Sulfur and Chlorine Play a Non-Acid Base Role in Periparturient Calcium Homeostasis

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ABSTRACT

The increased risk of periparturient hypocalcaemia through offering high-K feeds in the precalving period has been reported previously. Two experiments (experiment 1 and 2) investigated the effect of KCl fertilizer on pasture mineral concentration, the dietary cation-anion difference (DCAD), and the subsequent effect of this on periparturient plasma and urine mineral status. Experiment 2 examined the effect of precalving Mg source (MgO, MgSO₄, and MgCl₂) and postcalving Ca supplementation on the concentration of Ca and Mg in plasma and urine. Unexpectedly, pasture DCAD increased ($P = 0.06$) from 434 to 535 mEq/kg DM in experiment 1 as pasture K concentration decreased from 4.2 to 3.5%, primarily because of a corresponding and greater decrease in pasture Cl concentration (from 1.9 to 1.3%). Plasma Ca or Mg concentrations were not affected by pasture K concentration. A linear decline ($P < 0.10$) in urine Mg suggested a decline in Mg absorption as pasture K increased. In experiment 2, pasture DCAD decreased ($P < 0.05$) linearly from 403 to 350 mEq/kg DM as pasture K concentration decreased from 3.8 to 3.3%. However, precalving urine pH was not affected by the declining DCAD. Postcalving plasma Ca concentration was affected by precalving Mg source with MgSO₄ > MgCl₂ > MgO. Differences in acid-base balance do not explain the difference between Mg salts. These results indicate that precalving dietary S and Cl concentration plays an important role in Ca homeostasis, in addition to its role in acid-base balance. Supplementation with Ca postcalving increased plasma Ca concentration for 2 d postcalving. Milk production was not affected.

(Key words: hypocalcaemia, dietary cation-anion difference, potassium, magnesium)

Abbreviation key: Creat = Creatinine, DCAD = dietary cation-anion difference, ME = metabolizable energy.

INTRODUCTION

Parturient hypocalcaemia (blood Ca < 1.4 mmol/L) is a metabolic disease of extensive economic importance. It results from the sudden, increased demand placed on the plasma Ca pool by the onset of lactation. Subclinical hypocalcaemia (blood Ca < 2.0 mmol/L) is also important, reducing milk production (Block, 1984) and rumen and intestinal motility (Daniel et al., 1990; Hara et al., 2001). Many strategies are used to minimize the decline in blood Ca at parturition, including the feeding of a precalving diet containing a low Ca concentration (Wiggers et al., 1975; Littledike and Goff, 1987), administration of vitamin D approximately 1 wk precalving (McNeill et al., 2002) and the supplementation of Ca at calving (Roche, 1999). These treatments reflect the belief that dietary Ca concentration, either too great precalving or insufficient availability postcalving, is the principal determinant of a cow's susceptibility to parturient hypocalcaemia.

More recently, however, the relative importance of dietary Ca has come under review (Oetzel, 1991; Goff and Horst, 1997). It has been proposed that precalving dietary Ca may not be as important a risk factor as high intakes of K and subsequent effects on blood acid-base status (Goff and Horst, 1997). Changes in blood pH affect Ca metabolism (Block, 1984; Bushinsky et al., 1993; Roche, 1999). A prepartum diet, with a relative predominance of dietary macromineral anions to cations [a negative dietary cation-anion difference (**DCAD**)], has been shown to reduce blood pH (Stewart, 1981, 1983). Previous studies have shown a reduction in the incidence of hypocalcaemia and an increase in milk production when Na and K concentrations in the diet are reduced and/or Cl and S concentrations are increased to produce a negative DCAD (Block, 1984; Joyce et al., 1997).

Although fundamentally sound, the previous studies may not be wholly appropriate for pasture-based systems. Large-scale surveys have shown a 2 to 5% incidence of parturient paresis in pasture-fed cows (Caple, 1987; Mee, 1993; McDougall, 2001). Daniel et al. (1990) identified a 33% incidence of subclinical hypocalcaemia

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on the day of calving in cows grazing pasture in New Zealand. These are similar incidences to those reported by McLachlan et al. (2000) in Northern Australia. Although the concentration of dietary potassium ($>3.1\%$) and DCAD (>400 mEq/kg DM) is generally high in pasture-based diets, these incidences are no higher than incidences of milk fever reported in other countries where dietary K is much lower (Roche, 1999). Furthermore, Oetzel (1991) and Enevoldsen (1993) reported that S was the most important dietary constituent in determining the risk of hypocalcaemia, more important than either Cl or K. The absorption efficiency of S is known to be less than either Cl or K (Underwood and Suttle, 1999; NRC, 2001) and so, would not be expected to incur the same change in systemic pH. Its importance in hypocalcaemia prevention, therefore, does not fit with the current understanding of how manipulation of DCAD influences Ca homeostasis.

The dairy industries of southern Australia and New Zealand are based largely on fresh pasture and pasture silage. The variation in the DCAD of pasture (Roche et al., 2000) and the difficulty in accurately assessing DMI make an accurate reduction in DCAD difficult to achieve. Furthermore, the requirement for large amounts of "anionic salts" to reduce the DCAD sufficiently to alter systemic pH (Roche, 1999) make it an impractical method of improving Ca homeostasis. Nevertheless, anecdotal benefits in milk fever reduction to feeding insufficient quantities of "anionic salts" to alter systemic pH have been reported (McNeill et al., 2002).

The two experiments reported examined the effect of altering the pasture K concentration through fertilizer applications on the mineral concentration and DCAD of pasture and on periparturient plasma mineral concentrations. Experiment 2 also examined the effect of practical amounts of anionic salts precalving and Ca supplementation postcalving on periparturient plasma Ca concentrations and milk production.

MATERIALS AND METHODS

The experiments were conducted at the Whareroa Dexcel research farm, Taranaki, New Zealand ($174^{\circ}14'$ east; $39^{\circ}34'$ south), in July and August, 2000 and 2001.

Experimental Design and Treatments

Experiment 1. In May 2000, 40 paddocks (0.53 ha) were randomly allocated to one of four experimental farms (farmlets: K1, K2, K3, and K4) with 10 paddocks/farmlet. Each farmlet was balanced for soil P and K status. Muriate of potash (KCl; approximately 50% K) was applied at either 0, 75, 150, or 225 kg K/ha to K1, K2, K3, and K4, respectively, to establish four different

pasture (dietary) K concentrations according to a complete randomized block design.

Sixty-four Friesian cows (48 multiparous, 16 primiparous; 4.8 ± 2.37 yr; mean age $\pm$ SD), selected to calve over a 5-wk period (21 August 2000 $\pm$ 16.7 d; mean calving date $\pm$ SD), were allocated to four K farmlets (16 cows/farmlet). Mean calving BW (515 ± 75 kg) and BCS (3.0 ± 0.2) did not differ between farmlets. MgO (80 g/cow per d) was applied daily, on all farmlets, over the area to be grazed each day.

Experiment 2. The four farmlets already described in experiment 1 were used. Twelve primiparous cows replaced multiparous cows to maintain an age structure typical of what is seen on-farm in New Zealand (52 multiparous, 12 primiparous; 4.9 ± 2.35 yr; mean age $\pm$ SD). In May 2001, K3 and K4 farmlets received an additional 75 and 150 kg K/ha, respectively, as KCl. The 16 cows (9 August 2001 $\pm$ 17 d; mean calving date $\pm$ SD) in each K farmlet were randomly allocated to one of three sources of Mg supplementation precalving. Allocation was based on predicted calving date, age, previous lactation yield of milk and milk protein, and previous plasma concentration of Ca. Mean calving BW (500 ± 70 kg; mean $\pm$ SD) and BCS (2.9 ± 0.2 ; mean $\pm$ SD) did not differ between treatments. It was proposed that 20 cows receive 150 g MgCl_2 ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$; 100% purity; 17.9 g Mg), 20 cows receive 200 g MgSO_4 ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 98% purity; 19.3 g Mg), and 24 cows receive 35 g MgO (100% purity; 19.3 g Mg) for 21 d precalving orally, mixed with 1 L of warm water. On completion of the experiment, 19 cows had received MgCl_2 ; 19 cows had received MgSO_4 ; and 21 cows had received MgO for 25 ± 10 d (mean $\pm$ SD). Differences in planned and actual numbers of cows drenched were due to incorrect predicted calving dates for 6 cows.

Two cows in each K farmlet $\times$ precalving Mg treatment (24 cows) received supplementary Ca (150 g CaCO_3 /cow per d) for 4 d postcalving. The remaining cows (25 cows) did not receive postcalving Ca supplementation. All cows were supplemented with Mg postcalving through an application of 80 g MgO/cow per d to pastures to be grazed.

Grazing Management

The cows in the experiments described here were rotationally grazed. Cows had access to 10 paddocks (defined grazing area) of 0.53 ha within a farmlet, and these paddocks were grazed in a rotational order. As a result, cows had access to a fresh allocation of pasture daily and only returned to the same area when a minimum of two leaves had appeared on the majority ($>75\%$) of perennial ryegrass tillers.

The sward in both experiments consisted of approximately 65% perennial ryegrass (*Lolium perenne* L.), 30% other grasses (*Dactylus glomerata*, *Holcus lanatus*, and some *Poa* species), 2.5% white clover (*Trifolium repens*), and 2.5% weeds on a DM basis. The nutritive characteristics and mineral concentrations of the feeds offered are presented in Table 1.

The paddocks used in both experiments had 7 to 8 wk pasture regrowth. Pregrazing pasture mass averaged 3910 ± 300 kg DM/ha (3910 ± 410 , 3960 ± 230 , 3840 ± 410 , and 3920 ± 240 kg DM/ha, for K1, K2, K3, and K4, respectively) in experiment 1 and 4011 ± 359 kg DM/ha (4080 ± 330 , 4010 ± 280 , 3940 ± 260 , and 4030 ± 500 kg DM/ha, for K1, K2, K3, and K4, respectively) in experiment 2. Postgrazing residual pasture mass in experiment 1 averaged 1840 ± 140 (1840 ± 170 , 1810 ± 100 , 1900 ± 200 , and 1810 ± 120 kg DM/ha, for K1, K2, K3, and K4, respectively) and 1740 ± 330 (1690 ± 260 , 1730 ± 350 , 1690 ± 250 , and 1850 ± 420 kg DM/ha, for K1, K2, K3, and K4, respectively) in experiment 2. The average pasture mass across the four farmlets was 2570 ± 750 kg DM/ha (2560 ± 770 , 2610 ± 790 , 2560 ± 740 , and 2540 ± 760 kg DM/ha, for K1, K2, K3, and K4, respectively) and 2880 ± 1190 kg/DM (2880 ± 1230 , 2870 ± 1180 , 2810 ± 1160 , and 2940 ± 1190 kg DM/ha, for K1, K2, K3, and K4, respectively) in experiment 1 and 2, respectively.

Measurements

In both experiments, individual milk yields were recorded once every 2 wk for the first 2 mo of lactation. Individual p.m. and a.m. milk samples were collected on days of milk yield measurement for analysis of fat, protein, and lactose concentrations by FT120 (Helrod, Denmark).

Pasture allocations were visually assessed, and assessors were calibrated weekly through cutting a range of pasture yields, representative of pre- and postgrazing yields (O'Donovan, 2000). Precalving group DMI were calculated daily from pre- and postgrazing pasture masses (Roche et al., 1996).

Representative samples of pasture were collected by "plucking" pasture to grazing height from paddocks due to be grazed. Samples were bulked every 2 wk and duplicate samples were dried at either 105°C, for dry matter analysis, or 60°C for nutritive characteristic determination. All samples dried at 60°C were dried for 72 h, ground to pass through a 0.5-mm sieve, and analyzed for CP, NDF, ADF, NSC, fat, ash, digestibility, and metabolizable energy (ME) by near infra-red spectroscopy. Feed samples were bulked every 2 wk for mineral analysis by inductively coupled plasma emis-

sion spectroscopy. NE_L was calculated from ME by; NE_L = ME $\times$ 0.65 (Holmes and Wilson, 1987).

Sampling Regime and Analysis

In experiment 1, blood and urine was sampled on the day of calving and on each of the 3 d following calving at 0700 h. Both blood and urine were analyzed for Ca, Mg, and K concentration. Urine pH and urine creatinine concentration were also determined. In experiment 2, blood and urine was sampled a day prior to beginning Mg supplementation, on the day of calving, and on each of the first 4 d following calving at approximately 0700 h. Urine samples were also collected twice weekly prior to calving. Samples were bulked to provide a weekly sample.

Blood samples were analyzed for Ca, Mg, and NEFA concentration while urine pH, urine Ca, urine Mg, and urine creatinine concentration were also determined.

Urine sampling. Cows were manually stimulated to urinate, and a sample of urine, from midstream, was collected in a 30-ml container. Within 30 min of collection, pH was measured, and 30 ml of sample was frozen for subsequent analysis of Ca (o-Cresolphthalein complexone), Mg (xlidyl blue reaction), K (ion-selective electrodes), and creatinine (Jaffe reaction - alkaline picrate).

Blood sampling. Blood was collected by coccygeal venipuncture into heparinized vacutainers, placed on ice, and centrifuged for 10 min at $1120 \times g$ within 60 min of sampling. Plasma was analyzed for Ca (o-Cresolphthalein complexone), Mg (xlidyl blue reaction), K (ion-selective electrodes), and NEFA (colorimetric method).

All assays (blood and urine) were performed on the Hitachi 717 analyzer (Roche) at 30°C by Alpha Scientific, Ltd. (Hamilton, New Zealand). The interassay and intraassay coefficient of variation was <5% for all assays.

Calculations

The DCAD is the difference, in milliequivalents, between certain cations and anions in the diet. A diet with a negative cation-anion difference represents a diet where there is a relative predominance of macro-mineral anions to cations (Joyce et al., 1997). The most common equation used contains the cations Na and K and the anions Cl and S (Tucker et al., 1992).

$$\text{DCAD} = (\text{Na} + \text{K}) - (\text{Cl} + \text{S}) \text{ (mEq/kg DM)}$$

Urinary minerals (Ca, Mg, K) are expressed as ratios to creatinine concentration (Creat) to overcome variations in urine volume among animals (Dalley, 1994).

Statistical Analysis

All data were analyzed using the statistical procedures in Genstat 5.4.1 (Genstat V, 1997) software. All data pertaining to animal measurements were analyzed using cows as the experimental unit. Measurements related to pasture mass were analyzed using daily grazing area as the experimental unit, and feed data were analyzed using the fortnightly bulked sample as the experimental unit. The statistical model for experiment 1 included linear and quadratic effects for the four levels of K and a covariate for age. In experiment 2, the statistical model included linear and quadratic effects for four K farmlets, effects for three types of Mg supplement precalving, and two levels of postcalving Ca supplementation and any interactions. The model also included a covariate for age.

RESULTS

Experiment 1

Mean pasture K concentrations increased linearly ($P < 0.001$) with increasing rate of K fertilizer application (Table 1). The DCAD decreased linearly ($P = 0.06$) with increasing amount of K fertilizer applied, in association with a linear decrease ($P < 0.001$) in pasture Na concentration and an increase ($P < 0.001$) in pasture Cl concentration. The concentration of Ca in pasture also declined linearly ($P < 0.05$) with increasing application of K fertilizer, but pasture Mg concentration was unaffected.

Specific relationships were apparent between pasture K concentration and other pasture minerals. The concentration of Cl in pasture was positively correlated with K concentration in pasture ($P < 0.05$; $r^2 = 0.12$, $Cl = 0.188K + 0.88$), and there was a negative relationship between pasture K concentration and both pasture Ca ($P < 0.01$; $r^2 = 0.14$, $Ca = -0.047K + 0.60$) and pasture Na concentration ($P < 0.001$; $r^2 = 0.38$, $Na = -0.156K + 0.91$). Despite the reported linear decline in mean pasture DCAD as pasture K concentration increased, there was a positive relationship between pasture K concentration and DCAD ($P < 0.001$; $r^2 = 0.35$, $DCAD = 129.8K - 35$).

Plasma Ca concentrations at calving and the day following calving were unaffected by pasture K concentrations but increased linearly ($P < 0.05$) 2 d postcalving with increasing K concentration in the diet (Table 2). Approximately 3% of cows had plasma calcium concentrations < 1.4 mmol/L and the incidence of subclinical hypocalcaemia was 40%. The incidence did not differ between treatments. Plasma concentrations of Mg on the day of calving or d 1 and 2 postcalving were not affected by dietary K concentration. Plasma K concentration was unaffected by pasture K concentration.

There was a small but significant linear decline ($P < 0.05$) in urine pH (0.15 pH unit) as dietary K concentration increased and DCAD declined (Table 2). The Ca/Creat and K/Creat were unaffected by treatment, but there was a trend ($P < 0.10$) towards a decline in Mg/Creat as pasture K concentration increased. There was a strong positive relationship between Mg/Creat and Ca/Creat ($P < 0.001$; $r^2 = 0.67$, $Ca/Creat = 0.20$ Mg/Creat + 0.08).

Precalving group DMI were not affected by K farmlet and averaged 9.1 ± 0.8 kg DM/cow per d (1.8% BW). Average yield of milk, milk components, and milk composition were unaffected by treatment (Table 3). Mineral concentration of milk was similarly unaffected by precalving dietary K concentration.

Experiment 2

Mean pasture K concentrations and DCAD increased linearly ($P < 0.001$ and 0.05, respectively) with increasing rate of K fertilizer application. This was associated with a linear decrease ($P < 0.01$) in pasture Na concentration (Table 1). The concentrations of Ca, Mg, and S in pasture were not significantly affected by K fertilizer application.

Although pasture Cl concentration measured in this experiment was not significantly affected by the amount K fertilizer applied ($P = 0.17$), the concentration of Cl in pasture was positively correlated with K concentration ($P < 0.05$; $r^2 = 0.08$, $Cl = 0.146K + 1.19$). There was also a positive relationship between pasture K concentration and DCAD ($P < 0.001$; $r^2 = 0.61$, $DCAD = 14.69K - 15.37$). There was a negative relationship between pasture K concentration and pasture S concentration ($P < 0.001$; $r^2 = 0.18$, $S = -0.033K + 0.24$) and pasture Na concentration ($P < 0.01$; $r^2 = 0.13$, $Na = -0.110K + 0.77$). The relationship between pasture Ca concentration and pasture K concentration was not strong in this experiment.

Plasma Ca concentrations at calving and the day following calving were unaffected by pasture K concentrations but increased linearly ($P < 0.05$) on the second day postcalving with increasing K concentration in the diet (Table 4). Plasma concentrations of Mg increased linearly with increasing dietary K concentration on d 2 postcalving but were otherwise unaffected. Plasma NEFA concentration was unaffected by pasture K concentration. Urine pH was unaffected by DCAD or dietary K concentration either pre- or postcalving. The Ca/Creat increased ($P < 0.05$) linearly with decreasing DCAD and dietary K concentration precalving but was unaffected postcalving. The Mg/Creat ratio was unaffected by dietary K concentration, either pre- or postcalving. There was a strong relationship between Mg/

Table 1. Nutritive characteristics (% of DM) of pasture diets offered to cows on four farms (K1, K2, K3, and K4) differing in pasture K concentration (Experiments 1 and 2).

	K1	K2	K3	K4	SED	Trt	Linear
	<hr/>						<hr/>
	<i>P</i>						<hr/>
Experiment 1							
CP	26.4	26.4	25.4	23.8	1.52	0.31	0.09
NDF	45.0	43.4	44.3	46.9	2.00	0.36	0.31
ADF	27.0	26.8	27.6	27.2	1.44	0.95	0.78
NSC	4.1	3.9	3.7	4.6	1.62	0.95	0.75
Fat	4.1	3.7	3.4	3.8	0.30	0.22	0.28
Ash	13.8	13.8	13.6	12.6	0.68	0.26	0.10
NE _L ¹	1.65	1.66	1.66	1.63	0.014	0.18	0.12
K	3.52	3.76	4.12	4.22	0.215	0.008	<0.001
Ca	0.44	0.43	0.40	0.39	0.029	0.16	0.03
Mg	0.23	0.25	0.23	0.26	0.025	0.62	0.44
Na	0.44	0.30	0.25	0.23	0.050	<0.001	<0.001
Cl	1.26	1.50	1.79	1.90	0.082	<0.001	<0.001
S	0.33	0.32	0.33	0.33	0.009	0.89	0.52
DCAD ²	535	466	453	434	51.700	0.24	0.06
Experiment 2							
CP	25.6	26.2	25.8	25.0	1.19	0.81	0.57
NDF	45.3	45.2	44.3	46.0	1.02	0.43	0.77
ADF	23.2	23.3	22.4	23.2	0.78	0.69	0.81
NSC	5.9	5.4	6.3	5.7	1.11	0.86	0.91
Fat	4.6	4.6	4.8	4.6	0.18	0.81	0.95
Ash	11.8	12.1	11.9	11.7	0.31	0.65	0.77
NE _L ¹	1.73	1.70	1.70	1.69	0.026	0.62	0.23
K	3.32	3.51	3.74	3.83	0.148	0.004	<0.001
Ca	0.40	0.43	0.39	0.40	0.019	0.23	0.62
Mg	0.25	0.24	0.24	0.24	0.013	0.48	0.15
Na	0.44	0.39	0.32	0.33	0.047	0.04	0.008
Cl	1.67	1.71	1.70	1.79	0.082	0.49	0.17
S	0.36	0.37	0.36	0.35	0.012	0.28	0.32
DCAD ²	350	353	392	403	29.600	0.19	0.04

¹NE_L = Net Energy of Lactation (Mcal/kg DM).²DCAD = Dietary Cation-Anion Difference (mEq/kg DM).**Table 2.** Postcalving plasma Ca, Mg and K concentrations, mean urine pH and mean ratio of Ca, Mg and K to creatinine in urine of cows offered pasture from one of four farms (K1, K2, K3, and K4) differing in pasture K concentration (Experiment 1).

	K1	K2	K3	K4	SED	Trt	Linear
	<hr/>						<hr/>
	<i>P</i>						<hr/>
Plasma, mmol/l							
Calcium							
Calving	2.06	2.02	2.03	1.97	0.119	0.77	0.72
Day 1	2.08	2.15	2.18	2.14	0.116	0.87	0.58
Day 2	2.12	2.19	2.29	2.33	0.099	0.19	0.03
Magnesium							
Calving	0.71	0.67	0.73	0.65	0.060	0.74	0.91
Day 1	0.72	0.65	0.69	0.62	0.059	0.42	0.20
Day 2	0.68	0.63	0.66	0.58	0.062	0.44	0.18
Potassium							
Calving	4.74	4.83	4.76	4.59	0.185	0.59	0.16
Day 1	4.59	4.81	4.97	4.75	0.199	0.35	0.35
Day 2	4.65	4.68	4.69	4.64	0.216	1.00	0.98
Urine							
pH	8.07	7.96	8.00	7.92	0.059	0.06	0.02
Ca/Creat ¹	0.21	0.25	0.20	0.18	0.052	0.60	0.37
Mg/Creat ¹	0.68	0.82	0.53	0.58	0.111	0.05	0.10
K/Creat ¹	80.3	85.8	78.4	81.2	10.40	0.91	0.89

¹Creat = corrected for urinary volume using creatinine.

Table 3. Mean milk yield, milk component yield, milk component concentration and milk mineral concentration of dairy cows receiving pasture from one of four farms (K1, K2, K3, and K4) differing in pasture K concentration (Experiment 1).

Treatment	K1	K2	K3	K4	SED	Trt	Linear
Mean Yield, kg/cow/d						P	
Milk	22.4	22.2	20.1	22.2	1.93	0.57	0.66
Fat	1.04	1.03	0.91	1.01	0.089	0.40	0.53
Protein	0.78	0.76	0.69	0.75	0.066	0.48	0.49
Composition, g/kg							
Fat	46.2	46.7	45.0	55.6	1.64	0.83	0.36
Protein	34.7	34.3	34.6	33.9	0.74	0.74	0.26
Minerals, g/l							
Mg	0.11	0.11	0.12	0.11	0.008	0.24	0.51
Ca	1.23	1.27	1.29	1.24	0.070	0.91	0.87
Na	0.37	0.37	0.45	0.41	0.052	0.52	0.33
K	1.54	1.51	1.50	1.53	0.046	0.74	0.62

Creat and Ca/Creat precalving, ($P < 0.05$; $r^2 = 0.85$, Ca/Creat = $0.17 \text{ Mg/Creat} + 0.09$), on the day of calving ($P < 0.001$; $r^2 = 0.61$, Ca/Creat = $0.04 \text{ Mg/Creat} + 0.03$), and during the first 4 d postcalving ($P < 0.001$; $r^2 = 0.62$, Ca/Creat = $0.19 \text{ Mg/Creat} - 0.03$).

Precalving Mg source did not significantly affect the mean concentration of Ca, Mg, or NEFA in plasma on the day of calving. However, the incidence of clinical hypocalcaemia (plasma Ca concentration $< 1.4 \text{ mmol/L}$)

on the day of calving was lower in cows supplemented with MgSO_4 and MgCl_2 precalving (0 and 4%, respectively) than in cows offered MgO precalving (15%). The incidence of subclinical hypocalcaemia (plasma Ca concentration $< 2.0 \text{ mmol/L}$) was not different between treatments (35, 37.5, and 40% for MgSO_4 , MgO and MgCl_2 , respectively). Plasma Ca concentration d 1 postcalving was greater ($P < 0.001$) in cows supplemented with MgSO_4 or MgCl_2 precalving compared with those

Table 4. Plasma Ca, Mg, and NEFA concentration (mmol/L), mean urine pH, and mean ratio of Ca and Mg to creatinine in urine of cows offered pasture from one of four farms (K1, K2, K3, and K4) differing in pasture K concentration, either MgSO_4 , MgO, or MgCl_2 precalving, or one of two dietary Ca concentrations postcalving (Experiment 2).

	K				Mg			Ca		SED			P			
	K1	K2	K3	K4	MgCl_2	MgO	MgSO_4	Yes	No	K	Mg	Ca	K (trt)	K (linear)	Mg	Ca
Plasma Ca																
Calving	1.97	1.97	2.17	1.92	2.03	1.98	2.04	2.05	1.99	0.122	0.110	0.094	0.16	0.75	0.85	0.53
Day 1	2.19	2.18	2.10	2.12	2.21	1.98	2.27	2.25	2.08	0.107	0.077	0.065	0.79	0.42	<0.001	0.02
Day 2	1.97	2.26	2.20	2.23	2.12	2.09	2.27	2.23	2.11	0.095	0.084	0.071	0.01	0.02	0.09	0.10
Day 3	2.11	2.23	2.24	2.23	2.2	2.16	2.25	2.21	2.19	0.076	0.066	0.056	0.30	0.13	0.43	0.73
Day 4	2.25	2.37	2.27	2.30	2.32	2.22	2.37	2.28	2.31	0.069	0.057	0.049	0.31	0.85	0.04	0.51
Plasma Mg																
Calving	0.80	0.79	0.94	0.85	0.79	0.93	0.82	0.79	0.88	0.120	0.101	0.086	0.53	0.39	0.36	0.36
Day 1	0.65	0.68	0.88	0.79	0.72	0.8	0.74	0.62	0.84	0.096	0.078	0.067	0.07	0.04	0.57	0.002
Day 2	0.62	0.60	0.65	0.65	0.62	0.66	0.61	0.56	0.67	0.061	0.490	0.041	0.85	0.53	0.47	0.01
Day 3	0.69	0.54	0.64	0.61	0.67	0.61	0.58	0.55	0.67	0.103	0.087	0.074	0.54	0.64	0.59	0.13
Day 4	0.57	0.54	0.61	0.65	0.58	0.61	0.58	0.58	0.60	0.062	0.053	0.045	0.35	0.15	0.89	0.69
Plasma NEFA																
Calving	0.79	0.72	0.57	0.95	0.71	0.74	0.79	0.63	0.82	0.167	0.146	0.124	0.16	0.65	0.85	0.15
Day 1	0.52	0.51	0.51	0.68	0.55	0.61	0.49	0.51	0.57	0.112	0.096	0.082	0.38	0.22	0.52	0.47
Day 2	0.61	0.63	0.60	0.54	0.7	0.6	0.48	0.56	0.61	0.092	0.067	0.058	0.78	0.43	0.01	0.38
Day 3	0.69	0.57	0.59	0.66	0.67	0.63	0.59	0.66	0.61	0.098	0.085	0.072	0.53	0.75	0.63	0.47
Day 4	0.63	0.78	0.61	0.81	0.81	0.67	0.63	0.67	0.72	0.107	0.093	0.090	0.14	0.32	0.16	0.50
Precalving Urine																
pH	7.69	7.66	7.63	7.65	7.41	7.82	7.71	—	—	0.106	0.089	—	0.94	0.68	<0.001	—
Ca/Creat ¹	0.34	0.26	0.17	0.18	0.27	0.21	0.21	—	—	0.080	0.062	—	0.12	0.03	0.57	—
Mg/Creat ¹	0.88	0.62	0.92	0.71	0.76	0.75	0.84	—	—	0.124	0.107	—	0.04	0.71	0.64	—
Postcalving Urine																
pH	7.99	8.03	8.05	8.10	7.95	8.07	8.09	8.1	8.0	0.059	0.085	0.065	0.68	0.23	0.25	0.14
Ca/Creat ¹	0.07	0.12	0.09	0.09	0.07	0.07	0.15	0.14	0.06	0.071	0.055	0.046	0.89	0.28	0.22	0.09
Mg/Creat ¹	0.53	0.57	0.80	0.71	0.37	0.58	0.72	0.62	0.68	0.204	0.170	0.142	0.49	0.58	0.70	0.67

¹Creat = corrected for urinary volume using creatinine.

Table 5. Mean milk yield, milk component yield, and milk component concentration of cows offered pasture from one of four farms (K1, K2, K3, and K4) differing in pasture K concentration, either either MgSO₄, MgO, or MgCl₂ precalving, and one of 2 dietary Ca concentrations postcalving (Experiment 2).

	K				Mg			Ca		SED			P			
	K1	K2	K3	K4	MgCl ₂	MgO	MgSO ₄	Yes	No	K	Mg	Ca	K (trt)	K (linear)	Mg	Ca
Yield, kg/cow/d																
Milk	18.7	17.8	17.1	17.9	17.0	18.2	18.6	17.4	18.3	1.42	1.21	1.02	0.74	0.45	0.43	0.41
Fat	0.89	0.89	0.75	0.88	0.81	0.86	0.90	0.8	0.89	0.073	0.064	0.054	0.11	0.33	0.40	0.09
Protein	0.71	0.68	0.63	0.67	0.62	0.68	0.72	0.66	0.68	0.054	0.046	0.039	0.53	0.28	0.12	0.61
Lactose	0.91	0.86	0.84	0.88	0.82	0.89	0.91	0.85	0.89	0.730	0.061	0.052	0.81	0.55	0.37	0.37
Composition, %																
Fat	4.79	4.97	4.36	4.99	4.8	4.71	7.84	4.62	4.88	0.230	0.215	0.182	0.03	0.85	0.86	0.15
Protein	3.81	3.85	3.68	3.75	3.62	3.77	3.88	3.83	3.71	0.111	0.088	0.075	0.40	0.33	0.02	0.13
Lactose	4.87	4.83	4.88	4.87	4.83	4.87	4.89	4.85	4.87	0.620	0.055	0.046	0.86	0.73	0.51	0.57

who received MgO. Cows that received MgSO₄ compared with MgO precalving also had greater ($P < 0.05$) plasma Ca concentrations on d 2 and 4 postcalving and greater ($P = 0.06$) concentrations on d 2 postcalving than cows that received MgCl₂ precalving.

Plasma NEFA concentrations were consistently higher in cows receiving MgCl₂ precalving compared with MgSO₄ and the difference was significant ($P < 0.01$) on d 2 and approached significance ($P = 0.06$) on d 4 postcalving (mean concentrations of NEFA in plasma for the 4 d following calving were 0.55, 0.63, and 0.68 for cows supplemented with MgSO₄, MgO, and MgCl₂, respectively, before calving).

Supplementation of Ca postcalving, in the form of CaCO₃, increased plasma Ca concentration on d 1 ($P < 0.02$) and 2 ($P < 0.1$) postcalving. Continued supplementation on d 3 and 4 did not affect the concentration of Ca in blood. Plasma Mg concentration was inversely related to plasma Ca concentration on d 1 ($P < 0.001$; $r^2 = 0.26$, Plasma Mg = -0.45 Plasma Ca + 1.72) and 2 ($P < 0.05$; $r^2 = 0.10$, Plasma Mg = -0.19 Plasma Ca + 1.03) postcalving but there was a trend towards a positive relationship on d 3 postcalving ($P < 0.1$; $r^2 = 0.05$, Plasma Mg = 0.31 Plasma Ca - 0.05). Plasma NEFA was not affected by Ca supplementation.

Urine pH was lower ($P = 0.001$) precalving in cows receiving MgCl₂ compared with either MgSO₄ or MgO, which did not differ from each other. Urine mineral-to-creatinine ratio was not affected by precalving Mg source, either pre- or postcalving. There was a trend ($P < 0.1$) towards increased urinary output of Ca postcalving in cows supplemented with Ca postcalving.

Average yield of milk, milk components, and milk composition were unaffected by pasture K concentration, precalving Mg source, or postcalving supplementation of Ca (Table 5). Group DMI of precalving cows was not affected by pasture K concentration and averaged 7.4 ± 2.1 kg DM/c/per d (1.5% of BW).

DISCUSSION

The mineral contents of pastures eaten by cows in these experiments (Table 1) are within the ranges reported by Whitehead (1966; 1995) and Roche et al. (2000) for temperate pasture. Potassium fertilizer treatment resulted in different concentrations of macrominerals in pasture and a different pasture DCAD on different farmlets.

Grazed pasture is the major dietary component of dairy cows in many parts of the world and, in these situations is, therefore, the key determinant of the DCAD. Roche et al. (2000) reported that for a considerable part of spring and early summer the DCAD of pasture was in excess of +500 mEq/kg DM. A review of pasture mineral data in southeastern Australia, undertaken in 1997 to 1998 to examine the annual geographical range in pasture mineral concentrations (Jacobs and Rigby, 1999), supports these results. Thus, the DCAD offered to seasonal spring-calving dairy cows precalving on pasture-based diets is outside the range (<0 mEq/kg DM) that results in increased Ca absorption (Joyce et al., 1997; Roche, 1999; Goff, 2000).

The high pasture DCAD can mostly be attributed to a high K concentration. The other ions are present in relatively low amounts and are, therefore, not as influential, even though Cl concentration in temperate pasture is often higher than in other forages (up to 2.17%; Roche, 1999). The linear increase in pasture K concentration observed in both experiment 1 and 2 is a predictable response as the amount of K fertilizer applied is increased (Whitehead, 1995; Roche et al., 2000). As K is the major ion determining the DCAD of pasture, an increase in DCAD would be expected with increasing K concentration as was observed in experiment 2 and reported by Jacobs and Rigby (1999) and Roche et al. (2000). However, in experiment 1 the DCAD decreased as pasture K concentration increased due to correspond-

ing changes in pasture Na and Cl concentration. Whitehead (1966) and Roche et al. (2000) also reported an uptake of Cl associated with an uptake of K, probably to help maintain a cation-anion balance (Beaton and Sekhon, 1985). However, the difference in herbage K concentration is usually far greater than the difference in pasture Cl concentration (Roche et al., 2000). This was not the case in experiment 1 where a 0.5 percentage unit increase in pasture K concentration (+130 mEq/kg DM) was associated with 0.64 percentage unit increase in pasture Cl concentration (−180 mEq/kg DM). An additional decrease in pasture Na concentration (0.22 percentage units) as K concentration increased, further reducing DCAD. We are unaware of any other documented cases of DCAD declining as pasture K concentration increased, but an increase in pasture Cl and a decrease in pasture Na and Ca when pasture K concentration increases is well documented (Dibb and Thompson, 1985; Roche et al., 2000). Furthermore, published DCADs in grazing systems are scarce. The contradictory results in pasture DCAD in experiments 1 and 2 highlight the difficulty in consistently manipulating the DCAD for grazing animals.

Goff and Horst (1997) reported a significant increase in the incidence of milk fever and subclinical hypocalcaemia as dietary K concentration increased from 1.1 to 3.1%. Pasture K concentrations and DCAD in both experiment 1 and 2 were similar to the high dietary K concentrations used by Goff and Horst (1997), but the incidence of clinical (<1.4 mmol/L) and subclinical (<2.0 mmol/L) hypocalcaemia were much lower in the experiments reported here. The results reported by Goff and Horst (1997) may be partly due to the extreme population of animals used (older jersey cows). The incidences reported here are supported by Caple (1987), Daniel et al. (1990), and McDougall (2001) suggesting that dietary K concentration may not be as influential in Ca homeostasis in pasture-based systems or in herds with a normal age structure. This is supported by the linear increase in plasma Ca concentration on d 2 postcalving in both experiments as pasture K concentration increased.

Reducing DCAD from +400 to +350 mEq/kg DM in experiment 2 linearly increased ($P < 0.05$) Ca/Creat, indicating either increased intestinal absorption or bone resorption or a reduced renal reabsorption even though plasma Ca concentration was unaffected. Such an effect was unexpected because such a small decrease in DCAD did not affect systemic pH (as measured by urine pH) and would not be expected to influence Ca homeostasis (Roche, 1999; Underwood and Suttle, 1999; Goff, 2000). A larger change in DCAD in experiment 1 resulted in no such change in Ca/Creat, even though there was a linear decline in urine pH.

In experiment 2, supplementing cows with either 150 g MgCl₂ or 200 g MgSO₄ instead of 35 g MgO (isomagnesemic) significantly increased plasma Ca concentration in the immediate postpartum period and reduced the incidence of clinical hypocalcaemia (<1.4 mmol/L) on the day of calving (0, 4, and 15% for MgSO₄, MgCl₂, and MgO, respectively). The lack of a difference in periparturient plasma Mg concentration and Mg/Creat suggest that there was no difference in Mg absorption between the different sources (considering feces is not thought to be a significant route for excretion of Mg; Underwood and Suttle, 1999). A difference in absorbable Mg precalving is, therefore, unlikely to be the reason for the increased plasma Ca concentration observed postcalving. This suggests an effect of Cl and S on Ca homeostasis above that of Mg alone. The effect of Cl and S on acid-base balance and Ca absorption and excretion has been reported by several authors (Vagg and Payne, 1970; Schonewille et al., 1994; Roche, 1999; Goff, 2000). It is difficult to determine the true effects of Cl and S as many of these studies used Mg-based salts without accounting for the increase in dietary Mg (Dishington, 1975; Block, 1984; Joyce et al., 1997; Moore et al., 2000). The strong positive relationship between Mg/Creat and Ca/Creat in experiment 1 and 2 infers that Mg plays an important role in maintaining Ca homeostasis. This idea is supported by Sansom and Manston (1983) and Goff (2000).

Some authors (Oetzel et al., 1988) have used salts containing Cl and S but not Mg (ammonium-based salts) to reduce DCAD to −70 mEq/kg and yet observed similar benefits in reduced hypocalcaemia (13%) to those measured in experiment 2. However, the precalving supplement of MgCl₂ and MgSO₄ administered in experiment 2 would only reduce DCAD by 1.4 and 1.6 equivalents/cow per d, respectively (assuming a 100% absorption and a common acidity for both Cl and S). This would result in a final DCAD of approximately +140 to +200 mEq/kg DM, far higher than the −70 mEq/kg DM used by Oetzel et al (1988). Roche (1999) reported no decrease in either blood or urine pH and no increase in urine Ca concentration (an indicator of increased Ca absorption) unless DCAD was −150 mEq/kg DM. Goff (2000) recommended a similar value (−50 mEq/kg DM). Although the DCAD was reduced in experiment 2 by supplementing cows with MgCl₂ and MgSO₄, levels of +140 to +200 mEq/kg DM should not be sufficient to reduce systemic pH and increase Ca absorption or resorption. The lack of a difference in Ca/Creat before and after calving supports this, as does the lack of a difference in urine pH in cows supplemented with either MgSO₄ or MgO. The difference in urine pH between cows supplemented with MgCl₂ and MgSO₄, indicating less of an effect of MgSO₄ on acid-

base balance, also suggests an effect of treatment unrelated to DCAD. The extent of the decline in urine pH (7.82 to 7.41) in cows receiving MgCl_2 precalving is too small to suggest a change in blood pH (Roche, 1999; Goff, 2000), further supporting the idea that a reduction in blood pH through a reduced DCAD was not the reason for the improved periparturient Ca homeostasis observed in this experiment.

Studies from Australia (McNeill et al., 2002) have also reported reduced hypocalcaemia when anionic salts have not been supplemented in sufficient quantities to change systemic pH. The reason for the reduced incidence of hypocalcaemia in the Australian studies and in experiment 2 of the present study is unclear. Similarly, the consistently higher ($P = 0.08$) plasma Ca concentration in cows supplemented with MgSO_4 , as opposed to MgCl_2 , in experiment 2 does not fit with current understanding of Ca homeostasis. The absorption of S is known to be lower than Cl (Underwood and Suttle, 1999; NRC, 2001). Tucker et al. (1992) maintained that S should be included in the formula to calculate the potential acidogenic nature of the diet, but also claimed that the S component of the equation would need modification when the relative acidifying properties of Cl and S were more fully understood. This has led many authors (Oetzel et al., 1988; Goff, 2000) to attempt to place a weighting on the relative acidogenic effect of these two anions. Goff (2000) maintained that 0.25 was a suitable weighting for S, suggesting that S is only 25% as effective as Cl in reducing blood pH and, therefore, in preventing hypocalcaemia. Although this weighting may be appropriate from an absorption and acidogenic point of view, results from experiment 2 question the appropriateness of this weighting for hypocalcaemia prevention. Experiment 2 shows 26 g of S to have a greater effect on Ca homeostasis than 52 g of Cl or 50 g of K.

The apparently important role of S in hypocalcaemia was also identified by Oetzel (1991) who reported that dietary S concentration was the most important predictor of milk fever risk and was far more important than either dietary Cl or K. Enevoldsen (1993) reanalyzed Oetzel's dataset and agreed that sufficient information was present to suggest a strong link between dietary S concentration and the risk of milk fever and claimed that dietary Na, K, or Cl concentration and DCAD were poorly related to milk fever incidence.

These results question the currently accepted understanding of the processes involving DCAD and hypocalcaemia. Although the effects of a systemic acidosis on Ca absorption are now largely beyond dispute, the effect of dietary S on periparturient Ca homeostasis when absorption of S is low in comparison to Cl, Na, or K suggest that there are mechanisms involved that are

not related to acid-base balance. Further research is required to determine the effect of S on Ca homeostasis and the mechanism by which it works.

There was no significant effect of precalving DCAD or Mg source on the production of milk or milk components. This is supported by the milk production results of Roche (1999) but differs from the findings of Block (1984) and Beede et al. (1992), who reported increases in milk production of approximately 14 and 7% by preventing clinical and subclinical hypocalcaemia, respectively. However, the milk production of cows in the studies reported by Block (1984) and Beede et al. (1992) was substantially greater than the grazing cows reported here. It is possible that the negative milk production effects of hypocalcaemia are greater in high-yielding cows than low-yielding cows.

Supplementation of cows with Ca postcalving corrected, in part, the negative Ca balance created by the onset of lactation as reported by Roche (1999). However, unlike Roche (1999), who measured increased plasma Ca concentrations for 2 wk postcalving, plasma Ca in experiment 2 was only elevated for 2 d postcalving. This suggests that the critical time for Ca supplementation on pasture-based diets is during the 2 d immediately following calving. Although Ca supplementation postcalving increased plasma Ca concentration, it did not affect milk production. The milk production results presented by Roche (1999) support this conclusion.

CONCLUSIONS

Application of K fertilizer resulted in a DCAD ranging from 350 to 535 mEq/kg DM, but Ca homeostasis in dairy cows was not changed. Plasma Ca concentrations were increased, and the risk of clinical periparturient hypocalcaemia was reduced by MgCl_2 and MgSO_4 . However, improvements in Ca homeostasis were not the result of an altered systemic pH. Dietary S concentration was more important in the control of hypocalcaemia than either dietary K or Cl concentration. The mechanism by which acidogenic salts, and in particular SO_4^{2-} salts, influence Ca homeostasis requires further investigation.

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