Effect of dietary modifications of calcium and magnesium on reducing solubility of phosphorus in feces from lactating dairy cows

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ABSTRACT

Unintentional movement of P from dairy cow manure to off-farm locations has been an environmental concern for some time. The objective of this study was to evaluate the effects of increasing the dietary concentration and solubility of Ca and the dietary concentration of Mg on lactation performance and solubility of fecal P from lactating dairy cows receiving diets formulated to the same concentration of P (0.38% of dry matter). Eight dietary treatments were evaluated in a $2 \times 2 \times 2$ factorial arrangement involving 2 dietary sources of Ca (CaCO_3 having a moderate solubility of Ca vs. CaCl_2 having an excellent solubility of Ca) and 2 dietary concentrations of Ca (average of 0.64 and 0.95% of dry matter) and Mg (0.25 and 0.4% of dry matter). Twenty-four multiparous cows in mid lactation were fed the 8 diets in three 21-d periods. Dry matter intake and milk production were measured daily, and milk composition was measured on the last 6 milkings of each period. Fecal samples were collected twice a day during the last 5 d of each period, composited within cow, dried at 55°C, and subjected to 10 successive water extractions, and soluble P, Ca, and Mg were determined. Excretion of fecal P (g/d) was correlated positively with intake of P but not with intake of Ca or Mg. A smaller proportion of fecal P was extracted when dietary concentration of Ca increased (37.5 vs. 47.7%) and when CaCl_2 instead of CaCO_3 was fed (40.3 vs. 44.9%). Feeding more Mg reduced water-soluble P in feces but only when CaCO_3 and not CaCl_2 was fed. Increasing the amount of soluble Ca in the diet produced a relatively stable Ca-phosphate compound (hydroxylapatite) in ashed fecal samples, whereas feeding less soluble Ca resulted in a more soluble P phase (Mg-substituted whitlockite). Energy-dispersive X-ray elemental spectroscopy in conjunction with scanning electron microscopy showed spatial association between Ca-Mg and P. A reduction of approximately 5

g of soluble P/cow per d was detected when dietary concentration of Ca increased from an average of 0.64 to 0.95% of dry matter. Supplemental CaCO_3 would be a preferred source of Ca over CaCl_2 because cows fed CaCO_3 tended to produce more 4% fat-corrected milk, more milk fat, and milk with a greater concentration of fat and protein. Current prices would also favor feeding CaCO_3 over CaCl_2 . Increasing dietary intakes of Ca and Mg beyond current recommendations may increase formation of insoluble phosphate complexes (Ca-P rather than Ca-Mg-P associations), which result in decreased solubility of P in dairy-cow feces and reduce losses of P from agricultural areas where feces are applied.

Key words: phosphorus, calcium, environment

INTRODUCTION

Repeated application of manure to agricultural land can lead to a buildup of P in the soil over an extended period of time (Sentran and Ndayegamiye, 1995; Whalen and Chang, 2001; Toth et al., 2006). An accumulation of soil P increases the potential for involuntary off-site movement of water-soluble P, with potential detrimental effects on quality of surface water (Pote et al., 1996; van Es et al., 2004). Excess P is the most common cause of eutrophication of freshwater lakes, reservoirs, streams, and headwaters of estuarine systems (Correll, 1998).

Research has focused on decreasing concentrations of P in dairy-cow diets (Valk et al., 2000; Wu et al., 2000; Cerosaletti et al., 2004) and, therefore, excretion of P in feces, the main route of P excretion by dairy cows (Morse et al., 1992). Reductions in dietary concentration of P reduced concentrations of fecal P by up to 33% when used on commercial dairy farms (Cerosaletti et al., 2004). These decreases in P were particularly evident in the water-soluble fraction (Dou et al., 2002). However, reductions in concentrations of dietary and fecal P have practical limits. Milk production could not be sustained after 24 wk postpartum when cows were fed diets of 0.31% P (Wu et al., 2000) compared with cows fed diets of 0.4 or 0.48% P (DM basis). Dou et al. (2002) concluded that 0.31 to 0.37% P (DM basis)

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was adequate or near adequate for milk production of up to 50 kg/d. Phosphorus consumed in the phytate form is made available to the host animal through the phytase enzyme secreted by the ruminal microorganisms (Soares, 1995) and, therefore, should not be a factor affecting P solubility in ruminants.

Additional dietary strategies should be examined for their influence on P excretion and solubility in manure of lactating cows. Increased dietary Ca to sheep reduced the efficiency of P absorption (Field et al., 1983), likely as a result of reduced solubility of P in the gastrointestinal tract (**GIT**; Wan Zahari et al., 1990). Toor et al. (2005) reported reduced solubility of P in turkey manure having an increased ratio of Ca to P. The increased ratio of Ca to P was accompanied by a transformation of soluble Ca-P forms (dicalcium phosphate) into hydroxylapatite, a less soluble form of inorganic P. Magnesium-phosphate complexes are common in manure and, thus, influence solubility of P (Silveira et al., 2006). Therefore, changing the source and amount of Ca and Mg intake may influence P dynamics by formation of insoluble phosphates with these cations.

The inorganic Ca sources, CaCO_3 and CaCl_2 , differ in the extent and site of absorption of Ca in the GIT. The NRC (2001) assigns a Ca absorption coefficient of 0.95 to CaCl_2 and 0.70 to CaCO_3 in the limestone form. The Ca from CaCl_2 is thought to be primarily available in the rumen, whereas the Ca from CaCO_3 is thought to be primarily available in the small intestine after having been exposed to the acidic conditions of the abomasum. As a precaution during times of heat stress, dietary concentration of K is often increased to help replace K lost during sweating. Increased amounts of Mg are often fed to compensate for reduced Mg absorption due to increased intake of K (NRC, 2001).

This study tested the hypothesis that increased amount of soluble Ca in the GIT of lactating dairy cows will favor formation of Ca-P relative to generally more soluble Mg-P or Ca-Mg-P compounds, with the potential to reduce P solubility in feces. The objective was to evaluate the effects of 2 dietary concentrations of Ca and Mg and 2 dietary sources of Ca of different solubility on excretion and solubility of P in feces of lactating cows.

MATERIALS AND METHODS

Cows, Facilities, and Diets

Cows were managed according to guidelines approved by the University of Florida Animal Care and Use Committee before the start of the study. The experiment was conducted at the University of Florida Dairy Research Unit (Hague) from September to November.

Multiparous Holstein cows ($n = 24$), averaging 575 ± 60 kg of BW and 128 ± 21 DIM, 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 3 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) to measure DMI. Animals were fed a TMR in ad libitum amounts (5 to 10% orts) at 0600 and 1400 h. The amount of TMR offered and refused was recorded daily.

Eight dietary treatments were evaluated in a $2 \times 2 \times 2$ factorial arrangement involving 2 dietary sources of Ca (CaCO_3 from limestone with moderate absorbability vs. CaCl_2 having excellent absorbability) and 2 dietary concentrations each of Ca and Mg. Diets were formulated to contain 0.61% Ca (recommended Ca formulation; **RCa**; NRC, 2001) and 1.0% Ca (high Ca formulation; **HCa**) and 0.20% Mg (recommended Mg concentration; **RMg**) and 0.35% Mg (high Mg formulation; **HMg**) (DM basis). The purpose of using 2 Ca sources and concentrations was to achieve a range of Ca absorbability in the digestive tract. The low dietary Mg treatment required no inorganic Mg supplementation to meet the animal's Mg requirement (NRC, 2001). The HMg diet was achieved by feeding MgO. Diets were formulated to contain the same concentration of P (0.38% of DM), close to the dietary recommendation of 0.35% for cows in mid lactation (NRC, 2001). Eight mineral-vitamin premixes were prepared commercially (Lakeland Animal Nutrition, Lakeland, FL) and mixed with other dietary ingredients to prepare the 8 dietary treatments. The premixes were mixed with concentrate ingredients in 0.9-t amounts as needed and stored in 1.7-t-capacity metal bins. Concentrate, corn silage, and alfalfa hay were mixed as TMR for each feeding (Table 1). Silage was measured for DM weekly (Koster Crop Tester Inc., Brunswick, OH) to maintain the formulated ratio of forage to concentrate.

Cows were fed the 8 diets in three 21-d periods using an incomplete, partially balanced Latin square design. Each cow was assigned a new dietary treatment each period, and no treatment followed another treatment from the previous period more than once. The first 11 d of each period were for diet adaptation, and the last 10 d were used for data collection. Cows were weighed the first and last 2 d of each period at 0500 and 1700 h.

Sample Collection and Analysis

Samples of limestone, CaCl_2 , and MgO to be fed were analyzed for total and soluble Ca and Mg. Samples

Table 1. Ingredient composition of diets fed to lactating dairy cows

Ingredient	Amount, g/kg of DM
Corn silage	400
Alfalfa hay	120
Ground corn	220
Whole cottonseed	100
Soy plus ¹	60
Soybean meal	60
Minerals and vitamins premix ²	40

¹West Central Soy, Ralston, IA.

²Contained 6.7% CP, 0.9% P, 1.1% S, 4.1% K, 8.5% Na, 1,433 mg/kg of Zn, 513 mg/kg of Cu, 969 mg/kg of Fe, 1,038 mg/kg of Mn, 5.4 mg/kg of Co, 8.3 mg/kg of Se, 91,400 IU of vitamin A/kg, 22,915 IU of vitamin D₃/kg, and 683 IU of vitamin E/kg (DM basis). Concentration of Ca averaged 6.1 and 13.8% for the low and high Ca premixes, respectively, whereas concentration of Mg averaged 1.2 and 4.5% for the low and high Mg premixes, respectively (DM basis). Rice mill feed and cane molasses were sources of P.

were incubated for 24 h in filtered ruminal fluid collected from a ruminally cannulated cow fed a ration of 50% forage and 50% concentrate and in an HCl-pepsin solution (Tilley and Terry, 1963) separately. After centrifugation, supernatants were analyzed for Ca and Mg by atomic absorption spectroscopy. Blank tubes were used to correct values for Ca and Mg coming from ruminal fluid and microbial cells. Representative samples of concentrate mixes, corn silage, and alfalfa hay were collected weekly and composited by experimental period for chemical analyses. 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) before compositing. Composited and dried feed samples were analyzed for DM (105°C for 8 h), NDF using heat-stable α -amylase (Van Soest et al., 1991), ADF (AOAC, 1990), and N (Elementar Analysensysteme, Hanau, Germany). Protein was calculated by multiplying N $\times$ 6.25. Composites were analyzed by wet chemistry (Dairy One, Ithaca, NY) for Ca, P, Mg, K, Na, Zn, Cu, Mn, and Fe using the ignition method (Andersen, 1976); Cl was determined by titration with AgNO₃ 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., St. Joseph, MI).

Milk yield was measured at all milkings during the collection period. Milk composition (protein, fat, and somatic cells) was measured using 2 consecutive milkings collected on each of 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 DHIA (McDonough, GA) using infrared technologies (Bentley 2000, Bentley Instruments, Chaska, MN). Samples of water were

collected from water troughs every other day during the collection phase of each period, composited within period, and analyzed for Ca, Mg, and P as described previously.

Spot samples of urine were collected from each cow at 0500 and 1300 h during the last 3 d of each collection period, and pH was determined as samples were collected (Horiba twin pH meter B-213, Spectrum Technologies Inc., Plainfield, IL).

Fecal output was calculated using the marker ratio technique (Schneider and Flatt, 1975) with chromic oxide (Cr₂O₃) as an inert dietary marker. Cows were dosed orally via balling gun (Ideal Instruments Inc., Lexington, KY) with gelatin capsules (Torpac Inc., Fairfield, NJ) containing 10 g of Cr₂O₃ at 0500 and 1700 h from d 11 to 20 of each experimental period. Fecal grab samples were collected from each cow at the time of dosing Cr₂O₃ during the last 5 d of each period and composited within cow for each experimental period. Samples were dried at 55°C for 72 h and ground through a 2-mm screen of a Wiley mill (A. H. Thomas). Fecal samples were analyzed for P, Ca, and Mg by the ignition method (Andersen, 1976). Total Ca and Mg were measured by atomic absorption spectroscopy. Phosphorus was determined on a UV-visible recording spectrophotometer (Spectronic 1001, Bausch and Lomb, Rochester, NY) at 880-nm wavelength via the molybdate-blue colorimetric method (Murphy and Riley, 1962; US 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 (Schneider and Flatt, 1975).

Fecal samples were dried to standardize all samples to the same DM content so that the same amount of moisture and DM from each fecal sample would be mixed with distilled water for extraction. Drying is also effective for obtaining consistency in sample treatment, preserving samples, and avoiding DM variability in feces between animals and within animals in different sampling events. Furthermore, feces (or manure) often undergo a drying period upon application to soil. Previous research on the effect of drying on P solubility is contradictory. Ajiboye et al. (2004) reported an increase in water-extractable P (**WEP**) from dairy cow manure when samples were oven-dried at 105°C, whereas Chapuis-Lardy et al. (2004) reported a decrease in inorganic P soluble in water when dairy cow fecal samples were dried at 65°C. Drying feces at a low temperature is not likely to compromise relative treatment effects. Sample preservation without drying requires freezing to prevent microbial growth. The process of freezing and thawing may alter the original state of the sample,

e.g., by resulting in cellular lyses, a sample alteration in itself.

Ten successive water extractions were performed on 71 composited fecal samples (one cow was removed from the study in period 3 because of poor health) to simulate long-term effects of a wet environment on P movement from dairy feces. A water-to-feces ratio of 100:1 (vol:wt) was selected to maximize solubility and extraction of P. After 1 h of shaking, samples were centrifuged at $1,000 \times g$ for 5 min at 24°C. The supernatants were collected and filtered through a 0.45- μ m-pore filter. Supernatant solutions were analyzed for soluble reactive P, Ca, and Mg. Water-extractable Ca (**WECa**) and Mg (**WEMg**) were measured by atomic absorption spectroscopy (Varian, Varian, CA). Water-extractable P was determined on a UV-visible spectrophotometer via the molybdate-blue colorimetry method (Murphy and Riley, 1962).

Fecal samples from 3 cows fed the dietary treatment that resulted in greatest (RCa-RMg using CaCO_3) and least (HCa-HMg using CaCl_2) extent of WEP ($n = 6$) were selected to be analyzed for solid-state assessment of P speciation. Samples were sieved to pass a 53- μ m screen, deposited on a carbon mount, coated with a thin layer of C, and analyzed using a scanning electron microscope equipped with an energy-dispersive X-ray fluorescence elemental analysis system (model JSM-6400, Joel Ltd., Tokyo, Japan; equipped with a model 6506 EDS system for the X-ray fluorescence elemental analyses, Oxford Instruments, Witney, UK) at an accelerating voltage of 15 kV. Elemental dot maps were recorded at different magnifications, and elemental spectra were obtained from areas with a high P concentration. Three samples from those 2 diets plus 3 samples from 2 additional diets (RCa-HMg and HCa-HMg from CaCO_3) representing the range of dietary Ca and Mg concentrations were analyzed before and after ashing (550°C overnight) by powder X-ray diffraction (**XRD**) using a computer-controlled diffractometer (model I-2, Nicolet, Madison, WI) equipped with stepping motor and graphite crystal monochromator. Dried samples were scanned from 2 to 60° 2 θ after placement into a side-packed cavity mount that minimizes preferred orientation.

Solutions from all of the water-extracted fecal samples were modeled for chemical speciation (including prospective solid-phase stability evaluations) under an open system using Visual MINTEQ Version 2.51 (Gustafsson, 2006). In addition to P, Ca, and Mg measurements, generation of chemical speciation data required input of pH, electrical conductivity measured using a standard pH and conductivity meter, Na and K measured by atomic absorption, and dissolved organic and inorganic C determined using a carbon analyzer.

Statistical Analysis

Data were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The statistical model used to analyze the data was

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

where Y_{ijk} = 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.

The data set was complete except one cow consuming diet RCa-HMg in period 3 was removed from the experiment for health reasons. Daily milk yield and DMI during the collection days of each period were averaged before statistical analysis. The repeated water extractions of Ca, Mg, and P were analyzed using the PROC MIXED (Littell et al., 1996) procedure of SAS (Release 8.2) for repeated measures. Orthogonal contrasts were used to test main effects of dietary concentration of Ca, Mg, and source of Ca, as well as the 2- and 3-way interactions. Standard partial correlation analyses were performed by multivariate ANOVA (MANOVA) using PROC GLM of SAS and adjusted for treatments. Differences discussed in the text were significant at $P < 0.05$.

RESULTS AND DISCUSSION

Diet Composition and Intake

Upon analysis, the limestone contained 34.6% Ca and 0.3% Mg, CaCl_2 contained 28.3% Ca and 0.05% Mg, and MgO contained 60.5% Mg and 0.9% Ca (DM basis). Solubility in ruminal fluid and in pseudo-abomasal conditions was 0 and 100% for Ca in CaCO_3 , 68 and 100% for Ca in CaCl_2 , and 0.5 and 22% for Mg in MgO, respectively.

Diets were formulated to contain the same concentration of nutrients, with the exception of Ca, Mg, and Cl (Table 2). With the addition of inorganic Cl to the diets, average dietary concentration of Cl increased from 0.41 to 0.72 to 1.30% of DM. As a result, dietary DCAD ($\text{K} + \text{Na} - \text{Cl} - \text{S}$) decreased from 28.2 mEq/100 g of DM for the CaCO_3 diets to 17.7 mEq/100 g of DM for the RCa diets using CaCl_2 and to 4.8 mEq/100 g of DM for the HCa diets using CaCl_2 . Chemical composition of the diets met the minimum nutrient recommendations for cows in this study based on NRC (2001). Dietary concentrations of Ca and Mg (Table 2) varied with respect to the targeted values. The formulated RCa concentration was 0.61% Ca, and the analyzed concentration was, on average, 0.64% Ca (DM basis). The formulated HCa concentration was 1.0% Ca, and

Table 2. Chemical composition of diets fed to lactating dairy cows¹

Measure	CaCO ₃				CaCl ₂			
	Dietary concentration of Ca-Mg				Dietary concentration of Ca-Mg			
	R-R	H-R	R-H	H-H	R-R	H-R	R-H	H-H
CP, g/kg of DM	161	160	161	157	158	158	159	161
ADF, g/kg of DM	203	202	202	202	204	200	202	199
NDF, g/kg of DM	354	347	355	344	352	355	354	352
Lignin, g/kg of DM	35	34	35	34	35	34	35	34
Ether extract, g/kg of DM	35	35	35	35	35	35	35	35
Ash, g/kg of DM	57	60	66	74	63	72	68	87
NE _L , g/kg of DM	1.63	1.63	1.63	1.63	1.63	1.63	1.63	1.63
P, g/kg of DM	3.8	3.8	3.9	3.7	3.8	3.7	3.8	3.7
Ca, g/kg of DM	6.3	8.6	6.4	9.5	6.5	9.5	6.5	10.3
Mg, g/kg of DM	2.4	2.4	4.3	4.0	2.6	2.7	3.7	3.8
K, g/kg of DM	12.4	12.4	12.5	12.5	12.5	12.6	12.4	12.8
Na, g/kg of DM	4.9	4.3	4.5	5.1	4.3	5.1	4.2	4.8
S, g/kg of DM	2.0	2.0	2.2	2.0	2.0	2.0	2.1	2.0
Cl, g/kg of DM	4.0	3.8	4.4	4.1	6.6	14.3	7.6	11.8
Fe, mg/kg of DM	160	153	179	186	183	157	168	159
Zn, mg/kg of DM	137	119	183	154	279	112	144	100
Cu, mg/kg of DM	41	33	53	51	66	36	40	28
Mn, mg/kg of DM	92	80	120	119	140	83	107	70
DCAD, mEq/100 g of DM	29.0	27.6	25.8	30.3	19.8	1.8	15.6	7.9

¹Diets were formulated to contain recommended (R) and high (H) concentrations of Mg (0.25 and 0.40%, respectively) and Ca (0.64 and 0.95%, respectively) supplied by either CaCO₃ or CaCl₂ (NRC, 2001).

the analyzed concentration was from 0.86 to 1.03% Ca (DM basis). The RMg diets were expected to contain 0.2% Mg but averaged 0.25% Mg (DM basis) despite no addition of MgO. The HMg diets were formulated to contain 0.35% Mg, and the analyzed concentration was, on average, 0.40% Mg (DM basis). These differences between formulated and analyzed mineral concentrations were the product of the combined variability present in natural feedstuffs and in the concentrations of these 2 minerals in the different mineral mixes as well as errors associated with sampling and mixing. These differences did not affect the main objectives of the study.

Water contained 22.4 ± 5.0 mg/kg of Ca, 7.6 ± 2.0 mg/kg of Mg, and 0 mg/kg of P. Assuming water intake of approximately 100 L/cow per d, the intake of Ca, Mg, and P through water consumption was approximately 2, 1, and 0 g/d and contributed <2% of the total daily intake of each mineral. Therefore, only intake of Ca, Mg, and P from feed was considered relevant in this study.

Use of CaCl₂ 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 (Tucker et al., 1991; Goff and Horst, 1993; Pehrson et al., 1998). Metabolic acidosis produced by feeding CaCl₂ to dairy cows can be reflected in a reduction in urinary pH (Goff et al., 2004). Normal urinary pH of dairy cows is ≥ 8 . When cows were fed RCa diets, urinary pH was the same for cows

fed CaCO₃ or CaCl₂ (8.1 vs. 8.0). However, when cows consumed the HCa diet containing CaCl₂, urinary pH decreased compared with that of cows fed the HCa diet containing CaCO₃ (7.0 vs. 8.1; Ca source by Ca concentration interaction, $P < 0.0001$; Tables 3 and 4). Therefore, cows consuming diets of approximately 1.3% Cl (DM basis) resulting in a DCAD of approximately 6.0 mEq/100 g of DM had altered acid-base status. Likewise, when Hu et al. (2007) decreased DCAD from 22 to -3 mEq/100 g of DM by feeding CaCl₂ and removing NaHCO₃ and K₂CO₃ from the diet of lactating dairy cows, urinary pH decreased from 8.01 to 6.99.

Animals consuming CaCl₂ as the inorganic Ca supplement tended ($P = 0.08$) to eat less DM when expressed quantitatively (22.0 vs. 21.2 kg/d) but not when expressed as a percentage of BW (3.69 vs. 3.58%) compared with cows fed CaCO₃. Using empirical modeling techniques to evaluate cow responses to increasing dietary concentration of Cl from several studies, Sanchez et al. (1994) reported that DMI decreased dramatically in summer (about 2.5 kg/d) but was less affected during the winter season (about 0.5 kg/d) when dietary Cl increased from 0.4 to 1.0% (DM basis). The current study was conducted in the fall with reduced heat-stress effects on the cows; thus, cow response was similar to that reported for the winter season. Dry matter intake by lactating dairy cows decreased (Apper-Bossard et al., 2006; Hu et al., 2007) or was unchanged (Borucki Castro et al., 2004) by making DCAD more negative

Table 3. Dry matter intake, BW, milk production, milk composition, and urine pH of lactating dairy cows ($n = 9$ per treatment except $n = 8$ for R-H CaCO_3) fed diets formulated with either CaCO_3 or CaCl_2 and with recommended (R)¹ or high (H)² concentrations of Ca and Mg (NRC, 2001)

Measure	CaCO ₃				CaCl ₂				SEM
	Dietary concentration of Ca-Mg				Dietary concentration of Ca-Mg				
	R-R	H-R	R-H	H-H	R-R	H-R	R-H	H-H	
DMI, kg/d	22.5	21.8	21.7	21.9	22.6	20.4	21.0	20.7	0.64
DMI, % of BW	3.76	3.68	3.69	3.65	3.78	3.46	3.58	3.50	0.12
BW, kg	603	594	588	600	596	592	588	594	4.3
Milk yield, kg/d	35.9	34.0	34.5	34.9	37.6	33.2	33.7	33.2	0.9
Milk efficiency ³	1.60	1.58	1.60	1.61	1.67	1.62	1.61	1.64	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/d	1.11	1.08	1.12	1.07	1.12	1.00	0.96	1.02	0.05
4% FCM yield, kg/d	30.8	29.8	30.5	30.0	31.8	28.2	27.8	28.6	1.0
FCM efficiency ³	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/d	1.09	1.05	1.06	1.04	1.12	0.98	1.00	0.97	0.03
SCC, ×1,000	165	110	191	165	361	189	68	159	17.8
Urine pH	8.1	8.2	8.1	8.1	8.1	6.8	8.0	7.2	0.15

¹Average of 0.64% Ca and 0.25% Mg (DM basis).²Average of 0.95% Ca and 0.40% Mg (DM basis).³Kilograms of milk or FCM per kilogram of DMI.

through manipulation of dietary Cl. Intake of DM was not affected by other mineral formulations in the current study.

Milk Production

Source of supplemental Ca did not influence milk yield. Cows fed the RCa-RMg diets produced more milk compared with those fed the RCa-HMg diets (36.8 vs. 34.1 kg/d), whereas milk yield was unchanged by Mg feeding rate when diets contained a high concentration of Ca (33.6 vs. 34.1 kg/d for HCa-RMg vs. HCa-HMg; Ca concentration by Mg concentration interaction, $P = 0.01$; Tables 3 and 4). This response was likely due

to DMI changes because DMI followed the same pattern as milk yield. Production of 4% FCM tended ($P = 0.07$) to follow this same pattern (Tables 3 and 4). Efficiency of production of uncorrected milk and FCM (kg of milk production per kg of DMI) was not affected by dietary treatments, averaging 1.62 and 1.39, respectively (Table 3).

Concentration of milk fat tended to be less for cows fed CaCl_2 compared with cows fed CaCO_3 (3.00 vs. 3.15%, $P = 0.06$), which helped contribute to lower production of milk fat (1.02 vs. 1.09 kg/d, $P = 0.05$) and 4% FCM (29.1 vs. 30.3 kg/d, $P = 0.08$; Tables 3 and 4). According to Sanchez et al. (1994), increasing Cl in the diet from 0.4 to 1.0% (DM basis) decreased production

Table 4. P -values for orthogonal contrasts for DMI, BW, milk production, milk composition, and urine pH of lactating dairy cows fed diets formulated with either CaCO_3 or CaCl_2 and with recommended (R) or high (H) concentrations of Ca and Mg (NRC, 2001)

Measure	CaCO_3 vs. CaCl_2 Ca source	RCa vs. HCa Ca concentration	Ca source by Ca concentration interaction	RMg vs. HMg Mg concentration	Ca source by Mg concentration interaction	Ca concentration by Mg concentration interaction	Ca source by Ca concentration by Mg concentration interaction
DMI, kg/d	0.08	0.11	0.27	0.23	0.70	0.11	0.61
DMI, % of BW	0.15	0.12	0.39	0.41	0.86	0.37	0.57
BW, kg	0.22	0.71	0.95	0.26	0.84	0.01	0.41
Milk yield, kg/d	0.47	0.02	0.18	0.08	0.19	0.01	0.51
Milk efficiency	0.34	0.86	0.90	0.96	0.63	0.42	0.75
Milk fat, %	0.06	0.89	0.25	0.90	0.72	0.79	0.17
Milk fat, kg/d	0.05	0.34	0.86	0.29	0.32	0.22	0.15
4% FCM yield, kg/d	0.08	0.12	0.66	0.15	0.20	0.07	0.17
FCM efficiency	0.92	0.87	0.48	0.79	0.59	0.46	0.37
Milk protein, %	0.01	<0.01	0.18	0.31	0.54	0.44	1.00
Milk protein, kg/d	0.12	<0.01	0.39	0.07	0.13	0.05	0.49
SCC, $\times 1,000$	0.81	0.73	0.47	0.20	0.28	0.08	0.25
Urine pH	<0.0001	<0.01	<0.0001	0.67	0.66	0.34	0.25

of 4% FCM from approximately 20.8 to 19.7 kg/d in the summer but did not affect 4% FCM production in the winter season until Cl exceeded 1% of dietary DM. Others (Roche et al., 2005; Apper-Bossard et al., 2006; Hu et al., 2007) reported increasing concentration of milk fat with increasing concentration of DCAD but were unable to attribute the increase to increased DCAD alone because buffers such as sodium bicarbonate and sodium carbonate were fed to increase DCAD along with lessening of dietary Cl. The current study changed DCAD only through the addition of Cl and, therefore, indicates that a lower DCAD can negatively affect milk fat apart from the removal of traditional buffering supplements.

As with milk fat concentration, milk protein concentration was less when CaCl_2 was fed rather than CaCO_3 (2.97 vs. 3.03%, Tables 3 and 4; $P = 0.01$). In addition, concentration of milk protein was reduced from 3.03 to 2.96% as the dietary concentration of Ca increased ($P < 0.01$). This resulted in lower production of milk protein by cows fed the greater Ca diets (1.01 vs. 1.07 kg/d; $P < 0.01$). Hu et al. (2007) also reported a linear decrease in concentration of milk protein as DCAD decreased from 49 to 21 to -4 mEq/100 g of DM. A reduction in milk protein may occur if the overfeeding of Cl has a detrimental effect on microbial protein synthesis within the rumen.

Nutrient Digestibility

Apparent digestibility of DM and nutrients was not affected by dietary treatments. Mean values were 69.6% for DM, 64.8% for CP, 41.8% for ADF, and 47.2% for NDF. Apparent digestibility of P ranged from 57 to 63% across treatments and was not affected by dietary treatment. Values in the current study were generally greater than most other published values. Digestibility values of 28, 36, 42, and 46% have been reported for lactating cows fed diets of 0.46% P (Borucki Castro et al., 2004), 0.34% P (Taylor et al., 2009), 0.43% P (Ekelund et al., 2006), and 0.36% P (Knowlton et al., 2001), respectively. Two exceptions are that of Valk et al. (2002) and Dou et al. (2002), who reported apparent digestibility coefficients for P of 56 and 57% for dairy cows fed diets of 0.38 or 0.34% P (DM basis), respectively, which are similar to the current coefficients. Apparent digestibility of P can be affected by type of feedstuffs fed (Knowlton et al., 2001), week of lactation (Taylor et al., 2009), amount of milk produced (Valk et al., 2002), and parity (Knowlton et al., 2001). It is difficult to explain why the reported coefficients in the literature are so different from study to study because most fed similar proportions of forage to concentrate, accounted for parity, and did not use inorganic P sources in the

diets. Perhaps differences in recycling P through the saliva can account for differences in apparent digestibility among studies. The dietary Ca concentration and Ca source did not affect apparent digestibility of P in the current study. Taylor et al. (2009) reported no effect of increasing the dietary concentration of Ca from 0.52 to 1.03% (DM basis) on apparent digestibility of P by lactating dairy cows. Based on the lower urine pH, cows fed the high CaCl_2 diet likely mobilized some Ca and P from bone into the blood stream. However, absorption of P and Ca from the small intestine may not have been affected. Lomba et al. (1978) reported a low correlation (0.023) between digestible Ca and the DCAD equation $(\text{Na} + \text{K}) - (\text{Cl} + \text{S})$ when dairy cows were in a positive Ca balance compared with a correlation of 0.506 for cows in a negative Ca balance. The fact that increasing the dietary concentration of Ca with either CaCO_3 or CaCl_2 had similar effects on P dynamics indicates that the increased intake of Cl did not have a distinctive effect. Increasing the dietary concentration of Mg did not influence P digestibility.

Main dietary factors of Ca source, Ca concentration, and Mg concentration and their interactions did not influence apparent digestibility of Ca or Mg. Apparent Ca and Mg digestibility coefficients ranged from 23.4 to 39.6% for Ca and 22.3 to 41.2% for Mg and are similar to what other researchers have reported. Calcium digestibility coefficients were similar to those reported for lactating cows fed an alfalfa and corn silage-based diet (Martz et al., 1999), whereas Mg digestibility coefficients were comparable with those compiled from 8 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.

Water-Extractable P, Ca, and Mg

The 4 g/d increase in P intake (83 vs. 79 g/d, $P = 0.02$) was accompanied by a 4 g/d increase in fecal output of P (35 vs. 31 g/d) by cows fed the RCa diets compared with cows fed the HCa diets ($P < 0.01$; Tables 5 and 6). Therefore, the difference in P output due to dietary Ca concentration could be explained by the difference in P intake. Output of fecal P was correlated positively with intake of feed P across treatments ($r = 0.48$). Dietary Mg concentration did not influence intake of P or fecal excretion of P.

Feeding CaCl_2 resulted in a reduction in the proportion of fecal P that was solubilized in water after 10 water extractions compared with the feeding of CaCO_3 .

Table 5. Intake, fecal output, and fecal output of water-extractable P, Ca, and Mg from lactating dairy cows ($n = 9$ per treatment except $n = 8$ for R-H CaCO_3) fed diets formulated with either CaCO_3 or CaCl_2 and with recommended (R)¹ or high (H)² concentrations of Ca and Mg (NRC, 2001)

Measure ³	CaCO ₃				CaCl ₂				SEM
	Dietary concentration of Ca-Mg				Dietary concentration of Ca-Mg				
	R-R	H-R	R-H	H-H	R-R	H-R	R-H	H-H	
DMI, kg/d	21.8	21.9	21.6	22.2	23.1	20.9	20.6	20.2	0.8
P intake via feed, g/d	82	83	84	82	88	77	78	75	3
P output in feces, g/d	35	31	35	34	36	29	32	29	2
WEP output in feces, ⁴ % of P output	53.6	44.9	44.3	36.8	45.0	36.4	47.8	32.0	2.8
WEP output in feces, ⁴ g/d	18	14	15	12	17	11	15	9	1
Ca intake via feed, g/d	139	189	137	210	149	199	134	207	6
Ca output in feces, g/d	99	153	85	147	95	155	92	142	10
WECa output in feces, ⁴ % of Ca output	36.8	30.6	32.8	24.6	35.2	28.9	36.1	24.9	2.5
WECa output in feces, ⁴ g/d	34	43	28	36	33	44	32	35	3
Mg intake via feed, g/d	53	53	93	88	60	57	76	76	3
Mg output in feces, g/d	40	41	62	51	36	37	48	50	3
WEMg output in feces, ⁴ % of Mg output	80.1	72.0	80.7	81.8	70.9	88.0	78.6	69.2	4.5
WEMg output in feces, ⁴ g/d	31	28	50	42	25	33	38	34	2

¹Average of 0.64% Ca and 0.25% Mg (DM basis).²Average of 0.95% Ca and 0.40% Mg (DM basis).³WEP = water-extractable P; WECa = water-extractable Ca; WEMg = water-extractable Mg.⁴Based on 10 water extractions.

(40.3 vs. 44.9%, $P = 0.02$; Tables 5 and 6). This reduction was detected starting with the sixth water extraction (Ca source by water extraction number interaction, $P < 0.01$; Figure 1A). Less fecal P was extracted after 10 water extractions when dietary concentration of Ca increased from RCa to HCa (47.7 vs. 37.5%, $P < 0.001$). This reduction was detected starting with the third water extraction (Ca concentration by water extraction number interaction, $P < 0.0001$; Figure 1B). This 10-percentage-unit decrease in %WEP translated to 5 extra grams of WEP (16 vs. 11 g/cow per d; Table 5). When projected for a 500-lactating-cow dairy farm over a year's time, a reduction in 5 g of WEP/cow per d would result in 912 kg of P remaining on the farm rather than leaving if leaching or runoff was a serious problem.

Increasing the dietary concentration of Mg from 0.25 to 0.40% (DM basis) also decreased the proportion of WEP after 10 extractions (45.0 vs. 40.2%, $P = 0.02$), with the difference being significant with each sequential extraction (Figure 1C). The effect of dietary concentration of Mg on %WEP, however, was influenced by the source of supplemental Ca. Increasing dietary Mg reduced %WEP (after 10 extractions) only when CaCO_3 (49.2 vs. 40.5%) rather than CaCl_2 was fed (40.7 vs. 39.9%; Mg concentration by Ca source interaction, $P = 0.05$; Tables 5 and 6). Because the Mg from MgO was not soluble in the rumen, it was not available to bind with P. Insoluble Ca-P bonds may have formed

in the rumen when rumen-soluble CaCl_2 was fed. The addition of Mg becoming available in the GIT did not further reduce P solubility due to a lack of available P to form Ca-Mg-P bonds. Because CaCO_3 was insoluble in the rumen, the formation of Ca-P bonds was not as extensive. As a result, more P was available in the GIT along with additional Mg so that more Ca-Mg-P bonding occurred, resulting in a reduction of WEP in feces. Fecal output of P was not affected by dietary concentration of Mg (33 vs. 32 g/d), but fecal output of WEP was reduced ($P = 0.01$) by feeding more Mg (13 vs. 15 g/d). Increasing dietary concentration of Ca and Mg beyond the NRC recommendation using CaCO_3 and MgO reduced %WEP from 53.6 to 36.8%.

Cows supplemented with CaCO_3 or CaCl_2 did not differ in Ca intake (169 vs. 172 g/d), Ca output in feces (121 vs. 121 g/d), or WECa (after 10 extractions) as a proportion of total fecal Ca (31.2 vs. 31.3%; Table 5; Figure 2A). As expected, increasing Ca concentration in the diet increased ($P < 0.0001$) Ca intake (140 vs. 201 g/d) and Ca output in feces (93 vs. 149 g/d). The proportion of dietary Ca excreted in feces was 74.1% for cows fed HCa diets compared with 66.4% for cows fed RCa diets. Diets containing more Ca than needed by the animal result in a reduced proportion of dietary Ca absorbed (Horst, 1986; Reinhardt et al., 1988). The proportion of fecal Ca extracted after 10 water extractions was less ($P < 0.001$) for cows fed the HCa versus the RCa diets (27.2 vs. 35.2%; Tables 5 and 6). The

Table 6. *P*-values for orthogonal contrasts for intake, fecal output, and fecal output of water-extractable P, Ca, and Mg of lactating dairy cows fed diets formulated with either CaCO₃ or CaCl₂ and with recommended (R) or high (H) concentrations of Ca and Mg (NRC, 2001)

Measure ¹	CaCO ₃ vs. CaCl ₂ Ca source	RCa vs. HCa Ca concentration	Ca source by Ca concentration interaction	RMg vs. HMg Mg concentration	Ca source by Mg concentration interaction	Ca concentration by Mg concentration interaction	Ca source by Ca concentration by Mg concentration interaction
DMI, kg/d	0.19	0.30	0.11	0.10	0.10	0.21	0.54
P intake via feed, g/d	0.04	0.02	0.08	0.08	0.09	0.40	0.13
P output in feces, g/d	0.17	<0.01	0.38	0.15	0.15	0.15	0.73
WEP output in feces, ² % of P output	0.02	<0.001	0.30	0.02	0.05	0.43	0.29
WEP output in feces, ² g/d	<0.01	<0.0001	0.12	0.01	0.84	0.58	0.74
Ca intake via feed, g/d	0.39	<0.0001	0.99	0.50	0.11	<0.01	0.94
Ca output in feces, g/d	1.00	<0.0001	0.81	0.22	0.91	0.95	0.54
WECa output in feces, ²	0.97	<0.001	0.67	0.07	0.33	0.32	0.70
% of Ca output							
WECa output in feces, ² g/d	0.69	<0.001	0.71	<0.01	0.61	0.21	0.36
Mg intake via feed, g/d	<0.01	0.19	0.81	<0.0001	<0.0001	0.93	0.32
Mg output in feces, g/d	<0.01	0.39	0.10	<0.0001	0.33	0.09	0.11
WEMg output in feces, ²	0.53	0.96	0.26	0.95	0.10	0.17	<0.01
% of Mg output							
WEMg output in feces, ² g/d	<0.01	0.28	0.02	<0.0001	<0.01	0.02	0.34

¹WEP = water-extractable P; WECa = water-extractable Ca; WEMg = water-extractable Mg.

²Based on 10 water extractions.

WECa values for these 2 treatments were different at each water extraction (Figure 2B). Calcium supplied by the supplements was likely more soluble in the digestive tract than Ca coming from the other feed ingredients in the diet (NRC, 2001). Part of this increased soluble Ca consumed in the HCa diets likely complexed with P in the GIT, resulting in a reduction in WECa in the feces. This response is in agreement with that observed for %WEP; that is, increasing dietary Ca reduced %WEP in feces. Similar findings were reported in bird diets in which solubility of P in turkey manures was less when diets had an increased Ca:P ratio (Toor et al., 2005). In addition, less P was absorbed from the small intestine when the dietary concentration of Ca was increased from 1.9 to 3.56% for laying hens (Hurwitz and Bar, 1965). Sevilla and Ternouth (1981) reported an increase in P deficiency when lambs were fed Ca in excess of the animal's daily requirements. However, Deitert and Pfeffer (1993), working with milking goats over 2 lactations, reported no consistent effect on P balance when doubling the NRC recommendation for dietary Ca.

Amount of Mg intake did not affect amount of Ca consumed or excreted in feces. However, cows fed HMg diets produced feces tending ($P = 0.07$) to contain less %WECa than that of those cows fed RMg diets (29.6 vs. 32.9%; Tables 5 and 6). This difference was detected starting with the fourth water extraction (Figure 2C). This resulted in a reduced ($P < 0.01$) amount of WECa when HMg diets were fed (33 vs. 39 g/d). Because Ca can bind with Mg and P forming a Ca-Mg-P compound, feeding more Mg can reduce proportion of WECa in feces.

In general, output of Mg in feces was highly dependent on Mg intake; that is, as Mg intake increased, fecal output of Mg increased. For example, cows fed CaCl₂ consumed more Mg than cows fed CaCO₃, and these former cows also excreted in feces more Mg ($P < 0.01$). Likewise, cows fed HMg diets consumed more Mg (83 vs. 56 g/d) and excreted more Mg (53 vs. 38 g/d) than cows fed RMg diets ($P < 0.001$). Fecal Mg (mean of 78%) was substantially more water soluble after 10 extractions than was P (43%) or Ca (31%). In a similar fashion, dietary Mg was more soluble than dietary Ca and P in the rumen of dairy cows (Emanuele and Staples, 1994). When considering only the 10th water extraction, %WEMg was not affected by Ca source. However, when all 10 water extractions were included in the analysis, cows fed CaCl₂ produced feces of lower WEMg than those fed CaCO₃ by approximately 3 percentage units. Just as feeding more Mg reduced WECa, feeding a more water-soluble Ca source reduced WEMg. The main effects of Ca and Mg concentration did not affect WEMg; however, a 3-way interaction was detected for %WEMg (Table 6). The

Figure 1. Effect of feeding supplemental Ca carbonate or Ca chloride (A), diets with an average of 0.64 or 0.95% Ca (DM basis; B), and diets with an average of 0.25 or 0.40% Mg (DM basis; C) to lactating Holstein cows on cumulative extent of water-extractable P (WEP) from feces, expressed as a proportion of total fecal P, in each of 10 successive water extractions. Test of treatment by extract interaction was $P < 0.01$, $P < 0.0001$, and $P = 0.96$ for A, B, and C, respectively. *Means were different. The SEM was 1.9%.

HCa-RMg diet affected %WEMg differently depending on the Ca source. If the dietary Ca source was CaCO_3 , %WEMg in feces was the least (72.0%) coming from cows fed the 4 dietary treatments containing CaCO_3 , whereas if the Ca source was CaCl_2 , %WEMg in feces was the greatest (88.0%) coming from cows fed the 4 dietary treatments containing CaCl_2 (Ca source by Mg concentration by Mg concentration interaction, $P < 0.01$). The authors cannot offer a biological explanation for this 3-way interaction. Patterns of WEMg over the 10 extractions were not affected by treatments, with WEMg averaging 39% after 1 extraction to 78% after 10 extractions (figure not shown).

Partial correlation coefficients adjusted for treatment indicated that extent of fecal P extracted after the 10th water extraction was associated negatively with intake of feed Ca ($r = -0.46$; $P < 0.01$) and output of fecal Ca ($r = -0.48$; $P < 0.01$) and Mg ($r = -0.25$; $P < 0.05$). Feed intake of Mg or P was not correlated with WEP. Extent of WEP was correlated positively with WECa ($r = 0.43$; $P < 0.01$) but not with WEMg. However, extent of WEMg and WECa were positively correlated ($r = 0.64$; $P < 0.01$). Therefore, feeding more Ca resulted in greater fecal output of Ca, which was associated with reduced extent of WEP in feces. Calcium appeared to be more important than Mg in influencing WEP in feces of lactating dairy cows. Associated release of Ca with P and Mg is consistent with previous reports when using a high water-to-manure ratio (Kleinman et al., 2002) and when studying solubility of P from manure-amended soils (Hansen and Strawn, 2003; Josan et al., 2005; Silveira et al., 2006).

Chemical- and Solid-State Speciation Analyses

Chemical speciation analysis of the first water extraction across all treatments indicated that solutions were dominated by HPO_4^{2-} and H_2PO_4^- , which, in combination, represented at least 65% of the total P in solution. A Mg-P species represented ~20%, a Ca-P species represented ~10%, and K-P and Na-P species combined for <1% of total P 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 obtained from chemical speciation modeling (Visual-MINTEQ) indicated that solutions obtained after the first extraction were supersaturated with respect to whitlockite $[(\text{Ca,Mg})_3(\text{PO}_4)_2]$ and hydroxylapatite $[\text{Ca}_5(\text{PO}_4)_3(\text{OH})]$, signifying that these minerals would not have dissolved in the first water extraction. High concentrations of both Ca and Mg in solution likely suppressed dissolution of any Ca-Mg-P compounds present (e.g., whitlockite). Analysis of so-

lutions obtained from the seventh extraction onward indicated that Ca-P or Ca-P-Mg minerals were under-saturated (i.e., would eventually dissolve) in the extracting solutions, explaining associated release of these elements in latter extractions.

Evidence of a crystalline phosphate mineral (e.g., whitlockite or hydroxylapatite) was not detected by XRD analysis of dried fecal samples. However, when fecal samples were ashed to remove OM, phosphate minerals containing Ca or Ca and Mg were detected. Sylvite (KCl) and calcite (CaCO_3) were minerals common to all samples analyzed and, hence, were used as internal standards to correctly identify other peaks. A Mg-substituted whitlockite was the only P-containing mineral detected in fecal samples from animals fed the RCa using CaCO_3 , HMg diet (Figure 3a). Mainly whitlockite but some hydroxylapatite was detected in fecal samples from animals fed the RCa, RMg diet (Figure 3b). Feces from cows fed the HCa using CaCO_3 , HMg diet contained a predominance of hydroxylapatite with some whitlockite (Figure 3c), whereas hydroxylapatite was the only P-containing mineral detected in feces collected from dairy cows fed the HCa using CaCl_2 , HMg diet (Figure 3d). Hence, greater dietary intake of soluble Ca (increasing from RCa using CaCO_3 to HCa using CaCO_3 to HCa using CaCl_2) resulted in greater proportions of hydroxylapatite and reduced proportions of whitlockite in fecal samples (Figure 3). The proportion of WEP decreased as the proportion of hydroxylapatite increased, which is consistent with the lesser solubility of hydroxylapatite relative to whitlockite (Lindsay, 1979). As dietary supply of Ca increased, WEP decreased and the formation of hydroxylapatite increased. The %WEP decreased from a mean of 48.9 g/100 g of DM for fecal samples from cows fed the 2 recommended CaCO_3 diets to 36.8% for the HCa using CaCO_3 , HMg diet to 32.0% for the HCa using CaCl_2 , HMg diet (Table 5). It is uncertain as to whether hydroxylapatite and whitlockite were present in dried feces before ashing because they were not detected by XRD. Possibilities for lack of detection were a) they were present in crystalline form but in amounts too diluted by OM to be detected or b) they were present in noncrystalline form and, therefore, not detected by XRD before being crystallized at the elevated temperature of ashing. Whether these same P compounds are found in the same proportions in fresh feces is unknown. Effects of manure-handling variables such as storage conditions, rate of drying, type of application, and other similar effects were not evaluated in this study. Despite this uncertainty, the parallel between fecal ash mineralogy and P solubility is evidence that dietary modifications, particularly increased intake of soluble Ca, can affect the extent of binding of P to Ca, the chemical form of

Figure 2. Effect of feeding supplemental Ca carbonate or Ca chloride (A), diets with an average of 0.64 or 0.95% Ca (DM basis; B), and diets with an average of 0.25 or 0.40% Mg (DM basis; C) to lactating Holstein cows on cumulative extent of water-extractable Ca (WECa) from feces, expressed as a proportion of total fecal Ca, in each of 10 successive water extractions. Test of treatment by extract interaction was $P = 1.0$, $P = 0.53$, and $P = 0.66$ for A, B, and C, respectively. *Means were different. The SEM was 1.7%.

Figure 3. X-ray diffraction analysis of ashed fecal samples from dairy cows ($n = 3$ per diet) fed diets formulated to contain (a) recommended (R) concentrations of Ca (average of 0.64%, DM basis) using CaCO_3 and higher (H) than recommended concentrations of Mg (average of 0.4% Mg, DM basis), (b) RCa using CaCO_3 and RMg (average of 0.25% Mg, DM basis), (c) HCa using CaCO_3 (average of 0.95% Ca, DM basis) and HMg, or (d) HCa using CaCl_2 and HMg. The following mineral compounds were detected: calcite (CaCO_3 ; C), silvite (KCl ; S), whitlockite [$(\text{Ca,Mg})_3(\text{PO}_4)_2$; W], and hydroxylapatite [$\text{Ca}_5(\text{PO}_4)_3(\text{OH}, \text{Cl}, \text{F})$; A]. Each line in a graph represents a fecal sample from a different cow.

excreted P, and P solubility. These findings support the hypothesis that increasing the amount of soluble Ca in the GIT can help reduce solubility of fecal P.

Elemental dot maps (data not shown) and elemental spectra collected from areas of the maps showing greatest P concentrations verified spatial associations between Ca, Mg, and P. In some cases, the association was exclusively with Mg or Ca and in other cases with both, supporting the idea of a solid phase where Ca and Mg are combined with P either independently (P with Ca or Mg) or together (P with both Ca and Mg).

CONCLUSIONS

Although supplemental CaCl_2 was slightly more effective than CaCO_3 in reducing WEP in manure, CaCO_3 would be the preferred source of supplemental Ca because cows fed diets in which supplemental CaCO_3 replaced CaCl_2 tended to produce more 4% FCM, more milk fat, and milk with a greater concentration of fat and protein. Current mineral prices also would favor feeding of CaCO_3 over CaCl_2 . Apparent digestibility of DM, CP, NDF, ADF, Ca, Mg, and P was not affected by increased dietary concentrations of Ca or Mg or by the inorganic Ca source fed. Daily fecal excretion of P (g/cow) was correlated positively with P intake but unaffected by intake of Ca or Mg. Solubility of P in dairy-cow feces can be reduced preemptively by dietary control of Ca and Mg when cows are fed the recommended dietary concentration of P. Feeding increased amounts of soluble Ca and Mg reduced WEP in fecal samples; however, Ca was the driving factor reducing the amount of P solubilized from cow feces. A 5-g reduction in soluble P per cow per day was calculated to potentially leave the farm when dietary concentrations of Ca were increased from an average of 0.64 to an average of 0.95% (DM basis). Correlated release of Ca, Mg, and P in water extractions suggests that they were associated in feces. Spatial associations of Ca, Mg, and P using solid-state assessment of dried fecal samples and XRD using ashed fecal samples were detected. Supplying less soluble Ca in the diet produced a Ca-Mg-P mineral (whitlockite) in the fecal samples, whereas feeding more soluble Ca produced hydroxylapatite, which is less soluble. Feeding intermediate amounts of soluble Ca produced both minerals. The relative solubility and trends in minerals found in ashed fecal samples are consistent with observed WEP differences and support the idea that greater consumption of soluble Ca favors a more stable form of P in feces. Increasing dietary intakes of Ca and Mg beyond current recommendations can potentially increase the formation of insoluble phosphates, which may in turn decrease solubility of P in

dairy-cow feces and reduce losses of P from agricultural areas where feces are applied.

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