ABSTRACT

CASTEEL, SHAUN NATHAN. Phosphorus Dynamics from Broiler Breeder Diets in Manure, Soil, and Corn. (Under the direction of Drs. Daniel W. Israel and John T. Brake).

Studies of broiler breeder diet modifications to reduce phosphorus (P) excretion have evaluated bird performance, but no studies have quantified the effects of P in the manure and the impacts to soil and plant availability once soil-applied. Four diets were formulated by factoring two levels, 0.40 and 0.22% available P (NRC, Low, respectively), with or without phytase during the breeder laying phase (wk 22 to 64). Breeders fed phytase produced manures with 15% lower total P concentration, but did not change manure water-soluble P (WSP). However, P in the breeder manures was > 92% orthophosphate. The incubation of the four unique manures in samples of Portsmouth (Typic Umbraquult) and Wagram (Arenic Kandiudult) series generally did not differ in concentrations of Mehlich-3 P, soil WSP, total inorganic P, and total P. Phosphorus-based applications of breeder manures (NRC, Low) and triple superphosphate (TSP, $Ca[H_2PO_4]_2\ H_2O$) were applied to a P-deficient, Portsmouth soil in the greenhouse to determine the response of corn (*Zea mays*). Corn growth was equal among P sources in the initial study, but it tended to be greater in the soils amended with breeder manures in the residual study due to the P applied and the apparent liming effect of the soil. The NRC and Low breeder manures were applied at 39 kg P ha^{-1} in 2007 at Salisbury (Typic Rhodudult), Lewiston (Aquic Paleudult), and Plymouth (Typic Umbraquult), which ranged in soil P levels. Plymouth included TSP and an untreated control. Corn growth was equal among soils amended with the breeder manures NRC and Low in all site-years and grain P removal was equal five out of six site-years. Grain production, grain P removal, and applied P recovery were equal among P sources in 2007,

but the breeder manure treatments were greater than TSP in 2008. Breeder manures should be considered equivalent to TSP in P impacts to the soil and plant availability.

Phosphorus Dynamics from Broiler Breeder Diets in Manure, Soil, and Corn

by
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DEDICATION

I would like to dedicate this thesis to my wife and my family. Danae has been extremely supportive in my pursuit of this degree. My research has taken me away from her with long workdays from sunrise to sunset. She has sacrificed our time together allowing me to complete this dissertation to the best of my abilities. I thank God for leading us together as husband and wife and as best friends. Additionally, the love and encouragement given to me by my family has always been my foundation.

BIOGRAPHY

Shaun Nathan Casteel was born in Decatur, Illinois on 9 March 1979. His parents are Richard T., Jr. and Connie M. Casteel, and his two older sisters are Tonya and Carrie. He was raised on the Casteel family farm near Lovington, Illinois, where the principles and morals of his life were nurtured. As a young boy, Shaun was immersed into agriculture with his John Deere toys and his adventures to neighboring corn and soybean fields.

Shaun has enjoyed many broad experiences through his family, agriculture, education, and employment. The heart and soul of his education is rooted in the lessons learned from his dad and grandpa on the family farm. Every day after school, Shaun longed to be outside working on the farm rather than inside working on homework. Shaun found a happy median when he entered high school. His daily agriculture classes supplied the fuel for the days when he was restricted to class work. Shaun graduated from Lovington High School as Class Valedictorian and Illinois Scholar in May 1997. Some of his high school activities included FFA, basketball, baseball, and scholastic bowl.

Shaun's collegiate career began at Lake Land College, which he commuted to for two years, allowing him to stay on the farm. In 1999, he graduated Summa Cum Laude from Lake Land College with his Associate of Science degree in Agriculture. During the summer of 1998, Shaun was employed by American Cyanamid as a Field Sales Intern in the east central portion of Illinois. The following summer, he moved to Baraboo, Wisconsin, to work as a Field Biology Assistant for Zeneca Ag and to live on a dairy farm. Shaun majored in Crop Sciences at the University of Illinois in Urbana-Champaign and graduated with Highest Honors in 2001. He worked for Dr. Pederson in Soybean Pathology during his U of I school years and Novartis Seeds as a Seed Corn Production Intern in the summer of 2000. During the summer of 2001, he lived, learned, worked, and explored in Thessaloniki, Greece, as an International Student and Intern at the American Farm School.

Shaun desired to advise the American farmer with an unbiased and professional opinion, so he joined North Carolina State University in the Crop Science Department. He was an active member in the NC Cooperative Extension Cotton Program through research, weekly commentary, county talks, and professional meetings. God lead him to his soulmate, Danae, in Raleigh, whom he married 31 May 2003. He earned his M.S. in Crop Science with a focus in cotton physiology and management in May 2004. He continued serving the agriculture community as an Agronomist for the North Carolina Department of Agriculture in Raleigh for nearly two years. He was afforded the opportunity to earn his PhD in Soil Science at NC State University with specialization in nutrient management. He will be starting the next chapter of his career as the Extension Soybean Specialist at Purdue starting the 2009 Spring.

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CHAPTER 1

Literature Review of Phosphorus Management in Poultry Manure

Abbreviations: TP, total phosphorus; TSP, triple superphosphate; WSP_M, manure water-soluble phosphorus; WSP_S, soil water-soluble phosphorus.

Introduction

Agriculture was built on highly diversified farms that produced livestock, poultry, and crops. These forgone farms were cyclical systems that fed each component of production, i.e. crops were used to feed the livestock and manure from the livestock was used to supply plant-essential nutrients to the crops. No longer do the two agricultural sectors, livestock and crops, exist in the same proportions and locations as they once did. Animal production intensified in local regions (Kellogg et al., 2000) to maximize efficiencies in feeding, marketing, and processing during the 20th century (Hendrickson et al., 2008) while crop production centralized in other areas. In fact, less than 30% of corn grain stays on the farm where it was grown, which has led to a transfer of phosphorus (P) from the grain-producing areas to the animal-producing areas (Sharpley and Tunney, 2000). The intense animal production has created concentrated areas of manure production and subsequent land application. As a result, nutrient loading to the soil has caused an increased risk of nutrient loss to the environment and pollution to water bodies (Sharpley, 1996; Sims et al., 1998; Sims et al., 2000).

The Federal Water Pollution Control Act of 1972 and its amendment to the Clean Water Act in 1972 raised public and regulatory awareness of the pollution in national waters. The contamination of water was influenced by point and non-point sources of pollution. Various land activities contributed to non-point sources of pollution, but the majority was attributed to agriculture—crop fertilization, livestock grazing, and land application of animal manures. The United States Environmental Protection Agency identified runoff from 360 million hectares of agricultural land to be the leading cause of impairment in rivers and lakes and increased nutrients to be the greatest damaging factor in lakes, reservoirs, and ponds in the United States (USEPA, 2000). The primary contaminants of concern were P and nitrogen (N), which in excess can cause eutrophication (Carpenter et al., 1998). Nutrient enrichment of surface waters has multiple negative effects including increased algal blooms (toxic and non-toxic) and proliferation of aquatic weeds that deplete the oxygen in the waters as the biomass decomposes. Decreased biodiversity may result from the depletion of oxygen. Non-point sources have been difficult to measure and regulate, thus reduction in non-point source pollution must be achieved by focusing on land management and industry production practices, including manure characteristics.

Poultry Industry

The 20.9 billion dollar poultry industry could be used as one example of concentrated animal production with land application of manure. The majority of the United States poultry production has located in the Mid-South and Southeastern regions, of which North Carolina ranks fifth in broilers and second in turkeys (USDA, 2006). North Carolina broiler production increased from 540 million broilers in 1990 to 735 million broilers in 2005 having two counties that produced 25 percent of the state's broilers, and over half of the total broiler production was concentrated in ten counties (NCDA&CS, 2006).

Traditionally, applications of poultry manures were based on crop N removal rates with limited attention to P. The poultry manures typically supplied two to three times more P than the crop removed, which increased soil P concentrations, especially in the intense production areas (Mikkelsen, 2000). Loading soils above P saturation thresholds increased soil water-soluble P (WSP_S) and the potential for soluble P losses from agricultural fields (Van der Molen et al., 1998; Hooda et al., 2000; Pautler and Sims, 2000). Phosphorus-based applications of manures reduced P loading of soils, but more land was required to receive the manure. Many counties have a surplus of manure P due to limited crop acreage for P removal (Maguire et al., 2007). As a result, the poultry industry has been under scrutiny for nutrient loading, specifically P.

Dietary Phosphorus Modifications to Reduce Excreted Phosphorus

Typical poultry diets include soybean meal and corn in which phytate (organic P) composed over 60% of the total P in these ingredients (Nelson et al., 1968; Raboy et al., 1984). Poultry have been shown to be inefficient in utilizing phytate because younger birds such as broilers do not secrete sufficient phytase to completely hydrolyze phytate (McCuaig et al, 1972). Thus, mineral P has been supplemented to prevent dietary P deficiency and this has resulted in high P-concentrated manures and litters. Dietary non-phytate P (NPP) diets vary depending on the poultry type and typically start high and decrease as the birds age (i.e., feeding phases such as starter, grower, finisher, and withdrawal). Recommended dietary NPP content and ratios of dietary calcium (Ca) to NPP (from start to finish) are 0.45 to 0.30% NPP and 2.22 to 2.67 for broilers, respectively (Coon, 2002a), 0.40 to 0.25% NPP and 2.25 to 13.0 for layers, respectively (Coon, 2002b, and 2002c), and 0.45 to 0.40% NPP and 2.22 to 8.0 for broiler breeders, respectively (Coon, 2002d). Poultry have been shown to require Ca and P for bone formation during early development and higher amounts of Ca were required for egg-producing birds (i.e., for egg shell formation).

Significant advancements have been made through dietary P modifications to reduce total P excreted and therefore, address the concerns with the land application of poultry manure (CAST, 2002). Feed additions of phytase enhanced P digestibility by increasing phytate hydrolysis in the gut of poultry thereby reducing total P (TP, organic P, and inorganic P) fed and excreted (Cromwell et al, 1993; Coelho and Kornegay, 1996). Development of low phytate grains produced similar results in reduction of TP supplied and excreted by poultry (Raboy et al, 2000; Dorsch et al, 2003).

The effects of dietary P modifications on manure water-soluble P (WSP_M) were also critical, since WSP_M concentrations of various animal manures and municipal biosolids were positively correlated with surface-runoff P (Kleinman et al., 2007). Maguire et al. (2005) summarized multiple studies where dietary P modifications of broilers and turkeys had from no effect to a 83% reduction in WSP_M. However, some phytase additions in comparison to standard P diets increased WSP_M (1 to 18%) and WSP_M:TP (1 to 8%) in broiler litter (Maguire et al., 2004; Miles et al., 2003) and increased the WSP_M:TP (3 to 10%) in turkey litter (Maguire et al., 2004). The variable responses in WSP_M and WSP_M:TP raised environmental concerns about increased P movement to water bodies as a result of dietary P modifications and subsequent manure applications.

Phosphorus forms in manure were thought to determine the extent of P transport from land-applied manures to water bodies (Vadas et al., 2004) where inorganic P was relatively soluble compared to phytate (Anderson et al., 1974; Leytem et al., 2002). Increased phytate proportions in manure decreased the proportions of WSP_M:TP regardless of dietary P manipulation (Leytem and Maguire, 2007). Leytem et al. (2007, 2008) also reported that dietary Ca:P was a contributor of the variable results in WSP_M and WSP_M:TP from dietary P modifications. The increasing dietary Ca:P reduced soluble P independent of phytase supplementation.

Phosphorus Availability of Poultry Manure

State programs were developed to address soil P loading and to assess the risk of P loss from agricultural fields to watersheds in response to the contribution of P to the eutrophication of surface waters (Sharpley et al., 2003). Most programs ranked the risk with index values that considered numerous factors such as nutrient source (e.g., fertilizer vs. manure), soil P level, and application method (e.g., surface vs. incorporated). Another factor was the availability of the P in the manure although most states treated all poultry manures equally. Additionally, states assigned different P availability coefficients for the collective group of poultry manures as North Carolina used 0.80 (Crouse and Shaffer, 2006) while Pennsylvania used 1.0 (Weld et al., 2003).

Orthophosphate proportions of the TP in broiler litters were 30 to 40% (Leytem et al., 2007; Maguire et al., 2004) and in turkey litters were 50 to 70% (Maguire et al., 2004). Phosphorus availability would presumably be the lowest in the broiler litter and highest in turkey litter based on orthophosphate. Yet, all poultry manures were treated equally in nutrient management plans and P risk assessments, which raised environmental and agronomic concerns. For example, a P availability coefficient of 1.0 for broiler litters (low in orthophosphate) could be considered environmentally conservative, but could limit crop production due to an overestimation of P availability.

Physiological and management differences of broilers, broiler breeders, and layers would presumably influence manure P availability as well. Broilers have been reared for 6 to 9 wk, and broiler breeders have been maintained for approximately 64 wk (Scanes et al., 2004). Nutrient efficiency increases with age of the birds (Bell and Weaver, 2002), which would most certainly alter manure P characteristics of broiler breeders compared to broilers. Historically, broilers have been reared on litter to absorb excreta (e.g., wood chips high in carbon (C)), while excreta from broiler breeders and layers have been collected under slatted floors and cages, respectively (Bell and Weaver, 2002; Scanes et al., 2004). The C concentrations in the broiler litter, the breeder manure, and the layer manure would influence mineralization of organically bound nutrients (e.g., N, P). The high C in broiler litter would presumably have less available P than breeder or layer manures that were free of a litter substrate.

Recent studies have shown that the reduced P excreted from birds fed dietary P modifications often resulted in a reduced proportion of phytate in the manure (McGrath et al., 2005; Toor et al., 2005). No research has connected the manures produced from dietary P modifications to the interactions of manure-amended soil and plant uptake of P even though crop utilization of manures (e.g., N, P) was the foundation of nutrient management plans. Although studies of dietary P modifications of broiler breeders have evaluated bird performance (Berry et al., 2003), the manure P constituents and the response of soil and crops to soil applications of these manures have not been assessed. Understanding the P forms in all types of poultry manures and crop P utilization of these manures was necessary for accurate assessments of effects on soil sustainability and water quality.

Plant Response to Phosphorus-Based Applications of Poultry Manures

A few studies estimated plant P availability of poultry manures via Mehlich-3 P (M3P) extractions, WSP_S extractions, or plant response in the greenhouse. A Spodosol amended with poultry manure (free of wood chips) increased M3P concentrations 16% less than soils amended with potassium phosphate (KH_2PO_4) (i.e., the P in the poultry manure was 84% available or equivalent to potassium phosphate) (Griffin et al., 2003). Whereas, P applications of poultry layer manure (fresh, composted, pelletized) and triple superphosphate (TSP, $Ca[H_2PO_4]_2\ H_2O$) increased M3P at the same rate within two Ultisols and one Histosol of North Carolina (Montalvo, 2008). Pelletized broiler litter was equal to calcium phosphate ($CaHPO_4$) in biomass production, but annual ryegrass (*Lolium multiflorum*) and sorghum-sudan grass (*Sorghum X drummondii*) accumulated only 70% as much P from the litter as from calcium phosphate (Hammac et al., 2007). Composted poultry litter was generally equal to TSP in growth and P uptake in tall fescue (*Festuca arundinacea*) in the greenhouse (Sikora and Enkiri, 2005).

Phosphorus availability of poultry manures ranged from 30 to 70% based on the orthophosphate proportion in the manure TP, 84 to 100% based on M3P concentrations in amended soils, and 70 to 100% based on P accumulation of plants grown in the greenhouse. A major contributor to the variability of these P availability values was the type of birds that produced the manure (broiler, layer, turkey) and production practices (litter substrate, wood-free pure form, storage time). Phosphorus management of poultry manures needs to recognize the differences in P availability to ensure environmentally sound and agronomically productive recommendations.

A holistic approach to managing P in poultry manures integrates diet, manure, soil, and crop. Our studies fed modified-P diets to broiler breeders to assess the manure produced in terms of: (1) WSP_M and the proportions of phytate and orthophosphate, (2) P transformations in amended soils over time, (3) plant P availability to corn, and (4) P equivalency to TSP. We hypothesize that (1a) total P, WSP_M, and phytate in the breeder manures will decrease with the reduced dietary P regimes and phytase, (1b) phytate in breeder manures will be mineralized into orthophosphate as manure accumulation period increases, (2) P concentrations in the breeder manure-amended soils will be equivalent due to limited differences in orthophosphate proportions in the diet-modified breeder manures, and (3, 4) plant P availability and TSP equivalency will very high in breeder manures due to high orthophosphate proportions.

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CHAPTER 2

Dietary Phosphorus Modifications in Broiler Breeders:

Effects in Manure and Amended Soils

ABSTRACT

Dietary phosphorus (P) modifications have been employed to decrease total P (TP) of broiler and turkey litters; thereby, reducing the amount of P loading to soils in concentrated production areas. Our objectives were to characterize P constituents of broiler breeder manure produced from diet P modifications and to evaluate P transformation in soils amended with these manures. Four diets were formulated by factoring two P levels, 0.40 and 0.22% available P (AvP), with or without phytase during the breeder laying phase (22 to 64 wk of age). Forms of P in manure from these birds were characterized by solution ^{31}P nuclear magnetic resonance. Manures were incorporated in two Ultisols at 30 and 60 mg TP kg^{-1} and incubated for 12 wk. Manure TP decreased 28% when feeding 0.22 vs. 0.40% AvP and was not affected by phytase additions. Manure water-soluble P (WSP_M) decreased with phytase addition to the diet; however, decreases in dietary non-phytate P (NPP) and increases in dietary calcium to NPP ratio were highly correlated with WSP_M reduction ($r^2 = 0.98$ and 0.99, respectively). Soils amended with these diet modified-manures generally did not differ in Mehlich-3 P (M3P), soil WSP, total inorganic P, and TP. However, soil WSP was slightly higher in the Wagram soil at the high P rate of the breeder manures produced from the diets with 0.40% AvP. Increases of M3P averaged 40 to 48 and 90 to 99 mg kg^{-1} at wk 0 and stabilized at 30 and 62 to 68 mg kg^{-1} by wk 12. Breeder manures increased M3P to a much greater extent than has been reported for comparable applications of broiler and turkey litter. This was presumably due to large orthophosphate (92 to 96%) and small phytate (2 to 6%) proportions in the breeder manures. The high inorganic P proportion in the breeder manures may require greater land area for application since P availability would presumably be high.

Keywords: phytase, broiler breeder, phosphorus, phytate, orthophosphate, soil

Abbreviations: AvP, available phosphorus; CCE, calcium carbonate equivalent; M3, Mehlich-3; M3P, Mehlich-3 phosphorus; NPP, non-phytate phosphorus; PSR, phosphorus saturation ratio; TP, total phosphorus; TIP, total inorganic phosphorus; WSP_M, manure water-soluble phosphorus; WSP_S, soil water-soluble phosphorus.

INTRODUCTION

Animal production has changed dramatically in the past 30 years as large, confined animal feeding operations hasten nutrient loading of the land (Kellogg et al., 2000) and increase the risk of non-point nutrient loss to water bodies and subsequent eutrophication (Sharpley, 1996; Sims et al., 1998; Sims et al., 2000). Crop fertilization, livestock grazing, and land application of animal manures make agricultural land the greatest source of non-point pollution (USEPA, 2000). Most manure applications have been based on nitrogen (N) needs. Crop requirement or removal of N was greater than phosphorus (P) as demonstrated by the following N:P ratios—5:1 in corn and wheat, 6:1 in cotton, and 10:1 in coastal Bermuda hay (Osmond and Kang, 2008). Manure N:P ratios have rarely matched crop removal ratios and were approximately 1:1 in anaerobic swine sludge, 2:1 in broiler breeder manure, 3:1 in broiler and turkey litter, 4:1 in dairy slurry, and 6:1 in anaerobic swine effluent (Casteel et al., 2007). The imbalance of the N:P ratios in crop removal and manure supply sometimes results in manure P surplus (Maguire et al., 2007) and P accumulation in the soil, especially in these concentrated production areas where manure was land-applied (Mikkelsen, 2000). The degree of P saturation of soils has correlated well to soluble P and subsequent P losses from agricultural fields (Van der Molen et al., 1998; Hooda et al., 2000; Pautler and Sims, 2000). As a result, a need has developed to reduce soil P loading in high P soils.

Phosphorus loading from applications of poultry manure or litter can be reduced by applying these materials based on crop P removal and decreasing manure P concentrations through diet modifications (CAST, 2002). Poultry's limited ability to digest phytate from grain (McCuaig et al., 1972) and dietary “insurance” fortification of mineral P (e.g., oversupply of dicalcium phosphate) are primary sources of high concentrations of P in the manure. Dietary P reductions decreased total P (TP) and manure water-soluble P (WSP_M) in broiler litter (17 and 52%, respectively) and turkey litter (33 and 21%, respectively) (Maguire et al., 2004). Diets supplemented with phytase and concurrent mineral P reductions decreased TP and WSP_M in broiler litter (13 to 35% and 0 to 83%, respectively) (Angel et al., 2005; Applegate et al., 2003; Maguire et al., 2004, Miles et al., 2003; Smith et al., 2004) and turkey litter (7 to 45% and 1 to 83%, respectively) (Angel et al., 2005; Maguire et al., 2003, 2004; Penn et al., 2004). Leytem et al. (2008) reduced TP in broiler manure 50% by using low phytate soybean meal at the same dietary non-phytate-P (NPP) level. The use of high available P corn reduced TP and WSP_M in broiler litter (11 to 18% and 6 to 35%, respectively) and turkey litter (41 and 48%, respectively) (Miles et al., 2003; Smith et al., 2004). However, some phytase additions in comparison to standard P diets increased WSP_M (1 to 18%) and WSP_M:TP (1 to 8%) in broiler litter (Maguire et al., 2004; Miles et al., 2003) and increased WSP_M:TP (3 to 10%) in turkey litter (Maguire et al., 2004). The variable responses in WSP_M and WSP_M:TP raised environmental concerns about increased P movement to water bodies as a result of dietary P modifications and subsequent manure applications. However, Leytem et al. (2007, 2008) concluded that dietary calcium to P (Ca:P) was a contributor to the variance in the concentrations of WSP_M and the ratios of WSP_M:TP (i.e., increasing dietary Ca:P reduced soluble P independent of phytase additions).

The impacts of dietary P modifications on broiler breeder performance have been evaluated (Berry et al., 2003), but the effects of dietary P modifications on manure P have not been assessed. Physiological and management differences for broilers and broiler breeders would influence manure P characteristics. Broilers have typically been reared for 6 to 9 wk, and broiler breeders have been maintained for approximately 64 wk (Scanes et al., 2004). Nutrient efficiency of birds have been found to increase with age (Bell and Weaver, 2002). Improved nutrient utilization by broiler breeders (e.g., dietary P) would most certainly alter manure P characteristics in combination with dietary P manipulations. Historically, broilers have been reared on litter to absorb excreta (e.g., wood chips high in carbon (C)), while broiler breeders' excreta have been collected under slatted floors (Bell and Weaver, 2002; Scanes et al., 2004). Mineralization in high C:N material (e.g., broiler litter) was limited, while mineralization in lower C:N material with longer residence (e.g., broiler breeder manure) was greater.

Modification of broiler and turkey diets has decreased TP and in many cases soluble P in manures. This information has improved management of P when these manures were applied to cropping systems. Since broiler breeder production differed significantly from broiler production, information on the effects of dietary P modification on P concentrations, forms, and solubilities in broiler breeder manure was necessary. The influence of diet modifications on potential soil P losses and plant P availability upon applications of these manures to soil also needed to be evaluated. Our objectives were to characterize P constituents of manure produced by feeding broiler breeders lower dietary P rations with and without phytase and subsequent impacts of these manures on TP, P forms, and P transformations upon application to soil.

MATERIALS AND METHODS

Broiler Breeder Diets

A broiler breeder feeding trial was conducted to evaluate bird performance using diets that were modified to reduce P excretion (Plumstead et al., 2007). Each dietary P treatment was replicated four times, and reproductive efficiency was such that the total number of chicks produced per hen housed was not affected (Plumstead et al., 2007). The National Research Council (NRC, 1994) P feed recommendation for the breeder phase was 0.37% NPP, which was calculated to be equal to 0.40% available P (AvP, relative bioavailable P fraction) based on a slope assay with monocalcium phosphate as the reference (Apke et al., 1987; Soares, 1995). The two dietary P targets during the breeder phase were 0.40 and 0.22% AvP, which were formulated with and without phytase resulting in four diets (Table 1). The general industry practice has been to reduce dietary NPP by 0.1% with the simultaneous addition of 500 FTU (phytase activity units) kg^{-1} of feed (Van der Klis and Versteegn, 1996). Therefore, diets with 0.37% NPP and 0.27% NPP + phytase theoretically produced the same AvP of 0.40%, and henceforth will be referred to as NRC and NRC + phytase, respectively, for simplicity. Similarly, the low AvP diets were formulated with 0.19% NPP and 0.09% NPP + phytase to theoretically produce 0.22% AvP. The latter diet did not require supplemental dicalcium phosphate and the two will be referred to simply as Low and Low + phytase, respectively (Table 1). Allzyme SSF (Alltech, Nicholasville KY) was the phytase enzyme used with an analyzed activity of 1098 FTU g^{-1}. Additionally, dietary Ca was maintained at 2.7% of diet by weight in all diets, and dicalcium phosphate in the reduced P diets was substituted with limestone.

Broiler Breeder Diet Modified-Manure: Collection, Pelletization, and Characterization

Maguire et al. (2006) collected and analyzed manures under the slatted floor (drinker, feeder, common areas) and the scratch area to determine the influence of sampling location, manure moisture, and dietary modification on manure soluble P. Manure under the slatted floor (free of wood chips) was collected by dietary treatment (four replicates) and homogenized with a manure spreader. Homogenized manure was pelletized at the NCSU Animal Waste Management Center for evaluations in the laboratory, greenhouse, and field over several years. Manure was extruded through a die (0.63 cm diameter), heated for 10 min until pellets reached 115–124°C, cooled to ambient temperature, and then bagged at a moisture content of 6–8%. Individual manure pellet size was approximately 0.63 cm diameter by 0.9–1.3 cm in length. Bags of pelletized, breeder manure were stored in a storage shed for approximately one year until used in this study.

Pelletized manures were analyzed in triplicate for nutritional content according to the standard procedures of the NC Department of Agriculture & Consumer Services. Total P, K, Ca, Mg, S, Mn, Zn, and Cu were determined by microwave-assisted digestion of a 0.5-g dried manure sample with 10 mL of concentrated HNO_3 and 2 mL of 30% H_2O_2 (U.S. EPA Method 3051A, 2007), and analyzed with inductively coupled plasma-atomic emission spectroscopy (ICP-AES). Manure pH was determined by diluting dry manure 1:1 with deionized water with an equilibration time of 60 min. Calcium carbonate equivalence (CCE) was determined by boiling 1.0 g dry manure for 5 min in 50 mL of 0.5 *N* HCl, allowing to cool, and titrating to a pH of 7.0 with 0.25 *N* NaOH and phenolthalein (AOAC, 1990). Manure WSP was extracted in duplicate for each treatment replicate at 1:100 manure pellets (equivalent dry weight) to deionized water, horizontally shaken at 300 rpm for 1 h, centrifuged at 14 500 x g for 10 min, filtered through Whatman #40, and analyzed with ICP-AES (Wolf et al., 2008).

Solution ^{31}P Nuclear Magnetic Resonance Spectroscopy of Broiler Breeder Diet Modified-Manure Pellets

Manure pellets were dried and ground to pass a 0.5-mm sieve and then extracted in triplicate by shaking 2.00 ± 0.01 g of manure with 40 mL of 0.5 *M* NaOH + 0.05 *M* EDTA for 4 h at 20°C (Turner, 2004). Extracts were centrifuged at 10 000 x g for 30 min, and supernatant was subsampled for TP analysis by ICP–AES. The remaining supernatant from the triplicate extracts were combined, frozen rapidly at –80°C, lyophilized, and ground to a fine powder. Prior to ^{31}P nuclear magnetic resonance (NMR) spectroscopy, approximately 100 mg of each freeze-dried extract was redissolved in 0.9 mL of 1 *M* NaOH + 0.1 mL of D_2O (for signal lock) and transferred to a 5-mm NMR tube. The addition of NaOH adjusted the solution to a pH > 13, which was necessary to ensure consistent chemical shifts and optimum spectral resolution.

All of the pulsed field NMR experiments were performed on a Bruker AVANCE 500 MHz Spectrometer (1996) with Oxford Narrow Bore Magnet (1989), SGI INDY Host Workstation, XWINNMR Software version. The NMR probe was tuned to ^{31}P frequency that was 202.455 MHz in the 500 MHz spectrometer (^{1}H frequency -500.128 MHz). Samples were subjected to a 5-µs pulse (45°), a delay time of 4.0 s, an acquisition time of 2.0 s, and broadband proton decoupling. The relatively long delay time allowed sufficient spin–

lattice relaxation between scans for P compounds in these extracts with low paramagnetic ion concentrations (Turner, 2004). Each sample was scanned approximately 7 000 times over 12 h, and the spectra were plotted without line broadening. Chemical shifts of signals were determined in parts per million (ppm) relative to 85% (w/v) H_3PO_4 and assigned to individual P compounds or functional groups according to Turner et al. (2003). Concentrations of P forms were determined by integrating signal areas and multiplying the proportion of the signal area by the TP concentration as described previously. A strong signal near 5.75 ppm was assigned to inorganic orthophosphate, while signals between 4.0 and 5.7 ppm were assigned to orthophosphate monoesters (Fig. 1). A number of individual signals were detected within this region, including those at approximately 5.45, 4.45, 4.15, and 4.05 ppm in the ratio 1:2:2:1, which were assigned to phytate-P (Fig. 1). Other trace signals in this region probably represented lower-order inositol phosphate esters. A signal at approximately –4.7 ppm was assigned to pyrophosphate, a specific inorganic polyphosphate with chain length $n = 2$. Signals from orthophosphate diesters (usually occurring between 2 and –1 ppm), phosphonates (20 ppm), and long-chain inorganic polyphosphates (–4 ppm and –18 to –21 ppm) were not detected.

Soil Collection and Characterization

Samples of two Ultisols, Portsmouth fine sandy loam (Typic Umbraquult) and Wagram loamy sand (Arenic Kandiudult), were collected from the A horizon (0 to 20 cm) of a long-term P fertility study near Plymouth NC, and a forested area near Clayton NC, respectively. Soils low in Mehlich-3 P (M3P) were selected to evaluate soil P fractions and plant P availability (future greenhouse response studies) from P-based applications of breeder manures. Soil samples were air-dried, homogenized, sifted through 2-mm sieve, analyzed, and subsequently used for the incubation study.

Soil pH, humic matter, CEC, acidity, and Mehlich-3 extractable elements were analyzed by the standard procedures of the NC Department of Agriculture & Consumer Services (Mehlich, 1984). Soils were analyzed for P as follows:

(i) TP: 1 g of soil was ashed at 550° C overnight, diluted 1:25 with 0.5 *M* H_2SO_4, horizontally shaken at 300 rpm for 16 h, centrifuged for 10 min at 14 500 x g, and filtered through Whatman #1 filter paper (Kuo, 1996).

(ii) Total inorganic P (TIP): Soil was diluted 1:25 with 0.5 *M* H_2SO_4, horizontally shaken at 300 rpm for 16 h, centrifuged for 10 min at 14 500 x g, and filtered through Whatman #1 filter paper (Kuo, 1996).

(iii) M3P: Soil was diluted 1:10 with 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, horizontally shaken for 5 min, and filtered through Whatman #2 filter paper (Mehlich, 1984).

(iv) WSP_S: Soil was diluted 1:10 with deionized water, horizontally shaken at 300 rpm for 1 h, centrifuged for 10 min at 14 500 x g, and filtered through a 0.45-µm Millipore membrane.

Mehlich-3 extractable elements were analyzed with ICP-AES while all other P extracts were colorimetrically analyzed by the molybdate blue method (Murphy and Riley, 1962). Mehlich-3 P saturation ratio (M3-PSR) was calculated as: [M3P / (M3Al + M3Fe)] with values for P, Al, and Fe in mmol kg^{-1} (Maguire and Sims, 2002b; Sims et al., 2002).

Incubation Study

The Wagram soil was acidic (pH of 3.8) and was incubated with reagent grade $CaCO_3$ (1.8 g kg^{-1}) for 2 wk prior to initiating the study. Soil microorganisms were acclimated 5 d prior to application of manure where 125 g of air-dried soil (Portsmouth or Wagram) was placed in polyethylene cups for 72 h at 4°C, moistened, subject to water diffusion for 24 h at 4°C, and equilibrated for 24 h at 20°C (Franzluebbers et al., 1996; Shi, personal communication, 2006). Broiler breeder manure pellets were dried and ground to pass a 2-mm sieve and incorporated into the conditioned soil at rates of 30 and 60 mg TP kg^{-1} of dry soil (0.19 to 0.26 and 0.38 to 0.52 g of dry manure, respectively, per incubation cup). Manure source and TP rates were replicated four times and arranged in a randomized complete block design. Each shelf of the incubator represented a block and replication. Unamended controls were included for each soil to account for endogenous fluctuations of nutrients and pH. Amended and unamended soils were incubated at 50% container capacity (Cassel and Nielsen, 1986), 0.18 g g^{-1} for both soils, in polyethylene cups for 12 wk at 25°C. Four holes were cut in the incubation cup lids to preclude anaerobic conditions, and soil moisture was maintained gravimetrically with weekly additions of deionized water. Approximately 20-g subsamples of moist soil were collected from each cup at 0, 1, 2, 4, 8, and 12 wk after application to determine soil moisture, pH, TP, TIP, M3-extractable elements, and WSP_S.

Statistical Analyses

Nine treatments in each soil were partitioned into a factorial (4 breeder manures x 2 P rates) plus unamended control, which were crossed by 6 sample periods. Therefore, the main effects were manure, rate, and wk. Treatment effects were determined by analysis of variance using PROC GLM in SAS version 9.1 (SAS Institute, 2002) at a = 0.05 unless otherwise noted. Fisher's protected LSD was used to separate treatment means at a = 0.05. Treatment means, standard deviations, and standard errors were generated with JMP version 7 (SAS Institute, 2007), and regression and bar graphs were created with Sigma Plot 2001 (SPSS, Inc., 2001) and Microsoft Excel 2002 (Microsoft, 2002).

RESULTS AND DISCUSSION

Effects of Dietary Phosphorus Modification on Broiler Breeder Manure Pellets

Manure TP was not significantly reduced by the addition of phytase within the target dietary AvP of 0.40 (NRC) and 0.22% (Low) (Table 2), which concurs with the previous study that examined the same diets across manure sampling locations (Maguire et al., 2006). Vadas et al. (2004) also found no differences from the addition of phytase within target AvP diets. The addition of phytase numerically reduced manure TP 14% and 6% within NRC and Low diets, respectively. Reducing the target AvP from 0.40% to 0.22% decreased manure TP 28% and the addition of phytase decreased manure TP by 32% compared to NRC (Table 2). Several studies have demonstrated similar decreases in manure TP when dietary P was reduced and / or in combination with phytase additions (Ferguson et al., 1998; Perney et al., 1993; Vadas et al., 2004).

Birds fed diets with phytase produced manure with WSP_M reductions of 28% and 30% within NRC and Low diets, respectively (Table 3). Such reductions have been demonstrated for poultry litter (Applegate et al., 2003; McGrath et al., 2005; Smith et al., 2004) and swine manure (Baxter et al., 2003). In contrast, phytase additions in other studies have increased WSP_M (Miles et al., 2003; Vadas et al., 2004) or had no effect on WSP_M (Maguire et al., 2004; McGrath et al., 2005) in poultry manures and litters. Leytem et al. (2007, 2008) identified dietary Ca:P ratios as a major contributor to discrepancies in WSP_M where high dietary Ca:P (AvP or NPP) decreased phytate-P hydrolysis and absorption in poultry. This reduced the proportion of WSP_M presumably by forming Ca-P complexes. In our study, NPP was reduced (with and without phytase addition) and dietary Ca was maintained, which increased dietary Ca:NPP (Table 1). Manure WSP and WSP_M:TP decreased linearly as dietary NPP was reduced ($r^2 = 0.98$ and 0.99, respectively, Fig 2a). Increases in the ratio of dietary Ca to NPP decreased WSP_M in a first order relationship ($r^2 = 0.99$) and decreased WSP_M:TP in a quadratic fashion ($r^2 = 0.99$) (Fig. 2b). Manures produced from the dietary P modifications decreased in WSP_M largely due to dietary P diets and the subsequent ratios of dietary Ca to NPP instead of the phytase additions.

Manure concentrations of C, N, K, Ca, S, Mn, Zn, and Cu were not different among dietary treatments where N ranged from 25.5 to 27.8 g kg^{-1} and K ranged from 32.3 to 36.9 g kg^{-1} (Table 2). The ratios of C:N and C:P did not differ among treatments and ranged from 9.0 to 9.3 and 11.8 to 15.9, respectively (data not shown). Manure Ca:P was lowest in NRC (5.0) followed by NRC + phytase (6.6), Low (7.0), and Low + phytase (8.0) dietary treatments (Table 2). Breeder manure from the NRC diet yielded the lowest pH of 6.4 and

CCE of 32%. The remaining breeder manures were greater than NRC in pH (7.1 to 7.3) and CCE (34 to 37%) (Table 2). In other words, these breeder manures should have approximately one-third of the liming effect of pure calcium carbonate. If the dietary Ca:NPP had been maintained at the same level, we postulated that there would be minimal or no differences in Ca:P, pH, and CCE among manures produced from dietary P modifications.

Effects of Dietary Phosphorus Modification on Phosphorus Speciation in Broiler Breeder Manure Pellets

The main P species in the breeder manure pellets were orthophosphate and phytate-P (Fig. 1, Table 3) with limited proportions of lower esters of inositol P and pyrophosphate (data not shown). Total P recovery via NaOH-EDTA extraction was 91 to 99% compared to microwave-assisted digestion. Nuclear magnetic resonance (NMR) of ^{31}P was costly, so only one analysis was conducted on a composite sample of breeder manure that was produced from each of the four dietary P modifications to provide some descriptive data although statistical comparisons of the NMR spectra among the breeder manures could not be made. Orthophosphate proportions were very similar across diets (92 to 96%), and phytate-P proportion was 5 to 7% for NRC, Low, and Low + phytase, but 2% for NRC + phytase (Table 3). Pyrophosphate was approximately 1% and lower esters of inositol P were less than 0.5% for all manures (data not shown).

Other researchers have shown that the proportion of P species was very different in broiler litters, which were 30 to 40% orthophosphate and 50 to 65% phytate (Leytem et al., 2007; Maguire et al., 2004), and turkey litters were 50 to 70% orthophosphate and 33 to 41%

phytate-P (Maguire et al., 2004). Broilers have typically be reared for 6 to 9 wk on a litter substrate, and broiler breeders have been maintained for approximately 42 wk in the breeder laying phase (Scanes et al., 2004). The combination of greater manure residence time (~42 wk) and optimal C:N (~9 since it is free of wood chips) of breeder manure presumably allowed greater mineralization of organic complexes (e.g., phytate-P) than broiler litter, which has short manure residence time (~6 wk) and greater C:N due to the litter substrate (e.g., wood chips). Furthermore, mature poultry utilize nutrients more efficiently and thus breeders have a greater capacity to hydrolyze phytate-P than broilers (Bell and Weaver, 2002).

Manure TP of breeder manure replicates was multiplied by the ^{31}P NMR estimation of P species to obtain concentrations of P species. Orthophosphate was highest in NRC (20.4 g kg^{-1}) and lowest in Low + phytase (13.6 g kg^{-1}) (Table 3). Contrary to expectations, phytate-P in Low + phytase (0.837 g kg^{-1}) was higher than in NRC + phytase (0.430 g kg^{-1}), but elevated dietary Ca:NPP in Low + phytase (30) probably hindered phytate-P digestion and absorption. Increased concentrations of phytate-P and phytate-P:TP in manures have been correlated with large decreases in WSP_M presumably due to higher insolubility (Leytem et al., 2007), however, these predictors did not correlate in the broiler breeder diet modified-manure pellets (data not shown). Manure WSP declined as (1) manure TP decreased, (2) manure orthophosphate decreased, and (3) manure Ca:P increased (Table 3). Phosphorus from these breeder manures may be more soluble (i.e., due to greater orthophosphate content) and thus, more available to plants and more likely to move offsite with water than P from broiler and turkey litters.

Effect of Broiler Breeder Diet Modified-Manures on Mehlich-3 Phosphorus Levels in Amended Soils

The initial M3P concentrations of the Portsmouth (33.6 mg kg^{-1}) and the Wagram (13.5 mg kg^{-1}) soils were very low for agronomic production (Table 4). The application of the breeder manures caused similar increases of M3P within the low P rate (40 to 48 mg kg^{-1}) and the high P rate (90 to 99 mg kg^{-1}) in both soils (Fig. 3). Analysis of variance indicated that M3P changed by rate and over time in both soils (Table 5). Manures from different diets caused similar changes in M3P of the Wagram soil and the interaction of breeder manure by wk was significant in the Portsmouth soil (Table 5). Portsmouth soil amended with NRC was lowest in M3P changes after 1 wk; however, in the following wk the breeder manures did not differ (Fig. 3a). Litters from somewhat similar dietary P modifications of broilers (McGrath et al., 2005; Maguire et al., 2004) and of turkeys (Maguire et al., 2004) have been soil-applied and the impacts on M3P concentrations have also been variable over time. Mehlich-3 P declined over the following wk regardless of soil, manure, and rate (low P rate decreased 10 to 18 mg kg^{-1}, high P rate decreased 28 to 31 mg kg^{-1}). Mehlich-3 P was highest initially then within one wk soil amended with turkey litter decreased 5 to 21 mg M3P kg^{-1} (Maguire et al., 2005) and soil amended with poultry manure decreased 40 mg M3P kg^{-1} (Griffin et al., 2003). Decreases in M3P extractability with time were most certainly due to P sorption into Al and Fe hydroxides (Bolan et al., 1985) and P complexes with organic matter.

Mehlich-3 P concentrations were greater than TIP over the first 2 to 4 wk (limited P sorption) and stabilized by wk 8 and wk 12 (increased P sorption) where M3P:TIP averaged

0.96 in the Portsmouth soil and 0.84 in the Wagram soil (data not shown). Total inorganic P in the soils amended with breeder manures did not differ (Table 5). The Portsmouth soil averaged 22, 59, and 103 mg TIP kg^{-1}, and the Wagram soil averaged 16, 52, and 93 mg TIP kg^{-1} for unamended, low P rate, and high P rate, respectively. Total P in the soils amended with breeder manures did not differ over time (Table 5) where the Portsmouth soil averaged 91, 148, and 186 mg kg^{-1}, and the Wagram soil averaged 52, 91, and 136 mg kg^{-1} for unamended, low P, and high P, respectively.

For every 1 mg of breeder manure TP applied per kg of soil, M3P increased 1.33 to 1.65 mg kg^{-1} at wk 0 and 0.92 to 1.17 mg kg^{-1} by wk 12. Soils amended with turkey litter increased 0.97 to 1.82 mg M3P kg^{-1} for every 1 mg of TP applied per kg of soil (Maguire et al., 2005) and with poultry layer manure increased 1.2 µg M3P cm^{-3} for every 1 µg TP applied per cm^3 of soil (Montalvo, 2008). However, 1 µg TP applied per cm^3 of soil from poultry layer manure increased M3P by 0.90 µg per cm^3 of organic soil and 0.45 µg per cm^3 of piedmont soil (Montalvo, 2008). Mehlich-3 P increases from 1 mg of TP applied per kg of soil from turkey litter were 0.42 to 0.46 mg kg^{-1} (Maguire et al., 2004) and from broiler litter were 0.33 to 0.73 mg kg^{-1} (Leytem and Sims, 2005; Lucero et al., 1995; Maguire et al., 2004; Reddy et al., 1999). As previously mentioned, breeder manure TP was mainly orthophosphate (> 92%), which was presumably more M3-extractable than lower orthophosphate levels of broiler and turkey litters. When M3P changes were calculated for every 1 mg of orthophosphate applied per kg of soil, the ratio for broiler litters ranged from 1.0 to 1.3 mg kg^{-1} after 4 wks (Maguire et al., 2004), and the ratios for breeder manures in our study ranged from 0.95 to 1.4 mg kg^{-1} after 4 wks. Therefore, manure P species (i.e., orthophosphate, phytate-P) influenced M3 extractability, and plant P availability of these breeder manures may be greater than broiler litters that are lower in orthophosphate. Future research should include evaluating plant P availability from different poultry manures and litters that have varying proportions of orthophosphate.

Effect of Broiler Breeder Diet Modified-Manures on Water-Soluble Phosphorus Levels in Amended Soils

Soil WSP of the Portsmouth soil changed by the interaction of rate and wk; whereas, WSP_S of the Wagram soil was influenced by the interaction of manure, rate, and wk (Table 5). Initial WSP_S after low and high P applications averaged 2.8 and 7.3 mg kg^{-1} in the Portsmouth soil and 6.0 and 15.2 mg kg^{-1} in the Wagram soil. Soil WSP decreased 32 to 36% in the Portsmouth soil and 5 to 24% in the Wagram soil after 1 wk where it remained stable for the following wk (Fig. 3). Soil WSP did not differ among breeder manures in the Portsmouth soil at low and high P rates and the Wagram soil at the low P rate (Fig. 3) even though WSP_M and WSP_M:TP were greater in the NRC manure and lowest in the Low + phytase manure (Table 3). However, in the Wagram soil at the high P rate, NRC and NRC + phytase were greater than Low and Low + phytase by wk 8 and 12 (WSP_S difference of 2.0 mg kg^{-1} over wk 8 and 12, Fig. 4), which was not surprising since there was an application difference of 3.3 mg WSP_M kg^{-1} (data not shown).

Interestingly at wk 0, the Portsmouth soil WSP values (2.8 to 7.3 mg kg^{-1}) were lower than applied WSP_M, and the Wagram soil WSP values (6.0 to 15.2 mg kg^{-1}) were generally greater than applied WSP_M (low P rate: 3.3 to 6.0, high P rate: 6.6 to 12.0 mg kg^{-1}). The

Wagram soil was lower in concentrations of M3-Al and Fe than the Portsmouth soil, and thus, concentrations of WSP_S were greater in the Wagram soil due to limited P saturation (Table 4, Fig. 4). Mehlich-3 PSR was below 0.04 in both soils prior to applications (Table 4), and was raised to 0.09 and 0.15 in the Portsmouth soil and 0.13 and 0.25 in the Wagram soil (data not shown). Increasing M3-PSR has been associated with increased soluble P where M3-PSR change points of 0.14 and 0.21 were identified for dissolved reactive P in runoff of 17 soils from the Mid-Atlantic and in column leachate of 5 soils from Delaware, respectively (Sims et al., 2002).

Effect of Broiler Breeder Diet Modified-Manures on pH of Amended Soils

Soil pH decreased over the 12-wk incubation in the unamended controls of the Portsmouth soil (from 6.1 to 5.9) and the Wagram soil (from 5.4 to 5.0). Phosphorus-based applications incorporated varying amounts of breeder manures with 32 to 37% CCE (Table 2) and thus supplied 0.9 to 1.7 $cmol_c\ kg^{-1}$ at low P rate and 1.8 to 3.4 $cmol_c\ kg^{-1}$ at high P rate. Soil pH changed by manure source when averaged over rate and wk (Table 5) where Low and Low + phytase were greater than NRC and NRC + phytase in the Portsmouth soil (6.1 > 6.0) and the Wagram soil (5.6 > 5.5). Low and Low + phytase breeder manures supplied the most CCE. At the low P rate, the pH of soil amended with breeder manures did not differ from the unamended control of the Portsmouth soil and was generally greater than the unamended control of the Wagram soil (Fig. 5). At wk 12 and within the high P rate, the addition of breeder manures increased the pH of the Wagram soil compared to the unamended control (5.4 to 5.8 vs. 5.0, P = 0.021), and Low and Low + phytase increased the pH of the Portsmouth soil compared to the unamended control (6.2 vs. 5.9, P = 0.0009).

Poultry litters have been anecdotally regarded as having liming value. Maguire et al. (2006) noted layer manure increased pH of piedmont and coastal plain soils, and broiler litter increased pH of a piedmont soil but not a coastal plain soil 29 d after application. Tang et al. (2007) reported that poultry litter increased soil pH and reduced Al toxicity to wheat in a greenhouse study. The limited pH-buffering capacity of the Wagram soil (Table 4) allowed a greater soil pH decline in the unamended control, but this enabled a greater liming effect of breeder manures than the Portsmouth soil (Fig. 5). The Wagram soil increased 0.2 pH unit for each 1.0 $cmol_c$ from breeder manure applied to kg of $soil^{-1}$ (r^2 = 0.93) and the Portsmouth soil increased 0.1 pH unit for each 1.0 $cmol_c$ from breeder manure applied to each kg of $soil^{-1}$ (r^2 = 0.72) (Fig. 5). Breeder manures have an apparent liming effect once incorporated with the Portsmouth and the Wagram soils.

We postulated that if the dietary Ca:NPP was maintained there would be minimal CCE differences, if any, among P-modified diets of breeders. However, amended soil would most likely increase in pH compared to unamended soil, and N-based applications among the diet-modified manures would probably not differ in soil pH. Phosphorus-based applications of breeder manures from P-modified diets (i.e., decreased manure TP) would require more manure to supply the same amount of P as breeder manure produced from traditional diets. The increased mass of manure would also supply more CCE, which would increase soil pH more than the P application of breeder manure produced from traditional diets. Increasing soil pH has been found to be advantageous in acidic soils, but excessive increases in soil pH has been reported to limit micronutrient availability so this effect should be considered in the management of breeder manures from P-modified diets.

CONCLUSIONS

Phosphorus loading to soils from N-based applications of broiler breeder manure could be reduced by 28% if producers simply reduced the dietary P from 0.40 to 0.22% AvP. This should be extremely important in the concentrated poultry production regions that have high soil P concentrations and have been found to be more prone to P runoff and leaching. Dietary P modifications fed to broiler breeders need to maintain the dietary Ca:NPP to evaluate the WSP_M effects and the potential implications to the environment once those manures were land applied (i.e., soluble P losses).

The proportions of orthophosphate and phytate greatly influenced M3P extractability and presumably plant P availability. Regardless of dietary P regime, breeder manures were similar in the proportions of orthophosphate and subsequent M3P concentrations once applied to soils. Phosphorus-based applications of breeder manures (high in orthophosphate) would be highly P available to plants; whereas, plant availability of broiler litter would be less available due to the lower orthophosphate proportion. Nutrient management plans need to adjust the application rates of poultry manures based on inherent P differences to optimize production in P-limited soils and to protect soil from excessive P loading.

The apparent liming effect from breeder manure applications may also increase plant P availability in acidic soils like those of the NC Coastal Plain and Piedmont. Lime applications could be reduced approximately 30% when applying breeder manure based on calcium carbonate equivalence, which would be a cost savings to producers. Diet modifications to reduce excreted P from broiler breeders shows promise for reducing P loading of soils and potentially reducing soluble P, but plant P availability must be examined to determine the overall environmental and agronomic impact.

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Table 1. Broiler breeder diets used to produce typical and reduced amounts of manure P.

Diet	NPP†	Phytase	AvP‡	Total P	Ca	Ca:NPP
	%	FTU§ kg^{-1}	%	%	%	
NRC¶	0.37	0	0.40	0.63	2.7	7.3
NRC + phytase	0.27	500	0.40	0.53	2.7	10.0
Low	0.19	0	0.22	0.35	2.7	14.2
Low + phytase#	0.09	500	0.22	0.35	2.7	30.0

† NPP = Non-phytate P.

‡ AvP = Available P and assumes that 500 FTU kg^{-1} of feed released 0.1% of phytate P to available forms.

§ FTU, phytase unit where activity of phytase generated 1 µM of inorganic P min^{-1} from an excess of sodium phytate at pH 5.5 and 37°C thus equaling 1 FTU.

¶ NRC, National Research Council recommendation (1994).

No supplemental dicalcium phosphate was added.

Table 2. Selected properties of broiler breeder diet modified-manure pellets.

Manure from diet†	pH	CCE‡	Ca:P	Total in the Manure C	N	P	K	Ca	Mg	S	Mn	Zn	Cu
		%		g kg^{-1} dry matter							mg kg^{-1} dry matter		
NRC§	6.4b¶	32b	5.0c	253.6	27.8	21.7a	32.3	107.4	10.2b	6.6	1185	1033	135
NRC + phytase	7.1a	34ab	6.6b	247.6	26.8	18.6ab	35.3	121.2	12.9a	6.9	1297	917	125
Low	7.2a	37a	7.0ab	247.5	27.7	15.7b	36.9	109.2	14.1a	6.9	1301	932	127
Low + phytase	7.3a	36a	8.0a	235.6	25.5	14.8b	33.0	118.4	13.0a	6.1	1262	869	117
Significance	**	*	**	ns	ns	*	ns	ns	*	ns	ns	ns	ns
$LSD_{0.05}$	0.3	3	1.2	44.9	5.0	4.2	5.0	16.1	2.1	1.0	179	159	19
CV, %	2	4	9	9	9	12	7	7	9	8	7	9	8

* and ** Significance at the probability of 0.05 and 0.01, respectively, and no significance (ns) when a was greater than 0.05.

† Dietary non-phytate P levels were 0.37, 0.27, 0.19, and 0.09 % for NRC, NRC + phytase, Low, and Low + phytase, respectively. The addition of phytase theoretically increased available P to 0.40 and 0.22% for NRC + phytase and Low + phytase diets, which equaled NRC and Low diets, respectively. See Table 1 for further details.

‡ CCE, calcium carbonate equivalence.

§ NRC, National Research Council recommendation (1994).

¶ Values within same column followed by different letters are significantly different across diets using Fisher's Protected $LSD_{0.05}$.

Table 3. Phosphorus characterization of broiler breeder diet modified-manure pellets.

Manure from diet†	Total P	^{31}P Nuclear Magnetic Resonance Estimation: Orthophosphate	Phytate, sum‡	Phytate, C2 x 6§	Water-Soluble P	WSP_M:TP	Ca:P
	g kg^{-1} dry matter						
NRC¶	21.7a#	20.4 (94) ††	1.085 (5)	1.030 (5)	4.3a	0.20a	5.0c
NRC + phytase	18.6ab	17.9 (96)	0.430 (2)	0.370 (2)	3.1b	0.17ab	6.6b
Low	15.7b	14.4 (92)	0.987 (6)	1.050 (7)	2.3c	0.15b	7.0ab
Low + phytase	14.8b	13.6 (92)	0.837 (6)	0.703 (5)	1.6d	0.11c	8.0a
Significance	*	n/a‡‡	n/a	n/a	***	**	**
$LSD_{0.05}$	4.2				0.168	0.037	1.2
CV, %	12				3	12	9

*, **, and *** Significance at the probability of 0.05, 0.01, and 0.001, respectively.

† Dietary non-phytate P levels were 0.37, 0.27, 0.19, and 0.09 % for NRC, NRC + phytase, Low, and Low + phytase, respectively. The addition of phytase theoretically increased available P to 0.40 and 0.22% for NRC + phytase and Low + phytase diets, which equaled NRC and Low diets, respectively. See Table 1 for further details.

‡ Phytate proportion estimated by the sum of four signal peaks (1:2:2:1) at approximately 5.45, 4.45, 4.15, and 4.05 ppm (Fig. 1).

§ Phytate proportion estimated by the multiplying signal peak area C-2 by 6.

¶ NRC, National Research Council recommendation (1994).

Values within same column followed by different letters are significantly different across diets using Fisher's Protected $LSD_{0.05}$.

†† Values in parenthesis were the proportion (%) of total P for orthophosphate and phytate based on ^{31}P NMR peak integration. The total P concentrations of the manure replicates were multiplied by these ^{31}P NMR estimations.

‡‡ Statistical analysis of the ^{31}P NMR estimations were not conducted since only one ^{31}P NMR analysis was conducted per treatment.

Table 4. Selected properties of Portsmouth and Wagram soils used in the incubation study.

Soil Property	Portsmouth	Wagram†
pH	6.1	5.4
Humic Matter, %	2.30	0.87
	———$cmol_c\ kg^{-1}$———	
CEC	7.58	5.31
Acidity	0.71	1.36
Mehlich-3 Ca	4.67	3.35
	———$mg\ kg^{-1}$———	
Mehlich-3 Al	718.6	371.5
Mehlich-3 Fe	141.0	90.9
Mehlich-3 P	33.6	13.5
Mehlich-3 PSR‡	0.04	0.03
Total P	100.8	50.7
Total inorganic P	21.2	15.1
Water-soluble P	0.65	1.51

† Wagram was incubated with 1.8 g of reagent grade $CaCO_3$ kg^{-1} for 2 wk prior to analysis.

‡ PSR, Phosphorus saturation ratio, calculated as a molar ratio of [(M3P) / (M3Al + M3Fe)].

Table 5. Probability of significance within the ANOVA of the Portsmouth and the Wagram soils for Mehlich-3 P (M3P), water-soluble P (WSP_S), total inorganic P (TIP), total P (TP), M3-P saturation ratio (M3PSR), and pH.

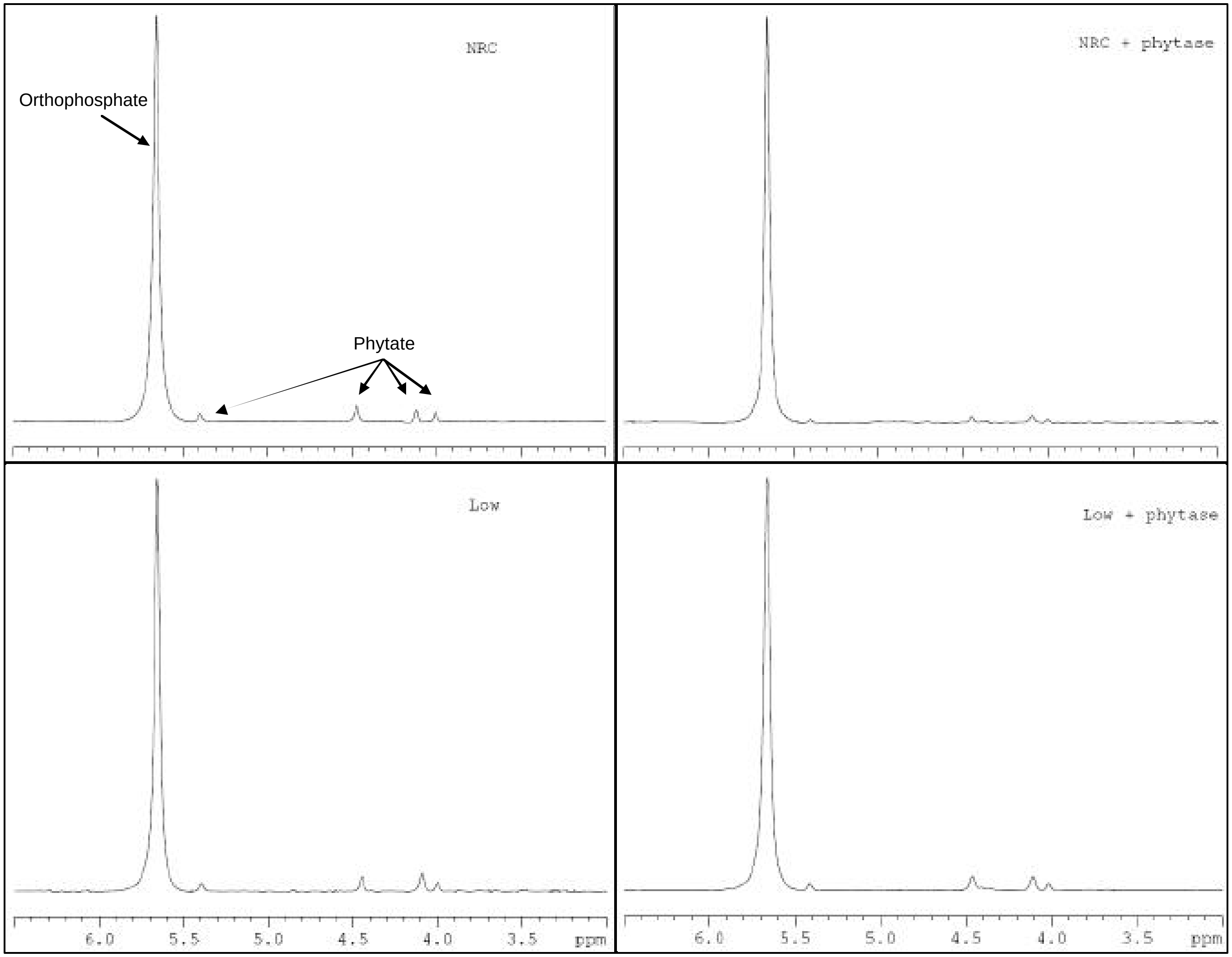
Source†	df	M3P‡	WSP_S‡	TIP	TP	M3-PSR	pH
Portsmouth							
Manure	3	***	ns	ns	ns	***	***
Rate	1	***	***	***	**	***	ns
Wk	5	***	***	ns	ns	***	ns
Manure x Rate	3	ns	ns	ns	ns	ns	ns
Manure x Wk	15	**	ns	ns	ns	*	*
Rate x Wk	5	**	***	ns	ns	**	ns
Manure x Rate x Wk	15	ns	ns	ns	ns	ns	ns
Wagram							
Manure	3	ns	*	ns	ns	ns	**
Rate	1	***	***	***	***	***	*
Wk	5	***	**	***	**	***	ns
Manure x Rate	3	ns	ns	ns	ns	ns	ns
Manure x Wk	15	ns	***	ns	ns	ns	ns
Rate x Wk	5	*	**	ns	ns	ns	*
Manure x Rate x Wk	15	ns	*	ns	ns	ns	ns

*, **, and *** Significance at the probability of 0.05, 0.01, and 0.001 respectively, and no significance (ns) when a was greater than 0.05.

† The unamended control was partitioned within the ANOVA and was not included in this summary.

‡ Fluctuations of the M3P and WSP_S in the unamended controls of each soil were subtracted prior to conducting ANOVA.

Figure 1. Solution ^{31}P nuclear magnetic resonance (NMR) spectra of manure pellets from four broiler breeder diets: NRC, NRC + phytase, Low, and Low + phytase. The signal at approximately 5.75 ppm was orthophosphate, and the four other strong signals at approximately 5.45, 4.45, 4.15, and 4.05 ppm represented phytate.


NRC
Orthophosphate
Phytate
NRC + phytase
Low
Low + phytase
6.0
5.5
5.0
4.5
4.0
3.5
ppm

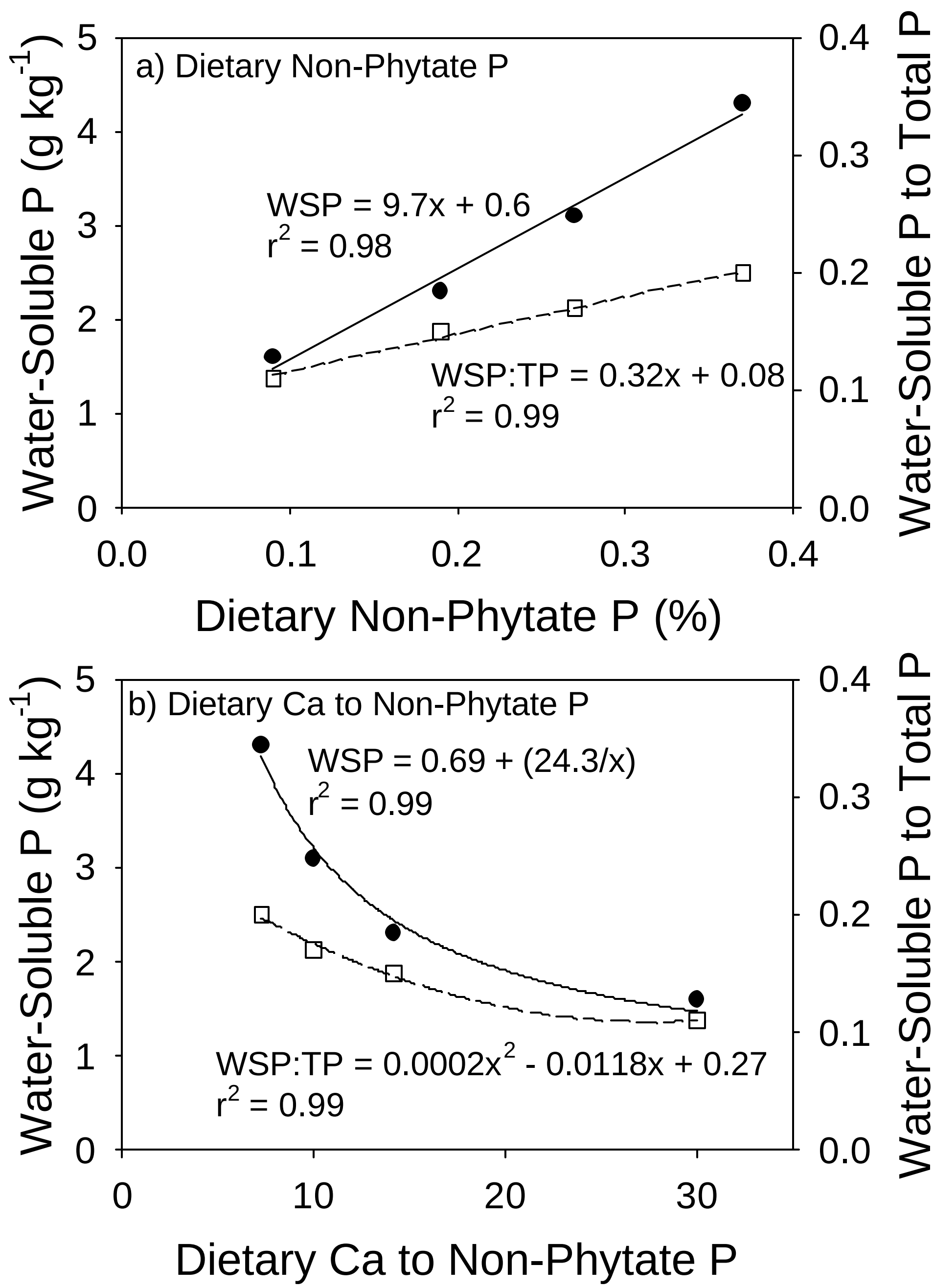


Figure 2. Water-soluble phosphorus (WSP) and WSP to total P (WSP:TP) in breeder manures as affected by (a) dietary non-phytate P rations and (b) ratios of dietary Ca to non-phytate P. Closed circles and solid regression lines were WSP, and open squares and dashed regression lines were WSP:TP.

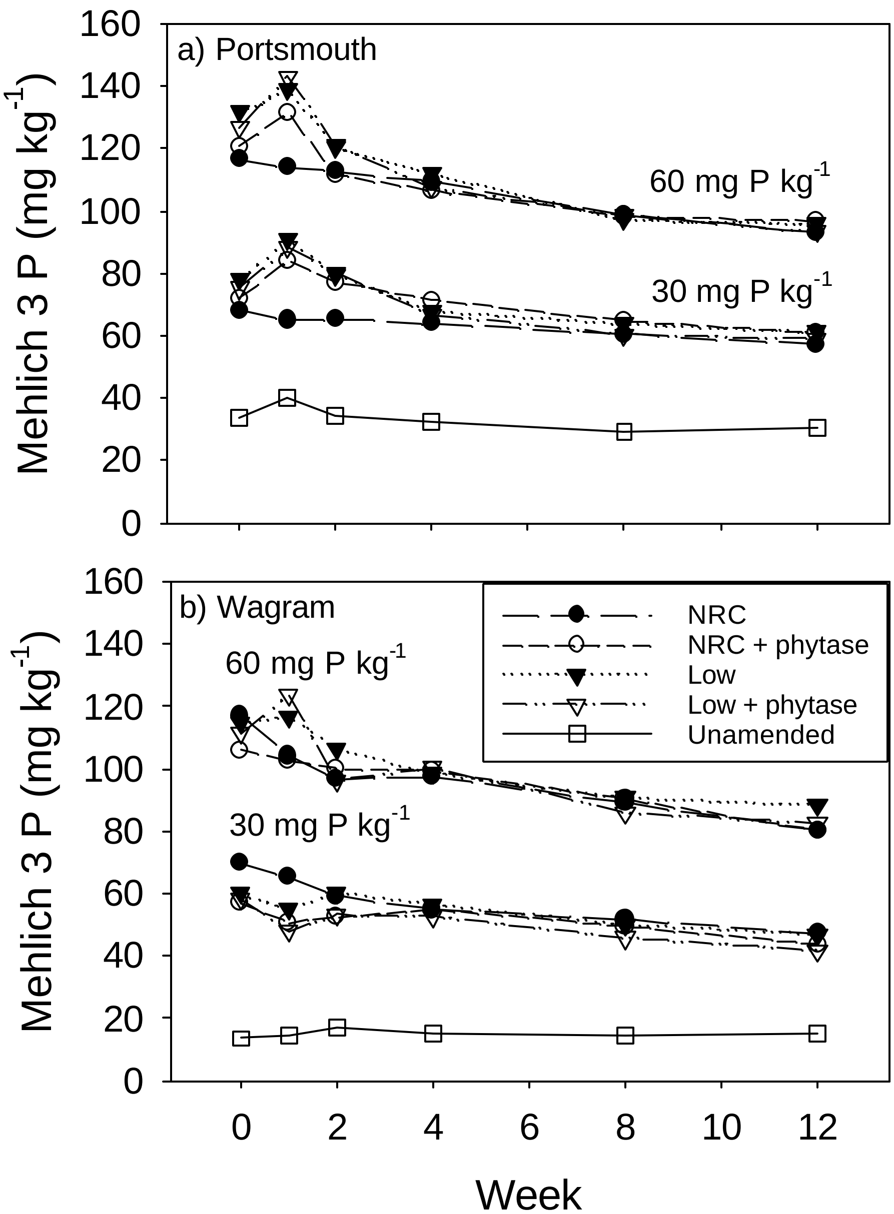


Figure 3. Mehlich-3 P changes over 12-wk incubation of (a) the Portsmouth soil and (b) the Wagram soil that were amended with four broiler breeder diet modified-manure pellets (NRC, NRC + phytase, Low, Low + phytase) at 30 and 60 mg TP kg^{-1} plus an unamended control. The standard error was 4.0 mg kg^{-1} in the Portsmouth soil and 8.5 mg kg^{-1} in the Wagram soil.

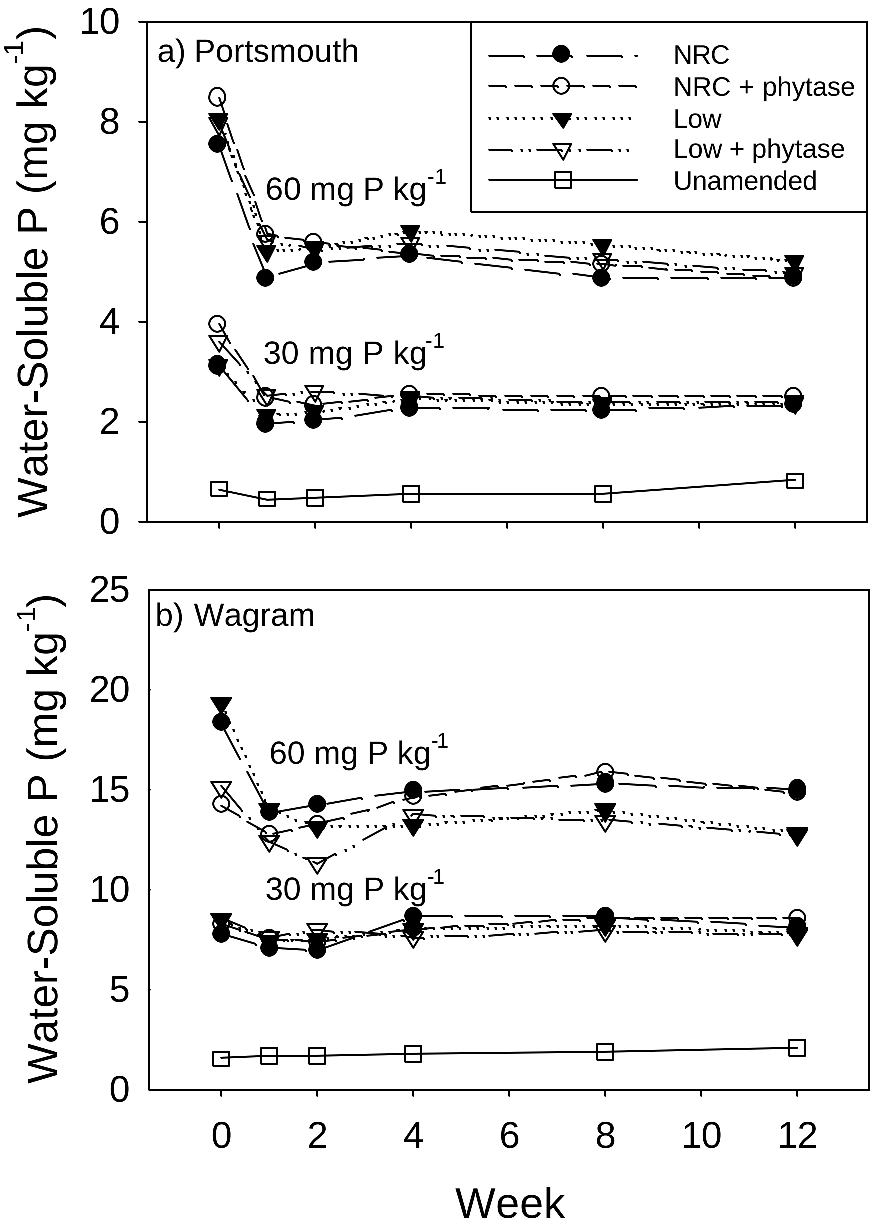
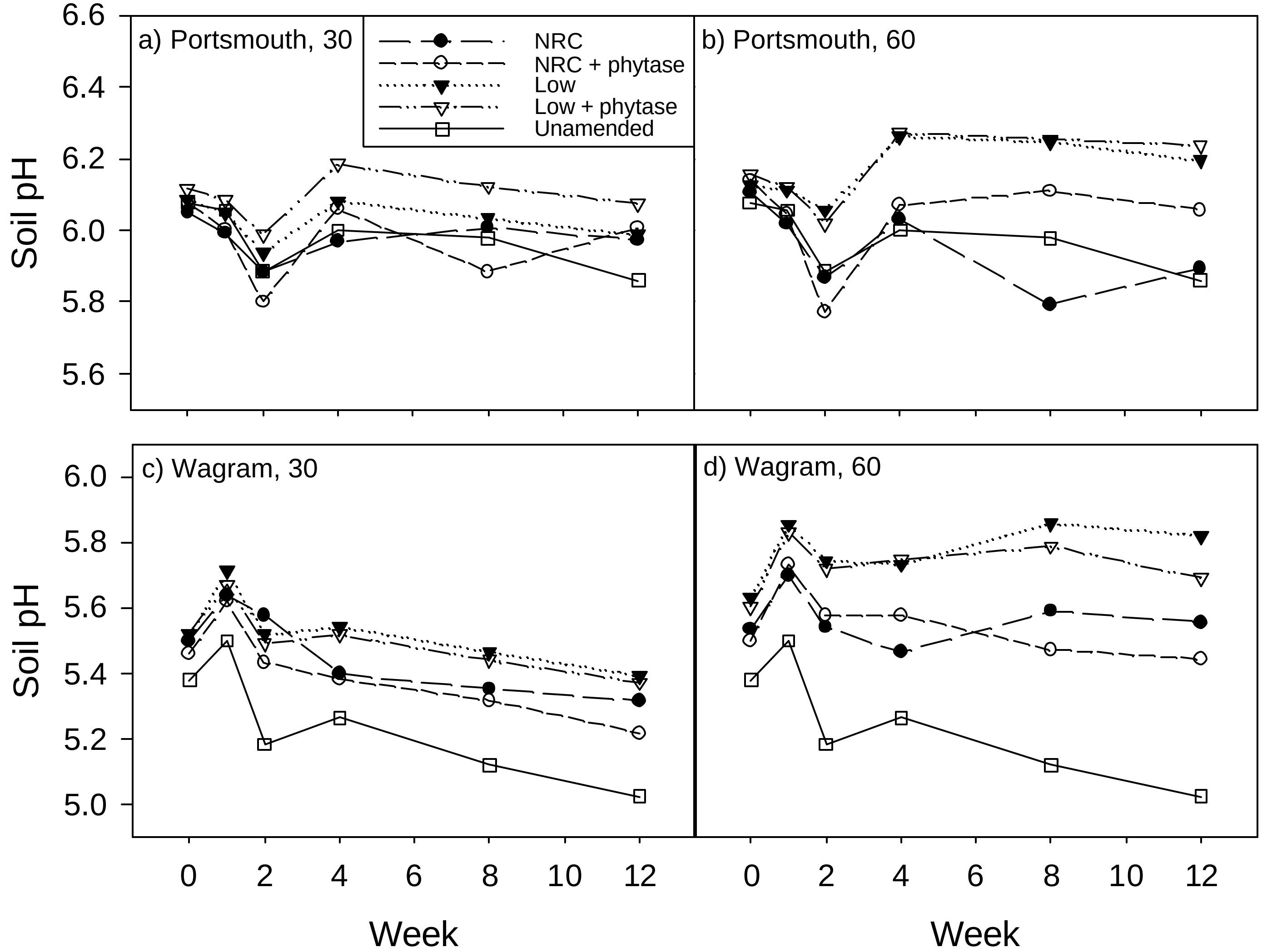


Figure 4. Soil WSP changes over 12-wk incubation of (a) the Portsmouth soil and (b) the Wagram soil that were amended with four broiler breeder diet modified-manure pellets (NRC, NRC + phytase, Low, Low + phytase) at 30 and 60 mg TP kg^{-1} plus an unamended control. Note the scale differences of the y-axis. The standard error was 0.2 mg kg^{-1} in the Portsmouth soil and 1.7 mg kg^{-1} in the Wagram soil.

Figure 5. Soil pH changes over 12-wk incubation of (a, b) the Portsmouth soil and (c,d) the Wagram soil amended with four broiler breeder diet modified-manure pellets (NRC, NRC + phytase, Low, Low + phytase) at 30 (a, c) and 60 (b, d) mg TP kg^{-1} plus unamended control. Note the scale differences of the y-axis. The standard error was 0.12 in the Portsmouth soil and 0.08 in the Wagram soil.



a) Portsmouth, 30
b) Portsmouth, 60
c) Wagram, 30
d) Wagram, 60
NRC
NRC + phytase
Low
Low + phytase
Unamended
Soil pH
Week
6.6
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
0
2
4
6
8
10
12

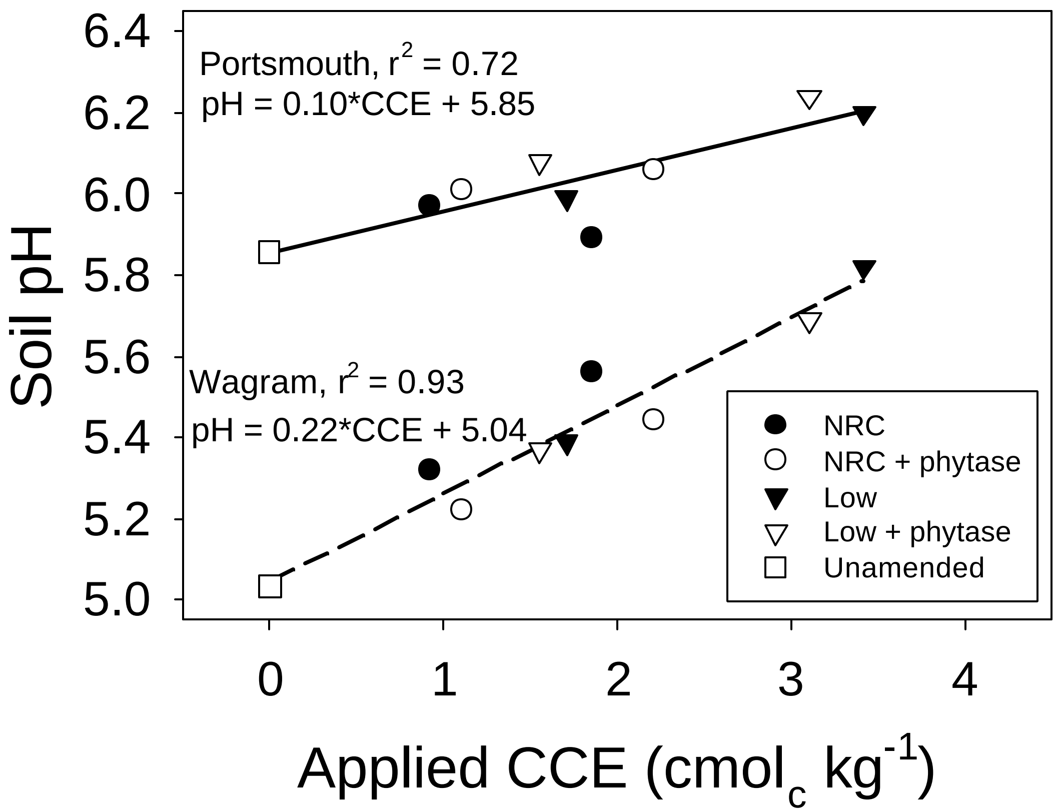


Figure 6. Broiler breeder diet modified-manure pellets influenced soil pH of the Portsmouth soil (solid line) and the Wagram soil (dashed line) after 12 wk of incubation. Low and high P rates were represented for each breeder manure within each soil and calcium carbonate equivalence (CCE, Table 2) was used to calculate applied CCE.

APPENDIX

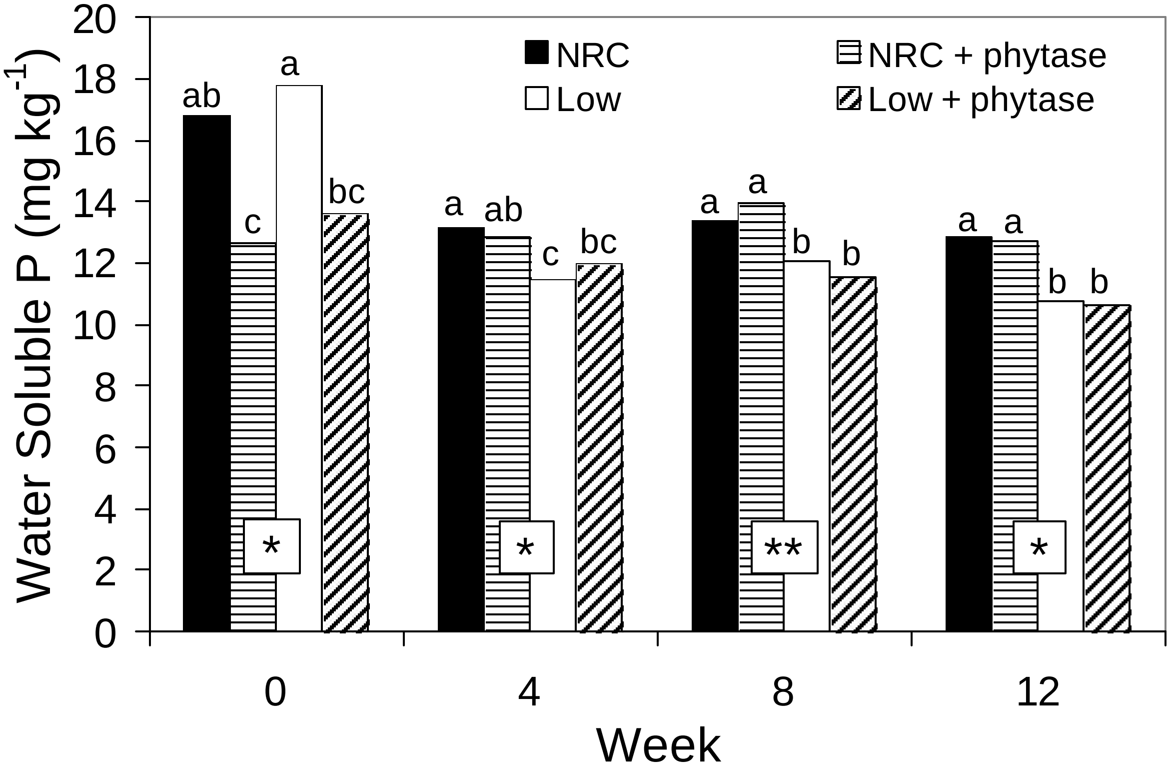


Figure A1. Soil WSP means separated within each sampling wk for the Wagram soil where broiler breeder diet modified-manures were applied at 60 mg TP kg^{-1} and unamended soil variations were subtracted from values for manure-amended soil. Significance at probability * = 0.05 and ** = 0.01. No significance (ns) when a was greater than 0.05. Fisher's Protected $LSD_{0.05}$ was used for wk 0, 4, 8, and 12.

CHAPTER 3

Broiler Breeder Manure Phosphorus Forms Are Affected by Diet, Location Within Pen, and Period of Accumulation

ABSTRACT

Phosphorus (P) modifications of poultry diets have successfully decreased total P (TP) in litters and manures, but impacts on water-soluble P (WSP_M) in broiler breeder manure have been unclear. Our objectives were to: (1) characterize P constituents of breeder manures as affected by dietary P modification, location within the pen, and manure accumulation period and (2) determine the impacts that P forms and moisture content in breeder manures had on the variability in WSP_M concentration. Two diets were formulated with and without phytase to attain 0.40% available P (AvP) during the breeder laying phase (wk 22 to 64). Manure was collected after accumulation periods of 48 h, 3 wk, and 39 wk in locations under the feeder, the drinker, and the common area between the drinker and feeder. Forms of P in manure from these breeders were characterized by solution ^{31}P nuclear magnetic resonance. Breeders fed phytase with simultaneous non-phytate P reduction produced manures with 15% lower TP, but did not change WSP_M when averaged over manure accumulation periods and locations within the pen. Manure WSP increased linearly as manure moisture increased regardless of diet, location within the pen, or accumulation period ($r^2 = 0.76$). After 39 wk of accumulation, orthophosphate was 91% and phytate was 6.7% of the manure TP. As manure accumulation periods increased (48 h < 3 wk < 39 wk), manure TP concentrations increased (11.9 < 13.2 < 17.3 g kg^{-1}), orthophosphate proportions increased (73.2% < 80.1% < 91.0%), and phytate proportions decreased (23.1% > 17.0% > 6.7%). The mineralization of phytate and other organic complexes presumably contributed to the increased orthophosphate and TP. Optimum phosphorus management of breeder manures should consider the impact of drinking water management on WSP_M, the manure sampling location on TP and WSP_M determinations, and the influence of P form (orthophosphate vs. phytate) on plant P availability.

Keywords: phytase, broiler breeder, phosphorus, phytate, orthophosphate,

Abbreviations: AvP, available phosphorus; NPP, non-phytate phosphorus; TP, total phosphorus; WSP_M, manure water-soluble phosphorus.

INTRODUCTION

Increased specialization of food animal management has led to regions of intensive production that have generated volumes of manure beyond the requirement of local crops (Maguire et al., 2007). Traditionally, manures were applied based on nitrogen (N) needs of the receiver crop, but this has led to phosphorus (P) rates in excess of crop needs with resultant P loading of soils (Mikkelson, 2000). Phosphorus has saturated many soils, which has resulted in increased P losses to surface waters and decreased water quality as well as negatively impacted aquatic ecosystems (Sharpley, 1996; Sims et al., 2000).

Manure management strategies have been developed to protect water quality and optimize manure resources. Phosphorus-based, instead of N-based, applications have addressed the P loading of soils, but the manure P surplus was considerable in many areas due to intense animal production and limited crop acreage (Maguire et al., 2007). The reexamination of poultry nutritional requirements has successfully reduced dietary P in rations while maintaining health and performance in broilers and turkeys (Maguire et al., 2004). Monogastric animals (swine and poultry) have been reported to not secrete enough phytase to completely hydrolyze phytate from soybean meal and corn (McCuaig et al., 1972). Thus, strategies to increase the efficiency of P have included feeding highly available P soybean meal and corn (i.e., low phytate) and/or supplementing feed with phytase (CAST, 2002). These strategies have reduced manure TP in swine (Baxter et al., 2003; Smith et al., 2004), in turkey litters (Angel et al., 2005; Maguire et al., 2004; Penn et al., 2004), in broiler litters (Applegate et al., 2003; Maguire et al., 2004; Miles et al., 2003), and in broiler breeder manures (Maguire et al., 2006).

Water-soluble P in manure (WSP_M) has become a proxy for environmental effects once manure was land applied as it was highly correlated with short-term effects in runoff P (Kleinman et al., 2007). In comparison to the standard diets, dietary P modifications of poultry has resulted in variable effects on WSP_M that have included decreases of up to 83% (Applegate et al., 2003; McGrath et al., 2005; Smith et al., 2004), increases of up to 48% (Miles et al., 2003; Vadas et al., 2004), as well as no changes (Maguire et al., 2004; McGrath et al., 2005). Leytem et al. (2007, 2008) determined that Ca:P ratios of broiler diets were a major contributor to WSP_M discrepancies as an increased dietary Ca:P ratio decreased WSP_M. Further, increased phytate proportions decreased the proportions of WSP_M to manure TP regardless of dietary P manipulation (Leytem and Maguire, 2007). Conversely, Maguire et al. (2006) suggested that less phytate in breeder manure decreased WSP_M because microbes had less material to mineralize into soluble P, but P forms were not determined in that study. In a breeder manure pellet study, phytate proportions were 2 to 6% with an inconsistent relationship to WSP_M (Casteel, 2009). Finally, a positive linear relationship between manure moisture and WSP_M was reported in breeder manures; however, the regression coefficient was low (Maguire et al., 2006). Therefore, dietary P modifications of broiler breeders needed more research to more fully characterize the effects on WSP_M and associated sources.

Orthophosphate proportions in breeder manures (92 to 96%) (Casteel, 2009) were higher than in broiler litters (30 to 40%) (Leytem et al., 2007; Maguire et al., 2004) and turkey litters (50 to 70%) (Maguire et al., 2004). Microbial-mediated mineralization of phytate was presumably higher in breeder manure due to the low C:N ratio in the breeder manure (free of wood chips) and greater time of manure storage (42 wk in breeder vs. as little as 6-9 wk in broiler). A fundamental understanding of why orthophosphate was higher and phytate was lower in breeder manure P compared to broiler and turkey litters was necessary. These differences in manure P forms may impact P management of land-applied poultry litters/manures in terms of soluble P loss and plant P availability.

Dietary P modifications with poultry have shown promise for reducing environmental impacts. However, there was limited research on the effects of dietary P modifications on broiler breeder manures. The relationship between WSP_M and P forms and moisture content in breeder manure remained unclear. Furthermore, a clear understanding of the differences among poultry litters/manures required closer examination of P forms and P stability in breeder manure. Our objectives were to: (1) characterize P constituents of breeder manures as affected by dietary P modification, location within the pen, and manure accumulation period, and (2) determine the impacts that P forms and moisture content in breeder manures have on the variability in WSP_M concentration.

MATERIALS AND METHODS

Broiler Breeder Diets

A broiler breeder feeding trial was initiated in 2006 to evaluate the variability in manure P by diet, location within the pen, and period of accumulation. The National Research Council (NRC, 1994) P feed recommendation for the breeder laying phase was 0.37% non-phytate P (NPP), which was equal to 0.40% available P (AvP, relative bioavailable P fraction) based on a slope assay with monocalcium phosphate as the reference

(Apke et al., 1987; Soares, 1995). Thus, the dietary P target during the breeder laying phase (42 wk from 22 to 64 wk of age) was 0.40% AvP formulated with and without phytase. These two diets (Table 1) were fed to 4 replicate pens of broiler breeders each. The general industry practice has been to reduce dietary NPP by 0.1% with the simultaneous phytase addition of 500 FTU (phytase activity units) kg^{-1} of feed (Van der Klis and Versteegn, 1996). Thus, diets with 0.37% NPP and 0.27% NPP + phytase theoretically produced the same AvP of 0.40%, and henceforth will be referred to as NRC and NRC + phytase, respectively. Allzyme SSF (Alltech, Nicholasville KY) was the phytase enzyme used with an analyzed activity of 1098 FTU g^{-1}. Dietary Ca was maintained at 2.7% of diet by weight with the substitution of dolomitic limestone for dicalcium phosphate in the reduced P diet but dietary Ca:P was not controlled as would be normal nutritional practice in laying diets (Table 1).

Broiler Breeder Manure Collection and Characterization

Six male and 60 female breeders were placed in breeding pens (4 x 4 m) from 22 through 64 wk of age to allow 42 wk of breeder production and manure accumulation. A “scratch area” covered with wood chips was in the front one-third of each pen where one feeder was provided for the males. The remaining area of each pen was an elevated wood “slat” floor with perforations that allowed excreta to pass down and accumulate on a concrete floor free of wood chips. Four feeders (for females) and two drinkers were evenly distributed on 1.5-m centers above the slat floor. A wooden tray (0.75 x 2.25 m) was covered with 0.5-mil thick plastic and divided into three sections (0.75 x 0.75 m) to collect manure from the areas under the feeder, the drinker, and the common location (the area between the feeder and the drinker). Manure was collected with sampling trays for 48 h (during wk 39) and 3 wk (from wk 40 through 42) of accumulation, since the digestion efficiency of the birds would be more uniform near the end rather than the initiation of the breeder phase. The birds were removed after 42 wk of breeder production. The third manure sample represented 39 wk of accumulation since sampling trays collected the last 3 wk of excreta. The 39-wk samples were collected with a PVC tube (10-cm diameter) under the feeder, the drinker, and in the common areas. Manure samples were homogenized in large plastic Ziploc bags immediately after collection. Manure was cooled to 4º C until subsamples were taken for analyses, and the remaining manure was frozen.

Manure moisture was determined by drying fresh subsamples overnight at 105º C. Manure WSP was extracted in duplicate for each treatment replicate at a ratio of 1:100 fresh manure (dry weight equivalent) to deionized water, horizontally shaken at 300 rpm for 1 h, centrifuged at 14 500 x g for 10 min, filtered through Whatman #40 paper, and analyzed with inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (Wolf et al., 2008). Three of the four replications of each diet, location within the pen, and sample period were dried, ground, and analyzed for nutritional content according to the standard procedures of the NC Department of Agriculture & Consumer Services. Total P, K, Ca, Mg, S, Mn, Zn, and Cu were determined by microwave-assisted digestion of a 0.5-g dried manure sample with 10 mL of concentrated HNO_3 and 2 mL of 30% H_2O_2 (U.S. EPA Method 3051A, 2007), and analyzed with ICP-AES. Total C and N were determined by oxygen combustion using a Perkin Elmer 2400 CHN elemental analyzer (Perkin Elmer Corp., Norwalk, CT). Manure pH was determined by diluting dry manure with deionized water at a ratio of 1:1 with 60 min of equilibration.

Solution ^{31}P Nuclear Magnetic Resonance Spectroscopy of Broiler Breeder Manure

Manure samples were dried and ground to pass a 0.5-mm sieve and then extracted in triplicate by shaking 2.00 $\pm$ 0.01 g of manure with 40 mL of 0.5 *M* NaOH + 0.05 *M* EDTA for 4 h at 20°C (Turner, 2004). Extracts were centrifuged at 10 000 x g for 30 min, and the supernatant was subsampled for TP analysis by ICP–AES. The remaining supernatant from the triplicate extracts were combined, frozen rapidly at –80°C, lyophilized, and ground to a fine powder. Prior to ^{31}P nuclear magnetic resonance (NMR) spectroscopy, approximately 100 mg of each freeze-dried extract was redissolved in 0.9 mL of 1 *M* NaOH + 0.1 mL of D_2O (for signal lock) and transferred to a 5-mm NMR tube. The addition of NaOH adjusted the solution to a pH > 13, which was necessary to ensure consistent chemical shifts and optimum spectral resolution.

All of the pulsed field NMR experiments were performed on a Bruker AVANCE 500 MHz Spectrometer (1996) with Oxford Narrow Bore Magnet (1989) and SGI INDY Host Workstation with XWINNMR Software version. The NMR probe was tuned to ^{31}P frequency that was 202.455 MHz in the 500 MHz spectrometer (^{1}H frequency -500.128 MHz). Samples were subjected to a 5-µs pulse (45°), a delay time of 4.0 s, an acquisition time of 2.0 s, and broadband proton decoupling. The relatively long delay time allowed sufficient spin–lattice relaxation between scans for P compounds in these extracts with low paramagnetic ion concentrations (Turner, 2004). Each sample was scanned approximately 7

000 times over 12 h, and the spectra were plotted without line broadening. Chemical shifts of signals were determined in parts per million (ppm) relative to 85% (w/v) H_3PO_4 and assigned to individual P compounds or functional groups according to Turner et al. (2003). Concentrations of P forms were determined by integrating signal areas and multiplying the proportion of the signal area by the TP concentration (g P kg^{-1} dry manure) that was determined by the microwave-assisted acid digestion. A strong signal appearing around 5.75 ppm was assigned to inorganic orthophosphate, while signals between 4.0 and 5.7 ppm were assigned to orthophosphate monoesters (Fig. 1). A number of individual signals were detected within this region, including those at approximately 5.45, 4.45, 4.15, 4.05 ppm in the ratio of 1:2:2:1, which were assigned to phytate-P (Fig. 1). Other trace signals in this region probably represented lower-order inositol phosphate esters. A signal at approximately –4.7 ppm was assigned to pyrophosphate, a specific inorganic polyphosphate with chain length n = 2. Signals from orthophosphate diesters (usually occurring between 2 and –1 ppm), phosphonates (20 ppm), and long-chain inorganic polyphosphates (–4 ppm and –18 to –21 ppm) were not detected.

Statistical Analyses

Treatment effects were determined by analysis of variance using PROC GLM in SAS version 9.1 (SAS Institute, 2002) at a = 0.05, 0.01, and 0.001. Fisher's protected LSD was used to separate treatment means at a = 0.05. Treatment means, standard deviations, and standard errors were generated with JMP version 7 (SAS Institute, 2007), and regression and bar graphs were created with Sigma Plot 2001 (SPSS, Inc., 2001) and Microsoft Excel 2002

(Microsoft, 2002). Main effects were reported whenever possible where the effects of: (1) diet were averaged over pen location and accumulation period, (2) location within pen were averaged over diet and accumulation period, and (3) accumulation period were averaged over diet and pen location.

RESULTS AND DISCUSSION

Effects of Diet, Location Within Pen, and Accumulation Period on Breeder Manure Characteristics:

Moisture and Water-Soluble Phosphorus

Manure produced from breeders fed phytase with simultaneous NPP reduction did not exhibit values of moisture or WSP_M that differed from the NRC diet (Table 2). Manure moisture averaged 40% for NRC and 37% for NRC + phytase diets after 39 wk of accumulation (data not shown). Maguire et al. (2006) reported no differences in moisture of breeder manures (41 to 46%) produced by similar dietary modifications of P when sampled after 42 wk of accumulation. However, manure produced from broiler breeders fed phytase were 28% lower than the traditional diet in the 42-wk samples of the Maguire et al. (2006) study. Dietary phytase additions of broilers decreased WSP_M from 10 to 50% (Applegate et al., 2003; Maguire et al., 2004), but other studies reported WSP_M increases from 1 to 48% (Miles et al., 2003; Vadas et al., 2004). Whereas, other research reported that dietary phytase additions had no effect on the WSP_M (Maguire et al., 2004) and the ratio of WSP_M:TP in broiler litter (Applegate et al., 2003; Miles et al., 2003; Smith et al., 2004). Similarly in our study, WSP_M did not differ among the manures produced from the NRC (4.0 g kg^{-1} dry matter) and the NRC + phytase (3.8 g kg^{-1} dry matter) diets when averaged over pen locations and accumulation periods (data not shown). Increases in the ratio of dietary Ca:P were a controlling factor in the discrepancies in dietary phytase effects on WSP_M, and when the ratio of dietary Ca:P was maintained phytase additions tended to increase the ratio of WSP_M:TP in broiler manure (Leytem et al., 2007, 2008). In our study, the dietary Ca:P increased from 7.3 to 10.0 with the addition of phytase (Table 1), which was a minimal change in comparison to previous studies.

Location within the pen and accumulation period influenced values of manure moisture and WSP_M (Table 2). Moisture was highest in the manure under the drinker (59%) followed by the common area between the drinker and feeder (55%) and then the feeder (36%) (Fig. 2). Spillage of water would account for the high moisture under the drinker, and spillage of feed provided material to absorb moisture under the feeder. Maguire et al. (2006) found that breeder manure sampled by location within the pen after 42 wk of accumulation averaged 39 to 69% moisture, which was somewhat higher than our samples taken after 39 wk of accumulation (26 to 48%). The differences between studies were most likely due to factors such as building ventilation, watering regimes, and environmental conditions (temperature and humidity).

Manure moisture and WSP_M were greatest after the 48 h accumulation period (63% and 4.2 g kg^{-1}, respectively) but stabilized over the 3-wk (41% and 3.4 g kg^{-1}, respectively) and the 39-wk accumulation periods (41% and 3.7 g kg^{-1}, respectively) (Fig. 2, 3). The decrease in moisture over time was most likely due to evaporation and microbial use. The patterns of manure moisture and WSP_M were very similar over accumulation periods, and in fact, WSP_M increased linearly as manure moisture increased (r^2 = 0.76, Fig. 4). Manure moisture was highly correlated with WSP_M regardless of the location within the pen and the accumulation period. For instance, the WSP_M concentrations in the manure that accumulated for 39 wk in the common area (48% moisture) and the manure that accumulated for 48 h under the feeder (49% moisture) were the same (3.6 g WSP_M kg^{-1} dry matter, Fig. 4). Dietary phytase additions, dietary Ca:P ratios, and the proportion of phytate in the manure were identified as the source of WSP_M variability in previous research, but rarely has manure moisture been reported. Future research should include manure moisture in relation to WSP_M as moisture could be the source of variation and / or the symptom of other factors (e.g., imbalance of dietary Ca:P). It was noteworthy that WSP_M in our breeder manures was approximately three to four times higher than previously reported (Maguire et al., 2006). We used the extraction ratio 1:100 that has been widely adopted for animal manures, which extracts more soluble P than the ratio of 1:10 (Kleinman et al., 2007) that was used by Maguire et al. (2006).

Poultry production practices that limit drinking water spillage should aid in the reduction of WSP_M and the subsequent impacts on land applications. In the short-term, soil surface runoff of P was highly correlated (r values from 0.79 to 0.93) to WSP_M concentrations of 15 wastes that included biosolids, chicken litter, turkey litter, beef feedlot manure, dairy manure, and swine slurry (Kleinman et al., 2007). The effects of broadcast-applied broiler litters modified in dietary P were transient and generally not detectable after multiple rainfall simulations (Maguire et al., 2005).

Total Phosphorus

Diet, location within pen, and accumulation period significantly influenced manure TP (Table 2). Birds fed phytase with simultaneous NPP reduction produced manure that was 15% lower in TP (15.0 > 12.8 g kg^{-1}), which was similar to the 14% numerical decrease reported by Maguire et al. (2006). Manure TP under the drinker (15.1 g kg^{-1}) and the common (14.7 g kg^{-1}) locations were higher than under the feeder (11.9 g kg^{-1}) presumably due to the dilution effect of spilled feed (Fig. 5). Total P concentration increased as the accumulation period increased: 48 h (11.9 g kg^{-1}) > 3 wk (13.2 g kg^{-1}) > 39 wk (17.3 g kg^{-1}) (Fig. 5). Microbial activity probably increased over accumulation period, which released carbon dioxide, decreased carbon content, and concentrated the P in the breeder manure.

Nitrogen-based applications of manures increased soil P levels due to the imbalance of crop removal rates and manure supply of N and P (Mikkelsen, 2000). Soil water-soluble P and the potential for soluble P losses from agricultural fields increased dramatically when soils exceeded the P saturation threshold (Van der Molen et al., 1998; Hooda et al., 2000; Pautler and Sims, 2000). Phosphorus-based applications of manures should reduce the risk of soluble P losses by limiting P buildup in the soil; however, greater land area will be needed to manage manure TP especially in areas of intense animal production (Maguire et al., 2007). Thus, the reduction of manure TP concentrations like those exhibited in our study (15%) and other dietary P modification studies (Maguire et al., 2005) would decrease the P loading in N-based applications of those litters/manures. In P-based management of manure, manure TP reductions would decrease the land area needed for applications and decrease the amount of supplemental N needed for the growing crop (i.e., the ratio of N:P in the manure would increase).

Phosphorus Forms Estimated by Solution ^{31}P Nuclear Magnetic Resonance and High-Performance Liquid Chromatography

Orthophosphate, phytate-P, lower esters of inositol P and pyrophosphate were the main P forms in the breeder manures (Fig. 1). Total P recovery via NaOH-EDTA extraction was equal to or 100% compared to microwave-assisted digestion. A composite sample of each diet, location within pen, and accumulation period was analyzed due to the high cost of NMR. Therefore, statistical comparisons of the NMR spectra among the breeder manures could not be made. Manure TP concentrations of breeder manure replicates were multiplied by the ^{31}P NMR estimation of P species to obtain concentrations of P species, which were used in regression models. Phytate concentrations were determined by high-performance liquid chromatography (HPLC) in manure that accumulated for 48 h within each treatment replicate and subjected to analysis of variance (Table 3). Unfortunately, phytate concentrations in the manures that accumulated for 3 wk and 39 wk were below the detection limits of the HPLC.

Dietary P modifications of broiler breeder influenced manure P forms in the short-term, but long-term differences were minimal. Dietary phytase additions with simultaneous reductions of NPP significantly decreased phytate concentrations in the breeder manure by 27% after 48 h of accumulation (Table 3). In similar dietary P modifications, phytate concentrations decreased 32 to 43% in broiler litters (Leytem et al., 2007; Maguire et al., 2004; McGrath et al., 2005) and 32% in turkey litters (Maguire et al., 2004) based on ^{31}P NMR estimation of single samples. Additionally, phytate proportions and concentrations in manure that accumulated for 48 h and 3 wk were slightly lower in NRC + phytase than NRC (Figs. 6, 7). However after 39 wk of accumulation, the proportions of orthophosphate and phytate between the manures produced from NRC and NRC + phytase were the same (~91% and ~7%, respectively, Fig. 6). Microbial activity presumably increased as the manure accumulation period increased (i.e., mineralization of organic compounds like phytate). Whereas, the proportion of P forms were very different in broiler litters as orthophosphate was 24 to 47% and phytate was 37 to 65% (Leytem et al., 2007; Maguire et al., 2004; McGrath et al., 2005), and turkey litters were 50 to 70% orthophosphate and 33 to 41% phytate (Maguire et al., 2004). In our study, pyrophosphate ranged from 1 to 2%, and lower esters of inositol P were approximately 1 to 2% after 48 h and 3 wk accumulation periods for all manures (data not shown). Lower esters of inositol P were less than 0.5% after 39 wk of accumulation.

Phytate concentrations were greater in the manures under the drinker (4.7 g kg^{-1}) and the common (5.2 g kg^{-1}) areas than manure under the feeder (2.7 g kg^{-1}) after 48 h of accumulation (Table 3). Spillage of feed probably diluted the mass of the manure and subsequently the P concentrations of the manure. After 48 h and 3 wk of manure accumulation, phytate proportions and concentrations were numerically lowest in the manures under the feeder, but highest after 39 wk (Figs. 8, 9). Orthophosphate proportions were numerically higher in the manure under the feeder after 48 h of accumulation, and it was lowest after 39 wk of accumulation (Fig. 8). Orthophosphate concentrations tended to be lowest in the manure under the feeder (Fig. 9). These data support the continued need for representative manure sampling for nutrient analysis, especially P.

The proportion of orthophosphate increased (73.2% < 80.1% < 91.0%) as the proportion of phytate decreased (23.1% > 17.0% > 6.7%) over longer manure accumulation periods (48h > 3 wk > 39 wk, Fig. 6). An example of the decreases of the phytate peaks was plotted in the ^{31}P NMR spectra of Fig. 1. This inverse relationship was also exhibited in the concentrations of phytate and orthophosphate over wk of manure accumulation (Figs. 7, 9). The concentrations of phytate decreased as wk of manure accumulation increased in the manures produced from the diets in a first order relationship (r^2 = 0.99, Fig. 7) and among the manures collected within the pen in an exponential relationship (r^2 = 0.99, Fig. 9). Concurrently, orthophosphate concentrations increased in a power regression fashion as the wk increased between the manures produced from the diets (r^2 = 0.95, Fig. 7) and among the manures collected within the pen (r^2 = 0.88, 0.96, 0.99; Fig. 9). Carbon concentrations also decreased exponentially as the duration of the accumulation period increased (Fig. 10), which indicated the process of mineralization where microorganisms used organic complexes (e.g., phytate) for growth and released carbon dioxide. The decline in phytate and carbon concentrations with the concurrent increase of orthophosphate concentrations indicated that organic P was transformed into inorganic P (i.e., mineralization of phytate). Furthermore, mineralization was highly favored throughout all the locations within pen and accumulation periods where C:N ranged from 5.8 to 10.3 (data not shown).

Phosphorus composition of manure has been shown to be critical in determining environmental implications as well as bioavailability. The WSP_M concentrations in manures that were broadcast-applied to the soil surface were highly correlated with runoff P (Kleinman et al., 2007). The proportion of WSP_M decreased as the phytate proportions in poultry manures increased (Leytem and Maguire, 2007), and thus, the potential for runoff P decreased. Increased proportions of orthophosphate and / or decreased proportions of phytate in manure would presumably increase bioavailability of P and further the potential environmental impacts from runoff P from land applications (i.e., eutrophication). Conversely, increased orthophosphate would presumably increase plant P availability and allow greater P uptake and removal by receiver crops.

CONCLUSIONS

Poultry production practices should emphasize drinking water management to limit spillage; thereby, reducing WSP_M and environmental concerns from broadcast applications. The strong correlation between WSP_M and moisture in the breeder manures ($r^2 = 0.76$) should require moisture content to be reported with future manure P studies in addition to maintaining similar dietary Ca:NPP ratios. Evidence from this study strongly confirmed the need for representative manure sampling for nutrient management. For example, sampling biased toward manure under the feeders would underestimate the WSP_M (up to 10%) and TP (up to 19%) when compared to sampling only the common areas. In other words, P-based applications of breeder manure that was characterized with feeder-biased manure sampling would over-apply TP by nearly 20% and increase the risk for soluble P losses.

The mineralization of phytate to orthophosphate explained the large difference in P forms of breeder manures compared to broiler and turkey litters. However, many manure P studies of broilers and turkeys were conducted over a single production cycle rather than multiple flocks on a single bed of litter, which has become the most common practice in the USA poultry industry. Further investigation of the influence of manure accumulation periods and associated moisture content on manure P mineralization in breeder manure, broiler litter, and turkey litter should be pursued. Additionally, high orthophosphate proportions in breeder manures would presumably be more plant available than broiler and turkey litters that may have higher phytate content. However, most P management plans have been based on TP applied with little attention to plant P availability differences among poultry manures/litters. Poultry manures and litters produced from dietary P modifications have altered proportions and concentrations of orthophosphate and phytate. These alterations may also change plant P availability and removal, which should be the foundation for nutrient management plans.

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Table 1. Broiler breeder diets used to produce typical and reduced amounts of manure P.

Diet	NPP†	Phytase	AvP‡	Total P	Ca	Ca:NPP
	%	FTU§ kg^{-1}		%		
NRC¶	0.37	0	0.40	0.63	2.7	7.3
NRC + phytase	0.27	500	0.40	0.53	2.7	10.0

† NPP, Non-phytate P.

‡ AvP, Available P that assumed 500 FTU kg^{-1} of feed releases 0.1% of phytate P to available forms.

§ FTU, phytase unit where activity of phytase generated 1 µM of inorganic P min^{-1} from an excess of sodium phytate at pH 5.5 and 37°C thus equaling 1 FTU.

¶ NRC, National Research Council (1994).

Table 2. Probability of significance within the ANOVA of the breeder manures that were produced by diet (NRC, NRC + phytase) and collected by location within pen (feeder, common, drinker) over different periods of accumulation (48 h, 3 wk, 39 wk). Moisture, water-soluble P (WSP_M), total P (TP), and carbon were evaluated.

Source	df	Moisture	Water-Soluble P	TP	Carbon
Diet	1	ns	ns	**	ns
Location	2	***	**	***	***
Acc.†	2	***	**	***	***
Diet x Location	2	ns	ns	ns	ns
Diet x Acc.	2	ns	ns	ns	ns
Location x Acc.	4	ns	ns	*	ns
Diet x Location x Acc.	4	ns	ns	ns	ns

*, **, and *** represented probability of significance at a = 0.05, 0.01, and 0.001, respectively, and no significance (ns) when a > 0.05.
† Acc., Manure accumulation period.

Table 3. Phytate concentrations in breeder manures collected after 48 h of accumulation at three locations within the pen crossed with two diets.

Manure from diet	Phytate Concentrations† After 48 h of Manure Accumulation			
	Locations Within the Pen			
	Feeder	Common	Drinker	Mean across pen locations‡
	g kg^{-1} dry matter			
NRC	3.4	6.0	5.0	4.8a
NRC + phytase	1.9	4.3	4.4	3.5b
Mean across diets‡	2.7b	5.2a	4.7a	

† Phytate determined by high-performance liquid chromatography for each treatment replicate and subjected to analysis of variance.

‡ Diet and Location were significant at a = 0.05, and Diet x Location was not significant.

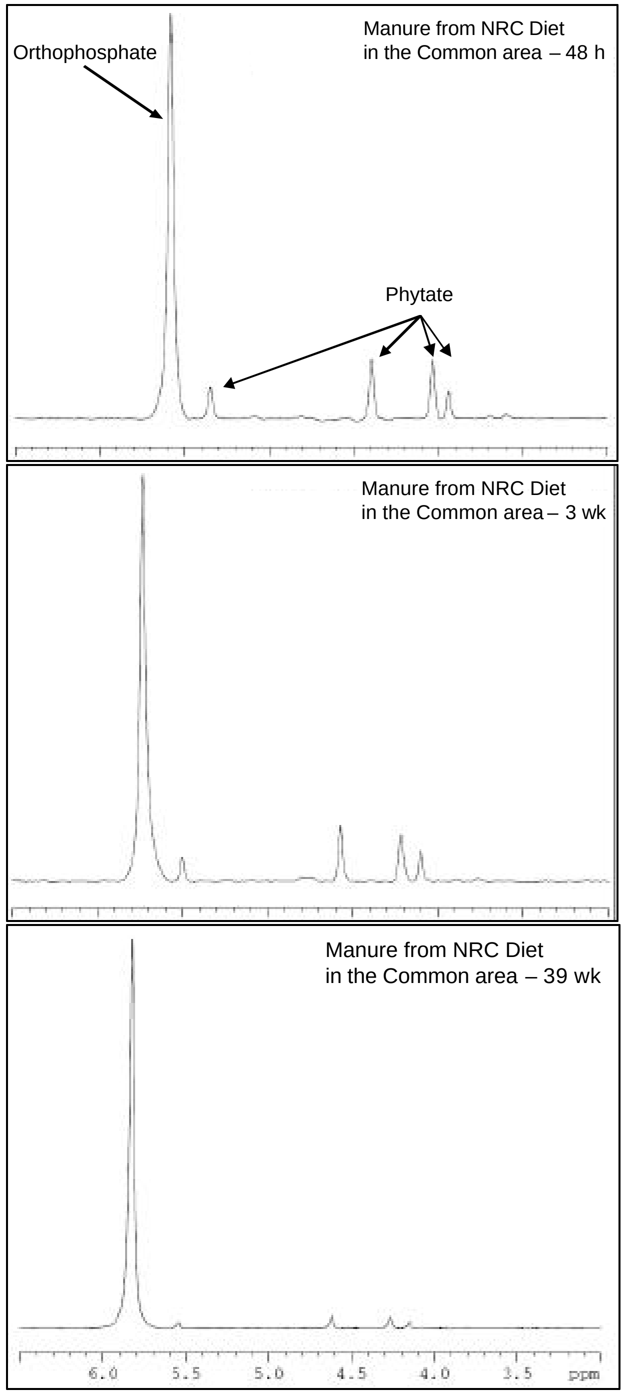

Figure 1. Solution ^{31}P nuclear magnetic resonance (NMR) spectra of breeder manure produced from the NRC diet (Table 1) and sampled in the common area after 48 h, 3 wk, and 39 wk of manure accumulation. The signal at approximately 5.75 ppm was orthophosphate, and the four other strong signals at approximately 5.45, 4.45, 4.15, and 4.05 ppm represented phytate.

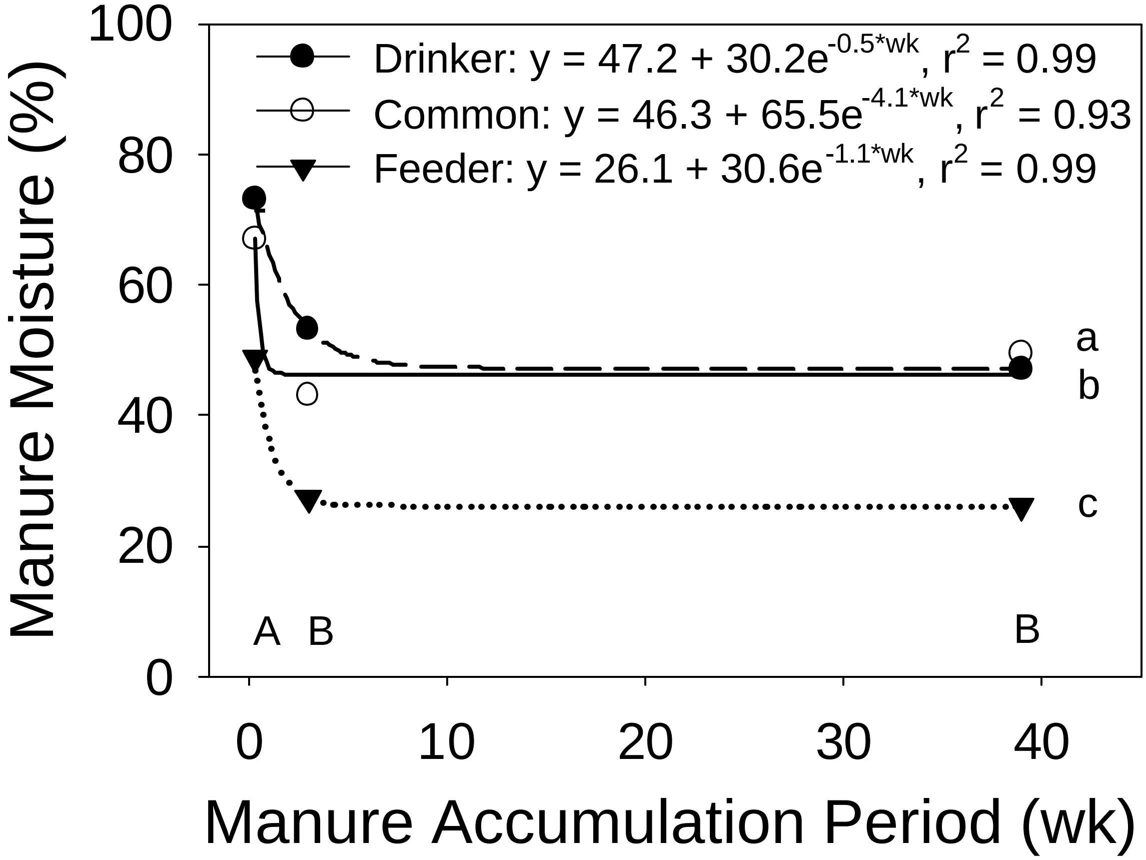

Figure 2. Manure moisture as affected by manure accumulation period and location within pen. Means separated according to Fisher's Protected $LSD_{0.05}$ among manure accumulation periods (upper case letters, averaged over diets and locations within pen) and among locations within pen (lower case letters, averaged over diets and manure accumulation periods). Means by manure accumulation: 48 h (63%) > 3 wk (41%) = 39 wk (41%). Means by locations within pen: drinker (59%) > common (55%) > feeder (36%).

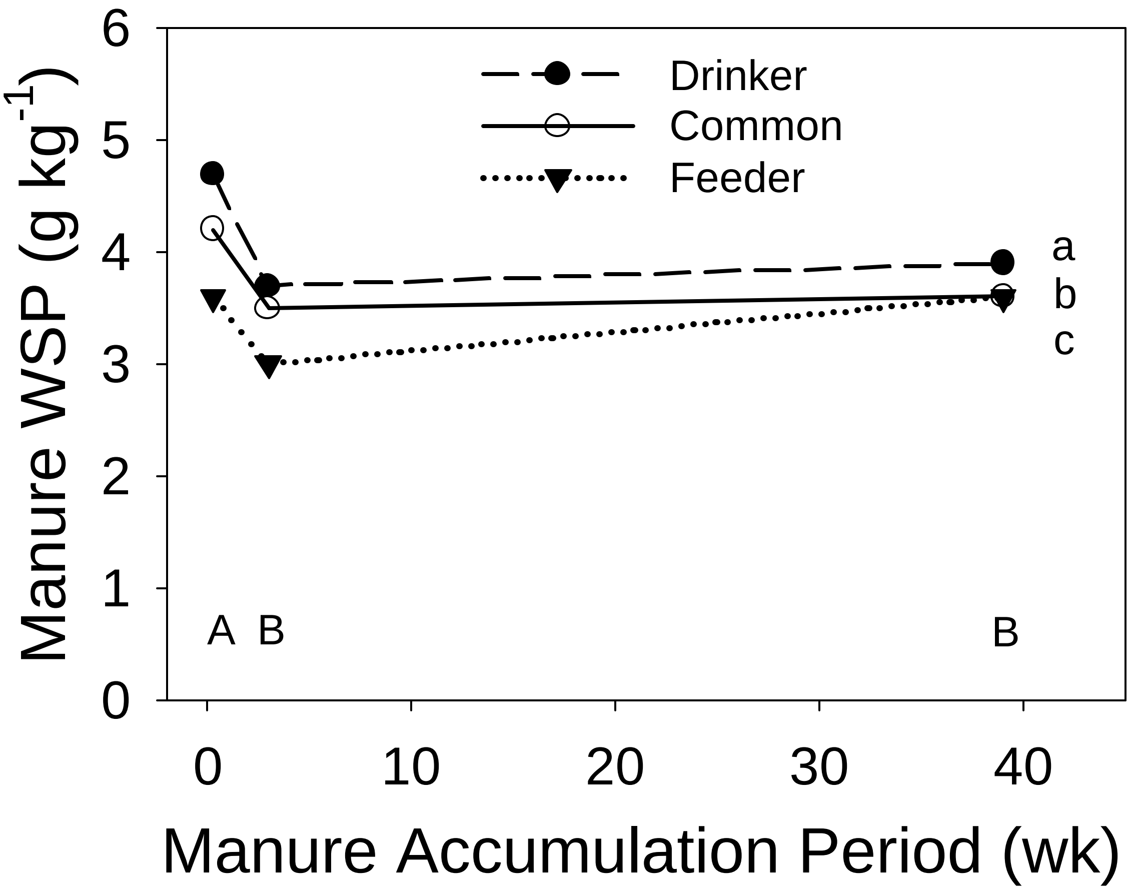

Figure 3. Manure water-soluble phosphorus (WSP_M) as affected by manure accumulation period and location within pen. Means separated according to Fisher's Protected $LSD_{0.05}$ among manure accumulation periods (upper case letters, averaged over diets and locations within pen) and among locations within pen (lower case letters, averaged over diets and manure accumulation periods). Means by manure accumulation: 48 h (4.2 g kg^{-1}) > 3 wk (3.4 g kg^{-1}) = 39 wk (3.7 g kg^{-1}). Means by locations within pen: drinker (4.3 g kg^{-1}) > common (3.9 g kg^{-1}) > feeder (3.4 g kg^{-1}).

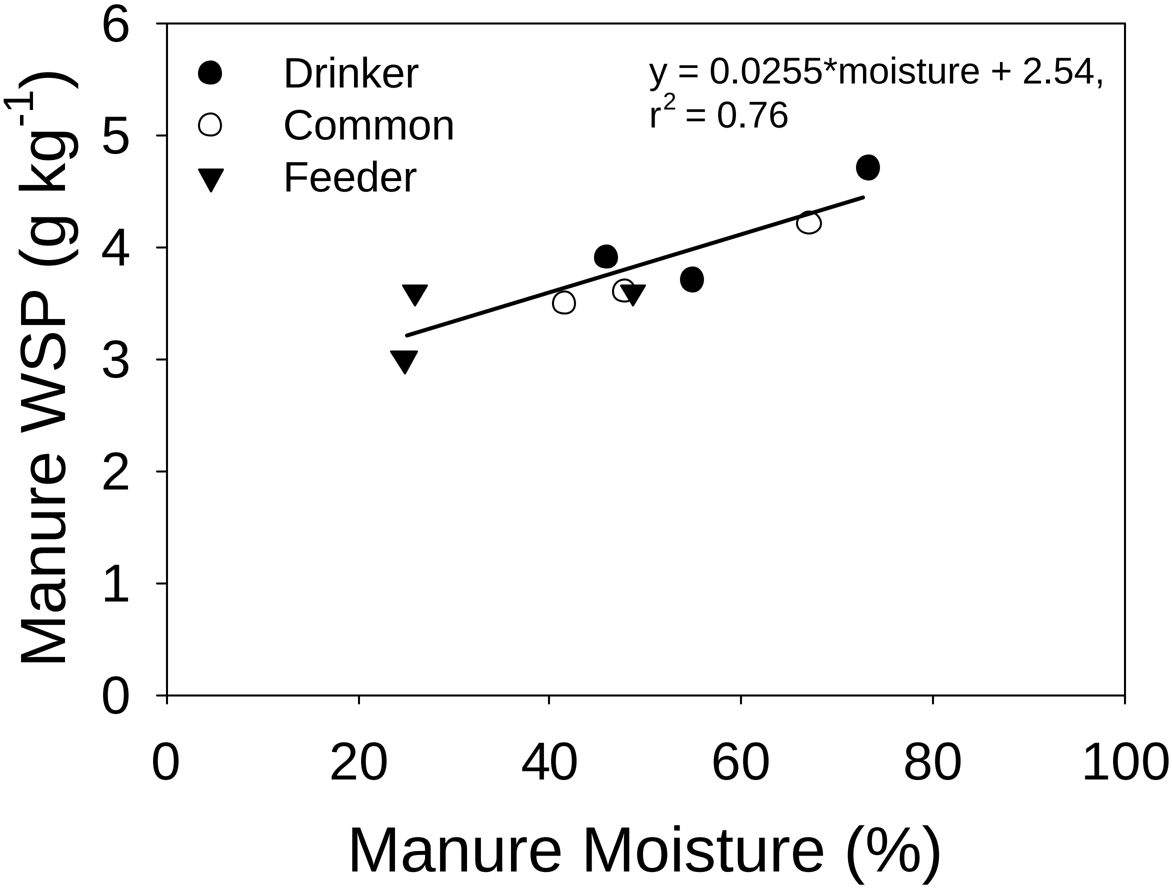

Figure 4. Manure water-soluble phosphorus (WSP) as affected by manure moisture from location within pen (drinker, common, feeder) after 48-h, 3-wk, and 39-wk manure accumulation periods and averaged over diet.

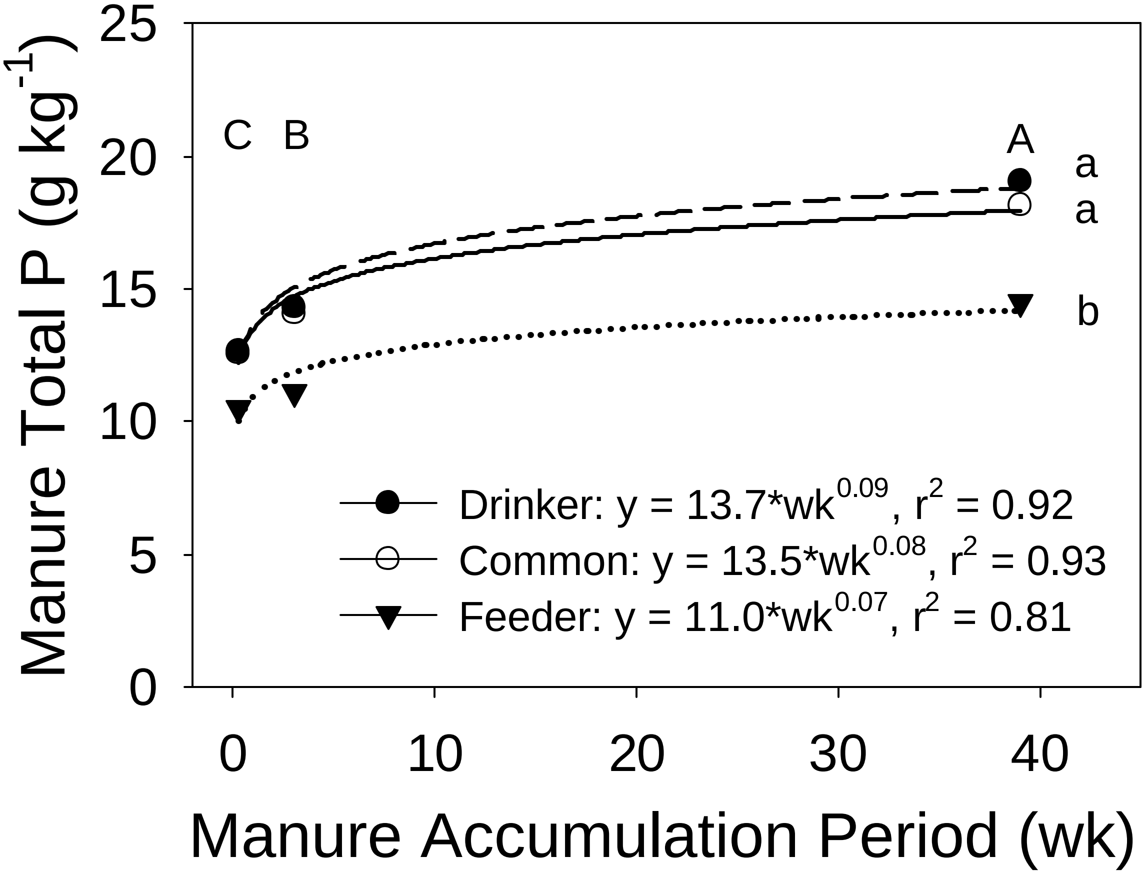

Figure 5. Manure phosphorus as affected by manure accumulation period and location within pen. Means separated according to Fisher's Protected $LSD_{0.05}$ among manure accumulation periods (upper case letters, averaged over diets and locations within pen) and among locations within pen (lower case letters, averaged over diets and manure accumulation periods). Means by manure accumulation: 48 h (11.9 g kg^{-1}) < 3 wk (13.2 g kg^{-1}) < 39 wk (17.3 g kg^{-1}). Means by locations within pen: drinker (15.1 g kg^{-1}) = common (14.7 g kg^{-1}) > feeder (11.9 g kg^{-1}).

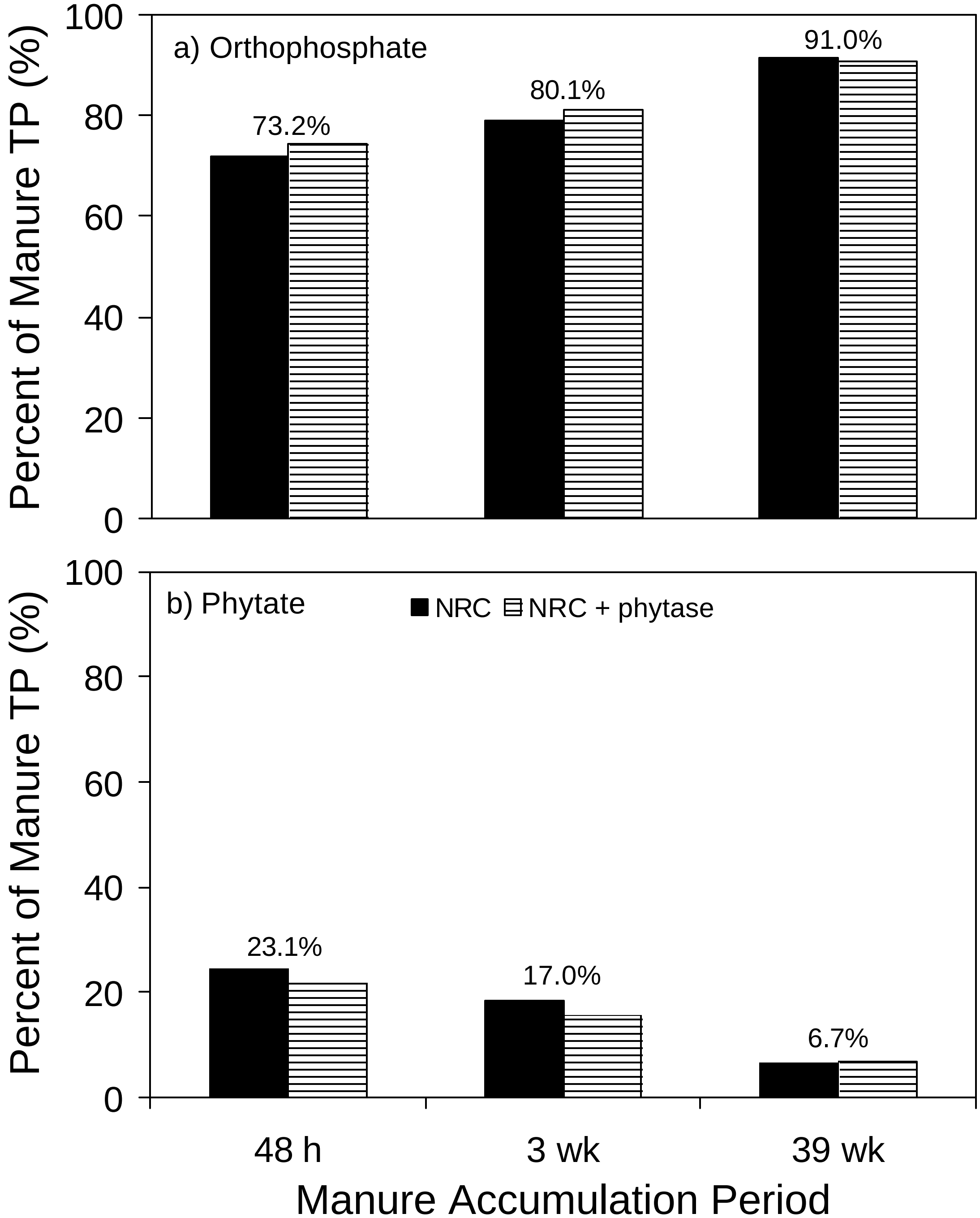

Figure 6. Percentage of (a) orthophosphate and (b) phytate in breeder manures that were produced from NRC and NRC + phytase diets (Table 1). Breeder manures were sampled after 48-h, 3-wk, and 39-wk accumulation periods, and P forms were averaged over locations within pen (feeder, common, drinker). Percentages displayed above the paired bars represented the average value for each manure accumulation period.

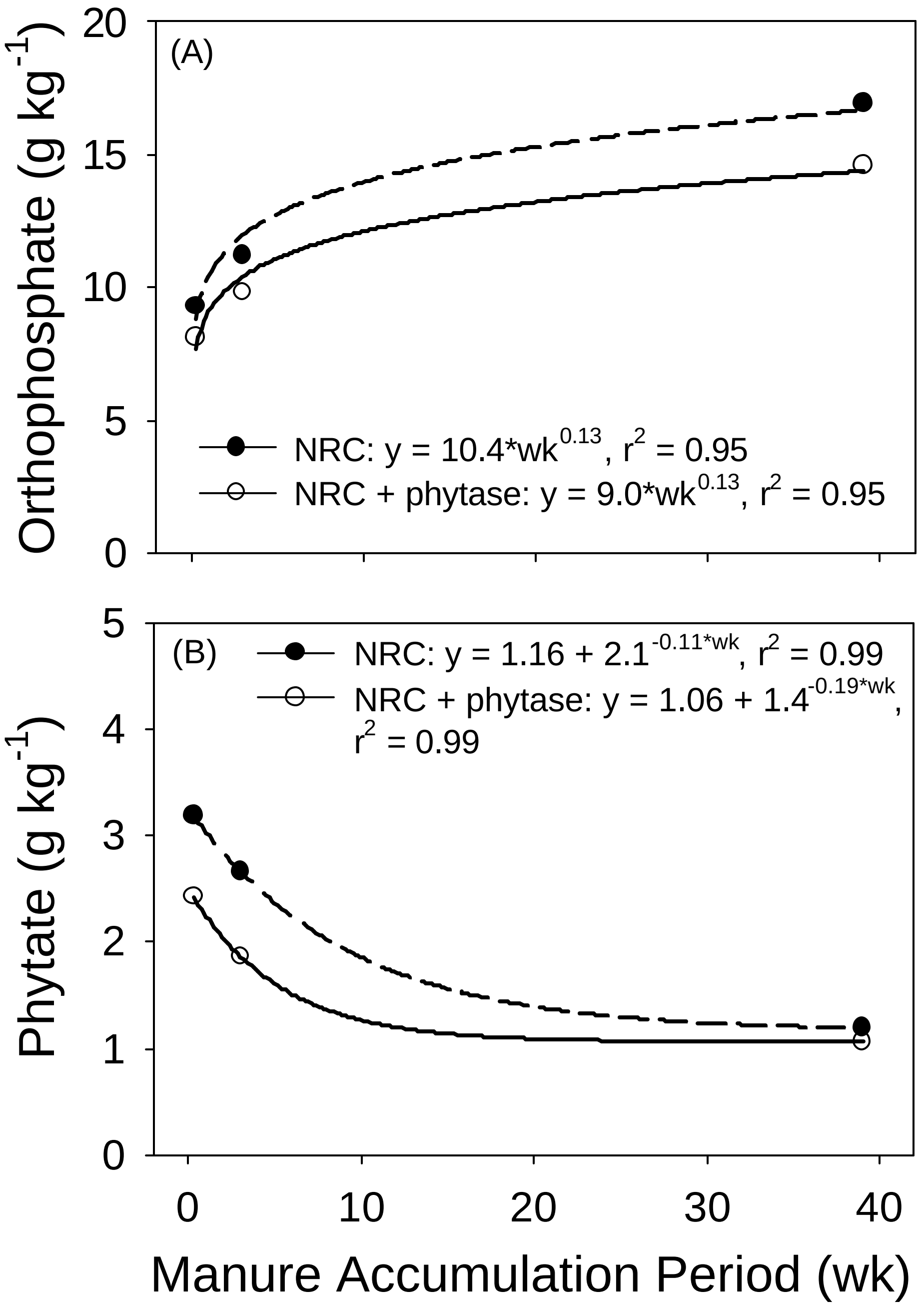

Figure 7. Concentrations of (a) orthophosphate and (b) phytate in breeder manures as affected by manure accumulation period and diet. The total P concentrations of the manure replicates were multiplied by the ^{31}P NMR estimations. Note scale differences of y-axis.

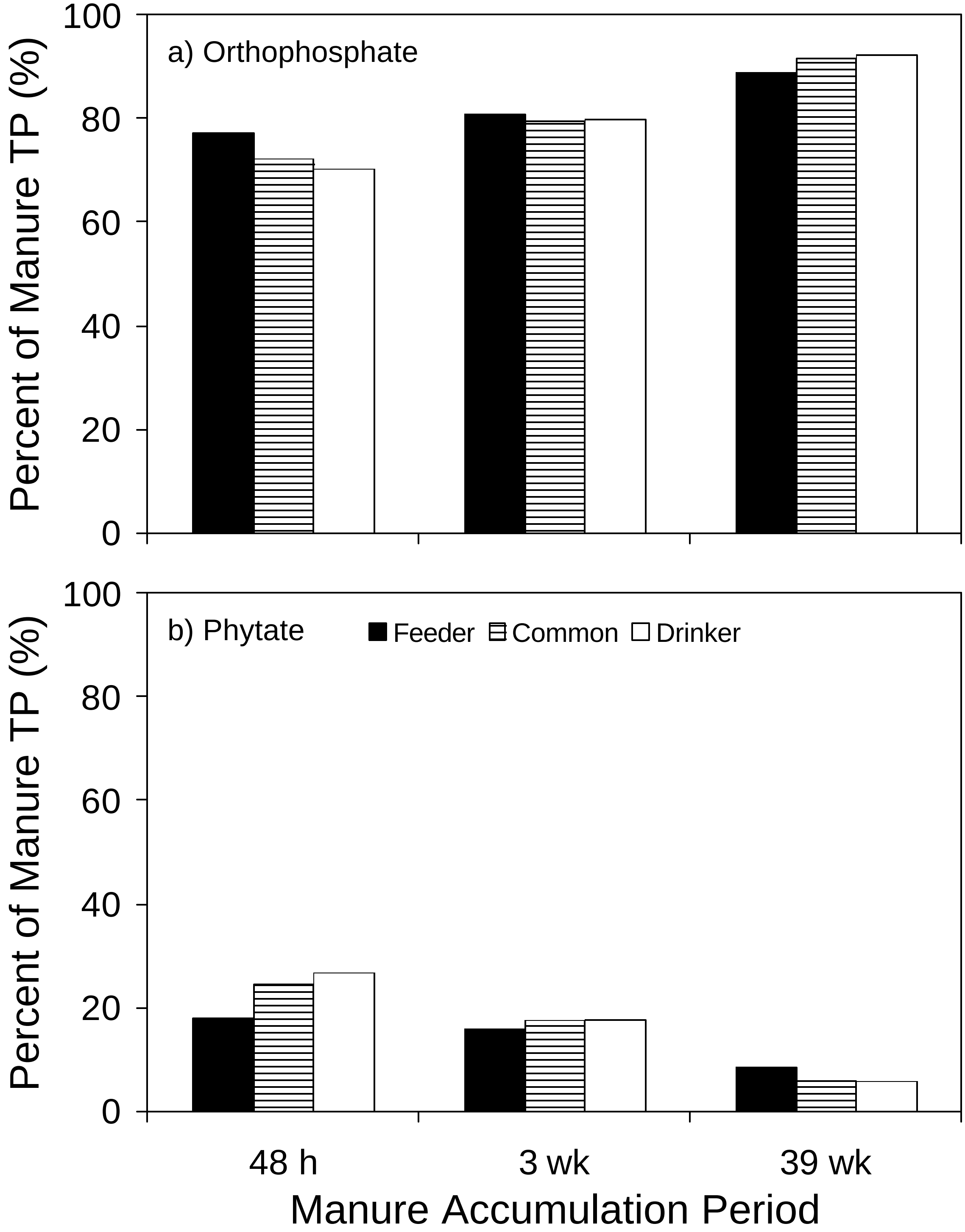

Figure 8. Percentage of (a) orthophosphate and (b) phytate in breeder manures that were sampled by locations within the pen. Breeder manures were sampled after 48-h, 3-wk, and 39-wk accumulation periods, and P forms were averaged over diets (NRC, NRC + phytase).

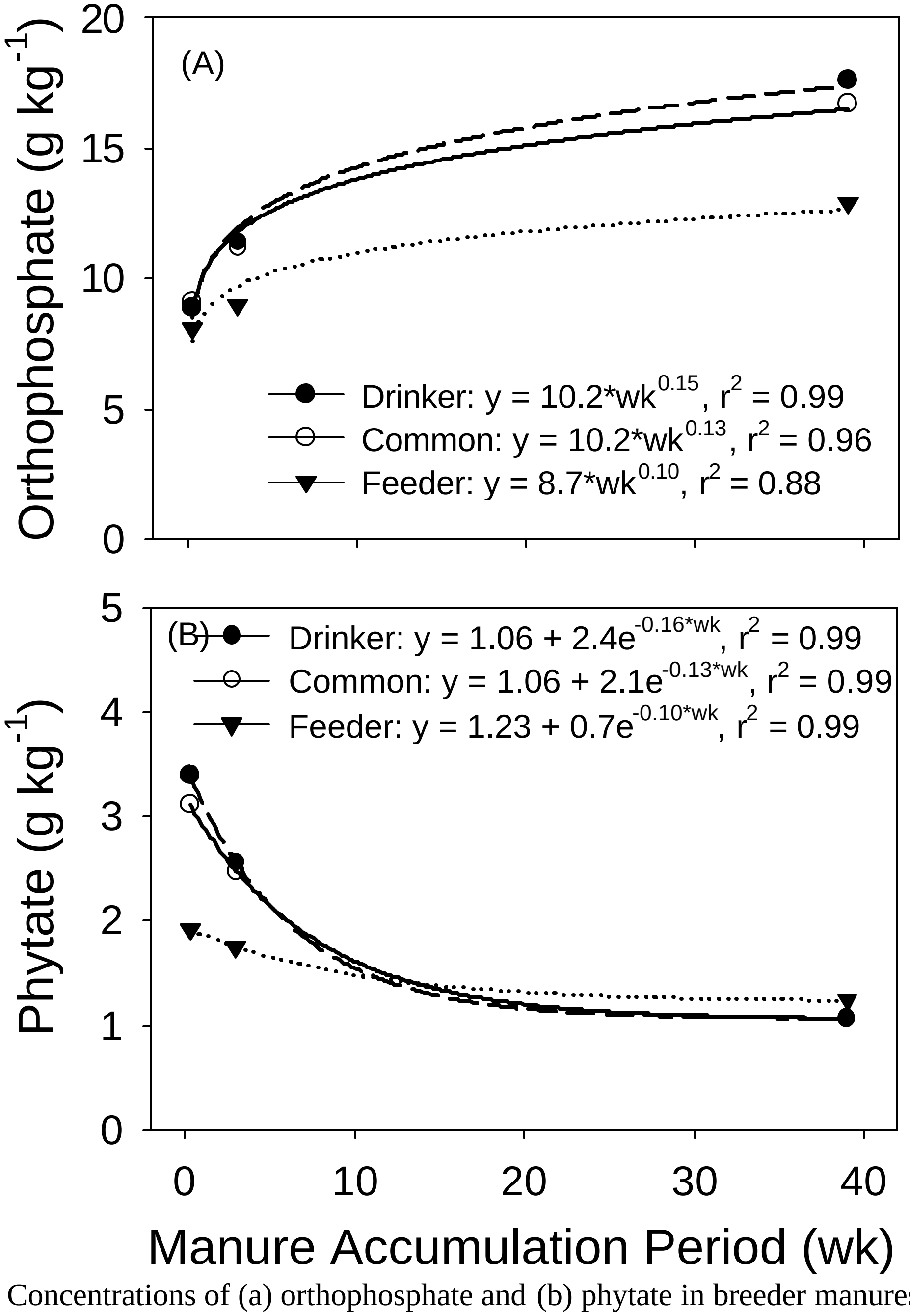

Figure 9. Concentrations of (a) orthophosphate and (b) phytate in breeder manures as affected by manure accumulation period and location within pen. The total P concentrations of the manure replicates were multiplied by the ^{31}P NMR estimations. Note scale differences of y-axis.

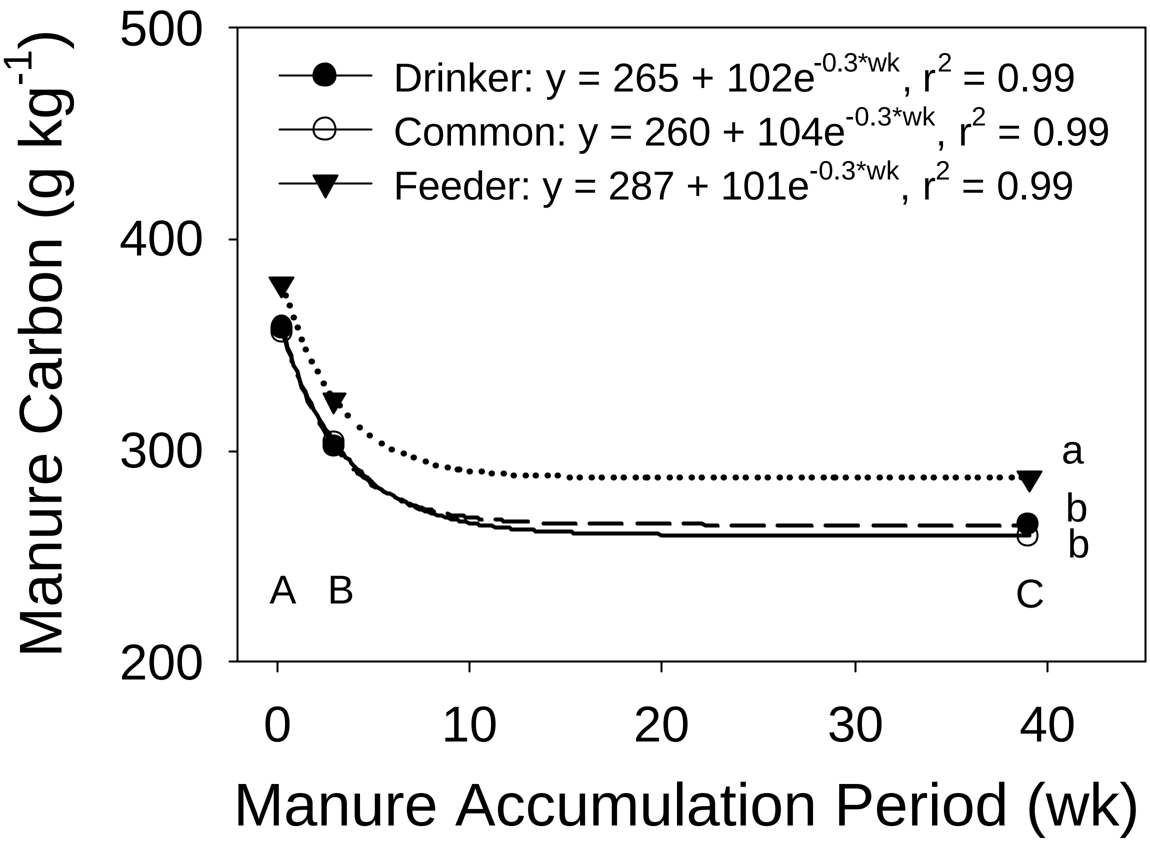

Figure 10. Manure carbon as affected by manure accumulation period and location within pen. Means were separated according to Fisher's Protected $LSD_{0.05}$ among manure accumulation periods (upper case letters, averaged over diets and locations within pen) and among locations within pen (lower case letters, averaged over diets and manure accumulation periods). Means by manure accumulation: 48 h (364 g kg^{-1}) > 3 wk (310 g kg^{-1}) > 39 wk (271 g kg^{-1}). Means by locations within pen: drinker (313 g kg^{-1}) = common (312 g kg^{-1}) < feeder (335 g kg^{-1}).

CHAPTER 4

Dietary Phosphorus Modification in Broiler Breeders:

Corn Response to Manure Phosphorus Rates in the Greenhouse

ABSTRACT

Plant phosphorus (P) availability of poultry manure/litters has not been well-defined, especially in manures produced from modern dietary P modifications. Our objectives were to: (1) determine P availability of breeder manures to corn in the greenhouse, (2) determine if breeder-dietary P modified manure was different in plant P availability than traditional breeder manure, and (3) determine the breeder manure equivalency to triple superphosphate (TSP). Manure was collected from broiler breeders that were fed two dietary P regimes (0.37 and 0.19% non-phytate P), which will be referred to as NRC and Low, respectively. The P sources were applied to a P-deficient, Portsmouth soil in pots at rates of 7.5, 15, 30 and 60 mg of P kg^{-1}, which would be approximately 18, 36, 72, and 144 kg P ha^{-1}, respectively. Corn (*Zea mays*) was grown in the same amended soils in two consecutive studies in the greenhouse and terminated 28 d after emergence in each study. Breeder manures maintained the soil pH at the low P rates and increased the soil pH at the higher P rates due to the calcium carbonate equivalence (i.e., liming value) of the manure. Plant height, leaf area, and shoot biomass were equivalent among P sources in study 1, but breeder manure amendments tended to produce taller plants with greater leaf area and shoot biomass in study 2, probably due to the apparent liming effect and P applied. Despite being applied on a P-basis, Mehlich-3 P concentrations were highest in soils amended with the NRC manure followed by the Low manure and TSP in both studies. Total P accumulation was equal among P sources at the low P application rates in study 1, study 2, and the sum of both studies. However, total P accumulation tended to be numerically higher in the manure-amended soil where the Low manure amendment produced plants that accumulated more P at the high P rate. The applied P recovery (APR, which corrected for soil P supply) in total plant P accumulation was significantly higher in the NRC (34.7%) and Low (35.8%) manures than TSP (30.6%) when both studies were summed. Plant growth, P accumulation, and APR in breeder manure-amended soils were equal to and or greater than TSP-amended soils probably due to the high orthophosphate proportion (92 to 94%) and the apparent liming effect of the breeder manures. Phosphorus management of poultry manures must collectively consider the impacts of dietary P modifications on manure properties, soil interactions, and plant availability.

Keywords: phytase, broiler breeder, phosphorus, phytate, orthophosphate, phosphorus availability

Abbreviations: APR, applied phosphorus recovery; DAE, days after emergence; M3, Mehlich-3; M3P, Mehlich-3 phosphorus; NPP, non-phytate phosphorus; TP, total phosphorus; TIP, total inorganic phosphorus; TSP, triple superphosphate; WSP_M, manure water-soluble phosphorus; WSP_S, soil water-soluble phosphorus.

INTRODUCTION

Animal production has become vertically integrated to maximize efficiencies in feeding, marketing, and processing during the 20th century (Hendrickson et al., 2008). Meanwhile, manure and nutrient loading of land in these concentrated production regions increased due to transportation logistics and limited crop removal (Kellogg et al., 2000). Manure management was traditionally based on crop nitrogen (N) removal rates with limited attention to phosphorus (P). For example, corn removal ratios of N and P were 5 to 1 (Osmond and Kang, 2008), and the application of broiler breeder manure supplied N and P at a ratio of 2:1 (Casteel et al., 2007), which provided 2.5 more times P than was required. Repeated manure applications and the imbalance of crop removal rates and manure supply of N and P increased soil P (Mikkelsen, 2000) and compromised the quality of surface and ground waters (Sims et al., 2000). Additionally, P-based applications of manures in many areas of intense animal production created a manure P surplus due to limited crop acreage (Maguire et al., 2007).

Manure total P (TP) applications have been shown to have long-term impacts on the ecosystem where soils above P saturation thresholds increased soil water-soluble P (WSP_S) and the potential for soluble P losses from agricultural fields (Van der Molen et al., 1998; Hooda et al., 2000; Pautler and Sims, 2000). In the short-term, P runoff from the soil surface was highly correlated (r values from 0.79 to 0.93) with the manure water-soluble P (WSP_M) of 15 wastes that included biosolids, chicken litter, turkey litter, beef feedlot manure, dairy manure, and swine slurry (Kleinman et al., 2007). Modification of animal P rations has been a strategy to reduce excreted P (CAST, 2002), and thus, to reduce manure TP applications to soil (Maguire et al., 2007). Dietary P modifications decreased manure TP and WSP_M in broiler litter from 13 to 35% and from 0 to 83%, respectively, (Angel et al., 2005; Applegate et al., 2003; Maguire et al., 2004, Miles et al., 2003; Smith et al., 2004) and in turkey litter from 7 to 45% and from 1 to 83%, respectively (Angel et al., 2005; Maguire et al., 2003, 2004; Penn et al., 2004). Some of these diet-modified litters were soil-applied and were evaluated for environmental impacts and reported plant P availabilities based on concentrations of WSP_S and Mehlich-3 P (M3P) (Maguire et al., 2004, 2005; McGrath et al., 2005; Smith et al., 2004; Vadas et al., 2004). No research has connected the manures produced from dietary P modifications to the interactions of manure-amended soil and plant uptake of P even though crop utilization of manures (e.g., N, P) was the foundation of nutrient management plans.

Plant response to poultry litter applications based on mass quantity and N rates have been reported, but P-based studies have been limited. Nitrogen- and mass-based applications of broiler litter increased the yield of bermudagrass [*Cynodon doctylon* (L.) Pers] (Evers, 1998; Franzluebbers et al., 2004; Sistani et al., 2008), corn (Z*ea mays* L.) (Brown et al., 1994; Wood et al., 1996), soybean (*Glycine max* L.) (Adeli et al., 2005), and cotton (*Gossypium hirsutum* L.) (Malik and Reddy, 2002; Mitchell and Tu, 2005; Tewolde et al., 2007a). Mass quantity-based applications of layer manures were used to calculate the supply of N and P to millet [*Urochloa ramosa* (L.) T. Q. Nguyen] in the greenhouse (Montalvo, 2008). Phosphorus extraction efficiencies of cotton were calculated based on mass quantity of broiler litter (Tewolde et al., 2007b). Pelletized broiler litter treatments produced biomass equal to calcium phosphate ($CaHPO_4$), but P recovery by annual ryegrass (*Lolium* *multiflorum*) and sorghum-sudan grass (*Sorghum X drummondii*) in litter treatments was only 70% of the $CaHPO_4$ treatment (Hammac et al., 2007). Growth and P uptake by tall fescue (*Festuca arundinacea*) were similar in composted poultry litter and triple superphosphate (TSP, Ca $[H_2PO_4]_2$ H_2O) treatments (Sikora and Enkiri, 2005).

Phosphorus characterization of poultry litter has provided links to poultry dietary P regimes and the environmental implications of the litter-amended soil (e.g., soluble P losses). Orthophosphate proportions of the P in broiler litters were 30 to 40% (Leytem et al., 2007; Maguire et al., 2004), in turkey litters were 50 to 70% (Maguire et al., 2004), and in broiler breeder manures were 92 to 95% (Casteel, 2009). Phosphorus availability would presumably be the lowest in the broiler litter and highest in broiler breeder manure based on orthophosphate. However, to our knowledge, no research has been published that addresses plant availability of P from these dietary P modifications. Additionally, nutrient availabilities of broiler breeder manure have not been published, to our knowledge. The objectives of this study were to: (1) determine P availability of breeder manures to corn in the greenhouse, (2) determine if breeder-dietary P modified manure was different in plant P availability than traditional breeder manure, and (3) determine the breeder manure equivalency to TSP.

MATERIALS AND METHODS

Soil Collection and Characterization

Portsmouth fine sandy loam (Typic Umbraquult) was collected from the A horizon (0 to 20 cm) of a long-term P fertility study near Plymouth, NC. Soil was air-dried, homogenized, sifted through a 2-mm sieve, and analyzed (Table 1). Initial soil samples were analyzed for soil pH, humic matter, CEC, acidity, and Mehlich-3 (M3) extractable elements by the standard procedures of the NC Department of Agriculture & Consumer Services (Mehlich, 1984), which included ICP-AES determination of M3 elements. Mehlich-3 P saturation ratio (M3-PSR) was calculated as: [M3P / (M3Al + M3Fe)] with values for P, Al, and Fe in mmol kg^{-1} (Maguire and Sims, 2002b; Sims et al., 2002). Phosphorus concentrations in soil extracts were determined colorimetrically by the molybdate blue method (Murphy and Riley, 1962) and included:

(i) Total P (TP): 1 g of soil was ashed at 550° C overnight, diluted 1:25 with 0.5 *M* H_2SO_4, horizontally shaken at 300 rpm for 16 h, centrifuged for 10 min at 14 500 x g, and filtered through Whatman #1 filter paper (Kuo, 1996).

(ii) Total inorganic P (TIP): Soil was diluted 1:25 with 0.5 *M* H_2SO_4, horizontally shaken at 300 rpm for 16 h, centrifuged for 10 min at 14 500 x g, and filtered through Whatman #1 filter paper (Kuo, 1996).

(iii) M3P: Soil was diluted 1:10 with 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, horizontally shaken for 5 min, and filtered through Whatman #2 filter paper (Mehlich, 1984).

(iv) WSP_S: Soil was diluted 1:10 with deionized water, horizontally shaken at 300 rpm for 1 h, centrifuged for 10 min at 14 500 x g, and filtered through a 0.45-µm Millipore membrane.

Greenhouse Study 1 – Initiation

Breeder manures produced from diets NRC [0.37% non-phytate P (NPP)] and Low (0.19% NPP) were pelletized and characterized by Casteel (2009, Table 2). These breeder manure pellets were compared to TSP for P availability to corn at 7.5, 15, 30, and 60 mg P kg^{-1} dry soil in the greenhouse, which would be approximately 18, 36, 72, and 144 kg P ha^{-1}, respectively, assuming a bulk density of 1.2 g cm^{-3} within a 20-cm depth. An untreated control (UTC) was included to assess soil P availability. Treatments of TSP and UTC were supplemented with NH_4NO_3 to supply 50 mg N kg^{-1} (90 mg N pot^{-1}). Nitrogen from the manures were assumed to be 50% plant available and supplemental NH_4NO_3 was applied to attain the target rate of 50 mg N kg^{-1} (90 mg N pot^{-1}). For example, at the rate of 7.5 mg P kg^{-1}, manure from NRC supplied 4.8 mg plant available N kg^{-1} (9.6 mg total N kg^{-1}) and NH_4NO_3 was added to supply the remaining 45.2 mg N kg^{-1} for a total of 50 mg N kg^{-1}. Amendments were thoroughly mixed with the Portsmouth soil (1.8 kg per replicate) in large plastic bags. The amended-soil was transferred to plastic pots (15 cm in diameter x 15 cm in height) and packed to a bulk density of approximately 1.2 g cm^{-3}. Thirteen treatments (3 P sources x 4 P rates + UTC) were arranged in a randomized complete block design with 4 replications.

Five seeds of corn (Pioneer 31G96) were planted to a depth of 4 cm, then the pots were moistened from the bottom (capillary rise) to a moisture content of 0.18 g g^{-1}, which was the same as the Portsmouth soil amended with these manures in a previous incubation study (Casteel, 2009). Soil moisture was maintained at this level daily by adding deionized water to the individual pots on a mass basis, which was adjusted for plant weight. Growing conditions were regulated with a wet wall and fan. Full sun was allowed to shine through greenhouse glass during early spring of 2007. Corn plants were thinned to 1 plant pot^{-1} 5 d after emergence (DAE).

Greenhouse Study 2 – Residual

Amended soils from study 1 were retained from each individual replicate (pot) and air-dried for study 2, which was conducted in the fall of 2007. Phosphorus was not added to treatments in study 2 so as to evaluate residual P supply. All treatments were supplemented with 90 mg N kg^{-1} (144 mg N pot^{-1}) from KNO_3 at a rate of 29 mg N kg^{-1} (46.4 mg N pot^{-1}) and from NH_4NO_3 at a rate of 61 mg N kg^{-1} (97.6 mg N pot^{-1}). Sulfur was supplied with K_2SO_4 at a rate of 25 mg S kg^{-1} (40 mg S pot^{-1}) to each treatment. Total potassium supplied was 80 mg K kg^{-1} (128 mg K pot^{-1}) from KNO_3 and K_2SO_4. Soil was sifted through a 2 mm screen, weighed to 1.6 kg, thoroughly mixed with fertilizer supplements, and then transferred to plastic pots (15 cm x 15 cm) to a bulk density of approximately 1.2 g cm^{-3}.

Data Collection – Plant and Soil

Plant heights and node counts (leaf collar method) were taken 10, 14, 21, and 28 DAE. Plants were cut 1 cm above the soil line 28 DAE and were partitioned into leaves and stalk (collectively called shoots). Leaf area was determined using Li-Cor LI-3100C Area Meter (Li-Cor Biosciences, Lincoln, Nebraska). Soil was passed through a 5.6 mm sieve where roots were collected and attached soil particles were washed off with water. Biomass partitions (leaves, stalks, and roots) were dried for 48 h at 80°C and weighed. Tissue concentrations of P and Ca were determined for all plant parts in both studies by dry ashing, digesting with 6N HCl, and analyzing by ICP-AES. The dry weight of the plant parts was multiplied by the nutrient concentrations of the respective plant part to calculate nutrient accumulations. Applied P recovery (APR) was calculated as: {[(P Accumulation of TRT – P Accumulation of UTC) / (soil weight)] / [P Applied]}*(100) where TRT was the P source x P rate, P Accumulation of UTC was 1.9 and 2.4 mg P plant^{-1} for study 1 and 2, respectively, and soil weight was 1.8 and 1.6 kg pot^{-1} in studies 1 and 2, respectively.

In study 1, leaves and roots produced from the P sources applied at 60 mg TP kg^{-1} plus the UTC were analyzed for total C and N by oxygen combustion using a Perkin Elmer 2400 CHN elemental analyzer (Perkin Elmer Corp., Norwalk, CT). Whereas, the biomass partitions for all treatments were analyzed for total C and N in study 2. Soil samples were taken after the plant termination in both studies, air-dried, ground ($\leq$ 2.00 mm), and analyzed for pH, M3P, TP, TIP, and WSP_S as described previously.

Statistical Analyses

Multiple linear regression was determined using PROC REG and the STEPWISE selection method for plant growth and P accumulation measurements in SAS version 9.1 (SAS Institute, 2002). Thirteen treatments in each study were partitioned into a factorial (3 P sources x 4 P rates) plus UTC where the main effects were reported as P source and P rate. Treatment effects were determined by analysis of variance using PROC GLM in SAS version 9.1 (SAS Institute, 2002) at a = 0.05, 0.01, and 0.001. Fisher's protected LSD was used to separate treatment means at a = 0.05. Treatment means, standard deviations, and standard errors were generated with JMP version 7 (SAS Institute, 2007), and regression and bar graphs were created with Sigma Plot 2001 (SPSS, Inc., 2001) and Microsoft Excel 2002 (Microsoft, 2002). Main effects were reported whenever possible where the main effect of: (1) P source was averaged over P rates and (2) P rate was averaged over P sources.

RESULTS AND DISCUSSION

Soil Effects

In study 1, WSP_S, M3P, and TIP concentrations 28 DAE increased as P rate increased (Fig. 1). At the rate of 60 mg P kg^{-1}, WSP_S concentrations were significantly greater in soil amended with TSP and the NRC manure (4.2 and 3.9 mg kg^{-1}, respectively) than the Low manure (3.3 mg kg^{-1}) (Fig. 1a). This was not surprising, as WSP_M was greater in manure produced from the NRC diet than the Low diet (4.3 > 2.3 g kg^{-1}, Table 2), and thus, the NRC manure supplied more WSP_M in P-based applications (i.e., higher ratio of WSP_M: P, Table 2). Mehlich-3 P concentrations were greatest in soil amended with the NRC manure followed by the Low manure and TSP (28.9 > 25.4 > 21.9 mg kg^{-1}, respectively, Fig. 1b) based on analysis of variance across P rates ($P < 0.01$); whereas, TIP concentrations did not differ among P sources (40.8 to 46.5 mg kg^{-1}, Fig. 1c). Concentrations of M3P and TIP did not differ among similar breeder manure applications in a soil incubation study (Casteel, 2009). Phosphorus applications of 0, 30, and 60 mg P kg^{-1} yielded 20.0, 42.7, and 69.9 mg TIP kg^{-1} in study 1, which were slightly lower than the incubation of breeder manure-amended soil (Casteel, 2009) at the same rates (22, 59, 103 mg TIP kg^{-1}, respectively) presumably due to plant P removal in study 1.

Soil WSP averaged 0.68 to 0.80 mg kg^{-1} among P sources in study 2, which were approximately half the values of study 1 (Figs. 1, 2) presumably due to plant P uptake in study 2 and soil drying between studies 1 and 2. Drying soil can increase the capacity of soil to adsorb P (Haynes and Swift, 1985) and thus, decrease the concentrations of WSP_S such as those seen in study 2. Concentrations of M3P and TIP in study 2 increased linearly as the initial P rate increased (Fig. 2). Among P sources in study 2, M3P concentrations were NRC $\geq$ Low $\geq$ TSP ($P < 0.05$), and TIP concentrations were NRC > Low = TSP ($P < 0.01$, Table 3).

Soil pH generally increased as P rate increased in both studies, especially in the manure amendments (Fig. 3). Greater N supply in study 2 probably increased nitrification (acidification process), especially in the treatments with less P applied (i.e., less breeder manure) (Fig. 3b). Soil pH effects among P sources followed the same trend in both studies (Low > NRC > TSP) (Fig. 3). Soil pH was higher in soil amended with the Low manure than the NRC manure after 12 wk of incubation (6.2 > 5.9) (Casteel, 2009). The calcium carbonate equivalence (CCE) of the Low manure (37%) was significantly higher than the NRC manure (32%) (Table 2), which was presumably due to the substitution of limestone for dicalcium phosphate in the Low diets (Casteel, 2009). Additionally, P-based applications incorporated varying amounts of these manures as the Low manure supplied 0.35 to 2.83 $cmol_c$ kg^{-1} and the NRC manure supplied 0.22 to 1.77 $cmol_c$ kg^{-1} (Fig. 4). Applied CCE (i.e., breeder manure applications from study 1) increased soil pH linearly in study 1 ($r^2 = 0.92$, Fig. 4a) and increased soil pH quadratically in study 2 ($r^2 = 0.96$, Fig. 4b). In the incubation study conducted by Casteel (2009), 1 $cmol_c$ applied from breeder manures increased soil pH by 0.22 units in the Portsmouth soil, which was similar to the 0.16 pH unit increase in the Portsmouth soil in study 1 (Fig. 4a).

Apparent liming effects were also reported for the applications of layer manure in piedmont and coastal plain soils and broiler litter in a piedmont soil (Maguire et al., 2006). Soil pH increased and extractable Al also decreased as poultry litter application rates increased in a wheat study (Tang et al., 2007). Liming has been a production practice that was used to manage constraints on plant growth such as acidity, Al toxicity, and macronutrient availability. Soils that maintain an optimal soil pH have a greater opportunity to maximize yield for agronomic goals as well as removing more nutrients (e.g., P in corn grain) from soils amended with manures, which was an environmental goal. Therefore, soils amended with the Low manure would presumably have better opportunity for plant growth, nutrient uptake, and removal due to the apparent liming effect in comparison to the NRC manure and TSP amendments. The impacts of the apparent liming effect from the breeder manures will be discussed more in the plant growth and P accumulation sections.

Plant Growth Effects

Plant height, leaf area, and shoot biomass were not influenced by P source in study 1 (Table 3) and ranged from 74 to 77 cm, 450 to 506 cm^2, and 2.8 to 3.1 g $plant^{-1}$, respectively (Fig. 5). Plant growth increased linearly as P rate increased (Table 4), which indicated that the corn was responsive to P applications and the soil was P-deficient. Plant height, leaf area, and shoot biomass were 48 to 94 cm, 151 to 726 cm^2, and 0.8 to 5.3 g $plant^{-1}$, respectively, for applications from 0 to 60 mg P kg^{-1} (Fig. 5). In P-based applications, shoot biomass accumulation of tall fescue was equal between poultry litter compost and TSP (Sikora and Enkiri, 2005) and of annual ryegrass and sorghum-sudangrass was equal between pelletized broiler litter and calcium phosphate (Hammac et al., 2007).

In study 2, plant growth curves increased to a plateau as P rate increased (rectangular hyperbola model) where TSP generally reached a plateau before the NRC and the Low manures (Fig. 6). Soils amended with the Low and the NRC manures tended to have taller plants with greater leaf area than soil amended with TSP (Fig. 6a, 6b). Plant growth differences among P treatments (source x rate) were most likely due to the additive effects of soil pH where small increases in macronutrient availability and uptake would collectively improve plant growth in the manure-amended soil. Plant height, leaf area, and shoot biomass increased linearly as soil pH and P rate increased in study 2 (R^2 = 0.81, 0.87, and 0.83, respectively) (Fig. 7). Multiple linear regression models of plant growth were marginally enhanced with the additions of soil pH and M3P in study 1, and the additions of P rate and M3P in study 2 (Table 4). Noteworthy, total N accumulation was generally the same among P sources and P rates in study 2, since high amounts of N were applied to override any residual N effects from the breeder manures that were applied in study 1 (Fig. A1).

Shoot biomass in study 2 was highly influenced by P rate (Table 3), but incremental increases of shoot biomass diminished as P rate increased (1.8 > 2.7 > 3.4 > 3.8 g plant^{-1}) similarly to plant height and leaf area (Fig. 6). Shoot biomass in study 2 was lower than study 1 at the P rate of 60 mg kg^{-1} (3.8 and 5.3 g plant^{-1}, respectively); whereas, plant height and leaf area across all treatments were greater in study 2 than study 1 (Figs. 5, 6). Study 2 was conducted in the early fall when cloudy days were fewer and overall temperatures were higher than study 1, which would promote rapid plant growth in study 2. These growing conditions could have dried out the soil of the high P rate treatments since pots in study 2 (1.6 kg) had less soil to hold water for corn plants than study 1 (1.8 kg). The limited water supply during hot growing days probably limited the biomass accumulation in the high P rate treatments.

Phosphorus Concentrations and Accumulations in Corn Plants

The interaction of source x rate was significant for total P accumulation in study 1, study 2, and the sum of both studies ($P < 0.001$, 0.05, and 0.001, respectively, Table 3). Phosphorus accumulated in shoots and roots was summed to obtain total P accumulation. In general, total P accumulation increased quadratically as P rate increased (Fig. 8). Total P accumulation was equal among P sources applied within P rates of 7.5, 15, and 30 mg kg^{-1} in study 1 (4.8 to 11.2 mg P $plant^{-1}$), study 2 (4.3 to 10.9 mg P $plant^{-1}$), and the sum of both studies (9.0 to 22.1 mg P $plant^{-1}$) (Fig. 8, Table 5). Sikora and Enkiri (2005) reported that cumulative P uptake of tall fescue was equal between TSP and poultry litter compost across similar P rates in a P-deficient Inceptisol. Cumulative P uptake of annual ryegrass and sorgum-sudangrass was equal among broiler litter pellets and calcium phosphate applied at approximately 15 and 30 mg P kg^{-1} to an Ultisol (Hammac et al., 2007).

In our studies, plants tended to accumulate more P in soil amended with the breeder manures than the TSP treatment (Fig. 8). At the P rate of 60 mg kg^{-1}, plants amended with the Low manure accumulated 42, 17, and 30% more P than TSP in study 1, in study 2, and in the sum of both studies, respectively (Fig. 8, Table 5). Plants amended with the Low manure accumulated 15% more P than the NRC manure at the P rate of 60 mg kg^{-1} in study 2 (Table 5). Soils amended with the Low manure were generally higher than the NRC manure and the TSP treatments in soil pH (Fig. 3), which presumably increased macronutrient availability (e.g., P, K, Ca) and P uptake. Further, total P accumulation was highly correlated with P rate where soil pH enhanced the multiple linear regression model in studies 1 and 2 (Table 4). Total P accumulation was generally equal between studies 1 and 2 (Table 5). However, at the P rate of 60 mg kg^{-1}, total P accumulation was 18.5 mg P $plant^{-1}$ in study 1 and 15.0 mg P $plant^{-1}$ in study 2 where root P accumulation contributed 49% (9.1 mg P $plant^{-1}$) and 25% (3.8 mg P $plant^{-1}$) of the total P, respectively (data not shown).

Applied P recovery (APR) was not influenced by P rate in study 1, but was influenced by P rate in study 2 and the sum of both studies (Table 3). Generally, the APR for total plant accumulation decreased as P rate increased where the lower P rates (34.5 to 36.4%) were greater than the P rate of 60 mg kg^{-1} (27.8%) in the sum of both studies (Table 5). In other words, less P was recovered in relation to the high P rate, which resulted in approximately 72% of the applied P still present in the soil. This would be beneficial for future crop uptake, but may contribute to soil P loading if repeated manure applications continued. The APR of total plant accumulation was equal among P sources in study 1 (15.2 to 19.5%) and in study 2 (15.4 to 16.3%) where the Low manure was greatest numerically (Fig. 9, Table 5). In the sum of both studies, the APR of total plant accumulation was higher in the Low (35.8%) and the NRC (34.7%) manures than TSP (30.6%) (Fig. 9, Table 5).

The APR of the NRC and Low manure amendments was equivalent to or greater than TSP in most measurements of P accumulation. Conversely, the P uptake by annual ryegrass and sorghum-sudangrass was greater in treatments of calcium phosphate than pelletized broiler litters at high P rates (Hammac et al., 2007). This difference may be due to the proportions of orthophosphate and phytate in the pelletized broiler litters. Broiler and turkey litters have been reported to have orthophosphate proportions from 30 to 40% (Leytem et al., 2007; Maguire et al., 2004) and 50 to 70% (Maguire et al., 2004), respectively. The lower orthophosphate and higher phytate proportions in the poultry litters would presumably decrease plant P availability. Comparative studies of broiler litter, turkey litter, broiler breeder manure, and layer manure will be needed in order to determine plant P availability to optimize P management for crop yield and environmental stability.

CONCLUSIONS

The breeder manures were equivalent to or greater than TSP in promoting plant growth, total P accumulation, and the APR of total plant accumulation in corn presumably due to the high orthophosphate proportion (92 to 94%) and the apparent liming effect once soil-applied. The breeder manures, especially the Low manure, maintained soil pH at the low application rates of P and increased soil pH at the high application rates of P (i.e., greater mass quantity applied). Lime applications in acidic soils, like those of the Coastal Plain of NC, have been necessary to manage soil pH for optimal crop growth including decreasing Al toxicity, increasing macronutrient availability (e.g., P, K, Ca), and maintaining microbial-mediated processes. Breeder manure applications would provide a two-fold benefit to producers where the manures would supply plant available P equivalent to TSP and reduce the amount of lime needed to manage soil pH, which would reduce input costs (i.e., lime, macronutrient).

Mehlich-3 P concentrations were highest in the soils amended with the NRC manure, and WSP_S was highest in soils amended with the NRC manure and TSP. According to assumptions in previous studies of manure-amended soils, plant P availability would be greatest in the soils amended with the NRC manure and TSP due the M3P and the WSP_S concentrations without considering other soil changes (i.e., apparent liming effects) that influence plant growth. Based on these short-term greenhouse response studies, breeder manure applications should be applied at the same P rate as TSP for optimal crop growth and P accumulation. As the application rate increased, plants could accumulate more P from the Low breeder manure than the traditional NRC breeder manure. Based on study 2, P-based applications of the traditional NRC breeder manure would require 15% more land area to remove the same quantity of P as the Low manure when applied at the same P rate (60 mg kg^{-1}). Field studies need to be conducted in order to determine if these early-season (28 DAE) differences persisted throughout the season to grain P removal. Phosphorus management in poultry manures need to collectively consider the effects of diets, manure characteristics, interactions once land applied, and plant dynamics.

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Table 1. Selected properties of Portsmouth soil used in the greenhouse response study.

Soil	pH	Humic Matter	CEC†	Acidity	Mehlich-3 Extractable Ca	Mehlich-3 Extractable Al	Mehlich-3 Extractable Fe	Mehlich-3 Extractable P	Total P	Total Inorganic P	WSP_S‡	PSR§
		%	$cmol_c\ kg^{-1}$			$mg\ kg^{-1}$						
Portsmouth	6.1	2.3	7.58	0.71	4.67	718.6	141.0	33.6	100.8	21.2	0.65	0.04

† CEC, Cation Exchange Capacity.

‡ WSP_S, Soil water-soluble P.

§ PSR, Phosphorus saturation ratio, calculated as a molar ratio of [(M3P) / (M3Al + M3Fe)].

Table 2. Selected properties of manure pellets that were produced by dietary phosphorus modifications of broiler breeders.

Manure from diet†	pH	CCE‡	N	K	Ca	P	Ca:P	WSP_M§	WSP_M:P	Orthophosphate¶	Phytate
		%	g kg^{-1}				g:g	g kg^{-1}	g:g	g kg^{-1}	
NRC	6.4b#	32b	27.8	32.3	107.4	21.7a	5.0b	4.3a	0.20a	20.4 (94)	1.085 (5)
Low	7.2a	37a	27.7	36.9	109.2	15.7b	7.0a	2.3b	0.15b	14.4 (92)	0.987 (6)

† The NRC (National Research Council, 1994) diet was 0.37% non-phytate P (NPP) with a dietary Ca:NPP ratio of 7.3. The Low diet was 0.19% NPP with a dietary Ca:NPP ratio of 14.2. See Casteel (2009) for further details of diets and manures produced.

‡ CCE, calcium carbonate equivalence.

§ WSP_M, manure water-soluble P.

¶ Values in parenthesis were the proportion (%) of total P for orthophosphate and phytate based on ^{31}P NMR peak integration. The total P concentrations of the manure replicates were multiplied by these ^{31}P NMR estimations.

Values within same column followed by different letters are significantly different between manures produced from dietary P modifications using Fisher's Protected $LSD_{0.05}$.

Table 3. Probability of significance within the ANOVA of study 1 (initial phosphorus applications) and study 2 (residual) for soil P concentrations, growth of corn, and P uptake.

Source	df	Soil WSP	Soil M3P	Soil TIP	Soil pH	Height 28 DAE	Leaf Area	Shoot Biomass	Total P Accumulation‡	Total APR§
Study 1 – Initiation										
Source	2	*	**	ns	**	ns	ns	ns	*	ns
Rate	3	***	***	***	***	***	***	***	***	ns
Source x Rate	6	*	ns	ns	***	ns	ns	ns	***	ns
Study 2 – Residual										
Source	2	ns	*	**	***	**	*	ns	ns	ns
Rate	3	ns	***	***	***	***	***	***	***	*
Source x Rate	6	ns	ns	ns	***	***	**	*	*	ns

*, **, and ** Significance at the probability of 0.05, 0.01, and 0.001 respectively, and no significance (ns) when a > 0.05.

† The unamended controls (UTC) were not included in the ANOVA.

‡ Total P Accumulation of studies 1 and 2 were summed and subjected to ANOVA: Source***, Rate***, and Source x Rate***.

§ APR, applied P recovery of studies 1 and 2 were summed and subjected to ANOVA: Source*, Rate*, and Source x Rate^{ns}.

Table 4. Predictors of the multiple linear regression models of height, leaf area, shoot biomass, and total phosphorus accumulation in studies 1 and 2.

	Height 28 DAE	Leaf Area	Shoot Biomass	Total P Accumulation
	Partial R^2†			
Study 1 – Initiation				
P Rate	0.78***	0.84***	0.95***	0.87***
Soil pH	ns	0.04***	ns	0.03***
Mehlich-3 P	ns	ns	0.01***	ns
Study 2 – Residual				
P Rate	ns	ns	ns	0.85***
Soil pH	0.81***	0.86***	0.81***	0.05***
Mehlich-3 P	0.02*	ns	ns	ns

† Partial R^2 represented the increase in the R^2 of the multiple linear regression model when the predictor was included.

*, **, and *** Significance of the predictors in the multiple linear regression model at the probability of 0.05, 0.01, and 0.001 respectively, and no significance (ns) when a > 0.05.

Table 5. Total phosphorus accumulation and applied P recovery of corn in study 1, study 2, and cumulatively.

P Source#	Total P Accumulation†‡ Applied P Rate (mg kg^{-1}) 7.5	15	30	60	Mean across rates	Applied P Recovery§¶ Applied P Rate (mg kg^{-1}) 7.5	15	30	60	Mean across rates
	mg P plant^{-1}					%				
Study 1										
NRC	4.6	7.1	12.0	20.7	11.1	20.2	19.3	18.7	17.4	18.9a
Low	5.4	6.5	11.7	20.5	11.0	25.9	17.0	18.1	17.2	19.5a
TSP	4.3	6.4	10.0	14.4	8.8	17.6	16.8	15.1	11.5	15.2a
Mean across P sources	4.8	6.7	11.2	18.5		21.2	17.7	17.3	15.4	
Study 2										
NRC	4.2	7.8	9.4	14.4	9.0	15.0	21.1	15.4	11.8	15.8a
Low	4.1	6.9	12.7	16.5	10.1	13.4	17.9	19.9	14.0	16.3a
TSP	4.5	6.6	10.6	14.1	8.9	17.3	16.5	16.3	11.6	15.4a
Mean across P sources	4.3	7.1	10.9	15.0		15.2	18.5	17.2	12.4	
Cumulative										
NRC	8.9	14.9	21.4	35.1	20.1	35.3	40.4	34.0	29.2	34.7a
Low	9.5	13.4	24.4	37.0	21.1	39.2	34.8	38.0	31.2	35.8a
TSP	8.8	13.0	20.6	28.4	17.7	34.9	33.3	31.4	23.1	30.7b
Mean across P sources	9.0	13.8	22.1	33.5		36.4	36.2	34.5	27.8	

† Phosphorus accumulation in the roots, stalks, and leaves where the products (dry weight x P concentration) were summed.

‡ The interaction of Source x Rate was significant for total P accumulation in study 1, study 2, and cumulatively (Table 3); therefore, means of the main effects were not separated. Treatment values were plotted in Figure 8 to report the interaction properly.

§ APR = {[(P Accumulation – P Accumulation of UTC) / (soil weight)] / [P Applied]}*(100). Total P accumulation of the untreated control (UTC) was 1.9 and 2.4 mg P plant^{-1} for study 1 and 2, respectively. Soil weight was 1.8 and 1.6 kg pot^{-1} in studies 1 and 2, respectively.

¶ For each study, means within same column followed by different letters were significantly different among P sources using Fisher's Protected $LSD_{0.05}$ (Table 3). Rate was only significant in cumulative calculations of APR (7.5 = 15 = 30 > 60).

P sources were the breeder manures, NRC and Low (Table 2; Casteel, 2009), and triple super phosphate (TSP).

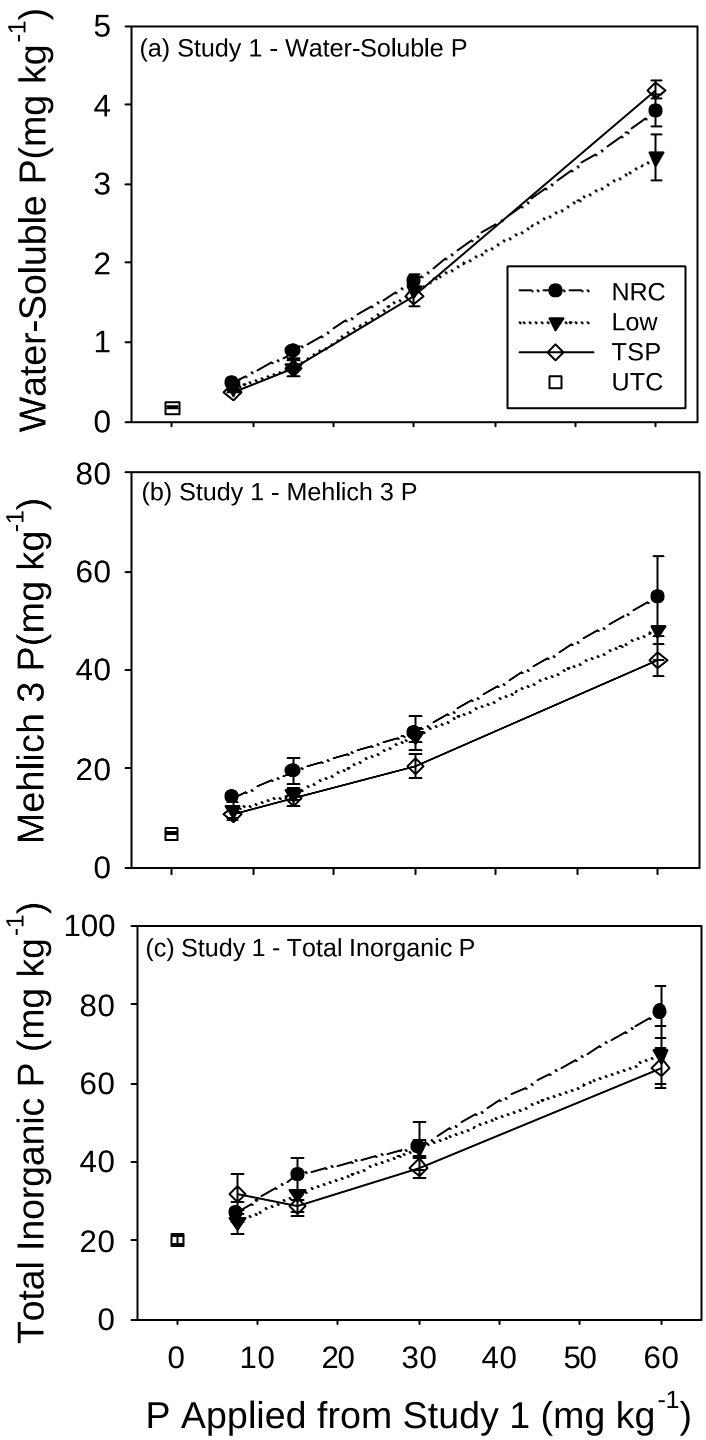

Figure 1. Effects of breeder manure sources (NRC, Low) and triple superphosphate (TSP) applied at four P rates plus an untreated control (UTC) on soil concentrations of (a) water-soluble phosphorus (P), (b) Mehlich-3 P, and (c) total inorganic P in study 1. Error bars equal standard errors.

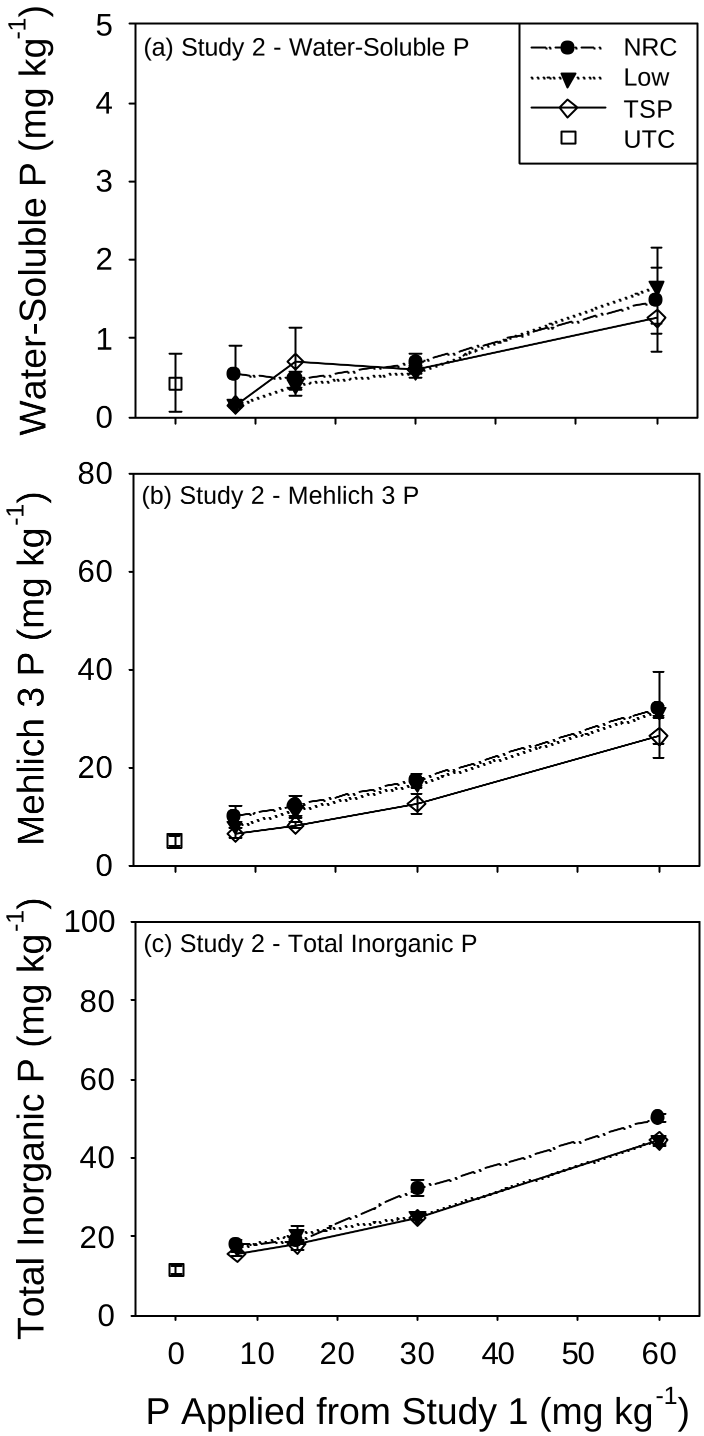

Figure 2. Residual effects of breeder manure sources (NRC, Low) and triple superphosphate (TSP) applied at four P rates plus an untreated control (UTC) on soil concentrations of (a) water-soluble phosphorus (P), (b) Mehlich-3 P, and (c) total inorganic P in study 2. Error bars equal standard errors.

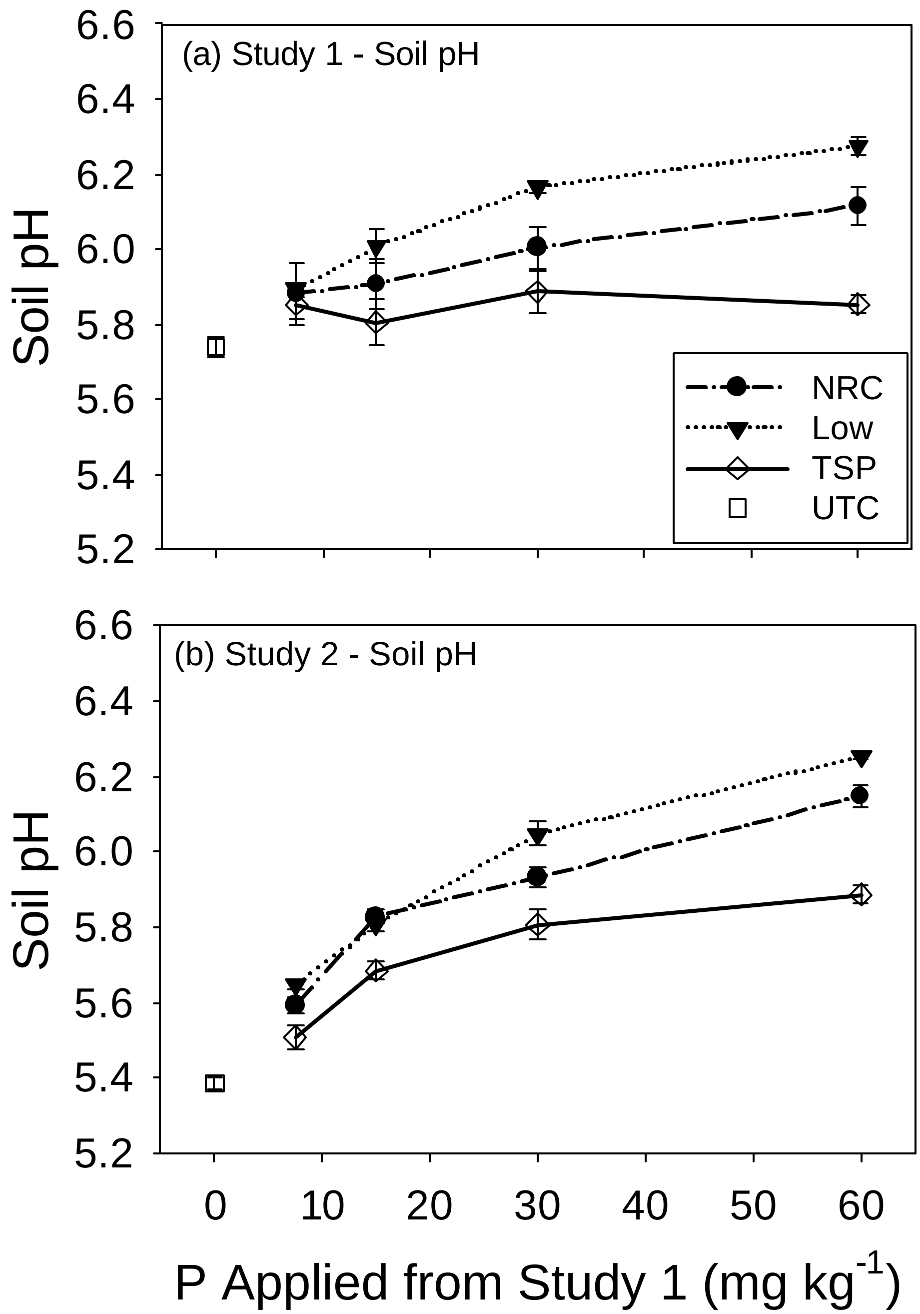

Figure 3. Soil pH effects of breeder manure sources (NRC, Low) and triple super phosphate (TSP) applied at four P rates plus an untreated control (UTC) in (a) study 1 – initiation and (b) study 2 – residual. Error bars equal standard errors.

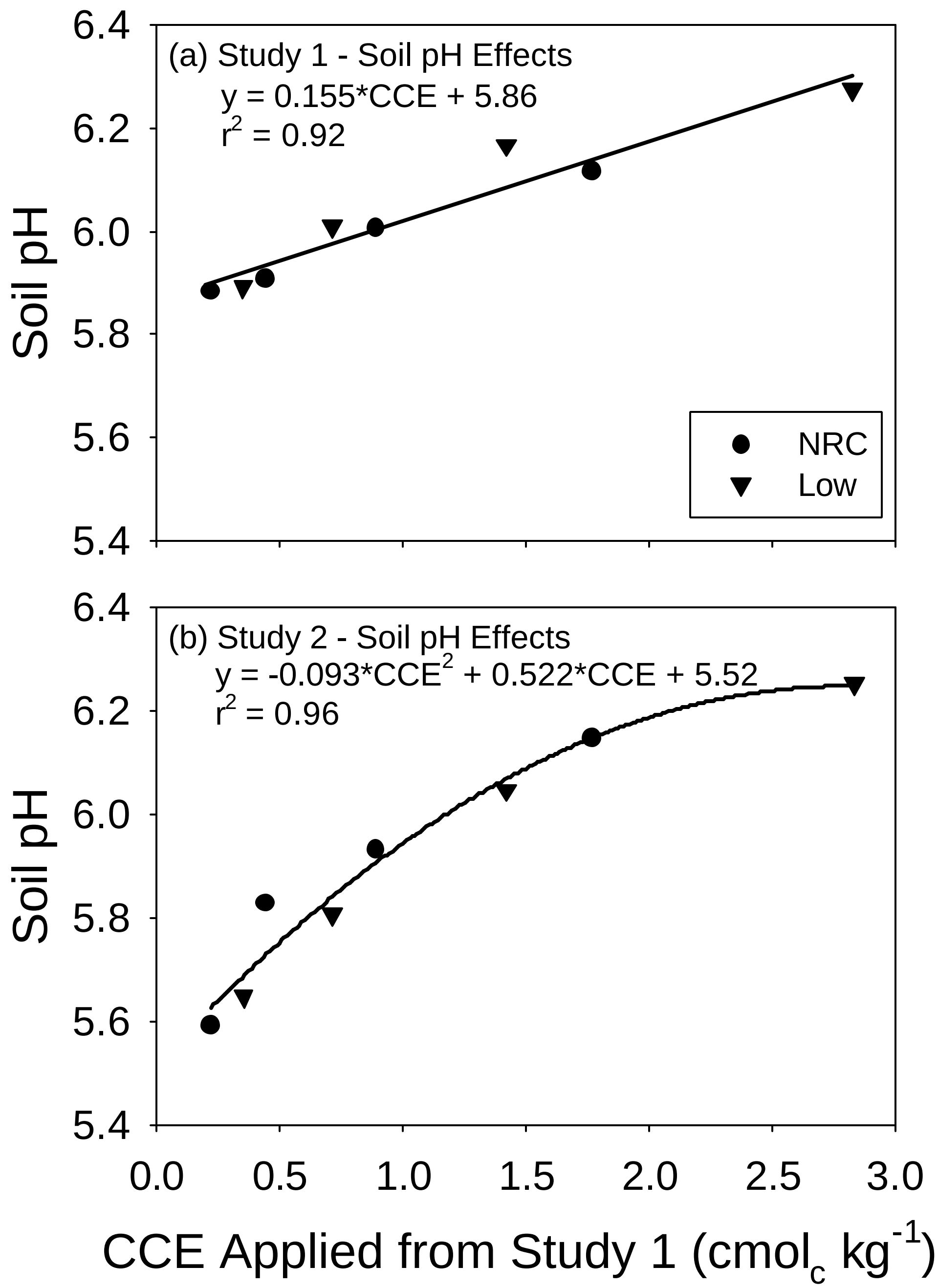

Figure 4. Soil pH effects of breeder manure sources (NRC, Low) applied at four P rates, which supplied eight levels of calcium carbonate equivalence (CCE) in (a) study 1 – initiation and (b) study 2 – residual.

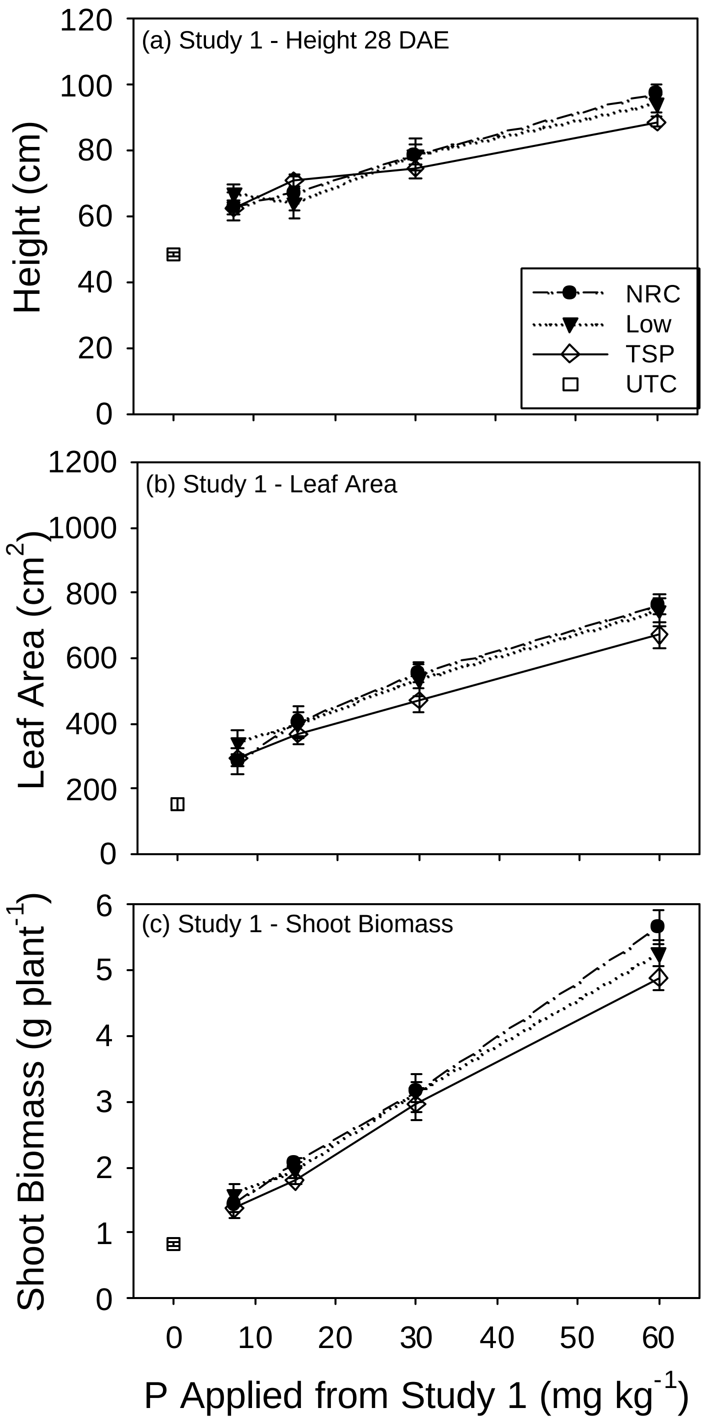

Figure 5. Effects of breeder manure sources (NRC, Low) and triple superphosphate (TSP) applied at four P rates plus an untreated control (UTC) on corn growth: (a) plant height, (b) leaf area, and (c) shoot biomass in study 1. Error bars equal standard errors.

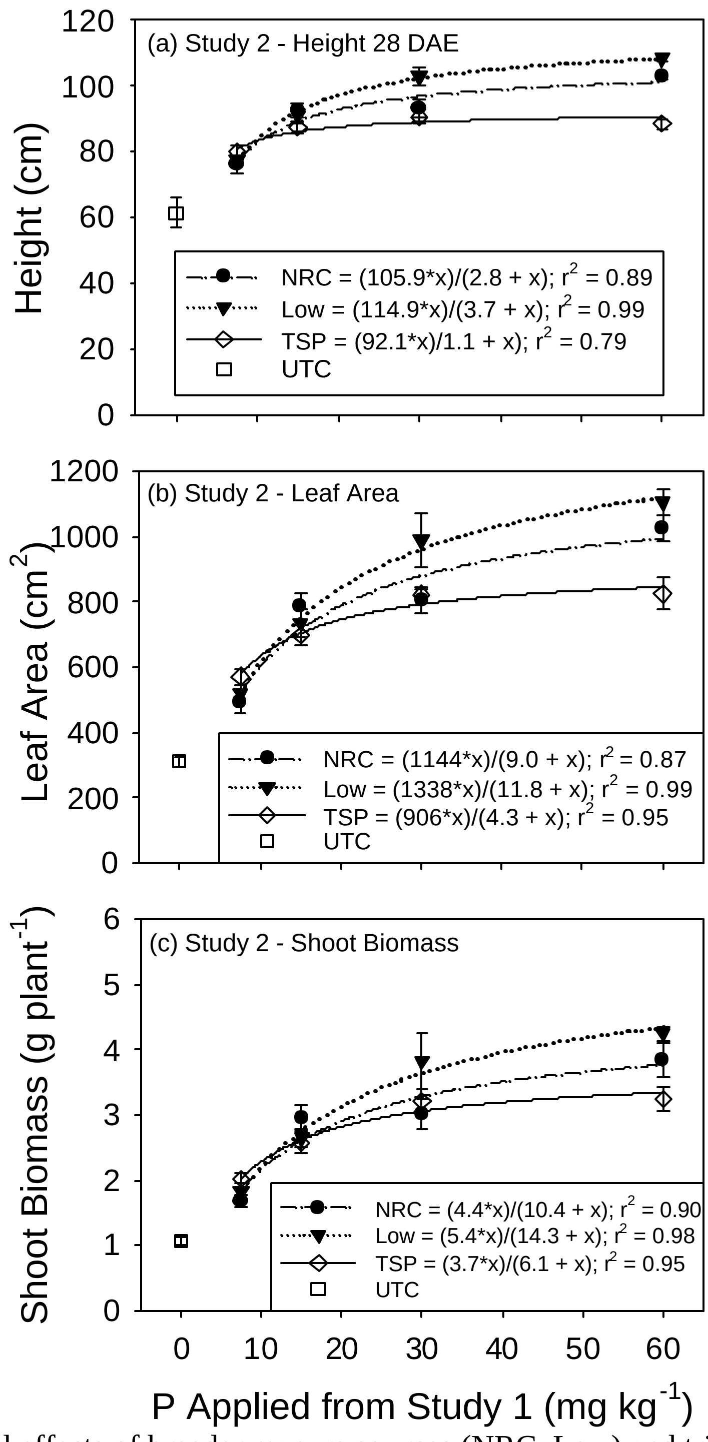

Figure 6. Residual effects of breeder manure sources (NRC, Low) and triple super phosphate (TSP) applied at four P rates plus an untreated control (UTC) on corn growth: (a) plant height, (b) leaf area, and (c) shoot biomass in study 2. Error bars equal standard errors.

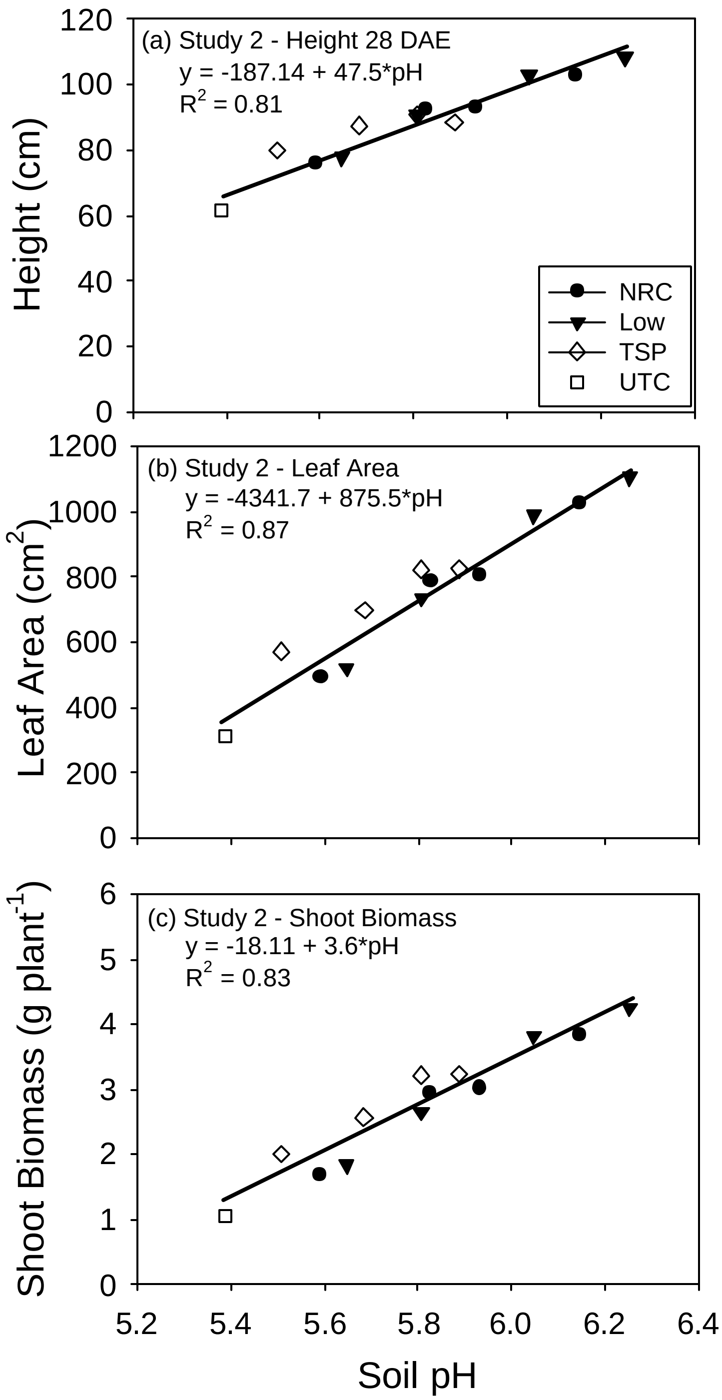

Figure 7. Effects of soil pH levels on corn growth: (a) plant height, (b) leaf area, and (c) shoot biomass in study 2 – residual. Multiple linear regression was used to determine the best predictors (Table 4). Treatments: breeder manures (NRC, Low), triple super phosphate (TSP), and untreated control (UTC).

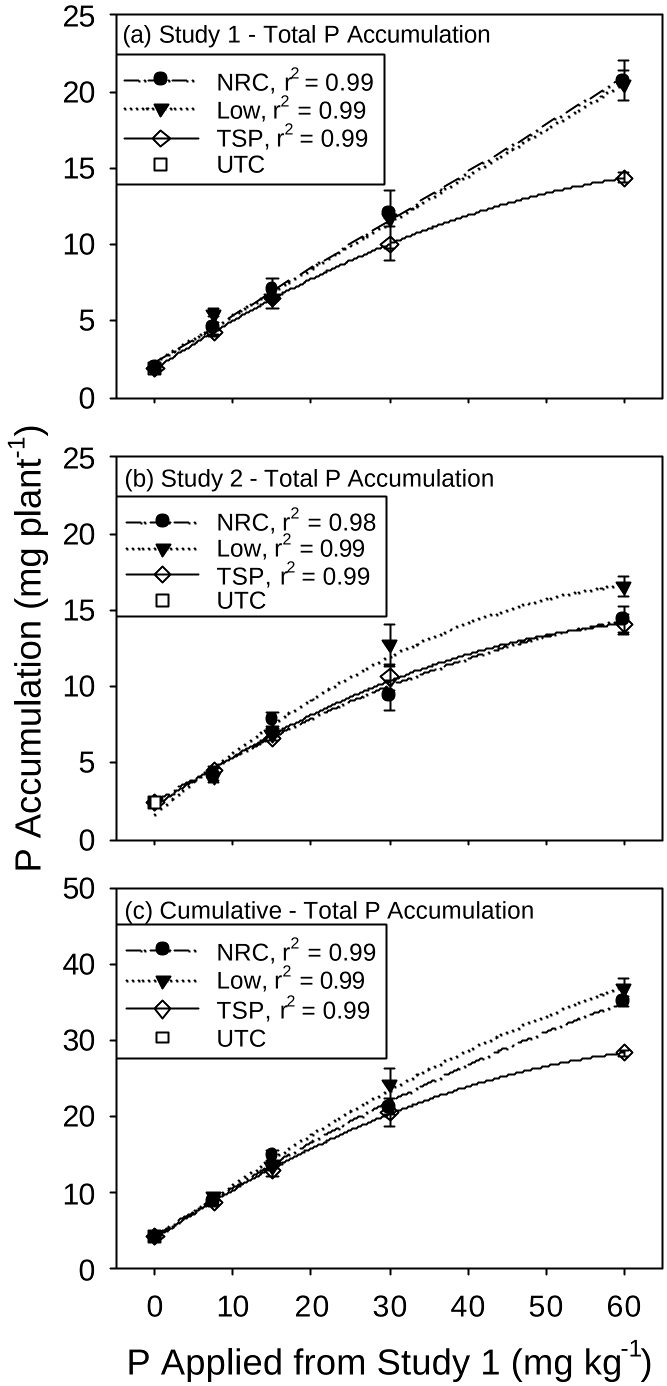

Figure 8. Effects of phosphorus (P) application rate on total P accumulation in (a) study 1, (b) study 2, and (c) cumulatively. Treatments: breeder manures (NRC, Low), triple superphosphate (TSP), and untreated control (UTC). Individual pots had 1.8 and 1.6 kg of soil in studies 1 and 2, respectively. Error bars equal standard errors.

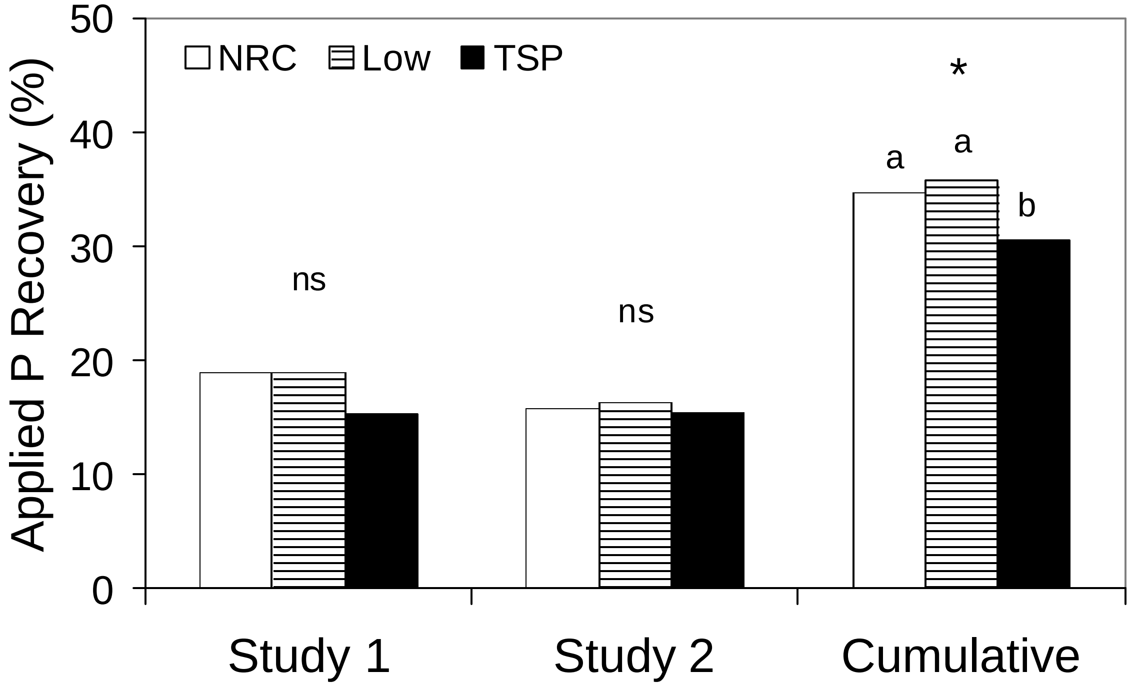

Figure 9. Applied phosphorus recovery (APR) of total plant uptake in study 1 – initiation, study 2 – residual, and cumulative. NRC manure, Low manure, and triple superphosphate (TSP) were averaged over P rates. Means were separated with Fisher's Protected $LSD_{0.05}$ within each category.

APPENDIX

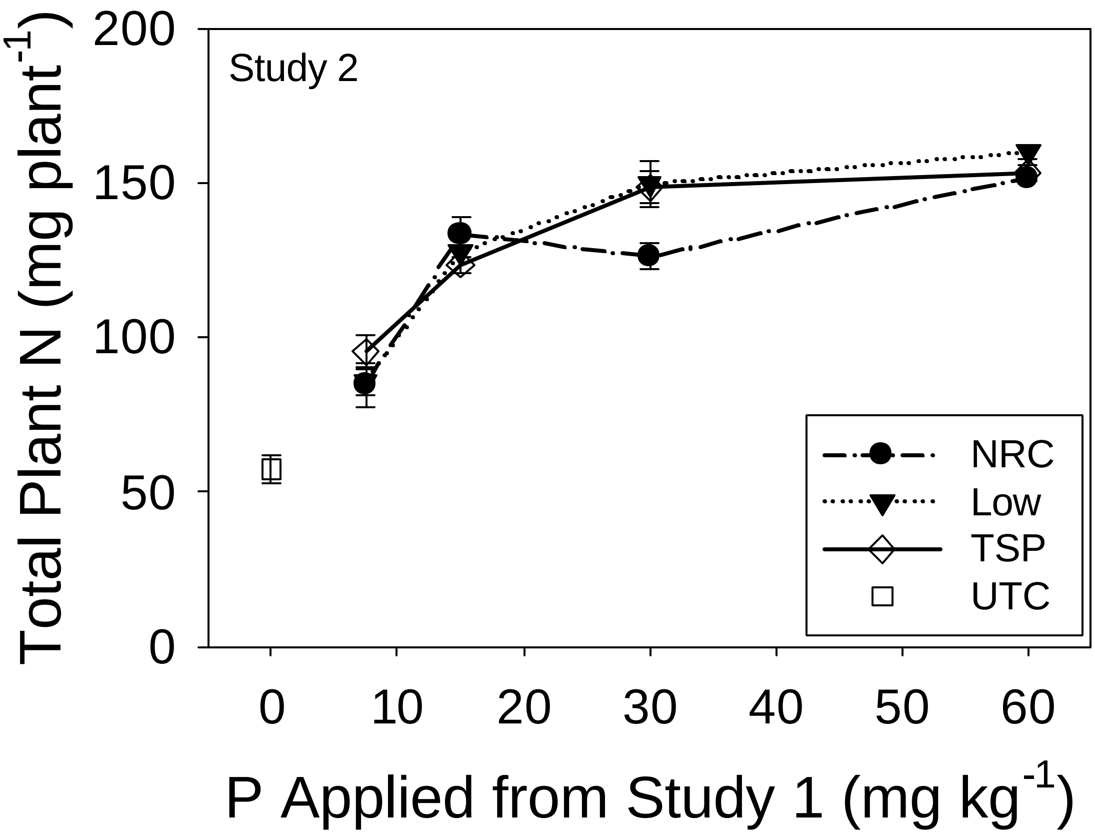

Figure A1. Effects of phosphorus (P) application rate by P sources on total N accumulation of corn grown in greenhouse study 2. Treatments: breeder manures (NRC, Low), triple superphosphate (TSP), and untreated control (UTC). Error bars equal standard errors.

CHAPTER 5

Dietary Phosphorus Modification in Broiler Breeders:

Manure Phosphorus Availability to Corn Across Soil Phosphorus Levels

ABSTRACT

Most nutrient management plans and P loss assessment programs have assumed that P availability of poultry manures were equal without considering manure P compositional differences. Pelletized manure produced from standard P (NRC) and reduced P (Low) diets of broiler breeders were applied to plots with a range of soil P levels to determine the soil P fluctuations and the P availability from these breeder manures relative to each other and triple super phosphate (TSP) over two growing seasons of corn (*Zea mays*). Treatments were applied at 39 kg P ha^{-1} in 2007 at Salisbury, Lewiston, and Plymouth in North Carolina. Applications of the NRC and the Low breeder manures equally increased the concentrations of Mehlich-3 P (M3P), total inorganic P (TIP), and soil water-soluble P (WSP_S) concentrations at Lewiston and Plymouth. Plant height and biomass production were equal among breeder manure-amended soils at all three locations. Grain P removal was equal between the breeder manures five out of six site-years. Soils amended with the NRC and the Low breeder manures increased M3P concentrations more than the TSP treatments at Plymouth (26.3 = 25.8 > 21.0 mg M3P kg^{-1}), but this difference was transient. Young corn plants produced more biomass and accumulated more P in soils amended with the Low breeder manure than with TSP in 2007 and 2008. Biomass production at tassel stage was equal among P sources in 2007 and 2008 where P accumulation in 2007 followed the order

NRC = Low > TSP. Grain production, grain P removal, and applied P recovery were equal among P sources in 2007, but these measures were greater for breeder manure treatments (NRC, Low) than for TSP treatments in 2008. The cumulative applied P recovery in the grain (sum of two seasons) followed the order: NRC (47.6%, a), Low (40.4%, ab) and TSP (36.0%, b). The dietary P modification fed to broiler breeders successfully reduced manure TP without altering soil P extractability and availability of P to corn. The P in breeder manures should be considered 100% available in nutrient management plans and P loss assessment programs.

Keywords: phytase, broiler breeder, phosphorus, phytate, orthophosphate, soil, phosphorus availability

Abbreviations: APR, applied phosphorus recovery; DAA, days after application; M3, Mehlich-3; M3P, Mehlich-3 phosphorus; NPP, non-phytate phosphorus; TP, total phosphorus; TIP, total inorganic phosphorus; TSP, triple super phosphate; WSP_M, manure water-soluble phosphorus; WSP_S, soil water-soluble phosphorus.

INTRODUCTION

Manure management has become a major issue in recent decades due to intense animal production in localized regions (Kellogg et al., 2000). Nitrogen-based applications of animal manures have supplied more phosphorus (P) than the receiver have crops removed; thereby, soils have been loaded with P (Mikkelsen, 2000) with increased the potential for soluble P losses when soils exceeded P saturation thresholds (Van der Molen et al., 1998; Hooda et al., 2000; Pautler and Sims, 2000). Phosphorus-based applications of manures reduced the amount of P added to the land; however, more land was required. Animals in many counties have produced more manure P than can be removed by crops grown in that county, and thus, longer transport distances have been required (Maguire et al., 2007). Another strategy to manage P in manures was to modify the dietary P to reduce the amount of P excreted (CAST, 2002). Maguire et al. (2005) summarized the effects of dietary P modifications where P in broiler litter was reduced 17% by feeding closer to the P requirement, 13 to 35% by adding phytase, and 11 to 18% by feeding low phytate corn (i.e., highly available P). Some broiler litters produced as a result of dietary P modifications increased manure water-soluble P (WSP_M) and increased the ratio of WSP_M to manure P (Maguire et al., 2004; Miles et al., 2003; Vadas et al., 2004). Increased concentrations of WSP_M were positively correlated to soil surface runoff of P (r values from 0.79 to 0.93) when 15 wastes were surface-applied (Kleinman et al., 2007). Thus, P management of poultry manures should account for crop uptake of P and the manure P forms and concentrations.

State programs were developed to assess the risk of P loss from agricultural fields to watersheds in response to the contribution of P to the eutrophication of surface waters

(Sharpley et al., 2003). Most programs ranked the risk with index values that consider numerous factors such as nutrient source (e.g., fertilizer vs. manure), soil P level, and application method (e.g., surface vs. incorporated). Most programs based application rates on total P or P_2O_5, but Arkansas (DeLuane et al., 2001) and Utah (Goodrich et al., 2000) chose application rates based on soluble P. Another factor was the availability of the P in the manure. All poultry manures were considered equally available in P, but the coefficient used in North Carolina was 0.80 (Crouse and Shaffer, 2006) while Pennsylvania was 1.0 (Weld et al., 2003). Orthophosphate proportions of the total P in broiler litters were 30 to 40% (Leytem et al., 2007; Maguire et al., 2004), in turkey litters were 50 to 70% (Maguire et al., 2004), and in broiler breeder manure pellets were 92 to 95% (Casteel, 2009a). Phosphorus availability would presumably be the lowest in the broiler litter and highest in broiler breeder manure pellets based on orthophosphate. Yet, all poultry manures were treated equally in P risk assessments and nutrient management plans, which raised environmental and agronomic concerns. For example, a P availability coefficient of 1.0 for broiler litters (low in orthophosphate) could be considered environmentally conservative, but it could limit crop production due to an overestimation of plant P availability. In contrast, a P availability coefficient of 0.80 for broiler breeder manure (high in orthophosphate) could increase P loading to the land and potential P losses to watersheds.

A few studies have estimated plant P availability of poultry manures via Mehlich-3 P (M3P) extractions, water-soluble P (WSP_S) extractions, or plant response in the greenhouse. A Spodosol amended with poultry manure (free of wood chips) increased M3P concentrations 16% less than soils amended with potassium phosphate (KH_2PO_4) (i.e., 84%

available P in poultry manure) (Griffin et al., 2003). In contrast, P applications of poultry layer manure (fresh, composted, pelleted) and triple super phosphate (TSP, Ca $[H_2PO_4]_2$ H_2O) increased M3P at the same rate within two Ultisols and one Histosol of North Carolina (Montalvo, 2008). In the greenhouse, P accumulation of annual ryegrass (*Lolium multiflorum*) and sorghum-sudan grass (*Sorghum X drummondii*) grown in soil amended with pelletized broiler litter was approximately 70% as much as soil amended with calcium phosphate ($CaHPO_4$) (Hammac et al., 2007). In the greenhouse, growth and P uptake in tall fescue (*Festuca arundinacea*) grown in soil amended with composted poultry litter was generally equal to soil amended with TSP (Sikora and Enkiri, 2005). However, P-based applications of poultry manures have not been evaluated for crop response in the field.

Phosphorus availability of poultry manures range from 30 to 95% based on the orthophosphate proportion of the manure P, i.e. 84 to 100% based on M3P concentrations in amended soils, and 70 to 100% based on P accumulation of plants grown in the greenhouse. A major contributor to the variability of these P availability values was the type of poultry that produced the manure (broiler, layer, breeder, turkey) and production practices employed (litter substrate, wood-free pure form, storage time). Phosphorus management of poultry manures should recognize the differences in P availability for reasons of optimum environmental and agronomic stewardship. Poultry manures produced as a result of dietary P modifications have been characterized and evaluated as a soil amendment in terms of environmental impacts, but no research has determined the crop response to soils amended with these manures. Additionally, nutrient availabilities of broiler breeder manure have not been published, to our knowledge. Two breeder manures produced from a traditional (NRC)

P diet and modified (Low) P diet were applied at a single P rate in the field across soil P levels to: (1) evaluate the temporal soil P changes over two growing seasons of corn (*Zea mays*), (2) determine P availability differences between the two breeder manures, and (3) determine P availability of the two breeder manures compared to TSP (i.e., P availability coefficient or TSP equivalency).

MATERIALS AND METHODS

Field Site Characterization

Three long-term (> 20 yr) P fertility sites (Salisbury, Lewiston, Plymouth) in North Carolina were selected for this study. Each site represented a specific physiographic region with a soil P gradient (Table 1). Salisbury was located in the Piedmont where the soil series was Hiwassee clay loam (fine, kaolinitic, thermic Typic Rhodudult). Lewiston was located in the Middle Coastal Plain where the soil series was Goldsboro loamy sand (fine-loamy, siliceous, subactive, thermic Aquic Paleudult). Plymouth was located in the Lower Coastal Plain where the soil series was Portsmouth fine sandy loam (fine-loamy, mixed, thermic Typic Umbraquult). Soil management was no-till at Salisbury and conventional till at Lewiston and Plymouth. Previous crops, established variables, and soil chemical properties are presented in Table 1.

Treatment Design

Mehlich-3 P concentrations varied at each location where three levels were evaluated at Salisbury (3.4, 3.5, 7.9 mg kg^{-1}) and at Lewiston (15.1, 26.5, 76.6 mg kg^{-1}) and will be referred to as soil A, B, and C within each location, respectively (Table 2). Four levels of M3P concentrations were evaluated at Plymouth (20.3, 24.8, 31.4, 46.1 mg kg^{-1}) and will be referred to as soil A, B, C, and D, respectively (Table 2). These pre-application concentrations were established through annual or biannual applications of P with incremental differences in the rate applied. Soil P levels were established in a randomized complete block design with four replications at Salisbury and Lewiston, and three replications at Plymouth. The subdivision of the main plots (soil P levels) at Salisbury (5.8 x 12.2 m) and Lewiston (7.3 x 13.7 m) were physically limited, so two breeder manures (NRC, Low) were randomly assigned as subplot factors to create the split-plot design. Breeder manures produced from diets NRC [0.37% non-phytate P (NPP)] and Low (0.19% NPP) were pelletized and characterized by Casteel (2009a) (Table 3). A P-deficient check was also monitored at Salisbury (2.3 mg M3P kg^{-1}) and Lewiston (5.9 mg M3P kg^{-1}), which did not have any P applied. Seven treatments were established at Salisbury and Lewiston: 3 soil P levels x 2 P sources + P-deficient check. The main plots at Plymouth (6.4 x 51.5 m) were divided into subplots where the NRC manure, the Low manure, TSP, and an untreated control (UTC) were randomly assigned, which totaled 16 treatments [(4 soil P levels) x (3 P sources) + (1 UTC for each soil P level)]. All P sources were applied at 39 kg P ha^{-1} that was a standard single-season application of P in a P-limited soil. Treatments were applied approximately 3 wk prior to planting corn in 2007 with no additional P over the following two growing seasons. Phosphorus sources were incorporated into the soil within 24 h of application at Lewiston and Plymouth; whereas, the breeder manures were surface-applied at Salisbury (no-till).

Field Site Management

Salisbury was planted April 10th in 2007 and 2008, Lewiston was planted April 10 2007 and April 17 2008, and Plymouth was planted April 23 2007 and April 30 2008. Plant populations were determined by counting plants within a 3-m section of rows 3 and 4. The final plant populations of the corn hybrid Pioneer 31G96 in 2007 and 2008 was 78 000 and 69 600 ha^{-1} at Salisbury, respectively; 52 600 and 64 000 at Lewiston, respectively; and 74 700 and 62 700 at Plymouth, respectively. A high rate of total nitrogen (224 kg N ha^{-1} per season) was applied to override any N effect from the broiler breeder manures at all locations. Pre-plant N rates were approximately 28 to 44 kg ha^{-1}over locations and years using ammonium nitrate, liquid N, or ammonium sulfate (only Plymouth in 2008), and the rest of the N was applied at side-dress with liquid N. Lime (1.1 Mg ha^{-1}) was applied to increase soil pH at Lewiston and Plymouth approximately 3 wk prior to planting in 2008. Practices recommended by the NC Cooperative Extension Service were followed in terms of weed, insect, and disease management.

Soil Characterization

Soil samples were air-dried, ground to pass through a 2-mm sieve, and analyzed (Table 1). Soil samples were analyzed for soil pH (1:1), humic matter, CEC, acidity, and Mehlich-3 (M3) extractable elements by the standard procedures of the NC Department of Agriculture & Consumer Services (Mehlich, 1984), which included ICP-AES determination of M3 elements. Mehlich-3 P saturation ratio (M3-PSR) was calculated as: [M3P / (M3Al + M3Fe)] with values for P, Al, and Fe in mmol kg^{-1} (Maguire and Sims, 2002b; Sims et al., 2002). Concentrations of M3P at Salisbury were below the detection limits of the ICP-AES and thus, M3P concentrations at Salisbury were determined colorimetrically by the molybdate blue method (Murphy and Riley, 1962). Additional soil P extracts were determined colorimetrically and included:

(i) Total P (TP): 1 g of soil was ashed at 550° C overnight, diluted 1:25 with 0.5 *M* H_2SO_4, horizontally shaken at 300 rpm for 16 h, centrifuged for 10 min at 14 500 x g, and filtered through Whatman #1 filter paper (Kuo, 1996).

(ii) Total inorganic P (TIP): Soil was diluted 1:25 with 0.5 *M* H_2SO_4, horizontally shaken at 300 rpm for 16 h, centrifuged for 10 min at 14 500 x g, and filtered through Whatman #1 filter paper (Kuo, 1996).

(iii) M3P: Soil was diluted 1:10 with 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, horizontally shaken for 5 min, and filtered through Whatman #2 filter paper (Mehlich, 1984).

(iv) WSP_S: Soil was diluted 1:10 with deionized water, horizontally shaken at 300 rpm for 1 h, centrifuged for 10 min at 14 500 x g, and filtered through a 0.45-µm Millipore membrane.

Plant and Soil Measurements

Soil samples were taken prior to the P applications in 2007, which represented the pre-application levels. In-season samples of soil and plant biomass were taken at three corn growth stages (~V-6, tassel, harvest) at each location and year. An additional soil sample was taken shortly after corn was planted in 2008 to evaluate changes in soil P status since the samples were taken at harvest in 2007. Soil samples were taken approximately 20 to 30 cm from the rows of corn to a depth of 10 cm at Salisbury (no-till), and a depth of 20 cm at Lewiston and Plymouth (conventional tillage). All soil samples were analyzed as described previously.

Plots were 7 to 8 rows wide depending on the location, where six representative plants were selected from rows 2 through 6 for height measurements at V-6 and at tassel. Biomass production was determined by collecting six representative plants from rows 2 and 5 at V-6, and four representative plants from rows 2 and 5 at tassel and harvest. Ears of corn were separated from the plants selected at harvest. Plants were dried at 60° C (48 to 72 h) and weighed to calculate biomass production, which was plant dry weight x plant population of the individual plot. Grain was harvested and weighed from rows 3 and 4 to measure fresh grain weight. Grain subsamples were taken to determine moisture and calculate dry weight yield. Grain subsamples were also dried at 80°C for 48 h and ground (< 1 mm) for tissue analysis (C, N, P, Ca). All biomass production was reported on a dry weight basis including grain. Harvest index was calculated as: (Biomass of grain) / (Biomass of total harvest) where total harvest was grain + stover. All plant biomass samples were subsampled and ground to pass through a 2-mm sieve and then analyzed for C and N by oxygen combustion using a Perkin Elmer 2400 CHN elemental analyzer (Perkin Elmer Corp., Norwalk, CT). Tissue concentrations of P and Ca were determined for all plant parts in all studies by dry ashing, digesting with 6N HCl, and analyzing on the ICP-AES. All tissue concentrations were multiplied by the measured biomass to calculate P accumulation at each growth stage. The applied P recovery (APR) was determined at Plymouth:

{[(P acc. in TRT) – (P acc. in UTC)] / (39 kg P ha^{-1})] x 100}, where P acc. was P accumulation, TRT was P source x soil P level, and UTC was the untreated control for each soil P level. These UTC's were essential for calculations of APR within each soil P level at Plymouth.

Statistical Analyses

The experimental design was a split plot where the main plots were initial soil P level and subplots were P sources. Treatment effects were determined via analysis of variance using PROC GLM in SAS version 9.1 (SAS Institute, 2002) at a = 0.05, 0.01, and 0.001. Fisher's protected LSD was used to separate treatment means at a = 0.05. Treatment means, standard deviations, and standard errors were generated with JMP version 7 (SAS Institute, 2007), and regression and bar graphs were created with Sigma Plot 2001 (SPSS, Inc., 2001) and Microsoft Excel 2002 (Microsoft, 2002). Main effects will be reported whenever possible where the main effect of: (1) P source was averaged over soil P levels and (2) soil P level was averaged over P sources.

RESULTS AND DISCUSSION

Soils Amended with Breeder Manure Pellets and Triple Superphosphate:

Effects on Mehlich-3 Phosphorus

Pre-application concentrations of M3P varied at each location where three levels were evaluated at Salisbury and at Lewiston (soil A, B, and C in Table 2), and four levels at

Plymouth (soil A, B, C, and D in Table 2). The application of 39 kg P ha^{-1} was estimated to equal a soil P application rate of 32.5 mg P kg^{-1} at Salisbury (10-cm depth) and 16.25 mg P kg^{-1} at Lewiston and Plymouth (20-cm depth) assuming a bulk density of 1.2 g cm^{3} for all three locations. Concentrations of M3P were highest approximately 60 d after application (DAA), which was the first sample after application when the corn was at the V-6 growth stage (Fig. 1). The increase in M3P concentrations due to the application of the NRC breeder manure (3.4 mg kg^{-1}) was greater than with the application of the Low breeder manure (1.7 mg kg^{-1}) at Salisbury ($LSD_{0.05}$, Table 2), which was very low in reference to the soil P application estimate of 32.5 mg P kg^{-1}. This was presumably due to P sorption by Al and Fe hydroxides (Bolan et al., 1985). The NRC breeder manure and the Low breeder manure increased M3P concentrations equally at Lewiston (17.5 to 19.7 mg kg^{-1}, Table 2), which were similar to the soil P application estimate of 16.25 mg P kg^{-1}. At Plymouth, the increase in the M3P concentrations due to the application of the NRC breeder manure (26.3 mg kg^{-1}) and the Low breeder manure (25.8 mg kg^{-1}) was greater than the application of TSP (21.0 mg kg^{-1}) ($LSD_{0.05}$, Table 2). These M3P concentrations were slightly higher than the soil P application estimate 16.25 mg P kg^{-1}. Fluctuations of the endogenous soil were not accounted for, which would alter the increase in M3P concentration due to the P application. However, soils amended with turkey litter have been found to increase 0.97 to 1.82 mg M3P kg^{-1} for every 1 mg of P applied kg^{-1} (Maguire et al., 2005), and soils amended with poultry layer manure increased 1.2 µg M3P cm^{-3} for every 1 µg P applied cm^{-3} (Montalvo, 2008).

Mehlich-3 P concentrations among P sources were generally equal during the remainder of 2007 and all of 2008 at Lewiston and Plymouth (Figs. 1b, 1c). The difference between M3P concentrations at harvest in 2007 and pre-application concentrations were greater in the NRC manure-amended soil (4.1 mg kg^{-1}) than the Low manure-amended soil (1.9 mg kg^{-1}) at Salisbury ($LSD_{0.05}$, Table 2). Regardless of P source, M3P concentrations at harvest in 2007 were greater than the pre-application concentrations by 6.7 to 8.9 mg kg^{-1} at Lewiston and 7.9 to 9.2 mg kg^{-1} at Plymouth (Table 2, Fig. 1). These differences indicated that the applied P was not completely taken up by the corn in 2007 (i.e., P input > P removal). Phosphorus accumulation by corn will be presented later.

Mehlich-3 P concentrations throughout the 2008 season were generally higher than pre-application concentrations at Salisbury and Plymouth (Figs. 1a, 1c); whereas, M3P concentrations were at or below the pre-application concentrations at Lewiston (Fig. 1b). In 2008 at harvest, breeder manure-amended soils had M3P concentrations that were generally equal to the pre-application concentrations at Salisbury and Plymouth (Table 2), which suggested that the single P application met the P needs for corn grown over two consecutive seasons. Breeder manure-amended soil averaged 7.5 to 9.7 mg kg^{-1} less M3P than the pre-application concentrations at Lewiston (Table 2). The P uptake by corn at Lewiston in 2007 and in 2008 was presumably greater than the amount of P applied and was presumably greater than P uptake at Salisbury and Plymouth (Table 2). Actual P accumulations and calculated APR will be discussed later.

Effects on Total Inorganic Phosphorus and Water-Soluble Phosphorus

Total inorganic P concentrations were higher in the soil at Salisbury (93.5, 102.2, 146.1 mg kg^{-1}) than at Lewiston (34.4, 55.5, 120.1 mg kg^{-1}) and at Plymouth (21.4, 26.3, 33.9, 58.9 mg kg^{-1}) (Table 1). Pre-application concentrations of TIP at Salisbury provided an indication of the P-supplying capacity of the soil even though the M3P concentrations were very low. The increase in TIP concentrations due to the NRC and the Low breeder manure applications was equal at Salisbury (25.7 to 26.4 mg kg^{-1}), at Lewiston (20.1 to 24.2 mg kg^{-1}), and at Plymouth (23.2 to 27.9 mg kg^{-1}) while TSP did not differ (data not shown). The surface application of the breeder manures at Salisbury delayed the maximum change in TIP concentrations compared to the soil-incorporated applications at Lewiston and Plymouth (i.e., maximum TIP concentration was 103 DAA at Salisbury vs. 61 DAA at Lewiston and 68 DAA at Plymouth) (Fig. 2). The increase in TIP concentrations at Salisbury was greater than the M3P concentrations, but approximately 25% less than the soil P application estimate (32.5 mg P kg^{-1}). A similar Ultisol from the Piedmont previously increased 0.45 µg M3P cm^{-3} for every 1 µg TP cm^{-3} applied from poultry layer manure (Montalvo, 2008).

Phosphorus loading of soil has raised environmental concerns with manure applications where WSP_S has been used to provide an indication of the potential for soluble P losses by runoff and leaching (Pautler and Sims, 2000). Soil WSP concentrations were very low at Salisbury where the initial samples were less than 0.20 mg kg^{-1} and the highest concentration over the two years was 0.36 mg kg^{-1} (Fig. 3a). The pre-application concentrations of WSP_S were very low at Plymouth (0.13 to 0.41 mg kg^{-1}) and moderate at Lewiston (0.36 to 2.66 mg kg^{-1}) (Table 1). The increase in WSP_S due to P application did not differ among P sources within soil P levels at Salisbury (0.02 to 0.20 mg kg^{-1}), at Lewiston (0.24 to 0.62 mg kg^{-1}), or at Plymouth (0.13 to 0.83 mg kg^{-1}). Therefore, the potential for soluble P losses were minimal at all locations based on the agronomic application of P across several soil P levels.

Soil WSP concentrations among sample periods were the greatest approximately 60 DAA (~V-6 growth stage of corn) and decreased throughout both seasons (Fig. 3). In general, WSP_S concentrations equaled the pre-application concentrations by the start of the 2008 season. In fact, WSP_S concentrations at Lewiston were much lower than the pre-application concentrations during 2008 (Fig. 3b), which followed the same trend observed in the concentrations of M3P (Fig. 1b) and TIP (Fig. 2b). The applications of the breeder manures and TSP equally influenced WSP_S in 2007 and 2008 at Plymouth, and thus, the potential for soluble P losses were equivalent. Similarly, WSP_S concentrations in a sandy loam and a silt loam soil were equal among dietary P-modified turkey litters and TSP (Maguire et al., 2005).

Effects on Soil pH

Initial soil pH values were 6.2 at Salisbury, 5.9 to 6.1 at Lewiston, and 5.5 to 5.6 at Plymouth (Table 1). In-season fluctuations in soil pH were most likely due to the N fertilization where the nitrification process acidified the soil shortly after the application. The soil pH values in soils amended with the NRC and the Low breeder manures did not differ from each other or the P-deficient check over 2007 and 2008 at Salisbury (Fig. 4a), since this soil was highly buffered. Soil pH values at Lewiston were in the order of Low > NRC > P-deficient check when averaged across soil P levels at harvest in 2007 (5.9 > 5.8 > 5.7) and at harvest in 2008 (6.3 > 6.2 > 5.8) ($LSD_{0.05}$). In fact, the P-deficient check at

Lewiston was generally lower than the breeder manure-amended soils from 101 DAA (tassel) until the end of the 2008 season (Fig. 4). Similarly, soil pH values at harvest in 2007 and 2008 at Plymouth followed the order Low = NRC > TSP > UTC ($LSD_{0.05}$).

Anecdotally, poultry manures and litters have been regarded as having a liming value through routine soil analyses. In this study, breeder manure applications appeared to assist in the management of soil pH, which would provide additional benefits in plant growth (e.g., macronutrient availability, reduction in Al toxicity) and reduce lime applications. Apparent liming effects were also reported for the applications of layer manure in piedmont and coastal plain soils and broiler litter in a piedmont soil (Maguire et al., 2006). Soil pH increased and extractable Al decreased as poultry litter application rates increased in a wheat study (Tang et al., 2007). The calcium carbonate equivalency of the Low breeder manure (37%) was higher than the NRC breeder manure (32%) (Table 3). Additionally, the P-based application supplied more manure and thus, more "calcium carbonate" from the Low breeder manure (919 kg CCE ha^{-1}) than the NRC breeder manure (575 kg CCE ha^{-1}), which would presumably maintain or increase soil pH. The soil pH values of two coastal plain soils also increased with breeder manure amendments where the Low breeder manure had the greatest apparent liming effect (Casteel, 2009a).

Effects on Corn Growth

Plant heights and biomass production of corn grown at Salisbury and Lewiston did not differ between the NRC and the Low breeder manure amendments (Table 4). Biomass production from early to mid-season was good in 2007 at Salisbury; however, limited water supply during reproductive growth and maturation hampered total biomass production. In 2007 and 2008, grain yields ranged from 5.6 to 6.1 and 8.6 to 9.6 Mg ha^{-1} at Salisbury, respectively, and the P-deficient check was 3.3 and 7.2 Mg ha^{-1}, respectively (Table 5). Grain yields at Lewiston were lower in 2007 (6.5 to 8.4 Mg ha^{-1}) than in 2008 (8.7 to 10.6 Mg ha^{-1}) across the range of soil P levels and P sources. The grain yield for the P-deficient check at Lewiston was 2.7 and 2.4 Mg ha^{-1} in 2007 and 2008, respectively (Table 5). Regardless of the breeder manures and soil P levels, the harvest indices were very similar at Salisbury in 2007 (0.44 to 0.48) and in 2008 (0.52 to 0.57); and at Lewiston in 2007 (0.51 to 0.55) and in 2008 (0.57 to 0.63) (data not shown).

At Plymouth in both years, breeder manure-amended soil produced taller plants than soils amended with TSP and the UTC, especially at the lower pre-application concentrations of P (Table 4, Fig. 7). In 2008 at Plymouth, young corn plants (V-7) were taller in breeder manure amended-soils (58 cm) than in soils amended with TSP (55 cm) and the UTC (54 cm) ($LSD_{0.05}$). Young corn plants (V-6) grown in the greenhouse were also taller in breeder manure-amended soils than TSP-amended soils in a residual P study (Casteel, 2009b). At tassel, corn plants in the breeder manure-amended soils tended to be taller than in TSP-amended soils in 2007, but similar in 2008 (Fig. 7).

Early-season biomass production was influenced by P source in 2007 and in 2008 at Plymouth (Table 4). In 2007, the Low manure-amended soil (0.93 Mg ha^{-1}) produced more biomass than soils amended with TSP (0.77 Mg ha^{-1}) and the UTC (0.41 Mg ha^{-1}) at V-7 growth stage ($LSD_{0.05}$). The NRC manure-amended soil and the TSP-amended soil both produced more biomass than the UTC in 2007 (data not shown). In 2008, young corn plants produced biomass in the order of NRC (0.30 Mg ha^{-1}) = Low (0.30 Mg ha^{-1}) > TSP (0.25 Mg ha^{-1}) > UTC (0.20 Mg ha^{-1}) ($LSD_{0.05}$). In contrast, shoot biomass production of young corn plants grown in the greenhouse were no different among soils amended with the NRC breeder manure, the Low breeder manure, and TSP (Casteel, 2009b). In P-based applications in the greenhouse, shoot biomass accumulation of tall fescue was equal between poultry litter compost and TSP (Sikora and Enkiri, 2005) while shoot biomass accumulation of annual ryegrass and sorghum-sudangrass was equal between pelletized broiler litter and calcium phosphate (Hammac et al., 2007).

In 2007 at Plymouth, biomass production at tassel and harvest was equal among the NRC breeder manure, the Low breeder manure, and TSP; which were all greater than UTC at the three lowest soil P levels (Fig. 8a, 8b). Biomass production was approximately 40% lower in 2008 at Plymouth due to the limited water supply. Biomass at tassel did not differ among the NRC breeder manure, the Low breeder manure, and TSP in 2008 at Plymouth (Fig. 8c). However, grain production in 2008 followed the order NRC (7.1 Mg ha^{-1}) = Low (6.6 Mg ha^{-1}) > TSP (6.0 Mg ha^{-1}) > UTC (4.5 Mg ha^{-1}) (data not shown, $LSD_{0.05}$). The harvest indices did not differ among P sources in 2007 at Plymouth (0.54 to 0.55), but the 2008 harvest indices were in the order NRC (0.59) > Low (0.54) = TSP (0.54) > UTC (0.48) ($LSD_{0.05}$, data not shown).

Soils Amended with Breeder Manure Pellets: Phosphorus Accumulation by Corn

Phosphorus accumulation at V-6 was higher in plants grown on soils amended with the NRC breeder manure (0.42 kg P ha^{-1}) than the Low breeder manure (0.37 kg P ha^{-1}) at

Salisbury in 2008. Conversely, young corn plants (V-6) accumulated 15% more P from soils amended with the Low breeder manure over the NRC breeder manure in a residual study in the greenhouse (Casteel, 2009b). In the present study, the early-season difference between corn grown in soils amended with the NRC and the Low breeder manures was transient. Otherwise, P accumulation at V-6 (0.50 to 3.04 kg P ha^{-1}) and at tassel (7.3 to 18.2 kg P ha^{-1}) was equal between the NRC and the Low breeder manures within each year and location (Table 4; Figs. 5, 6, 9). Phosphorus accumulation at tassel represented approximately 50% of the total P accumulated at harvest, except in 2007 at Salisbury (~90%) and Lewiston (~60%) (Figs. 5, 6, 9)

In 2007 at Salisbury, P accumulation in the grain was 6% higher in soils amended with the NRC breeder manure (17 kg P ha^{-1}) than in those amended with the Low breeder manure (16 kg P ha^{-1}) (Fig. 5) presumably due to greater soil P availability (i.e., differences in the M3P concentrations after P applications, Table 2). Breeder manure-amended soils did not differ in P accumulation at harvest at the remaining locations in either year (Table 4, Figs. 5, 6, 9). Grain P removal among soils amended with the various P sources ranged from 22.1 to 27.1 kg P ha^{-1} under good growing conditions (Salisbury 2008, Lewiston 2007-8, Plymouth 2007) and ranged from 11.8 to 17.0 kg P ha^{-1} under water stress conditions (Salisbury 2007, Plymouth 2008) (Figs., 5, 6, 9). Grain P removal in the P-deficient checks was 6.0 to 12.3 kg P ha^{-1} at Salisbury and 5.4 to 5.7 kg P ha^{-1} at Lewiston (Table 5). Regardless of the growing conditions during these studies, grain P removal represented 84 to 92% of the total P accumulated at harvest (Figs. 5, 6, 9).

Breeder manures supplied adequate plant available P for corn growth and grain P removal in P-limited soils. The yields at Salisbury were lower in 2007 than in 2008; whereas, the yields at Plymouth were higher in 2007 than in 2008. Thus, the P accumulation in the corn plants over the two years at Salisbury and Plymouth was generally equal to the single P application (39 kg P ha^{-1}) in terms of grain P removal and the M3P concentrations (Table 2, Figs. 5, 9). The single P application of the breeder manures at 39 kg P ha^{-1} probably would not supply adequate P in a P-deficient soil over two years in good growing conditions. In fact, corn at Lewiston yielded above average in both years and removed approximately 52 kg P ha^{-1} in the grain, which decreased the M3P concentrations below the pre-application concentrations of M3P (Table 2, Fig. 6).

Phosphorus Accumulation and Applied Phosphorus Recovery of Corn Grown in Soils Amended with Breeder Manure Pellets and Triple Superphosphate

Phosphorus sources applied at the same rate (39 kg P ha^{-1}) differed in P accumulation and the applied P recovery (APR) at Plymouth in both years where there was no interaction of soil P level x P source (Figs. 9, 10). Phosphorus accumulation at all stages of growth were greater in soils amended with the various P sources than the UTC in 2007 and in 2008 (Fig. 9). In 2007 and 2008, young corn plants accumulated more P from soils amended with the Low breeder manure (3.0 and 0.87 kg P ha^{-1}, respectively) than soils amended with TSP (2.44 and 0.69 kg P ha^{-1}, respectively) and the UTC (1.2 and 0.5 kg P ha^{-1}, respectively) (Fig. 9). Early-season P accumulation was equal between soils amended with NRC and TSP in 2007, but early-season P accumulation was 33% greater in soils amended with NRC than

TSP in 2008 (Fig. 9). Young corn plants recovered a higher percentage of applied P in breeder manure-amended soils than from TSP-amended soil at V-7 in 2007 and 2008 (Fig. 10). Young corn plants also recovered more P from breeder manures (~35%) than TSP (31%) when two consecutive studies in the greenhouse were summed (Casteel, 2009b).

The early-season differences in P accumulation and APR were present at tassel in 2007 where the P accumulation was: NRC = Low > TSP > UTC (Figs. 9, 10). However, P sources were equal in grain P removal in 2007 (Fig. 9), and the APR was 28 to 32% in the grain (Table 6, Fig. 10). In the lower yielding environment of 2008 (water stress), grain P removal was in the order of NRC (14.9 kg P ha^{-1}) = Low (13.6 kg P ha^{-1}) > TSP (11.8 kg P ha^{-1}) > UTC (8.8 kg P ha^{-1}) (Fig. 9b) and the APR was NRC = Low > TSP (Table 6, Fig. 10). Cumulative APR in the grain was greatest in the NRC breeder manure amendments, lowest in the TSP treatments, and intermediate with the Low breeder manure (Table 6).

Several factors probably contributed to the differences in biomass production (reported in previous sections), P accumulation, and APR between the breeder manures and TSP. First, soil pH values tended to be higher in the breeder manure-amended soil, which could increase plant growth by increasing macronutrient uptake and reducing acidity effects. Corn grown in the greenhouse accumulated more P from breeder manure-amended soils than TSP due to the P applied and the apparent liming effect of the breeder manures (macronutrient availability) (Casteel, 2009b). Secondly, breeder manures supplied both macro- and micro-nutrients in addition to the P applied, which could have had an additive effect on plant growth and P accumulation. In fact, at harvest in 2008, concentrations of M3-K followed the order of: Low $\geq$ NRC = TSP = UTC (140 $\geq$ 127 = 117 = 115 mg M3-K, respectively), which was still adequate for corn production. However, the concentrations of M3-Zn were near the critical concentration for corn production at harvest in 2008 and followed the order of: Low > NRC > UTC = TSP (1.27 > 1.17 > 0.97 = 0.96 mg M3-Zn kg^{-1}, respectively). Thirdly, the TSP-amended soils tended to be numerically lower in M3P concentrations over the 2008 growing season (Fig. 1c), which would presumably limit corn growth and P uptake. A final possible explanation could be the water-holding capacity in soils amended with breeder manures was increased above the TSP-amended soil, especially in the limited water conditions in 2008.

The breeder manures were equal to or greater than TSP in promoting growth of field-grown corn. In 2007, in-season differences in plant growth (height and biomass) and P accumulation among P sources did not persist to harvest (Figs., 7a, 8a, 8b, 9a). However, in 2008 breeder manure-amended soils produced more biomass that accumulated more P and increased the APR above the TSP treatments (Figs. 8d, 9b, 10b). The TSP equivalency in biomass production and grain P removal was approximately 1.3 for the NRC manure and 1.1 for the Low manure when 2007 and 2008 studies were summed (Table 6). Phosphorus-based applications of breeder manures should assume that all of the P was plant available, irrespective of whether a traditional diet (NRC) or a modified diet (Low) was fed.

CONCLUSIONS

Breeder manure P provided adequate P for corn growth across several soil types and soil P levels (P-limited to P-sufficient) in North Carolina. The P-modified diets fed to broiler breeders successfully reduced total P in the manure without altering soil P extractability and plant P availability once soil applied. The NRC and the Low breeder manure application generally increased M3P concentrations equally where in-season fluctuations were similar over two years. The NRC and the Low breeder manures were equally available for corn uptake of P in soils of the Piedmont, the Middle Coastal Plain, and the Lower Coastal Plain of North Carolina. The increase in M3P concentrations was approximately equal to the P application (based on soil P application estimate) at Lewiston and Plymouth, which suggested that nearly all of the applied P from the NRC and the Low breeder manures was M3P extractable. Nutrient management plans should treat these breeder manures equally in terms of P availability for crop growth.

Breeder manure applications based on P needs supplied the P requirement of corn with no more risk for soluble P losses during the growing season than did the TSP application. Soils amended with the NRC and the Low breeder manures were equal to or greater than TSP in corn growth, biomass production, and P accumulation presumably due to the high orthophosphate proportions (92 to 94%) and the additional characteristics of the breeder manures (e.g., apparent liming effect, nutrients like K and Zn). The P availability of the breeder manures should be considered equal to TSP in terms of nutrient management and risk assessments of P loss.

Many nutrient management plans and P loss risk programs have considered all poultry manures equal in P availability, and the P availability coefficient was 0.80 in North Carolina. This research led to the conclusion that P-based applications of breeder manures in North Carolina may have been loading soils with plant available P at a 20% faster rate based on the difference in P availability coefficients. If the P availability coefficient of breeder manures was increased from 0.8 to 1.0, P-based applications would have required 20% more land for the same volume of manure. However, the land increase of 20% could have been offset if broiler breeder producers adopted dietary P modifications. For example, the land requirement would decrease based on the manure TP reductions in this study (i.e., 20% increase in P availability coefficient – 28% reduction in manure TP = 8% less land needed for P-based application of breeder manure).

Phosphorus-response field studies will be needed to verify P availability of broiler litter, turkey litter, and layer manure, which have various orthophosphate proportions. Additionally, litters and manures produced from birds (broiler, turkey, layer) fed dietary P modifications need to be evaluated for any changes in P availability to crops grown in the field.

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Table 1. Selected characteristics of the three long-term phosphorus (P) fertility sites (Salisbury, Lewiston, Plymouth) used in the determination of P availability of breeder manure pellets to corn.

Selected Site Characteristics		Location		
		Salisbury	Lewiston	Plymouth
Physiographic Region		Piedmont	Middle Coastal Plain	Lower Coastal Plain
Soil Series		Hiwassee	Goldsboro	Portsmouth
Soil Texture		clay loam	loamy sand	fine sandy loam
Soil Subgroup		Typic Rhodudult	Aquic Paleudult	Typic Umbraquult
Long-Term Variables		P, K	P, K, N, pH	P
Tillage		no-till	conventional	conventional
Previous Crops		corn, soybean, wheat, rye	corn, soybean, peanut, cotton	corn, soybean
Soil Chemical Properties†				
Soil pH		6.2	5.9 – 6.1	5.5 – 5.6
Humic Matter	%	0.13 – 0.23	0.89 – 1.04	3.48 – 3.62
CEC‡	$cmol_c\ kg^{-1}$	5.2 – 6.0	4.7 – 4.9	7.4 – 7.7
Acidity	$cmol_c\ kg^{-1}$	0.7 – 0.8	0.8 – 1.0	1.6 – 1.8
M3-Ca§	$cmol_c\ kg^{-1}$	2.7 – 3.3	2.8 – 3.1	4.1 – 4.4
M3-Al	$mg\ kg^{-1}$	921.2 – 947.3	680.1 – 742.9	953.4 – 992.4
M3-Fe	$mg\ kg^{-1}$	39.7 – 43.0	130.5 – 153.9	143.8 – 155.2
M3-P	$mg\ kg^{-1}$	3.4 – 7.9	15.1 – 76.6	20.3 – 46.0
Total P	$mg\ kg^{-1}$	216.3 – 254.6	101.3 – 183.4	123.7 – 173.0
Total Inorganic P	$mg\ kg^{-1}$	93.6 – 146.1	34.4 – 120.1	21.6 – 58.4
Water-Soluble P	$mg\ kg^{-1}$	0.06 – 0.18	0.36 – 2.66	0.13 – 0.41
M3-PSR¶		0.005 – 0.018	0.018 – 0.086	0.018 – 0.0415

† Values represented the range across the soil P levels tested. Note: The other long-term variables were not included in the characterization, which included the P-deficient soils.

‡ CEC, cation exchange capacity.

§ M3, Mehlich-3 extractable elements.

¶ PSR, Phosphorus saturation ratio, calculated as a molar ratio of [(M3P) / (M3Al + M3Fe)].

Table 2. Mehlich-3 phosphorus (P) fluctuations due to the application of breeder manures (NRC, Low) and triple superphosphate (TSP) and corn P uptake at Salisbury, Lewiston, and Plymouth over the 2007 and 2008 growing seasons.

	Pre-application conc. of M3P†			M3P increase due to P source (~V6 in 2007 - pre-app.)				M3P change at 2007 harvest (2007 harvest - pre-app.)				M3P change at 2008 harvest (2008 harvest - pre-app.)			
	P sources‡			P sources			Mean across P sources§	P sources			Mean across P sources	P sources			Mean across P sources
	NRC	Low	TSP	NRC	Low	TSP		NRC	Low	TSP		NRC	Low	TSP	
	mg kg^{-1}														
Salisbury															
A	3.8	3.0	-	1.7	1.2	-	1.5	1.2	1.3	-	1.2	-0.6	-0.1	-	-0.3
B	3.4	3.5	-	4.3	2.1	-	3.2	6.1	1.9	-	4.0	1.5	-0.3	-	0.6
C	7.5	8.2	-	4.2	1.7	-	2.9	4.9	2.6	-	3.8	1.4	-1.0	-	0.2
Mean across soil P levels¶				3.4a	1.7b	-	-	4.1a	1.9b	-	-	0.8a	-0.4b	-	-
Lewiston															
A	13.9	16.3	-	17.0	16.9	-	16.9	9.1	10.3	-	9.7	-1.4	-0.9	-	-1.1a
B	26.7	26.3	-	23.6	17.6	-	20.6	9.7	8.5	-	9.1	-7.6	-6.2	-	-6.9a
C	81.9	71.4	-	18.5	17.9	-	18.2	7.8	1.3	-	4.6	-20.1	-15.3	-	-17.7b
Mean across soil P levels				19.7	17.5	-	-	8.9	6.7	-		-9.7	-7.5	-	-
Plymouth															
A	21.1	20.7	19.5	24.3	19.0	17.1	20.1	7.4	7.8	9.8	8.3	2.1	2.8	3.2	2.7
B	25.1	22.4.	26.3	21.0	19.2	16.8	19.0	7.6	7.0	4.3	6.3	0.7	0.1	-0.6	0.1
C	31.2	32.3	32.4	30.0	35.5	21.6	29.0	9.1	9.5	8.0	8.9	1.5	-2.2	-3.7	-1.5
D	48.4	44.4	44.4	30.0	29.6	28.5	29.4	7.5	12.5	13.3	11.1	-5.1	-2.2	-2.2	-3.2
Mean across soil P levels				26.3a	25.8a	21.0b	-	7.9	9.2	8.9	-	-0.2	-0.4	-0.8	-

† conc. of M3P, concentration of Mehlich-3 phosphorus.

Table 2. Continued.

‡ P sources were breeder manures (NRC and Low, Table 2) and TSP (triple superphosphate) that were applied at 39 kg P ha^{-1} prior to planting corn in 2007. No additional P was applied over the two growing seasons.

§ Values within same column (mean across P sources) followed by different letters were significantly different among soil P levels within each location using Fisher's Protected $LSD_{0.05}$.

¶ Values within same row (mean across soil P level) followed by different letters were significantly different among P sources within each location using Fisher's Protected $LSD_{0.05}$. The soil P x P source interaction was not significant at any location.

Table 3. Selected properties of manure pellets that were produced by dietary phosphorus modifications of broiler breeders.

Manure from diet†	pH	CCE‡	N	K	Ca	P	Ca:P	WSP_M§	WSP_M:P	Orthophosphate¶		Phytate	
		%	g kg^{-1}				g:g	g kg^{-1}	g:g	g kg^{-1}			
NRC	6.4b#	32b	27.8	32.3	107.4	21.7a	5.0b	4.3a	0.20a	20.4	(94)	1.085	(5)
Low	7.2a	37a	27.7	36.9	109.2	15.7b	7.0a	2.3b	0.15b	14.4	(92)	0.987	(6)

† The NRC (National Research Council, 1994) diet was 0.37% non-phytate P (NPP) with a dietary Ca:NPP ratio of 7.3. The Low diet was 0.19% NPP with a dietary Ca:NPP ratio of 14.2. See Casteel (2009a) for further details of diets and manures produced.

‡ CCE, calcium carbonate equivalence.

§ WSP_M, Manure Water-Soluble P.

¶ Values in parenthesis were the proportion (%) of total P for orthophosphate and phytate based on ^{31}P NMR peak integration. The total P concentrations of the manure replicates were multiplied by these ^{31}P NMR estimations.

Values within same column followed by different letters are significantly different between manures produced from dietary P modifications using Fisher's Protected $LSD_{0.05}$.

Table 4. Probability of significance of corn growth, biomass production, and phosphorus (P) accumulation as affected by soil P level and P source at Salisbury, Lewiston, and Plymouth in 2007 and 2008.

		~ V-6†			Tassel			Grain		Total Harvest‡	
Source	df	HT§	Biomass	P Acc.¶	HT	Biomass	P Acc.	Biomass	P Acc.	Biomass	P Acc.
Salisbury – 2007											
Soil P	2	ns	ns	ns	ns	ns	ns	ns	*	ns	*
Source	1	ns	ns	ns	ns	ns	ns	ns	**	ns	*
Soil P x Source	2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Salisbury – 2008											
Soil P	2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Source	1	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
Soil P x Source	2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Lewiston – 2007											
Soil P	2	*	**	*	ns	ns	ns	ns	*	ns	*
Source	1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Soil P x Source	2	ns	*	*	ns	ns	ns	ns	ns	ns	ns
Lewiston – 2008											
Soil P	2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Source	1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Soil P x Source	2	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
Plymouth – 2007											
Soil P	3	**	ns	ns	***	**	***	*	**	**	**
Source#	3	***	***	***	***	***	***	***	***	***	***
Soil P x Source	9	***	ns	ns	***	**	ns	***	ns	***	ns
Plymouth – 2008											
Soil P	3	ns	ns	ns	*	ns	*	**	***	**	**
Source	3	***	***	***	***	*	**	***	***	***	***
Soil P x Source	9	ns	ns	ns	*	*	ns	ns	ns	*	ns

*, **, and ** Significance at the probability of 0.05, 0.01, and 0.001 respectively, and no significance (ns) when a > 0.05.

Table 4. Continued.

† Leaf growth stage was approximately V-6 (6 exposed corn leaf collars) over the locations and years.

‡ Total Harvest was the sum of grain and stover accumulations of biomass and P.

§ HT, height of corn plant at the specified growth stage.

¶ P Acc., Phosphorus accumulation at each growth stage where tissue P concentration was multiplied by the biomass accumulation.

The unamended controls (UTC) were included in the analysis of variance at Plymouth in 2007 and 2008.

Table 5. Corn growth, biomass production, and phosphorus (P) accumulation in the P-deficient checks at Salisbury and Lewiston in 2007 and 2008. Means reported with standard deviations.

	Height		Biomass Production				Phosphorus Accumulation			
	V-6†	Tassel	V-6	Tassel	Grain	Total Harvest‡	V-6	Tassel	Grain	Total Harvest
	cm		Mg ha^{-1}				kg P ha^{-1}			
Salisbury										
2007	51 ± 4	242 ± 19	0.26 ± 0.07	6.2 ± 1.3	3.3 ± 0.9	7.9 ± 1.6	0.44 ± 0.15	7.9 ± 3.2	6.0 ± 1.8	6.9 ± 2.0
2008	48 ± 5	238 ± 22	0.13 ± 0.03	5.8 ± 1.0	7.2 ± 2.4	12.7 ± 3.6	0.30 ± 0.08	6.4 ± 1.5	12.3 ± 4.4	13.5 ± 5.0
Lewiston										
2007	29 ± 3	140 ± 24	0.03 ± 0.01	2.2 ± 0.7	2.7 ± 1.0	5.1 ± 1.7	0.06 ± 0.02	2.1 ± 0.8	5.4 ± 2.3	5.9 ± 2.4
2008	31 ± 2	108 ± 9	0.06 ± 0.01	1.3 ± 0.2	2.4 ± 0.2	4.8 ± 1.5	0.10 ± 0.02	1.5 ± 0.2	5.7 ± 0.1	5.5 ± 3.0

† Leaf growth stage was approximately V-6 (6 exposed corn leaf collars) over the locations and years.

‡ Total Harvest was the sum of grain and stover accumulations of biomass and P.

Table 6. Applied phosphorus recovery of corn grain produced in 2007, 2008, and cumulatively at Plymouth as affected by P sources and soil P levels.

	Applied Phosphorus Recovery in Grain†				
	Soil P Levels				Mean across soil P levels‡
	A	B	C	D	
	%				
2007					
NRC	42.2	35.5	29.7	20.0	31.8
Low	36.2	38.4	25.0	12.4	28.0
TSP	37.4	37.8	25.0	12.3	28.1
Mean across P sources§	38.6a	37.2a	26.6ab	14.9c	
2008					
NRC	19.7	15.4	13.0	15.1	15.8a
Low	14.8	12.0	7.9	14.9	12.4a
TSP	9.8	12.7	7.2	2.0	7.9b
Mean across P sources	14.8	13.3	9.4	10.7	
Cumulative					
NRC	61.9	50.8	42.7	35.1	47.6a
Low	51.0	50.4	32.9	27.3	40.4ab
TSP	47.2	50.5	32.2	14.3	36.0b
Mean across P sources	53.4	50.6	35.9	25.6	

† Applied phosphorus recovery was calculated as: {[(P acc. in trt) – (P acc in UTC)] / (39 kg P ha^{-1})] x 100}, where P acc. was P accumulation, trt was P source x soil P level, UTC was the untreated control within the soil P level, and 39 kg P ha^{-1} was P rate applied prior to planting in 2007. No additional P was applied over the two growing seasons.
‡ Values within same column (mean across soil P levels) followed by different letters were significantly different among P sources within 2007, 2008, and cumulative categories according to Fisher's Protected $LSD_{0.05}$.
§ Values within same row (mean across P sources) followed by different letters were significantly different among soil P levels within 2007, 2008, and cumulative categories according to Fisher's Protected $LSD_{0.05}$. The soil P x P source interaction was not significant in 2007, 2008, or cumulatively.

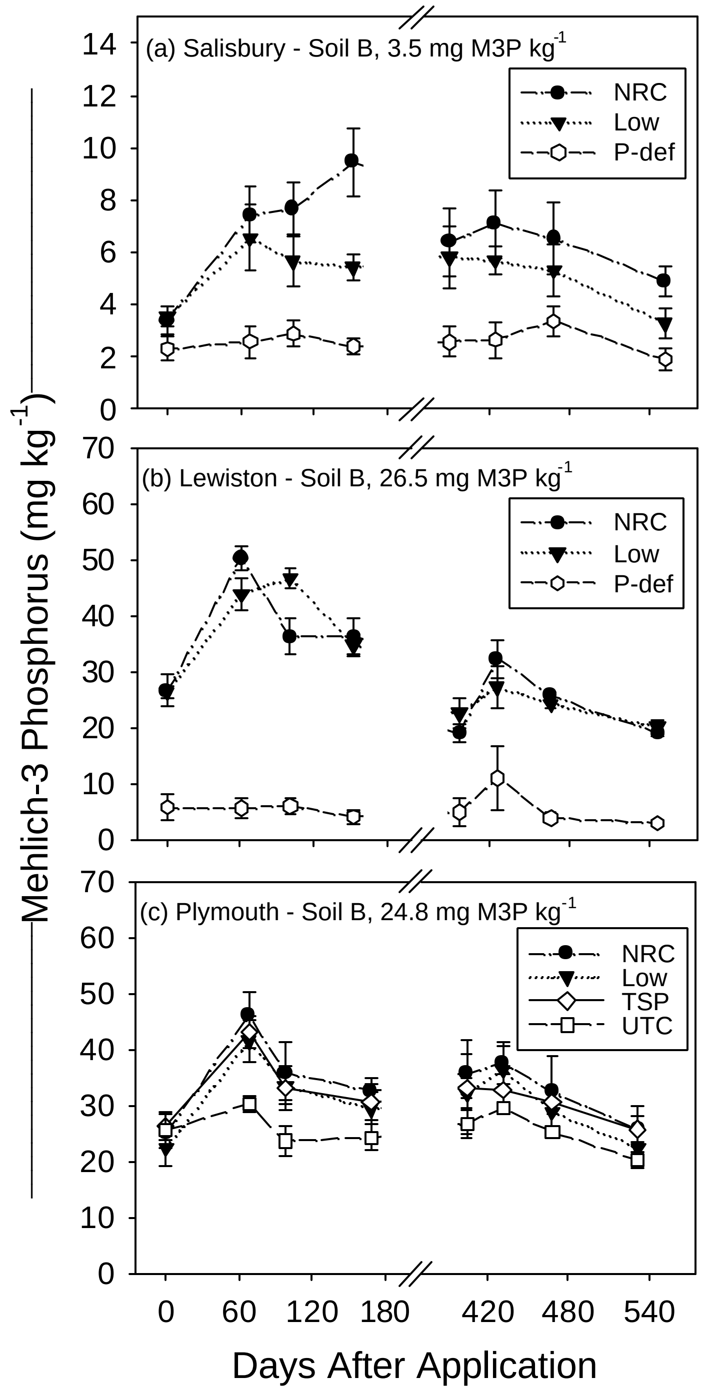

Figure 1. Mehlich-3 phosphorus P concentrations in Soil B at (a) Salisbury, (b) Lewiston, and (c) Plymouth over two growing seasons of corn as affected by a single P application of breeder manures (NRC, Low) and triple superphosphate (TSP). M3P, Mehlich-3 P; P-def, P-deficient check; UTC, untreated control. Note scale differences of the y-axis. Standard errors presented as individual error bars.

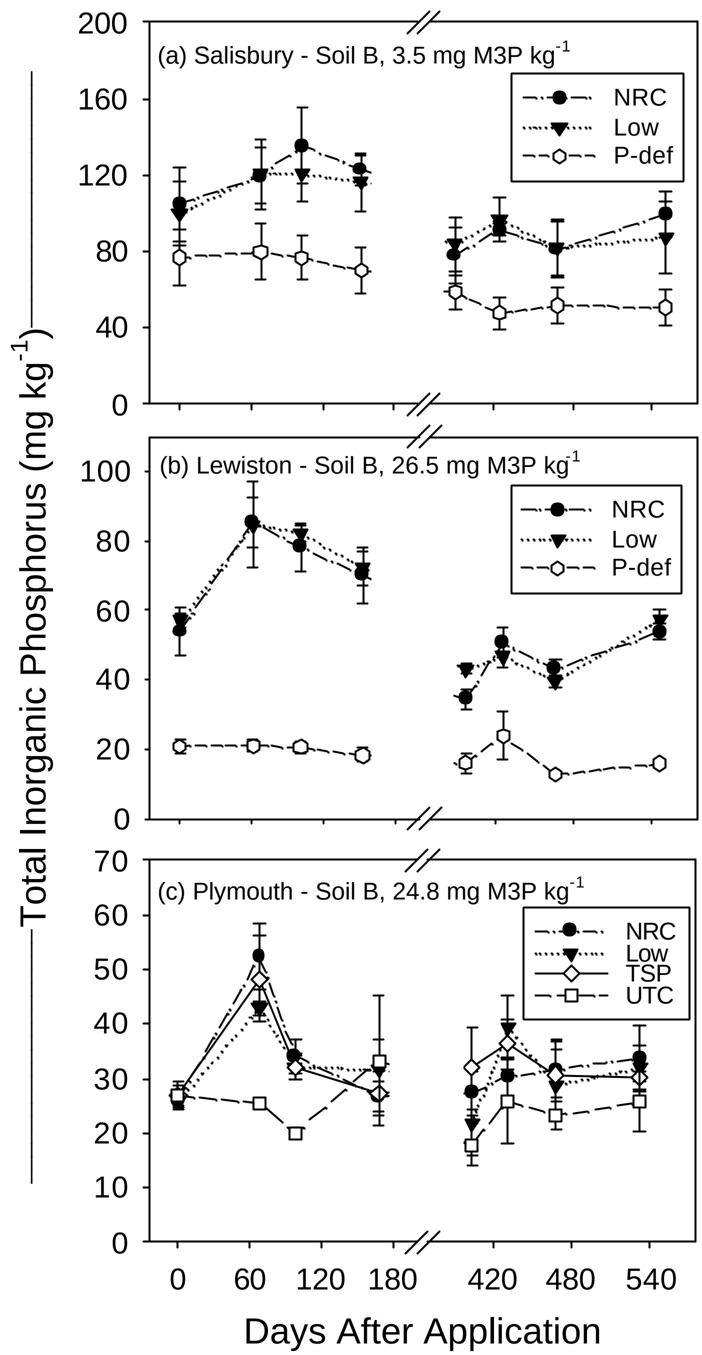

Figure 2. Total inorganic phosphorus (P) concentrations in Soil B at (a) Salisbury, (b) Lewiston, and (c) Plymouth over two growing seasons of corn as affected by a single P application of breeder manures (NRC, Low) and triple super phosphate (TSP). M3P, Mehlich-3 P; P-def, P-deficient check; UTC, untreated control. Note scale differences of the y-axis. Standard errors presented as individual error bars.

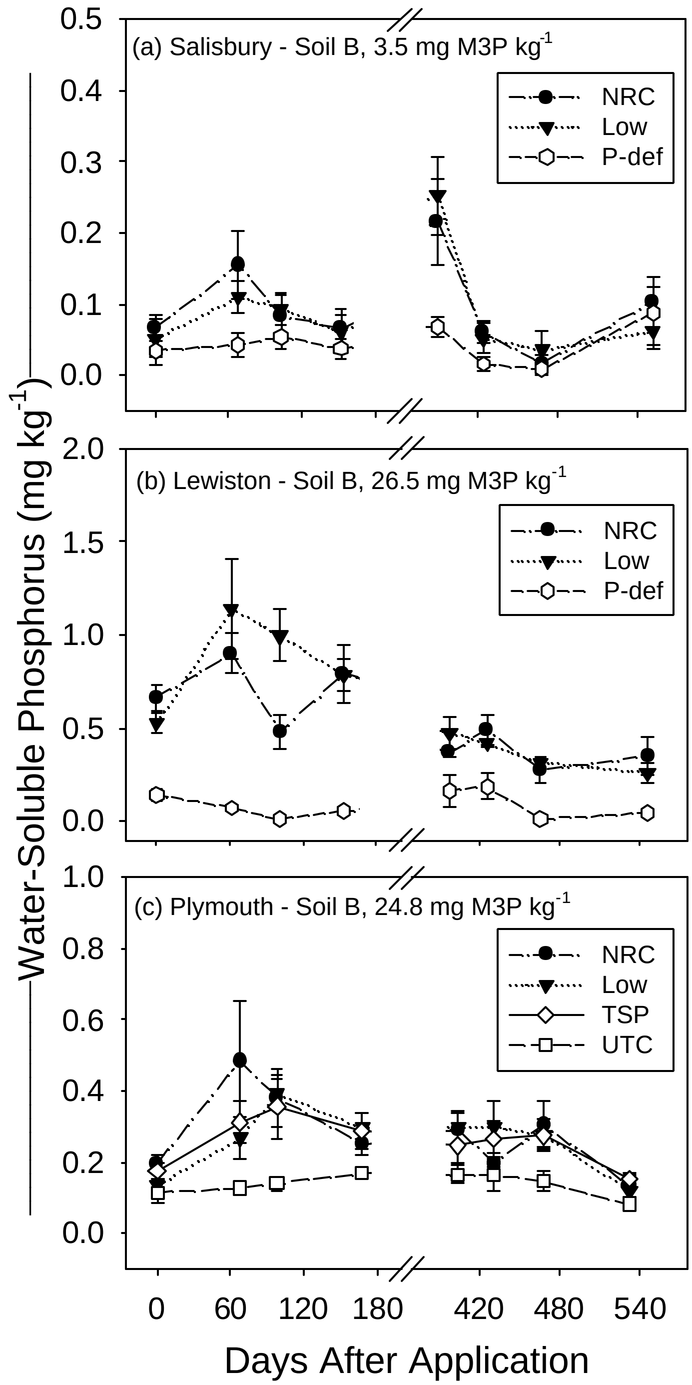

Figure 3. Soil water-soluble phosphorus (P) concentrations in Soil B at (a) Salisbury, (b) Lewiston, and (c) Plymouth over two growing seasons of corn as affected by a single P application of breeder manures (NRC, Low) and triple super phosphate (TSP). M3P, Mehlich-3 P; P-def, P-deficient check; UTC, untreated control. Note scale differences of y-axis. Standard errors presented as individual error bars.

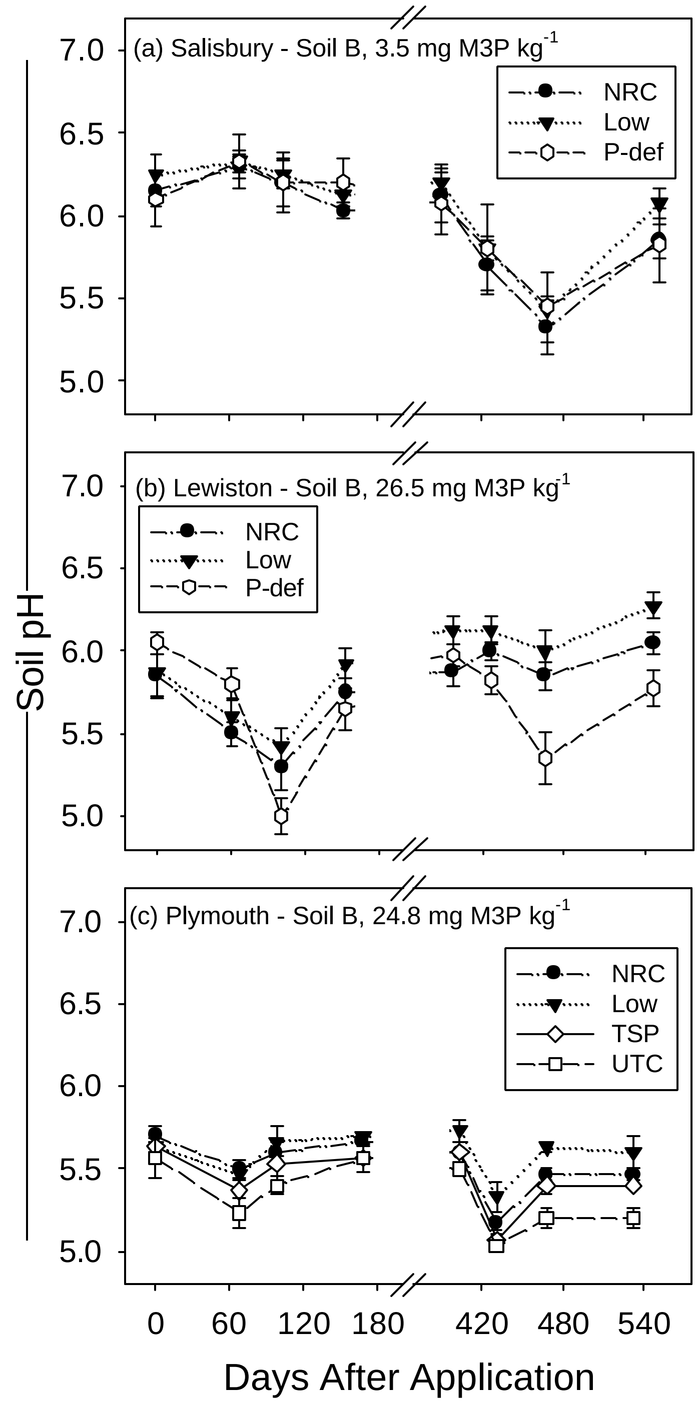

Figure 4. Soil pH values in Soil B at (a) Salisbury, (b) Lewiston, and (c) Plymouth over two growing seasons of corn as affected by a single phosphorus application of breeder manures (NRC, Low) and triple super phosphate (TSP). M3P, Mehlich-3 P; P-def, P-deficient check; UTC, untreated control. Standard errors presented as individual error bars.

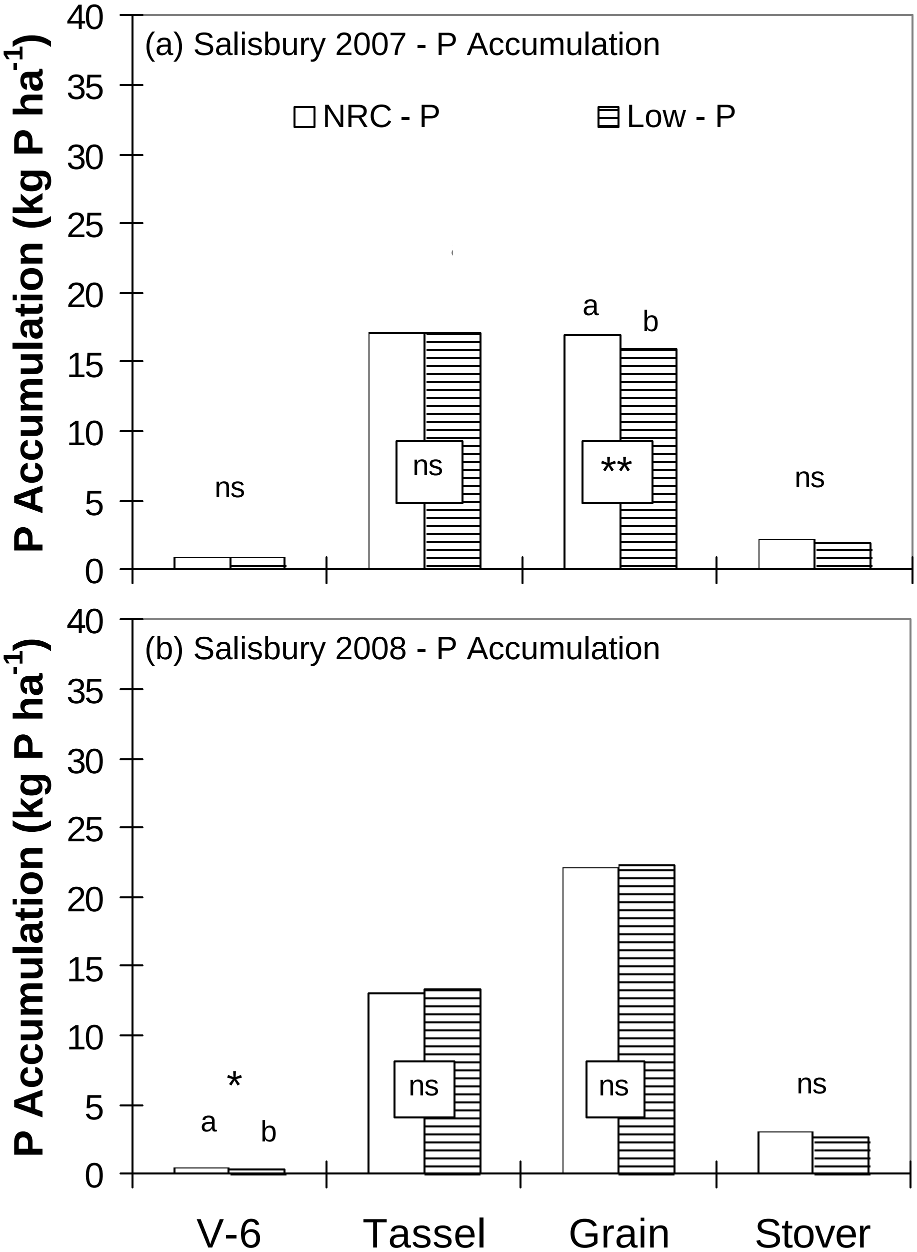

Figure 5. Phosphorus accumulation of corn sampled at V-6, tassel, and harvest (grain, stover) in (a) 2007 and (b) 2008 at Salisbury. Phosphorus sources were averaged over soil P levels, since there was no interaction of soil P x P source (Table 3). Means were separated according to Fisher's Protected $LSD_{0.05}$ within each growth stage for P accumulation. Significance at probability of 0.05, 0.01, and 0.001 was represented by *, **, and ***, respectively. No significance (ns) when a > 0.05.

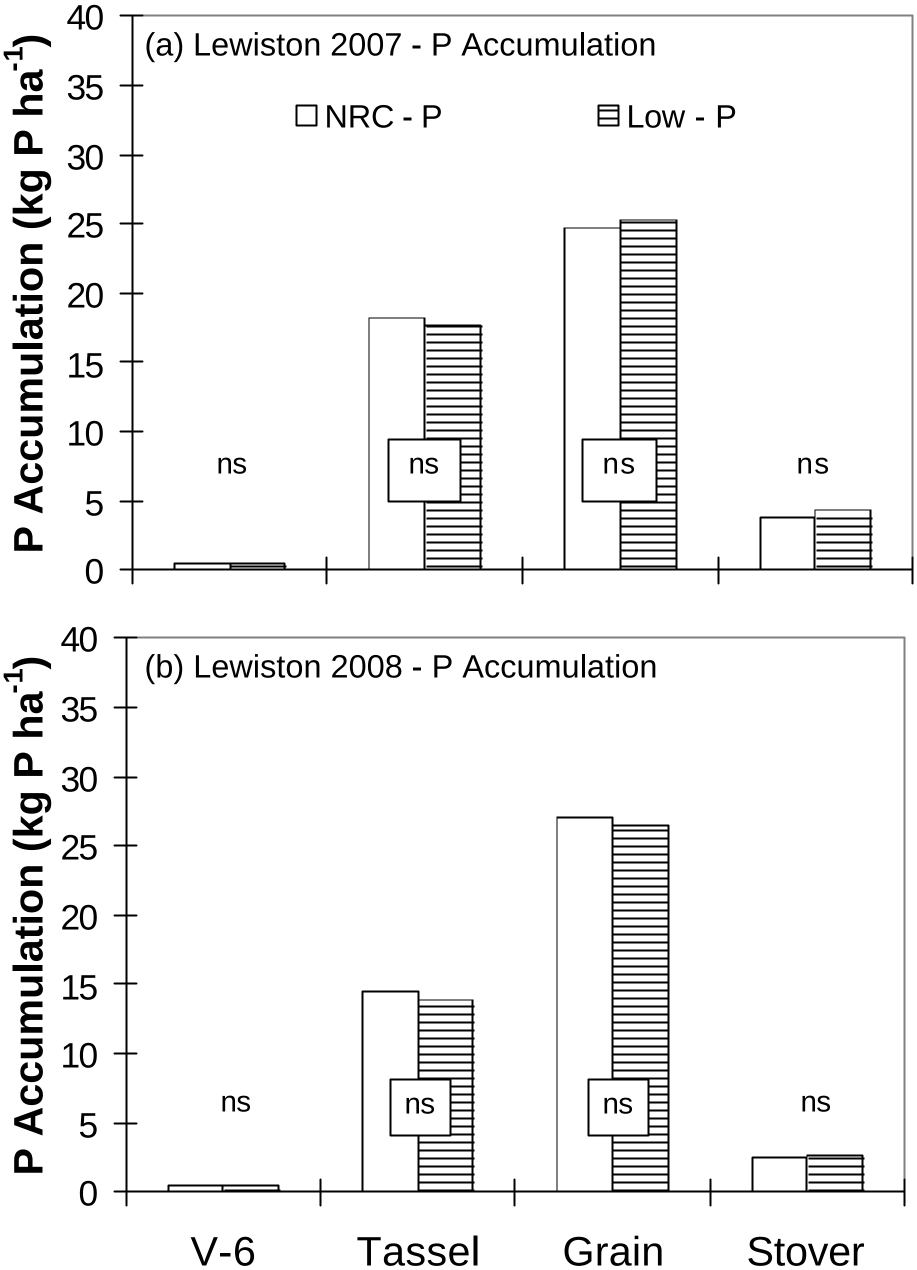

Figure 6. Phosphorus accumulation of corn sampled at V-6, tassel, and harvest (grain, stover) in (a) 2007 and (b) 2008 at Lewiston. Phosphorus sources were averaged over soil P levels, since there was no interaction of soil P x P source (Table 3). Means were separated according to Fisher's Protected $LSD_{0.05}$ within each growth stage for P accumulation. Significance at probability of 0.05, 0.01, and 0.001 was represented by *, **, and ***, respectively. No significance (ns) when a > 0.05.

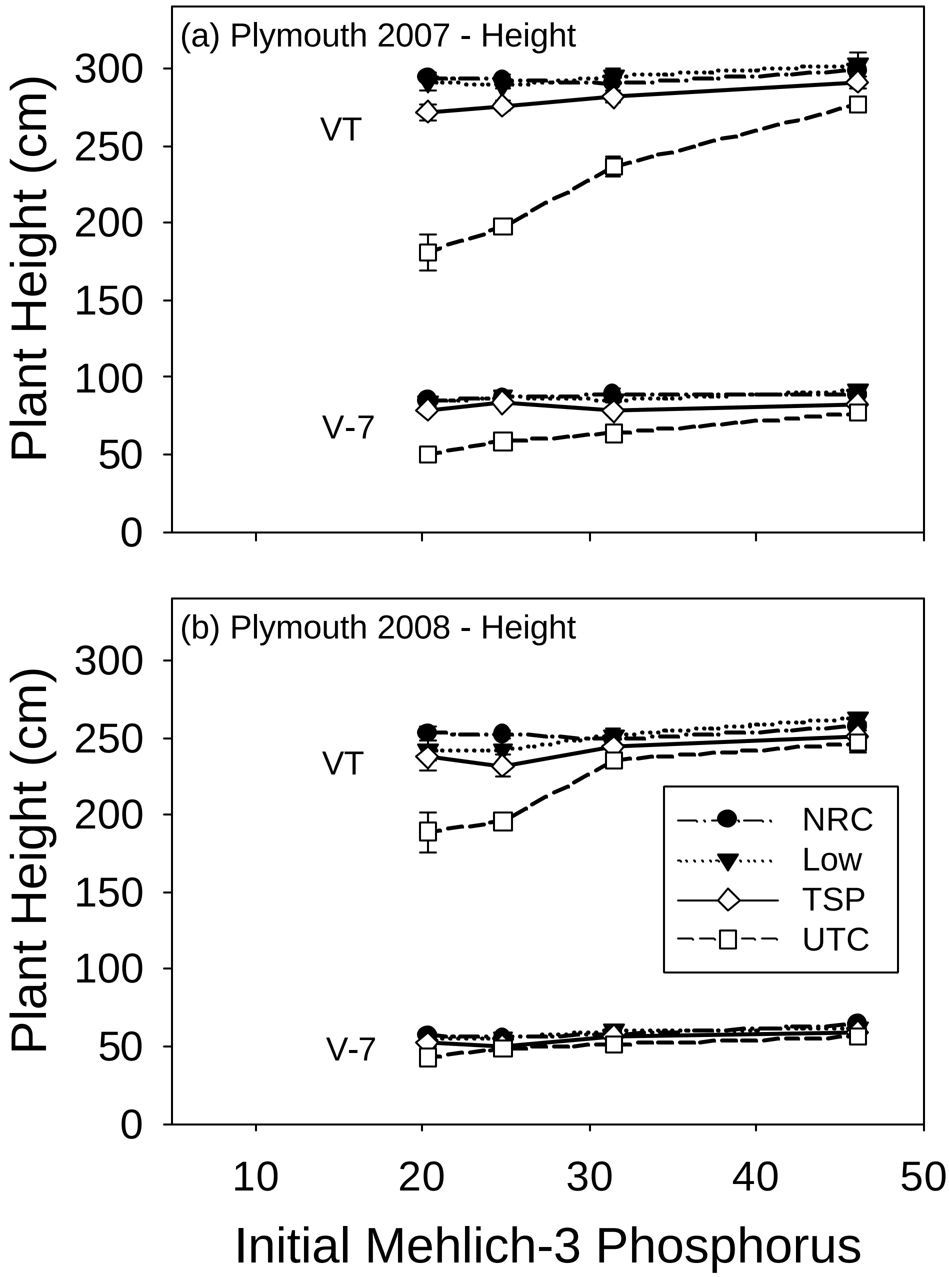

Figure 7. Plant height at corn growth stages V-7 and VT (tasseling) at Plymouth in (a) 2007 and (b) 2008. Initial Mehlich-3 phosphorus (P) represented the pre-application soil levels A, B, C, and D. Phosphorus sources were breeder manures (NRC and Low) and triple super phosphate. The untreated control (UTC) represents the soil that was not amended with P. Standard error bars are presented. Standard errors presented as individual error bars.

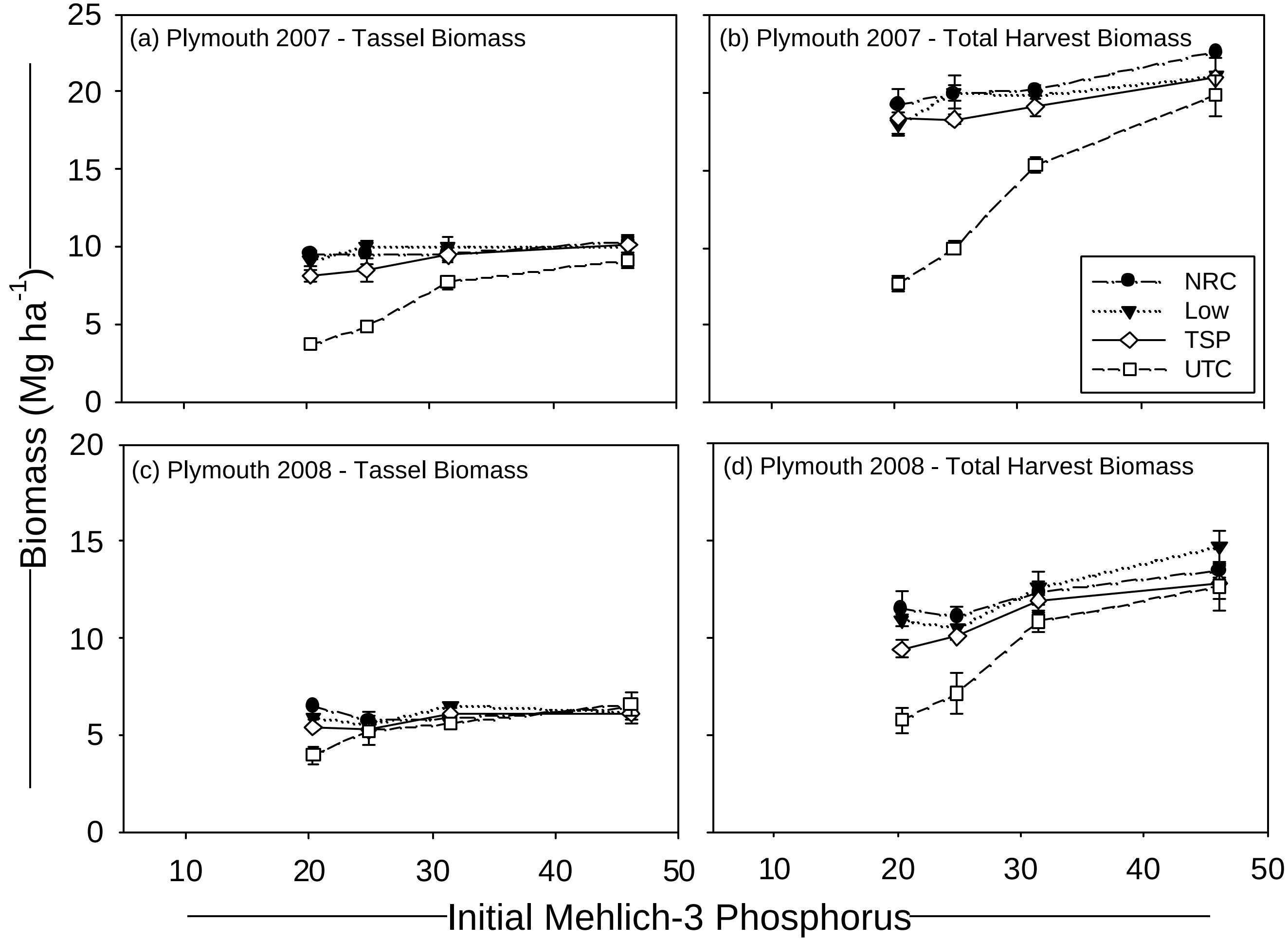

Figure 8. Biomass production of corn sampled at tassel and harvest (grain, stover) in (a) 2007 and (b) 2008 at Plymouth. Note scale differences of the y-axis. Standard errors presented as individual error bars.

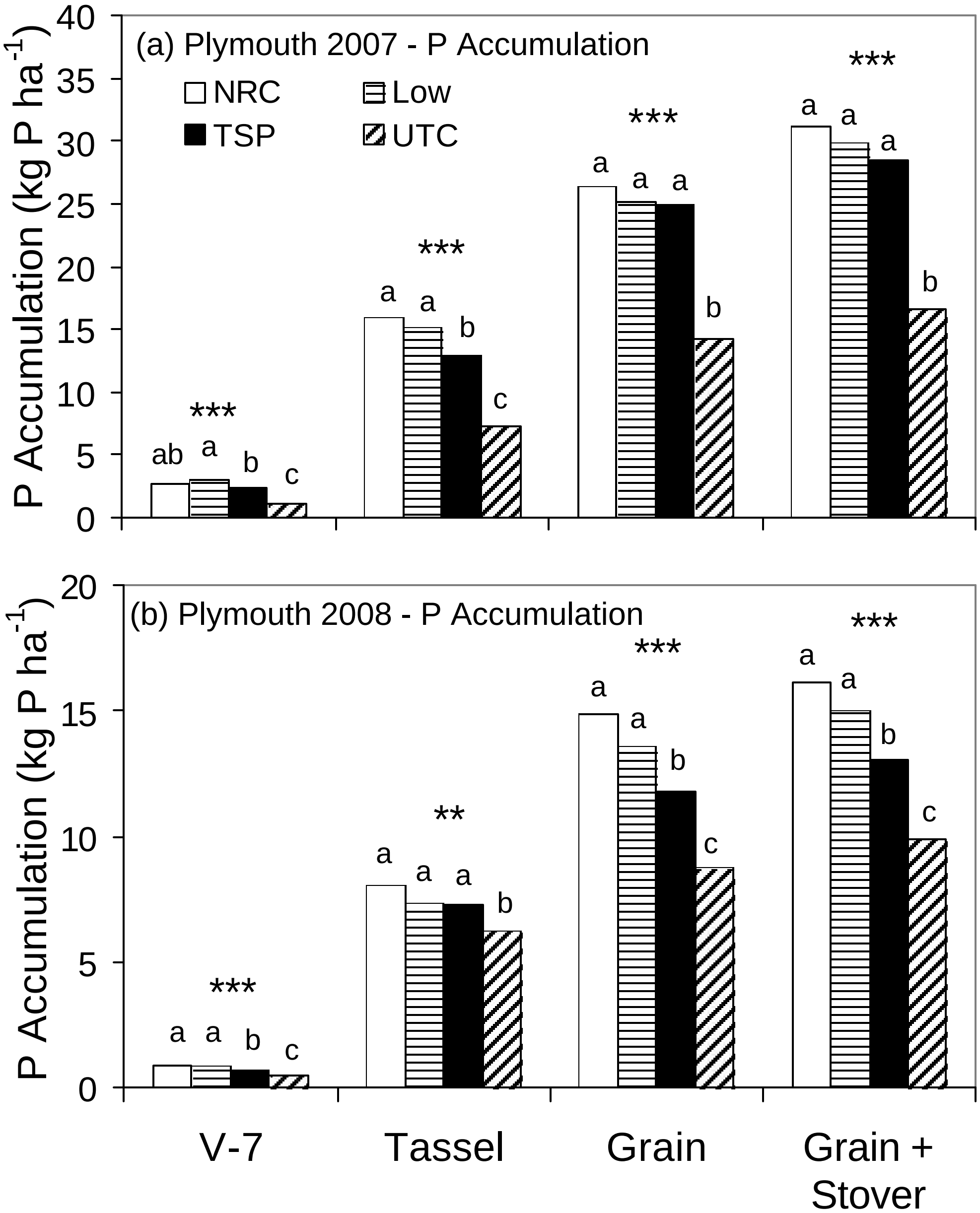

Figure 9. Phosphorus (P) accumulation in corn sampled at V-7, tassel, and harvest (grain, stover) in (a) 2007 and (b) 2008 at Plymouth. Phosphorus sources were averaged over soil P levels, since there was no interaction of soil P x P source (Table 4). Means were separated according to Fisher's Protected $LSD_{0.05}$ within each growth stage. Significance at probability of 0.05, 0.01, and 0.001 was represented by *, **, and ***, respectively. No significance (ns) when a > 0.05. Note scale differences of the y-axis.

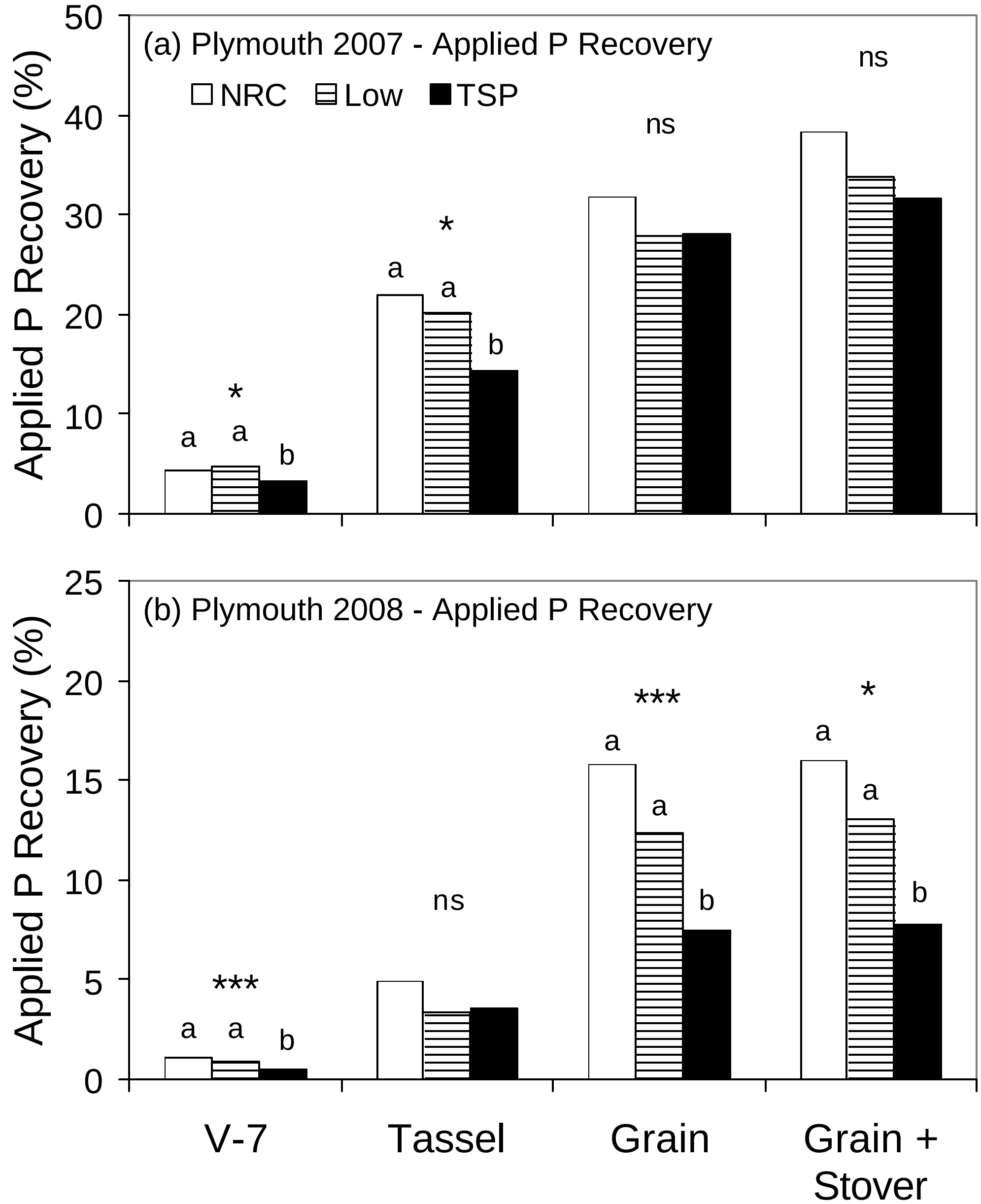

Figure 10. Applied phosphorus (P) recovery (APR) in corn sampled at V-7, tassel, and harvest (grain, stover) in (a) 2007 and (b) 2008 at Plymouth. Breeder manures (NRC, Low) and triple super phosphate (TSP) were averaged over soil P levels, since there was no interaction of soil P x P source. Means were separated according to Fisher's Protected $LSD_{0.05}$ within each growth stage. Significance at probability of 0.05, 0.01, and 0.001 was represented by *, **, and ***, respectively. No significance (ns) when a > 0.05. Note scale differences of the y-axis. The APR was calculated for P sources as follows: {[(Accumulated P by Source) – (Accumulated P by the untreated control)] / [39 kg P ha^{-1}] x 100}.

CHAPTER 6

General Conclusions

Intense poultry production regions creates a manure phosphorus (P) surplus due to the limited ability of poultry to digest phytate (i.e., the result is P-concentrated manure) and the limited P removal by crops grown in these regions (i.e., P input > P removal). Dietary P modifications successfully reduced P in broiler and turkey litters by adjusting the dietary P ration with and without phytase. However, production practices of broiler breeders are substantially different than broilers or turkeys and thus, a clear understanding of breeder manure P and associated effects of dietary P modifications are needed. Broiler breeder production is critical in North Carolina, since it represents approximately 20% of the USA broiler breeder production.

Phosphorus reduction in breeder manure P was generally successful with dietary P modifications. Phytase addition with a simultaneous reduction in dietary P ration produced broiler breeder manure that was 15% lower in total P. In another study, manure total P decreased 28% when broiler breeders were fed 0.22% available P (NRC) vs. 0.40% available P (Low) and was not affected by phytase additions. Manure water-soluble P (WSP) concentrations were closely related to the moisture content of fresh broiler breeder manure. Efficient water management in broiler breeder production should assist in the reduction of immediate environmental implications from the land-application of breeder manures. In other words, the reduction of water spillage in broiler breeder production would reduce water-soluble P in the manure and thus, decrease potential for soluble P losses following a surface application to soil. Improved water management in other poultry production systems (e.g., broiler, turkey, layer) should also be a goal to reduce short-term implications to the environment.

Two Ultisols were amended with breeder manures produced from four dietary P modifications. These amended soils generally did not differ in Mehlich-3 P (M3P), soil WSP, total inorganic P, and total P. Breeder manures increased M3P to a much greater extent than comparable applications of broiler and turkey litter. Further investigation revealed inherent differences in manure P forms of broiler breeder manure compared to broiler and turkey litters. Breeder manure P was approximately 73% orthophosphate and 23% phytate after 48 h of manure accumulation. Phytate was mineralized as the manure accumulation period increased (48 h < 3 wk < 39 wk), and thus, orthophosphate proportions increased. Orthophosphate proportions were approximately 90% in breeder manures; whereas, orthophosphate was 30 to 40% in broiler litter and 50 to 70% in turkey litter. The increase in M3P based on the orthophosphate application rates of our breeder manures and previous broiler litters produced similar changes. Plant P availability of the breeder manures should be high in comparison to the broiler and turkey litters.

Plant P availability of breeder manures produced from dietary modifications (NRC, Low) were generally equal to each other and triple superphosphate (TSP) in the greenhouse and the field. Corn (*Zea mays*) growth was equal among P sources in greenhouse study 1 (initiation), but plant growth tended to be greater in the breeder manure-amended soils in greenhouse study 2 (residual) due to the P applied and the apparent liming effect from the breeder manures. Total P accumulation was equal among P sources at the low P application rates in greenhouse studies 1 and 2. In the field, P-based applications of the NRC and the Low breeder manures equally increased the concentrations of M3P, total inorganic P, and soil WSP at Lewiston and Plymouth. Breeder manure-amended soils were equal in plant growth in all site-years and grain P removal five out of six site-years. Grain production, grain P removal, and applied P recovery were equal among P sources in 2007, but the NRC and the Low breeder manure treatments were greater than TSP in 2008. This difference was presumably due to the combination of the apparent liming effect of the breeder manures and the additive effects of other nutrients supplied from the breeder manures (e.g., K, Zn).

The characteristics of breeder manure reported in this research did not include the scratch litter, which would marginally dilute the nutrient concentrations. The P in breeder manure that accumulated under a slat floor (free of wood chips) should be considered 100% plant available. Phosphorus management of breeder manure will need to consider the influence of the scratch litter on the plant P availability of the mixture of breeder manure + scratch litter, since all the manure and litter are traditionally removed from breeder houses. Accumulated manure under the slat floors would represent approximately 85% of the total volume of breeder manure + scratch litter mixture: 66% of pen area (16 m^2) x 0.15 m thickness (visual observation). Scratch litter would represent approximately 15% of the total volume of breeder manure + scratch litter mixture: 33% of pen area (16 m^2) x 0.05 m thickness (visual observation). Additionally, P concentration in the scratch litter would be approximately 50% as much as the breeder manure under the slat floor. Therefore, the mixture of breeder manure + scratch litter would be approximately 7% less in total P volume compared to the breeder manure under the slat floor [(30 g P kg^{-1} in breeder manure)*(0.85)

+ (15 g P kg^{-1} in scratch litter)*(0.15) = 27.8 g P kg^{-1}]. The density of the breeder manure would be greater than the scratch litter, which would decrease the impact the scratch litter would have on the mixture of breeder manure + scratch litter. The addition of scratch litter would reduce total P, but the dilution would be minimal and plant P availability would still be very high.

Many nutrient management plans and P loss risk programs consider all poultry manures equal in P availability, and the P availability coefficient is 0.80 in North Carolina. This research leads to the conclusion that P-based applications of breeder manures in North Carolina have been loading soils with plant available P at a 20% faster rate based on the difference in P availability coefficients. If P availability coefficient of breeder manures is increased from 0.8 to 1.0, P-based applications will require 20% more land for the same volume of manure. The land increase of 20% could be offset if broiler breeder producers adopt dietary P modifications. For example, the land requirement would decrease based on the manure TP reductions in this study (i.e., 20% increase in P availability coefficient – 28% reduction in manure TP = 8% less land needed for P-based application of breeder manure).

Phosphorus-response studies are needed in the field to verify P availability of broiler litter, turkey litter, and layer manure, which have various orthophosphate proportions. Field evaluation of the breeder manure + scratch litter mixture would beneficial as well. Additionally, litters and manures produced from birds (broiler, turkey, layer) fed dietary P modifications need to be evaluated for any changes in P availability to crops grown in the field.