ROLES OF DIETARY CALCIUM AND MAGNESIUM IN CONTROLLING DAIRY FECAL PHOSPHORUS SOLUBILITY

By

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A mi familia.

My parents are an example of hard work, I am always amazed of their vision and effort to provide their kids with the tools to succeed in life, particularly their encouragement to study and be the best we can possibly be. My siblings were like parents to me for most of my life, most of all, they are my friends and they know how much their children mean to me. I always felt their full support, particularly in difficult times such as during my knee surgeries. My nieces and nephews were always the best excuse to go home. This dissertation is for you, my success is yours!

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ROLES OF DIETARY CALCIUM AND MAGNESIUM IN CONTROLLING DAIRY FECAL PHOSPHORUS SOLUBILITY

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Soils receiving dairy manure (mixture of feces with urine) over long periods of time can be a non-point source of phosphorus (P) that potentially can degrade water quality. My research objectives were to evaluate the combined effect of physiological state and diet on dairy-feces P solubility and to test the hypothesis that fecal P solubility can be decreased by increasing available calcium (Ca) relative to magnesium (Mg) in the diet of lactating dairy cows. Feed and fecal samples were collected from animals in different physiological states and experimental multiparous cows (24) were fed eight diets with a 2x2x2 factorial arrangement involving two dietary Ca sources, two dietary Ca and Mg concentrations. Feed and fecal samples were collected and chemically analyzed for nutritive parameters, total and water-extractable P, Ca and Mg. The combined effect of physiological state and diet yielded fecal samples with different water-extractable P (WEP), despite comparable dietary P concentrations. Dietary treatments had little practical effects on animal performance parameters, digestibility of nutrients, and overall P balance of lactating dairy cows; however addition of $CaCl_2$ had a tendency to decrease dry matter intake (DMI). Fecal samples with higher Ca and Mg concentrations showed reduced WEP; possibly, high Ca and Mg concentrations mutually suppressed dissolution of Ca,Mg-P forms by the common ion effect. This finding was supported by consecutive extraction data,

SEM solid-state analysis and by XRD results from ashed fecal samples where hydroxyapatite (HAP), HAP plus Ca,Mg-P, and Ca,Mg-P were the P forms found in ashed fecal samples for high, intermediate, and low dietary available Ca, respectively. Increasing Ca concentration in the diet of lactating dairy cows preemptively reduced P release from incubated feces-soil mixtures. This effect was most pronounced in soils with low P retention capacity. No further P stabilization effect of high available dietary Ca was observed over a 42 week period in soil-feces incubations. This lack of a time effect suggests that Ca, Mg, and P interactions in the gastro intestinal tract (GIT) may be the major determinant of the subsequent environmental fate of fecal P; formation of stable Ca-P forms are determined in the GIT and changes in P forms upon application of feces to soils are unlikely.

CHAPTER 1
INTRODUCTION

Phosphorus (P) is an essential element for living organisms. Increased P concentration in diets of lactating dairy cows has been shown to positively affect feed intake, milk production (Call et al., 1987), and reproductive performance (Scharp, 1979, Steevens et al., 1971). This perception of improved economical benefits associated with increased P feeding has led farmers to overfeed P to dairy cattle (Tallam et al., 2005). However, feeding P in excess of National Research Council (NRC) (2001) recommendations has not shown evidence of improved animal performance over a 2 year period (Wu and Satter, 2000).

Intensive dairy production often requires import of feedstuffs to meet nutrient requirements of animals and maximize milk production. Import of feedstuffs creates a nutrient imbalances for the farm system; nutrient inputs (feed) exceed outputs (milk and meat). Up to 72% of the P imported can accumulate on the farm (Klausner et al., 1998). Manure from these operations is the end point where excess nutrients are collected. This manure is frequently applied to soil in spray fields. High intensity areas (HIA) close to areas where animals queue for milking receive high fecal loading as well.

An increase in soil P (soil test P) concentration has been documented where manure applications to soils have exceeded crop removal for long periods of time (Daniel and Lemunyon, 1998; Sharpley, 1996; Sims et al., 2000; Whalen and Chang, 2001). Soils receiving dairy manure can become a source of P for offsite movement. This P may cause eutrophication, which can eventually lead to a decrease in the aesthetic and recreational value of water bodies (Ebeling et al., 2002; Sharpley et al., 1994). Phosphorus movement from agricultural areas has become an increasingly environmental concern. According to the United States Environmental

Protection Agency (USEPA, 2000), agriculture is the leading source of water quality impairments of rivers and lakes in the USA.

Application of manure to crops based on P concentrations is an approach that can minimize P buildup in soils around confined animal feeding operations, but this approach restricts manure application areas or increases costs due to transportation (Dou et al., 2003). Another research focus to optimize P balance in farms is to decrease P in animal diets and therefore P concentration in feces, the main route of P excretion (Morse et al., 1992). Lowering dietary P has successfully decreased fecal-P concentrations as dietary P concentration is one of the dominating factors affecting fecal P excretion (Chapuis-Lardy et al., 2004; Toor et al., 2005a; Wu et al., 2001). Dietary modifications have reduced fecal P concentrations up to 33% when used in commercial dairy farms (Cerosaletti et al., 2004), particularly decreasing the water-soluble P fraction (Dou et al., 2002) with no detrimental effects on animal productivity (Wu and Satter, 2000).

There is a limit to which dietary and therefore manure P can be reduced because dairy cows have a threshold for P excretion. Based on NRC recommendations, P should be fed to lactating dairy cows at approximately 3.7 g kg^{-1} of dietary DM (NRC, 2001). Contrasting findings on animal performance have been reported when feeding P at concentrations below NRC recommendations. In different experiments; Wu et al., (2000), documented negative impacts on animal performance when feeding 3.1 g of P kg^{-1} of dietary DM, whereas Dou et al. (2002) concluded that 3.1 to 3.7 g of P kg^{-1} of dietary DM was adequate or near adequate for milk production. Irrespective of dietary P concentration, fecal P concentrations remained above 4.0 g kg^{-1} of feces DM even when cows were fed diets containing P at 3.1 g kg^{-1} of dietary DM (Wu et al., 2000).

Differences in concentrations and release characteristics of P in manure, independent of dietary P intake, also have been documented when comparing animals of different type or physiological state (Nair et al., 2003). Therefore, both animal requirement and dietary P concentration are relevant factors that should be accounted for when considering P excretion via feces.

To optimize P balance in farms, emphasis should be placed not only on reducing dietary and fecal P concentrations but also on P stability in the long term. Feces-derived components can play an important role in the fate of P once applied to the soil, particularly in soils with low P sorbing capacity (Josan et al., 2005).

Interactions between Ca and Mg with P can play a major role in P nutrition and therefore excretion. Dietary concentrations of Ca and Mg, number of days of lactation and manure pH have been shown to influence excretion and solubility of fecal P from ruminants (Chapuis-Lardy et al., 2004; Nair et al., 2003).

Ruminants maintain Ca homeostasis with remarkable precision, particularly their blood concentration because of the importance of Ca in muscle contraction (heart) and nerve transmission. Contrary to Ca, homeostasis control of P and Mg is less rigorous. Interactions between Ca, Mg and P in combination with the effect of parathyroid hormone, calcitonin and vitamin D play an important role in homeostasis of Ca, P, and Mg; for instance, excessive dietary Ca can negatively impact Mg and P absorption (Reinhardt et al., 1988) and Cu liver storage (Huber and Price, 1971). Adequate dietary concentrations of Ca and P, their chemical forms, and ratio of Ca:P are the primary factors influencing their absorption. A dietary Ca:P ratio between 1:1 and 2:1 is usually recommended; however, ruminants can tolerate a wider range, particularly when the animal has adequate supply of vitamin D (McDowell, 2003).

Metabolism of Ca, Mg and P in domestic animals is "extremely complex" (Littledike and Goff, 1987). Calcium absorption is highly regulated in ruminants (with exception of aged lactating cows). Homeostasis mechanisms allow for a certain degree of dietary manipulation without negatively impacting animal heath. Although dietary Ca:P ratios greater than 2:1 are above NCR (2001) recommendations, they are often fed to lactating dairy cows in research experiments (Dann et al., 2006) and are a common practice in rations for pregnant non-lactating pregnant cows (far-off or close-up) (Chan et al., 2006, Tucker et al., 1991) with no evidence of negative effects on P metabolism.

Diets offered to dairy cows in the last days of pregnancy (close-up animals) can be formulated to contain additional anions such as Cl and S. These are referred to as negative DCAD diets (dietary cation-anion difference) based on the equation (Na+K-Cl-S). These diets are formulated to promote Ca mobilization from bone so as to reduce the incidence of milk fever once the animal is in lactation. Feeding negative DCAD diets (-5 to -10 meq/100 g of DM) increased H^+ fluxes as a companion cation upon higher absorption of Cl and S, causing mild metabolic acidosis. To buffer and maintain blood pH, the animal responds by increasing bone resorption, mobilizing bicarbonate; along with it, Ca is also mobilized and therefore the animal is metabolically prepared to respond to the increased Ca demand from milk production (Chan et al., 2006, Goff and Horst, 1993, Goff et al., 2004, Tucker et al., 1991). Calcium chloride is one of the "anionic salts" used in formulation of negative DCAD diets. While these diets are fed to animals in a specific physiological state and for short periods of time, they provide evidence that an increased dietary Ca:P ratio is not detrimental to animal health or performance.

Lactating dairy cows in the southeastern United States experience heat stress, particularly between June 1st and September 30th (West, 2003). Sweating and accelerated respiration are

means utilized by ruminants to dissipate heat and maintain body temperature. These mechanisms increase the animal's requirements for Na and K (Sanchez et al., 1994). Increased dietary K has a suppressant effect on Mg absorption which can lead to hypomagnesemia (grass tetany); however, it can be effectively counteracted with additional Mg supplementation (Jittakhot et al., 2004). The NRC (2001) recommends increased Mg concentration in diets of lactating dairy cows when fed high dietary K. The main drawback of increased dietary Mg is reduced dry matter intake (DMI), particularly above 4.0 g kg^{-1} of DM (Sanchez et al., 1994), mainly as salts of sulfate and chloride. Ruminants can excrete large amounts of Mg via urine therefore Mg toxicity is not a practical problem in dairy cows (NRC, 2001).

Although the small intestine is the main absorption site for Ca (Horst et al., 1994) and P (Grace et al., 1974) and the reticulo-rumen is for Mg (Tomas and Potter, 1976), interaction between these elements in the animal has been hypothesized to play a role in the inorganic form in which P is excreted and therefore in its solubility in manures of monogastric animals, particularly poultry manures (Cooperband and Good, 2002; Toor et al., 2005b); additionally, manures from turkey fed a Ca:P ratio above 2 experienced a transformation of more soluble (dicalcium phosphate) to less soluble P compounds (hydroxylapatite) in their manure (Toor et al., 2005b).

Manure-impacted soils often have conditions associated with increased P stability because dairy manure has (with respect to soils) elevated concentrations of P and Ca and high pH; however, no solid state crystalline phosphate minerals have been identified in these soils (Harris et al., 1994) or in (dairy) manure-soil incubations (Cooperband and Good, 2002). Instead, P remains highly soluble, even after years of abandonment (Josan et al., 2005; Nair et al., 1995). High concentrations of Mg and dissolved organic carbon have been proposed as potential

inhibitors of more stable calcium-phosphate minerals (Ca-P) in manure-amended soils (Harris et al., 1994; Josan et al., 2005).

From an animal nutrition standpoint, large intakes of Ca and Mg are of concern because of their effect on decreasing P absorption by the formation of insoluble phosphates in the gastro intestinal tract (GIT) (McDowell, 2003). However from an environmental perspective and given the importance on reducing P losses from agricultural areas (Sims et al., 2000; USEPA, 2000), interactions of Ca and Mg with P to decrease P solubility in dairy manures is a desired characteristic. Thus, the question before us is: Can dietary Ca be increased to reduce P solubility in feces from lactating dairy cows without negatively impacting animal health and performance?

My study tests the following hypotheses:

H1: The combination of physiological state of the dairy cow and diet found on dairy farms results in feces with different P release characteristics.

H2: Higher availability of dietary Ca relative to Mg can decrease fecal P solubility by favoring formation of more stable calcium phosphates, with no detrimental effects on animal health and performance.

CHAPTER 2
PHOSPHORUS RELEASE FROM DAIRY HEIFER AND COW FECES INFLUENCED BY PHYSIOLOGICAL STATE AND DIET

Abstract

The objective of this study was to evaluate the combined effect of diet and physiological state of Holstein animals on concentration and solubility of P, Ca, and Mg in feces, using standard diets of comparable P concentration. Dietary ingredients and fecal samples from heifers, pregnant, nonlactating cows within three weeks of calving; and lactating dairy cows were collected and analyzed for Ca, Mg, and P concentration. Solubility of these three minerals in feces was determined using repeated water extractions at a 1:50 feces:water ratio. Increased dietary concentrations of P, Ca and Mg did not result in increased total concentrations of those nutrients in feces among physiological states. Total P (TP) concentration in feces did not relate to water-extractable P (WEP), but WEP differed among physiological groups ($P < 0.006$). Of the three groups, feces from heifers had the highest P solubility despite having the lowest TP fecal concentrations. Increased ratio of P:(Ca+Mg) resulted in increased WEP. Release of P from fecal samples was highly associated with release of Ca and Mg; the correlation coefficient was greater for Mg than Ca. Total concentration of Ca and Mg in feces as well as the form in which those nutrients are present may be important when considering potential for off-site P movement. Feeding dairy animals close to their requirements reduces both total and particularly water-extractable P concentrations in fecal samples.

Introduction

Phosphorus (P) movement from agricultural areas has increasingly become an environmental concern. According to United States Environmental Protection Agency (USEPA, 2000), agriculture is the leading source of water quality impairments in rivers and lakes in the USA. Intensive dairy production requires import of feedstuffs to complement those produced on

the farm, if any, to meet nutrient requirements of animals and maximize milk production. With the import of feedstuffs into the system, a nutrient imbalance is created on farm where nutrient inputs (feed) exceed outputs (milk and meat). Up to 72% of the P imported can accumulate on the farm (Klausner et al., 1998). Manure from these operations is the end point where excess nutrients are collected. An increase in soil P (soil test P) concentration has been documented where manure applications to soils have exceeded crop removal for long periods of time (Daniel and Lemunyon, 1998; Sharpley, 1996; Sims et al., 2000; Whalen and Chang, 2001). Soils receiving dairy manure can become a source of P for offsite movement. This P may cause eutrophication, which can eventually lead to a decrease in the aesthetic and recreational value of water bodies (Ebeling et al., 2002; Sharpley et al., 1994).

To minimize P release from dairy- manure-impacted soils, a common approach has been to decrease dietary concentrations of P fed to dairy cows (Cerosaletti et al., 2004; Dou et al., 2003). Lowering P in the diet results in decreased fecal-P concentrations as dietary P concentration is one of the dominating factors affecting fecal P excretion (Chapuis-Lardy et al., 2004; Toor et al., 2005; Wu et al., 2001). However, this approach has a baseline limit below which animal performance is negatively affected; feeding P below that point is questionable, as pointed out by Chapuis-Lardy et al. (2004). Differences in P concentrations and P release characteristics of manure, independent of dietary P intake, also have been documented when comparing animals of different type or physiological state. Dietary concentrations of Ca and Mg, days in milk and manure pH also have been shown to influence excretion and solubility of fecal P (Chapuis-Lardy et al., 2004; Nair et al., 2003).

Recent studies hypothesized that Mg in feces, in addition to Ca, may play a role in promoting P solubility in manure-amended soils due to the greater solubility of Mg phosphates

relative to Ca phosphates (Josan et al; 2005; Nair et al., 2003). The potential influence of Mg may be dictated ultimately by dietary factors. Diets routinely formulated for animals in different physiological states contain varying concentrations of Mg and Ca, which could affect P solubility in feces regardless of total P in the diet. Objectives of this study were (i) to compare P solubility of dairy feces from animals in different physiological states consuming diets tailored to their respective needs and (ii) to relate P release from feces to Ca and Mg release.

Materials and Methods

Five Holstein animals in each of three physiologically different groups - heifers, close-up cows (pregnant, nonlactating dairy cows within three weeks of calving) and lactating cows - were used at the University of Florida Dairy Research Unit (DRU). The heifer group consisted of animals between 14-16 months of age and fed to gain 0.9 kg d^{-1}. Lactating group was composed of three multiparous and two primiparous cows, with 13 (± 8) days in milk; close-up group had two multiparous and three primiparous animals and averaged 260 (± 10) days of gestation; detailed information on animals is given in Table 2-1.

Ingredient and chemical composition of diets fed to each group (Table 2-2) represent those used by commercial dairy farms in Florida. Diets differed in P, Ca and Mg concentrations because they were formulated to meet requirements of animals in different physiological states. The diet for the close-up group was formulated to contain more Ca, Mg, S, and Cl in order to establish metabolic acidosis and reduce the risk of pariparturient paresis.

All ingredients, except forages (Table 2-2) were mixed together to form a concentrate mix. The concentrate mix and forages were then mixed and fed as a total mixed ration daily. Individual samples of forages and concentrate mixes were collected 3 d prior to the days of fecal collection and composited. Composited and dried feed samples were analyzed for DM (105°C for 8 h), neutral detergent fiber (NDF) using heat-stable α-amylase (Goering and Van Soest,

1970; Van Soest et al., 1991), acid detergent fiber (ADF) (AOAC, 1990), and total nitrogen (N); crude protein (CP) was calculated by multiplying N x 6.25(Elementar Analysensysteme, Hanau, Germany). Crude protein was calculated by multiplying N x 6.25. In addition, a composites were sent to a DHIA Forage Testing Laboratory (Dairy One, Ithaca, NY) where samples were analyzed by wet chemistry for Ca, P, Mg, K, Na, Zn, Cu, Mn, and Fe by the ignition method (Andersen, 1976); Cl was determined by titration with $AgNO_3$ using Brinkman Metrohm 716 Titrino Titration Unit with silver electrode (Metrohm Ltd., C-H-9101 Herisau, Switzerland) and S by oxidation (Leco Model SC-432, Leco Instruments, Inc).

Animals were accustomed to each diet for at least 10 d prior to fecal collection. To obtain a composited fecal sample per animal, approximately 1000 g of wet feces were collected twice in 1 d from the rectum of each of five animals in the three physiological groups. Fecal samples were dried at 55°C in a forced-air oven and ground to pass the 2-mm screen of a Wiley mill (A.H. Thomas, Philadelphia, PA).

Fecal samples were analyzed in quadruplicate for total P, Ca and Mg by the ignition method (Andersen, 1976). Calcium and Mg were measured by atomic absorption spectroscopy; P was determined on a UV-visible recording spectrophotometer at 880 nm wave-length via the molybdate-blue colorimetric method (Murphy and Riley, 1962).

Dried fecal samples were mixed with distilled water at a ratio of 1:50 g of DM:mL, shaken for 1 h, and centrifuged at 1000x g for 5 min. The supernatants were collected and filtered through a 0.45 µm filter. Ten successive extractions were carried out at room temperature (~25°C). Supernatant solutions were analyzed for soluble reactive P, Ca, and Mg, using the same methods specified above for total determination of these elements in fecal samples.

Fecal samples were dried to standardize all samples to the same DM content so that the same amount of moisture+DM from each fecal sample would be mixed with distilled water for extraction. Drying is also effective to obtain consistency in sample treatment, preserve samples, and avoid DM heterogeneity in feces between animals and within animals in different sampling events. Furthermore, feces (or manure) often undergo a drying period upon application to soil. We are aware of the possible effects on P solubility when fecal samples are dried. Previous research has reported contradictory findings, Ajiboye et al. (2000) reported an increased in water-extractable P (WEP) from dairy cow feces when samples were oven-dried at 105 ºC whereas Chapuis-Lardy et al. (2004) showed a decrease in inorganic P soluble in water when dairy cow fecal samples were dried at 65 ºC. We believe that relative treatment effects would not be compromised by drying at low temperature, as Dou et al. (2000) reported that ~70% of P in dairy cow manure was water soluble and most of it was extracted in the initial steps of repeated water extractions.

Statistical Analyses. Single data measurements of Ca, Mg and P were analyzed using the GLM procedure of SAS whereas Mixed Linear Model was used for repeated measures from consecutive extractions of the same elements; slice option was used to detect differences in individual extractions. Treatment differences were evaluated using the F-protected least significant difference test. Correlations and nonlinear regressions were used to describe relationships between elements in our analysis. Differences discussed in the text were significant at $P \leq 0.05$ unless otherwise indicated.

Results and Discussion

Diets differed in nutrient concentrations since each was customized to meet the nutrient requirements or to initiate a desired physiological response of animals at a given physiological state (Table 2-2). These dietary differences among groups of animals were large for Ca and Mg,

but minimal for P. Concentrations of P, Ca, and Mg were greater in feces than in the diet (Fig. 2-1) although no statistical analysis was performed for this comparison because feed samples were not replicated. Increased concentration of minerals in feces as compared to the diet suggests greater digestibility of other feed components, protein for example, compared to that of the minerals measured.

Although the concentrations of dietary P were similar for each animal group (Table 2-2), P concentration in fecal samples from close-up cows differed from that of lactating cows but P in fecal samples from heifers did not differed from any of the other two groups (Fig. 2-1). Dietary P requirements for heifers, close-up and lactating animals based on NRC (2001) recommendations are 2.4, 3.0-4.0, and 3.7 g kg^{-1} of dietary DM respectively. Phosphorus concentration of diets fed in this study ranged from 3.4 – 3.7 g kg^{-1} of dietary DM (Table 2-2). Therefore the heifer group was fed a diet greater in P concentration (3.4 g kg^{-1}) than recommended (2.4 g kg^{-1}). The P concentration in the diet of close-up cows was increased intentionally so that when feed intake decreases around the time of parturition, intake of P is adequate to meet tissue and fetal needs. However close-up cows in this study were several days away from parturition and therefore, feed intake had not decreased resulting in greater P intake than needed by tissues and fetus. Dietary P concentrations above animal requirements markedly increased P fecal concentration as observed in feces from heifers (5.8 g kg^{-1}) and close-up cows (6.4 g kg^{-1}) (Fig. 2-1), suggesting that dietary P concentration is not the only factor affecting total fecal P concentration but also dietary concentration with respect to animal requirements.

Increased fecal P concentration with increased concentration of dietary P has been documented widely when working with animals in the same physiological state (e.g. lactating dairy cows), particularly when animals were fed above their P requirement. For example, Dou et

al. (2002) reported that increasing dietary P concentrations positively correlated with higher P concentrations in feces; others have reported similar results (Dou et al., 2003; Toor et al., 2005a; Valk et al., 2002; Wu et al., 2000). Results of the present study confirm the importance of considering animal P requirements and P intake as factors affecting P content of feces. However, fecal P concentrations do not represent total output of fecal P. Lactating cows fed diets of adequate P concentration (mg kg^{-1}) may have greater total P output (g d^{-1}) because of high feed consumption compared to heifers fed diets containing somewhat excessive dietary P concentrations.

Diets with increased Ca or Mg concentration did not coincide with increased Ca or Mg concentration in feces, respectively. Diet for close-up cows contained a greater Mg concentration than that of lactating cows (Table 2-2) whereas the opposite was true for fecal Mg concentrations (Fig. 2-1c). Heifers had the lowest Mg concentration in diet and feces; the same trend observed for dietary and fecal Mg concentrations among groups was also documented for Ca. In the case of Mg, the inorganic Mg supplement can be a factor influencing the results. Concentration of Mg in the mineral supplement of pregnant nonlactating and lactating cows was similar (3.0 and 2.9 g kg^{-1} respectively) but the source of Mg differed. Lactating cows were fed only MgO, whereas pregnant nonlactating cows were fed MgO and $MgSO_4$. The absorption coefficient for Mg in MgO is 0.7 and in $MgSO_4$ is 0.9 (NRC, 2001). Higher availability of Mg would mean higher absorption and therefore reduced fecal excretion, helping to account for the fact that there was reduced Mg in feces of animals receiving $MgSO_4$ in their diet. Heifers received no inorganic Mg supplement; an absorption coefficient of 0.16 was assigned for Mg from natural feedstuffs (NRC, 2001).

Feces from Holstein females in different physiological states not only differed in mineral concentration but also in water-extractable mineral (Table 2-3). Water-extractable P made up 86% of the total fecal P of heifers but only 47 and 42% of that of close-up and lactating cows, respectively (Table 2-3). Water-extractable Ca and Mg as a proportion of total Ca and Mg was lower for lactating cows compared with the other two groups (20% vs. ~42% for Ca and 56% vs. ~87% for Mg); indicating Ca is present in a less soluble form, consistent with the solubility of P from feces from lactating cows, suggesting an increased association of P with Ca rather than Mg.

Absorption of P in the gastrointestinal tract of the animal is influenced by amount of P intake, source of P, intestinal pH, age of animal, intestinal parasitism, and intakes of Ca, Fe, Al, Mn, K and Mg (McDowell, 2003). Cumulative water-extractable P was weakly correlated with fecal P concentration ($r^2 = 0.22$ P=0.08). Although different ratios were used in previous research, positive correlation between TP and WEP in manure has been reported (e. g., Chapuis-Lardy et al., 2004; Dou et al., 2002; Dou et al., 2003). For example He et al. (2004), using a 1:100 manure to water ratio, reported a r^2=0.62 between H_2O-extracted P_i and TP in manure. This relationship could be influenced by dietary concentration with respect to animal requirements. The heifer group was fed P above their requirements that can increase the water soluble P portion in manure as shown previously by Dou et al. (2002).

Fecal samples from three physiological groups differed in WEP at the first extraction and thereafter in cumulative WEP ($P < 0.05$) (Fig. 2-2). Fecal samples from the heifer group had the greater WEP both in concentration and as percent of TP dissolved at every extraction. With three cumulative extractions over 50% of the total extracted P was solubilized. At this point WEP from fecal samples of heifers had solubilized 57% of the total dissolved compared with 53% for close-up and lactating cows. Three extractions can be considered enough to evaluate relative

differences in WE minerals among fecal samples; however, ten extractions were done as an indication of the long term solubility of minerals. Feces with high P concentration also had the greater WEP:TP ratio in feces (see heifers in Table 2-3). For Ca and Mg however, increased total concentration in feces was not related to greater soluble proportion (Table 2-3).

Decreased ratio of P:(Ca+Mg) in feces increases the possibility of interaction of these cations with P; this interaction may result in a less soluble form of P in feces. This ratio was greatest in feces from heifers (0.31), followed by close-up cows (0.22) and lactating cows (0.10) (Fig. 2-1). The ratio of P:(Ca+Mg) in feces influenced WEP. Fecal samples with the lowest ratio (from lactating cows) had the lowest concentration of WEP (1655 mg kg^{-1}) (Table 2-3; Fig 2-1). These results are in agreement with previous reports where it has been suggested that increased Mg and Ca concentrations in manure and manure-amended soils (Nair et al., 2003; Sharpley et al., 2004) could be the reason for the increase in either Mehlich-1 or Mehlich-3 extractable P in samples despite low WEP concentrations. They suggested that the presence of Ca-P complexes was responsible for such behavior. This idea is consistent with results from our experiment when WEP was compared in a regression analysis to total concentrations of either Ca or Mg in feces (Fig. 2-3b).

Concentrations of water-extractable Ca+Mg were highly correlated with WEP in feces of Holstein dairy animals in three different physiological states (Fig. 2-3a). Close association between these elements suggests that a Ca-P, Mg-P and/or Ca,Mg-P solid phase controls the initial release of P from feces. However, it is evident based on feces analysis by animal group that the relationship of water-extractable Ca and Mg to WEP is not the same across fecal sources. To further explore the association of Ca and Mg with WEP, total Ca and Mg concentrations in feces were plotted against the fraction of fecal TP that was water extractable (WEP:TP) (Fig.

2-3b). Increased Ca and Mg concentrations in feces were negatively correlated with WEP:TP, a relationship ($P < 0.001$) best described by a power function. We recognize this evidence is not conclusive, as other forms of Ca ($CaCO_3$ vs. $CaCl_2$) and Mg (MgO vs. $MgSO_4$) can influence the relationship of these two elements with P. Pronounced differences between heifers and adult animals (pregnant nonlactating and lactating) are apparent in the relationship between WEP:TP ratio and total Ca or Mg (Fig. 2-3b). The heifer group had the higher proportion of TP as WEP and clustered separately from the other two groups. Data points for heifers are circled in Figure 2-3b. When heifer data were removed, increased total Ca or Mg concentrations in feces of adult animals was not associated with a distinct decrease in WEP:TP.

Water extractable Ca and Mg showed a strong power function relationship with WEP for all physiological states; however r^2 values were consistently higher for WEMg than WECa across physiological states (Fig. 2-4 a, b and c). These findings confirm a close association between P release and that of Ca and Mg, and suggest the presence of a Mg,Ca-P phase associated with P release from fecal samples. Similar results have been reported in dairy, layer chicken and swine manures (Kleinman et al., 2005) and in dairy manure-impacted soils (Josan et al., 2005).

Summary and Conclusions

Total P fecal concentrations did not relate to P extracted after 10 consecutive water extractions. Calcium and Mg in feces seem to play an important role in controlling WEP. Increased ratio of P:(Ca+Mg) in feces corresponded to increased WEP. The proportion of TP that was water-extractable also was reduced by increased Ca and Mg in feces, although this may have been influenced by the physiological state of the animals. Animals in different physiological states and fed different diets produced feces with different P release characteristics. Release of P from feces as measured using successive water-extractions strongly related to release of Mg and

Ca, with the relation between Mg and P being stronger. The proportion of TP solubilized after ten consecutive extractions went from ~42% for adult lactating cows fed diets matching their P requirements to ~86% for growing heifers fed a diet containing P at 140% of their requirement.

Results of this study indicate that, dietary P concentration with respect to animal P requirements, and dietary and fecal concentrations of Ca and Mg influence P solubility in feces of Holstein dairy cattle.

Table 2-1. Body weight, dry matter intake (DMI) and milk yield of three physiological groups: heifers, pregnant nonlactating (close-up), and lactating animals.

	Body Weight	DMI	Milk Yield
	kg	--- kg day^{-1} ---	
Heifers	380 (±15)	10.2 (±1.5)	--
Close-up			
Primiparous	570 (±23)	8.0 (±1.2)	--
Multiparous	723 (±18)	11.0 (±0.5)	--
Lactating			
Primiparous	550 (±17)	14 (±0.6)	26 (±0.7)
Multiparous	680 (±24)	16 (±1.8)	29 (±1.4)

Table 2-2. Ingredient and nutrient content of diets fed to the three physiological groups: heifers, pregnant nonlactating (close-up), and lactating animals.

	Heifers	Close-up	Lactating
Ingredient	---------- g kg^{-1} ----------		
Corn silage	363	450	375
Bermudagrass hay	300	150	--
Alfalfa hay	--	--	100
Ground corn	41	146	220
Citrus pulp	131	50	50
Cottonseed hulls	87.0	--	20.0
Minerals and vitamins premix†	17.0	65.0	50.0
Extruded Soybean meal	--	--	70.0
Soybean meal	61.0	125.0	100.0
Sunflower oil	--	14.0	15.0
Chemical composition‡			
Crude Protein	105.0	134.0	151.0
Neutral Detergent Fiber	500.0	390.0	318.0
Acid Detergent Fiber	283.0	208.0	188.0
Ether extract	31.0	46.0	43.0
Ca	4.5	15.6	7.8
P	3.4	3.1	3.7
Mg	1.5	3.3	2.8
K	14.0	14.0	15.0
Na	1.5	2.2	5.0
S	3.1	4.2	2.3
Cl	4.6	8.1	4.4
Fe	0.15	0.3	0.2
Zn	0.04	0.04	0.1
Cu	0.01	0.02	0.02
Mn	0.04	0.03	0.07

† Ingredients used for the Minerals and vitamins premix are specified;
Heifers = Corn Meal, monocalcium phosphate, dicalcium phosphate, ammonium sulfate, salt, copper sulfate, sodium selenite, ethilenediamine dihydriodite, manganous sulfate, zinc sulfate, vitamin A supplement, vitamin E supplement, and stabilized feed fat. Pregnant nonlactating= Calcium carbonate, monocalcium phosphate, dicalcium phosphate, magnesium oxide, salt, potassium sulfate, magnesium sulfate, sodium selenite, cobalt sulfate, copper sulfate, zinc sulfate, manganous oxide, calcium iodate, ammonium chloride, calcium sulfate, vitamin A supplement, vitamin D_3 supplement, vitamin E supplement, rice mill byproduct (vitamin carrier), and stabilized feed fat. Lactating= Calcium carbonate, sodium sesquicarbonate, urea, dicalcium phosphate, monocalcium phosphate, roughage products, processed grain byproducts, magnesium oxide, salt, magnesium sulfate, potassium sulfate, potassium chloride, niacin supplement, zinc sulfate, manganese sulfate, vitamin E supplement, ferrous sulfate, copper sulfate, vitamin A supplement, cobalt carbonate, vitamin D_3 supplement, ethilenediamine dihydriodite, sodium selenite.

Table 2-3. Cumulative water extractable (WE) P, Ca, and Mg after 10 successive extractions in feces from Holstein heifers, pregnant nonlactating cows (close-up), and lactating cows.

	Heifers	Close-up	Lactating
	---------- mg kg^{-1} ----------		
P	5034[a]†	2943[b]	1655[c]
Ca	6289[b]	9110[a]	6583[b]
Mg	3543[b]	5538[a]	4731[a]
	---------- WE mineral : Total Mineral ----------		
P	0.86[a]	0.47[b]	0.42[b]
Ca	0.44[a]	0.40[a]	0.20[b]
Mg	0.91[a]	0.84[a]	0.56[b]

† For each element, mean values within a row followed by a different letter were significantly different ($P < 0.05$) as determined by F-protected least significant difference test.

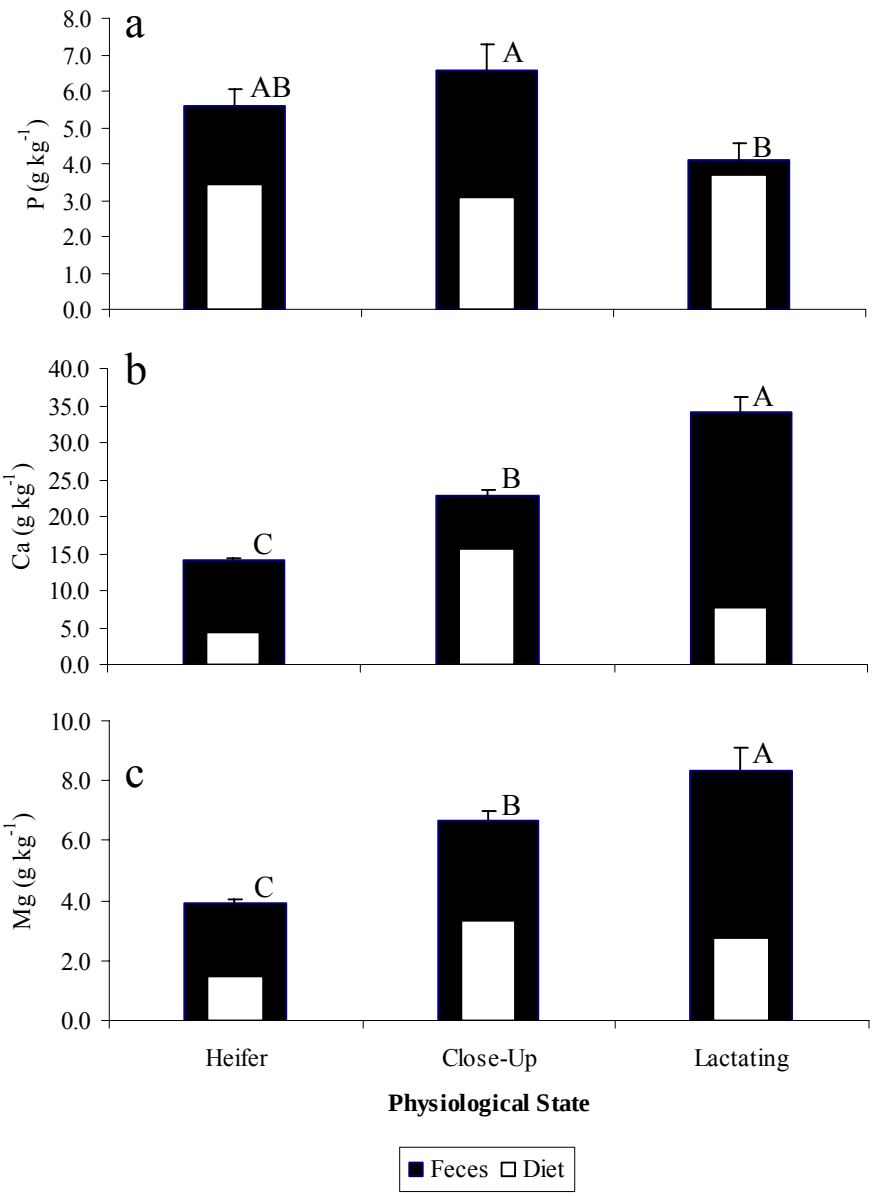

Figure 2-1. Concentration of P (a), Ca (b), and Mg (c) in diet (% of diet dry matter) and feces (+ standard error bars) of heifers, pregnant nonlactating (close-up), and lactating dairy cattle. Columns with different letter superscripts within an element were different ($P < 0.05$).

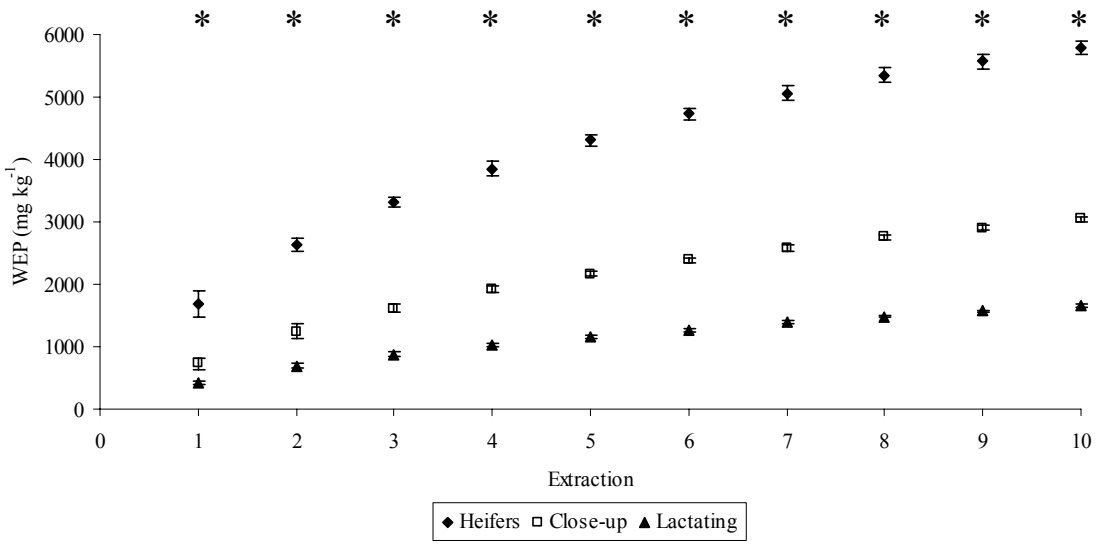

Figure 2-2. Cumulative water extractable P (WEP) concentrations (mg kg^{-1}) (±SD) with sequential extractions of feces from dairy animals in three physiological states; heifers, pregnant nonlactating (close-up), and lactating.
Asteris (*) indicates differences ($P < 0.05$) in cumulative WEP among physiological states.

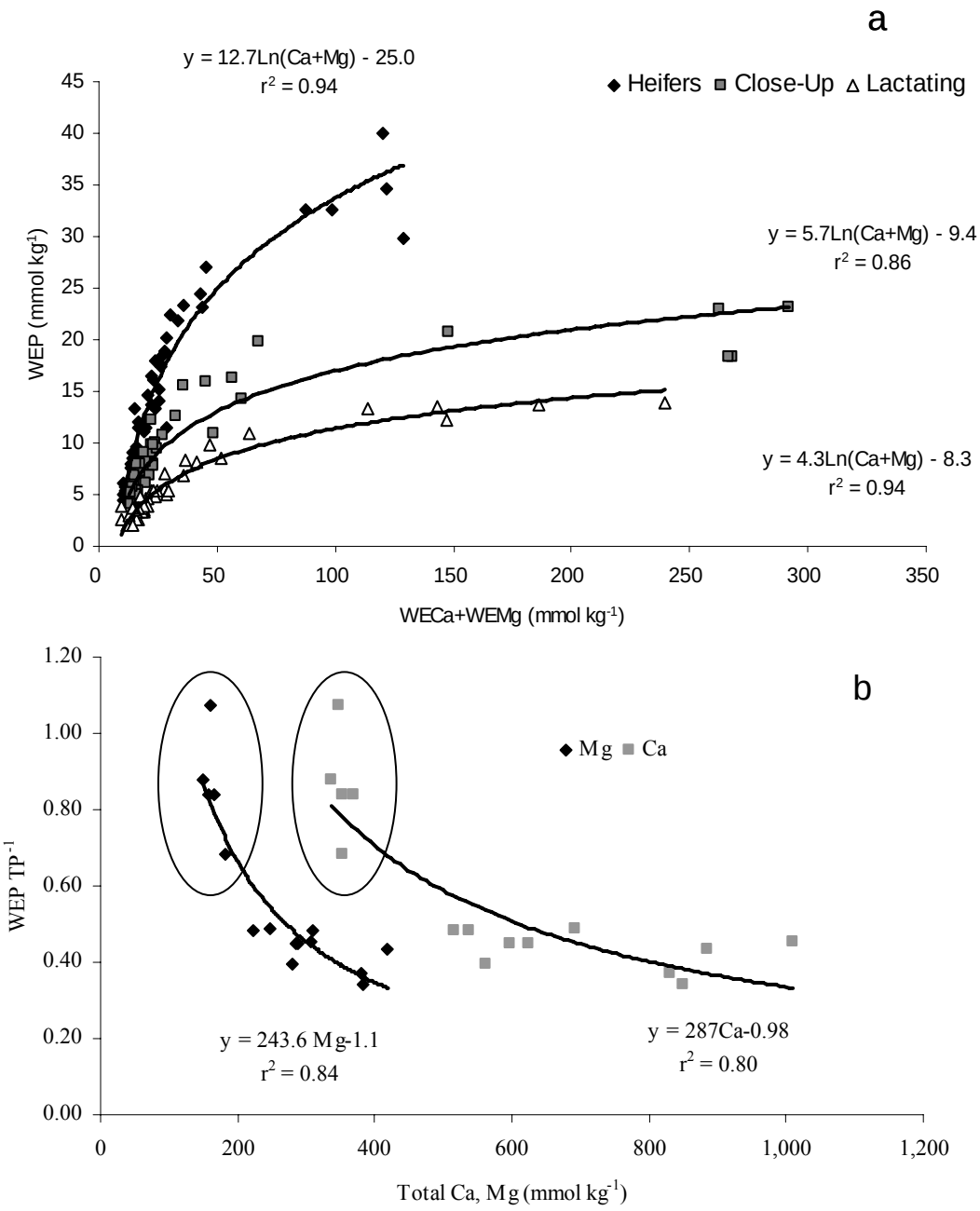

Figure 2-3. Relationship between (a) water-extractable P (WEP)and WECa+ WeMg over 10 extractions in feces of Holstein dairy animals in three different physiological states and (b) proportion of fecal TP that was water-extractable (WEP:TP) with total Ca and Mg content in feces of Holstein animals. Circled data points represent those of the heifer group.

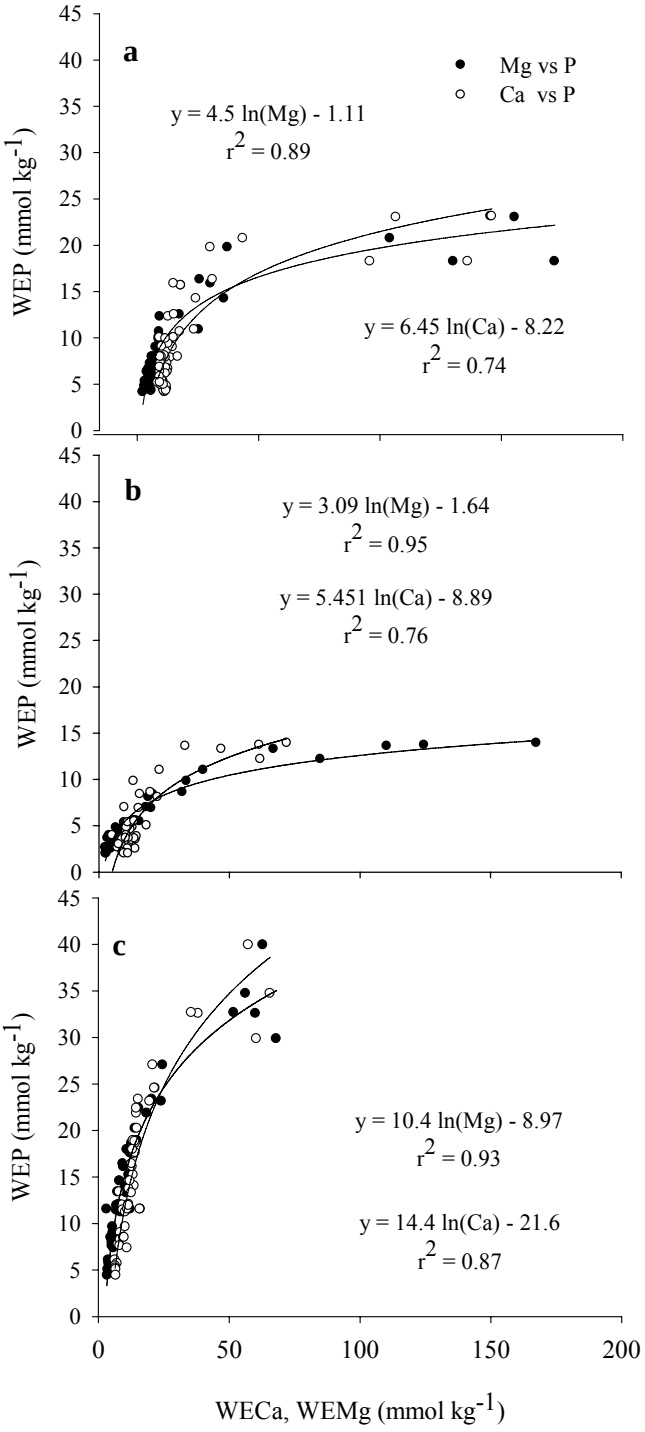

Figure 2-4. Relationship between water extractable P and water extractable Ca or Mg from feces of Holstein dairy animals in three different physiological states (a) heifers, (b) pregnant nonlactating (close-up) and (c) lactating.

CHAPTER 3
EFFECT OF DIETARY MODIFICATIONS OF CALCIUM AND MAGNESIUM ON PERFORMANCE OF LACTATING DAIRY COWS

Introduction

Offsite movement of P from agricultural soils receiving manure has been well documented (Eghball et al., 1996; Sims et al., 1998). Excess P is the most common cause of eutrophication of freshwater lakes, reservoirs, streams, and headwaters of estuarine systems (Correll, 1998). Application of manure to soils to satisfy P crop requirements is an approach that can minimize P buildup in soils around confined animal feeding operations; however, this approach restricts manure application areas or increases costs due to transportation (Dou et al., 2003).

A common and successful approach to reduce P concentrations in dairy cow manure has been to reduce the concentration of dietary P (Cerosaletti et al., 2004; Valk et al., 2000; Wu et al., 2000), which reduces the most soluble P forms in manure. From a practical standpoint and based upon both recent research (Wu et al., 2000) and the NRC (2001) recommendations, P should be fed to lactating dairy cows at approximately 3.7 g of P kg^{-1} of dietary DM, a rate at which no detrimental effects on production or reproductive performance due to low dietary P have been reported (Wu and Satter, 2000). Other dietary strategies should be examined in order to reduce the extent of P loss from manure. Dietary Ca and Mg can interact with P to reduce availability of P in the gastrointestinal tract, mainly by the formation of insoluble Ca and Mg phosphates which are therefore unavailable to the animal (McDowell, 2003). However, the effect of the dietary source of Ca and the amount of Ca and Mg consumed on P balance and animal performance of lactating dairy cows has not been evaluated.

This experiment tested the hypotheses that changing Ca source and both Ca and Mg concentrations in diet will not: (i) have detrimental effects on animal performance parameters, (ii) affect digestibility of other nutrients and (iii) significantly alter P balance in cows. The specific objectives were to evaluate effects of controlling the concentration of Ca and Mg in the diet with two different Ca sources on: (i) animal performance parameters, (ii) nutrient digestibility, and (iii) overall P intake and output of lactating dairy cows.

Materials and Methods

Cows, Diets, and Facilities

The experiment was conducted at the University of Florida Dairy Research Unit (Hague) from September to November of 2005. Experimental multiparous cows (# = 24) 575 ± 60 kg of body weight; (128 ± 21 days in milk) were housed in a sand-bedded freestall barn with grooved concrete floors, and fans and sprinklers that operated when the temperature exceeded 25°C. Animals had continuous access to water, were exposed to continuous lighting during night hours, and were milked three times daily at 0100, 0900, and 1700 h. Prior to the start of the study, cows were trained to use electronic feed gates (Calan gates; American Calan Inc., Northwood, NH) in order to measure dry matter intake (DMI). Cows were weighed the first and last 2 d of each period at 0500 and 1700 h. Animals were fed a total mixed ration (TMR) twice daily at 0600 and 1400 h, in ad libitum amounts (5-10% orts) silage was measured for DM weekly (Koster, Koster Crop Tester, Inc.) in order to maintain the formulated ratio of forage to concentrate.

Eight dietary treatments were evaluated in a 2x2x2 factorial design involving 2 dietary sources of Ca ($CaCO_3$ vs. $CaCl_2$), 2 dietary concentrations of Ca (6.0 -LoCa- vs. 10.0 -HiCa-g kg^{-1} DM basis), and 2 dietary concentrations of Mg (2.0 -LoMg- vs. 3.5 g -

HiMg- kg^{-1} DM basis). Diets were formulated to contain the same P concentration (3.7 g kg^{-1} DM basis). The lower dietary Ca concentration was selected based on NRC (2001) recommendations for lactating dairy cows. The increased dietary Ca concentration was chosen to limit intake of $CaCl_2$ below 215 g d^{-1}, therefore minimizing possible negative effects on DMI. The low dietary Mg concentration required no inorganic Mg supplementation to meet the animal's Mg requirement (NRC, 2001). The greater dietary Mg concentration is used in Florida to compensate for reduced Mg absorption when increased dietary concentrations of K are fed during heat stress conditions. List of dietary ingredients and dietary chemical analysis is provided in Table 3-1 and Table 3-2, respectively.

Calcium availability in the GIT was modified by: (a) using 2 Ca sources ($CaCO_3$ or $CaCl_2$) with different Ca availability coefficient and (b) using two calcium concentrations in the diet. Inorganic Ca supplements selected were the industry standard ($CaCO_3$) and an anionic salt ($CaCl_2$). Calcium chloride has been used in diet formulation to obtain a negative anion-cation balance, specific for pregnant nonlactating dairy cows close to parturition. Nutritionally, the main difference between these two Ca sources is their extent and site of availability. The NRC (2001) assigns an availability coefficient of 0.95 to $CaCl_2$ and 0.75 to $CaCO_3$. The digestive site of solubility was evaluated with an *in-vitro* study using the method developed by Moore and Dunham (1971). Results demonstrated that both sources are dissolved to 100%, but the site of availability is different. In the rumen all of $CaCl_2$ dissolved whereas 68% of $CaCO_3$ was soluble in the rumen incubation, the remaining Ca from $CaCO_3$ was soluble in post-rumen digestion (upon addition of HCl and pepsin).

Treatments (diets) were assigned randomly to cows in three 21-d periods. During the experiment each cow received a treatment only once and no treatment followed another treatment from the previous period more than once. During the first 11 d of each period cows were adjusted to a new diet and the last 10 d were used for data collection.

Sample Collection and Analysis

The daily DMI was measured for individual cows by recording the amount of TMR offered and refused. A concentrate mix containing the formulated mineral treatments was prepared in 0.9 tonne amounts as needed and stored in 1.7 tonne capacity metal bins. Concentrate, silage, and hay were mixed as TMR for each feeding. Representative samples of concentrate mixes, corn silage, and alfalfa hay were collected weekly and composited by experimental period for chemical analysis. Corn silage and alfalfa hay samples were dried at 55°C in a forced-air oven and ground to pass a 1-mm screen of a Wiley mill (A.H. Thomas, Philadelphia, PA) prior to compositing. Composited and dried feed samples were analyzed for DM (105°C for 8 h), neutral detergent fiber (NDF) using heat-stable α-amylase (Goering and Van Soest, 1970; Van Soest et al., 1991), acid detergent fiber (ADF) (AOAC, 1990), and total nitrogen (N) (Elementar Analysensysteme, Hanau, Germany); crude protein (CP) was calculated by multiplying N x 6.25. Crude protein was calculated by multiplying N x 6.25. In addition, composites were sent to a DHIA Forage Testing Laboratory (Dairy One, Ithaca, NY) where samples were analyzed by wet chemistry for Ca, P, Mg, K, Na, Zn, Cu, Mn, and Fe by the ignition method (Andersen, 1976); Cl was determined by titration with $AgNO_3$ using Brinkman Metrohm 716 Titrino Titration Unit with silver electrode (Metrohm Ltd., C-H-9101 Herisau, Switzerland) and S by oxidation (Leco Model SC-432, Leco Instruments, Inc).

Milk yields were measured for all milkings during the collection period and milk composition (protein, fat, and somatic cells) was measured on two consecutive milkings during the last 3 d of each period (n=6). Somatic cell scores were generated as described by Norman et al. (2000) for statistical analysis of SCC. Samples were analyzed by Southeast Dairy Labs (McDonough, GA) by infrared technologies (Bentley 2000, Bentley Instruments, Chaska, MN). Average daily milk production was used to calculate average daily water intake using the equation developed by Murphy et al. (1983). The equation is as follows:

Free water intake (kg d^{-1}) = 15.99 + (1.58 x DMI, kg d^{-1}) + (0.9 x milk, kg d^{-1}) + (0.5 x Na intake, g d^{-1}) + (1.20 x min temp C). Temperature values used for the calculation were taken from the Florida Agricultural Weather Network (FAWN) weather station in Alachua, FL.

Water samples were collected from water troughs every other day during the collection phase of each period and composited within period. Urine samples were collected from each cow at 0500 and 1300 h during the last 3 d of each collection period and composited within cow and period. Determination of urine pH was done as samples were collected (Horiba twin pH meter B-213, Spectrum Technologies, Inc, Plainfield, IL).

Water and urine samples were frozen until analysis then filtered using Whatman 42 filter paper. Calcium and Mg were measured by atomic absorption (220 FS, Varian Inc.). Phosphorus was determined on a UV-visible recording spectrophotometer at 880 nm wave-length via the molybdate-blue colorimetric method (Murphy and Riley, 1962) (U.S. EPA, 1993; method 365.1). Colorimetric determination of creatinine in urine was done

based on the procedure described by Vagnoni et al. (1997). Urine volume was calculated based on concentration of creatinine in urine concentration using the equation developed by Valadares et al. (1999). The equation is as follows:

Urine output (kg d^{-1}) = animal weight in kg x (29.0 = creatinine concentration in urine as mg L^{-1}) x (0.9595, L kg^{-1})

Fecal output was calculated using the marker ratio technique with chromic oxide (Cr_2O_3) as an inert dietary marker. Cows were dosed orally via balling gun (Ideal Instruments, Inc.) with gelatin capsules (Torpac Inc.) containing 10 g of Cr_2O_3 at 0500 and 1700 h from d 11 to 20 of each experimental period. At the time of dosing Cr_2O_3, fecal grab samples were collected from each cow twice daily during the last 10 d of each period and composited within cow for each experimental period. Samples were dried at 55ºC for 72 h and ground through a 2-mm screen of a Wiley mill (A.H. Thomas, Philadelphia, PA). Fecal samples were analyzed in triplicates for total P, Ca and Mg by the ignition method (Andersen, 1976). Total Ca and Mg were measured by atomic absorption spectroscopy; P was determined on a UV-visible recording spectrophotometer at 880 nm wave-length via the molybdate-blue colorimetric method (Murphy and Riley, 1962) (U.S. EPA, 1993; method 365.1). Feces were analyzed for Cr by atomic spectrophotometry (Williams et al., 1962). Apparent digestibility of Ca, Mg, P, CP, NDF, ADF and DM were calculated using the marker ratio technique.

Intake of Ca, Mg, and P from water was calculated based on water intake and mineral concentration measured in water samples. Urine output of Ca, Mg, and P was calculated based on urine volume and mineral concentration measured in urine samples.

Blood was collected (~10 ml) by coccygeal venipuncture at 1800 h into heparinized tubes (Vacutainer Company) from individual cows on day 20 of each period. Blood was centrifuged immediately after collection at 1,000 x g and 4°C for 15 min to separate plasma which was stored at −5°C until analyzed. Concentrations of plasma glucose and urea nitrogen were determined with an automated colorimetric procedure, which utilized an autoanalyzer (AutoAnalyzer II, Bran+Luebbe, Buffalo Grove, IL). The glucose analysis is based upon the procedure described by Gochman and Schmitz (1972). The procedure for determination of urea nitrogen (Industrial Method US-339-01, Bran+Luebbe, Buffalo Grove, IL) is based on that of Coulombe and Favreau (1963).

Overall P, Ca and Mg balance was calculated by adding together intake in feed and water, then subtracting excretion in feces, urine and secretion in milk. Milk P, Ca and Mg concentrations used to calculate mineral output in milk were: 0.93, 1.19, and 0.013 g kg^{-1} of milk, respectively (National Dairy Council, 1993).

Statistical Analysis

Treatments were arranged in a 2x2x2 factorial design using an incomplete, partially balanced Latin square. Data was analyzed using the MIXED procedure of SAS. Orthogonal single degree of freedom contrasts were used to detect main effect of Ca source, Ca concentration, and Mg concentration as well as 2- and 3-way interactions. Differences discussed in the text were significant at $P \leq 0.05$ unless otherwise indicated. The statistical model used to analyze the data was:

$$Y_{ijk} = \mu + \alpha_i + b_j + c_k + e_{ijk}$$

Where:
Y_{ikj} = observed response,
μ = overall mean,
α_i = fixed effect of treatment $_i$,
b_j = random effect of cow $_j$,

c_k = fixed effect of period $_k$, and
e_{ijk} = residual error.

Before completion of the third period, cow 4499 consuming diet 3, was removed from the experiment for health reasons.

Results and Discussion

Diet Composition and Intake

Diets were formulated to contain the same concentration of nutrients with the exception of Ca and Mg (Table 3-2). Chemical composition of the diets met the minimum nutrient recommendations for cows in this study based on NRC Nutrient Requirements of Dairy Cattle Software (2001). Dietary Ca and Mg concentrations varied with respect to the targeted values. Desired LoCa concentration was 6.0 and averaged 6.4 g Ca kg^{-1} diet DM and targeted HiCa concentration was 10 and ranged from 8.6 to 10.3 g Ca kg^{-1} diet DM; whereas LoMg diets were formulated to contain 2.0 but averaged 2.5 despite no addition of inorganic Mg source. The HiMg was targeted to contain 3.5 g Mg kg^{-1} diet DM and the actual concentration ranged from 3.7 to 4.3 g Mg kg^{-1} diet DM. Measured dietary concentration of Ca and Mg reflect differences with respect to the formulated rations. These differences were product of the combined variability present in natural feedstuffs and concentration of these two minerals in the different mineral mixes. These differences did not affect the main hypothesis or the objective of the study.

Use of $CaCl_2$ as a dietary anionic salt in the late prepartum period of dairy cows to induce metabolic acidosis and promote Ca mobilization from bone is a well-documented practice (Goff and Horst, 1993; Goff et al., 2004; Pehrson et al., 1998; Tucker et al., 1991). Metabolic acidosis produced by feeding $CaCl_2$ to dairy cows can be reflected in a reduction in urinary pH (Goff et al., 2004). Feeding $CaCl_2$ reduced urine pH only in the

HiCa diets. There was a dietary Ca source by Ca concentration interaction (P <0.0001); When cows were fed LoCa diets urine pH was the same for both Ca sources. However, when cows consumed the HiCa diet containing $CaCl_2$ urine pH decreased compared with that of animals fed the high $CaCO_3$ diet (8.1 vs. 7.0). Therefore cows consuming $CaCl_2$ at 9.9 g kg^{-1} of DM were more likely experiencing metabolic acidosis.

Animals consuming $CaCl_2$ as the inorganic Ca supplement tended (P = 0.0816) to experience reduced DMI when expressed quantitatively (22.0 vs. 21.2 kg d^{-1}) but not when expressed as a proportion of BW (3.69 vs. 3.58%) compared to those fed $CaCO_3$. Feeding $CaCl_2$ increased the dietary concentration of Cl from 0.4 to a mean of 1.0% (DM basis). Using empirical modeling techniques to evaluate cow responses to increasing dietary Cl concentration from several studies, Sanchez et al. (1994) reported that DMI decreased dramatically (about 2.5 kg d^{-1}) during the summer season but decreased very little during the winter season (about 0.5 kg d^{-1}) when dietary Cl increased from 0.4 to 1%. The current study was conducted in the fall without heat stress effects on the cows; thus cow response was similar to that reported for the winter season.

Glucose is the major carbohydrate source for mammalian cells to produce energy. Because of its importance, ruminants finely regulate stable plasma glucose concentrations; nevertheless, plasma glucose can reflect the animal's energy status. Average plasma glucose concentrations of animals in this experiment (63.8 mg dL^{-1}) were in the expected range (Dukes and Swenson, 1984), and well above those reported for hypoglycemic dairy cows (<20 mg dL^{-1}) (Hayirli et al., 2002). Coinciding with lower DMI, cows fed $CaCl_2$ tended (P = 0.061) to have lower concentrations of plasma glucose (64.7 vs. 63.0 mg dL^{-1}). Effects of feeding $CaCl_2$ at concentrations used in HiCa diets in

this experiment should be evaluated over longer periods of time to assess milk production and reproductive performance.

Blood urea N (BUN) can be used as an indicator of rumen N (protein) utilization. Concentrations of BUN were positively associated with intakes of ruminally degradable and undegradable protein and negatively associated with intake of net energy (DePeters and Ferguson, 1992). No differences among treatments were detected for BUN using the orthogonal single degree of freedom contrasts. The overall mean value (10.2 mg/dl) is within the expected range and consistent with values others have reported for lactating dairy cows in confinement (Colmenero and Broderick, 2006; Gressley and Armentano, 2007) and grazing with TMR supplementation (Gehman et al., 2006).

Body weight and body weight change were affected by the interaction between Ca and Mg dietary concentration. Cows fed diets of greater concentration of Mg gained less BW when dietary concentrations of Ca were at 0.64% but not when they were at 0.95% (Ca concentration by Mg concentration interaction, $P = 0.003$; Table 3-3; Figure 3-1). As a result, mean BW of cows fed this combination of high Mg and low Ca was lighter as well ($P = 0.01$; Table 3-3; Figure 3-1). Cows fed LoCa-LoMg diets had greater BW (599 vs. 588 kg) and greater BW gain (3.77 vs. 3.64 kg $21d^{-1}$) than those receiving LoCa-HiMg diets whereas dietary concentration of Mg did not influence BW (593 vs. 597 kg) or BW gain (3.57 vs. 3.58 kg per 21 d) when dietary concentration of Ca was high (Mg concentration by Ca concentration interaction, $P < 0.01$; Table 3-3; Figure 3-1). Therefore better gains in BW due to treatment led to heavier cows.

Milk Production Parameters

Cows fed the LoCa diets produced more milk when fed the LoMg diets compared with those fed the HiMg diets (36.8 vs. 34.1 kg d^{-1}) whereas milk yield was unchanged

by Mg diets when the diets contained a high concentration of Ca (34.1 vs. 33.6 kg d^{-1}; dietary Ca concentration by dietary Mg concentration interaction Table 3-3; Figure 3-1; *P* =0.0136). Production of 4% fat-corrected milk tended (*P* = 0.0665) to follow this same pattern (Table 3-3). The observed trend in milk yield was significantly correlated (r^2=0.27) with DMI, a factor closely associated with milk production. Efficiency of production of uncorrected or fat-corrected milk (milk production/feed intake) was not affected by dietary treatments, averaging 1.62 and 1.39, respectively (Table 3-3).

Concentration of milk fat tended to be lower for cows fed $CaCl_2$ (3.00 vs. 3.15%, Table 3-3; *P* = 0.0621). As a result, daily production of milk fat tended to be lower (0.57 vs. 0.60 kg d^{-1}, Table 3-3; *P* = 0.0528) as well as production of 4% fat-corrected milk (64.2 vs. 66.7 kg d^{-1}, Table 3-3; *P* = 0.0798) by cows fed $CaCl_2$ compared to those fed $CaCO_3$. According to Sanchez et al. (1994), increasing Cl in the diet from 0.4 to 1.0% decreased production of 4% fat-corrected milk from about 20.8 to 19.7 kg d^{-1} in the summer but did not affect 4% fat-corrected milk production significantly in the winter season until Cl concentration exceeded 1% of dietary DM.

As with milk fat concentration, milk protein concentration was lowered when $CaCl_2$ was fed rather than $CaCO_3$ (2.97 vs. 3.03%, Table 3-3; *P* = 0.0127). In addition, concentration of milk protein was reduced from 3.03 to 2.96% as the dietary concentration of Ca increased from 0.64 to 0.95% (Table 3-3; *P* = 0.0057). This resulted in lower production of milk protein by cows fed the greater Ca diets (1.01 vs. 1.07 kg d^{-1}, Table 3-3; *P* = 0.0026).

Nutrient Digestibility

Apparent digestibility values of DM, protein, ADF and NDF measured in this experiment are in the expected range for diets fed to lactating dairy cows (Ruiz et al.,

1995; Staples et al., 1997). Apparent DM and NDF digestibilities were not affected by main dietary factors of Ca source, Ca concentration, and Mg concentration. Apparent digestibility of dietary CP increased from 64.2% to 65.3% when dietary Mg was increased from 0.25 to 0.39% (Table 3-4; $P = 0.046$). Increasing Mg concentration in the diets containing $CaCO_3$ increased ADF digestibility (42.5 vs. 44.8%); however, no change was detected in ADF apparent digestibility when increasing Mg concentration in the diet of animals fed $CaCl_2$ (40.0 vs. 40.0%; Ca source by Mg concentration interaction; Table 3-4; $P = 0.032$). These results confirm our hypothesis that changes in Ca and Mg concentrations or Ca source in the diet as used in this experiment would not have a major impact on apparent digestibility of other dietary nutrients.

Apparent P digestibility ranged from 57.2 to 63.4% and was not affected by any of the main dietary treatments (dietary Ca source and dietary concentrations of Ca and Mg) as expected. Increased Mg concentration in diets used in this experiment are comparable to those often used to feed commercial lactating dairy cows during summer months to prevent potential negative effects of increased dietary K concentration on Mg absorption. These dietary Mg concentrations have not been reported to affect P nutrition (Jittakhot et al., 2004). Dietary concentrations of Ca can negatively affect P absorption; however, the ratio of Ca:P is a key point with respect to absorption of both elements by the animal (McDowell, 2003). In an experiment using a Ca:P ratio similar to the highest used in our experiment (2.32 vs. 2.78), Weiss (2004) reported no signs of P deficiency when evaluating Mg digestibility. Values of apparent P digestibility obtained in our study (overall mean of 59.6%) are similar to those reported by Martz et al. (1999) in a trial with nonlactating Holstein cows and with those reported by Valk et al. (2002) in their

second year of study (59.4%), yet greater than they reported for their first year of study (43.5%) in which they used slightly different dietary ingredients and P concentrations. Their results are also consistent with those of Wu et al. (2000) who reported apparent P digestibility values ranging between 45 and 50% when animals were fed P close to their P requirements.

Main dietary factors of Ca source, Ca concentration, and Mg concentration and their interactions did not influence apparent digestibility of Ca and Mg. Apparent digestibility values of both Ca and Mg can range widely depending on dietary concentration of energy, water, fatty acids, P, Ca, Mg,, as well as ruminal pH, animal condition and age. Vitamin D status is particularly important for Ca as is dietary concentration of Ca and K for Mg (Reinhardt et al., 1988). Apparent Ca and Mg digestibility values in our experiment are similar to what other researchers have reported. Calcium values were similar to those reported for lactating cows fed an alfalfa and corn silage-based diet (Martz et al., 1989); whereas Mg values were comparable with those compiled from eight experiments involving 39 dietary treatments and 162 lactating Holstein cows (Weiss, 2004).

Changes in dietary Ca concentration did not affect apparent Mg digestibility, consistent with results reported by Weiss (2004) in which similar concentrations of both Ca and Mg in diets of lactating dairy cows were used. This contrasts with the idea that Ca and Mg are antagonistic in the GIT because they compete for absorption sites in the small intestine (Alcock and Macintyre, 1962). However, it has been documented that the reticulum-rumen is the main absorption site for Mg (Tomas and Potter, 1976) whereas Ca is absorbed mainly by the small intestine (Horst et al., 1994)

Balance of P, Ca and Mg

Daily intake of P, Ca, and Mg was quantified taking into account that supplied by the feed and water. No differences among dietary treatments were detected for daily DMI, water intake, fecal output and urine output (Tables 3-6 and 3-7). Feed ingredients provided all of the P and most of the Ca and Mg intake, with water supplying a minimal proportion. Because there was no difference in DMI among treatments and the proportion of P, Ca, and Mg provided by water was both low with respect to total and similar among treatments, concentrations of these minerals in the diet were the dominant factors dictating P, Ca and Mg intake. Intake (g d^{-1}) of P, Ca and Mg reported in this study are consistent with other published research where dairy cows were fed similar concentrations of those nutrients (Knowlton and Herbein, 2002; Knowlton et al., 2001; Weiss, 2004; Weiss and Wyatt, 2004).

Total P intake was affected by source and concentration of Ca in the diet. A greater P intake was detected for animals fed $CaCO_3$ vs. $CaCl_2$ (83.1 vs. 79.1 g d^{-1}; $P = 0.011$) and for cows fed the low vs. high Ca diets (83.5 vs. 78.7 g d^{-1}; $P = 0.004$; Tables 3-6 and 3-7), an effect mainly driven by the negative effect of Hi $CaCl_2$ diets on DMI as discussed previously. The 5 g d^{-1} increase in P intake by cows fed the low Ca diets was accompanied by a 5 g d^{-1} increase in fecal output of P by this same treatment (34.5 vs. 30.5 g d^{-1}; $P = 0.005$). As a result, P balance was unchanged by dietary concentration of Ca. Increased dietary P above animal requirements resulted in higher fecal P excreted, consistent with the idea that feeding P closer to animal requirements increases its digestibility (retention) and therefore reduces its excretion (Dou et al., 2002). Phosphorus secreted in milk represented nearly as much P as that excreted in feces; however, feces

were the main excretion route for P in agreement with what Morse et al. (1992) had previously reported.

Main dietary treatments and their interactions did not affect overall P balance. On average, cows excreted 32.5 g of P d^{-1}, a value lower than the 47 g d^{-1} reported by Weiss and Wyatt (2004) and almost half of what Borucki Castro et al. (2004) reported (62 g d^{-1}) when feeding similar dietary P concentrations in diets with different DCAD values. Furthermore, we compared P excretion data from our experiment with the equations developed by Weiss and Wyatt (2004).

Equation A: manure P (g d^{-1}) = -2.5 + 0.64 x P intake (g d^{-1});

Equation B: manure P (g d^{-1}) = 7.5 + (0.78 x P Intake (g d^{-1})) – (0.702 x milk yield (kg d^{-1})).

Correlation coefficients (r^2) of 0.28 and 0.24 were obtained for equations A and B, respectively, between our experimental data and the predicted values using these equations. Differences in apparent P digestibility between our data and that generating the equations may account for the low correlation coefficients.

Calcium source had no effect on Ca intake. Greater Ca concentrations were present in the $CaCl_2$ diets; however, cows fed the $CaCl_2$ diets had a tendency to eat less DM. Increased concentration compensated for the reduced DMI, thus there was no difference in total Ca intake when comparing two Ca sources. Urinary concentration of Ca (0.19 vs. 0.01 g kg^{-1}) and excretion of Ca in the urine (7.0 vs. 1.0 g d^{-1}) was greater for cows fed the diets containing the greater concentration of $CaCl_2$ compared with cows fed diets with the lower $CaCl_2$ concentration, whereas these measures were not different when cows were fed the low or high concentrations of $CaCO_3$, illustrating the greater solubility of Ca

in the $CaCl_2$ form in the digestive tract compared with that of $CaCO_3$ (Ca source by Ca concentration interaction, $P < 0.0001$). Once absorbed, excess Ca can be filtered by the kidney and excreted via urine as a means to maintain Ca homeostasis; however, it is possible that a portion of that Ca (urinary excretion) can come from bone mineralization, a documented effect when anionic salts (like $CaCl_2$) are fed to lactating dairy cows (Block, 1984; Oetzel et al., 1991). Nevertheless, the balance of Ca was not different between cows fed the 2 sources of Ca because urinary Ca was such a small part of the total Ca excretion.

As expected, increasing Ca concentration in the diet increased Ca intake (200 vs. 139 g d^{-1}; $P < 0.0001$) and fecal output (142 vs. 95 g d^{-1}; $P < 0.0001$). As a result cows fed diets of 0.95% Ca tended to excrete 12 g d^{-1} more Ca than cows fed diets of 0.64% Ca (57 vs. 45 g d^{-1}; $P = 0.057$; Tables 3-8 and 3-9). Calcium homeostasis principles indicate that animals consuming Ca below its Ca requirement will increase the proportion of dietary Ca absorbed. However, diets containing more Ca than needed result in a reduced proportion of dietary Ca absorbed (Horst, 1986; Reinhardt et al., 1988). Based on NRC (2001) recommendations, Ca concentrations in the high Ca diets were in excess of cows' requirements.

Cows consuming more Mg were in a more positive Ca balance (16.6 vs. 3.6 g d^{-1}; $P = 0.034$; Tables 3-8 and 3-9) but this 13-g difference simply may have been due to the tendency of a greater intake of Ca by cows fed the diets of greater Mg concentration (173 vs. 166 g d^{-1}; $P = 0.103$).

Consistent with previous reports (Knowlton et al., 2001), feces was the main excretion route for Mg. Urinary excretion of Mg was greater, proportionally, than urinary

excretion of both P and Ca, results consistent with other reports on lactating (Knowlton et al., 2001) and nonlactating (Jittakhot et al., 2004) dairy cows, possibly because Mg absorbed in excess of Mg requirements is mainly excreted via urine (McDowell, 2003; Wang and Beede, 1992). Excess intake of P is recycled via saliva and increased in plasma. Excess Ca absorption triggers a set of mechanisms to both excrete excess Ca (either via urine or feces) and to decrease its absorption from the GIT (Horst, 1986).

Consuming more Mg resulted in greater Mg retention (22 vs. 9 g d^{-1}; $P < 0.0001$); however, this increase in Mg retention was greater when cows also were consuming $CaCO_3$ (27 vs. 9 g d^{-1}) rather than $CaCl_2$ (17 vs. 10 g d^{-1}; Ca source by Mg concentration interaction, $P = 0.006$; Tables 3-10 and 3-11, Figure 3-1). This was likely due to the greater concentration of dietary Mg in the $CaCO_3$ diets (4.15 vs. 2.4 mg kg^{-1}) vs. the Mg in the $CaCl_2$ diets (3.75 vs. 2.65 mg kg^{-1}; Ca source by Mg concentration interaction, $P < 0.0001$). This led to a moderately greater intake of Mg by cows fed the high Mg diets containing $CaCO_3$ (91 vs. 54 g d^{-1}) compared with cows fed the high Mg diets containing $CaCl_2$ (77 vs. 56 g d^{-1}; Ca source by Mg concentration interaction, $P < 0.0001$).

Conclusions

Increasing the Cl concentration of the diet from 4.0 to 10.0 g kg^{-1} (DM basis) using $CaCl_2$ reduced urine pH from 8.1 to 7.0, indicating that lactating cows were in a metabolic acidotic state. Cows fed diets in which supplemental $CaCO_3$ replaced $CaCl_2$ tended to produce more fat-corrected milk, more milk fat, and milk with a greater concentration of fat, as well as to produce milk with a greater concentration of protein. The Ca in $CaCl_2$ appeared to be more available than the Ca in $CaCO_3$ based upon the increased concentration of Ca in the urine of cows fed $CaCl_2$. Milk production was greatest for cows fed diets of 6.4 g of Ca kg^{-1} DM and 2.5 g of Mg kg^{-1} DM. Apparent

digestibility of DM, CP, NDF, and ADF were not affected by Ca source, Ca concentration, or Mg concentration. Retention of P was not affected by diet. Cows that consumed more Ca and Mg than required excreted and retained more of these minerals daily. Addition of $CaCl_2$ to rations of lactating dairy cows warrants long term studies because of the tendency to decrease DMI and associated implications observed in this study.

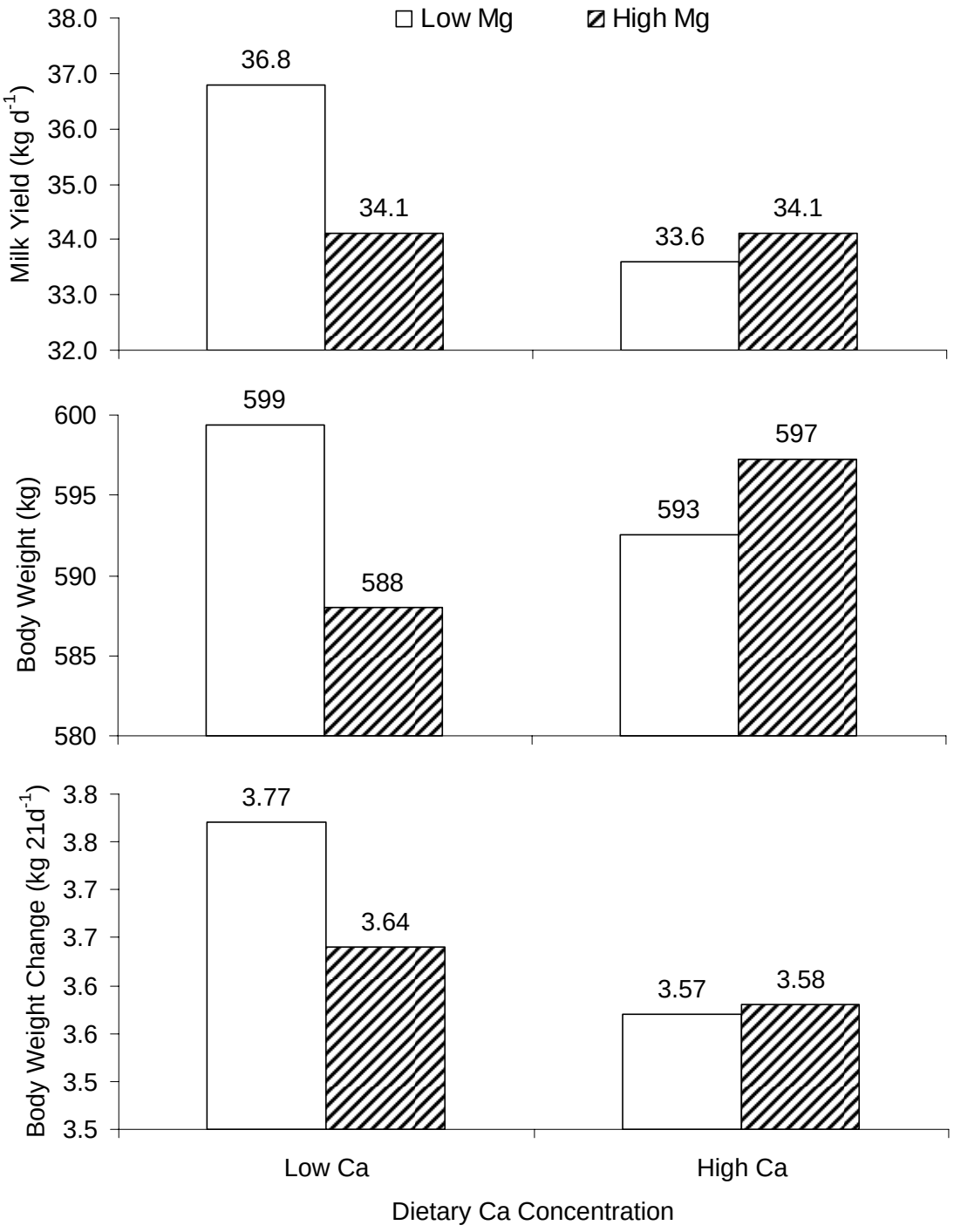

Figure 3-1. Interaction effect of Ca by Mg dietary concentrations in a) milk yield, b) body weight, and c) body weight change.

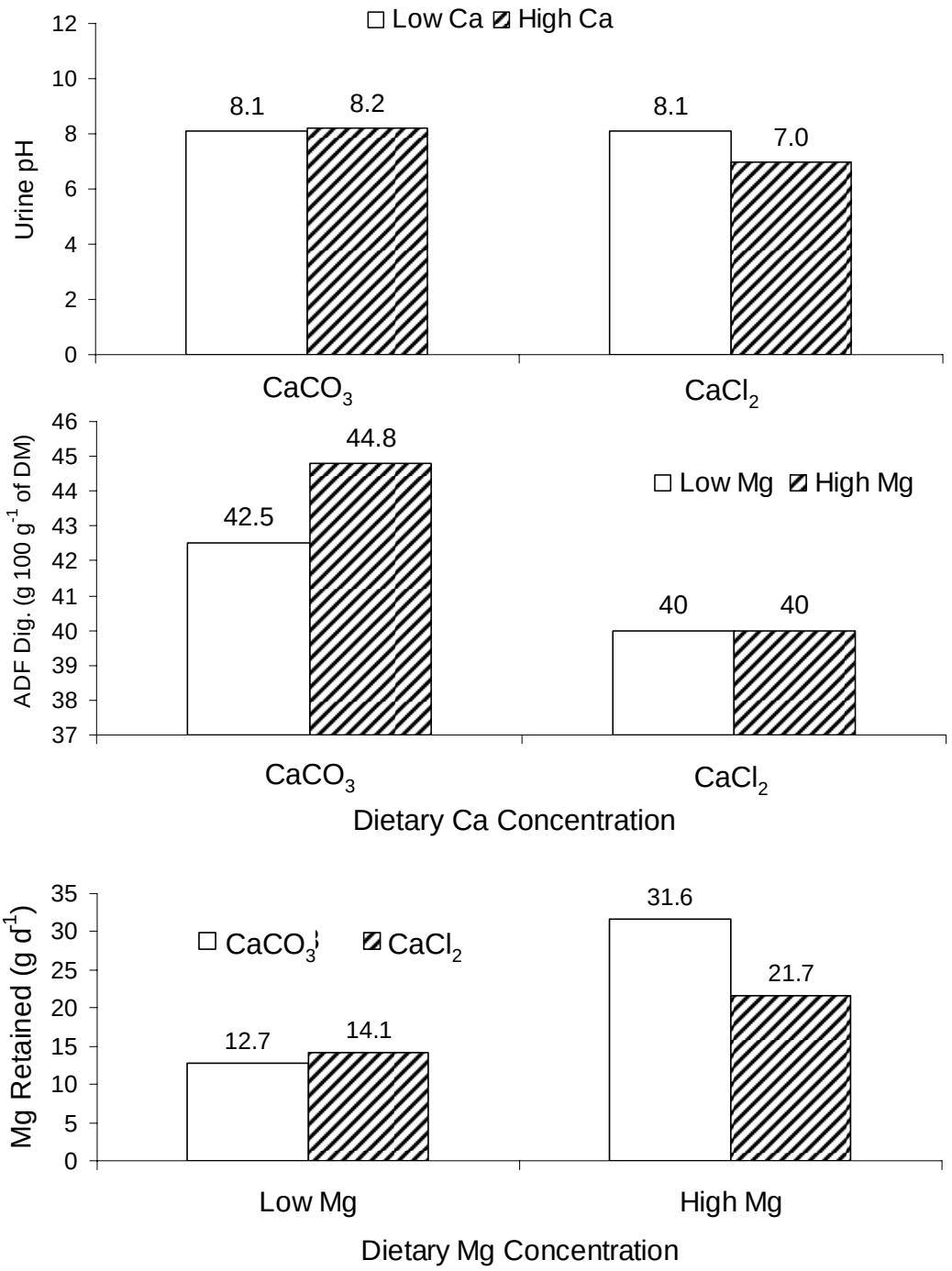

Figure 3-2. Interaction effect of a) Ca source by Ca concentration on urine pH and Ca source by Mg concentration on b) acid detergent fiber (ADF) digestibility, and c) Mg balance (Mg retained).

Table 3-1. Ingredients and their concentration in dry matter basis used in formulation of diets fed to lactating dairy cows.

Ingredient	g kg^{-1} DM
Corn silage	400
Alfalfa hay	120
Ground corn	220
Whole cottonseed	100
Extruded soybean meal	60
Soybean meal	60
Minerals and vitamins premix†	40

† Vitamin and vitamin premixes were added in the same proportion to all diets.

Table 3-2. Chemical composition of diets fed to lactating dairy cows.

Measure	Source of Ca, concentration of Ca, and concentration of Mg in diets							
	$CaCO_3$				$CaCl_2$			
	Dietary Ca (g kg^{-1} of DM)				Dietary Ca (g kg^{-1} of DM)			
	6.3	8.6	6.4	9.5	6.5	9.5	6.5	10.3
	Dietary Mg (g kg^{-1} of DM)				Dietary Mg (g kg^{-1} of DM)			
	2.4	2.4	4.3	4.0	2.6	2.7	3.7	3.8
Chemical Composition┼								
CP, g kg^{-1} DM	161	160	161	157	158	158	159	161
ADF, g kg^{-1} DM	203	202	202	202	204	200	202	199
NDF, g kg^{-1} DM	354	347	355	344	352	355	354	352
Non-fiber carbo-hydrates, g kg^{-1} DM	394	398	384	390	393	381	385	365
Ash, g kg^{-1} DM	57	60	66	74	63	72	68	87
P, g kg^{-1} DM	3.7	3.8	3.9	3.8	3.8	3.7	3.8	3.7
Ca, g kg^{-1} DM	6.3	8.6	6.4	9.5	6.5	9.5	6.5	10.3
Mg, g kg^{-1} DM	2.4	2.4	4.3	4.0	2.6	2.7	3.7	3.8
K, g kg^{-1} DM	12.0	12.0	13.0	13.0	13.0	13.0	12.0	13.0
Na, g kg^{-1} DM	5.0	4.0	4.0	5.0	4.0	5.0	4.0	5.0
S, g kg^{-1} DM	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Cl, g kg^{-1} DM	4.0	4.0	4.0	4.0	7.0	14.0	8.0	12.0
Fe, mg kg^{-1} DM	160	153	179	186	183	157	168	159
Zn, mg kg^{-1} DM	137	119	183	154	279	112	144	100
Cu, mg kg^{-1} DM	41.2	32.9	52.8	51.4	66.4	35.7	40.0	28.1
Mn, mg kg^{-1} DM	91.7	79.6	120	119.0	244.0	82.9	107.0	70.1

Table 3-2. Least squared means and standard error of the mean for animal production parameters of lactating dairy cows receiving two Ca sources, two Ca concentrations, and two Mg concentrations.

	Source of Ca, concentration of Ca, and concentration of Mg in diets								
	$CaCO_3$				$CaCl_2$				
	Dietary Ca (g kg^{-1} of DM)				Dietary Ca (g kg^{-1} of DM)				
	6.3	8.6	6.4	9.5	6.5	9.5	6.5	10.3	
Measure	Dietary Mg (g kg^{-1} of DM)				Dietary Mg (g kg^{-1} of DM)				
	2.4	2.4	4.3	4.0	2.6	2.7	3.7	3.8	SEM
DMI, kg day^{-1}	22.5	21.8	21.7	21.9	22.6	20.4	21.0	20.7	0.64
DMI, % of BW	3.8	3.7	3.7	3.7	3.8	3.5	3.6	3.5	0.12
Body Weight, kg	603	594	588	600	596	592	588	594	4.3
BW Change, kg 21 d^{-1}	19.8	6.6	-1.0	17.3	6.0	10.9	-9.2	16.3	5.9
BUN, mg $100ml^{-1}$	10.8	9.5	10.6	10.9	10.2	9.4	10.6	10.0	0.8
Glucose, mg $100ml^{-1}$	65.1	65.4	64.7	63.6	62.0	62.1	62.8	65.0	1.3
Urine pH	8.1	8.2	8.1	8.1	8.1	6.8	8.0	7.2	0.15
Milk yield, kg day^{-1}	35.9	34.0	34.5	34.9	37.6	33.2	33.7	33.2	0.9
Milk Efficiency	1.6	1.6	1.6	1.6	1.7	1.6	1.6	1.6	0.5
Milk fat, %	3.12	3.16	3.27	3.05	3.02	3.03	2.89	3.08	0.11
Milk Fat, kg day^{-1}	0.61	0.59	0.62	0.59	0.62	0.55	0.53	0.57	0.03
Fat Corrected Milk (FCM)	67.9	65.6	67.3	66.1	70.2	62.2	61.3	63.0	2.1
FCM Efficiency	1.40	1.37	1.42	1.38	1.42	1.38	1.33	1.41	0.49
Milk Protein, %	3.07	2.99	3.08	2.96	3.00	2.98	2.98	2.92	0.03
Milk Protein, kg day^{-1}	1.09	1.05	1.06	1.04	1.12	0.98	1.00	0.97	0.03
Somatic cell count (x1000)	165	110	191	165	361	189	68	159	17.8

Table 3-3. *P* values for animal production parameters of lactating dairy cows receiving two Ca sources, two Ca concentrations, and two Mg concentrations.

	$CaCO_3$ vs. $CaCl_2$ (1: Ca Source)	High vs. Low Ca (2: Ca Conc.)	Interaction 1 by 2	High vs. Low Mg (3: Mg Conc.)	Interaction 1 by 3	Interaction 2 by 3	Three way interaction 1 by 2 by 3
DMI, kg day^{-1}	0.0816	0.1122	0.2728	0.2318	0.6965	0.1135	0.6064
DMI, % of BW	0.1483	0.1163	0.3867	0.4143	0.8613	0.3666	0.5656
Body Weight, kg	0.2239	0.7018	0.9532	0.2574	0.8449	0.01	0.4133
BW Change, kg 21 d^{-1}	0.2707	0.0458	0.1563	0.2251	0.9881	0.0032	0.5436
BUN, mg $100ml^{-1}$	0.4411	0.2842	0.8294	0.3194	0.9688	0.379	0.5153
Glucose, mg $100ml^{-1}$	0.0607	0.6623	0.3953	0.6744	0.1278	0.8494	0.3278
Urine pH	<.0001	0.0002	<.0001	0.6658	0.6604	0.3374	0.2451
Milk yield, kg day^{-1}	0.4704	0.0156	0.1825	0.0764	0.1904	0.0136	0.5104
Milk Efficiency	0.3374	0.8568	0.8959	0.9257	0.6298	0.4118	0.7512
Milk fat, %	0.0621	0.8932	0.2468	0.896	0.7129	0.7893	0.1708
Milk Fat, kg day^{-1}	0.0528	0.3357	0.8632	0.2912	0.3158	0.216	0.1458
Fat Corrected Milk	0.0798	0.1179	0.6606	0.152	0.1959	0.0665	0.1665
FCM Efficiency	0.922	0.8689	0.4805	0.7903	0.5864	0.4608	0.3695
Milk Protein, %	0.0127	0.0057	0.176	0.3078	0.5411	0.436	0.9999
Milk Protein, kg day^{-1}	0.1165	0.0026	0.3866	0.0743	0.1305	0.0518	0.4912
Somatic cell count	0.8076	0.7327	0.4744	0.200	0.280	0.0773	0.2469

Table 3-4. Least squared means and standard error of the mean for nutrient digestibility of lactating dairy cows receiving two Ca sources, two Ca concentrations, and two Mg concentrations.

Measure	Ca Source, dietary Ca concentration, and dietary Mg concentration								
	$CaCO_3$				$CaCl_2$				
	Dietary Ca (g kg^{-1} of DM)				Dietary Ca (g kg^{-1} of DM)				
	6.3	8.6	6.4	9.5	6.5	9.5	6.5	10.3	
	Dietary Mg (g kg^{-1} of DM)				Dietary Mg (g kg^{-1} of DM)				
	2.4	2.4	4.3	4.0	2.6	2.7	3.7	3.8	SEM
Digestibility (%)									
DM	70.3	69.6	70.6	70.4	69.0	68.9	68.4	69.8	2.6
Protein	65.0	64.4	66.8	64.7	64.3	63.1	65.3	64.6	4.1
ADF	42.4	42.5	44.7	44.9	37.1	43.0	36.8	43.1	3.5
NDF	46.8	47.1	49.0	49.1	44.9	48.1	43.7	49.1	1.3
Phosphorus	57.7	63.4	58.1	58.7	57.2	61.4	59.1	61.2	1.4
Calcium	23.4	28.7	39.6	29.5	30.9	23.5	29.7	31.2	3.1
Magnesium	22.3	31.0	37.3	41.2	35.7	34.1	37.0	35.0	2.7

Table 3-5. *P* values for nutrient digestibility of lactating dairy cows receiving two Ca sources, two Ca concentrations, and two Mg concentrations.

	$CaCO_3$ vs. $CaCl_2$ (1: Ca Source)	High vs. Low Ca (2: Ca Conc.)	Interaction 1 by 2	High vs. Low Mg (3: Mg Conc.)	Interaction 1 by 3	Interaction 2 by 3	Three way interaction 1 by 2 by 3
DM	0.887	0.100	0.987	0.715	0.433	0.327	0.706
Protein	0.613	0.369	0.927	0.046	0.397	0.566	0.055
ADF	0.316	0.373	0.128	0.008	0.032	0.602	0.675
NDF	0.175	0.917	0.566	0.670	0.807	0.579	0.797
Phosphorus	0.338	0.255	0.853	0.234	0.917	0.789	0.628
Calcium	0.105	0.172	0.195	0.597	0.600	0.942	0.979
Magnesium	0.427	0.250	0.308	0.596	0.585	0.779	0.781

Table 3-6. Least squared means and standard error of the mean for dry matter and water intake, urinary and fecal output and overall P retention (g d^{-1}) of lactating dairy cows receiving two Ca sources, two Ca concentrations, and two Mg concentrations.

	Ca Source, dietary Ca concentration, and dietary Mg concentration								
	$CaCO_3$				$CaCl_2$				
	Dietary Ca (g kg^{-1} of DM)				Dietary Ca (g kg^{-1} of DM)				
	6.3	8.6	6.4	9.5	6.5	9.5	6.5	10.3	
Measure	Dietary Mg (g kg^{-1} of DM)				Dietary Mg (g kg^{-1} of DM)				
	2.4	2.4	4.3	4.0	2.6	2.7	3.7	3.8	SEM
DMI, kg d^{-1}	22.5	21.8	21.7	21.9	22.6	20.4	21.0	20.7	0.64
Water intake, kg d^{-1}	104	101	96.3	105	98.0	103	104	96.5	3.8
Fecal output, kg d^{-1}	6.7	6.5	6.3	6.4	6.9	6.3	6.6	6.2	0.2
Urine output, kg d^{-1}	40.3	49.5	51.5	38.5	48.8	42.8	33.2	36.2	9.9
Phosphorus									
Feed, g kg^{-1}	3.8	3.8	3.9	3.7	3.8	3.7	3.8	3.7	0.0
Water, g kg^{-1}	--	--	--	--	--	--	--	--	--
Feces, g kg^{-1}	5.3	4.6	5.5	5.2	5.3	4.7	5.0	4.8	0.2
Urine, g kg^{-1}	--	--	--	--	--	--	--	--	--
Intake in feed, g d^{-1}	85.0	83.0	84.0	81.0	86.0	75.0	79.0	76.0	2.1
Intake in water, g d^{-1}	--	--	--	--	--	--	--	--	--
Output in feces, g d^{-1}	36.0	30.0	34.0	34.0	36.0	29.0	32.0	29.0	2.0
Output in urine, g d^{-1}	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0
Output in milk, g d^{-1}	32.2	31.1	30.3	34.3	31.4	32.8	30.8	30.5	2.3
Retention, g d^{-1}	17.2	21.9	19.2	12.9	17.1	13.0	16.0	16.3	3.4

Table 3-7. *P* values for dry matter and water intake, urinary and fecal output and overall P balance (g d^{-1}) of lactating dairy cows receiving two Ca sources, two Ca concentrations, and two Mg concentrations.

	$CaCO_3$ vs. $CaCl_2$ (1: Ca Source)	High vs. Low Ca (2: Ca Conc.)	Interaction 1 by 2	High vs. Low Mg (3: Mg Conc.)	Interaction 1 by 3	Interaction 2 by 3	Three way interaction 1 by 2 by 3
DMI, kg d^{-1}	0.082	0.112	0.273	0.232	0.697	0.114	0.606
Water intake, kg d^{-1}	0.584	0.710	0.422	0.625	0.719	0.917	0.038
Fecal output, kg d^{-1}	0.863	0.071	0.176	0.114	0.747	0.391	0.991
Urine output, kg d^{-1}	0.507	0.815	0.980	0.424	0.461	0.633	0.292
Phosphorus							
Feed, g kg^{-1}	0.033	0.001	0.807	0.996	0.887	0.040	0.007
Water, g kg^{-1}	--	--	--	--	--	--	--
Feces, g kg^{-1}	0.202	0.009	0.941	0.442	0.147	0.191	0.910
Urine, g kg^{-1}	0.231	0.408	0.801	0.281	0.257	0.540	0.728
Intake in feed, g d^{-1}	0.011	0.004	0.194	0.199	0.620	0.286	0.130
Intake in water, g d^{-1}	--	--	--	--	--	--	--
Output in feces, g d^{-1}	0.247	0.005	0.505	0.812	0.331	0.076	0.968
Output in urine, g d^{-1}	0.469	0.907	0.358	0.186	0.948	0.676	0.939
Output in milk, g d^{-1}	0.706	0.539	0.798	0.804	0.516	0.580	0.318
Retention, g d^{-1}	0.364	0.577	0.830	0.612	0.369	0.473	0.134

Table 3-8. Least squared means and standard error of the mean for dry matter and water intake, urinary and fecal output and overall Ca retention (g d^{-1}) of lactating dairy cows receiving two Ca sources, two Ca concentrations, and two Mg concentrations.

	Ca Source, dietary Ca concentration, and dietary Mg concentration								
	$CaCO_3$				$CaCl_2$				
	Dietary Ca (g kg^{-1} of DM)				Dietary Ca (g kg^{-1} of DM)				
	6.3	8.6	6.4	9.5	6.5	9.5	6.5	10.3	
Measure	Dietary Mg (g kg^{-1} of DM)				Dietary Mg (g kg^{-1} of DM)				
	2.4	2.4	4.3	4.0	2.6	2.7	3.7	3.8	SEM
DMI, kg d^{-1}	22.5	21.8	21.7	21.9	22.6	20.4	21.0	20.7	0.64
Water intake, kg d^{-1}	104	101	96.3	105	98.0	103	104	96.5	3.8
Fecal output, kg d^{-1}	6.7	6.5	6.3	6.4	6.9	6.3	6.6	6.2	0.2
Urine output, kg d^{-1}	40.3	49.5	51.5	38.5	48.8	42.8	33.2	36.2	9.9
Calcium									
Feed, g kg^{-1}	6.0	8.6	6.5	9.5	6.4	9.5	6.4	10.2	0.15
Water, g kg^{-1}	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.00
Feces, g kg^{-1}	15.6	20.2	13.2	22.8	14.8	23.6	14.9	23.4	0.86
Urine, g kg^{-1}	0.03	0.03	0.00	0.02	0.02	0.19	0.01	0.18	0.02
Intake in feed, g d^{-1}	141	188	140	209	142	195	135	209	5.61
Intake in water, g d^{-1}	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	0.18
Output in feces, g d^{-1}	105	132	80	146	99	148	96	143	6.36
Output in urine, g d^{-1}	1.0	1.0	1.0	1.0	1.0	8.0	1.0	6.0	0.89
Output in milk, g d^{-1}	41.2	39.7	38.8	43.9	40.2	42.0	39.4	39.0	2.9
Retention, g d^{-1}	-3.9	17.8	19.8	21.4	2.2	-1.5	1.7	23.6	8.7

Table 3-9. *P* values for dry matter and water intake, urinary and fecal output and overall Ca retention (g d^{-1}) of lactating dairy cows receiving two Ca sources, two Ca concentrations, and two Mg concentrations.

	$CaCO_3$ vs. $CaCl_2$ (1: Ca Source)	High vs. Low Ca (2: Ca Conc.)	Interaction 1 by 2	High vs. Low Mg (3: Mg Conc.)	Interaction 1 by 3	Interaction 2 by 3	Three way interaction 1 by 2 by 3
DMI, kg d^{-1}	0.082	0.112	0.273	0.232	0.697	0.114	0.606
Water intake, kg d^{-1}	0.584	0.710	0.422	0.625	0.719	0.917	0.038
Fecal output, kg d^{-1}	0.863	0.071	0.176	0.114	0.747	0.391	0.991
Urine output, kg d^{-1}	0.507	0.815	0.980	0.424	0.461	0.633	0.292
Calcium							
Feed, g kg^{-1}	<.0001	<.0001	0.003	<.0001	0.170	0.006	0.607
Water, g kg^{-1}	0.927	0.519	0.523	0.997	0.205	0.636	0.059
Feces, g kg^{-1}	0.045	<.0001	0.236	0.907	0.938	0.054	0.044
Urine, g kg^{-1}	<.0001	<.0001	<.0001	0.391	0.733	0.644	0.889
Intake in feed, g d^{-1}	0.879	<.0001	0.572	0.103	0.413	0.008	0.952
Intake in water, g d^{-1}	0.798	0.745	0.752	0.743	0.259	0.444	0.177
Output in feces, g d^{-1}	0.200	<.0001	0.857	0.275	0.899	0.035	0.037
Output in urine, g d^{-1}	0.001	<.0001	<.0001	0.096	0.431	0.708	0.798
Output in milk, g d^{-1}	0.706	0.539	0.798	0.804	0.516	0.580	0.318
Retention, g d^{-1}	0.243	0.103	0.840	0.034	0.912	0.821	0.082

Table 3-10. Least squared means and SEM for dry matter and water intake, urinary and fecal output and overall Mg retention (g d^{-1}) of lactating dairy cows receiving two Ca sources, two Ca concentrations, and two Mg concentrations.

	Ca Source, dietary Ca concentration, and dietary Mg concentration								
	$CaCO_3$				$CaCl_2$				
	Dietary Ca (g kg^{-1} of DM)				Dietary Ca (g kg^{-1} of DM)				
	6.3	8.6	6.4	9.5	6.5	9.5	6.5	10.3	
Measure	Dietary Mg (g kg^{-1} of DM)				Dietary Mg (g kg^{-1} of DM)				
	2.4	2.4	4.3	4.0	2.6	2.7	3.7	3.8	SEM
DMI, kg d^{-1}	22.5	21.8	21.7	21.9	22.6	20.4	21.0	20.7	0.64
Water intake, kg d^{-1}	104	101	96.3	105	98.0	103	104	96.5	3.8
Fecal output, kg d^{-1}	6.7	6.5	6.3	6.4	6.9	6.3	6.6	6.2	0.2
Urine output, kg d^{-1}	40.3	49.5	51.5	38.5	48.8	42.8	33.2	36.2	9.9
Magnesium									
Feed, g kg^{-1}	2.3	2.5	4.4	4.0	2.6	2.7	3.7	3.8	0.04
Water, g kg^{-1}	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00
Feces, g kg^{-1}	6.1	5.6	9.4	7.9	5.3	5.8	7.5	8.1	0.28
Urine, g kg^{-1}	0.10	0.11	0.12	0.15	0.22	0.17	0.25	0.27	0.03
Intake in feed, g d^{-1}	54.0	53.5	95.0	87.8	57.1	54.5	76.9	76.5	2.0
Intake in water, g d^{-1}	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.1
Output in feces, g d^{-1}	41	36	59	51	36	36	48	50	1.9
Output in urine, g d^{-1}	3.0	4.0	5.0	6.0	6.0	6.0	6.0	8.0	0.8
Output in milk, g d^{-1}	4.5	4.3	4.2	4.8	4.4	4.6	4.3	4.3	0.3
Retention, g d^{-1}	6.7	9.9	27.1	27.1	10.1	8.9	19.3	15.4	2.6

Table 3-11. *P* values for dry matter and water intake, urinary and fecal output and overall Mg retention (g d^{-1}) of lactating dairy cows receiving two Ca sources, two Ca concentrations, and two Mg concentrations.

	$CaCO_3$ vs. $CaCl_2$ (1: Ca Source)	High vs. Low Ca (2: Ca Conc.)	Interaction 1 by 2	High vs. Low Mg (3: Mg Conc.)	Interaction 1 by 3	Interaction 2 by 3	Three way interaction 1 by 2 by 3
DMI, kg d^{-1}	0.082	0.112	0.273	0.232	0.697	0.114	0.606
Water intake, kg d^{-1}	0.584	0.710	0.422	0.625	0.719	0.917	0.038
Fecal output, kg d^{-1}	0.863	0.071	0.176	0.114	0.747	0.391	0.991
Urine output, kg d^{-1}	0.507	0.815	0.980	0.424	0.461	0.633	0.292
Magnesium							
Feed, g kg^{-1}	<.0001	0.958	0.001	<.0001	<.0001	<.0001	0.002
Water, g kg^{-1}	0.925	0.509	0.440	0.969	0.179	0.794	0.110
Feces, g kg^{-1}	0.005	0.313	0.001	<.0001	0.175	0.280	0.141
Urine, g kg^{-1}	<.0001	1.000	0.532	0.055	0.544	0.335	0.564
Intake in feed, g d^{-1}	<.0001	0.077	0.444	<.0001	<.0001	0.515	0.178
Intake in water, g d^{-1}	0.970	0.798	0.664	0.741	0.259	0.545	0.317
Output in feces, g d^{-1}	0.002	0.044	0.009	<.0001	0.271	0.949	0.271
Output in urine, g d^{-1}	0.001	0.206	0.556	0.013	0.415	0.348	0.542
Output in milk, g d^{-1}	0.706	0.538	0.798	0.804	0.516	0.580	0.318
Retention, g d^{-1}	0.024	0.813	0.269	<.0001	0.007	0.409	0.943

CHAPTER 4
DIETARY CONTROL OF CALCIUM AND MAGNESIUM AS A MEANS OF REDUCING PHOSPHORUS SOLUBILITY IN DAIRY FECES

Abstract

Large dietary intakes of calcium (Ca) and magnesium (Mg) can reduce phosphorus (P) absorption by the formation of insoluble phosphate inside the gastro intestinal tract; however, from an environmental perspective their interaction with P may decrease P solubility in dairy feces and minimize losses from agricultural lands. The objective of this study was to evaluate effects of dietary Ca and Mg concentrations and solubility of Ca source on P concentration and solubility in feces of lactating cows receiving diets with the same P concentration. Twenty four multiparous cows in mid lactation were fed eight different diets in three-21 d periods. Fecal samples were collected twice a day during the last 10 d of each period, composited within cow, and dried at 45 C. Successive water extractions (100:1 water:feces ratio, shaken for 1 h) were performed for all dried fecal samples. Dietary treatments had no effect on fecal P concentrations. Increased Ca concentration in both diet and feces reduced water extractable P (WEP) from fecal samples. Increased fecal concentrations of Mg and Ca in feces mutually suppressed the dissolution of each element, suggesting their association in a solid phase. Fecal samples with higher Ca and Mg concentrations showed reduced WEP; possibly, Ca and Mg mutually suppressed dissolution of Ca,Mg-P forms by the common ion effect. Calcium and Mg dietary modifications effectively reduced P solubility in fecal samples of lactating dairy cows despite the same dietary and fecal P concentration.

Introduction

Intensively managed animal operations import feedstuffs to satisfy dietary requirements and maximize animal production. Manure generated from these operations is often applied to soils in excess of crop requirements for phosphorus (P), leading to buildup of soil P

concentrations in the long term (Sentran and Ndayegamiye, 1995; Toth et al., 2006; Whalen and Chang, 2001), increasing the potential for offsite P movement with detrimental effects on surface water quality (Pote et al., 1996; van Es et al., 2004).

Manure-impacted soils often have conditions associated with increased P stability (elevated concentrations of P and Ca and high pH), but no solid state crystalline phosphate minerals have been identified in these soils (Harris et al., 1994) or in (dairy) manure-soil incubations (Cooperband and Good, 2002). Instead, P remains highly soluble, even after years of abandonment (Josan et al., 2005; Nair et al., 1995), where high concentrations of Mg and dissolved organic carbon have been proposed as inhibitors of more stable calcium-phosphate minerals (Ca-P) in manure-amended soils (Harris et al., 1994; Josan et al., 2005).

Research efforts have focused on decreasing P in animal diets and therefore P concentration in feces, the main route of P excretion (Morse et al., 1992). Dietary modifications have successfully reduced fecal P concentrations up to 33% when used in commercial dairy farms (Cerosaletti et al., 2004), particularly decreasing the water-soluble P fraction (Dou et al., 2002), with no detrimental effects on animal productivity (Wu and Satter, 2000). However, all of these experiments where significant reduction in manure P has been documented, initial dietary P was well above animals' requirements; furthermore fecal P concentrations remain above 4.0 g kg^{-1} of DM even when cows were fed with diets containing P at 3.1 g kg^{-1} of DM.

There is a threshold point to which dietary and therefore manure P can be reduced. Based on National Research Council recommendations, P should be fed to lactating dairy cows at approximately 3.7 g kg^{-1} of diet DM (NRC, 2001). Contrasting findings on animal performance have been reported when feeding P at concentrations below 3.7 g kg^{-1} of diet DM. In different experiments Wu et al. (2000) documented negative impacts on animal performance when

feeding P at 3.1 g kg^{-1} of diet DM, whereas Dou et al. (2002) concluded that 3.1 to 3.7 g of P kg^{-1} of dietary DM was adequate or near adequate for milk production.

Emphasis thus far has been placed on reducing dietary and fecal P concentrations, but the solubility and long-term stability of P in manure may be of equal or greater importance. Feces-derived components can play an important role in the fate of P in soils, particularly soils with low P sorbing capacity (Josan et al., 2005).

Phosphorus availability (digestibility or absorption) -and therefore excretion- in the gastro intestinal tract (GIT) of ruminants is influenced by several factors. Dietary concentration and source of P are most influential (Chapuis-Lardy et al., 2004); however, intestine pH, animal age, intestinal parasitism and intakes of Ca, Fe, Al, Mn, K and Mg can also affect P absorption. Increased concentration of cations in the diet has been shown to decrease P availability in the GIT and thereby to increase P excretion (McDowell, 2003); however, effects of those cations on fecal P solubility has not been evaluated.

Calcium and P nutrition are often considered together because they are closely related; excess of one can result in poor absorption of the other. From an animal science perspective, large intakes of Ca and Mg (as well as Fe and Al, usually low in dairy cow diets) are of concern because of their effect on decreasing P absorption by the formation of insoluble phosphate in the GIT (McDowell, 2003). From an environmental perspective and given the importance of reducing P losses from agricultural lands (Sims et al., 2000; USEPA, 2000), decreased P solubility in dairy manures is a desired characteristic.

This study tested the hypothesis that increased Ca availability in the GIT of lactating dairy cows will favor Ca-P formation with potential to reduce P solubility in feces. The objective was

to evaluate effects of 2 dietary concentrations of Ca and Mg and solubility of dietary Ca source on P concentration and solubility in feces of lactating cows.

Materials and Methods

The experiment was conducted at the University of Florida Dairy Research Unit from September to November of 2005. Cows were managed according to the guidelines approved by the University of Florida Animal Care and Use Committee. Eight different diets were fed to a group of 24 multiparous cows averaging 128 (±21) days in milk (DIM) and a production of 39.5 (±8.2) kg of milk a day. Cows were in a freestall barn with grooved concrete floors, and fans and sprinklers that operated when the temperature exceeded 25°C. Animals had continuous access to water and individual pens bedded with sand and with continuous lighting during night hours, and were milked mechanically three times a day at 0500, 1300 and 2100 h. While receiving the standard farm diet, cows were trained for ten days to use electronic feed gates (Calan gates; American Calan Inc., Northwood, NH) to allow measurement of dry matter intake (DMI) by individual cows. Before completion of the third period, one of the cows was removed from the experiment for health reasons.

Diets offered were formulated to contain the same P concentration (3.7 g P kg^{-1} of DM). Increased Ca availability in the GIT tract was achieved by: (a) using 2 Ca sources ($CaCO_3$ or $CaCl_2$) each having a different Ca availability coefficient and (b) using two calcium concentrations in the diet. Inorganic Ca supplements selected were the industry standard ($CaCO_3$) and an anionic salt ($CaCl_2$). Calcium chloride is used in diet formulation to obtain desired anion-cation value, for pregnant nonlactating dairy cows close to parturition. Desired Ca concentrations were 6.0 (LoCa) and 10.0 (HiCa) g kg^{-1} of dietary DM. The former was selected based on NRC (2001) recommendations for lactating dairy cows; the latter, chosen to increase Ca concentration in the diet while maintaining $CaCl_2$ at a concentration below 27.5 mg kg^{-1} of

the diet DM and prevent possible negative effects on DMI. Consideration was also given to the Ca:P ratio in the diet. Desired Mg concentrations were 2.0 (LoMg) and 3.5 (HiMg) g kg^{-1} of diet DM. The lower Mg concentration requires no inorganic Mg supplementation while meeting animal requirements; the high Mg concentration is used in Florida to minimize negative impacts of heat stress on lactating dairy cows during the summer months.

Dietary ingredients (and proportions) for all diets were: corn silage (40%); alfalfa hay (12%), ground corn (22%), whole cottonseed (10%), soybean meal (6%), extruded soybean meal (6%), and vitamin and mineral premix (4%) on dry matter basis. Treatments (diets) were assigned to cows in three 21-d periods. During the experiment each cow received a treatment only once and no treatment followed another treatment from the previous period more than once. During the first 11-d of each period cows were adjusted to a new diet. Fecal grab samples were collected twice a day from day 12 to day 21 to obtain a composite sample per cow per period. Individual feed ingredient were sampled once a week during each period and composited per period. Composited feed and fecal samples were dried at 45°C in a forced-air oven and ground to pass the 2-mm screen of a Wiley mill (A.H. Thomas, Philadelphia, PA). Fecal output was calculated using the marker ratio technique with chromic oxide (Cr_2O_3) as an inert dietary marker. Cows were dosed with 10 g of Cr_2O_3 twice a day from d 11 to 20 of each experimental period.

Fecal samples were dried to standardize all samples to the same DM content so that the same amount of moisture+DM from each fecal sample would be mixed with distilled water for extraction. Drying is also effective to obtain consistency in sample treatment, preserve samples, and avoid DM variability in feces between animals and within animals in different sampling events. Furthermore, feces (or manure) often undergo a drying period upon application to soil.

We are aware of the possible effects on P solubility when fecal samples are dried. Previous research has reported contradictory findings. Ajiboye et al. (2000) reported an increased in water extractable P (WEP) from dairy cow manure when samples were oven-dried at 105 ºC whereas Chapuis-Lardy et al. (2004) showed a decrease in inorganic P soluble in water when dairy cow fecal samples were dried at 65 ºC. We believe that relative treatment effects would not be compromised by drying feces at low temperature, as Dou et al. (2000) found that a ~70% of P in dairy manure was water soluble and most of it was extracted in the initial steps of repeated water extractions.

Ten successive water extractions were performed on all 71 fecal samples. A mixture of 100:1 water:feces ratio was selected to maximize P solubility and extraction and detect differences in WEP among treatments in the long term. After 1h of shaking, samples were centrifuged at 1000x g for 5 minutes. The supernatants were collected and filtered through 0.45 µm filter. Extractions were carried out at room temperature (~25 °C). Collected supernatant solutions were analyzed for soluble reactive phosphorus, Ca, and Mg. Fecal samples were analyzed in triplicates for total P, Ca and Mg by the ignition method (Andersen, 1976). Total and water-extractable Ca and Mg were measured by atomic absorption (AA) spectroscopy; P was determined on a UV-visible recording spectrophotometer at 880 nm wave-length via the molybdate-blue colorimetric method (Murphy and Riley, 1962) (U.S. EPA, 1993; method 365.1). Composited and dried feed samples were analyzed for DM (105°C for 8 h), neutral detergent fiber (NDF) using heat-stable α-amylase (Goering and Van Soest, 1970; Van Soest et al., 1991), acid detergent fiber (ADF) (AOAC, 1990), and total nitrogen (N); crude protein (CP) was calculated by multiplying N x 6.25(Elementar Analysensysteme, Hanau, Germany). Crude protein was calculated by multiplying N x 6.25. In addition, a composites were sent to a DHIA

Forage Testing Laboratory (Dairy One, Ithaca, NY) where samples were analyzed by wet chemistry for Ca, P, Mg, K, Na, Zn, Cu, Mn, and Fe by the ignition method (Andersen, 1976); Cl was determined by titration with $AgNO_3$ using Brinkman Metrohm 716 Titrino Titration Unit with silver electrode (Metrohm Ltd., C-H-9101 Herisau, Switzerland) and S by oxidation (Leco Model SC-432, Leco Instruments, Inc) (Table 4-1).

Fecal samples from 3 cows from each of the two treatments that resulted in highest and lowest WEP were selected to be analyzed by Scanning Electron Microscope (SEM). Samples were first sieved to pass a 53 μm screen. Samples were placed on a SEM carbon mount and then coated with a thin layer of carbon. The sample was analyzed with a JSM 6400 Scanning Electron Microscope unit using an accelerating voltage of 15 kV. A general dot map was first obtained from the sample; then an elemental spectral was obtained from areas with high P concentration. Those samples and three samples from two other diets ($LoCaCO_3$, Hi Mg and Hi $CaCO_3$, HiMg) were analyzed by X-ray diffraction in a dry powder mount preparation.

Visual-MINTEQ Version 2.51 was used to calculate chemical speciation (including solid-phase equilibrium forms) under an open system for data from the first extraction (Gustafsson, 2005). In addition to P, Ca and Mg measurements for all extracts, chemical speciation data included pH and electrical conductivity (EC) using a standard pH and conductivity meter; Na and K by AA; and dissolved organic C determined using a carbon analyzer (TOC-5050A; Shimadzu, Kyoto, Japan) (Method 5310A; American Public Health Association, 1992).

Statistical Analyses: Treatments were arranged in a 2x2x2 factorial design using an incomplete, partially balanced Latin square. Data was analyzed using the GLM procedure of SAS. Orthogonal single degree of freedom contrasts were used to detect main effect of Ca source, Ca concentration, and Mg concentration as well as 2- and 3-way interactions.

The statistical model used to analyze the data was:

$Y_{ijk} = \mu + \alpha_i + b_j + c_k + e_{ijk}$
Where:
Y_{ikj} = observed response,
μ = overall mean,
α_i = fixed effect of treatment,
b_j = random effect of cow,
c_k = fixed effect of period, and
e_{ijk} = residual error.

Correlations and nonlinear regressions were used to describe relationships between elements in our analysis. Differences discussed in the text were significant at $P \leq 0.05$ unless otherwise indicated.

Results and Discussion

Dietary and Total Fecal Concentrations of Phosphorus, Calcium, and Magnesium

Several factors have been shown to affect mineral digestibility and therefore excretion; intake amount and source, intestine pH, age of animal, intestinal parasitism and intake level of other elements are among the most influential (McDowell, 2003). In this experiment fecal concentration of P was not affected by dietary concentration of Ca and Mg. Dietary concentration of Ca and Mg did not affect fecal concentration of each other. Therefore, of the measured parameters, dietary concentration was the most influential factor controlling fecal concentration of each respective mineral. No differences in concentration of dietary or fecal P were detected based on the single degree of freedom contrasts used (Table 4-2 and 4-4). This is consistent with other reports when dietary and fecal P have been measured in lactating dairy cows (Morse et al., 1992) and more recently (Wu et al., 2000).

Increasing dietary concentration of Mg did not affect fecal P concentration (Table 4-4). However, feeding HiCa diets, irrespectively of source, reduced concentration of P in feces (4.8 vs. 5.3 g kg^{-1}). Contrary to our results, Steevens et al. (1971) reported that higher dietary Ca

concentrations resulted in decreased P absorption (or increased P excreted) by lactating dairy cows. However, Borucki Castro et al. (2004) reported no differences in fecal or urine P concentrations with changes in dietary anion-cation balance for lactating dairy cows. Deitert and Pfeffer (1993), working with milking goats over two lactations, found no consistent effect when doubling dietary Ca NRC recommendations on P balance. Additionally, Wise et al. (1963) found no detrimental effects on growth or feed efficiency of calves when dietary Ca:P ratios were between 1:1 and 7:1. Although the exact mechanism for increased fecal P with decreased dietary Ca is not clear, no detrimental effects on animal performance due to reduced P availability or absorption were observed with dietary Ca:P ratios used in this experiment.

Dietary Ca and Mg concentrations had higher variability than expected from ration formulation calculations (Table 4-1). However, based on NRC (2001) recommendations, measured Ca and Mg concentrations in the diet were in a safe range to meet animal requirements without interfering with absorption of other nutrients. Variability of those elements in the diet was reflected by an interaction between dietary Ca source by Ca concentration on total Mg concentration in feces ($P = 0.001$), though fecal Mg concentrations closely related to dietary Mg concentration. Effects of dietary concentrations of Ca, P and Mg on Mg fecal concentrations in this experiment are consistent with those reported by Weiss (2004) when measuring digestibility of Mg in lactating dairy cows. Weiss found that Ca concentrations in manure reflected its dietary amounts; Ca source affected total Ca concentrations in manure, but this effect was driven by higher dietary Ca in diets containing $CaCl_2$ as the inorganic Ca source.

Increased fecal Ca concentrations (14.2 to 23.5 g kg^{-1}) were greater, proportionally, than its counterparts in the diet (6.4 vs. 9.5 g kg^{-1}), suggesting a decrease in apparent digestibility (absorption efficiency) with increased dietary Ca. Calcium concentrations in the HiCa diets are

above the animal requirement, such that additional intake is not absorbed as efficiently in the digestive tract. Contrary to Ca and P, apparent Mg digestibility increased with increased dietary Mg concentration, possibly because of greater availability of inorganic Mg supplement (added to HiMg diets) as opposed to the Mg present in organic feedstuffs (i.e. corn silage).

Effect of Dietary and Fecal Concentrations of Ca and Mg on Water Extractable P, Ca, and Mg

Water extractable P data were analyzed as concentration (mg kg^{-1}) and standardized to TP in feces (WEP:TP). There were no differences in fecal TP among treatments, so statistical results from WEP and WEP:TP were essentially equivalent. Therefore, only concentration data will be discussed.

Water extractable P was reduced when $CaCl_2$ was the inorganic Ca source in the diet (as opposed to $CaCO_3$) (1.98 vs. 2.31 g kg^{-1}). Increasing dietary Ca concentrations, regardless of the Ca source reduced WEP (1.80 vs. 2.49 g kg^{-1})) (Fig. 4-1a). Increased Ca availability, not only concentration, also had an effect on reducing WEP (r^2=0.37). Results from our experiment suggest that increased Ca delivered to the GIT, either by increased solubility (source effect) or increased concentration (HiCa vs LoCa) reduced WEP in feces from cows receiving those treatments, perhaps because of increased association of Ca with P. Increased Mg concentration in diet and feces did not have an effect on WEP (2.26 vs. 2.03 g kg^{-1}) (Fig. 4-1).

Cumulative water extractable Mg in feces was consistent with dietary and fecal Mg concentrations. Although interactions of dietary Ca source by Ca concentration treatments and Ca concentration by Mg concentration in the diet affected fecal WEMg, these interactions reflected the variability in dietary concentrations of both Ca and Mg.

Cumulative water extractable Ca concentration from feces was greater in samples from cows receiving HiCa diets, consistent with TCa in fecal samples. However, LoCa diets had a

greater proportion extracted when data were normalized to TCa concentration in feces (WECa:TCa). Fecal samples from animals receiving HiMg diets had lower WECa compared to those fed LoMg diets (5.10 vs. 5.81 g kg^{-1}), whereas diets containing $CaCl_2$ reduced total and WEMg (5.06 vs. 5.85 g kg^{-1}) concentration in feces. Therefore, increased fecal concentrations of Mg and Ca in feces had a synergistic effect on reducing solubility of each other, suggesting their association in a solid phase and limited dissolution because of the common ion effect.

Water extractable P, Ca, and Mg concentrations were also analyzed cumulatively after each individual extraction; there was no effect of dietary Ca concentration and source on cumulative WEP for the first seven extractions, after the seventh extraction, cumulative WEP from fecal samples from cows receiving HiCa diets was lower than WEP from cows fed the LoCa diets (Fig 4-2a). Increases in WEP after the seventh extraction were smaller for fecal samples from HiCa treatments, suggesting that remaining P in the sample has increased stability; chemical speciation modeling indicated that Ca-P or Ca,Mg-P minerals were undersaturated based on extract solution concentrations. Dietary Ca concentration was also the main variable affecting WECa; feces from cows receiving HiCa diets had increased WECa throughout the ten extractions. The same was true for WEMg from feces and dietary Mg concentrations (Fig. 4-2).

Relationship between Fecal WEP with WEMg and WECa Concentrations in Fecal Samples

Release of P in water extractions from fecal samples was associated with that of Ca and Mg (Table 4-3). This relationship was negative for the first extraction where increased concentrations of Ca and Mg (mmol kg^{-1} of feces) reduced WEP in all treatments (r^2=0.23); this trend did not change when data were analyzed by Ca source, and Ca or Mg concentration in the diet. The presence of soluble Ca and Mg salts might be responsible for the high concentration of those two elements in the first extraction. Chemical speciation analysis of the first extraction showed that concentration of P species in solution was dominated by HPO_4^{-2} and $H_2PO_4^-$

representing 65% or more of TP in solution; Mg-P represented ~20% and Ca-P ~10%; K and Na-P combined for less than 1% of TP in solution. These results are consistent with leachate data from manure amended soils using a 1:10 soil to solution ratio (Silveira et al., 2006). Saturation indices showed solutions to be supersaturated with respect to whitlockite (Ca,Mg-P) and hydroxylapatite (Ca-P), indicating that these minerals, if present, would not have dissolved in the first extract. Possibly, Ca and Mg together suppressed dissolution of Ca,Mg-P forms (e.g., whitlockite) by the common ion effect.

Concentrations of Ca and Mg in solution after the first extraction were greatly reduced compared to the first extraction (Fig. 4-2); we suspect that most of the readily-soluble Ca and Mg salts were dissolved in the first extraction because of the high solution to feces ratio used. From the second extraction onward, relationship of WEP with WECa and WEMg from fecal samples changed to a positive one, possibly due to dissolution of Ca-Mg phosphate. These results are consistent with previous reports when using high water:manure ratio (Kleinman et al., 2002) and when studying P release from manure-amended soils (Hansen and Strawn, 2003; Josan et al., 2005; Silveira et al., 2006).

Regression coefficients for the association of WEP with WECa, WEMg, or WECa+WEMg (Table 4-3) were calculated using data for extractions 2-10. Water extractable P variability was better explained by Mg release from fecal samples than Ca, a behavior consistent across diets with or without the inclusion of data from the first extraction in the equation.

Interactions observed between WECa and WEMg, and the observed associated release between P, Ca, and Mg led us to use solid state assessments in fecal samples to explore reasons for the significant differences in WEP among feces (treatments) with similar TP content.

No evidence of a crystalline Ca and/or Mg phosphate mineral was detected by X-ray diffraction analysis in dried fecal samples. However, when these samples were ashed to remove organic matter, different minerals (Ca/Mg phosphate among them) were observed. sylvite (KCl) and calcite ($CaCO_3$) were common minerals to all samples analyzed and used as internal standards. Whitlockite $(Ca,Mg)_3(PO_4)_2$ was the only P-containing mineral detected in fecal samples from animals being fed $LoCaCO_3$, HiMg diet (Fig. 4-3.1); whitlockite and some hydroxyl-apatite $Ca_5(PO_4)_3(OH, Cl, F)$ were detected in ashed fecal samples from animals receiving diet with $LoCaCO_3$, LoMg (Fig. 4-3.2). Feces from cows fed HiCa diets behaved differently, those from cows fed $HiCaCO_3$, HiMg showed a dominance of hydroxyl-apatite with some withlockite (Fig. 4-3.3) whereas hydroxyl-apatite was the only P-containing mineral observed in ashed feces from dairy cows fed $HiCaCl_2$, HiMg diets (Fig. 4-3.4). Under water-extraction conditions used in this experiment (and most environmental conditions) hydroxyl-apatite is a mineral with greater stability (reduced solubility) than withlockite (β-Tri-Ca/Mg phosphate) (Lindsay, 1979). Mineral sequence documented in fecal samples is consistent with the observed differences in WEP from those samples and leads us to believe dietary modifications, particularly increased dietary Ca availability, affected the inorganic form in which P is excreted and therefore its solubility.

There is uncertainty as to whether these minerals were present in feces prior to ashing as they were not detected by X-ray diffraction. We suspect that (a) they may have been present in concentrations below detection, (b) they were not crystalline and therefore not detected by XRD or (c) they were not present at all. Despite this uncertainty, there is no doubt fecal samples from cows fed HiCa diets had lower cumulative WEP in correspondence with the expected solubility of the minerals found in those samples.

Dot maps and elemental spectral data showed high spatial associations between Ca, Mg and P in areas first identified with high P concentrations; in some cases the association was mainly with Mg, or Ca and in other cases with both (Fig 4-4). This trend was consistent in samples from the two diets analyzed, lowest and highest WEP. These findings support the idea of a solid phase where both Ca and Mg are combined with P, independently (P with Ca or Mg) or together (P with both Ca and Mg).

Impact of Dietary Modifications on Fecal-P Solubility

Dietary Ca concentration affected fecal TP concentration. Cows consuming HiCa diets had lower fecal TP than cows being fed LoCa diet (4.8 vs. 5.3 g P kg^{-1} feces; $P = 0.009$). This difference resulted in increased total excretion of P from cows fed LoCa diets (34.5 vs. 30.5 g P cow^{-1} d^{-1} feces; $P = 0.005$). As a result of these differences and the effect of increasing dietary Ca on fecal WEP, fecal samples from cows under HiCa diets had lower cumulative WEP after ten extractions. Increasing dietary Ca concentration reduced cumulative WEP (15.9 vs. 11.7 g P cow^{-1} d^{-1}; $P <.0001$).

Main dietary treatments of Ca source and Mg concentrations had no effect on fecal TP concentration or output (Table 4-5). However, feeding $CaCl_2$ reduced cumulative WEP from feces and daily WEP when fecal excretion was factored in (Table 4-6). We believe feeding $CaCl_2$, a Ca source of greater solubility in the rumen than $CaCO_3$ (100 vs. 68%) provided a more steady flow of Ca to the small intestine, site where Ca and P may first interact. Once in the small intestine food is not subject to acidic pH conditions and therefore any Ca-P formed may be excreted in that condition. Based on XRD data of ashed fecal samples, formation of less soluble Ca-P is expected in the GIT of cows fed higher available Ca, reason for the reduction in cumulative WEP of fecal samples from cows being fed the $CaCl_2$ diets (15.1 vs. 12.5 g WEP cow^{-1} d^{-1}).

Differences in WEP factored in with fecal output provide an opportunity to better observe the impact of dietary modifications on the overall P balance in a dairy farm. Increasing available dietary Ca reduced the P solubility and its potential for offsite movement.

Conclusions

Increasing Ca availability in the GIT either by increased dietary concentration or by feeding a more soluble Ca form reduced WEP from feces, but dietary Mg concentrations did not have an effect on WEP. High correlation coefficients in the release of WECa and WEMg with WEP in water extractions suggest Ca, Mg and P are associated in feces. This inference is supported by solid-state evidence of spatial presence of Ca, Mg, and P in fecal samples regardless of the dietary treatment and by XRD results from ashed fecal samples. Phosphorus solubility for dairy cows feces can be reduced by dietary control of Ca and Mg when the same dietary P concentration is fed; the effect is driven by increased dietary Ca availability.

Table 4-1. Nutrient content of diets with different Ca and Mg concentrations and two calcium sources fed to the lactating dairy cows.

Measure	Ca Source, dietary Ca concentration, and dietary Mg concentration							
	$CaCO_3$				$CaCl_2$			
	Dietary Ca (g kg^{-1} of DM)				Dietary Ca (g kg^{-1} of DM)			
	6.3	8.6	6.4	9.5	6.5	9.5	6.5	10.3
	Dietary Mg (g kg^{-1} of DM)				Dietary Mg (g kg^{-1} of DM)			
	2.4	2.4	4.3	4.0	2.6	2.7	3.7	3.8
Chemical Composition[†]								
CP, g kg^{-1} DM	161	160	161	157	158	158	159	161
ADF, g kg^{-1} DM	203	202	202	202	204	200	202	199
NDF, g kg^{-1} DM	354	347	355	344	352	355	354	352
Lignin, g kg^{-1} DM	35	34	35	34	35	34	35	34
Non-fiber carbo-hydrates, g kg^{-1} DM	394	398	384	390	393	381	385	365
Ash, g kg^{-1} DM	57	60	66	74	63	72	68	87
P, g kg^{-1} DM	3.8	3.8	3.9	3.8	3.8	3.7	3.8	3.7
Ca, g kg^{-1} DM	6.3	8.6	6.4	9.5	6.5	9.5	6.5	10.3
Mg, g kg^{-1} DM	2.4	2.4	4.3	4.0	2.6	2.7	3.7	3.8
K, g kg^{-1} DM	12.0	12.0	13.0	13.0	13.0	13.0	12.0	13.0
Na, g kg^{-1} DM	5.0	4.0	4.0	5.0	4.0	5.0	4.0	5.0
S, g kg^{-1} DM	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Cl, g kg^{-1} DM	4.0	4.0	4.0	4.0	7.0	14.0	8.0	12.0
Fe, mg kg^{-1} DM	160	153	179	186	183	157	168	159
Zn, mg kg^{-1} DM	137	119	183	154	279	112	144	100
Cu, mg kg^{-1} DM	41.2	32.9	52.8	51.4	66.4	35.7	40.0	28.1
Mn, mg kg^{-1} DM	91.7	79.6	120	119	244	82.9	107	70.1

[†]CP= Crude Protein; NDF= Neutral Detergent Fiber; ADF= Acid Detergent Fiber; DM= Dry Matter.

Table 4-2. *P* value for orthogonal single degree of freedom contrast for total dietary concentrations of phosphorus (P), calcium (Ca), and magnesium.

	$CaCO_3$ vs. $CaCl_2$ (1: Ca Source)	High vs. Low Ca (2: Ca Conc.)	Interaction 1 by 2	High vs. Low Mg (3: Mg Conc.)	Interaction 1 by 3	Interaction 2 by 3	Three way interaction 1 by 2 by 3
TP, g kg^{-1}	0.1345	0.1345	0.5811	1.0000	0.9723	0.6047	0.3075
TCa, g kg^{-1}	0.0244	<.0001	0.0915	0.0473	0.7310	0.0790	0.9838
TMg, g kg^{-1}	0.1291	0.8452	0.0814	<.0001	0.0009	0.2285	0.2444

Table 4-3. Total (T) fecal and water-extractable (WE) concentrations of P, Ca, and Mg in lactating dairy cows feces after ten successive extractions with water.

	Ca Source, dietary Ca concentration, and dietary Mg concentration							
	$CaCO_3$				$CaCl_2$			
	Dietary Ca (g kg^{-1} of DM)				Dietary Ca (g kg^{-1} of DM)			
	6.3	8.6	6.4	9.5	6.5	9.5	6.5	10.3
Measure	Dietary Mg (g kg^{-1} of DM)				Dietary Mg (g kg^{-1} of DM)			
	2.4	2.4	4.3	4.0	2.6	2.7	3.7	3.8
TP, g kg^{-1}	5.3	4.6	5.5	5.2	5.3	4.7	5.0	4.8
TCa, g kg^{-1}	15.6	20.2	13.2	22.8	14.8	23.6	14.9	23.4
TMg, g kg^{-1}	6.1	5.6	9.4	7.9	5.3	5.8	7.5	8.1
WEP, g kg^{-1}	2.8	2.1	2.4	2.0	2.4	1.68	2.3	1.5
WECa, g kg^{-1}	5.0	6.7	4.5	5.5	4.8	6.9	4.8	5.6
WEMg, g kg^{-1}	4.6	4.7	7.8	6.4	3.6	5.2	5.7	5.7
	---------- WE mineral : Total Mineral ----------							
WEP, g $100g^{-1}$	53.4	45.2	44.4	37.9	43.7	36.2	47.3	32.3
Ca, g $100g^{-1}$	34.0	33.6	33.8	25.0	33.7	31.0	34.5	24.5
Mg, g $100g^{-1}$	77.3	82.7	84.2	81.4	70.0	91.4	78.5	69.8

Table 4-4. *P* values for total (T), cumulative water-extractable (WE), and water-extractable:Total concentration of phosphorus (P), calcium (Ca), and magnesium (Mg) in feces of lactating dairy cows receiving two Ca sources, two Ca concentrations, and two Mg concentrations.

	$CaCO_3$ vs. $CaCl_2$ (1: Ca Source)	High vs. Low Ca (2: Ca Conc.)	Interaction 1 by 2	High vs. Low Mg (3: Mg Conc.)	Interaction 1 by 3	Interaction 2 by 3	Three way interaction 1 by 2 by 3
TP, g kg^{-1}	0.202	0.009	0.941	0.442	0.147	0.191	0.910
TCa, g kg^{-1}	0.045	<.0001	0.236	0.907	0.938	0.054	0.044
TMg, g kg^{-1}	0.005	0.313	0.001	<.0001	0.175	0.280	0.141
WEP, g kg^{-1}	0.002	<.0001	0.366	0.073	0.436	0.759	0.462
WECa, g kg^{-1}	0.853	<.0001	0.847	0.016	0.735	0.096	0.643
WEMg, g kg^{-1}	0.004	0.738	0.0143	<.0001	0.053	0.009	0.869
			---------- WE mineral : Total Mineral ----------				
P, g $100g^{-1}$	0.014	<.0001	0.376	0.053	0.075	0.479	0.295
Ca, g $100g^{-1}$	0.684	0.002	0.616	0.036	0.656	0.019	0.887
Mg, g $100g^{-1}$	0.194	0.208	0.407	0.612	0.197	0.003	0.099

Table 4-5. Correlation coefficient (r^2) between water-extractable P (WEP) and water-extractable Ca (WECa), water-extractable Mg (WEMg) or water-extractable Ca+ water-extractable Mg in fecal samples from lactating dairy cows fed diets differing in Ca source, Ca concentration and Mg concentration.

	Ca Source, dietary Ca concentration, and dietary Mg concentration							
	$CaCO_3$				$CaCl_2$			
	Dietary Ca (g kg^{-1} of DM)				Dietary Ca (g kg^{-1} of DM)			
	6.3	8.6	6.4	9.5	6.5	9.5	6.5	10.3
Correlation	Dietary Mg (g kg^{-1} of DM)				Dietary Mg (g kg^{-1} of DM)			
	2.4	2.4	4.3	4.0	2.6	2.7	3.7	3.8
WEP vs. WECa	0.60	0.43	0.20	0.12	0.46	0.35	0.51	0.11
WEP vs.WEMg	0.81	0.61	0.55	0.65	0.53	0.65	0.67	0.55
WEP vs.WECa + WEMg	0.77	0.61	0.49	0.50	0.55	0.35	0.69	0.45

Table 4-6. Least squared means and SEM for fecal output, P concentration in the feces, P excretion via feces and the cumulative WEP per kilogram of feces, per day per cow and its implications in a 1000 head farm of lactating dairy cows receiving two Ca sources, two Ca concentrations, and two Mg concentrations.

	Ca Source, dietary Ca concentration, and dietary Mg concentration								
	$CaCO_3$				$CaCl_2$				
	Dietary Ca (g kg^{-1} of DM)				Dietary Ca (g kg^{-1} of DM)				
	6.3	8.6	6.4	9.5	6.5	9.5	6.5	10.3	
Measure	Dietary Mg (g kg^{-1} of DM)				Dietary Mg (g kg^{-1} of DM)				
	2.4	2.4	4.3	4.0	2.6	2.7	3.7	3.8	SEM
Phosphorus Excretion									
Fecal output, kg d^{-1} cow^{-1}	6.7	6.5	6.3	6.4	6.9	6.3	6.6	6.2	0.2
P conc.in feces, g kg^{-1}	5.3	4.6	5.5	5.2	5.3	4.7	5.0	4.8	0.2
P out in feces, g $d^{-1}cow^{-1}$	35.5	30.1	34.2	33.5	36.3	28.9	32.1	29.6	1.9
Phosphorus Solubility									
Cumulative WEP, g kg^{-1} of feces	2.7	2.2	2.4	2.0	2.3	1.8	2.2	1.3	0.14
Cumulative WEP, g d^{-1} cow^{-1}	17.9	14.5	15.3	12.7	15.6	11.4	14.7	8.28	0.15
WEP, g 100 g^{-1} of TP	53.4	45.2	44.4	37.9	43.7	36.2	47.3	32.3	2.9
Impact 1000 head dairy									
TP Excreted, kg d^{-1}	35.5	30.1	34.2	33.5	36.3	28.9	32.1	29.6	1.9
Cumulative WEP, kg d^{-1}	17.9	14.5	15.3	12.7	15.6	11.4	14.7	8.28	0.15

Table 4-7. *P* values for fecal output, P concentration in the feces, P excretion via feces and the cumulative water-extractable P per kilogram of feces, per day per cow and its implications in a 1000 head farm of lactating dairy cows receiving two Ca sources, two Ca concentrations, and two Mg concentrations.

	$CaCO_3$ vs. $CaCl_2$ (1: Ca Source)	High vs. Low Ca (2: Ca Conc.)	Interaction 1 by 2	High vs. Low Mg (3: Mg Conc.)	Interaction 1 by 3	Interaction 2 by 3	Three way interaction 1 by 2 by 3
Phosphorus Excretion							
Fecal output, kg d^{-1}	0.863	0.071	0.176	0.114	0.747	0.391	0.991
P conc.in feces, g kg^{-1}	0.202	0.009	0.941	0.442	0.147	0.191	0.910
P output in feces, g d^{-1}	0.247	0.005	0.505	0.812	0.331	0.076	0.968
Phosphorus Solubility							
Cumulative WEP, g kg^{-1} of feces	0.0018	<.0001	0.3657	0.0731	0.4763	0.7593	0.4622
Cumulative WEP, g d^{-1} cow^{-1}	0.0114	<.0001	0.1910	0.0331	0.6379	0.3684	0.5154
WEP, g 100 g^{-1} of TP	0.0142	<.0001	0.3756	0.0525	0.0753	0.4789	0.2951

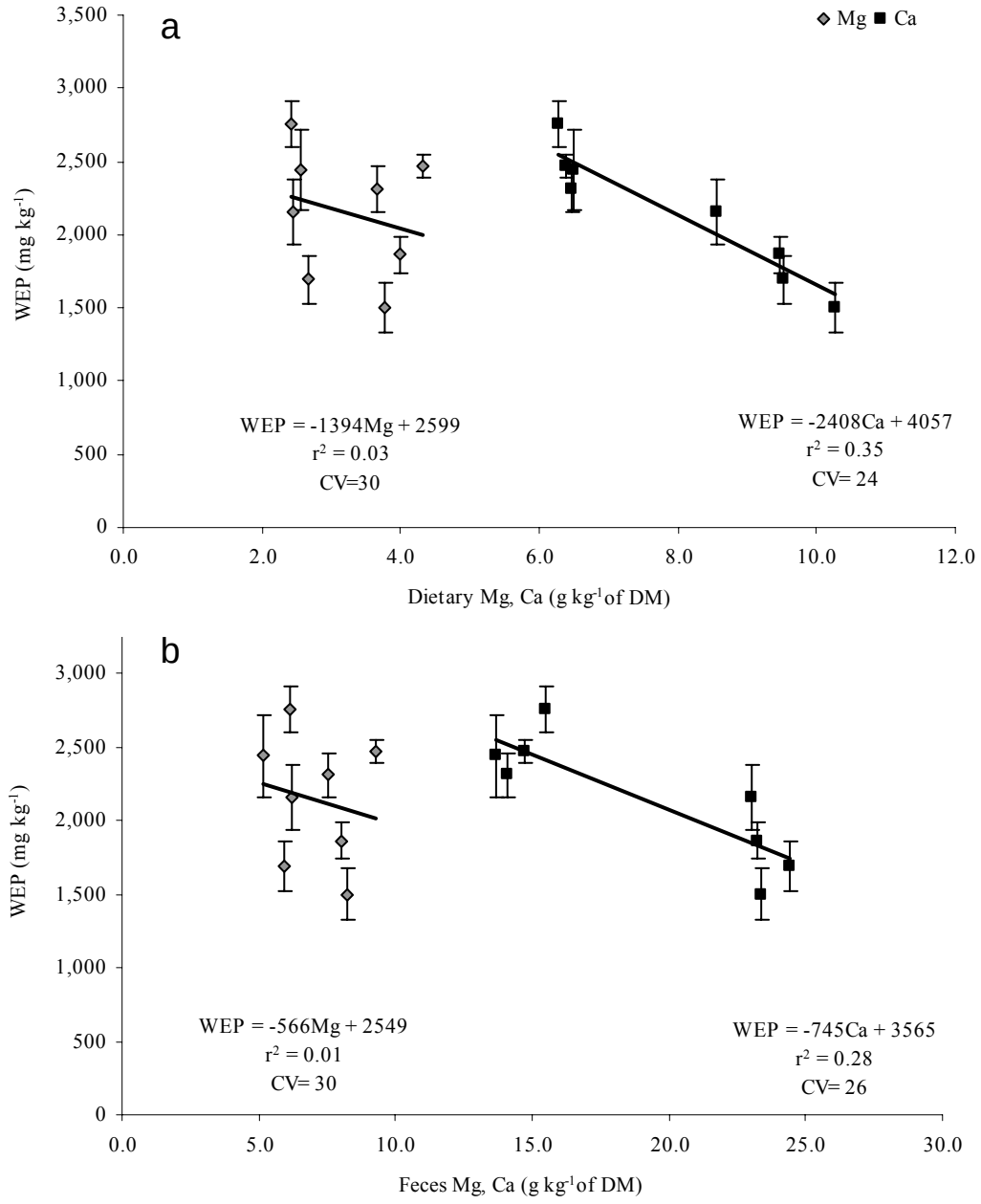

Figure 4-1. Variation in water extractable P with respect to changes in dietary and fecal concentrations of Mg and Ca.

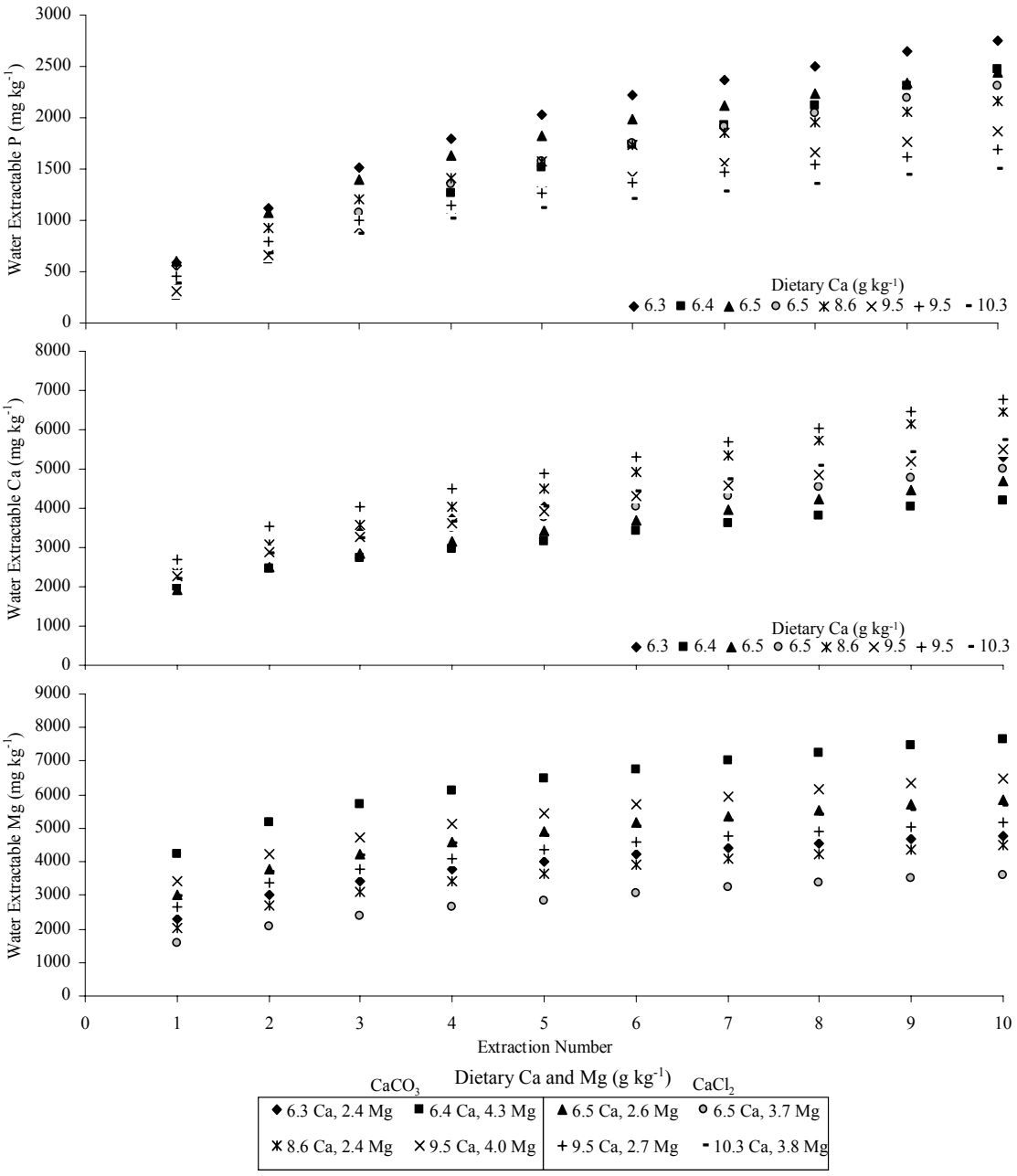

Figure 4-2. Cumulative water extractable P, Ca and Mg with changes in dietary concentrations of Ca and Mg.

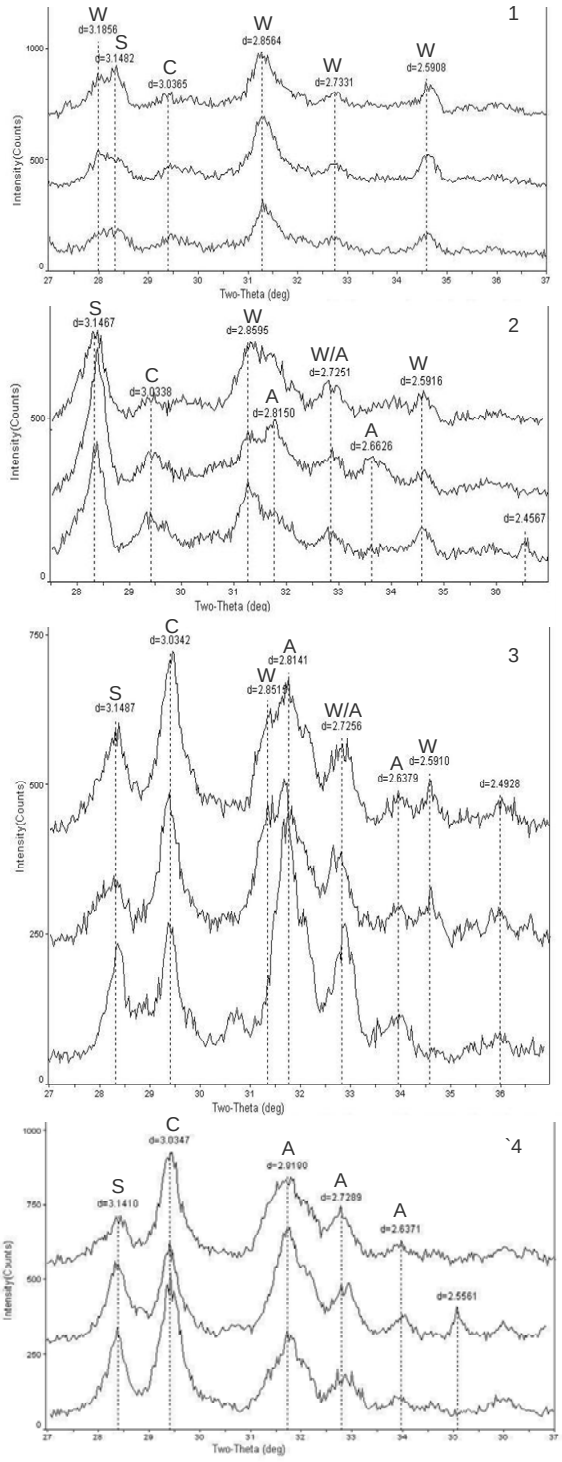

Figure 4-3. X-ray diffraction analysis of ashed fecal samples of randomly selected dairy cows receiving (1) $LoCaCO_3$, HiMg; (2) $LoCaCO_3$, LoMg; (3) $HiCaCO_3$, HiMg; and (4) $HiCaCl_2$, HiMg. Minerals present are: C: calcite ($CaCO_3$); S: silvite (KCl); W: whitlockite $(Ca,Mg)_3(PO_4)_2$; and A: hydroxyl-apatite $Ca_5(PO_4)_3(OH, Cl, F)$. LoCa refers to low dietary Ca concentration whereas HiCa is high dietary Ca concentration; same applies to Mg. Each line within a graph represents a different fecal sample.

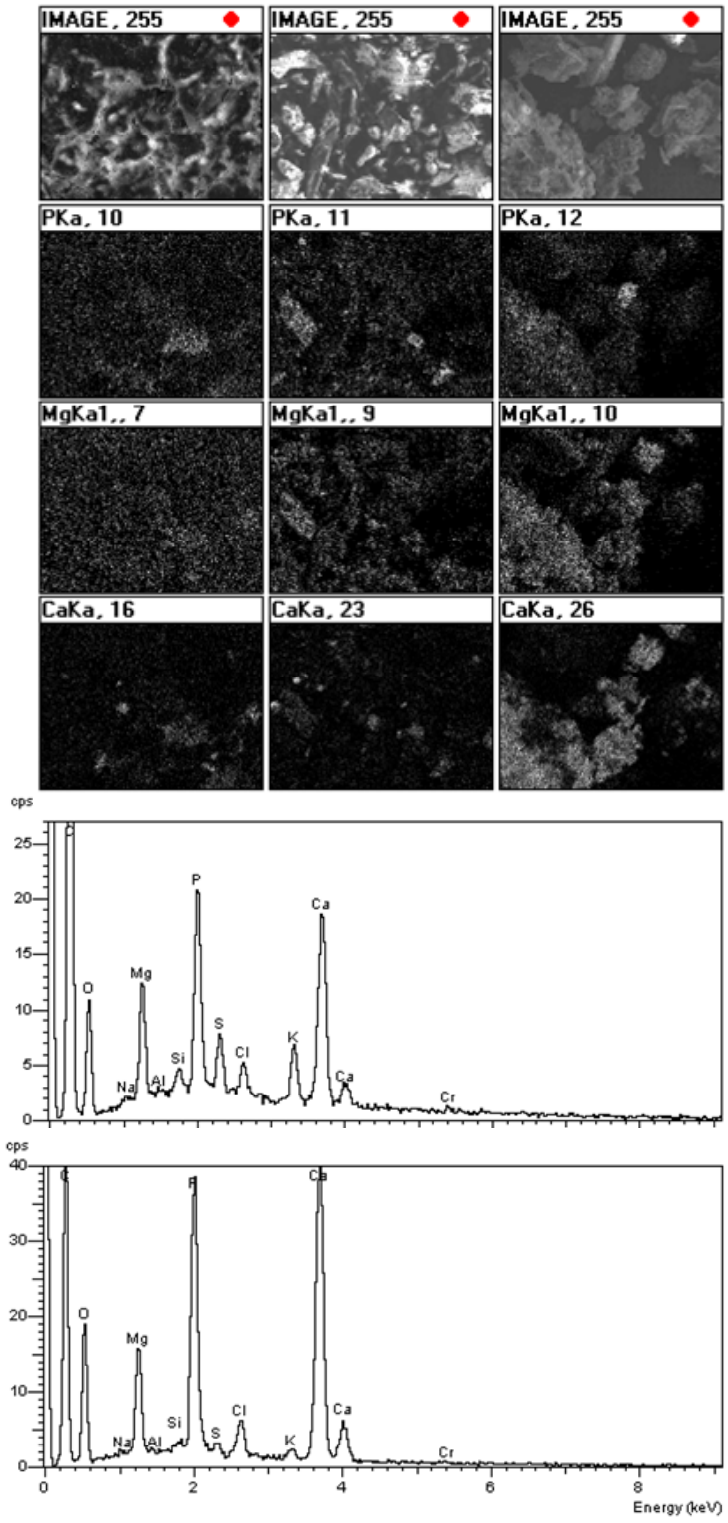

Figure 4-4. Dot maps and P, Ca and Mg elemental spectra from areas identified with high P concentrations of randomly selected fecal samples of cows being fed low Ca and high Mg concentrations or high Ca and high Mg concentration in their diets.

CHAPTER 5
DIETARY CALCIUM AND MAGNESIUM EFFECT ON PHOSPHORUS SOLUBILITY OF DAIRY FECES IN THREE DIFFERENT SOILS; AN INCUBATION STUDY

Introduction

Animal waste from dairy farms is commonly applied to soils as manure (mixtures of feces, bedding materials, water, etc.). Farm management strategies often include areas of animal rest where considerable amounts of feces can accumulate on the soil surface. Differences in P solubility and chemical forms between manure (mix of feces and urine) and feces have been documented (Toor et al., 2005), but risk assessment tools (P Indexes) make no distinction for P application based on -dairy- waste characteristics. It is assumed that most P is highly soluble or becomes plant available soon after application (Cooperband and Good, 2002). Surface soils where dairy manure accumulates over long periods of time are a source of P for offsite movement (Sharpley et al., 2001, Sims et al., 1998). This P reaches water bodies and promotes eutrophication, negatively impacting water quality (Ebeling et al., 2002).

Research efforts on controlling P balances at the farm level have focused on understanding P movement (Beauchemin et al., 1998, Turner and Haygarth, 2000), soil P retention capacity (Khiari et al., 2000, Rhue et al., 2006), reducing P concentrations in the feed to minimize P excretion (Dou et al., 2003, Wu and Satter, 2000), or evaluating manure chemical and physical characteristics, with (Dou et al., 2002) or without (He et al., 2004) dietary control. However, little emphasis has been placed on dietary modifications to increase long term P stability in soils where feces-derived components can play a major role in ultimate P solubility.

Cooperband and Good (2002) showed in incubation experiments of poultry manure with soil that sparingly soluble Ca and Mg phosphates minerals control soil solution P concentrations. Dairy manure impacted soils, on the other hand often have conditions associated with increased P stability by its association with Ca (high pH and elevated concentrations of P and Ca) yet solid

state crystalline phosphate minerals have not been identified in these soils (Harris et al., 1994) or in (dairy) manure-soil incubations (Cooperband and Good, 2002). Instead, Josan et al. (2005) and Nair et al. (1995) demonstrated that P remains highly soluble, even after years of abandonment. Elevated concentrations of Mg and dissolved organic carbon have been proposed as inhibitors of more stable calcium-phosphate minerals (Ca-P) in manure amended soils (Harris et al., 1994; Josan et al., 2005).

The objective of this study was to test the following hypotheses: (i) Increased Ca availability in the GIT of lactating dairy cows will favor Ca-P formation in feces therefore reduce P solubility in soil-feces incubations, and (ii) reduction in P solubility with time for soil-feces mixtures is favored by higher available Ca in the diet. The approach was to evaluate effects of dietary Ca and Mg concentrations and of solubility of Ca source on P concentration and solubility in soils incubated with feces from lactating cows.

Materials and Methods

Selected dairy fecal samples were incubated with the surface horizon (A_p) of three soil orders (Table 5.1) in a laboratory setting. Dry feces were added to each soil to supply a TP concentration of 1000 mg P kg^{-1}, rate in the low range of typical heavy intensity areas in dairy farms. Samples were kept under controlled conditions at room temperature for 6 months. Moisture content was checked every 2 weeks and maintained at 30% by weight. Consecutive water extractions at a ratio of 1:100 feces:water were used as a indication of long term solubility of P in the incubations.

Soils from the three dominant soil orders of Florida (Spodosol, Entisol, and Ultisol) were collected. The soils classified at the subgroup level as Alaquod, Quartzipsamment, and Paleudult, respectively. Soil morphological, physical, and chemical characteristics (Tables 5.1 and 5.2) were reasonably representative of these suborders as they occur in Florida based on comparison

with data collected under the Florida Soil Survey Program. They were collected from forested areas in Alachua and Levy Counties, Florida, where recent agricultural impact was minimal. None were elevated in P. Content of clay, Al, and Fe followed a typical trend among these soils: Spodosol <Entisol<Ultisol (Table 5.2).

Soils were analyzed for total by the ignition method (Andersen, 1976) and Mehlich-1 extractable concentrations of metals and P. Total and Mehlich-1 extractable Al, Fe, Ca, and Mg were determined by atomic absorption (AA) spectroscopy; P was determined on a UV-visible recording spectrophotometer at 880 nm wave-length via the molybdate-blue colorimetric method (Murphy and Riley, 1962) (U.S. EPA, 1993; method 365.1). Relative P sorption capacity of the soils was determined by the single-point P absorption isotherm as described by Harris et al. (1996). Organic matter was determined by loss-on-ignition (Schulte, 1995); pH was determined at 1:1 soil to water ratio (USDA, 2004) and particle size distribution determined by the pipette method (USDA, 2004).

Fecal samples were obtained from an experiment conducted at the University of Florida Dairy Research Unit. Twenty four cows were fed eight different diets. Diets offered were formulated to contain the same P concentration (3.7 g P kg^{-1} of DM) with two sources of Ca ($CaCO_3$ and $CaCl_2$) and two concentrations of Ca (6.0 and 10.0 g kg^{-1}) and Mg (2.0 and 3.5 g kg^{-1}). Treatments (diets) were randomly assigned to cows in three 21-d periods. During the experiment each cow received a treatment only once and no treatment followed another treatment from the previous period more than once. During the first 11-d of each period cows were adjusted to a new diet. Fecal grab samples were collected twice a day from day 11 to day 21 to obtain a composite sample per cow per period. Composited fecal samples were dried at 45°C in a forced-air oven and ground to pass the 2-mm screen of a Wiley mill (A.H. Thomas,

Philadelphia, PA). Measured concentrations of P, Ca and Mg in fecal samples varied with respect to formulations (Table 5.3); however, these differences did not alter the objectives of the study.

Composited fecal samples from 4 cows (replications) from each of four treatments (out of eight) were selected to incubate with soils. Treatments selected represent both concentrations of Ca and Mg and two sources of Ca. Treatments chosen differed in WEP but did not represent the extremes (highest and lowest WEP). To minimize effects from differences in organic matter application, individual fecal samples chosen had a TP concentration between 4500 to 5500 mg kg-1 (Table 5-3). Samples from animals receiving high diet Ca concentrations are referred as HiCa whereas HiMg is used for high dietary Mg. Fecal samples (treatments) selected were from animals being fed: (i) $LoCaCO_3$, HiMg; (ii) $HiCaCO_3$, HiMg; (iii) $LoCaCl_2$, LoMg; (iv) $HiCaCl_2$, LoMg.

Mixture of incubated soils and fecal samples will be discussed on the basis of the Ca source and concentration of Ca and Mg used in the feeding experiment; for example, animals receiving $CaCO_3$ at 6.4 g Ca kg^{-1} and 4.3 g Mg kg^{-1} of dietary DM will be referred as $LoCaCO_3$-HiMg.

Successive water extractions at room temperature (~25 °C) were performed for all soil-feces mixes after 1, 25, and 42 weeks of incubations at a 100:1 water:soil+feces ratio. Incubated samples were air dried prior to extraction. After 1 h of shaking time, samples were centrifuged at 4000x g for 5 minutes. Samples incubated for 25 weeks were consecutively extracted seven more times for a total of eight extractions. Supernatants were filtered (0.45 μm) and analyzed for extractable phosphorus (WEP), Ca (WECa), and Mg WEMg. Water-extractable Ca and Mg were measured by atomic absorption spectroscopy; P was determined on a UV-visible recording

spectrophotometer at 880 nm wave-length via the molybdate-blue colorimetric method (Murphy and Riley, 1962) (U.S. EPA, 1993; method 365.1).

Two samples from each of two treatments (highest and lowest WEP) were analyzed by x-ray diffraction (XRD). Samples were first sieved to pass a 53 μm screen, then clay- and silt size fractions were mounted on a porous ceramic tile for the XRD scan.

Statistical Analyses: The statistical model used was a 3 x 4 factorial design with treatment (four diet), soils (three soils), and replications (four per treatment). Single data measurements of Ca, Mg and P were analyzed using the GLM procedure of SAS whereas the Mixed Linear Model was used for repeated measures from consecutive extractions of the same element; the slice option was used to detect differences in individual extractions. Orthogonal single degree of freedom contrasts and Tukey-Kramer test were used to detect differences among main treatment effects and least squared means of individual treatments, respectively. Correlations and nonlinear regressions were used to describe relationships between elements in our analysis. Differences discussed in the text were significant at $P \leq 0.05$ unless otherwise indicated.

Results and Discussion

Differences in chemical and physical characteristics of the three soils used in the incubations (Table 5-2) are consistent with WEP results. The A horizon of Spodosol had the lowest concentration of P and cations (Ca, Mg, Fe and Al), both as total and Mehlich-1 extractable, and the lowest pH, organic matter and clay content. Extreme depletion of metals and clay is characteristics of Spodosol surface horizons due to the intense podzolization that defines soils of the Spodosol order. The Spodosol A horizon also had the lowest P retention capacity (Table 5-2), which is also typical (Nair et al., 1999, Yuan and Lavkulich, 1994) due to the lack of P-retaining components (clay-sized metal oxides) and therefore the highest WEP at all times (Fig. 5-1) regardless of treatment when compared with its counterpart in either of the other two

soils used. The A horizon of the Ultisol, on the other hand, had the highest concentration of metals and clay and the highest P retention capacity, consistent with the tendency towards a reduction in WEP over time (25 and 42 weeks). Water-extractable P after 25 and 43 weeks from feces incubated with the Entisol's A horizon showed an intermediate P release behavior which would be expected from its intermediate properties (metal and clay content; P retention capacity) relative to the other 2 soils.

Because of the soil by time interaction (Table 5.4) it was not possible to test the effect of incubation time on WEP; however, it is apparent that there was no reduction of WEP during the 42 weeks (Fig. 1). In effect, hypothesis (ii) that high available Ca in the diet would result in greater P stabilization over time was not supported for the 42 week period.

Interaction of time by soil affected the overall WEP (average of all diets within time) (Table 5.4). This interaction occurred between samples incubated with the Ultisol and Entisol. At time zero the Ultisol had higher WEP across diets than the Entisol (263.5 vs. 247.8 mg kg^{-1}), but after 6 months of incubation WEP from the Ultisol was lower than the Entisol (260.5 vs. 300.7 mg kg^{-1}). Samples incubated with Spodosol had higher WEP vales at all times (Fig. 5-1).

Effect of dietary treatments on WEP from fecal samples observed in chapter 4 was also documented in feces-soil incubations. Increased dietary Ca concentration from 6.4 to 9.5 g $kg^{-1}$of diet DM reduced WEP in feces-soil incubations (369 vs 228 g kg^{-1} of mix) (Fig. 5-2), supporting hypothesis (i). This represents a reduction in WEP based on dietary modifications from 35.1 % to 21.7% of the TP in the soil-feces mix; although these values are pooled means across soils the same trend was documented in each soil type at all times (no soil by diet interaction). The reduction in WEP from fecal samples of animals receiving higher dietary Ca suggests the

formation of a more stable (i.e. less soluble) form of P that likely formed in the GIT of the animal.

Chemical conditions in the animal's GIT before the small intestine do not favor the formation of such a phase. In the fore-stomach (reticulum-rumen) of lactating dairy cows, under most circumstances, pH ranges from 5.8 to 6.5; with ≥5.8 considered desirable as this is the threshold point for subclinical ruminal acidosis (Yang et al., 2001). A pH of 6.5 favors formation of Ca-P, but nutrients remain in the reticulo-rumen only temporarily, then undergo an acidic stage at the abomasum (true-stomach) where the pH can be below 2.0 due to the release of HCl (Barry and Manley, 1984). The acidity of the feed (known as chyme) when it enters the small intestine stimulates secretions from the pancreas. These secretions contain bicarbonate, intended to neutralize HCl present in the chyme. At this point (or thereafter) is where a stable P compound can form and ultimately be excreted. Given fecal sample handling and feces-soil mixes used in this experiment, P solubility reduction in manure-impacted soils was achieved preemptively via dietary control; Ca concentration was an effective dietary factor when cows were fed at the recommended dietary P concentration (NRC, 2001).

There was an effect of soil and diet on cumulative WEP after eight consecutive extractions from fecal samples after 25 weeks of incubation at a 1:100 feces-soil mix to water ratio (Table 5.6); interaction between soil and diet did not affect cumulative WEP. Dietary effect was consistent with that observed for fecal samples and for feces-soil mix single extractions at 1, 25 and 42 weeks on WEP.

There was no effect of dietary Ca source or the interaction of dietary Ca source by dietary Ca concentration. Dietary Ca concentration affected WEP; HiCa diets (in contrast to LoCa diets) reduced WEP from fecal samples incubated with the Ap horizon of both Entisol and Spodosol;

however, there was no dietary Ca concentration effect on WEP from fecal samples incubated with the Ap horizon of Ultisol (Table 5.8) because of the greater P retention capacity present in this soil, therefore reducing both the overall P solubility and the treatment effects. Fecal samples incubated with Ap horizon from Ultisol showed the lowest cumulative WEP concentrations (Fig. 5.3), behavior in agreement with the expected P retention capacity (relative to the other two soils) based on physical and chemical characteristics of the Ultisol, particularly soil texture and total and Mehlich-1 extractable concentrations of Al (Table 5.2). These findings led us to believe soil solution P in Ultisol is interacting with the soil's active sites for P retention. Therefore solution concentrations are being controlled by sorption-desorption mechanisms, particularly P associated with amorphous Fe and Al forms. These P fixing mechanism may be the reason for the reduction in WEP with time, as observed by Sartain (1980) who documented a reduction in extractable P with multiple extractants; during his experiment, soil TP concentration remained constant. Cooperband and Good (2002) also proposed sorption-desorption as the processes controlling P solubility from dairy-manure amended Mollisols.

The Entisol and Spodosol showed increased cumulative WEP over eight extractions (Table 5-8). This observation agrees with soil characteristics (Table 5.2). In soils with low P retention capacity, like the Entisol and Spodosol in this experiment, fecal (manure) components and characteristics are expected to play a major role in the overall soil P release. Increasing dietary Ca concentration from 6.5 to 9.5 g Ca kg^{-1} dietary DM effectively reduced WEP from feces incubated with Entisol (834.0 vs. 634.5 g WEP kg^{-1} soil) and Spodosol (1014.1 vs. 774.7 g WEP kg^{-1} soil) (Table 5-7). This treatment effect on cumulative WEP after 25 weeks of incubation is similar to those observed in single water extractions at three different times of incubation (Fig 5-2).

Dairy-manure impacted soils have been shown to continue releasing high P concentrations (up to 100 mg P kg^{-1} soil; 1:10 soil to water extractions) even after years of abandonment (Josan et al., 2005). After fractionation extractions it has been shown that P in the A horizon is mainly associated with Ca and Mg (Nair et al., 1995) and it has been proposed that sparingly-soluble Ca- and/or Mg-P phases may control release of P (Josan et al., 2005). This idea is consistent with the high correlation observed between the release of Ca and Mg (individually or collectively) with that of P from consecutive extractions (Fig. 5-3) and with x-ray diffraction results from ashed fecal samples where crystalline forms of Ca and Mg were found; a more stable Ca-P -apatite- was present in feces with lower WEP concentrations whereas a more soluble Ca(Mg)-P form -withlockite- was found in feces with higher WEP. As discussed in chapter 4, these P forms do not necessarily exist in fresh or dried fecal samples, but it is likely that their stoichiometric precursors that reflect their relative solubility are present in the feces. These changes in P chemical composition and structural arrangement are likely the result of dietary modifications, particularly increased Ca concentration, and the main reason for the differences in WEP in soil-feces incubations (Table 5-7).

Cumulative release of WECa and WEMg were associated with WEP (Fig 5-3). Associated release differed with changes in diet and soils; however, correlations were significant in all cases when analyzed within soil and diet. Correlation of cumulative WEP with cumulative WECa+WEMg from fecal samples incubated with the Spodosol showed the influence of dietary modifications. The main difference, as discussed above, was between Lo and HiCa diets; however, the effect of Mg was more clearly observed in this correlation. When diets contained HiCa concentrations, addition of Mg (HiMg) did not make a difference, but when diets contained LoCa concentrations, increasing dietary Mg increased WE concentrations of both P and the

combination of Ca and Mg (Fig. 5-3). This suggests a higher association of P with Mg, resulting in their higher WE concentration. This dietary effect was not observed in fecal samples incubated with the Ultisol and Entisol because of their higher P retention capacity.

Conclusions

Increasing Ca concentration in the diet of lactating dairy cows from 6.5 to 9.5 g Ca kg^{-1} dietary DM preemptively reduced P release from incubated feces-soil mixtures. This effect was most pronounced in Spodosol, probably because it had minimal components to retain the P released from the feces. Soil characteristics should be taken into account when considering P loading rates; soils used in this experiment differed in WEP concentrations. Changes in dietary Ca source and Mg concentrations had no effect on WEP. No further P stabilization effect of high available dietary Ca was observed during a 42 week period. This lack of a time effect suggests that Ca, Mg, and P interactions in the GIT may be the major determinant of the subsequent environmental fate of fecal P. However, a longer incubation time may ultimately confirm a greater stabilization with time for the high Ca diets.

Table 5-1. Morphological summary of soils from which surface horizon samples were collected for the incubation experiment.

	Horizon	Lower Depth	Texture	Color		
Ultisol				Hue	Value	Chroma
	Ap	20	Sand	10YR	4	2
	A/E	38	Sand	10YR	4/5	2/3
	E	51	Sand	10YR	5	4
	Bt1	63	Loamy Sand	10YR	5	6
	Bt2	92	Sandy Loam	10YR	5	6
	Btg	110+	Sandy Clay Loam	10YR	6	2
Spodosol						
	Ap	8	Sand	10YR	4	1
	E1	20	Sand	10YR	5	1
	E2	52	Sand	10YR	7	1
	Bh1	59	Sand	10YR	2	1
	Bh2	66	Sand	10YR	3	2
	Bw	134	Sand	7.5YR	4	3
	Btg	144+	Sandy Clay Loam	10YR	6	2
Entisol						
	Ap	6	Sand	10YR	4	2
	E1	50	Sand	10YR	5	4
	E2	100+	Sand	10YR	6	6

Table 5-2. Chemical and physical characteristics of the surface horizon (Ap) from each soil type used for the incubation.

	Ultisol	Spodosol	Entisol
Total (mg kg^{-1})			
P	46.2	8.2	133.3
Ca	191.2	130.0	271.3
Mg	106.3	10.6	87.2
Fe	851.3	124.9	796.9
Al	4877	1210	2490
Mehlich-1 (mg kg^{-1})			
P	2.0	1.5	6.9
Ca	95.8	47.4	202.4
Mg	19.8	6.4	25.7
Fe	15.2	5.9	21.3
Al	371.4	38.1	145.3
RPA┼	91	11	66
pH	4.3	4.2	5.3
Organic Matter (g kg^{-1})	40.9	8.0	21.6
Particle Size Dist.			
Sand (g kg^{-1})	931	969	979
Silt (g kg^{-1})	46	25	8
Clay (g kg^{-1})	23	6	13

┼: Relative P absorption as determined by single-point P absorption isotherm as measured in the A horizon of each soil order collected.

Table 5-3. Chemical characteristics of fecal samples used for the incubation with three different soils orders.

Ca Source	$CaCO_3$		$CaCl_2$	
Dietary Ca (g kg^{-1} DM)	6.4	9.5	6.5	9.5
Dietary Mg (g kg^{-1} DM)	4.3	4.0	2.7	2.7
Mineral	---------- mg kg^{-1} ----------			
P	5170 ± 380	5012 ± 354	4940 ± 508	4795 ± 308
Mg	8925 ± 430	7980 ± 733	4894 ± 490	5958 ± 1068
Ca	12432 ± 1027	23539 ± 2004	12633 ± 1249	24894 ± 4025
Cumulative WEP*	2370 ± 225	1931 ± 356	2364 ± 568	1808 ± 484

*: Cumulative WEP after 10 successive extractions using a 1:100 feces to water ratio.

Table 5-4. ANOVA table of water-extractable P (WEP) from soil-feces at different times of incubation (1, 25, and 42 weeks).

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Replication	3	99092	33030	8.65	0.0134
Soil	2	366575	183287	48.0	0.0002
Error 1†	6	22914	3819	--	--
Diet	3	760066	253355	71.2	<.0001
Soil x Diet Int.	6	19064	3177	0.89	0.5138
Error 2†	27	96054	3557	--	--
Time	2	40738	20369	5.59	0.0055
Soil x Time Int.	4	80755	20188	5.54	0.0006
Diet x Time Int.	6	26042	4340	1.19	0.3204
Soil x Diet x Time Int.	12	31129	2594	0.71	0.7345
Error 3†	72	262164	3641	--	--
Corrected Total	143	1804600			

† Error term 1 = Rep x Soil Interaction, Error term 2 = Rep x Soil x Diet Interaction, Error term 3 = Rep x Soil x Diet x Time Interaction.

Table 5-5. Water extractable P from a single extraction at 1:100 soil-feces mix to water ratio at different times of incubation and the respective standard error of the mean (SEM).

Ca Source	$CaCO_3$		$CaCl_2$		
Dietary Ca (g kg^{-1} DM)	6.4	9.5	6.5	9.5	
Dietary Mg (g kg^{-1} DM)	4.3	4.0	2.7	2.7	SEM
	----- Water Extractable P (mg kg^{-1}) -----				
One week					
Ultisol	287.7	211.9	351.0	203.3	16.2
Spodosol	357.5	275.8	431.6	246.6	18.9
Entisol	260.6	208.2	325.0	197.3	16.1
25 weeks					
Ultisol	302.5	211.7	325.4	202.3	22.2
Spodosol	446.8	339.8	494.7	320.6	42.0
Entisol	345.2	198.3	435.8	223.5	36.1
42 weeks					
Ultisol	290.8	142.7	260.3	119.7	10.5
Spodosol	393.9	294.3	510.0	266.3	46.1
Entisol	412.7	242.8	408.4	205.4	28.7

Table 5-6. ANOVA table of cumulative water-extractable P (WEP) after eight extractions of at 1:100 soil-feces to water ratio after 25 weeks of incubation.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Soil	2	448509	224255	13.4	<.0001
Diet	3	358381	119460	7.14	0.0008
Soil x Diet Interaction	6	92315	15386	0.92	0.4935
Replication	3	134878	44959	2.69	0.0624
Error	33	552186	16733		
Corrected Total	47	1586270			

Table 5-7. Cumulative water extractable P (WEP) from ten consecutive extractions at 1:100 soil-feces mix to water ratio after six months of incubation and the respective standard error of the mean (SEM).

Ca Source	$CaCO_3$		$CaCl_2$		
Dietary Ca (g kg^{-1} DM)	6.4	9.5	6.5	9.5	
Dietary Mg (g kg^{-1} DM)	4.3	4.0	2.7	2.7	SEM
	----- Water Extractable P (mg kg^{-1}) -----				
Time = 25 weeks					
Ultisol	698.5[a]†	464.5[a]	701.5[a]	606.75[a]	66.5
Spodosol	1040.8[a]	813.3[b]	987.5[a]	736.0[b]	83.4
Entisol	786.0a	624.8b	882.0a	643.5b	54.2

† For each property, mean values within a row followed by a different letter were significantly different ($P < 0.05$) as determined by Duncan's mean separation procedure.

Table 5-8. *P* values of water extractable P (WEP) for single degree of freedom contrasts based on dietary variables from where fecal samples were collected.

	High vs. Low Ca	$CaCO_3$ vs. $CaCl_2$	Ca Source by Ca Conc. Interaction
	---------- *P* values ----------		
Time = 25 weeks			
Ultisol	0.2213	0.6268	0.7107
Spodosol	0.0079	0.4587	0.8687
Entisol	0.061	0.8183	0.5081

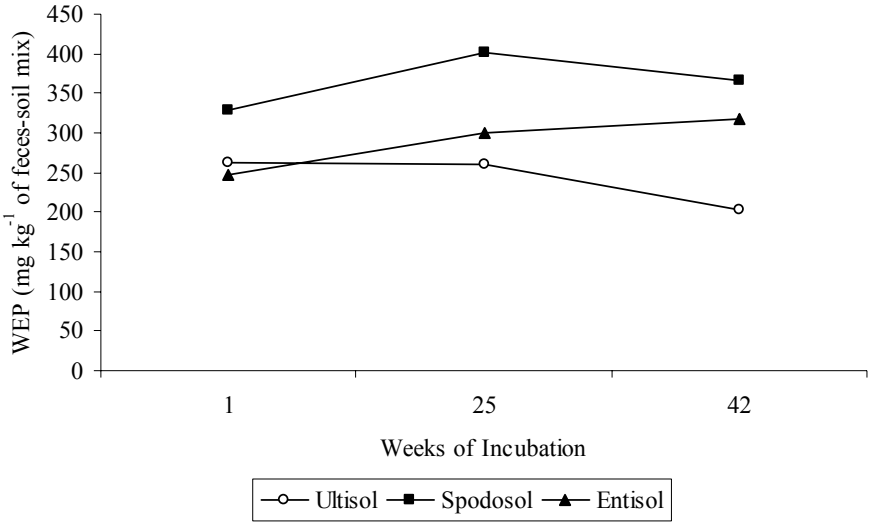

Figure 5-1. Interaction effect of soil type and time on water-extractable P (WEP) single extractions at 1:100 water to soil-feces incubations. Data points are averaged across diets.

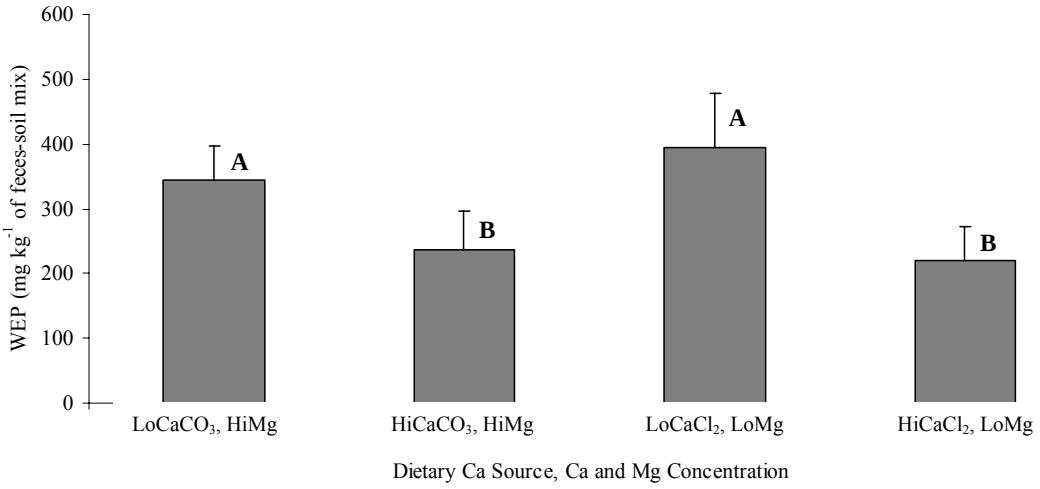

Figure 5-2. Averaged water-extractable P (WEP) at three different times (1, 25, and 42 weeks) from single extractions at 1:100 ratio of water to soil-feces incubations.

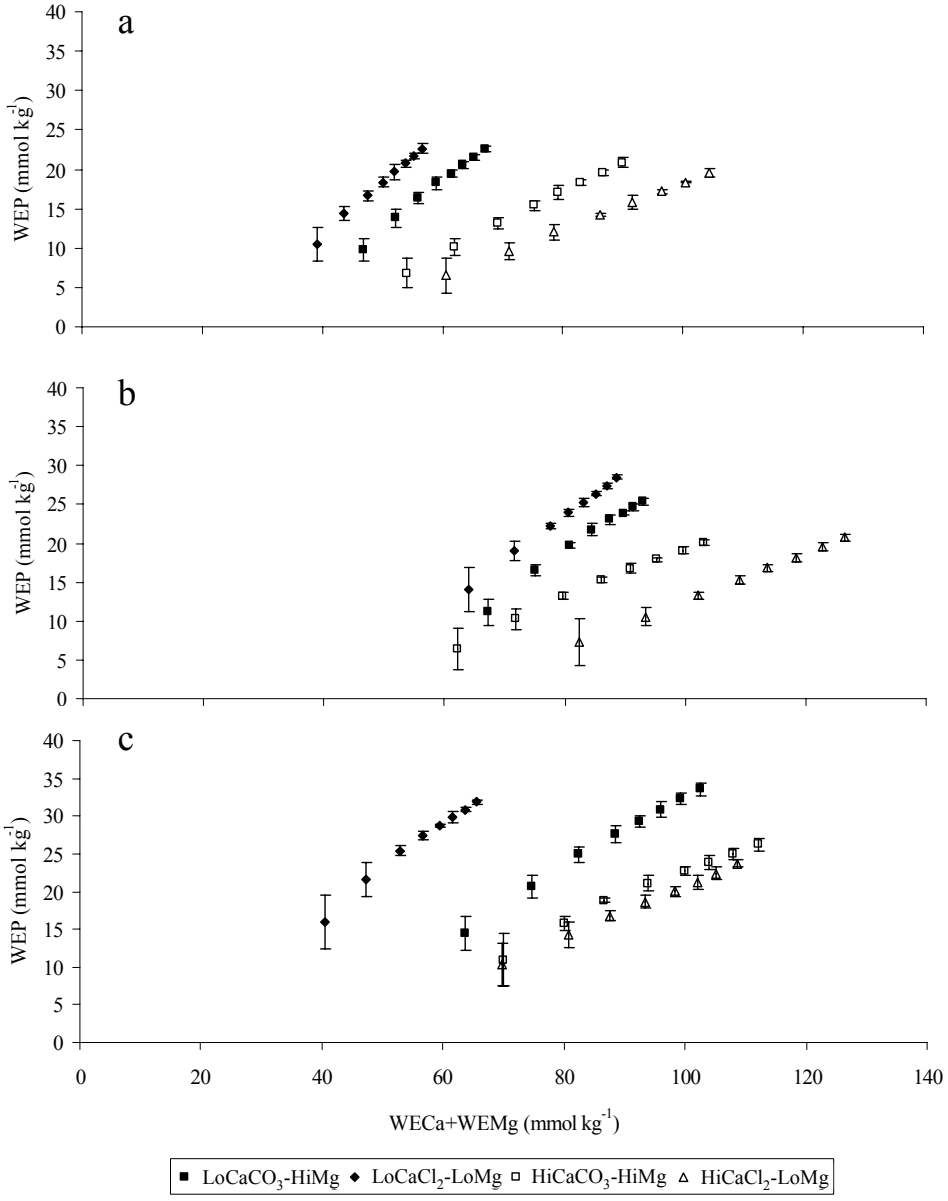

Figure 5-3. Relationship between water-extractable P (WEP) with WECa+WEMg from eight consecutive extractions at 1:100 soil-feces to water ratio. Fecal samples were incubated for 25 weeks with a) Ultisol, b) Entisol, and c) Spodosol. Data points shown are average of four replications ± standard error.

CHAPTER 6
SUMMMARY AND CONCLUSIONS

Dairy manure is a resource used by farmers to satisfy crop nutrient demands. Application of manure at rates to meet plant N requirements results in excess P with respect to plant needs. Over time, this excess P builds up in the soil and becomes more susceptible to offsite movement, thereby posing a potential eutrophication risk to water bodies. These risks are directly proportional to the solubility of manure P and soil's P retention capacity. This dissertation addressed the efficacy of dietary manipulations that would reduce P solubility in dairy manure by means other than dietary P reductions alone. It documented effects of dietary Ca and Mg on solubility of P in dairy feces and soil-feces mixtures incubated over time, and on animal health and performance, and probed physiological and chemical mechanisms responsible for those effects.

Reducing dietary P concentration is a starting point to optimize P balance in dairy farms and minimize the potential for negative environmental impact of accumulated P; however, feeding P at the NRC recommended dietary concentration (3.7 g P kg^{-1} diet DM for lactating dairy cows) is the most viable and safe alternative to ensure adequate animal productivity and reproductive performance. Excess dietary P has been shown to increase manure TP, particularly the most soluble fraction. In soils with low P retention capacity, manure components (Ca, Mg, dissolved organic carbon) determine P solubility and stabilization in soils; therefore, increasing available Ca concentration in the diet of lactating dairy cows seems like an alternative to promote formation of stable Ca-P, as opposed to less stable Mg-P or Ca, Mg-P forms, and hence reduce manure-P solubility in soils.

Large dietary intakes of Ca and Mg can reduce P absorption by the animal due to the formation of insoluble phosphates inside the gastro intestinal tract; however, from an

environmental perspective; however, their interaction with P may decrease P solubility in dairy feces and minimize losses from dairy manure-impacted soils. Dietary manipulations to increase dietary Ca (and Mg) with an environmental objective should take into account animal nutrient requirements and possible detrimental effects of such dietary changes on animal health and performance.

Total P fecal concentrations did not relate to P extracted after 10 consecutive water extractions in fecal samples from animals in different physiological states. Calcium and Mg in feces seem to play an important role in controlling WEP. Increased ratio of P:(Ca+Mg) in feces corresponded to increased WEP. Animals in different physiological states and fed different diets produced feces with different P release characteristics; overfeeding P to heifers resulted in fecal samples with the greatest cumulative WEP concentrations but not the highest fecal TP concentration. The proportion of TP solubilized after ten consecutive extractions went from ~42% for adult lactating cows fed diets matching their P requirements to ~86% for growing heifers fed a diet containing P at 140% of their requirement. Results of this study indicate that physiological state of the animal, dietary P concentration with respect to animal requirements, and dietary and fecal concentrations of Ca and Mg influence P solubility in feces of Holstein dairy cattle.

Use of two Ca sources and two concentrations of Ca and Mg in the diet of lactating dairy cows had no effect on milk yield, milk efficiency, fat corrected milk, fat corrected milk efficiency, digestibility of nutrients and overall P balance of lactating dairy cows. There was an effect on milk fat production (% and kg d^{-1}) and milk protein concentration (%). Addition of $CaCl_2$ to rations of lactating dairy cows warrants long term studies because of the tendency to decrease DMI and associated implications observed in this study.

Main dietary treatments of Ca source, Ca concentration and Mg concentration had no effect on fecal P concentrations. Increased Ca concentration in both diet and feces reduced water extractable P (WEP) from fecal samples. Fecal samples with higher Ca and Mg concentrations showed reduced WEP; possibly, Ca and Mg mutually suppressed dissolution of Ca,Mg-P forms by the common ion effect.

Increasing Ca activity in the GIT either by increased concentration or by feeding a more soluble Ca form reduced WEP from feces, but dietary Mg concentrations did not affect WEP. Increased fecal concentrations of Mg and Ca in feces mutually suppressed the dissolution of each element, suggesting that Ca, Mg and P are associated in feces. This is supported by (i) consecutive extraction data; (ii) SEM/EDS analysis where close association between Ca, Mg, and P in fecal samples regardless of the dietary treatment were documented; and (iii) XRD results from ashed fecal samples where hydroxyapatite (HAP), HAP plus Ca,Mg-P (Whitlockite), and Ca,Mg-P are the P forms for high, intermediate, and low dietary available Ca, respectively. Phosphorus solubility in dairy cow feces can be reduced by dietary control of Ca and Mg when the same dietary P concentration is fed; the effect is driven by increased dietary Ca availability. Inhibitory effect of Mg on formation of stable Ca-P can be preempted by sufficient available dietary Ca.

Increasing Ca concentration in the diet of lactating dairy cows from 6.5 to 9.5 g Ca kg^{-1} dietary DM preemptively reduced P release from incubated feces-soil mixtures. This effect was most pronounced in the Spodosol, probably because lack of P sorbing components in this soil resulted in almost exclusive control of P release by fecal components (as product of dietary treatments). Soil characteristics should be taken into account when considering P loading rates; soils used in this experiment differed in WEP concentrations. Changes in dietary Ca source and

Mg concentrations had no effect on WEP from feces and soil feces incubations. No further P stabilization effect of high available dietary Ca was observed over a 42 week period. This lack of a time effect suggests that Ca, Mg, and P interactions in the GIT may be the major determinant of the subsequent environmental fate of fecal P. However, a longer incubation time may ultimately confirm a greater stabilization over time for the high Ca diets.

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BIOGRAPHICAL SKETCH

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