EFFECTS OF DIETARY PHOSPHORUS AND PHYTATE-PHOSPHORUS ON ENVIRONMENTALLY RELEVANT PHOSPHORUS EXCRETION IN HORSES

By

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To My Mom and Dad

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1xNRC-HighPP	100% NRC requirement for phosphorus and high amount of phytate
1xNRC-LowPP	100% NRC requirement for phosphorus and low amount of phytate
3xNRC-HighPP	300% NRC requirement for phosphorus and high amount of phytate
3xNRC-LowPP	300% NRC requirement for phosphorus and low amount of phytate
ATP	Adenosine triphosphate
BW	Body Weight
Ca	Calcium
Ca:P	Calcium to phosphorus ratio
CP	Crude Protein
Cu	Copper
DE	Digestible Energy
DM	Dry Matter
DMI	Dry Matter Intake
DRP	Dissolved Reactive Phosphorus
Mg	Magnesium
N	Nitrogen
NRC	National Research Council
P	Phosphorus
TP	Total Phosphorus
WEP	Water Extractable Phosphorus
WSP	Water Soluble Phosphorus
Zn	Zinc

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Elevated phosphorus (P) levels in surface water bodies can lead to eutrophication and other impairments to water quality. Livestock manure has been identified as a significant source of the phosphorus (P) responsible for water quality degradation. Total and water soluble phosphorus (WSP) excretion has been shown to be highly correlated with daily P intake in livestock and poultry, but this relationship has not been evaluated in horses. The objectives of the research presented in this thesis were to: 1) characterize total P and WSP fractions in horse manure, and 2) determine how dietary intake of total P and phytate-P affect the fractional distribution of P in manure. We hypothesized that feeding horses above minimum P requirements would result in greater total P and WSP excretion, thereby presenting the greatest environmental risk. Because it is unclear how well horses utilize phytate-P in common feedstuffs, we further speculated that diets with a higher level of phytate-P would reduce excretion of WSP in manure. Eight mature (mean ± SE, 540 ± 22 kg) geldings were used in a 4 X 4 Latin square design study to examine 4 diets differing in total P and phytate P levels. Diets contained either 100% or 300% of current NRC (2007) requirements for P, with either a low or high portion of the P as phytate-P. All 4 diets evaluated had the same 2:1 calcium

to P ratio. Each study period consisted of an 11-day diet adaptation phase, followed by a 3-day collection of all feces and urine. The main route of P excretion in horses was through the feces, whereas only a small quantity of P was excreted via the urine. On an as-excreted basis, horse feces contained 0.03 – 0.19% total P and 0.02 – 0.09% WSP, resulting in the daily excretion of 16.8 to 58.8 mg total P and 9.3 to 24.2 mg WSP per kg body weight. Although urinary P excretion was not influenced by dietary intake, total P and WSP excretion in feces was affected by both the P and phytate-P concentration in the diet. Feeding horses at 3-times their P requirement resulted in 2.5 to 3 times the excretion of total P and WSP. Inclusion of higher amounts of phytate-P in the diet resulted in further increases in fecal total P and WSP, as well as increased fecal excretion of calcium, magnesium and zinc compared to diets providing a similar level of total P intake with lower amounts of phytate-P. Results indicate that overfeeding P to sedentary horses results in higher P excretion, of which 40 – 58% is water-soluble. Because WSP is the most mobile P fraction, it poses the greatest risk for contaminating storm water runoff and leaching into groundwater. In addition to properly handling and storing manure, feeding horses in excess of their P requirements should be avoided whenever possible to reduce risk for water quality degradation.

CHAPTER 1
INTRODUCTION

Animal production in the United States is valued at more than $100 billion and has consolidated significantly over the past 20 years, with a large number of animals being produced on an increasingly smaller land base (Kellogg et al., 2000). Animal agriculture plays a large part in nonpoint sources of pollution because the feces and urine excreted daily from the animal has the potential to come in contact with runoff during a storm event. This manure-contaminated runoff is eventually deposited into lakes, rivers, wetlands, and coastal waters, and may even leach into underground sources of drinking water.

Research has indicated that the water soluble P (WSP) fraction in manure is the most susceptible to loss in the environment (Kleinman et al., 2000; Sharpley and Moyer, 2000). Sharpley and Moyer (2000) found a strong correlation between WSP in dairy, poultry, and swine manures and the amount of P leached from soil following simulated rainfall events. Manure WSP has been shown to be highly correlated with daily P intake in livestock and poultry (Dou et al., 2002). As a result, one of the fundamental methods for altering both total P and WSP in livestock and poultry manures has been through diet modification. Dou et al. (2002) reported that decreasing dietary P in lactating dairy cow rations not only reduced the concentration of total P in feces, but also decreased the amount and proportion of P that was water-soluble. A meta-analysis of digestibility trials conducted in mature, idle horses demonstrated a positive linear correlation between dietary P intakes and P excretion in manure (Lawrence et al., 2003). More recently, Westendorf and Williams (2011) reported an increase in the WSP concentration of feces in horses fed mixed hay/grain diets that were heavily supplemented with monosodium phosphate, providing fed 4-times the current P requirement. These researchers did not measure fecal output, however, so daily excretion of WSP was not quantified. In horses fed all-forage diets, 50-90% of the P excreted in manure was water-soluble (Warren et al., 2011).

The concentration and proportion of WSP in manure, as well as its potential for loss in surface runoff differs between species. Kleinman et al. (2000) observed that WSP was greatest in swine slurry, intermediate in layer chicken manure, and lowest in dairy manure. Differences in diet composition and digestive physiology between species make extrapolations from other livestock manure to horses inaccurate. In addition the calcium and phytate-P content of horse manure may affect P solubility, as has been shown in other species (Herrera et al., 2010; Leytem et al., 2007). Characterization of the forms in which P is excreted in horse manure and how diet composition affects P excretion would provide more meaningful information to assess the potential release of P into the environment. More importantly, horse-specific information is necessary given that regulatory agencies may use such values when setting threshold limits form equine operations.

Cereal grains, oilseed meals, and inorganic P sources provide the largest source of P in equine diets, with digestibility ranging from 32 to 47% (Schryver et al., 1971). One factor limiting P availability from plant sources is its association with phytate (myo-inositol hexakisphosphate). Approximately 65-70% of the P in cereal grains, grain byproducts and oilseeds is organically bound in the form of phytate-P (O'dell et al., 1972). Monogastric animals do not produce the phytase enzyme needed to cleave P from the phytate complex and instead rely on microbial breakdown of phytate in the gastrointestinal tract to varying degrees. In horses, availability of P from phytate-P is thought to be approximately half that from inorganic sources such as monosodium phosphate (Hintz et al., 1973). Although phytate-P may result in greater P excretion in manure, it may also reduce the proportion of P that is water-soluble (Leytem et al., 2007), and could potentially be a viable dietary strategy for reducing P loss to the environment.

The objectives of this study were to: 1) characterize total and water-soluble P excretion in horses, and 2) determine how dietary intake of total P and phytate-P affect the fractional distribution of P in manure. We hypothesized that feeding horses above minimum P requirements would result in greater total P and WSP excretion, thereby presenting the greatest environmental risk. Based on the low-solubility of phytate-P, we further speculated that diets with a higher level of phytate-P would reduce excretion of WSP in manure.

CHAPTER 2
LITERATURE REVIEW

Impact of Animal Agriculture on the Environment

Animal production in the United States is valued at more than $100 billion and has consolidated significantly over the past 20 years, with a large number of animals being produced on an increasingly smaller land base (Kellogg et al., 2000). Such consolidation of animal production where nutrient imports in feed and mineral often exceed nutrient exports can generate regional and farm scale nutrient surpluses that can potentially contribute to nonpoint source nutrient pollution of water bodies. (Sharpley et al., 1994; Sims et al., 1998). Nonpoint source pollution, unlike pollution from industrial and sewage treatment plants, comes from many different sources. Animal agriculture plays a large part in nonpoint sources of pollution because the manure, feces and urine that is excreted daily from the animal has the potential to come in contact with runoff during a storm event. This manure-contaminated runoff is eventually deposited into lakes, rivers, wetlands, and coastal waters, and may even leach into underground sources of drinking water. Overall the excreta from livestock animals, such as cattle, swine, poultry and horses, can most certainly have impacts on the environment around them.

Animal Manure

The storage and disposal of animal manure is under increasing scrutiny because of growing public concern over the long term effect on phosphorus and nitrogen accumulation in soils and its contribution to water quality degradation. While nitrogen is a large concern, the focus of this literature review and thesis is on phosphorus.

One of the major concerns with animal manure and the environment is its use as fertilizer. When stockpiled manure is broadcast applied to land, soils will retain most of the phosphorus (P) applied in excess of crop uptake. Of existing manure application methods, broadcasting generally results in the greatest potential for water-soluble P losses in runoff (Romkens et al., 1973; Mueller et al., 1984; Andraski et al., 1985; Zhao et al., 2001). This is due in part to unfavorable nitrogen/phosphorus (N/P) ratios in manure relative to the uptake of these nutrients by most crops, which results in over-application of P when manures are applied to meet the N requirement of the crop (Mikkelsen et al., 2000). As a result, long-term manure application to agricultural land often leads to soil P accumulation, which has the potential to accelerate P transfer in runoff to water bodies (Leytem, 2006). A survey of U.S. horse operations (USDA-APHIS, 1998) found that over half land applied horse manure and stall waste on pastures and fields as their primary means of disposal.

Phosphorus Interactions in the Soil

The forms of P present in soil can include organic, soluble or “bound” forms. Organic phosphorus is the principal form in the manure of most animals. The relative amounts of organic phosphorus vary considerably. Decomposition (mineralization) of organic matter converts organic forms of P to inorganic plant-available forms. Organic P is released more rapidly in warm, well-aerated soils, such as those found in Florida (Schulte, 1996).

Soluble P consists mostly of inorganic P, but can include small amounts of organic P, as well as orthophosphate, the form taken up by plants. It is also the form subject to loss by dissolution in runoff and to a lesser extent leaching. When manure is added to the soil, the soil's pool of soluble P increases. With time, soluble P is transformed slowly to lesser soluble forms, a process known as adsorption.

Attached P is inorganic P that is unavailable to plants. A large amount of the soil's P is bound in compounds that are formed when the anionic forms of dissolved P become attached to cations, such as iron, aluminum and calcium (Wiederholt and Johnson, 2005).

The sequence and time interval between manure application to soil and a runoff event plays a key role in the magnitude of observed P loss (Westerman et al., 1980; Sharpley et al., 1997). Immediately following manure broadcasting, the potential for P loss peaks and then declines over time, as water-soluble P applied in the manure increasingly interacts with soil and is converted to recalcitrant forms (Edwards et al., 1993). Phosphorus occurs in the soil solution as the negatively charged phosphate ion H_2PO_4- in acid soils or HPO_4- in alkaline soils. These ions react readily with iron, aluminum, and manganese compounds in acid soils and with calcium compounds in neutral and alkaline soils. They become strongly attached to the surfaces of these compounds or form insoluble phosphate precipitates (Schulte, 1996). Mueller et al. (1984) reported declining dissolved reactive phosphorus (DRP) concentration (from 0.94-0.26 mg/L) in runoff from no-till plots broadcast with dairy manure over two months of the growing season.

Plant-Available Forms of Phosphorus

Although P is widely distributed in nature, P is not found by itself in elemental form. Elemental P is extremely reactive and will combine with oxygen when exposed to the air. In natural systems P will exist as phosphate. Orthophosphate (PO_4^{3-}) is readily available to the plant (Busman et al., 2002). Phosphorus availability needs to be defined with respect to an external sink, such as a plant, plant community, or crop. Plants differ in their ability to extract P from soils due to differences in rooting systems, mycorrhizal associations, and growth rates (Tiessen and Moir, 1993).

Interactions of Phosphorus with Water

The Environmental Protection Agency (USEPA, 1996) has identified eutrophication as the most widely spread water quality impairment in the United States. Identification of P as one of the main polluting nutrients in fresh water systems has focused attention on P losses from agricultural land, with manure applications and soils that test high in P identified as risk factors (USEPA, 2000; Sims et al., 2000). With few exceptions surface waters receive most of their P in surface water rather than in groundwater, since phosphates bind to most soils and sediments. The exception are where watersheds are of volcanic origin or where soils are water-logged and anoxic (Correll et al., 1998).

Eutrophication is the process by which a body of water acquires a high concentration of nutrients, especially phosphates and nitrates. These typically promote excessive growth of algae. As the algae die and decompose, high levels of organic matter and the decomposing organisms deplete the water of available oxygen, causing the death of other organisms, such as fish. Eutrophication is a natural, slow-aging process for a water body, but human activity greatly speeds up the process (Art et al., 1993).

Phosphorus cycles through the environment, changing form as it does so. Aquatic plants take in dissolved inorganic P and convert it to organic P as it becomes part of their tissues. Various P compounds may be chemically or enzymatically hydrolyzed to orthophosphate, which is the only form of P that can be assimilated by bacteria, algae, and plants. Particulates may be deposited in the bottom sediments, where microbial communities gradually use many of the organic constituents of the sediments, ultimately releasing much of their P contents back to the water column as orthophosphate (Correll et al., 1998).

Surface water concentrations of inorganic P and total P between 0.01 and 0.02 mg/L are considered critical values above which eutrophication is accelerated (Sawyer, 1947; Vollenweider, 1968). These values are an order of magnitude lower than P concentrations in soil solution critical for plant growth (0.2-0.3 mg/L) emphasizing the disparity between critical lake and soil P concentrations and the importance of controlling P losses to limit eutrophication (Tisdale et al., 1985).

Water quality criteria for P have been established (USEPA, 1986). For example, to control eutrophication, total P should not exceed 0.05 mg/L in streams entering lakes/reservoirs, nor 0.025 mg/L within lakes/reservoirs.

Phosphorus as a Dietary Nutrient

Main Functions of Phosphorus in the Body

Phosphorus is the second most abundant macro mineral in the body, after calcium. Approximately 85% of P is found in bones and teeth, while the other 15% is found in soft tissues. Structurally, P occurs as phospholipids, which are a major component of most biological membranes, and as nucleotides and nucleic acids. The functional roles of P include: (1) the buffering of acid or alkali excesses, hence helping to maintain normal pH; (2) the temporary storage and transfer of the energy derived from metabolic fuels (e.g., ATP); and (3) by phosphorylation, the activation of many catalytic proteins. Since phosphate is not irreversibly consumed in these processes and can be recycled indefinitely, the actual function of dietary P is first to support tissue growth (either during individual development or through pregnancy and lactation) and, second, to replace excretory and dermal losses (DRI, 1997).

The main function of P in the body is in the formation of bones and teeth. It plays an important role in the body's utilization of carbohydrates and fats and in the synthesis of protein for the growth, maintenance, and repair of cells and tissues. It is also crucial for the production of ATP, a molecule the body uses to store energy. Phosphorus as phosphate is a major buffer of acid in urine by virtue of its monovalent, divalent, and trivalent forms. Phosphate helps to protect blood systemic acid/base balance, acts as temporary store and transport mechanism for energy and helps in activating catalytic proteins (Diem, 1970).

Phytate-Phosphorus

Phytic acid (myo-inositol hexaphosphoric acid) is an abundant plant constituent, comprising 1-5% of edible legumes, cereals, oil seeds, pollens, and nuts (Graf and Empson, 1987). Phytate (myo-inositol hexakisphosphate) is the primary storage form of P found in cereal grains and constitutes a large portion of the total P contained in seed-based diets (Ravindran, 1994). Approximately 65-70% of the total P in grains and grain byproducts is organically bound in the form of phytate-P (O’dell et al., 1972). This form is relatively unavailable to monogastric animals, such as swine and poultry, because they lack the necessary enzyme to break the bond between phytate and P. Moreover, phytate chelates other minerals and thus makes zinc and iron, and to a lesser extent, calcium and magnesium, unavailable as well.

The enzyme, phytase, catalyzes the hydrolysis of the phosphate monoester from phytic acid resulting in the stepwise formation of *myo*-inositol pentakisphosphate, up to monophosphates, and release of P (Jendza et al., 2009). The stepwise removal of P from phytate usually results in increased P digestibility (Mroz et al., 1994; Traylor et al., 2001; Adeola et al., 2004).

Phosphorus and Phytate-Phosphorus Interactions with Other Nutrients

Phosphorus and calcium

The absolute intakes of calcium (Ca) and P by horses must be adequate, but secondarily it is important to evaluate the Ca:P ratio. If Ca intake is less than P intake (ratio less than 1:1), Ca absorption may be impaired. (NRC, 2007). Hintz (1997) cautioned that horse owners need to be aware of feeding excess P, particularly if horses are fed large amounts of grain-based feedstuffs, such as wheat bran or oats. While a low Ca:P ratio can be detrimental, ratios as high as 6:1 in the growing horse may be acceptable if P intake is adequate (Jordan, 1975).

Minerals such as Ca and other di- and trivalent cations supplemented to diets can form stable complexes with phytate resulting in reduced hydrolysis of phytate-P (Leytem et al., 2007). The formation of stable Ca-phytate complexes that are resistant to hydrolysis by phytase has been thought to be the mechanism whereby Ca reduces the disappearance of phytate from the small intestine of broilers (Maenz et al., 1999; Angel et al., 2002). Such larger complexes may not be available for hydrolysis by phytase enzymes (both endogenous and supplemented) either as a result of changes to the phytate-P structure that preclude it from binding to the substrate-binding site of the enzyme; or due to reduced solubility of the Ca-phytate complex that causes it to precipitate out of solution (Leytem et al., 2007).

In addition to inhibiting phytate hydrolysis, the addition of Ca to diets can cause the precipitation of insoluble Ca-P complexes in poultry litter therefore making the P less soluble (Leytem et al., 2007). Toor et al. (2005) showed that as dietary Ca increased there was an increase in insoluble Ca-P precipitates in poultry manures and litters.

Leytem et al. (2007) observed that an increase in dietary Ca generally decreased phytate-P hydrolysis leading to greater excretion of phytate-P. Also as the dietary Ca:P ratio increases, water soluble phosphorus (WSP) decreased due to an increase in both phytate excretion and formation of insoluble CaP complexes. Warren et al. (2011) observed a higher total P excretion with a proportionally lower water-soluble P content when horses were fed a perennial peanut hay compared to a grass hay, which they attributed to the higher Ca intake from the legume forage.

Phosphorus in Equine Diets

Digestibility of Different Phosphorus Sources

The availability of P from plant sources is fairly low in horses, ranging from 32-42% (Schryver, 1974). Since P availability from these sources may not be enough to meet requirements, additional amounts are generally supplied in the form of inorganic P. However, inorganic sources of P, including dicalcium phosphate, bone meal, and monosodium phosphate, are only slightly more available (44-47%) to the horse than organic sources (Schryver et al., 1974).

Phosphorus Requirements

Endogenous losses of P by the mature horse have been estimated at 10 mg/kg BW/d (Schryver, 1971). Combined with a 35% absorption efficiency, the 1989 NRC estimated the maintenance P requirements for a 500-kg to be 14.3 g (0.028 g/kg BW). Pagan (1994) estimated endogenous phosphorus loss to be 4.7 g/d for a 550 kg horse (about 8.5 mg/kg BW), which is slightly lower than the 1989 NRC estimates. Further, in

Pagan's study, the true digestibility of P was estimated to be 25.2%, which is also lower than the 35% used by the 1989 NRC. However, the resulting requirement for a 500kg horse was estimated by Pagan to be 17 g P/d which is similar to 1989 NRC values. This emphasizes the importance of knowing the availability of a P source when formulating rations as the absorption efficiency can greatly influence the amount of P needed in the diet (NRC, 2007). It is worth noting that the most recent NRC (2007) elected to retain the lower P requirements used in the earlier 1989 edition.

Pattern of Phosphorus Excretion in the Horse

The small intestine has been shown to be the major site of P absorption in ruminants (Pfeffer, 1970), swine (Moore, 1955), and dogs (Bergeim, 1926). However, the phosphate concentration of the fluid of the large colon of the horse is much higher than that of the small intestine and terminal colon (Alexander, 1962), suggesting that phosphate may be secreted to buffer the volatile fatty acids produced in the horse's large intestine (Alexander, 1970). Alexander (1972) further suggested that phosphate may be the more important buffer system in the dorsal colon. Moreover, the P concentration of the large intestinal contents increased at a rate different from that of the water removal of the digesta. These findings suggest that the intestinal absorption and secretion of P by the horse may be regulated (Schryver, 1972).

The renal excretion of P seems to be the most important mechanism in the maintenance of P homeostasis in ponies. The renal excretion of P was small when intake was low, but increased when P intake was increased (Schryver, 1971). Nonetheless, several studies have confirmed that the primary route of P excretion in horses is through the feces (Schryver et al., 1971, 1972; van Doorn et al., 2004; Warren et al., 2011).

Prevalence of Overfeeding Phosphorus

In dairy cattle, several studies indicate a direct link between P intake and P excretion (Morse, 1992; Wu and Satter, 2000; Knowlton et al., 2004, and Knowlton, 2002). Overfeeding of dietary P is common in the industry. Phosphorus is often fed to dairy cattle 20 to 40% in excess of requirements (Shaver and Howard, 1995; Sink, 2000). In dairy cows, the most common explanation for overfeeding of dietary P is the perception that high P diets improve reproductive performance. This perception originates from the observation that severe P deficiency impairs reproductive performance in cattle (Knowlton et al., 2004). There is no research to suggest a benefit from feeding P to dairy cows in excess of NRC requirements (Brodison et al., 1989; Brintrup, 1993; Wu and Satter, 2000). Phosphorus is also overfed because of the inclusion of feeds in the diet that are naturally high in P. Koelsch and Lesoing (1999) constructed nutrient balances for Nebraska livestock farms and found that producers who used corn ethanol byproducts had greater imbalances between P inputs and outputs than producers who did not use corn ethanol byproducts.

Swine manure contains high levels of P, the result of poor digestibility of phytate-P and feeding above the P requirement (Knowlton et al., 2004). Kornegay et al., (2001) presented data from several surveys conducted in the late 1980's through the mid-1990's, indicating that P was being fed at 110 to 155% of requirement as listed in the NRC (1998). The result of such overfeeding is a diet that is more expensive and results in a larger concentration of P in manure (Knowlton et al., 2004).

As in other species, some over-formulation of dietary P occurs in poultry production. The importance of P has led nutritionists to build in a significant margin of safety to reduce the likelihood of problems due to inadequate P (Knowlton et al., 2004).

The nutrient levels provided to horses in practical feeding situations are often much higher than amounts recommended. The National Animal Health Monitoring System, Equine '98 Study (USDA-APHIS, 1998) reported that over 58% of horse operations in the United States fed a vitamin-mineral or protein-vitamin-mineral supplement. Although it is reasonable to assume some of this supplementation may be necessary to meet minimum nutrient requirements, it is also very common for horse owners to provide supplements without regard to the nutrient levels provided by the basal diet (Warren, 2006).

Dietary Strategies for Reducing Phosphorus Loss to the Environment

Concentrated animal agriculture has been identified as a significant source of P contamination of surface water (median contribution = 7 to 48% of total P loads, depending on watershed) (Smith et al., 2000). Greater pressure on states from federal clean water regulations has significantly increased the level of regulatory pressure felt by farmers (Knowlton et al., 2004). The federal Concentrated Animal Feeding Operation regulations to address water pollution call for site-specific decisions on whether N- or P-based manure application limits are needed to protect water quality (EPA, 2001).

Reducing Phosphorus Intake

Dou et al. (2002) reported how varying P concentrations in lactating cow diets affects the amount as well as the chemical forms and fractional distribution of P in fecal excretion. Fecal samples were collected from individual cows in three independent feeding trials each employing diets with varying P concentrations. Across all three experiments, concentrations of total P in fecal samples increased with increasing dietary P. In all feeding trials, the base (control) diets appeared to provide adequate amounts of P. Lowering dietary P concentrations by minimizing unnecessary mineral P supplementation will reduce the excretion of not only total P in feces, but more importantly the most environmentally vulnerable P fraction.

Exogenous Phytase Supplementation

Phytase is often added to diets of non-ruminant animals in order to reduce the need for supplementation with inorganic P (Beegle et al., 2000). Nelson et al.(1968) demonstrated the ability of exogenous phytase to release P bound to phytic acid. However, only recently has a commercially available phytase preparation been approved for use in swine diets. Addition of exogenous (microbial) phytase has been shown to catalyze the hydrolysis of the phytate molecule, releasing bound phytate P (Jongbloed et al., 1992; Cromwell et al., 1993; Lei et al., 1993).

Microbial phytase has been shown to affect performance of pigs fed low-P diets by increasing average daily gains primarily due to an increased feed intake (Simons et al., 1990; Jongbloed et al., 1992; Yi and Kornegay, 1996). The addition of microbial phytase also decreases P excretion by 25 to 50% (Simons et al., 1990; Cromwell et al., 1993; Lei et al., 1993) by increasing P digestibility or retention and by decreasing the need for supplemental inorganic P.

Harper (1997) did two experiments using 413 crossbred growing-finishing pigs to assess the use of a commercial microbial phytase in corn-soybean meal diets to improve phytate-P bioavailability, and thus reduce inorganic P supplementation and fecal P excretion. Results from both experiments indicate that growth performance of pigs fed the low-P corn-soy diets can be restored by supplementing phytase. Overall enhancement of apparent P digestibility with phytase supplementation was 33% in experiment 1 and 44% in experiment 2. The estimates of reduced P excretion with phytase was about 21.5% relative to the pigs fed the 100% NRC (1998) levels. The supplemented phytase brought about an approximate 30% increase in P digestibility.

A few studies have evaluated the effect of diet on ruminal phytase activity. Yanke et al. (1998) observed a linear increase in ruminal phytase activity with decreasing forage: concentrate ratio in steers fed either 100% hay, 55% barley: 45% hay, or 90% barley: 10% hay.

A small number of studies have investigated the addition of phytase to the diets of horses; however, no improvements in phosphorus digestion were observed (Morris-Stoker et al., 2001; Patterson et al., 2002; van Doorn et al., 2004). While it is believed that microbes in the hindgut of the horse can produce some phytase (Hintz et al., 1973), a more likely explanation for the lack of response to phytase observed in these studies was that the diets tested were already sufficient in P or were fortified with inorganic P sources (Warren et al., 2011). In swine and poultry, effects of supplemental phytase are minimized or even eliminated if the basal diet already meets the animal's P requirement (Jongbloed et al., 2000). The magnitude of the response is also dependent upon the type of diet, phytate-P content of the diet, Ca: P ratio, and the age and physiological state of the animal (Knowlton et al., 2004). The use of exogenous phytase in horses deserves further research.

Soluble vs. Insoluble Phosphorus Excretion

According to Spiekers (1993), fecal P can be categorized into three components: (i) unavailable dietary P, referring to dietary P that cannot be absorbed under any conditions; (ii) endogenous P loss, consisting of microbial residue P and metabolic P; and (iii) regulated dietary P, a component that varies according to P intake relative to animal requirement. The regulated P fraction is of primary interest concerning dietary P status. If dietary P is deficient, the animal will utilize as much feed P as possible, and regulated P in feces will be negligible. If dietary P is marginal, some small amount of regulated P in feces is likely. But if dietary P exceeds animal needs, much of the surplus P will be excreted in feces as regulated P (Wu and Satter, 2000).

Dou et al. (2002) reasoned that the unavailable dietary P in feces is largely organic and water-insoluble plant cell wall residues. The endogenous P loss includes P in microbial residues, sloughed gut tissue, and digestive secretions (Wu and Satter, 2000). Most of the P in this fraction would be organic and relatively insoluble, although the smaller portion of P in digestive secretions would be water soluble. In contrast, regulated P in feces would be largely, if not completely, water soluble. The readily soluble P measured in a single water extract of fecal material would provide a relative measure of the regulated P component. If P is fed at the requirement level, readily soluble P will reflect the soluble part of the unavailable plus some small amount of regulated P; as more P is fed in excess of the requirement, it will reflect more of the regulated P. According to this relationship, readily soluble P in feces will increase as more P is fed, and the increase should account for most of the increased fecal P concentration (Dou et al., 2002).

Dissolved organic P, not readily adsorbed on soil particles, may be more mobile than inorganic or orthophosphate, thereby serving as a greater potential threat to water quality (Chardon, 1997). Dou et al. (2002) found that organic P concentrations in the filtrates of a single water extraction of animal manures (i.e. readily soluble P) generally tended to increase as more mineral P was added to the diet. In other words, eliminating or decreasing the amount of mineral P supplementation is likely to provide the benefit of decreasing soluble organic P in feces as well.

If diet manipulation leads to increasing water soluble phosphorus (WSP) to total P ratios (despite decreasing manure total P) and manures are applied at a P-based rate, then the greater WSP application could lead to increased concerns about dissolved reactive phosphorus (DRP) losses in runoff immediately following manure application (Maguire et al., 2005).

Although some studies indicated that feed strategies increased the WSP to TP ratio of manure produced, there was no consistent trend for any feeding strategy that suggested this strategy increase DRP losses in runoff following land application of manure according to a P based nutrient management plan. However, the variability in the data suggest that there is still work to be done on refining diets to ensure that diet modification to reduce manure P consistently decreases P losses in runoff immediately following manure applications (Maguire et al., 2005).

The concentrations and proportions of WSP in manure, as well as its potential for surface P loss to runoff differs between species. Kleinman et al. (2000) observed that WSP and runoff DRP were greatest in swine slurry, intermediate in layer chicken manure, and lowest in dairy manure. Differences in diet composition and digestive physiology between species can make extrapolations from other livestock manure to horses inaccurate. Characterization of the forms in which P is excreted in horse manure and how diet composition affects P excretion would provide more meaningful information to assess the potential release of P into the environment. More importantly, horse-specific information is necessary given that regulatory agencies may use such values when setting threshold limits on equine operations.

The objectives of the research presented in this thesis were to characterize the P fractions in horse manure in terms of their environmental relevance, and to determine how dietary intake of total P and phytate-P affect the fractional distribution of P in horse manure.

CHAPTER 3
MATERIALS AND METHODS

Horses

Eight mature (mean ± SE, 540 ± 22 kg) geldings (4 Thoroughbred, 4 Quarter Horse) were used to evaluate the effects of dietary phosphorus and phytate-phosphorus concentrations on total and water-soluble phosphorus excretion. Horses were housed in individual 3.7 m x 3.7 m box stalls at the Institute of Food and Agricultural Sciences (IFAS) Horse Teaching Unit in Gainesville, FL. Horses also had access to a 3.7 m x 12 m grass-free exercise area attached to each individual stall. Access to the exercise area was permitted for 8 h/d during the diet adaptation phase and limited to two periods of 20 min each during the collection phase. All horses received routine healthcare, including vaccination, anthelmintic treatment and hoof care established in the standard operating procedures for the IFAS Horse Teaching Unit. All animal protocols and procedures were reviewed and approved by the IFAS Animal Care Committee at the University of Florida.

Dietary Treatments

Horses were fed 4 diets that differed in total phosphorus (P) and phytate-P concentrations. Each diet contained one of two levels of total P: 100% or 300% of NRC (2007) requirements for P for horses at maintenance. In addition, each diet contained one of two levels of phytate-P: low phytate-P (≤ 2% of total P in the phytate-P form) or high phytate (≥ 25% of total P in the phytate-P form). Collectively, the two levels of total P and two levels of phytate-P resulted in the following 4 diet combinations: 1) 100% NRC P with low phytate-P (1xNRC-LowPP); 2) 100% NRC P with high phytate-P (1xNRC-HighPP); 3) 300% NRC P with low phytate-P (3xNRC-LowPP); and 4) 300% NRC P with high phytate-P (3xNRC-HighPP). Diet composition and mean daily nutrient intake for each diet are provided in Table 3-1. Diets were isonitrogenous and isocaloric, and contained a similar Ca:P ratio. Daily rations were split into two equal-sized feedings fed at 0700 and 1900 h.

Experimental Design

The 4 experimental diets were fed to 8 horses according to a 4 x 4 Latin square design. In each period, 2 horses were randomly assigned to each of the 4 diets. At the conclusion of each period, dietary treatments were switched until all horses received all diets, giving 8 observations for each experimental diet.

Each period consisted of an 11-d dietary adjustment phase, followed by a 3-d total fecal and urine collection. During each 3-d collection phase, horses were fitted with harnesses specifically designed to facilitate collection of voided urine and feces (Stablemaid PTY, LTD, Melbourne, Australia). The harness permitted the horse to move about freely within the confines of the stall, as well as allowed the horse to lie down to rest. Although the primary route of P excretion in the horse is through the feces, urinary P losses have been shown to increase with the addition of inorganic P sources to the diet (Lavin et al., 2009; Schryver et al., 1971). Therefore, both urine and feces were collected to more accurately assess the effect of diet on total and fractional P excretion in the horse.

Harnesses were emptied of their contents at 8-h intervals (0700, 1500, and 2300 h). Total urine and feces voided were weighed and recorded and a 10% representative sample from each collection was stored at -20ºC until further analysis. Feed offered at each feeding was weighed and recorded. In addition, body weights were obtained prior to the start of the study and at the end of each period and used to adjust dietary intake, as needed.

Sample Preparation and Analyses

All feedstuffs were analyzed by a commercial laboratory (Dairy One, Ithaca, NY) for nutrient composition prior to the start of the study. Feed samples obtained during the collection phase of each period were analyzed for DM content by drying in a 60ºC in a forced-air oven for 48 h. During the collection phase, any feed refused was collected once daily immediately prior to the 0700 h feeding. Orts were dried at 60ºC in a forced-air oven for 48 h and weight change was recorded. Calculations of daily nutrient intake were adjusted, where appropriate, to account for feed refusals.

For each horse in each period, fecal and urine samples were thawed, thoroughly mixed and composited into samples that represented each 24-h cycle within a 3-d collection. For example, the day 1 composite began with the samples obtained at 1500 h on the first day of the collection phase and ended with the samples obtained at 0700 h on the second day of the collection phase. This resulted in 3 fecal and 3 urine samples per horse per period. Once the 24-h composited samples were processed and analyzed (as described below), data were averaged resulting in one mean for each nutrient or variable of interest per horse per diet.

Fecal samples were dried at 60ºC in a forced-air oven for 48 h and weight change was recorded. Dried feces were then finely ground to pass a 1 mm screen using a Wiley mill. A sub-sample of ground feces was further oven-dried at 105ºC for 24 h for determination of DM content. To determine mineral content of feces, dried samples were first ashed overnight in a muffle furnace at 600ºC and digested in triplicate with 5% hydrochloric acid. Fecal total P was determined colorimetrically and quantified with standards of known concentration using the molybdate blue method (Harris and Popat, 1954) and read at 660 nm on a PowerWave XS microplate reader (BioTek Instruments, Winooski, VT). Fecal calcium, magnesium, copper and zinc concentrations were determined in triplicate on previously ashed and acid-digested samples by atomic absorption spectrometry (AA800, PerkinElmer Corp., Norwalk, CT). Fecal water-soluble P (WSP) was determined using a modification of the method described by Self-Davis and Moore (2000). Briefly, a 50 mL volume of a 1:20 slurry of ground, oven-dried feces and deionized water was created. The mixture was shaken at a rate of 180 excursions/min for 20 min and then centrifuged at 1250 *g* for 20 min. The supernatant was passed through a 0.45 μm membrane and the WSP content of the filtrate was quantified colorimetrically using the molybdate blue method.

Urine total P was determined on thawed, 24-h composited liquid samples using the molybdate blue method. It was assumed that 100% of the total P in urine was also water-soluble; thus a separate determination of urine WSP was not made. Urinary calcium, magnesium, copper and zinc concentrations were determined in duplicate on liquid samples by atomic absorption spectrometry.

Calculations

Phosphorus excretion was evaluated as fecal total P, fecal WSP, and urinary total P. In addition, P excretion was evaluated as total P and WSP excretion in manure according to Equations 3-1 and 3-2. Total P, WSP, Ca, Mg, Zn and Cu excretion in feces, urine and manure were compared among dietary treatments as g/d and mg/(kg $BW \cdot d^{-1}$). In addition, the concentration (% or mg/kg DM) of total P, WSP, Ca, Mg, Zn, and Cu in feces and urine were compared.

$$\text{Manure total P excretion} = \text{fecal total P} + \text{urinary total P} \qquad (3\text{-}1)$$

$$\text{Manure soluble P excretion} = \text{fecal soluble P} + \text{urinary total P} \qquad (3\text{-}2)$$

Apparent digestibility of P and P balance were calculated according to Equations 3-3 and 3-4, respectively.

$$\%\ \text{Apparent digestibility} = [(\text{P intake} - \text{fecal P excretion}) / \text{P intake}] \times 100 \qquad (3\text{-}3)$$

$$\text{P Balance (g/d)} = \text{P intake} - \text{fecal P excretion} - \text{urinary P excretion} \qquad (3\text{-}4)$$

Statistics

To determine differences between dietary treatments, all data were analyzed using one-way ANOVA as part of the PROC MIXED procedure of SAS (Version 9.3, SAS Institute Inc., Cary, NC.). Horse, period, and diet were evaluated as fixed effects. Treatment means were compared after Dunnett's adjustment of the LSMEANS. A second ANOVA was used to evaluate the effects of dietary P and phyate-P concentrations separately (as opposed to the 4 diet combinations), with horse, period, dietary P level (100% vs. 300% of NRC P requirements), dietary phytate-P level (high vs. low phytate-P) and the interaction of P and phytate-P level included in the model as fixed effects. In all ANOVA, individual treatment means were compared using a Dunnett's adjustment of the LSMEANS statement. Because intake of magnesium, copper, and zinc varied between diets (Table 3-1), fecal and urinary excretion of these minerals was compared between diets using intake as a covariate. All data are expressed as the LSMEAN ± SE. Differences were considered significant at $P \leq 0.05$.

Table 3-1. Composition of the four experimental diets and mean daily nutrient intake for each diet.

Feedstuff or nutrient	Diet[1] 1xNRC-LowPP	1xNRC-HighPP	3xNRC-LowPP	3xNRC-HighPP
	---------------- Diet composition, % of daily DMI ----------------			
Coastal bermudagrass hay	94.47	61.70	86.66	52.55
Oats, whole	0	13.46	9.12	7.36
Corn, cracked	0	22.44	0	7.36
Wheat middlings	0	0	0	28.38
Corn oil	4.97	0	0.91	0
Molasses	0	1.33	0.68	1.60
Calcium carbonate	0	0.39	0.87	1.89
Calcium monophosphate	0	0	1.19	0.26
Trace mineral mixture[2]	0.28	0.67	0.32	0.19
Salt	0.28	0	0.26	0.42
	-------------Mean daily nutrient intake[3] -------------			
DM, kg/d	9.1 [b]	8.9 [b]	10.4 [a]	9.3 [b]
DE, Mcal/d	20.9	21.8	21.2	21.5
CP, kg/d	877.0 [b]	815.9 [b]	994.9 [a]	1148.8 [a]
Ca, g/d	38.6 [b]	41.7 [b]	97.4 [a]	103.9 [a]
Mg, g/d	17.0 [c]	14.5 [d]	19.8 [b]	24.3 [a]
P, g/d	20.7 [b]	22.3 [b]	51.8 [a]	53.4 [a]
Phytate-P, g/d	0.2 [c]	5.3 [b]	1.4 [c]	26.4 [a]
Ca:P ratio	1.86	1.87	1.88	1.94
Cu, mg/d	178.8 [a]	197.4 [a]	207.3 [a]	145.7 [b]
Zn, mg/d	416.5 [b]	458.3 [a]	483.8 [a]	474.5 [a]

[1] Diets contained either 100% (1xNRC) or 300% (3xNRC) of the P requirement for horses at maintenance (NRC, 2007) and either low (LowPP) or high (HighPP) phytate-P.

[2] Trace mineral mixture contained (g/kg): 60 calcium, 10 phosphorus, 650 sodium chloride, 1.0 potassium, 1.0 magnesium, 3.0 sulfur, 0.05 cobalt, 1.5 copper, 0.21 iodine, 0.5 manganese, 0.04 selenium, and 3.0 zinc.

[3] Mean daily nutrient intake reflects actual intake for all nutrients.

[a,b,c] Means in the same row differ ($P < 0.05$).

CHAPTER 4
RESULTS

Total Phosphorus

As expected, P intake differed between diets ($P < 0.0001$). Daily P intake was lower in 1xNRC diets compared to 3xNRC diets ($P < 0.0001$), but did not differ between 1xNRC-LowPP and 1xNRC-HighPP, nor between 3xNRC-LowPP and 3xNRC-HighPP (Table 3-1).

On an as-excreted basis, the concentration of total P in feces was affected by diet ($P < 0.0001$), where 1xNRC-LowPP had the lowest concentration ($P < 0.01$) and 3xNRC-HighPP had the highest concentration ($P < 0.0001$) compared to the other diets (Table 4-1). Fecal total P concentration was intermediate in 1xNRC-HighPP and 3xNRC-LowPP, which did not differ from each other ($P = 0.093$) (Table 4-1).However, the difference between these latter two diets became significant when fecal P concentration was expressed on a DM basis. On a DM basis, fecal P concentration differed among all diets ($P < 0.0001$), with the highest concentration noted in 3xNRC-HighPP ($P < 0.0001$), followed by 3xNRC-LowPP ($P < 0.01$), 1xNRC-HighPP ($P < 0.05$), and the lowest concentration of total P in feces observed in the 1xNRC-LowPP diet ($P < 0.05$) (Table 4-1).

Daily fecal excretion of total P, expressed as either grams per day ($P < 0.0001$; Table 4-2) or as milligrams per kilogram of BW ($P < 0.0001$; Figure 4-1) was also affected by diet. On an absolute and BW basis, daily excretion of total P in feces was the greatest with 3xNRC-HighPP ($P < 0.001$), followed by 3xNRC-LowPP ($P < 0.0001$). The lowest daily total P excretion in feces was observed when horses received either

1xNRC-HighPP or 1xNRC-LowPP, which did not differ from each other (*P* =0.372) (Table 4-2 and Figure 4-1).

In contrast to feces, diet had no effect (*P* = 0.389) on the concentration of total P in urine on an as-excreted basis (Table 4-1). There was an overall effect of diet on daily P excretion in urine when expressed as grams per day (*P* = 0.031), yet there were no identifiable differences between specific diets (Table 4-2). When urinary P excretion was expressed on a BW basis, there was no differences between diets (*P* = 0.062; Figure 4-2).

Feces was the primary route of P excretion for all diets, thus it had the biggest influence on total P excretion in manure (Table 4-2). Similar to that observed for fecal total P excretion, daily excretion of total P in manure, expressed as either grams per day (*P* < 0.0001; Table 4-2) or as milligrams per kilogram of BW (*P* < 0.0001; Figure 4-3) was affected by diet. On an absolute and BW basis, daily excretion of total P in manure was the greatest with 3xNRC-HighPP (*P* < 0.01), followed by 3xNRC-LowPP (*P* < 0.0001). Excretion of total P in the combined manure was lowest when horses received either the 1xNRC-HighPP or 1xNRC-LowPP diets, which did not differ from each other (Table 4-2 and Figure 4-3).

Water-Soluble Phosphorus

On an as-excreted basis, the concentration of WSP in feces was affected by diet (*P* < 0.0001) (Table 4-1). Similar to the pattern noted for total P, the percent WSP in feces was highest with 3xNRC-HighPP (*P* < 0.0001) and lowest when horses received the 1xNRC-LowPP (*P* < 0.01) (Table 4-1). Fecal WSP concentration was intermediate within the 3xNRC-LowPP and 1xNRC-HighPP diets, which did not differ from each other

(*P* = 0.529). Similar differences between diets were observed when fecal WSP concentration was expressed on a DM basis (Table 4-1).

Daily fecal excretion of WSP, expressed as either grams per day (*P* < 0.0001; Table 4-2) or as milligrams per kilogram of BW (*P* < 0.0001; Figure 4-4) was also affected by diet. On an absolute and BW basis, daily fecal excretion of WSP was highest with 3xNRC-HighPP (*P* < 0.01), followed by 3xNRC-LowPP (*P* < 01). The lowest daily WSP excretion in feces was observed when horses received either 1xNRC-HighPP or 1xNRC-LowPP, which did not differ from each other (Table 4-2 and Figure 4-4).

The quantity of WSP in feces exceeded the amount of P excreted in urine; thus, WSP excretion in manure followed a similar pattern as that observed for WSP excretion in feces (Table 4-2, Figure 4-5). Daily excretion of WSP in manure, expressed as either grams per day (*P* < 0.0001; Table 4-2) or as milligrams per kilogram of BW (*P* < 0.0001; Figure 4-5) was affected by diet. On an absolute and BW basis, daily excretion of manure WSP was highest with 3xNRC-HighPP (*P* < 0.01), followed by 3xNRC-LowPP (*P* < 0.01). Excretion of WSP in the collective manure was lowest when horses received either the 1xNRC-HighPP 1xNRC-LowPP diets, which did not differ from each other (Table 4-2 and Figure 4-5).

The proportion of total P in manure that was water-soluble was also affected by diet (*P* = 0.004) (Table 4-2). Further analysis demonstrated an effect of dietary P level (*P* = 0.0009), but not phytate-P level (*P* = 0.088) on fractional P excretion. Diets providing 100% of the NRC requirements for P resulted in a greater (P < 0.001) proportion of P excretion in soluble form compared to diets providing 300% of NRC recommendations (Table 4-2).

Apparent Phosphorus Digestibility and Balance

Phosphorus apparent digestibility was affected by diet ($P = 0.003$) (Table 4-3). The effect of diet was predominantly due to phytate-P intake ($P < 0.0001$), where both diets with low amounts of phytate-P had a greater ($P < 0.05$) apparent P digestibility compared to diets with high amounts of phytate-P. In contrast, dietary P level had no effect on P digestibility ($P = 0.079$), as there was no difference between 1xNRC-HighPP and 3xNRC-HighPP ($P = 0.430$) nor 1xNRC-LowPP and 3xNRC-LowPP ($P = 0.435$).

All horses exhibited positive P balance during the study. Phosphorus balance was affected by diet ($P < 0.0001$) (Table 4-3). Diets containing 100% of NRC requirements for P had lower ($P < 0.001$) P balance than diets containing 300% of NRC requirements. Phosphorus balance was similar for 1xNRC-LowPP and 1xNRC-HighPP ($P = 0.710$) and between 3xNRC-LowPP and 3xNRC-HighPP ($P = 0.061$).

Fecal and Urinary Excretion of Other Minerals

Calcium

To maintain similar Ca:P ratios among diets, dietary Ca supply paralleled dietary P intake. As expected, dietary Ca intake was higher ($P < 0.0001$) in the 3xNRC diets (300% of NRC Ca requirements) compared to the 1xNRC diets (100% of Ca requirements). Calcium intake did not differ between 1xNRC-LowPP and 1xNRC-HighPP ($P = 0.771$) or between 3xNRC-LowPP and 3xNRC-HighPP ($P = 0.147$) (Table 3-1).

When expressed either on an as-excreted basis ($P < 0.0001$) or DM basis ($P < 0.0001$) , the concentration of Ca in feces was affected by diet (Table 4-4). Fecal Ca concentration differed among all diets, where the highest concentration of fecal Ca was noted for 3xNRC-HighPP ($P < 0.0001$), followed by 3xNRC-LowPP ($P < 0.01$), 1xNRC-HighPP ($P < 0.01$), and the lowest concentration of fecal Ca was observed in 1xNRC-LowPP ($P < 0.01$) (Table 4-4). Daily excretion of Ca in feces, expressed as either grams per day ($P < 0.0001$; Table 4-4) or milligrams per kilogram of BW ($P < 0.0001$; Figure 4-6) was also affected by diet. On an absolute and BW basis, daily excretion of Ca in feces was highest with 3xNRC-HighPP ($P < 0.0001$), followed by 3xNRC-LowPP ($P < 0.0001$), then 1xNRC-HighPP ($P < 0.05$), and lowest when horses received the 1xNRC-LowPP diet ($P < 0.05$).

The concentration of Ca in urine was affected by diet ($P = 0.0004$), with 3xNRC-HighPP exhibiting the highest ($P < 0.05$) urine Ca concentration compared to all other diets (Table 4-5). Daily excretion of Ca in urine, expressed as either grams per day ($P = 0.001$; Table 4-5) or milligrams per kilogram of BW ($P = 0.001$; Figure 4-10) was also affected by diet. Urinary Ca excretion was primarily affected by Ca intake ($P < 0.0001$) rather than phytate-P intake ($P = 0.432$) and, as a result, was higher ($P < 0.05$) in both 3xNRC diets compared to the 1xNRC diets on both an absolute (Table 4-5) and BW basis (Figure 4-10).

Magnesium

Magnesium intake differed between diets ($P < 0.0001$), with the highest intake observed for 3xNRC-HighPP ($P < 0.01$), followed by 3xNRC-LowPP ($P < 0.01$), 1xNRC-LowPP ($P < 0.01$), and the lowest daily Mg intake observed in 1xNRC-HighPP ($P < 0.01$). As a result of these unintentional differences, Mg intake was used as a covariate in the statistical model when determining differences in fecal and urinary Mg excretion between the four dietary treatments.

The concentration of Mg in feces, expressed on an as-excreted basis ($P < 0.0001$) or DM basis ($P < 0.0001$) was affected by diet (Table 4-4). Further analysis showed that dietary phytate-P level ($P < 0.0001$), rather than dietary P level ($P = 0.403$ and $P = 0.229$ for as-excreted and DM basis, respectively) accounted for the differences in fecal Mg concentration. Fecal Mg concentration was highest in 3xNRC-HighPP ($P < 0.05$) compared to 1xNRC-LowPP and 3xNRC-LowPP. Fecal Mg concentration did not differ between 1xNRC-LowPP, 1xNRC-HighP and 3xNRC-LowPP (Table 4-4).The concentration of Mg in urine ($P = 0.925$; Table 4-5) and daily excretion of Mg in feces ($P = 0.224$; Table 4-4 and Figure 4-7) and urine ($P = 0.881$; Table 4-5 and Figure 4-11) were not affected by diet.

Zinc

Zinc intake differed between diets ($P = 0.005$), with intake being lower ($P < 0.05$) in 1xNRC-LowPP compared to all other diets (Table 3-1). Because of these differences, Zn intake was used as a covariate to determine differences in fecal and urinary Zn excretion.

The concentration of Zn in feces, expressed on an as-excreted basis ($P < 0.0001$) or DM basis ($P < 0.0001$) was affected by diet (Table 4-4). Further analysis showed that dietary phytate-P level ($P < 0.0001$), rather than dietary P level ($P = 0.399$ and $P = 0.172$ for as-excreted and DM basis, respectively) accounted for the differences in fecal Zn concentration. Fecal Zn concentration differed among all diets where it was highest in 3xNRC-HighPP ($P < 0.001$), followed by 1xNRC-HighPP ($P < 0.01$), 3xNRC-LowPP ($P < 0.001$), and lowest in 1xNRC-LowPP ($P < 0.01$) (Table 4-4). Daily excretion of Zn in feces, expressed as either milligrams per day ($P < 0.0001$; Table 4-4) or milligrams per kilogram BW per day ($P < 0.0001$; Figure 4-8) was also affected by diet. Fecal Zn excretion on an absolute and BW basis was greatest when horses received 3xNRC-HighPP ($P < 0.0001$) and lowest with 1xNRC-LowPP ($P < 0.0001$) compared to all other diets. Fecal zinc excretion was intermediate in 1xNRC-HighPP and 3xNRC-LowPP, but did not differ between these two diets ($P = 0.916$) (Table 4-4 and Figure 4-8). Neither the concentration of Zn in urine ($P = 0.473$; Table 4-5) nor the daily excretion of Zn in urine ($P = 0.804$; Figure 4-12) were affected by diet.

Copper

Copper intake differed between diets ($P = 0.002$), with intake being lower ($P < 0.05$) in 3xNRC-HighPP compared to all other diets (Table 3-1). Because of these differences, Cu intake was used as a covariate to determine differences in fecal and urinary Cu excretion.

The concentration of Cu in feces, expressed on an as-excreted basis ($P < 0.0001$) or DM basis ($P < 0.0001$) was affected by diet (Table 4-4). Similar to fecal Mg and Zn, further analysis showed that dietary phytate-P level ($P < 0.0001$), rather than dietary P level ($P = 1.000$ and $P = 0.816$ for as-excreted and DM basis, respectively) accounted for the differences in fecal Cu concentration. Fecal Cu concentration was higher in 1xNRC-HighPP ($P < 0.001$) and lowest in 1xNRC-LowPP ($P < 0.001$). The 3xNRC diets contained intermediate concentrations of fecal Cu and did not differ from each other ($P = 0.187$; Table 4-4). A similar pattern of differences existed for fecal Cu excretion, expressed on an absolute basis (Table 4-4) and BW basis (Figure 4-9). In contrast, urinary Cu concentration ($P = 0.135$; Table 4-5) and excretion (P = 0.519; Table 4-5 and Figure 4-13) were unaffected by diet.

Table 4-1. Concentration of total phosphorus and water-soluble phosphorus (WSP) in feces and urine on an as-excreted and DM basis when horses were fed diets containing either 100% (1xNRC) or 300% (3xNRC) of phosphorus requirements and either low (LowPP) or high (HighPP) amounts of phytate-phosphorus.

	Diet					*P*-values		
Item	1xNRC-LowPP	1xNRC-HighPP	3xNRC-LowPP	3xNRC-HighPP	SEM	Diet	Period	Horse
Feces total P, % as-excreted	0.033[c]	0.066[b]	0.086[b]	0.157[a]	0.006	<0.0001	0.372	0.669
Feces total P, % DM basis	0.15[d]	0.26[c]	0.37[b]	0.65[a]	0.02	<0.0001	0.207	0.272
Feces WSP, % as-excreted	0.020[c]	0.035[b]	0.040[b]	0.065[a]	0.003	<0.0001	0.289	0.526
Feces WSP, % DM basis	0.09[c]	0.14[b]	0.17[b]	0.27[a]	0.01	<0.0001	0.189	0.349
Urine total P, % as-excreted	0.0023	0.0030	0.0027	0.0028	0.0003	0.389	0.085	0.339

[a,b,c,d] Means in the same row with different superscripts differ ($P < 0.01$).

Table 4-2. Daily total and water-soluble phosphorus excretion in feces and urine when horses were fed diets containing either 100% (1xNRC) or 300% (3xNRC) of phosphorus requirements and either low (LowPP) or high (HighPP) amounts of phytate-phosphorus.

	Diet					*P*-values		
Item	1xNRC-LowPP	1xNRC-HighPP	3xNRC-LowPP	3xNRC-HighPP	SEM	Diet	Period	Horse
Fecal total P, g/d	9.75 [c]	12.22 [c]	22.63 [b]	31.78 [a]	0.77	<0.0001	0.783	0.085
Urinary total P, g/d	0.14	0.21	0.14	0.22	0.04	0.031	0.148	0.117
Manure total P, g/d	9.90 [c]	12.44 [c]	22.77 [b]	32.00 [a]	0.77	<0.0001	0.810	0.085
Fecal water-soluble P, g/d	5.68 [c]	6.67 [c]	10.84 [b]	13.37 [a]	0.32	<0.0001	0.053	0.003
Manure water-soluble P, g/d	5.82 [c]	6.88 [c]	10.98 [b]	13.59 [a]	0.33	<0.0001	0.046	0.002
Water-soluble P as % of total P	58.3 [a]	54.4 [a]	48.1 [b]	43.0 [b]	2.4	0.004	0.065	0.503

[a,b,c] Means in the same row with different superscripts differ ($P < 0.01$).

Table 4-3. Apparent digestibility and balance of phosphorus when horses were fed diets containing either 100% (1xNRC) or 300% (3xNRC) of phosphorus requirements and either low (LowPP) or high (HighPP) amounts of phytate-phosphorus.

	Diet					*P*-values		
Item	1xNRC-LowPP	1xNRC-HighPP	3xNRC-LowPP	3xNRC-HighPP	SEM	Diet	Period	Horse
P Digestibility, %	50.3^{a}	40.6^{b}	51.0^{a}	39.5^{b}	2.3	0.003	0.390	0.839
P Balance, g/d	8.8^{c}	8.2^{c}	24.5^{a}	21.3^{b}	1.0	<0.0001	0.531	0.792

^{a,b,c} Means in the same row with different superscripts differ ($P < 0.01$).

Table 4-4. Concentration and daily excretion of calcium, magnesium, zinc, and copper in feces when horses were fed diets containing either 100% (1xNRC) or 300% (3xNRC) of phosphorus requirements and either low (LowPP) or high (HighPP) amounts of phytate-phosphorus.

	Diet					*P*-values		
	1xNRC-LowPP	1xNRC-HighPP	3xNRC-LowPP	3xNRC-HighPP	SEM	Diet	Period	Horse
Calcium, % as-excreted	0.12 [d]	0.32 [c]	0.46 [b]	0.75 [a]	0.03	<0.0001	0.123	0.174
Calcium, % DM basis	0.56 [d]	1.23 [c]	1.96 [b]	3.11 [a]	0.10	<0.0001	0.109	0.222
Calcium, g/d	31.2 [d]	57.7 [c]	123.2 [b]	155.6 [a]	3.6	<0.0001	0.516	0.005
Magnesium, % as-excreted	0.09 [c]	0.12 [a,b]	0.10 [b]	0.19 [a]	0.02	<0.0001	0.022	0.928
Magnesium, % DM basis	0.42 [c]	0.48 [a,b]	0.43 [b]	0.78 [a]	0.08	<0.0001	0.033	0.760
Magnesium, g/d	23.9	25.2	26.3	36.2	4.9	0.098	0.017	0.555
Zinc, mg/kg as-excreted	15.3 [d]	45.9 [b]	29.8 [c]	60.5 [a]	2.5	<0.0001	0.519	0.033
Zinc, mg/kg DM	74.6 [d]	176.1 [b]	131.1 [c]	250.7 [a]	11.1	<0.0001	0.835	0.213
Zinc, mg/d	475.1 [c]	818.7 [b]	817.3 [b]	1233.3 [a]	32.5	<0.0001	0.216	0.018
Copper, mg/kg as-excreted	3.2 [c]	16.2 [a]	7.4 [b]	9.9 [b]	0.9	<0.0001	0.091	0.065
Copper, mg/kg DM	14.9 [c]	62.5 [a]	32.5 [b]	41.0 [b]	3.5	<0.0001	0.231	0.299
Copper, mg/d	87.5 [d]	278.6 [a,c]	189.0 [b]	228.5 [b,c]	18.5	<0.0001	0.698	0.242

[a,b,c,d] Means in the same row with different superscripts differ ($P < 0.05$)

Table 4-5. Concentration and daily excretion of calcium, magnesium, zinc, and copper in urine when horses were fed diets containing either 100% (1xNRC) or 300% (3xNRC) of phosphorus requirements and either low (LowPP) or high (HighPP) amounts of phytate-phosphorus.

	Diet					P-values		
	1xNRC-LowPP	1xNRC-HighPP	3xNRC-LowPP	3xNRC-HighPP	SEM	Diet	Period	Horse
Calcium, % as-excreted	0.27 [b]	0.24 [b]	0.37 [a]	0.30 [a]	0.02	0.0004	0.830	0.004
Calcium, g/d	15.6 [b]	15.5 [b]	24.3 [a]	22.8 [a]	1.6	0.001	0.925	0.095
Magnesium, % as-excreted	0.07	0.06	0.06	0.05	0.02	0.099	0.690	0.100
Magnesium, g/d	4.1	3.9	3.9	3.9	0.9	0.925	0.731	0.039
Zinc, mg/kg as-excreted	0.59	0.65	0.61	0.54	0.07	0.473	0.433	0.020
Zinc, mg/d	3.6	4.1	4.1	3.8	0.50	0.756	0.956	0.027
Copper, mg/kg as-excreted	0.01	0.03	0.03	0.01	0.01	0.135	0.025	0.445
Copper, mg/d	0.09 [d]	0.16	0.11	0.09	0.05	0.544	0.052	0.445

[a,b] Means in the same row with different superscripts differ ($P < 0.05$)

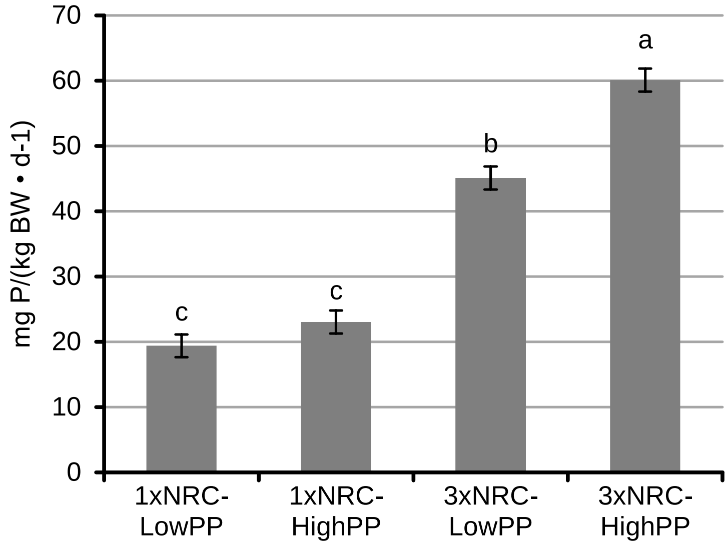

Figure 4-1. Mean (± SE) daily excretion of total phosphorus in feces when horses were fed diets containing either 100% (1xNRC) or 300% (3xNRC) of phosphorus requirements and either low (LowPP) or high (HighPP) amounts of phytate-phosphorus. Overall effect of diet ($P < 0.0001$), period ($P = 0.818$) and horse ($P = 0.327$). [a,b,c]Means with different letters differ ($P < 0.001$).

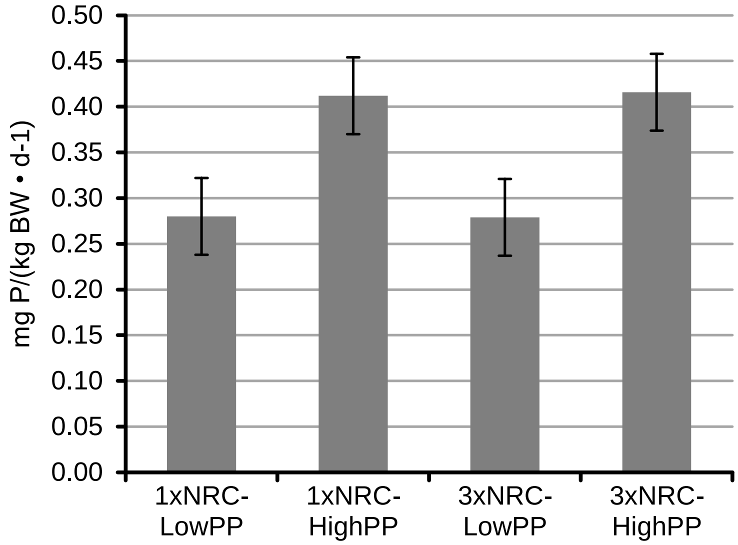

Figure 4-2. Mean (± SE) daily excretion of total phosphorus in urine when horses were fed diets containing either 100% (1xNRC) or 300% (3xNRC) of phosphorus requirements and either low (LowPP) or high (HighPP) amounts of phytate-phosphorus. Overall effect of diet (P = 0.062), period (P = 0.158) and horse (P = 0.064).

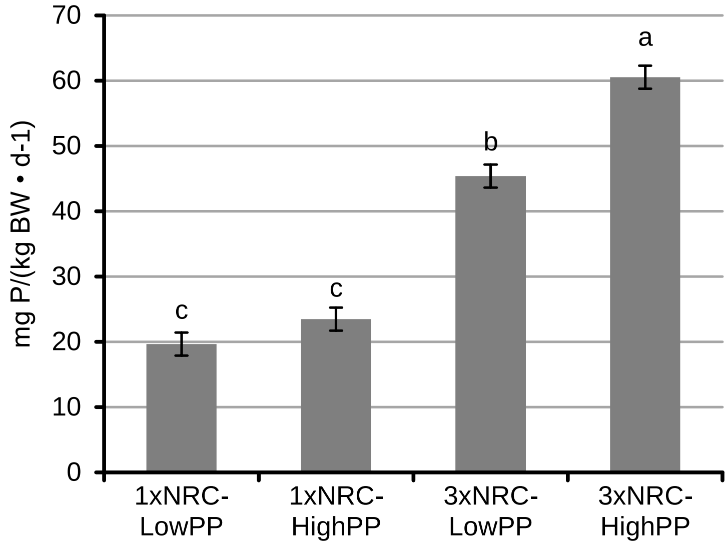

Figure 4-3. Mean (± SE) daily excretion of total phosphorus in manure when horses were fed diets containing either 100% (1xNRC) or 300% (3xNRC) of phosphorus requirements and either low (LowPP) or high (HighPP) amounts of phytate-phosphorus. Overall effect of diet (P < 0.0001), period (P = 0.823) and horse (P = 0.309). [a,b,c]Means with different letters differ (P < 0.001).

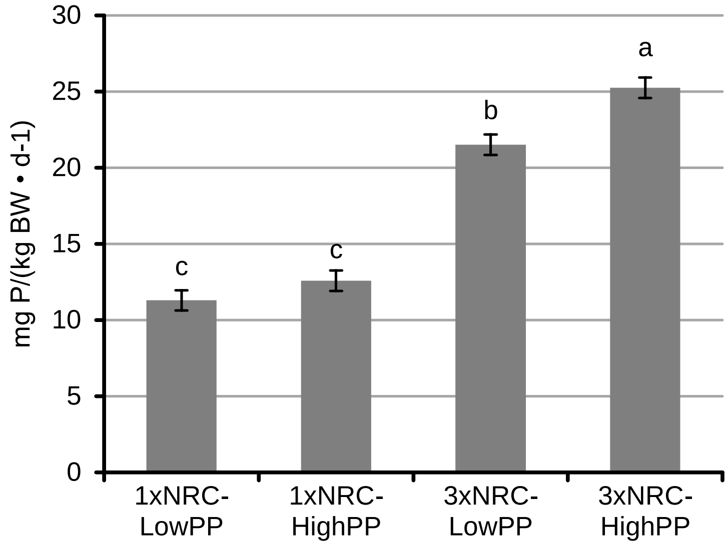

Figure 4-4. Mean (± SE) daily excretion of water-soluble phosphorus in feces when horses were fed diets containing either 100% (1xNRC) or 300% (3xNRC) of phosphorus requirements and either low (LowPP) or high (HighPP) amounts of phytate-phosphorus. Overall effect of diet ($P < 0.0001$), period ($P = 0.100$) and horse ($P = 0.025$). [a,b,c]Means with different letters differ ($P < 0.01$).

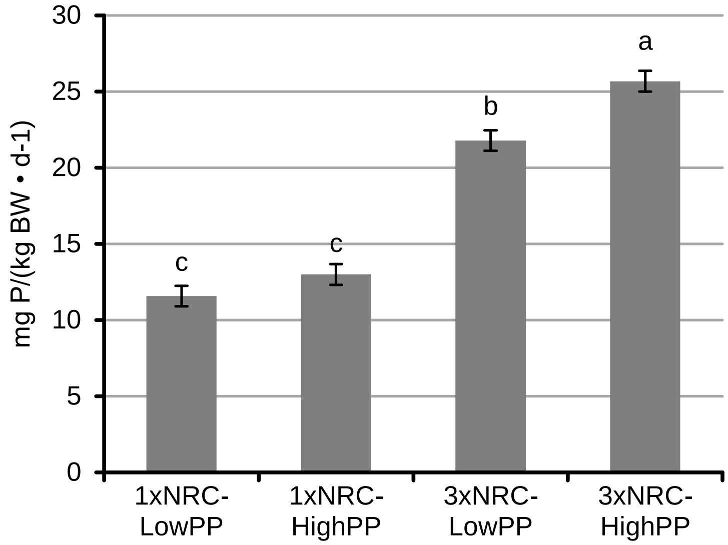

Figure 4-5. Mean (± SE) daily excretion of water-soluble phosphorus in manure when horses were fed diets containing either 100% (1xNRC) or 300% (3xNRC) of phosphorus requirements and either low (LowPP) or high (HighPP) amounts of phytate-phosphorus. Overall effect of diet ($P < 0.0001$), period ($P = 0.081$) and horse ($P = 0.021$). [a,b,c]Means with different letters differ ($P < 0.01$).

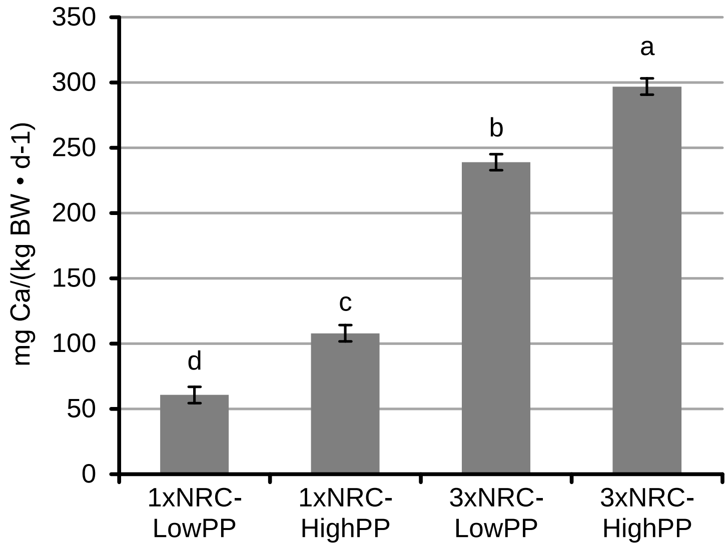

Figure 4-6. Mean (± SE) daily excretion of calcium in feces when horses were fed diets containing either 100% (1xNRC) or 300% (3xNRC) of phosphorus requirements and either low (LowPP) or high (HighPP) amounts of phytate-phosphorus. Overall effect of diet ($P < 0.0001$), period ($P = 0.595$) and horse ($P = 0.056$). [a,b,c,d]Means with different letters differ ($P < 0.05$).

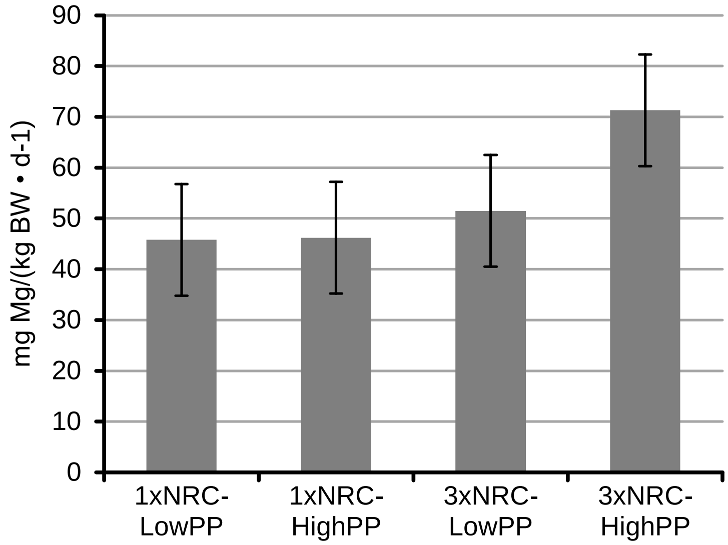

Figure 4-7. Mean (± SE) daily excretion of magnesium in feces when horses were fed diets containing either 100% (1xNRC) or 300% (3xNRC) of phosphorus requirements and either low (LowPP) or high (HighPP) amounts of phytate-phosphorus. Overall effect of diet (*P* =0.224), period (*P* = 0.042) and horse (*P* = 0.482).

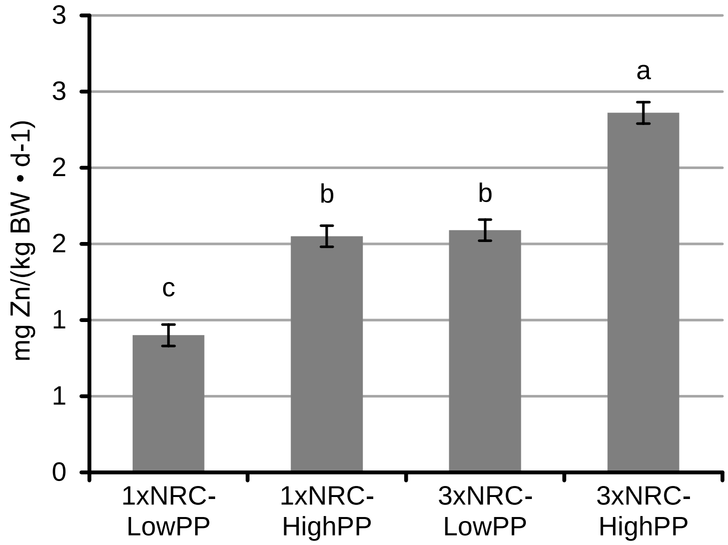

Figure 4-8. Mean (± SE) daily excretion of zinc in feces when horses were fed diets containing either 100% (1xNRC) or 300% (3xNRC) of phosphorus requirements and either low (LowPP) or high (HighPP) amounts of phytate-phosphorus. Overall effect of diet ($P < 0.0001$), period ($P = 0.023$) and horse ($P = 0.038$). [a,b,c] Means with different letters differ ($P < 0.001$).

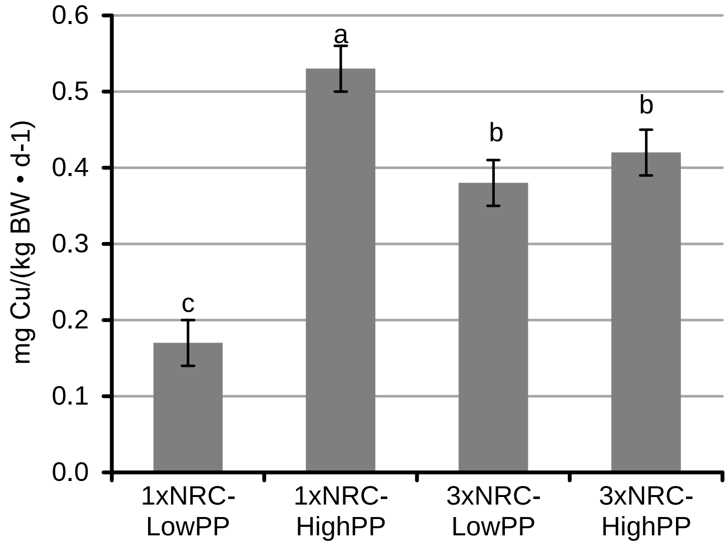

Figure 4-9. Mean (± SE) daily excretion of copper in feces when horses were fed diets containing either 100% (1xNRC) or 300% (3xNRC) of phosphorus requirements and either low (LowPP) or high (HighPP) amounts of phytate-phosphorus. Overall effect of diet ($P < 0.0001$), period ($P = 0.820$) and horse ($P = 0.030$). [a,b,c] Means with different letters differ ($P < 0.05$).

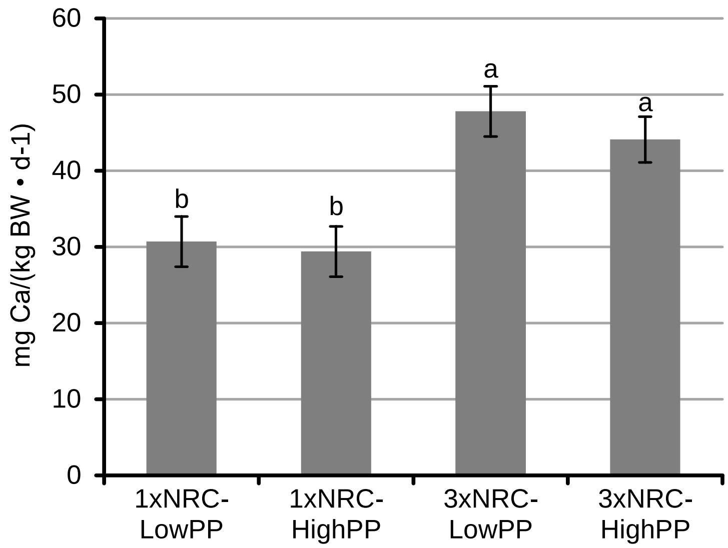

Figure 4-10. Mean (± SE) daily excretion of calcium in urine when horses were fed diets containing either 100% (1xNRC) or 300% (3xNRC) of phosphorus requirements and either low (LowPP) or high (HighPP) amounts of phytate-phosphorus. Overall effect of diet (P = 0.001), period (P = 0.861) and horse (P = 0.03). [a,b]Means with different letters differ (P < 0.05).

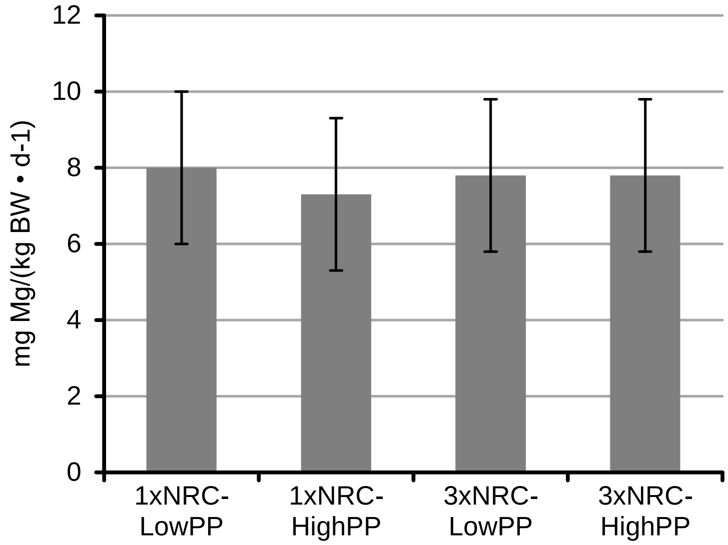

Figure 4-11. Mean (± SE) daily excretion of magnesium in urine when horses were fed diets containing either 100% (1xNRC) or 300% (3xNRC) of phosphorus requirements and either low (LowPP) or high (HighPP) amounts of phytate-phosphorus. Overall effect of diet (*P* =0.868), period (*P* = 0.639) and horse (*P* = 0.013).

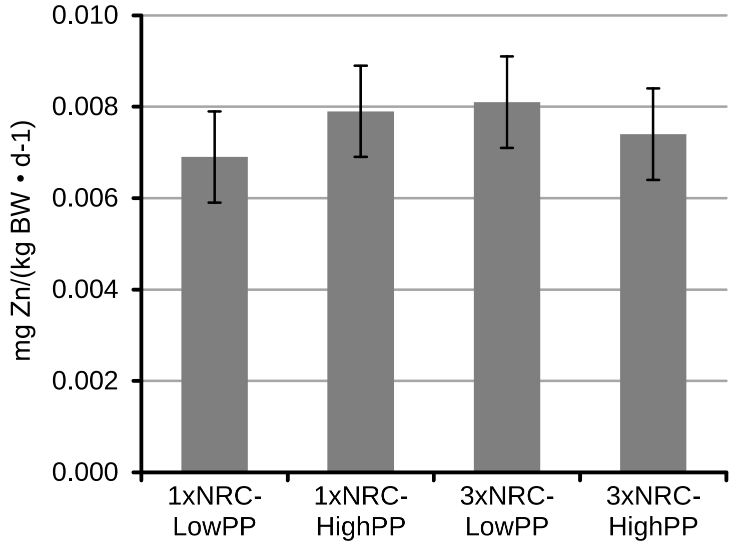

Figure 4-12. Mean (± SE) daily excretion of zinc in urine when horses were fed diets containing either 100% (1xNRC) or 300% (3xNRC) of phosphorus requirements and either low (LowPP) or high (HighPP) amounts of phytate-phosphorus. Overall effect of diet (*P* = 0.892), period (*P* = 0.863) and horse (*P* = 0.804).

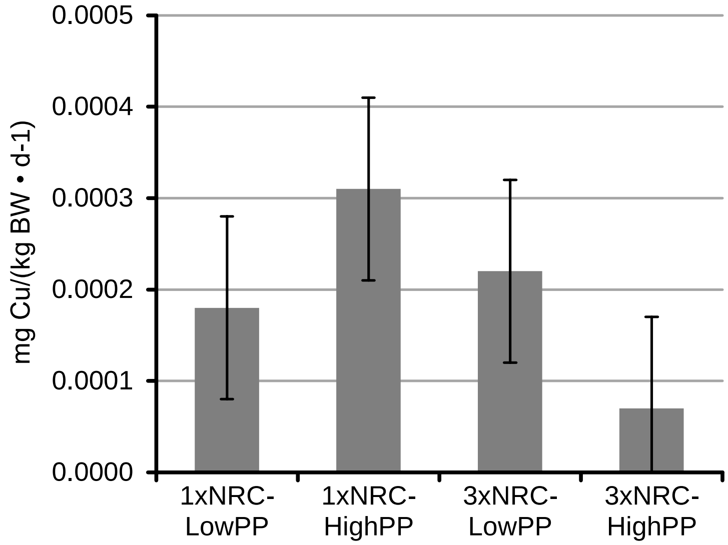

Figure 4-13. Mean (± SE) daily excretion of copper in urine when horses were fed diets containing either 100% (1xNRC) or 300% (3xNRC) of phosphorus requirements and either low (LowPP) or high (HighPP) amounts of phytate-phosphorus. Overall effect of diet (P = 0.519), period (P = 0.028) and horse (P = 0.537).

CHAPTER 5
DISCUSSION

The key findings of this study were: 1) feces was the primary route of P excretion in horses; 2) urinary P excretion was not affected by P intake; 3) total and water-soluble P excretion in feces was greater with increasing concentration of dietary P; 4) fecal total and water-soluble P were further increased when phytate-P made up a higher proportion of dietary P; and 5) dietary phytate-P appeared to increase fecal excretion of calcium, magnesium and zinc.

The results of the current study confirmed that feces is the main route of phosphorus excretion in horses (Lavin et al., 2009; Schryver et al. 1971, 1972; van Doorn et al., 2004; Warren et al., 2011). By comparison, urinary P excretion was quite low (0.1 – 0.4 g/d) and was not affected by either P or phytate-P intake. The lack of diet effect on urinary P contradicts earlier work done by Schryver et al. (1971) who found that urinary excretion of P was linearly related to intake in ponies fed diets containing 0.2% to 1.19% P. However, Hintz and Schryver (1972) did not observe differences in urinary P excretion in ponies fed diets with 0.21 to 0.35% P from various sources. Van Doorn et al. (2004) found that urinary excretion of P increased as P intake increased, but was dependent upon the type of P supplemented. When horses were fed at NRC requirements for P, urinary P excretion was negligible, whereas it was 0.3 and 1.0 g/d when horses were fed diets that provided 4-times their P requirement from either monocalcium phosphate supplementation or use of high phytate-P feedstuffs, respectively. In the current study, the urinary P excretion was similar regardless of P or phytate-P intake. Discrepancies between these studies and the current study may be related to many factors, including dietary P source, relative P intake, and intake of other minerals such as calcium. Although urinary P has been shown to increase with increasing dietary intake, daily excretion of P in urine remains a very minor proportion of the total P and WSP excretion in manure.

In contrast to urine, total P excretion in feces was affected by P concentration in the diet, which agrees with others (Schryver et al., 1971, 1972; van Doorn et al., 2004; Westendorf and Williams, 2011). The concentration of total P in feces ranged from 0.03 to 0.19% as-excreted (0.14 to 0.81% on DM basis). Westendorf and Williams (2011) reported fecal total P concentrations of 0.36% and 0.81% on a DM basis for horses receiving approximately 2-times or 4-times the current NRC (2007) P requirement, respectively. The current study also found that inclusion of higher amounts of phytate-P in the diet resulted in an even further increase in fecal total P. Regardless of whether horses received 100 or 300% of NRC P requirements, fecal excretion of total P was 25 to 40% higher when a significant portion of the dietary P was in the form of phytate-P (9.8 and 12.2 g P/day for 1xNRC-LowPP and 1xNRC-HighPP, respectively and 22.6 and 31.8 g P/day for 3xNRC-LowPP and 3xNRC-HighPP, respectively). In contrast, van Doorn et al. (2004) found no difference in fecal total P excretion between low phytate-P diets and high phytate-P diets.

In the current study, the concentration of WSP in feces ranged from 0.015 to 0.084% as-excreted (0.09 – 0.36% WSP on DM basis). This agrees with Westendorf and Williams (2011) who reported fecal WSP concentrations of 0.02% and 0.07% on an as-excreted basis for horses receiving approximately 2-times or 4-times the current NRC (2007) P requirement, respectively. Warren et al. (2011) reported an average fecal WSP concentration of 0.32% on a DM basis in horses consuming all-forage diets that were right at or slightly below the minimum P requirement for sedentary horses. The fecal WSP value reported by Warren et al. (2011) is almost four times as high as the concentration noted for the 1xNRC-LowPP diet in the current study (0.09%), which is the closest in composition to the all-forage diets used by Warren et al. (2011). The discrepancy in fecal WSP concentration between Warren et al. (2011) and the present study may be related to the methodology used to determine WSP in feces. Warren et al. (2011) determined WSP on fresh feces, whereas fecal material was dried to a constant weight prior to WSP determination in the present study. Herrera et al. (2010) discussed the importance of drying fecal samples to standardize all samples to the same DM content so that the same amount of moisture and DM from each fecal sample would be mixed with distilled water for extraction. In addition, they noted that drying is also effective for obtaining consistency in sample treatment, preserving samples, and avoiding DM variability in feces between animals and within animals in different sampling events.

An increase in fecal WSP when increasingly higher amounts of P are fed was shown in dairy cattle with P intakes ranging from 97-171 g P/d (Dou et al., 2002). The ratio of P intake to WSP excretion in dairy cattle was approximately 4.0 when P intake was on the low end of the range fed, and dropped to down to 1.8 when P intake increased. Applying this ratio to the data in the current study results in a value of 3.2 for the 1xNRC diets and 4.5 for the 3xNRC diets. Thus, it appears that WSP excretion as a proportion of dietary P intake may be higher in dairy cattle consuming high amounts of P compared to horses fed excess P. Dou et al. (2002) also reported that the proportion of total P excreted in cattle feces that was water-soluble ranged from 56-64%. In the current study, fecal WSP made up 41 to 58% of total P excreted. Greater excretion of WSP as a proportion of intake and total P output in dairy cattle compared to horses is likely related to greater microbial phytase activity in the rumen vs. the hindgut of the horse.

According to Spiekers et al. (1993), fecal P can be categorized into three components: 1) unavailable dietary P, referring to dietary P that cannot be absorbed under any conditions; 2) endogenous P loss, consisting of microbial residue P and metabolic P; and 3) regulated dietary P, a component that varies according to P intake relative to animal requirements. The regulated P fraction is of primary interest concerning dietary P status. If dietary P is deficient, the animal will utilize as much feed P as possible, and regulated P will be negligible. If dietary P is marginal, some small amount of regulated P is likely, but if dietary P exceeds animal needs, much of the surplus P will be excreted in feces as regulated P (Wu et al., 2000). Dou et al. (2002) reasoned that the unavailable dietary P is largely organic and water-insoluble plant cell wall residues. The endogenous P loss includes P in microbial residues, sloughed gut tissue, and digestive secretions (Wu et al., 2000). Most of the P in this fraction would be organic and relatively insoluble, although the smaller portion of P in digestive secretions would be water soluble. In contrast, regulated P would be largely, if not completely, water soluble. The readily soluble P measured in a single water extract of fecal material, as was performed in the current study, is believed to provide a relative measure of the regulated P component. If P is fed at the requirement level, readily soluble P will reflect the soluble part of the unavailable P, plus some small amount of regulated P; as more P is fed in excess of the requirement, it will reflect more of the regulated P. According to this relationship, readily soluble P in feces will increase as more P is fed, and the increase should account for most of the increased fecal P concentration (Dou et al., 2002). The above may explain why we observed a greater WSP excretion when P intake increased. The majority of the surplus P fed to horses in the 3xNRC diets would be a part of the regulated portion of P, which is largely water-soluble.

In the current study, we observed that as phytate in the diet increased, so did the excretion of calcium in the feces. In the 3xNRC diets we were feeding 3 times the amount of phosphorus, we also had to feed 3 times the amount of calcium to keep the Ca:P similar (2:1). In the HighPP diets, the amount of Ca excreted was higher compared to the LowPP diets. Field et al. (1983) found that feeding higher levels of Ca to sheep reduced the efficiency of P absorption, likely as a result of reduced solubility of P in the gastrointestinal tract (Wan Zahari et al., 1990). Toor et al. (2005) reported reduced solubility of P in turkey manure having an increased ratio of Ca to P. Herrera et al. (2010) looked at the effects of increasing the dietary concentration and solubility of Ca and the dietary concentration of Mg on lactation performance and solubility of fecal P from lactating dairy cows receiving diets formulated to the same concentration of P. They found that increasing dietary intakes of Ca and Mg beyond current recommendations may increase formation of insoluble phosphate complexes which result in decreased solubility of P in the feces of dairy cattle. The increased ratio of Ca to P was accompanied by a transformation of soluble Ca-P forms (dicalcium phosphate) into hydroxylapatite, a less soluble form of inorganic P (Herrera et al., 2010). Magnesium-phosphate complexes are common in manure and, thus, influence solubility of P (Silveria et al., 2006). Therefore Herrera et al. (2010) suggested that changing the source and amount of Ca and Mg intake may influence P dynamics by formation of insoluble phosphates with these cations. Further research is needed in this area in horses.

In the current study, most of the emphasis in diet formulation was based on controlling P and Ca intake, whereas lesser attempts were made minimize differences in the intake of Mg, Cu and Zn. Although diets were formulated to meet or slightly exceed Mg, Cu and Zn requirements, there were some differences in daily intake between treatments (Table 3-1). As a result, intake of Mg, Cu or Zn was used as a covariate in the statistical model when determining differences in fecal and urinary excretion of these minerals between the four dietary treatments. Despite these differences in dietary intake, diets with a higher level of phytate-P resulted in a higher fecal concentrations of Ca, Mg and Zn and greater daily excretion of Ca and Zn in feces. Phytic acid has a strong binding affinity to several minerals, such as Ca, Mg, Zn and iron. When a mineral binds to phytic acid, it becomes insoluble, precipitates and will be nonabsorbable in the intestines (Pallauf and Rimbach, 1997). While Ca intake was tightly controlled in this study, differences in Mg and Zn intake existed between dietary treatments. Therefore, the effect of dietary phytate on Mg and Zn excretion should be more systematically evaluated in horses to confirm the results presented here.

In the current study, apparent digestibility of P ranged from 39 – 51%. This agrees with previous studies that have investigated the digestion and metabolism of P in horses and ponies where P digestibility ranged from 30 to 45% (Kichura et al., 1983; Schryver et al., 1971). The availability of P from plant sources is fairly low, ranging from 32-42% (Schryver, 1974). Inorganic sources of phosphorus are slightly more available (44-47%)

to the horse than organic sources (Schryver, 1974). In the present study, this could explain why diets with lower amounts of phytate-P (1xNRC-LowPP and 3xNRC-LowPP) had a greater apparent P digestibility than diets that contained higher levels of phytate-P (1xNRC-HighPP and 3xNRC-HighPP), despite different total P concentrations in the diets. In contrast, Van Doorn et al. (2004) found no effect of phytate-P on P digestibility; however, the apparent P digestibility values reported by these authors for diets containing high amounts of inorganic P supplementation or high levels of phytate-P were much lower than other published values (2.4 to 15.2%).

Schryver et al. (1972), found that the major sites of net phosphorus absorption from all feed sources were the dorsal large colon and the small colon. Thus, the horse differs from sheep (Pfeffer et al., 1970), pigs (Moore et al., 1955), and dogs (Bergeim et al., 1926) in which there is little phosphorus absorption from the large intestine. Alexander and Hickson (1970) suggested that the large amounts of phosphate found in the cecum and colon of the horse (Alexander, 1962) serve to buffer the volatile fatty acids produced by bacterial fermentation.

In conclusion, it is clear that overfeeding P to horses can lead to higher levels of total P and WSP excretion. Such manures may be at greater risk for loss to runoff or leaching into groundwater. Horse owners should be encouraged to evaluate current feeding programs to avoid excessive P supplementation beyond that established for meeting nutrient requirements.

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BIOGRAPHICAL SKETCH

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