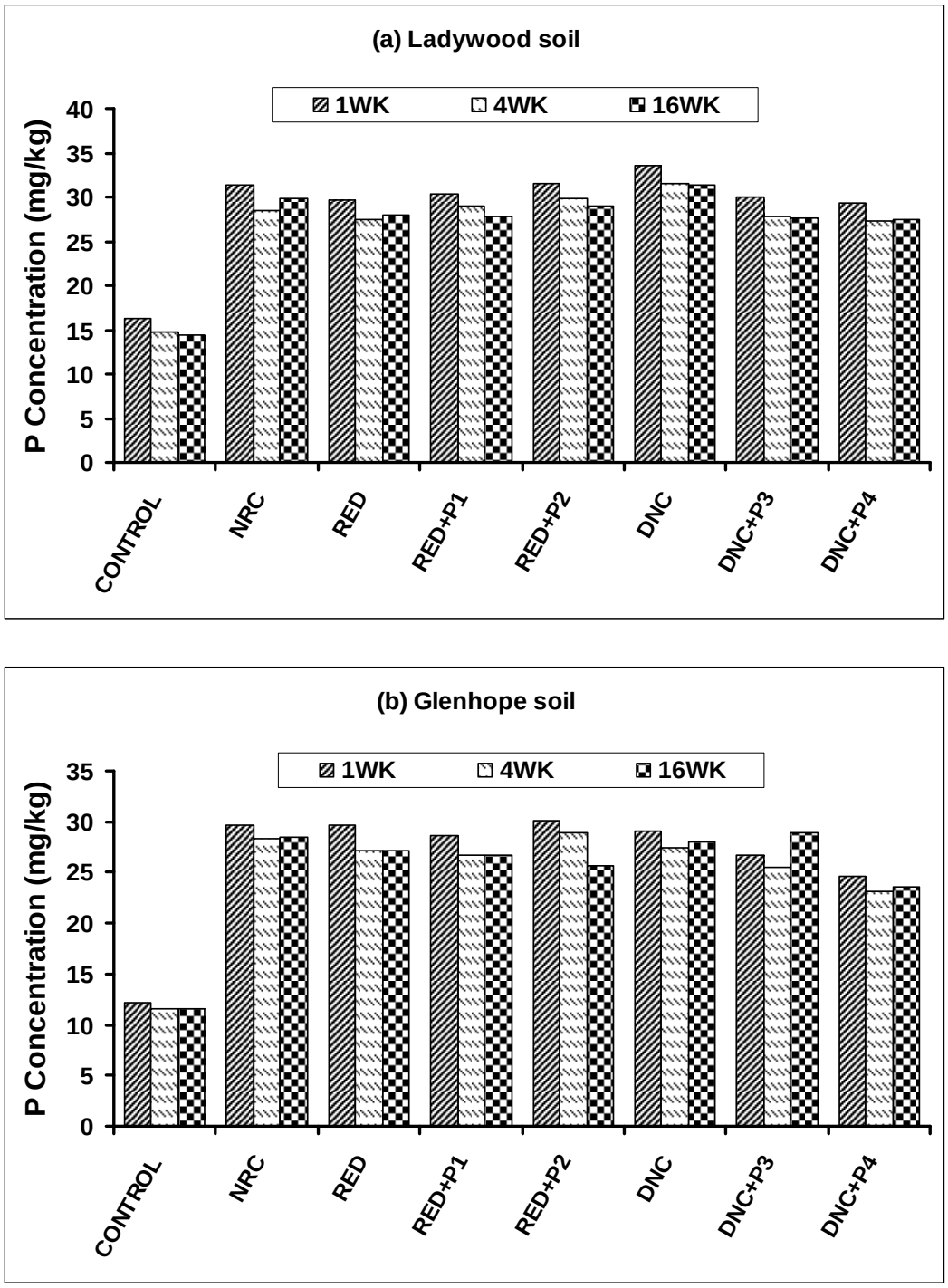

Figure 3.8 Effects of incubation time on Mehlich-3 P solubility in (a) Ladywood and (b) Glenhope soil

here also favors sorption due to the short reaction time and low concentration of extractable P.

Comparing the effects of Mehlich-3 extractable P in these two soils with incubation time, we observed no difference in the extractable P as it declined between 1 and 4 wk and then remained relatively unchanged after 4 wk of incubation. In contrast to what was observed in the water extraction, changes in extractable P with time were not visible in Mehlich-3 extraction, probably because it extracts a larger pool of P (Sharpley 1996; Atia and Mallarino, 2002).

3.5 Summary and Conclusions

The addition of phytase to swine diets did not have a consistent effect on the solubility of P in manure amended soils. This implies that phytase addition should not contribute to P losses into water bodies. In our study, the absence of inorganic P in the DNC diets in form of dicalcium P increased the solubility of manure P in calcareous soils. This shows that although diets containing no supplemental inorganic P may reduce manure total P excretion, the use of manure from such diets may increase manure P solubility in calcareous soils. The mechanisms for this increase in extractable P and its magnitude in different calcareous soils, however, warrant further studies.

Soil texture played a significant role in the solubility of manure P with incubation time in the soils. In fine textured soils, the water extractable P increased after 4 wk and then decreased between 4 wk and 16 wk of incubation. However, in the coarse textured soils, the reverse was the case as the solubility of P in water decreased significantly after 4 wk and then increased between week 4 and week 16 of incubation. The differences with respect to time could be because coarse textured soils have higher sand content which may increase solubility. Moreover, P sorption capacity of coarse textured soils is low and this may enhance P solubility in a longer time period say 16 wk as observed in our study

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4. OVERALL SYNTHESIS

The swine industry is faced with the problem of high nutrient composition in manure and the potential loss to the environment. Because of the recent growth in animal agriculture, dietary manipulation has been tailored towards reducing the P content of manure and possibly, the manure P accumulation in soils. Phytase supplementation in diets of animals reduces P excretion in manure; however, the reactivity of the manure P from phytase supplemented diets has not been well documented. This raises the question if the strategies (such as phytase inclusion in diets) used to decrease P concentration in manure will likewise minimize the loss of P from agricultural land into the adjacent water bodies.

This was a collaborative and interdisciplinary study between animal scientists and soil scientists to find a common solution to the problem of high P content in swine manure. The animal scientists formulated the seven diets used for this study from three basal diets which were: (a) NRC diet containing P at the NRC (1998) recommendations (b) RED diet with 0.1 percentage unit reduction in the available P values of NRC. (c) DNC diet which contained no added inorganic P. This diet was formulated to examine if the supplementation of inorganic P in animal diets could be eliminated through phytase addition. Two other diets were formulated from the RED diet by adding 500 or 1000 U of phytase per kg of diet. The remaining two diets were formulated by adding two higher levels of phytase (2000 or 4000 U kg^{-1} diet) to the DNC diet. The performance of the pigs fed these diets is addressed in a companion study. The soil science aspect of the study

characterized the P forms in manure collected from phytase supplemented swine diets as a first step in determining the fate of manure, and then investigated its reactivity in amended Manitoba soils. The broad objective of this research study was fulfilled in two experiments. The first experiment investigated and characterized the forms of P in swine feces and manures. It provided information on the fractions of P in swine feces and manures through the use of the modified sequential extraction procedure and how they have been modified by phytase addition. Our findings showed that phytase supplementation to swine diets reduced the total P in both feces and manure samples. From the sequential extraction of both feces and manure, addition of the moderate levels of phytase (500 or 1000 U) to RED diets reduced the total P in the feces. The reduction was not significant at 500U of phytase, but was significant ($p \leq 0.05$) at 1000 U of phytase addition. Significant reduction ($p \leq 0.05$) was also observed at 2000 U of phytase addition to DNC diet; however, this became non-significant when 4000 U of phytase was added to DNC diets.

In manure samples, significant reduction ($p \leq 0.05$) was observed when 2000 U of phytase was added to DNC diets. And as we had in the feces, phytase addition at 4000 U to DNC diets resulted in non-significant and increase in the total P. This effect indicated that 2000 U of phytase is the best level of supplementation to the DNC diets in our study.

Unlike the feces and manure samples, the urine total P increased in the phytase supplemented diets compared to diets without phytase. Although, the percentage P in urine is small when compared to that of feces and manure, however, by considering the urine, the impact of phytase addition was less substantial in manure compared to the feces. Phytase addition reduced both the H_2O- and $NaHCO_3$- P fractions which form the

labile P fractions especially in the feces. This reduction in labile P of manure and feces following phytase supplementation is desirable for the environment since the labile fraction is the one prone to runoff and leaching losses. These manure P fractions, when expressed as a percentage of total P increased slightly in phytase supplemented diets. Therefore, phytase addition reduced the absolute amount of labile P concentrations, thus reducing the potential detrimental effect on the adjacent water bodies and subsequent eutrophication, however, it increased the percentage of total P that is labile.

The second experiment was conducted to examine the solubility of manure P in soils. The main objective of the second experiment was to investigate the reactivity or solubility of manure P collected from pigs fed phytase supplemented diets in the first study in different Manitoba soils. We examined the solubility of manure P with time using various extracting solutions. Addition of manure based on the total P content implies that manures containing different WSP fractions were added to the soils. We observed that phytase addition to diets generally did not affect the solubility of manure in amended soils. This indicated that nutritional management strategies (such as use of phytase) will not contribute to the enrichment of surface waters with P and subsequent eutrophication.

We observed that the absence of inorganic Ca-P in the DNC diets increased the solubility of P in amended calcareous soils. This implies that caution should be taken in using higher levels of phytase to eliminate inorganic Ca-P as this might contribute to P loss in some types of soils. Besides, textural characteristics of a soil played an important role in the solubility of manure P in soils. Manure P solubility was increased in the coarse

textured soils used for this experiment with time and this could contribute to P leaching losses in such soils.

The implications of the findings from this research are that phytase supplementation into swine diets has the potential to reduce total and labile P fractions in the feces of swine and as thus, reduce the negative environmental impact associated with intensive swine production. Moreover, the addition of manure P from phytase supplemented swine diets to soils did not have significant effect on the solubility of P in soils. Although, these results were obtained from laboratory studies, they gave an idea of what is likely to happen on a field scale, through the effects of physico-chemical properties of soils if such experiments were to be conducted on the field. Thus, phytase supplementation in diets is not likely to contribute to the losses of P from manure amended soils. However, caution should be exercised in dietary modification in a quest to eliminate the inorganic Ca-P supplementation through phytase addition. Our results showed that manure from diets with no inorganic Ca-P with and without phytase addition actually increased P solubility in calcareous soils, probably due to the absence of calcium. Exchangeable Ca^{2+} is a candidate of P precipitation, and its absence in diets increased the solubility of manure P in calcareous soils. This effect suggests that the addition of Ca-based by products such as lime and gypsum to manure could be used to further reduce P solubility. It would be of great interest to investigate the solubility of such lime treated manure P in different soils of Manitoba.

Future studies in this area could investigate the mechanism of the increased manure P solubility from diets containing no supplementation of inorganic P in calcareous soils. Also, in diet modifications, there could be an addition of lower level of

phytase (say 500 or 1000 U) to the DNC diets in order to obtain comparable results of phytase supplementation in manure from both RED and DNC diets. Moreover, in the manure P solubility experiments in soils, it may be necessary to add manure at different rates and incubate at longer periods of time (say 32 wk) in order to examine the fate of P in soils over a longer time period. It may also be of great importance to take this laboratory study to the field and confirm these results.

Overall, our results showed that phytase addition in diets does not contribute to the solubility of manure P in soils and potential loss to adjacent environments. For a hog farmer, with good consideration of the economy of costs of phytase and also the gains in animal performance, phytase supplementation in diets may be a cost-effective means of improving utilization of dietary P. It could also be a preventive technological measure that decreases the P concentration in manure from intensive animal agriculture and minimizes P loss in amended soils. Information from this research will be beneficial to many swine producers in reducing the contribution of animal agriculture to P loss in the environment. Hence, the overall objective of this research study to characterize the forms of manure P from phytase supplemented swine diets and to examine the solubility of the same in soils was accomplished.

5. APPENDICES

Appendix A

RESULTS OF THE SEQUENTIAL EXTRACTION EXPERIMENT OF THE FIRST STUDY

Total P (g/kg) in the urine, feces and manure samples

	Urine	Manure	Feces
NRC	0.649	18.44	21.54
NRC	0.769	17.22	18.78
NRC	0.573	20.44	23.39
NRC	0.570	20.44	25.12
RED	0.059	17.34	24.76
RED	0.027	13.44	20.72
RED	0.050	11.78	18.23
RED	0.038	13.09	20.36
RED+P1	0.220	10.78	10.70
RED+P1	0.201	13.22	18.77
RED+P1	0.122	13.34	18.49
RED+P1	0.052	13.16	18.11
RED+P2	0.117	15.97	17.43
RED+P2	0.285	11.22	16.28
RED+P2	0.034	10.56	13.76
RED+P2	0.125	11.63	18.05
DNC	0.016	12.94	14.18
DNC	0.013	14.25	21.06
DNC	0.025	14.06	20.65
DNC	0.035	13.72	17.61
DNC+P3	0.055	9.56	12.86
DNC+P3	0.021	11.22	14.05
DNC+P3	0.023	11.88	15.85
DNC+P3	0.099	8.38	11.22
DNC+P4	0.042	15.88	23.24
DNC+P4	0.020	11.28	18.38
DNC+P4	0.012	9.50	12.25
DNC+P4	0.060	9.53	18.03

P extracted (% of total P) by various extractants in the freeze dried manure samples

	H_2O	$NaHCO_3$	NaOH Pi	NaOH Po	HCl Pi	HCl Po	Residual
NRC	69.43	12.30	6.29	4.17	3.49	2.85	1.47
NRC	59.09	13.08	9.01	5.98	5.37	5.61	1.86
NRC	66.84	14.18	7.18	2.55	5.02	2.59	1.64
NRC	60.48	16.58	7.77	1.80	7.31	3.76	2.29
RED	58.93	20.86	6.34	2.17	7.49	2.46	1.75
RED	62.68	17.49	7.31	3.34	3.52	3.96	1.71
RED	65.91	16.45	7.12	4.98	1.88	2.29	1.38
RED	66.19	18.48	6.21	2.73	2.80	2.08	1.51
RED+P1	82.15	6.95	0.99	5.66	0.60	1.38	2.26
RED+P1	65.00	15.27	6.74	4.01	4.20	2.90	1.89
RED+P1	67.38	15.45	5.82	4.33	3.31	2.01	1.70
RED+P1	65.42	17.99	5.47	3.93	3.11	2.17	1.91
RED+P2	59.37	14.58	6.85	7.31	4.53	5.03	2.32
RED+P2	63.17	13.06	5.94	8.73	2.88	4.14	2.08
RED+P2	61.66	9.60	5.79	12.33	3.05	5.23	2.34
RED+P2	66.75	18.06	4.63	4.88	1.57	1.98	2.12
DNC	76.67	14.46	1.78	2.57	1.54	1.94	1.04
DNC	58.78	28.88	2.92	4.98	1.65	1.07	1.72
DNC	62.55	25.48	3.28	3.64	2.07	1.55	1.43
DNC	64.22	24.19	2.44	4.78	1.72	1.54	1.12
DNC+P3	73.87	14.03	1.43	6.82	1.00	1.05	1.81
DNC+P3	62.60	25.04	3.12	4.77	1.28	1.61	1.59
DNC+P3	75.66	13.26	1.52	5.36	1.54	0.96	1.71
DNC+P3	81.07	8.13	1.23	4.46	0.81	2.26	2.04
DNC+P4	61.71	18.29	5.79	3.60	4.60	3.81	2.20
DNC+P4	77.19	9.63	1.54	5.78	0.90	2.37	2.58
DNC+P4	78.25	9.78	1.44	5.22	1.07	1.98	2.26
DNC+P4	76.18	10.84	1.70	5.81	1.30	1.84	2.34

P extracted (% of total P) by various extractants in the freeze dried feces samples

	H_2O	$NaHCO_3$	NaOH Pi	NaOH Po	HCl Pi	HCl Po	Residual
NRC	55.28	16.49	5.88	6.25	3.08	11.07	1.94
NRC	52.33	17.31	7.76	6.79	2.05	11.63	2.13
NRC	58.13	19.39	8.13	3.72	2.36	6.02	2.26
NRC	50.76	18.29	8.25	3.37	12.59	4.11	2.63
RED	57.52	21.45	6.20	2.95	2.90	7.21	1.78
RED	60.60	18.05	6.67	4.70	3.97	3.88	2.12
RED	61.49	18.52	7.10	5.61	2.33	3.27	1.68
RED	62.54	19.88	6.01	3.78	3.69	2.18	1.91
RED+P1	70.53	14.74	1.44	7.22	0.76	2.88	2.44
RED+P1	61.90	19.73	5.31	5.82	2.31	3.18	1.76
RED+P1	60.25	19.98	7.09	3.27	4.44	3.13	1.84
RED+P1	59.07	21.54	5.48	5.23	2.98	3.64	2.07
RED+P2	57.15	17.21	5.92	8.68	3.46	5.09	2.49
RED+P2	57.44	17.20	5.25	10.76	2.28	4.92	2.16
RED+P2	56.77	12.76	4.84	17.81	1.52	4.02	2.27
RED+P2	67.55	18.29	3.57	4.94	1.30	2.30	2.06
DNC	77.90	12.75	1.15	5.30	1.00	0.75	1.16
DNC	71.23	16.57	1.65	4.94	1.48	2.35	1.78
DNC	67.61	21.07	1.94	4.75	1.25	1.63	1.73
DNC	70.94	18.71	1.23	5.06	0.82	1.42	1.82
DNC+P3	76.85	9.19	1.00	8.15	0.81	2.67	1.33
DNC+P3	74.71	14.62	1.34	5.05	0.87	1.40	2.01
DNC+P3	73.79	13.68	1.26	5.98	0.84	2.22	2.22
DNC+P3	75.66	11.08	0.94	6.08	0.46	3.04	2.74
DNC+P4	55.25	21.00	5.80	4.75	7.38	3.51	2.31
DNC+P4	72.15	14.65	1.29	6.57	0.80	2.04	2.51
DNC+P4	71.75	13.88	1.19	7.21	0.90	2.11	2.96
DNC+P4	69.47	14.27	1.56	8.24	1.40	2.60	2.45

Appendix B

RESULTS FROM THE EXTRACTABLE PHOSPHORUS IN THE SOIL INCUBATION STUDY OF EXPERIMENT II

Extractable P (mg kg^{-1}) from the Osborne soils after 1 wk of incubation

	Mehlich-3	Kelowna	Olsen	Water
NRC	84.75	62.80	37.88	8.45
NRC	77.25	52.50	32.38	6.80
NRC	82.63	58.00	33.63	7.65
NRC	73.13	51.50	30.88	6.55
RED	77.25	55.20	32.63	7.25
RED	79.00	55.40	29.88	7.35
RED	83.75	58.30	32.13	8.13
RED	79.75	55.80	31.88	7.53
RED+P1	77.25	53.10	29.63	6.75
RED+P1	83.38	60.70	33.13	7.43
RED+P1	78.38	55.20	31.38	7.25
RED+P1	77.00	55.20	30.63	7.03
RED+P2	79.50	56.20	32.63	7.45
RED+P2	84.00	57.70	33.13	7.88
RED+P2	81.50	56.10	32.88	7.40
RED+P2	83.50	61.90	35.13	8.05
DNC	98.25	68.10	36.63	7.68
DNC	104.00	66.70	38.13	7.75
DNC	110.75	74.20	44.13	8.95
DNC	108.75	76.10	40.63	9.45
DNC+P3	100.50	66.90	38.13	7.18
DNC+P3	106.25	72.00	39.38	7.95
DNC+P3	106.50	70.90	43.38	8.15
DNC+P3	106.25	71.00	42.13	8.05
DNC+P4	98.25	65.30	37.88	7.38
DNC+P4	100.00	68.10	35.38	7.45
DNC+P4	97.50	66.70	36.88	7.08
DNC+P4	103.25	68.20	35.88	7.10
CONTROL	86.50	52.50	27.78	5.55
CONTROL	90.50	57.30	28.48	5.40
CONTROL	89.50	57.90	29.48	5.73
CONTROL	88.25	60.90	29.08	5.80

Extractable P (mg kg^{-1}) from the Osborne soils after 4 wk of incubation

	Mehlich-3	Kelowna	Olsen	Water
NRC	80.00	57.20	29.40	7.68
NRC	78.75	54.80	30.30	7.68
NRC	80.25	55.10	36.50	8.08
NRC	82.00	56.50	31.10	8.32
RED	79.75	58.60	32.10	8.17
RED	78.50	53.50	28.40	8.03
RED	75.75	53.10	32.80	7.52
RED	81.50	55.00	33.80	8.52
RED+P1	80.50	56.20	32.00	8.37
RED+P1	78.25	57.90	32.40	8.05
RED+P1	78.25	54.20	28.00	7.90
RED+P1	81.75	54.70	33.50	8.25
RED+P2	79.75	54.50	28.30	7.82
RED+P2	77.50	56.80	29.00	7.88
RED+P2	82.00	59.70	30.30	8.18
RED+P2	76.25	55.40	29.50	7.63
DNC	105.75	71.20	39.20	9.30
DNC	95.75	62.60	34.10	8.17
DNC	106.25	72.30	36.90	9.27
DNC	103.25	68.20	35.60	8.75
DNC+P3	106.50	71.80	39.10	8.80
DNC+P3	101.75	71.90	38.10	8.47
DNC+P3	104.50	74.60	36.60	8.43
DNC+P3	97.75	63.40	35.10	7.57
DNC+P4	101.50	67.40	36.70	8.17
DNC+P4	98.50	66.40	34.10	8.02
DNC+P4	98.00	64.00	33.60	7.83
DNC+P4	101.50	65.30	36.00	8.05
CONTROL	89.50	57.60	28.60	6.32
CONTROL	93.00	61.20	29.60	6.38
CONTROL	91.25	60.40	29.90	6.40
CONTROL	87.75	56.00	29.30	6.40

Extractable P (mg kg^{-1}) from the Osborne soils after 16 wk of incubation

	Mehlich-3	Kelowna	Olsen	Water
NRC	82.50	62.60	33.43	7.63
NRC	83.00	62.70	33.23	7.80
NRC	85.25	62.00	35.93	7.85
NRC	89.00	65.30	36.23	7.85
RED	83.50	61.90	33.93	7.65
RED	79.75	59.50	34.03	7.35
RED	78.00	62.00	32.93	7.17
RED	84.50	62.90	33.43	7.82
RED+P1	78.50	57.50	31.03	6.93
RED+P1	80.25	59.00	32.93	7.37
RED+P1	81.50	59.70	32.73	7.55
RED+P1	79.75	57.50	33.13	7.02
RED+P2	83.25	59.80	32.63	7.45
RED+P2	81.75	58.70	33.03	7.33
RED+P2	79.25	58.70	32.63	7.50
RED+P2	83.50	60.60	33.53	7.68
DNC	106.75	76.20	40.13	8.25
DNC	102.25	72.00	39.13	7.67
DNC	99.25	71.90	42.43	7.70
DNC	111.25	76.10	41.13	8.23
DNC+P3	100.50	71.20	39.23	7.45
DNC+P3	99.75	67.30	39.83	7.27
DNC+P3	100.75	66.90	39.73	7.30
DNC+P3	107.50	73.80	40.93	7.63
DNC+P4	102.25	74.30	41.63	8.17
DNC+P4	107.00	73.20	43.93	8.18
DNC+P4	103.75	74.00	40.53	8.15
DNC+P4	105.00	74.50	40.83	8.10
CONTROL	94.25	61.60	35.13	6.20
CONTROL	92.25	62.40	35.23	6.37
CONTROL	93.25	62.60	36.33	6.43
CONTROL	92.50	62.00	35.03	6.10

Extractable P (mg kg^{-1}) from the Red River soils after 1 wk of incubation

	Mehlich-3	Kelowna	Olsen	Water
NRC	61.63	36.60	36.08	3.23
NRC	64.00	38.10	36.68	3.18
NRC	60.75	35.50	36.38	3.03
NRC	59.50	35.00	35.78	3.10
RED	61.63	36.20	35.58	3.18
RED	64.13	37.00	38.98	3.35
RED	64.50	37.20	38.18	3.38
RED	63.75	37.60	37.98	3.45
RED+P1	59.13	34.60	35.58	3.00
RED+P1	62.00	35.50	39.98	3.20
RED+P1	63.25	35.10	36.68	3.30
RED+P1	62.88	35.70	37.08	3.08
RED+P2	62.00	38.60	36.28	3.13
RED+P2	67.63	38.70	38.58	3.65
RED+P2	59.75	35.30	35.38	3.13
RED+P2	65.75	36.70	39.38	3.45
DNC	60.75	34.70	34.78	3.18
DNC	61.50	35.30	35.78	3.15
DNC	61.50	34.90	35.38	2.78
DNC	61.25	33.90	36.38	2.80
DNC+P3	60.25	33.80	36.28	2.53
DNC+P3	62.00	34.80	37.18	2.75
DNC+P3	62.88	34.90	36.88	3.02
DNC+P3	61.63	35.80	37.08	3.02
DNC+P4	61.50	35.50	36.48	3.05
DNC+P4	63.88	36.80	38.38	3.22
DNC+P4	66.75	40.60	40.68	3.48
DNC+P4	63.00	35.60	38.08	3.13
CONTROL	56.00	35.40	33.98	2.45
CONTROL	53.13	36.60	33.18	2.33
CONTROL	54.50	40.40	33.68	2.27
CONTROL	51.75	36.70	31.98	2.27

Extractable P (mg kg^{-1}) from the Red River soils after 4 wk of incubation

	Mehlich-3	Kelowna	Olsen	Water
NRC	59.75	35.90	34.60	4.22
NRC	61.25	38.90	35.00	4.18
NRC	65.00	43.30	37.10	4.68
NRC	60.75	36.60	35.30	4.03
RED	65.75	39.10	36.30	4.62
RED	62.75	39.20	36.60	4.45
RED	64.25	39.60	36.00	4.65
RED	59.75	36.50	35.00	4.43
RED+P1	54.75	33.50	32.00	3.77
RED+P1	53.75	34.10	29.90	3.60
RED+P1	56.50	33.20	31.10	3.82
RED+P1	57.25	34.80	31.80	3.80
RED+P2	63.00	41.05	36.20	4.13
RED+P2	59.75	41.30	33.50	4.07
RED+P2	56.00	38.10	31.40	3.92
RED+P2	59.00	41.45	34.10	3.95
DNC	58.75	36.10	33.90	3.97
DNC	62.00	37.80	35.30	4.23
DNC	61.75	38.90	38.40	4.30
DNC	65.75	41.60	40.20	4.63
DNC+P3	58.00	35.30	34.40	3.72
DNC+P3	57.75	35.50	35.00	3.75
DNC+P3	56.25	34.60	35.00	3.75
DNC+P3	55.25	33.40	35.80	3.53
DNC+P4	60.00	44.00	41.40	4.83
DNC+P4	60.00	38.80	36.50	4.55
DNC+P4	61.00	40.10	40.50	4.48
DNC+P4	56.25	37.20	35.00	3.93
CONTROL	50.25	31.90	31.50	3.63
CONTROL	50.50	31.20	30.30	3.48
CONTROL	52.50	32.20	30.70	3.68
CONTROL	51.75	32.00	32.10	3.60

Extractable P (mg kg^{-1}) from the Red River soils after 16 wk of incubation

	Mehlich-3	Kelowna	Olsen	Water
NRC	62.75	41.20	45.43	4.00
NRC	61.75	40.60	43.23	4.12
NRC	62.00	40.40	44.53	4.42
NRC	63.00	40.50	44.83	4.12
RED	64.50	42.60	44.23	4.70
RED	63.25	41.00	43.73	4.88
RED	63.50	43.20	44.83	4.55
RED	62.25	42.30	43.33	4.68
RED+P1	65.75	42.20	45.13	3.55
RED+P1	65.25	43.90	45.63	3.23
RED+P1	69.00	44.00	47.23	3.77
RED+P1	72.75	44.10	48.03	3.68
RED+P2	59.25	40.30	46.13	3.17
RED+P2	61.50	39.20	44.73	2.88
RED+P2	62.50	40.60	44.03	4.68
RED+P2	62.25	40.20	42.53	3.55
DNC	65.50	44.10	51.13	3.23
DNC	61.75	44.00	50.13	3.77
DNC	67.00	43.90	48.83	3.68
DNC	72.25	45.50	50.63	3.17
DNC+P3	63.50	40.80	46.13	2.88
DNC+P3	63.00	39.70	45.13	3.12
DNC+P3	62.50	38.20	44.93	3.00
DNC+P3	64.00	43.20	45.63	3.17
DNC+P4	64.75	42.90	47.13	3.43
DNC+P4	64.25	42.20	46.13	3.17
DNC+P4	63.50	43.50	46.43	3.35
DNC+P4	68.50	45.40	46.63	3.48
CONTROL	60.75	38.90	41.43	2.75
CONTROL	58.75	38.10	41.73	2.82
CONTROL	58.75	38.50	41.13	2.77
CONTROL	58.50	38.00	41.13	2.70

Extractable P (mg kg^{-1}) from the Ladywood soils after 1 wk of incubation

	Mehlich-3	Kelowna	Olsen	Water
NRC	31.75	14.90	16.38	3.70
NRC	31.00	14.60	17.18	4.08
NRC	32.25	13.80	16.28	4.20
NRC	30.63	15.40	19.63	4.12
RED	30.50	14.20	15.24	2.70
RED	29.00	13.50	15.28	4.18
RED	29.25	14.20	15.73	4.05
RED	30.00	13.90	15.23	4.40
RED+P1	29.88	21.30	14.88	4.67
RED+P1	29.63	14.80	15.43	4.57
RED+P1	30.25	15.80	20.13	4.70
RED+P1	31.63	15.50	15.88	4.42
RED+P2	31.88	15.80	15.83	4.75
RED+P2	31.63	16.20	15.78	4.82
RED+P2	31.00	16.00	15.88	4.68
RED+P2	31.75	11.40	15.53	4.82
DNC	34.25	18.60	16.88	5.03
DNC	33.38	17.45	15.83	5.00
DNC	33.75	16.20	16.03	5.43
DNC	32.63	16.50	15.93	5.42
DNC+P3	30.63	16.10	14.93	4.47
DNC+P3	30.00	14.30	14.08	4.35
DNC+P3	29.75	14.65	13.78	4.37
DNC+P3	29.88	14.20	14.33	4.45
DNC+P4	28.25	14.20	13.38	4.00
DNC+P4	32.88	15.90	12.93	3.83
DNC+P4	28.00	14.05	13.98	4.18
DNC+P4	28.50	15.35	13.23	4.03
CONTROL	16.88	8.25	6.43	2.57
CONTROL	17.13	8.05	6.78	2.52
CONTROL	16.50	7.25	6.13	2.58
CONTROL	14.63	7.25	6.71	2.50

Extractable P (mg kg^{-1}) from the Ladywood soils after 4 wk of incubation

	Mehlich-3	Kelowna	Olsen	Water
NRC	27.88	11.65	12.85	3.00
NRC	29.00	13.75	13.85	2.57
NRC	29.25	14.00	14.35	0.53
NRC	28.00	13.35	12.85	0.43
RED	27.75	12.50	13.45	0.65
RED	26.63	14.05	16.45	1.45
RED	27.25	14.10	12.50	0.98
RED	28.00	14.75	12.95	1.63
RED+P1	27.50	18.90	12.40	3.62
RED+P1	29.25	26.10	12.85	0.62
RED+P1	28.88	19.40	13.15	1.05
RED+P1	30.00	24.45	13.85	0.68
RED+P2	29.75	17.50	14.05	2.18
RED+P2	29.75	15.85	14.20	1.80
RED+P2	29.88	16.40	14.45	0.47
RED+P2	30.00	15.60	14.00	0.88
DNC	31.13	16.60	14.60	0.81
DNC	31.00	14.70	13.40	0.59
DNC	32.00	14.40	14.55	0.37
DNC	31.88	15.45	14.10	1.16
DNC+P3	27.88	11.95	13.30	0.47
DNC+P3	28.00	15.90	12.45	1.26
DNC+P3	28.00	10.95	14.30	0.44
DNC+P3	27.38	13.55	12.65	2.44
DNC+P4	27.25	12.60	11.75	0.37
DNC+P4	27.75	15.70	12.65	0.87
DNC+P4	27.13	11.80	11.85	1.70
DNC+P4	26.88	16.00	11.30	1.08
CONTROL	14.85	8.95	6.10	1.63
CONTROL	15.15	10.05	6.20	0.26
CONTROL	14.45	9.45	6.00	0.26
CONTROL	14.80	9.70	5.35	0.19

Extractable P (mg kg^{-1}) from the Ladywood soils after 16 wk of incubation

	Mehlich-3	Kelowna	Olsen	Water
NRC	30.00	18.00	9.68	3.43
NRC	29.70	18.20	11.53	3.32
NRC	29.25	18.50	11.83	3.40
NRC	30.10	17.60	11.98	3.43
RED	28.25	16.95	10.48	3.43
RED	27.80	17.35	11.28	3.30
RED	27.75	17.05	11.08	3.40
RED	27.95	17.30	11.63	3.33
RED+P1	28.95	17.15	11.28	3.27
RED+P1	27.60	16.35	11.18	3.25
RED+P1	26.30	16.45	11.03	3.30
RED+P1	28.00	17.70	11.63	3.43
RED+P2	28.35	17.15	11.78	3.37
RED+P2	29.50	17.70	11.93	3.28
RED+P2	30.05	18.30	12.03	3.67
RED+P2	28.20	17.10	11.53	3.62
DNC	31.35	19.00	12.28	3.70
DNC	30.90	18.55	12.08	3.98
DNC	31.60	18.75	12.13	4.05
DNC	31.35	18.35	12.33	3.98
DNC+P3	27.85	16.75	11.03	3.42
DNC+P3	27.40	16.95	10.63	3.40
DNC+P3	27.45	17.20	10.48	3.53
DNC+P3	27.70	16.70	11.58	3.22
DNC+P4	27.85	17.65	11.63	3.10
DNC+P4	27.40	18.05	10.93	3.25
DNC+P4	26.70	17.05	11.58	2.93
DNC+P4	27.80	17.40	10.88	3.12
CONTROL	14.80	8.15	5.48	1.52
CONTROL	14.35	7.75	5.03	1.43
CONTROL	13.65	7.70	5.68	1.30
CONTROL	14.80	8.15	4.93	1.43

Extractable P (mg kg^{-1}) from the Glenhope soils after 1 wk of incubation

	Mehlich-3	Kelowna	Olsen	Water
NRC	30.00	18.75	17.73	2.55
NRC	29.25	17.50	17.78	2.48
NRC	29.75	16.05	16.93	2.50
NRC	29.75	17.50	17.38	2.50
RED	29.38	17.20	16.08	1.83
RED	30.13	16.55	15.68	2.28
RED	29.00	14.85	16.43	2.32
RED	29.88	14.90	15.68	2.52
RED+P1	28.38	14.95	16.53	2.32
RED+P1	27.63	13.25	15.38	2.58
RED+P1	28.75	15.80	14.48	2.25
RED+P1	29.50	14.85	22.78	2.45
RED+P2	30.00	15.00	16.88	2.60
RED+P2	29.50	14.60	21.28	2.32
RED+P2	30.13	14.70	16.83	2.40
RED+P2	30.75	16.80	16.53	2.42
DNC	29.00	17.05	15.53	2.62
DNC	29.00	16.85	15.68	2.47
DNC	29.25	15.65	15.63	2.52
DNC	28.75	17.05	15.53	2.38
DNC+P3	26.38	15.00	15.68	2.25
DNC+P3	26.75	15.60	15.43	2.70
DNC+P3	27.13	15.75	15.78	2.58
DNC+P3	26.63	15.55	15.98	2.30
DNC+P4	24.25	13.70	12.88	2.02
DNC+P4	24.75	15.35	13.38	1.97
DNC+P4	24.00	15.25	13.43	2.30
DNC+P4	25.38	14.45	13.98	2.42
CONTROL	12.50	6.20	5.33	1.38
CONTROL	11.75	6.40	5.18	1.52
CONTROL	12.25	6.45	5.53	1.35
CONTROL	12.25	8.15	5.53	1.52

Extractable P (mg kg^{-1}) from the Glenhope soils after 4 wk of incubation

	Mehlich-3	Kelowna	Olsen	Water
NRC	28.30	18.45	15.70	0.23
NRC	29.20	18.20	15.85	0.49
NRC	27.70	20.35	15.00	0.59
NRC	28.05	17.50	15.95	0.31
RED	26.95	16.25	15.55	0.26
RED	27.55	21.00	16.00	0.36
RED	27.00	17.65	15.90	0.27
RED	27.15	15.25	15.95	0.23
RED+P1	26.42	15.50	15.25	0.29
RED+P1	26.92	16.75	14.55	0.40
RED+P1	26.58	19.80	15.05	0.30
RED+P1	26.67	18.65	19.20	0.24
RED+P2	29.38	15.85	15.30	0.74
RED+P2	28.38	19.00	15.80	0.29
RED+P2	29.38	17.70	15.50	0.34
RED+P2	28.63	17.80	15.30	0.67
DNC	27.55	17.45	14.95	1.06
DNC	27.05	18.15	15.05	0.27
DNC	27.80	17.70	17.40	0.34
DNC	27.25	18.15	16.05	0.46
DNC+P3	25.05	16.90	13.70	0.34
DNC+P3	25.30	17.05	14.65	1.16
DNC+P3	26.20	16.35	16.15	0.36
DNC+P3	25.35	17.65	15.15	0.31
DNC+P4	22.75	16.50	13.10	0.30
DNC+P4	23.25	16.35	13.80	1.39
DNC+P4	23.40	15.40	13.35	0.93
DNC+P4	23.20	15.30	13.80	1.37
CONTROL	11.40	7.95	5.00	0.23
CONTROL	12.05	9.10	5.10	0.21
CONTROL	11.30	6.50	5.55	0.64
CONTROL	11.30	6.45	7.45	0.26

Extractable P (mg kg^{-1}) from the Glenhope soils after 16 wk of incubation

	Mehlich-3	Kelowna	Olsen	Water
NRC	28.75	18.75	15.18	1.27
NRC	27.90	18.95	15.63	1.23
NRC	28.80	19.90	16.18	1.21
NRC	28.45	20.30	16.48	1.20
RED	26.80	18.25	15.68	0.98
RED	27.30	18.30	15.98	0.92
RED	27.50	18.55	16.38	4.73
RED	27.15	18.40	16.08	3.88
RED+P1	26.85	17.90	15.63	3.85
RED+P1	26.50	17.85	16.03	4.02
RED+P1	26.35	18.95	15.58	4.07
RED+P1	26.90	17.75	15.63	4.82
RED+P2	25.85	17.35	14.83	3.98
RED+P2	25.70	17.70	14.73	3.87
RED+P2	26.60	17.80	15.13	3.98
RED+P2	24.45	17.25	14.53	4.05
DNC	27.60	18.70	15.33	4.50
DNC	27.25	18.10	15.33	4.28
DNC	28.20	18.45	16.63	4.53
DNC	28.95	19.15	16.43	4.52
DNC+P3	29.30	18.80	17.88	4.50
DNC+P3	28.75	18.95	17.98	4.28
DNC+P3	28.70	18.90	17.98	4.67
DNC+P3	29.20	18.45	17.88	4.78
DNC+P4	23.45	15.25	13.33	3.77
DNC+P4	23.60	15.60	13.38	3.72
DNC+P4	23.40	16.15	13.33	3.88
DNC+P4	23.70	16.50	13.48	3.93
CONTROL	11.55	7.05	5.78	3.42
CONTROL	11.65	7.15	5.48	3.23
CONTROL	11.40	7.45	5.33	3.35
CONTROL	11.70	7.35	5.93	3.23