10 Environmental Implications of Inositol Phosphates in Animal Manures

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Animal production in the USA is valued at more than \$100 billion and has consolidated significantly during the last 20 years, with a larger number of animals being produced on an increasingly smaller land base (Kellogg *et al.*, 2000). Manure generated from animal production is currently estimated to exceed 335 million t of dry matter per year in the USA, while global manure production is estimated at ~13 billion t of dry matter per year (Mullins *et al.*, 2005). Manures contain significant amounts of phosphorus, with values between 6.7 and 29.1 g P/kg on a dry weight basis reported for several species of animals (Barnett, 1994). This phosphorus includes inorganic and organic forms, with the latter constituting between 10% and 80% of the total (Peperzak *et al.*, 1959; Gerritse and Zugec, 1977). Inositol phosphates are one of the primary organic phosphorus species found in manures, with *myo*-inositol hexakisphosphate typically being the most abundant (Peperzak *et al.*, 1959; Barnett, 1994; Turner and Leytem, 2004).

The environmental fate of phosphorus in animal manures is determined in part by the chemical composition of the phosphorus, yet few studies have fully characterized manure phosphorus and determined the effect of the various phosphorus compounds on phosphorus behaviour in soil. The various forms of organic phosphorus differ in the extent of their sorption when applied to soils, with *myo*-inositol hexakisphosphate being strongly bound while other organic phosphorus compounds such as nucleotides, DNA and glucose phosphates are more mobile (Celi and Barberis, 2005). Phosphorus applied to soil as manure may also behave differently from mineral phosphate fertilizer, due to other chemical characteristics of the manure. Organic matter in manure can complex iron and aluminium via organic ligands, which decreases the precipitation of inositol phosphates with these metals. It also competes for sorption sites in soil, increasing the concentration of phosphate in solution (Iyamuremye *et al.*, 1996). Inositol phosphates in manure can also disperse soil colloids and therefore increase the potential for particulate phosphorus transport in runoff (see Celi and Barberis, Chapter 13, this volume). Based on this evidence, more detailed information on the forms of phosphorus in manures, as well as those manure characteristics that influence phosphorus sorption, may shed light on the potential for off-site losses of phosphorus from land application of manure.

This chapter addresses environmental issues concerning phosphorus and inositol phosphates in animal production. We summarize studies on the phosphorus composition of manures, including those using traditional extraction procedures and the more recent application of nuclear magnetic resonance (NMR) spectroscopy. Finally, we review how dietary modification and storage alters the phosphorus composition of manures, and explore the impact of such alterations on phosphorus solubility in soils and the potential for phosphorus transfer in runoff.

Why is Manure Phosphorus an Environmental Concern?

Consolidation of animal production can generate regional and farm-scale nutrient surpluses where nutrient imports in feed and mineral fertilizer exceed nutrient exports in crops and animal products (Sharpley *et al.*, 1994; Sims *et al.*, 1998). These nutrient surpluses can in turn increase the risk of nutrient loss to the environment and pollution of water bodies (Sharpley, 1996; Sims *et al.*, 1998, 2000). Nutrients in manures can be recycled by application to cropland, which reduces the need for commercial fertilizers. Unfortunately, large amounts of manure produced in localized areas, coupled with the high cost of effective nutrient utilization strategies in an unbalanced system, favour manure disposal via land application in excess of crop nutrient needs, rather than utilizing manure in areas with nutrient deficiencies (Sharpley *et al.*, 1998).

Phosphorus is a particular concern, because it can accumulate in soil to concentrations greater than those needed for optimum crop production. This is due in part to unfavourable nitrogen/phosphorus ratios in manures relative to the uptake of these nutrients by most crops, which results in overapplication of phosphorus when manures are applied to meet the nitrogen requirement of the crop (Mikkelsen, 2000). As a result, long-term manure application to agricultural land leads to soil phosphorus accumulation and greater potential for phosphorus transfer in runoff to water bodies. This can contribute to eutrophication in freshwater ecosystems, and numerous examples of water quality impairment associated with phosphorus pollution from animal operations now exist (Burkholder and Glasgow, 1997; US Geological Survey, 1999; Boesch *et al.*, 2001). There is therefore an urgent need to understand and reduce the impact of animal manures on the pollution of water bodies. This demands a mechanistic understanding of the behaviour of manure phosphorus in soils and its potential for phosphorus transfer in runoff. Important aspects include the manure characteristics that determine phosphorus behaviour following land application and the potential changes induced by dietary modification.

Phosphorus Composition of Animal Manures

Investigation of the dynamics of manure phosphorus following application to soils requires information on the phosphorus composition of the manure. One of the earliest studies of manure characterization was performed by Funatsu (1908), who used sequential extraction techniques to fractionate the phosphorus in guano. The procedure involved dilute acid to extract inorganic phosphate, inositol phosphates and other organic forms, followed by ether and alcohol to extract phospholipids, with the residue (unextracted fraction) being labelled as nucleic acid. Variations of this procedure were subsequently used by others to characterize manures from pigs fed a variety of feed rations (Rather, 1918), poultry and mixed farmyard manure (Ghani, 1941), sheep manure (McAuliffe and Peech, 1949) and fresh manure from horses, cattle, sheep, pigs and hens (Kaila, 1948). Organic phosphorus in these studies ranged between 18% and 50% of the total phosphorus, with the acid-soluble organic phosphorus (which typically included inositol phosphates) constituting between 0% and 86% of the total organic fraction.

Peperzak *et al.* (1959) used a similar sequential extraction procedure to determine the phosphorus composition of a variety of manures. Total phosphorus concentrations ranged between 4 and 30 g P/kg dry weight, with the inorganic fraction constituting 53–95% of total phosphorus (Table 10.1). In this procedure, *myo*-inositol hexakisphosphate was isolated from the acid extract and was found to represent between 1% and 22% of total phosphorus, with other acid-soluble organic phosphorus forms constituting between 3% and 44%. The alcohol-soluble fractions were small (0.4–1.3%) while residual phosphorus values ranged between 2% and 27% of total phosphorus. When manures of different ages were examined from a stockyard, the general trend was a decrease in organic phosphorus from 49% to 32% of total phosphorus over 20 years, with a

Table 10.1. Concentrations of phosphorus compounds in sequential extracts of animal manures. (From Peperzak *et al.*, 1959.)

Animal	Total P (g P/kg)	Phosphate	*myo*-Inositol hexakisphosphate	Other acid-soluble P	Alcohol-soluble P	Residual P
		% of total P				
Chick	13–23	53–56	ND[a]	17–44	0.6–1.0	2–27
Hen	7–30	54–81	12–22	3–11	0.1–0.6	5–12
Sheep	12	63	2	19	0.4	16
Sow	11	83	0.6	13	0.5	3
Horse	4–7	73–95	1–2	14	0.8	2–20
Steer	8–12	60–64	7–10	12–13	1.0	13–19
Bull	9	76	0.5	8	0.7–1.0	14
Cow	4–7	67–87	1–5	7–25	1.3	3–14
Calf	5	62	3	17	0.4–1.3	17

[a]ND = not detected.

concomitant decrease in *myo*-inositol hexakisphosphate from 3.9% to 1.5% of total phosphorus.

Barnett (1994) published the most recent comprehensive study on organic phosphorus compounds in animal manures using conventional sequential fractionation techniques. Organic phosphorus in a variety of manures was fractionated into phospholipids, nucleic acids, acid-soluble organic phosphorus, inorganic phosphate and residual phosphorus. Inorganic phosphate constituted the greatest proportion of the total phosphorus, followed in descending order of magnitude by residual phosphorus, acid-soluble organic phosphorus and small amounts of phospholipids. In this study the *myo*-inositol hexakisphosphate content was not directly measured, but the acid-soluble organic phosphorus fraction, which typically includes the inositol phosphates, ranged between 7.8% and 53.4% of the total phosphorus.

Interest in the environmental fate of manure phosphorus prompted recent studies to adopt the Hedley fractionation (Dou *et al.*, 2000; Sharpley and Moyer, 2000; Weinhold and Miller, 2004). This procedure was originally developed to assess phosphorus solubility in soil (Hedley *et al.*, 1982) and involves sequential extraction with water, sodium bicarbonate, sodium hydroxide and hydrochloric acid. Phosphorus extracted in water and bicarbonate is considered readily soluble, while that extracted in sodium hydroxide (assumed to be associated with amorphous iron/aluminium and organic matter) and hydrochloric acid (assumed to be calcium phosphates) is considered poorly soluble. However, several problems compromise the suitability of the Hedley fractionation for manures. In particular, phosphorus chemistry differs markedly between soils and manures, being controlled commonly by iron and aluminium oxides and calcium carbonate in soils (Hedley *et al.*, 1982), and by association with calcium and magnesium in manures (Cooperband and Ward Good, 2002).

Turner and Leytem (2004) used solution ^{31}P NMR spectroscopy to unequivocally identify phosphorus compounds in the various fractions of the Hedley extraction scheme as applied to poultry, swine and cattle manures. Two main groups of phosphorus compounds were determined with this procedure: a readily soluble fraction extracted with water and sodium bicarbonate and a stable fraction extracted with sodium hydroxide and hydrochloric acid. Organic phosphorus in the readily soluble fraction included DNA, phospholipids and simple phosphate monoesters. Organic phosphorus in the stable fraction consisted mainly of *myo*-inositol hexakisphosphate. Since there was considerable overlap between the extracts, the authors recommended a simpler procedure consisting of extraction with sodium bicarbonate to remove the readily soluble fraction (which would be most susceptible to transport in runoff), followed by extraction with a solution containing sodium hydroxide and ethylenediamine tetraacetate (EDTA) to recover the more stable fraction. This method gave near-quantitative recovery of phosphorus from swine and poultry manure (Turner, 2004; Turner and Leytem, 2004).

Solution ^{31}P NMR spectroscopy has been used to quantify the phosphorus composition of a wide variety of manures (Leinweber *et al.*, 1997; Leytem *et al.*, 2004; Maguire *et al.*, 2004; Turner, 2004; Turner and Leytem, 2004; McGrath *et al.*, 2005). These studies indicate that manure phosphorus is predominately inorganic phosphate, followed in descending order by phosphate monoesters, phosphate diesters (nucleic acids and phospholipid), pyrophosphates and, in some cases, phosphonates. Concentrations of *myo*-inositol hexakisphosphate ranged from non-detectable to 80% of the total phosphorus in manures from a variety of ruminant (cattle and sheep) and monogastric animals (poultry, swine; Table 10.2). Solid-state ^{31}P NMR spectroscopy has also been applied to manures (e.g. Hunger *et al.*, 2004), but cannot accurately assess the organic phosphorus fraction.

As demonstrated by both sequential fractionation and solution ^{31}P NMR spectroscopy, the *myo*-inositol hexakisphosphate content of manures can vary widely, both among and within species (Table 10.2). There are physiological differences between ruminant and monogastric animals that can account for these differences. The diets of monogastric animals often include large amounts of cereal grains, in which much of the phosphorus occurs as salts of *myo*-inositol hexakisphosphate (phytate); for example, approximately two-thirds of the phosphorus in maize and soybeans is in this form (see Raboy, Chapter 8, this volume). As monogastric animals do not possess ample gut phytase (McCuaig *et al.*, 1972), manures from poultry and pigs can contain large amounts of undigested phytate (although see Leytem *et al.*, 2004). In contrast, ruminant animals have the capacity to hydrolyse inositol phosphates in their diet, and manures from animals fed grass or lucerne-based diets contain little phytate. However, there is evidence that for ruminants fed a grain-based diet, metal complexation can prevent extensive hydrolysis of *myo*-inositol hexakisphosphate and allow it to pass through the animal intact (see Dao, Chapter 11, this volume).

Dietary effects are also evident within a given species. For example, manure from laying hens fed maize with varying levels of non-phytate phosphorus, with and without phytase additions, can contain a wide range of *myo*-inositol hexakisphosphate concentrations (35–80% of total phosphorus, whereas manure from broilers fed a diet consisting mainly of barley contains closer to 10% of total phosphorus in this form (Table 10.2). This indicates the importance of determining dietary impacts on the composition of manure phosphorus excreted from the animal to assess the potential behaviour of manure phosphorus once applied on land. Since it has been demonstrated that inositol phosphates can sorb strongly to soils (see Celi and Barberis, Chapter 13, this volume), changes in the concentration of *myo*-inositol hexakisphosphate in manure could be of concern from an environmental standpoint (discussed later).

Impact of Dietary Manipulation on *myo*-Inositol Hexakisphosphate in Manure

As monogastric animals cannot fully utilize phytate in cereal grains, mineral phosphate supplements are commonly added to their diets to prevent phosphorus deficiency. As described above, this increases phosphorus concentrations in manure and can lead to phosphorus accumulation in soils when manure phosphorus is applied in excess of crop phosphorus removal (Sims *et al.*, 2000).

To address concerns regarding surplus phosphorus in manure, strategies involving dietary manipulation are being widely adopted to reduce manure phosphorus concentrations (see Lei and Porres, Chapter 9, this volume). By reducing phosphorus excretion, manures with nitrogen/ phosphorus ratios more closely matching the nutrient needs of crops can be generated, thereby reducing overapplication of phosphorus and build-up of soil phosphorus. For monogastric animals that have a limited ability to digest phytate, dietary strategies include the isolation of mutant grains that store most of the total phosphorus in the grain as inorganic phosphate and less as phytate (Raboy *et al.*, 2000; Dorsch *et al.*, 2003, see Raboy, Chapter 8, this volume), thereby enhancing phosphorus uptake by the animal and reducing the excreted phosphorus (Spencer *et al.*, 2000; Veum *et al.*, 2002; Jang *et al.*, 2003; Klunzinger *et al.*, 2005). Supplementation of animal feeds with microbial phytase is

Table 10.2. Concentrations of phosphorus compounds in extracts of manures from a selection of animals determined by solution ^{31}P NMR spectroscopy. (From Leytem *et al.*, 2004, 2005, 2006 and unpublished data; Maguire *et al.*, 2004.)

Manure	Total phosphorus[a]	Phosphate[b]	Phosphate monoesters[b]	Pyro-phosphate[b]	*myo*-Inositol hexakisphosphate[b]
	g P/kg dry wt				
Swine manure, fresh (barley feed)	13.46 (97)	13.02 (94)	0.67 (5)	0.13 (1)	Tr
Swine lagoon liquid	30.00 (99)	29.15 (97)	0.75 (3)	0.09 (<1)	ND
Broiler manure (barley feed)	6.36 (99)	4.46 (70)	1.92 (30)	ND	0.74 (12)
Broiler manure (standard maize diet)	15.61 (96)	7.21 (46)	8.19 (53)	0.21 (1)	7.61 (49)
Broiler manure (maize, low NPP[c])	9.49 (99)	1.22 (28)	8.17 (86)	0.10 (1)	7.62 (80)
Broiler manure (maize, low NPP + phytase)	9.61 (98)	5.33 (56)	4.04 (42)	0.13 (1)	3.39 (35)
Broiler litter (maize, high NPP)	13.90 (98)	5.71 (41)	8.38 (60)	0.06 (<1)	7.83 (56)
Broiler litter (maize, high NPP + phytase)	10.40 (96)	5.05 (49)	5.74 (55)	ND	4.88 (47)
Turkey litter (maize, high NPP)	15.40 (87)	10.90 (71)	6.74 (44)	0.14 (1)	5.09 (33)
Turkey litter (maize, high NPP + phytase)	12.80 (94)	8.56 (67)	4.82 (38)	0.14 (1)	3.45 (26)
Dairy lagoon liquid	8.80 (93)	7.93 (90)	0.82 (9)	0.06 (<1)	0.37 (4.2)
Dairy compost	2.50 (98)	2.28 (91)	0.22 (9)	0.004 (<1)	0.03 (1)
Beef manure (maize-fed)	4.20 (99)	2.51 (60)	1.60 (38)	0.09 (2)	0.34 (8)
Beef manure (pasture-fed)	4.10 (83)	2.65 (65)	1.0 (25)	0.25 (6)	ND
Sheep (barley-fed)	8.45 (91)	5.52 (65)	1.68 (20)	0.41 (5)	0.47 (6)

[a]Values are total phosphorus extracted by sodium hydroxide and ethylenediaminetetraacetate (EDTA), and values in parentheses are the proportion (%) of the total manure phosphorus determined by microwave digestion.
[b]Values in parentheses are the proportion (%) of the extracted phosphorus.
[c]NPP = non-phytate phosphorus.
Tr = trace; ND = not detected.

also used to increase phytate hydrolysis in the gut, thereby enhancing phosphorus utilization by the animal (Cromwell *et al.*, 1993; Coelho and Kornegay, 1996; see Lei and Porres, Chapter 9, this volume). The combination of low-phytate grains with phytase additions is also utilized to further reduce phosphorus excretion.

In addition to reducing the concentrations of phosphorus in manure, dietary modification is expected to influence manure phosphorus composition, which may have implications for the environmental fate of manure phosphorus (Turner *et al.*, 2002). Potentially the greatest impact of diet modification in monogastric animals on phosphorus forms in manure is likely to be changes in the amount of phytate excreted, with a corresponding increase in the proportion of the manure phosphorus that occurs as water-extractable phosphate. Thus, as diet modification reduces the proportion of the manure phosphorus occurring as *myo*-inositol hexakisphosphate, the proportion of water-extractable phosphate in the manure increases as a fraction of total phosphorus, even though the total phosphorus concentration may be reduced. This is particularly evident for poultry manures (Fig. 10.1) and may be important when manures are applied to land on the basis of phosphorus content, as is now common in several states in the USA.

Feeding low-phytate grains

Mutant grains that contain substantially less phytate than the wild-type equivalent that has traditionally been fed to animals (Raboy *et al.*, 2000; Dorsch *et al.*, 2003; see Raboy, Chapter 8, this volume) have recently been developed. At present there are low-phytate varieties of maize, barley and soybean meal that can be used in feed formulations. Low agronomic yields of these mutant grains have prevented wide adoption, but future improvements are likely, and these grains will be useful for developing strategies to reduce phosphorus excretion by monogastric animals. Large reductions in total phosphorus excretion can be achieved using these grains (Spencer *et al.*, 2000; Li *et al.*, 2001; Veum *et al.*, 2002; Jang *et al.*, 2003; see Lei and Porres, Chapter 9, this volume), although only a few studies have determined their impact on phosphorus composition in manure. Toor *et al.* (2005) reported a decrease of only 10% in excreted total phosphorus from broilers fed diets containing normal maize vs. low-phytate maize, although there was a 47% reduction in the amount of *myo*-inositol hexakisphosphate excreted by the birds. Baxter *et al.* (2003) saw the same trend for swine fed low-phytate maize; total phosphorus excretion was only slightly reduced, but *myo*-inositol hexakisphosphate excretion was reduced by almost 50%.

When low-phytate barleys were included in broiler diets, manure total phosphorus concentrations were reduced by 14–24% (Leytem *et al.*, 2006b; Table 10.3). However, *myo*-inositol hexakisphosphate concentrations in manures from all dietary treatments constituted only 3–12% of the total phosphorus in the manure, even when as much as 91% of total phosphorus in the feed was phytate. This same trend was also reported for swine in a similar study; total phosphorus excretion was reduced by ~33% when animals were fed low-phytate diets, yet *myo*-inositol hexakisphosphate was excreted only in trace amounts (Leytem *et al.*, 2004; Table 10.3). This indicates that even though monogastric animals do not possess sufficient phytase to hydrolyse phytate in the part of the digestive tract where phosphorus sorption takes place, the phytate is not necessarily excreted by the animal.

A possible explanation is that barley diets contain high intrinsic phytase activity (see Lei and Porres, Chapter 9, this volume), which might lead to phytate hydrolysis in the animal. However, in a study of swine manure from animals fed diets containing wild-type and low-phytate maize, which contains little intrinsic phytase, most of the excreted phosphorus (~80% of total phosphorus) was inorganic phosphate and there was little difference in the manure fractions across dietary treatments (Weinhold and Miller, 2004). A more likely explanation, therefore, is that phytate is hydrolysed in the hindgut by intestinal microflora, even though the animals derive little nutritional benefit from this process in the lower intestine.

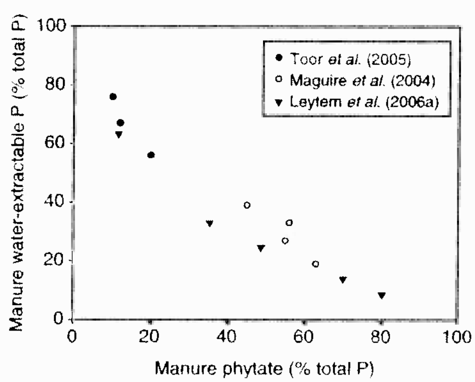

Fig. 10.1. The effect of phytate concentration on water-extractable phosphorus in manures from modified poultry diets. (From Maguire *et al.*, 2004; Toor *et al.*, 2005; Leytem *et al.*, 2006.)

Table 10.3. Phosphorus concentrations in poultry and swine manure fed either a wild-type barley (Copeland and CDC Bold) or mutant barley with reduced amounts of grain phytic acid content (M 422, M 635, M 955). Phosphorus concentrations were determined by extraction in sodium hydroxide and ethylenediaminetetraacetate (EDTA) and solution ^{31}P NMR spectroscopy. Means in the same column (for each animal type) followed by the same letter do not differ significantly ($P > 0.05$). (From Leytem *et al.*, 2004; Leytem, A.B., Thacher, P.A. and Turner, B.L., 2006, unpublished data.)

		NaOH–EDTA extractable P (g P/kg dry wt)			
Grain type	Feed phytate (% total phosphorus)	Total P[a]	Phosphate[b]	Phosphate monoesters[b,c]	*myo*-Inositol hexakis-phosphate[b]
Poultry (broiler chicks)					
Copeland	91	6.36 (99)a	4.46 (70)a	1.92 (30)a	0.74 (12)a
M 422	40	4.48 (93)c	3.42 (69)c	1.53 (31)b	0.34 (7)ab
M 635	37	4.93 (92)bc	3.20 (72)c	1.29 (29)b	0.34 (8)ab
M 955	<1	5.15 (92)b	3.88 (75)b	1.24 (24)b	0.14 (3)b
Swine (barrows)					
CDC Bold	55	13.46 (97)a	13.02 (94)a	0.67 (5)a	Tr
M 422	50	8.55 (95)b	7.77 (86)b	1.08 (12)a	Tr
M 635	26	8.05 (91)b	7.59 (86)b	1.08 (12)a	Tr
M 955	3	8.36 (95)b	7.78 (88)b	0.91 (11)a	ND

[a]Values in parentheses are the proportion (%) of the total manure phosphorus determined by microwave digestion.
[b]Values in parentheses are the proportion (%) of the NaOH–EDTA extracted phosphorus.
[c]Values for phosphate monoesters include *myo*-inositol hexakisphosphate and other monoesters.
Tr, trace; ND, not detected.

Feeding microbial phytase as a supplement

There are several different types of phytase enzymes (see Mullaney and Ullah, Chapter 7, this volume), although they all catalyse the release of phosphate residues from *myo*-inositol hexakisphosphate. Phytase supplements are now a common component of animal diets and have been successful in reducing phosphorus concentrations in manures (see Lei and Porres, Chapter 9, this volume). However, the effects on manure phosphorus composition and therefore manure phosphorus behaviour in soils are poorly understood.

It would be expected that manures from diets that included phytase would have less *myo*-inositol hexakisphosphate than equivalent diets without phytase. This was the case in a study of manures from swine fed diets with and without phytase (Baxter *et al.*, 2003). Concentrations of *myo*-inositol hexakisphosphate in fresh swine manure were decreased by 2.0–3.9 g P/kg by adding phytase to the feed. However, during storage of manure from the normal diet for 150 days, *myo*-inositol hexakisphosphate as a percentage of total phosphorus decreased from 15.5% to 8.5%, which was attributed to microbial degradation. For the phytase-amended diet the decrease in *myo*-inositol hexakisphosphate during storage was only between 9.1% and 9.8%, indicating hydrolysis by the added phytase prior to excretion (Baxter *et al.*, 2003). Therefore, after 150 days of storage, there was no significant difference in *myo*-inositol hexakisphosphate concentrations in swine manures from the two diets.

Maguire *et al.* (2004) grew three flocks of broilers and two flocks of turkeys on the same bed of litter using diets that were 'high' and 'low' in non-phytate phosphorus with and without phytase additions. Concentrations of *myo*-inositol hexakisphosphate in both broiler and turkey litters from diets that included phytase were consistently lower than in litters from equivalent non-phytase diets (Table 10.4). Inorganic phosphate levels in the broiler and turkey litters

Table 10.4. Dietary studies where phytase has been used to reduce the total phosphorus concentrations in poultry manures and the influence on manure phytate content. Means followed by the same letter (within each column and for each study) are not significantly different ($P > 0.05$).

Animal	Diet, non-phytate P (%)	Phytase addition	Manure characteristics (g P/kg dry wt) Total P	WSP[a]	Phytate	WSP/total P ratio	Phytate P/total P ratio	References
Turkey	0.56	No	17.8a	6.4a	5.09	0.36	0.28	Maguire *et al.* (2004)
Turkey	0.48	Yes	13.5b	6.3a	3.45	0.47	0.26	
Turkey	0.42	No	11.8c	5.1b	4.89	0.43	0.41	
Turkey	0.34	Yes	11.0d	5.0b	3.65	0.45	0.33	
Broiler	0.36	No	14.1a	4.7a	7.83	0.33	0.56	Maguire *et al.* (2004)
Broiler	0.26	Yes	10.8c	4.2a	4.88	0.39	0.45	
Broiler	0.29	No	11.7b	2.2b	7.32	0.19	0.63	
Broiler	0.20	Yes	9.7d	2.6b	5.35	0.27	0.55	
Broiler	0.36	No	13.6a	1.1a	7.8	0.08	0.57	McGrath *et al.* (2005)
Broiler	0.26	Yes	10.7bc	1.0a	5.4	0.09	0.50	
Broiler	0.29	No	11.2b	0.9a	7.3	0.08	0.65	
Broiler	0.23	Yes	9.6c	0.6a	4.9	0.06	0.50	

[a]WSP = water-soluble phosphate in manure.

were largely unaffected by dietary phytase. This was most likely due to the benefit of decreased dietary inorganic phosphate supplements being cancelled out by the increased phytate hydrolysis by dietary phytase.

McGrath *et al.* (2005) determined *myo*-inositol hexakisphosphate in litters from broilers fed a variety of diets with and without phytase addition, and found that concentrations were lower in litter from diets containing phytase than from diets without phytase (Table 10.4). Toor *et al.* (2005) analysed turkey manure and broiler litter samples from diets with and without phytase using X-ray absorption near-edge structure spectroscopy. Although detection of organic phosphates was difficult using this technique, the authors concluded that dietary phytase addition decreased *myo*-inositol hexakisphosphate concentrations in manures and litters, and that dicalcium phosphate was the most abundant form of phosphorus present.

There has been some discussion in the literature as to whether residual dietary phytase will continue to hydrolyse *myo*-inositol hexakisphosphate in manures following excretion, hence making phosphorus more water-soluble. Angel *et al.* (2005) used combinations of boiling poultry and swine manures, or added antibiotics, to show that dietary phytase supplementation had no effect on phytate hydrolysis following excretion. These authors concluded that the 'increase in water-soluble phosphorus as a percent of total phosphorus post excretion is a function of excreta microbial activity and not dietary phytase addition' (Angel *et al.*, 2005). McGrath *et al.* (2005) stored broiler litters generated from diets 'high' and 'low' in phosphorus, with and without phytase, at two different moisture contents for 440 days. By comparing the interactions of storage time and moisture, they showed that *myo*-inositol hexakisphosphate concentrations decreased through time only in litter that was stored 'wet'. This was unrelated to dietary phytase and was instead attributed to enhanced microbial activity in the wet litter (McGrath *et al.*, 2005). Maguire *et al.* (2006) fed broiler breeders diets 'high' and 'low' in dietary non-phytate phosphorus, with and without phytase. Soluble phosphorus was similar in manure from under the feeder as in a clean area, indicating no effect of spilled feed whether or not it included phytase. However, under the drinker, manure moisture and soluble phosphorus were higher irrespective of the diet, presumably due to increased microbial activity breaking down *myo*-inositol hexakisphosphate into more soluble forms. The effects of manure-derived phytase in soils are unknown, although discussion of the interactions of phytase with soil constituents can be found elsewhere in this volume (see George *et al.*, Chapter 14).

Combining low-phytate grains and phytase

In addition to research on low-phytate grains or phytase alone, a few studies have investigated a combination of low-phytate grains and phytase. Baxter *et al.* (2003) reported that such a combination decreased *myo*-inositol hexakisphosphate in fresh swine manures more than either approach individually (Table 10.5). This trend was also seen in broiler litters, in which *myo*-inositol hexakisphosphate decreased from 20% of total phosphorus in a normal maize diet to 12% and 10% in diets containing low-phytate maize and low-phytate maize plus phytase, respectively (Toor *et al.*, 2005; Table 10.5). Other studies combined phytase and low-phytate grains in poultry diets and reported reductions of 27–45% of total phosphorus and 27–49% of water-extractable phosphate in the litter, although none determined *myo*-inositol hexakisphosphate directly (Applegate *et al.*, 2003; Miles *et al.*, 2003; Penn *et al.*, 2004).

Manure phosphorus composition and phosphorus solubility in soil

Manipulating the diets of monogastric animals can have a large impact on the amount of *myo*-inositol hexakisphosphate excreted from swine, poultry and fish. In addition, storage of manure prior to land application can also influence inositol phosphate concentrations by promoting microbial degradation. This raises an important question: Do differences in inositol phosphate concentrations influence the solubility and potential transport of manure phosphorus to water bodies following application to soil?

Release of soluble phosphorus from manure-amended soil varies considerably depending on the source of the manure applied (i.e. animal

Table 10.5. Dietary studies utilizing low-phytate grains with and without the addition of phytase and the effect on manure phytate content. Means followed by the same letter (within column for each study) are not significantly different at $P = 0.05$.

Animal	Diet	Phytase	Total P	WSP[a]	WSP/total P ratio	Phytate/total P ratio	Reference
			g P/kg dry weight				
Broiler	Normal maize	No	22.4a	12.6	0.56	20	Toor *et al.* (2005)
Broiler	Low-phytate maize	No	20.1b	13.5	0.67	12	
Broiler	Low-phytate maize	Yes	15.7c	12.0	0.76	10	
Swine	Normal maize	No	25.5a	11.9a	0.47	15	Baxter *et al.* (2003)
Swine	Low-phytate maize	No	20.7b	10.8a	0.52	8	
Swine	Low-phytate maize	Yes	15.2c	7.9b	0.52	5	

[a]WSP = water-soluble phosphate in manure.

species, diets fed, manure handling and storage). This is primarily due to differences in the concentrations of total and soluble phosphorus in the manure (Sharpley and Moyer, 2000; Kleinman *et al.*, 2002a,b; Vadas *et al.*, 2004), but may also be due in part to variability in other physical and chemical properties of the manure. Inorganic phosphate is relatively soluble in soils compared to *myo*-inositol hexakisphosphate, which is strongly retained and unlikely to be lost as soluble phosphorus in runoff (Anderson *et al.*, 1974; Leytem *et al.*, 2002). Therefore, variability of the phosphorus composition of manures, either due to differences in species, manure-handling techniques or through dietary manipulation, could increase phosphorus transport from land-applied manures to water bodies (Vadas *et al.*, 2004).

When a variety of manures (swine, dairy and beef cattle manures that were handled/stored differently) were incorporated into semiarid calcareous soils, there was no significant correlation between *myo*-inositol hexakisphosphate content (ranging between 0% and 8% of total phosphorus) and soil phosphorus solubility (Leytem and Westermann, 2005; Fig. 10.2a). In this instance, the small amounts of *myo*-inositol hexakisphosphate in the manures were probably insufficient to influence phosphorus solubility in the soil. Instead, phosphorus solubility was clearly influenced by the amount of carbon added to the soil (Fig. 10.2b).

When poultry manures were added to a similar calcareous soil, the amount of *myo*-inositol hexakisphosphate in the manures, which ranged between 35% and 80% of total phosphorus, was strongly and negatively correlated with bicarbonate-extractable soil phosphate, following manure application (Fig. 10.3a). Manures were applied at the same total phosphorus rate, so this correlation was almost certainly due to the greater proportion of water-soluble phosphate added in manure with lower *myo*-inositol hexakisphosphate concentrations. However, the relationship was transient, becoming insignificant after 9 weeks of incubation (Fig. 10.3b). This demonstrates clearly that when manures are applied on the basis of phosphorus content, the proportion of *myo*-inositol hexakisphosphate, and therefore of water-soluble phosphate, has a strong influence on the solubility of the manure phosphorus soon after application.

Extractable phosphate concentrations increased between the second and ninth week of incubation and were correlated with the amount of *myo*-inositol hexakisphosphate in the manures. In other words, manures with more *myo*-inositol hexakisphosphate caused greater increases in extractable soil phosphate over time. Analysis of

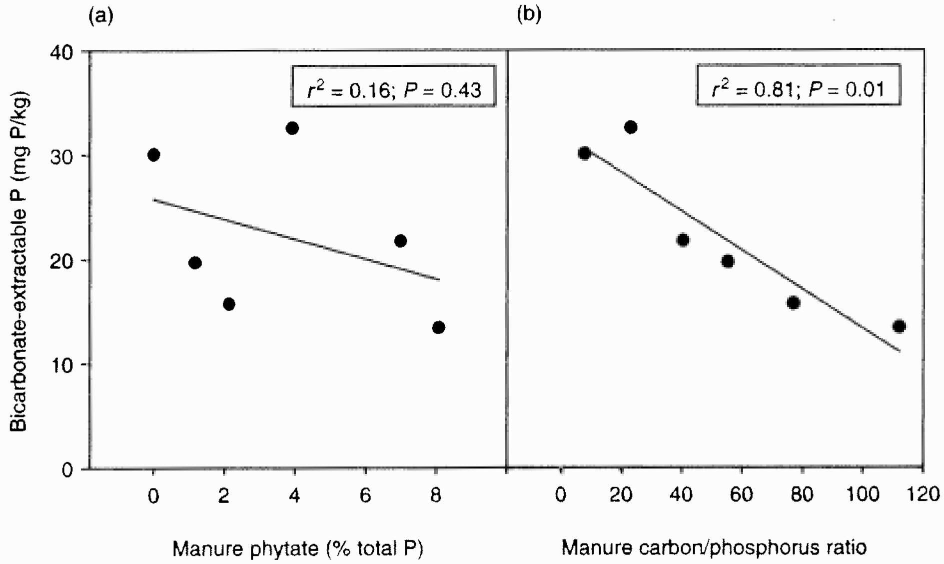

Fig. 10.2. Relationship between bicarbonate-extractable phosphate and (a) manure phytate concentration and (b) manure carbon/phosphorus ratio for six manures of varying origin added to a calcareous arable soil (Portneuf silt loam) from Idaho, USA, containing 0.75% organic carbon, pH 7.6 and 18% clay. (From Leytem and Westermann, 2005.)

the manure-amended soils immediately following incorporation (Fig. 10.4a) and after 9 weeks of incubation (Fig. 10.4b) using solution ^{31}P NMR spectroscopy demonstrated the hydrolysis of *myo*-inositol hexakisphosphate in the soil, strongly suggesting that this was responsible for the increase in extractable phosphate.

Although *myo*-inositol hexakisphosphate is strongly bound in soils, microbes in the semiarid calcareous soil were able to break it down into

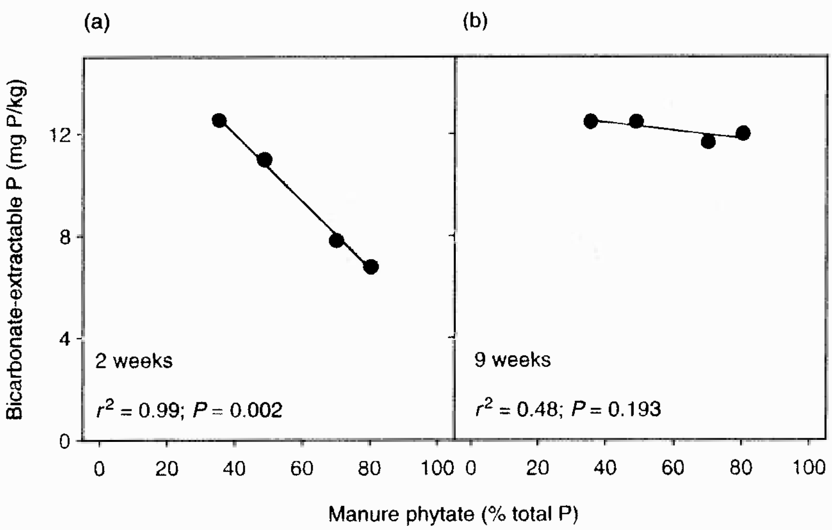

Fig. 10.3. Relationship between the phytate concentration in poultry manure and the bicarbonate-extractable phosphate in manure-amended soil following (a) 2 weeks of incubation and (b) 9 weeks of incubation. The soil was a calcareous arable soil (Portneuf silt loam) from Idaho, USA, containing 0.75% organic carbon, pH 7.6 and 18% clay. (From Leytem *et al.*, 2006.)

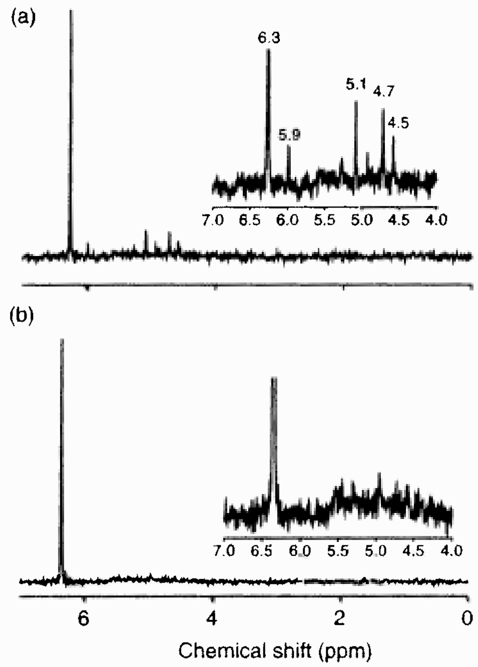

Fig. 10.4. Solution ^{31}P nuclear magnetic resonance (NMR) spectra of extracts of a soil amended with poultry manure (a) immediately following incorporation and (b) after 9 weeks of incubation. The peak at 6.3 ppm is inorganic phosphate, while the other four labelled signals are from *myo*-inositol hexakisphosphate. The spectra demonstrate the relatively rapid hydrolysis of manure-derived *myo*-inositol hexakisphosphate in soil. (From Leytem *et al.*, 2006.)

inorganic phosphate within a few weeks. It would therefore not be expected to accumulate in these soils following successive manure applications. This confirms the evidence for the relative bioavailability of inositol phosphates in calcareous soils (Turner *et al.*, 2003) and may explain why some contain no detectable phytate (see Turner, Chapter 12, this volume). In contrast, the same manures applied to an acidic soil showed no correlation between added manure *myo*-inositol hexakisphosphate and extractable soil phosphate (Mehlich-3 extraction) on any of the sampling dates, with only the manure carbon/phosphorus ratio being correlated to the extractable phosphate concentrations ($r^2 = 0.84$ at 2 weeks of incubation; data not shown).

The solubility of phosphorus in manure-amended soils seems to be influenced by the characteristics of the manure applied. In the short term, manures with large concentrations of *myo*-inositol hexakisphosphate can demonstrate lower phosphorus solubility on calcareous soils, although this trend does not seem to hold true for acidic soils. However, due to microbial breakdown of *myo*-inositol hexakisphosphate in applied manures and concurrent release of soluble phosphate, these differences are likely to become insignificant over time. Other manure properties, particularly the carbon content, seem to exert a large influence on phosphorus solubility following application to both calcareous and acidic soils (Leytem *et al.*, 2005), presumably due to stimulation of the microbial biomass and fixation of phosphorus in microbial tissue. This means that the addition of manure results in a lower soluble phosphorus concentration than would be expected from mineral phosphate fertilizer application. It therefore follows that in the long term the most important factor to consider for land application of manures is total phosphorus, rather than the form of the phosphorus applied.

An important impact of manure inositol phosphates on the loss of phosphorus to water bodies involves erosion and transport of particulate phosphorus. Erosion can be severe on agricultural land and is potentially responsible for the movement of large amounts of inositol phosphates to water bodies (see McKelvie, Chapter 16, this volume). Erosion can be promoted by inositol phosphates in manures due to the dispersion of soil colloids following sorption to soil components (see Celi and Barberis, Chapter 13, this volume). There is almost no information on inositol phosphate transport in particulate material from agricultural land, and it is not discussed further here. However, several stereoisomeric forms of inositol hexakisphosphate have been reported from riverine-suspended solids (Suzumura and Kamatani, 1995). More information can be found in a detailed review of organic phosphorus transfer from soils to water bodies (Turner, 2005).

Dietary Manipulation and the Environmental Fate of Manure Phosphorus

Manures from low-phytate feed

Although the total phosphorus excreted from monogastric animals fed a variety of low-phytate grains has been shown to be significantly reduced, the impacts of these manures on potential phosphorus losses following long-term application to agricultural land have not been studied. One of the primary reasons for this is the lack of sufficient quantities of manure needed for field-scale assessments, particularly multi-year projects. Investigation is therefore limited to laboratory-scale studies.

Gollany *et al.* (2003) showed a 10% reduction in manure phosphorus availability when manure from swine fed low-phytate maize-based diets vs. normal maize diets was incorporated into a silt loam soil. Leytem *et al.* (2005) incorporated manure from swine fed a variety of low-phytate barley-based diets and found no significant relationship between the amount of *myo*-inositol hexakisphosphate added in the manures and bicarbonate-extractable phosphate in soil (Fig. 10.5a). However, as with previous studies, there was a strong relationship between the amounts of carbon added with the manures and the bicarbonate-extractable phosphate (Fig. 10.5b). As the amount of phosphorus excreted by animals fed low-phytate grains is reduced, there is a corresponding increase in the manure carbon/phosphorus ratio, which can enhance the stabilization of phosphorus in manure-amended soils compared with soils amended with manures from normal grain-based diets. Therefore, even when applied on the same total phosphorus basis, there is a potential environmental benefit to feeding low-phytate grains when the subsequent manures are land-applied, at least in the short term.

Manures from phytase-amended feed

Studies have consistently shown reductions in manure total phosphorus and *myo*-inositol hexakisphosphate from swine and poultry that have been fed diets with phytase, but only when, as recommended, inorganic phosphate supplementation is reduced to account for enhanced phosphorus availability due to phytase addition. However, there has been some disagreement over the effect of added phytase on manure water-extractable phosphate, which is important because it is linked directly to phosphorus losses in runoff (Maguire *et al.*, 2005a). Dietary phytase addition can decrease total manure phosphorus concentrations by as much as 45% for poultry and 40% for swine (see Lei and Porres, Chapter 9, this volume). These reductions are important, as total phosphorus determines build-up or decline in soil test phosphorus following land application of manures. This is particularly true where manure is applied on the basis of nitrogen content – the effects of changes in manure

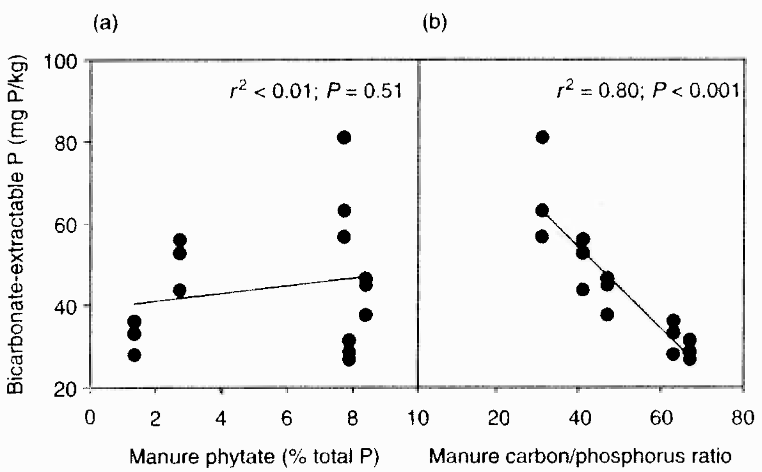

Fig. 10.5. The relationship between bicarbonate-extractable phosphate and (a) manure phytate concentration or (b) manure carbon/phosphorus ratio for manures from swine fed low-phytate grain diets applied to a calcareous arable soil (Portneuf silt loam) from Idaho, USA, containing 0.75% organic carbon, pH 7.6 and 18% clay. (From Leytem *et al.*, 2005.)

phosphorus composition are therefore only likely to become relevant when manure is applied on the basis of phosphorus content.

Several studies have surface-applied manures and litters derived from phytase-amended diets and measured phosphorus in runoff. Smith *et al.* (2004a) reported that although dietary phytase additions decreased the water-extractable phosphate in swine manure, this had no significant effect on soluble phosphorus losses in runoff from manured soils, relative to manure from a non-phytase-amended diet. This was surprising because equivalent weights of manures were applied, so manures with smaller concentrations of water-soluble phosphate (i.e. from phytase-amended diets) were expected to yield less soluble phosphate in runoff. In a similar study, however, soluble phosphate concentrations in runoff immediately following the application of poultry litter from a phytase-amended diet were lower than from soils that received litter from a normal diet (Smith *et al.*, 2004b). Again, manure was applied on a weight basis and, importantly, the effect became insignificant when three consecutive rainfall events were included. It should be noted that in both studies the application of alum (aluminium sulphate) to the litters considerably reduced soluble phosphate in litter and in runoff following litter application to soil. In one study in which dietary phytase significantly increased manure water-extractable phosphate, Vadas *et al.* (2004) reported no significant differences in soluble phosphate concentrations in runoff between soils amended with poultry manures from phytase and non-phytase-amended diets, even when manures were applied at the same total phosphorus rate.

Using turkey and broiler litters from equivalent phytase- and non-phytase-amended diets, Maguire *et al.* (2004, 2005b) found that dietary phytase decreased *myo*-inositol hexakisphosphate in litters, but generally had little effect on manure inorganic phosphate or soluble phosphate losses in runoff when manures were incorporated into soil prior to rainfall. This occurred whether litter was applied on the basis of nitrogen or phosphorus content. Where more than one runoff event was conducted, soluble phosphate losses decreased as the number of runoff events increased, and the effects of diet and manure characteristics became less significant. These data highlight the point that the soluble phosphorus in manure has a greater impact on runoff soluble phosphate concentrations in the short term than in the long term (Penn *et al.*, 2004; Smith *et al.*, 2004b; Maguire *et al.*, 2005b). However, we still must consider the fact that long-term land application of manures results in the accumulation of a large pool of phosphorus, which may be available for release to runoff water over time. The reduction in total manure phosphorus with phytase additions has the long-term benefit of reducing total phosphorus additions to fields receiving continual nitrogen-based manure applications that overapply phosphorus compared to crop needs.

Manures from low-phytate grains and phytase-amended feeds

As already discussed, combining low-phytate grains and phytase was shown to result in greater reductions in manure total phosphorus than either strategy on its own. It has also been shown to reduce water-extractable phosphate by 27–49% (Maguire *et al.*, 2005a). Smith *et al.* (2004b) reported that adding phytase to poultry diets containing low-phytate maize led to less soluble phosphate in runoff compared to that from a normal diet, but was not different to soluble phosphate in runoff from diets containing phytase or low-phytate maize on their own when manures were surface-applied at the same total phosphorus rate. Penn *et al.* (2004) observed similar concentrations of soluble phosphate in runoff from soils receiving surface application of turkey manure (same total phosphorus applied) from normal or low-phytate maize plus phytase diets. As there are only a limited number of studies measuring runoff from soils amended with these manures, it is too early to draw firm conclusions. However, the consistent reduction in total phosphorus and water-extractable phosphate in the manures suggests a clear benefit in terms of water quality.

Summary

Research to date has shown manure composition to be heavily dependent on both animal species and diet. In particular, differences in feed composition and phytase supplementation mean that manures from monogastric animals contain a wide range of *myo*-inositol hexakisphosphate concentrations. However, manure tends to be stored for long periods of time prior to land application, which allows microbial activity to break down a large fraction of the *myo*-inositol hexakisphosphate. This creates manures that have low *myo*-inositol hexakisphosphate concentrations when they are eventually land-applied. An important consequence is that other manure characteristics, such as the carbon/phosphorus ratio, may have a greater influence on subsequent phosphorus solubility in the short term than the phosphorus composition of the manure upon excretion from the animal. This must be considered when assessing the effects of dietary manipulation on the environmental impact of manure phosphorus.

When manure is applied to soil, a variety of factors can influence the phosphorus solubility and the potential for phosphorus transport to water bodies. In the case of surface-applied manure, the water-extractable phosphate concentration has the greatest influence on soluble phosphate losses when rainfall immediately follows manure application. When manures are incorporated into soils, other factors control phosphorus solubility and the potential for phosphorus losses to water bodies. In calcareous soils with low organic matter contents, phosphorus sorption can be influenced in the short term by the *myo*-inositol hexakisphosphate content of the manure, because manure with large concentrations of *myo*-inositol hexakisphosphate lead to small increases in soil phosphate solubility compared with manures dominated by inorganic phosphate. However, this effect is reduced as *myo*-inositol hexakisphosphate undergoes hydrolysis and contributes to the extractable phosphate pool, at which point other factors, such as the manure carbon/phosphorus ratio, determine differences in phosphate solubility. In contrast, when manures are applied to acidic soils, there seems to be no influence of *myo*-inositol hexakisphosphate content on extractable soil phosphate, and other manure characteristics may have a greater influence on phosphorus solubility. In situations where phosphorus losses are dominated by soil erosion and particulate phosphorus losses, the phosphorus concentration in the soil will overwhelm any influence of the applied manure phosphorus forms.

Concern has been expressed about the potential negative environmental implications of diet alteration on phosphorus losses from manure-amended soils, but given the urgent requirement to reduce total phosphorus concentrations in manures in areas of high livestock density, dietary manipulation is overwhelmingly beneficial. Such manipulation may increase the proportion of the manure phosphorus that is soluble in water, but this is likely to have negative environmental consequences only when manure is applied on a phosphorus basis and without prolonged storage prior to land application. If manures are applied on an equivalent weight or nitrogen basis, diet modification will result in less total phosphorus being added to soils and therefore a reduction in soil test phosphorus build-up over time. This in turn decreases the risk of phosphorus transfer to water bodies. In addition, most research indicates a reduction or no increase in phosphorus losses in runoff from soils amended with manures from modified diets compared with normal diets, when these are applied on an equivalent phosphorus basis (surface application or incorporation of manures). It therefore seems likely that in most cases there is no enhanced environmental risk from dietary modification and associated changes in manure phosphorus composition.

Future Research Needs

There is an increasing body of research aimed at understanding the influence of manure phosphorus composition on the potential environmental impacts related to land application of manure. At present, few studies have determined manure phosphorus composition using techniques such as solution ^{31}P NMR spectroscopy, yet this information provides valuable insight into the behaviour of phosphorus in manure after land application and can help identify the potential risks of modifying manures through diet manipulation.

The study of dietary impacts on manure phosphorus composition and subsequent environmental risk is becoming more important. There are few studies that have detailed the impacts of altering animal diets on manure phosphorus composition, and these have focused primarily on phosphorus in feeds (i.e. non-phytate phosphorus levels and the use of phytase). Dietary components, such as the calcium/phosphorus ratio in feeds, micronutrient additions and carbon

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composition of feeds, can alter the manure phosphorus composition and influence water-extractable phosphate, but have not been investigated in detail (see also Dao, Chapter 11, this volume). There is evidence that calcium and other divalent cations often found in micronutrient supplements can bind with *myo*-inositol hexakisphosphate, making both less available during digestion (Maenz *et al.*, 1999). Future studies should therefore look beyond just dietary phosphorus in order to understand the extent to which we can alter the phosphorus composition in manures and maximize the benefits of dietary manipulation.

The use of low-phytate grains in animal feeding operations has received considerable interest (see Raboy, Chapter 8, this volume) and further research will be necessary as new grains become available, especially as these become economically viable. Low-phytate grains have an advantage over phytase addition, because they minimize the interference that dietary inputs (such as calcium and other micronutrients) may have on phytate digestion and phytase efficacy. An important drawback at this point to using low-phytate grains is the issue of identity preservation (ability to keep low-phytate grains separate from other grains during processing), which will hopefully be overcome in the future.

Now that modified diets (phytase additions, low-phytate grains and lower phosphorus) are widely implemented, there is a need for long-term studies to determine the environmental effects of manure application resulting from these diets and the effects on soil phosphorus forms. There are no long-term trials studying the effect of land-applied manures from low-phytate diets on soil organic matter, soil phosphorus availability and forms, or phosphorus losses in runoff. Given the importance of understanding the impact of intensive animal operations on the phosphorus pollution of water bodies, such studies are urgently required.

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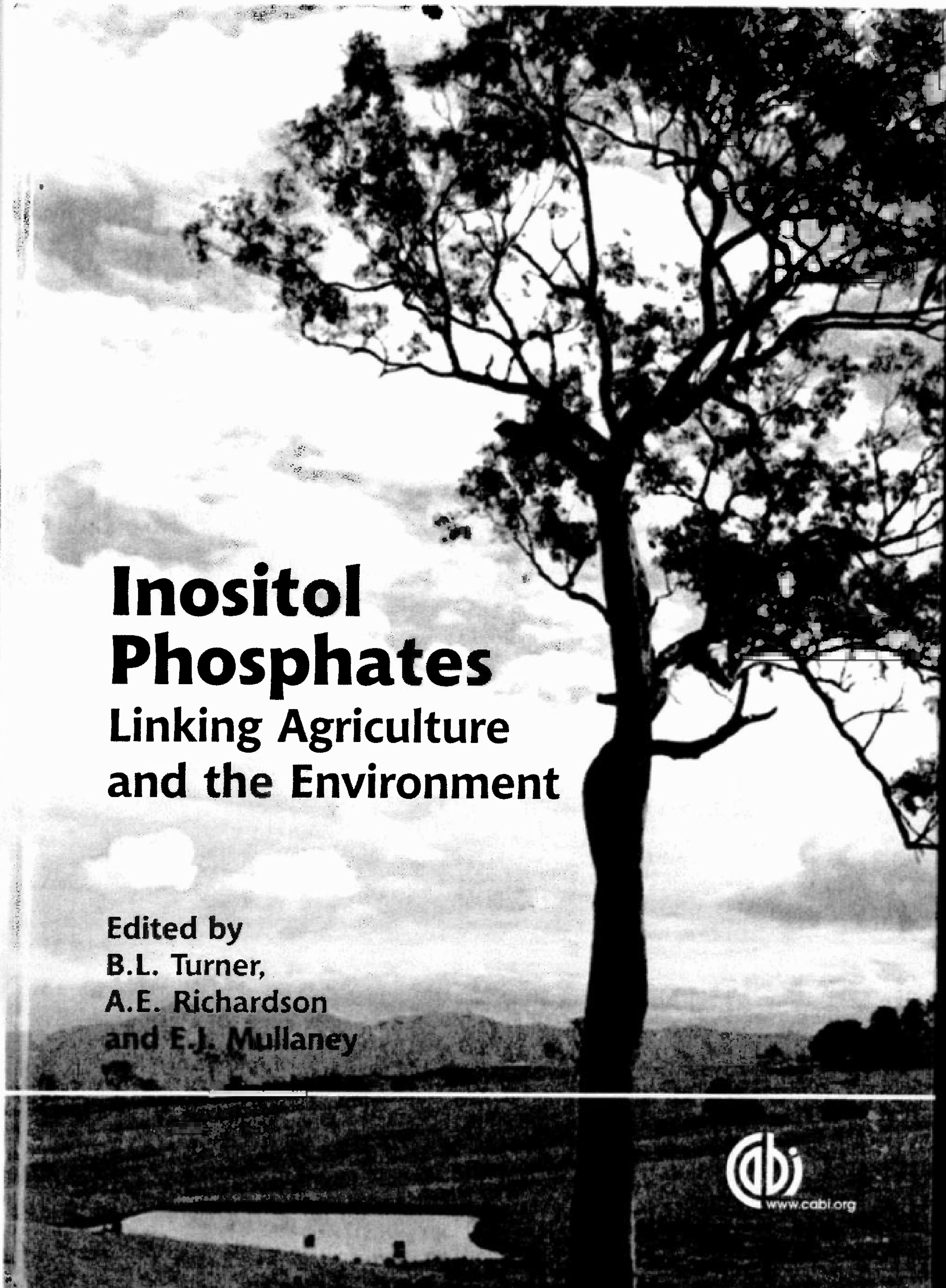
Inositol
Phosphates
Linking Agriculture
and the Environment
Edited by
B.L. Turner,
A.E. Richardson
and E.J. Mullaney
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