Carryover effects of potassium supplementation on calcium homeostasis in dairy cows at parturition

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ABSTRACT

The purpose of this study was to test whether supplementation with K improves bone mineral density (BMD) in older cows so that by parturition their bone is better able to mobilize Ca. Twenty-four Holstein Friesian cows (6 mo pregnant, lactating, and in their third or later lactation) were allocated to 2 equal groups and individually fed twice daily a total diet comprising low K oaten hay plus a pelleted concentrate fortified with or without K₂CO₃ to achieve 3.12% K/kg of DM in the total diet of the K-supplemented (KS) cows compared with 1.50% K/kg of DM for the control cows. The cows were fed their respective diets from the beginning of their sixth month of pregnancy until 2 wk before the expected date of parturition. The strategy was to use K to stimulate a mild increase in extracellular pH to potentially improve BMD well before parturition, when high K contents in the diet are considered safe, but cease supplementing in the few weeks prepartum, when high intakes of K are known to be problematic. The expectation was that the effect of the denser bone would carry through to benefit the cow's plasma Ca, P, and Mg status at parturition. Prior to the period of K supplementation, the cows were part of a commercial pasture-based herd, to which they were returned at the end of the supplementation period and treated as 1 group from at least 11 d prepartum until the end of the study at d 42 of the next lactation. Supplementation with K successfully induced a sustained increase of urinary pH throughout late lactation and into the dry period, as expected. The KS cows consistently averaged a urine pH 0.25 ± 0.10 U higher than the controls. However, there was no significant effect of K supplementation on BMD, bone mineral concentrations, plasma osteocalcin, urinary deoxypyridinoline:creatinine plasma Ca, or

plasma P concentrations during or immediately after the cessation of supplementation, nor where there any carryover effects during parturition or by d 42 of lactation. Instead, there was an unexpected decrease in the concentration of Mg in plasma of the KS cows compared with the control cows that extended from 0.5 to 2.5 d postpartum. The timing of the decline in plasma Mg was paralleled by declines in plasma concentrations of 1,25 dihydroxy-vitamin D₃ and urinary excretion of Ca and Mg, whereas urinary excretion of P increased; all changes were consistent with a hypomagnesemia that could increase the risk of hypocalcemia. These data suggest that, in addition to the well-documented negative effects of K when fed immediately at parturition, the effects of high dietary K diets can carry over for at least 11 d to trigger a mild hypomagnesemia at parturition. Because K supplementation did not improve BMD prepartum, it was not possible to conclude for or against an ability of denser bone to reduce the risk of hypocalcemia in older cows at parturition.

Key words: bone health, parturition, hypocalcemia, hypomagnesemia

INTRODUCTION

Diets rich in K are strongly implicated as a cause of periparturient hypocalcemia in the dairy cow, and the period of greatest risk is in the days immediately prepartum (Goff and Horst, 1997; Lean et al., 2006; Roche and Berry, 2006; DeGaris and Lean, 2008; Goff, 2008). Two hypotheses relate high K intakes to hypocalcemia at this time. High K diets can 1) slightly alkalinize the extracellular fluid of the cow, which in turn reduces the rate of synthesis of the hormone 1,25 dihydroxy-vitamin D₃ (**1,25 OH vit D₃**) by the kidney, needed to drive the absorption of Ca from the small intestine, and 2) reduce the rate of absorption of Mg from the rumen, leading to a hypomagnesemia, which in turn reduces the sensitivity of kidney and bone tissue to parathyroid hormone (**PTH**). Parathyroid hormone would normally stimulate the synthesis of 1,25 OH vit D₃ and, consequently, Ca absorption, as well as promote the mobilization of bone Ca to improve the flow of Ca into the blood stream.

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Yet, beyond the immediate prepartum period, the risk of a high K diet causing metabolic problems is thought to recede and may even be advantageous (Sanchez and Beede, 1996). Studies in humans of osteoporosis indicate that diets rich in K can improve bone tissue in terms of bone mineral density (BMD) and turnover (Sebastian et al., 1994; New et al., 1997, 2000; Tucker et al., 1999; New, 2000; Jehle et al., 2006). The proposed mechanism linking dietary K to BMD is that higher K diets can lead to a mild increase, within physiological limits, in the pH and alkalinity of extracellular fluid, which in turn could improve BMD (McNeill et al., 2002). Just as the DCAD literature argues that acidifying the diet stimulates the mobilization of bone mineral (Goff, 2006, 2008; DeGaris and Lean, 2008), alkalinizing the diet could stimulate bone mineral accretion. Jehle et al. (2006) have recently confirmed this in osteopenic women supplemented with an alkalinizing (potassium citrate) compared with a nonalkalinizing (KCl) form of K over a 12-mo period.

High K diets that successfully alkalinize metabolism could similarly improve BMD in dairy cows. That is, the increased turnover of bone Ca, P, or Mg to be expected from denser bones, if coinciding with parturition, could improve Ca homeostasis at that critical time. Studies in both cattle and sheep indicate dramatic challenges to BMD from one parturition to the next, as reviewed by McNeill et al. (2002). Bone Ca reserves can fluctuate by as much as 10 to 15%, falling throughout early lactation and replenishing in late lactation, and likely result in a general declining trend in BMD over the lifetime of the cow (Kronfeld et al., 1976; Braithwaite, 1983; Gibb et al., 1992). Such reports, while highlighting the natural process of bone loss in early lactation, also highlight the need to provide a diet that promotes the repletion of bone tissue in late lactation and into the dry period.

To investigate the potential advantages of high K diets, we tested the hypothesis that an alkalinizing supplement, K_2CO_3 , fed throughout late lactation and into the dry period increases BMD and the capacity of the cow to maintain calcium homeostasis at parturition. Care was taken to cease the addition of K_2CO_3 by 14 d prepartum to remove the risk of any immediate negative effects of K on Ca homeostasis at parturition.

MATERIALS AND METHODS

Experimental Animals, Feeding, and Management

Two groups of cows were matched for number of days pregnant, number of lactations, milk yield of previous lactation, and liveweight in their sixth month (140 d before their expected date of parturition) of their third or later pregnancy, and randomly allocated into either

a control or a K-supplemented (KS) group of 11 animals each. The study was conducted in a year-round calving herd at the University of Sydney's Mayfarm and Corstorphine research dairies in Camden, New South Wales, Australia (150°39'E, 34°3'S). The cows were assembled in 3 batches from July 2003 to January 2004, paired on their predicted date of parturition, and allocated to either group randomly. Final means for the control and KS groups, respectively, at randomization were as follows: number of days pregnant = 152 ± 3 and 157 ± 5 d; number of lactations = 5.0 ± 0.5 and 5.0 ± 0.5 ; average milk yield of previous lactation = 24.6 ± 3.4 and 21.5 ± 2.1 L of milk/d; liveweight = 608 ± 25 and 574 ± 11 kg.

The cows were individually fed a diet of oaten hay plus pelleted concentrate fortified with or without K, as K_2CO_3 , from d -140 until 14 d before the expected date of parturition. The level of K added was designed to reflect that seen in quality pasture. From 14 d prepartum, all cows were moved into a commercial herd where they were offered a hay and concentrate diet until parturition. After parturition, they were offered a pasture-based diet supplemented with a pelleted concentrate until the study ended at 42 d postpartum to test for carryover effects on milk production.

From d -140 to d -14, the cows were kept in a 0.5-ha bare paddock adjacent to the milking shed with constant access to full shade and water. They were walked less than 50 m, twice daily following milking, to be fed individually in head bales in 2 equal portions per day (at 0830 and 1630 h) a combination of a low K (1.7% DM) long oaten hay and a pelleted concentrate (George Weston Foods Ltd., Chatswood, New South Wales, Australia) with or without added K in the form of feed grade K_2CO_3 . Control cows received the basal pellets with a K concentration of 1.3% DM compared with the KS cows, where the K was added to lift the concentration in the pellets to 4.2% DM, resulting in dietary concentrations of K for the entire diets of 3.12% and 1.50 to 1.57% for the KS and control cows, respectively (Table 1). From 140 to 70 d before their expected date of parturition, all cows were offered 9.0 kg of DM of oaten hay and 9.0 kg of DM of pellets per day. After lactation ceased, all were fed 8 kg of DM of oaten hay and 4 kg of DM of pellets per day until 14 d before their expected date of parturition; this timing proved to be, in relation to actual date of parturition (mean $\pm$ SE), 11.7 ± 1.6 d for the control cows and 11.6 ± 1.4 d for the KS cows. During late lactation approximately 500 g/d of K_2CO_3 was mixed with the pelleted portion of the KS diet; this was reduced to 200 g/d for the nonlactating period. The K_2CO_3 was mixed with the KS pellets immediately before each feeding by dissolving it in 200 mL of water and stirring it with

Table 1. Composition of the experimental diets for the periods of K supplementation (DM basis)¹

Item	Late lactation		Early dry period	
	Control	KS	Control	KS
Component (g/kg)				
Oaten hay	500	486	667	656
Pellets	500	486	333	328
K ₂ CO ₃	0	27	0	16
Analysis (% unless noted)				
CP	13.9	13.9	11.7	11.7
ADF	19.1	19.1	22.7	22.4
NDF	36.9	37.0	43.2	42.8
NFC	11.6	11.7	17.4	17.4
Total digestible nutrient	70.6	71.0	69.9	70.0
ME (MJ)	10.8	10.8	10.6	10.6
Ca	0.92	0.90	1.02	1.00
P	0.44	0.42	0.36	0.35
Mg	0.41	0.42	0.41	0.45
K	1.50	3.12	1.57	3.12
Na	0.23	0.21	0.16	0.18
Cl	0.52	0.49	0.61	0.59
S	0.23	0.20	0.17	0.18
DCAD ² (mEq/100 g)	+19	+62	+19	+60

¹Late lactation = from 140 to 70 d before the expected date of parturition; early dry period = from 70 to 14 ± 5 d before the expected date of parturition; control = cows (n = 11) not supplemented with K; KS = cows (n = 11) supplemented with K.

²DCAD, mEq/100 g = (Na %/0.023 + K %/0.039) – (Cl %/0.0355 + S %/0.016).

the pellets until absorbed. The hay and pellets (mixed or not mixed with K₂CO₃) were weighed for each cow into individual bins on the previous day for feeding for the next day.

At 14 d before their expected date of parturition, all cows were transported 3 km to the University of Sydney's Corstorphine commercial dairy, where they were combined with the commercial herd of dry cows and offered, as a group, 1.5 kg of DM of proprietary pellets per day (George Weston Foods Ltd.) and oaten hay ad libitum (Table 1). Actual intakes of the pellets and hay per group or individual cow were not measured from this point onwards because it was considered that the period of treatment to manipulate bone tissue had ceased and that the cows were now into the period of carryover, during which the intention was to mimic commercial conditions for a pasture-based system.

Following parturition, the cows entered the commercial milking herd of approximately 250 cows, which were grazed on a predominately kikuyu (*Pennisetum clandestinum*) pasture offered ad libitum and supplemented with approximately 6 kg of DM of corn silage [analysis on DM basis: CP, 14.2%; ADF, 40.3%; NDF, 63.1%; NFC, 14.2%; total digestible nutrient, 51%; ME, 8.9 MJ/kg (NE_L = 1.12 Mcal/kg); Ca, 0.67%; P, 0.34%; Mg, 0.49%; K, 2.34%; Na, 0.65%; and Cl, 1.84%] and 6 kg of DM of pellets/cow per day. The pellets were from the same batch as those fed from d –14 to parturition and so were considered to have a similar compositional value (presented previously), and were fed at the time

of milking in 2 equal portions per day. The corn silage was offered on a feed pad between the morning and afternoon milking.

Sampling

Prior to d –14, the oaten hay was sampled 3 times per week and the pellets, with or without the added K₂CO₃, were sampled weekly. Feed samples were combined on monthly basis, dried at 60°C for 48 h, ground through a 0.5-mm sieve, and sent for wet chemistry proximate and mineral analysis to Dairy One Forage Laboratory (Ithaca, NY).

Pasture samples were not taken during the period from parturition to d 42 of lactation; it was considered that an approximation of the nutritional profile of the study was all that was needed because the methodology was to impose K treatment before d –14 and then treat all cows similarly thereafter. A typical nutritive profile of the kikuyu pasture that the cows were grazing can be derived from the pasture study of Fulkerson et al. (2007), conducted at the same time on a site adjacent to the paddocks grazed and on the same soil type [analysis on DM basis: CP, 25.3%; ADF, 25.9%; NDF, 50.3%, ME, and 9.9 MJ/kg (NE_L = 1.52 Mcal/kg)]. A typical mineral profile for kikuyu is Ca, 0.30%; P, 0.42%; Mg, 0.28%; K, 3.88%; Na, 0.10%; and Cl, 1.59% (McNeill et al., 2002).

Blood and urine samples were taken immediately before the initiation of K supplementation at 140 d

before the expected date of parturition, and thereafter weekly, between 0800 and 0900 h, during the morning feeding until 14 d prior, and then within 2 to 6 h after parturition, 12 h after that, and at 2, 2.5, and 42 d postpartum. Other than the samples on the day of parturition, all blood samples were taken immediately after the morning milking or before the morning feed, chilled and centrifuged at $1,700 \times g$ for 10 min within 30 min of sampling, and frozen at -20°C for later analysis. Five-milliliter subsamples of urine were taken immediately at sampling into an ice bath and frozen at -20°C for mineral and creatinine analysis.

Bone biopsy samples were collected on 3 occasions: from the thirteenth rib on the right side immediately before the initiation of the treatment, from the thirteenth rib on the left side at 14 d before the expected date of parturition, and from the twelfth rib on the right side at 42 postpartum. The cows were restrained in a cattle crush and sedated with Dormosedan (detomidine HCl, 10 mg/mL; Pfizer Animal Health, New York, NY) at the dose rate of 0.1 mL i.v./100 kg of BW. An area (approximately 20×20 cm) approximately halfway along and centered over the twelfth rib was clipped and scrubbed for surgery. Twenty-five to 30 mL of local anesthetic (lignocaine HCl, 20 mg/mL; Troy Laboratories (Australia) Pty. Ltd., New South Wales, Australia) was applied subcutaneously in multiple injections along the lines of the anterior and dorsal borders of the clipped area to form an inverted L nerve block for the site of incision at the center of the clipped area. A unicortical biopsy was taken using a 14-mm trephine by boring through the lateral cortex and extending into the medullary cavity of the rib until the resistance of the medial cortex was felt, the trephine was withdrawn, and the core was prised from the rib using blunt surgical scissors. A second biopsy sample was taken about 1 to 1.5 cm dorsal to the first biopsy site. One of each pair of biopsy cores was fixed in 10% formalin (pH 7) and the other was fixed in 80% alcohol for subsequent measurement of BMD and composition (Ca, P, and Mg).

Measurements

Liveweight was measured weekly throughout the experimental period on the first day of each week and immediately before the morning feeding at approximately 0830 h. Body condition scores were measured using a 1- to 5-point scale (1 = poor to 5 = good) at the start of the experiment, 14 d before parturition, immediately after parturition, and 42 d after parturition (Edmonson et al., 1989).

Urine pH was measured immediately after sampling using a handheld electronic pH meter (pH-mV-Temp Meter, model WP-80, TPS Pty Ltd., Brisbane, Aus-

tralia) and used to indicate the potential for changes in acid-base balance of the cows throughout the study (Horst et al., 1997; Goff, 2000; Oetzel, 2000; Seifi et al., 2004).

Concentrations of Ca, P, Mg, and K in plasma and urine were analyzed by inductively coupled plasma emission spectrophotometry. Because the total 24-h urine output could not be measured, the ratio of mineral to creatinine concentration was used as an index of excretion (Asai et al., 2005).

Plasma was assayed for the concentration of 1,25 OH vit D₃ (radioimmunoassay kit IDAA-54F2, IDS Laboratories, Abacus Diagnostics, Brisbane, Australia) and the biochemical marker for bone formation, osteocalcin (osteocalcin enzyme immunoassay kit, Nichols Advantage, Nichols Diagnostics, San Juan Capistrano, CA). The monoclonal antiosteocalcin antibody used in the osteocalcin kit was raised against bovine osteocalcin.

Urine was assayed for the concentration of the marker for bone resorption deoxypyridinoline (DPD; pyrilinks-D immunoassay kit, Immulite, Diagnostics Products Corp., Los Angeles, CA) using an Immulite 2000 analyzer; as for the urinary minerals, the ratio of DPD to creatinine concentration was used as an index of excretion.

Bone mineral density in the rib biopsies was measured using a peripheral quantitative computed tomography scanner (Picker International, Cleveland, OH). The biopsies from all sampling times for all cows were scanned in one continuous run. The samples were scanned perpendicularly to the lateral cortex once every 2 mm such that 5 measures were taken for each biopsy and then averaged for a final BMD per biopsy.

The mineral composition of the bone biopsies was measured after drying to a constant weight at 100°C and then grinding to a fine powder with a mortar and pestle. Approximately 100 mg of each homogenized sample was weighed and ashed in a muffle furnace at 550°C for 8 h. The ash was then dissolved into concentrated hydrochloric acid, made up to 100 mL using nanopore deionized water in volumetric flasks, and sent to the Department of Chemical Engineering, University of Sydney, Australia, for analysis by inductively coupled plasma emission spectrophotometry for the concentrations of Ca, P, and Mg.

The feeding, management, surgical procedures and sampling regimens used on the animals in this experiment were approved by the Animal Ethics Committee, University of Sydney.

Calculations and Statistical Analysis

The plasma, urine, and bone measurement, liveweight, and condition score data across the entire trial

Figure 1. Mean urine pH values of the cows in the control (●) and K-supplemented (■) groups from 140 d before the expected date of parturition until 42 d postpartum (SEM is shown as vertical bars).

were analyzed by repeated measures ANOVA using Genstat (v. 8.0, VSN International, Hemel Hempstead, UK). Both the main effects (treatment, time) and the interaction effects (treatment $\times$ time) were examined. When the ANOVA suggested a significant treatment or time effect, means were compared with Fischer's least significant difference test. Daily liveweight and condition score changes were determined using the generalized linear regression component of Genstat for periods d -140 to -70 , d -70 to 0 , d 0 to 2.5 , and d 2.5 to 42 , with d 0 being the expected date of parturition.

RESULTS

Throughout the period of K supplementation, feed refusals were minor ($<1\%$) and therefore not recorded. Consequently, the daily DMI of the KS cows, not including the weight of the added K_2CO_3 , matched that of the control cows until supplementation ceased. Between d -140 and -14 , DMI was 18.0 kg/d for the control cows plus 0.50 kg/d of K_2CO_3 to equal 18.5 kg of DM/d for the KS cows. Between d -70 and -14 , DMI was 12.0 kg/d for the control cows plus 0.20 kg/d of K_2CO_3 to equal 12.2 kg of DM/d for the KS cows. Compared with the control cows, the KS cows consumed 0.31 and 0.19 kg more K/cow per day for periods d -140 to -70 and d -70 to -14 , respectively.

Compared with the targeted 14 d prepartum, the mean actual number of days prepartum that supplementation ceased was 11.7 d for the control cows and 11.6 d for the KS cows [standard error of the difference (SED) = 1.09 d; $P = 0.641$].

Table 2. Liveweight and BCS means for the cows in the control (no K supplementation) versus K-supplemented (KS) groups relative to the expected date of parturition¹

Item	Treatment		SED ²	P-value
	Control	KS		
Liveweight (kg)				
d -140	611.8	589.0	30.36	0.470
d -70	671.6	646.1	32.56	0.443
d -14	735.7	704.8	29.53	0.308
d 0	660.2	618.6	29.82	0.179
d 7	631.6	584.6	25.78	0.084
d 42	584.8	544.6	27.37	0.158
BCS ³ (U)				
d -140	3.0	2.7	0.19	0.246
d -70	NA ⁴	NA	NA	NA
d -14	3.9	3.8	0.14	0.439
d 0	3.2	3.2	0.260	0.900
d 7	NA	NA	NA	NA
d 42	2.0	1.8	0.14	0.203

¹Period of K supplementation for the KS cows was from d -140 to -14 .

²Standard error of the difference.

³On a scale from 1 (poor) to 5 (good).

⁴Not measured at this time.

Throughout the period of K supplementation, the KS cows consistently averaged a urine pH 0.25 U higher than that of the control cows (d -140 to -14 : control = 8.23 U, KS = 8.48 U; SED = 0.034 pH units; $P < 0.001$; Figure 1). Prior to the start of supplementation, the urine pH of all cows averaged 8.36 ± 0.04 U and decreased erratically until the end of the supplementation period, when both groups converged to average 8.21 ± 0.03 U by 14 d before the expected date of parturition (d -14 : control = 8.23 U, KS = 8.24 U; SED = 0.023 pH units; $P = 0.773$). At the beginning and end of the supplementation period, the treatment groups diverged (control) or converged (KS) within a week of the change in the DCAD of their diets. Thereafter, for the last 14 d of the dry period and the first 42 d of lactation, the urine pH of the treatment groups remained similar and steady, staying between 8.2 and 8.3 U.

Cows in both treatment groups gained liveweight and condition score steadily and similarly over the period of K supplementation and until parturition ($P > 0.05$; Tables 2 and 3). Over this period, the liveweight (mean $\pm$ SE) of all cows increased from an average of 581 ± 23 kg at -140 d to 700 ± 20 kg at parturition. Mean BCS of all cows were 2.8 , 3.8 , and 3.2 U at -140 d, -14 , and parturition, respectively. From parturition, mean liveweights and body condition scores of all cows declined by 79 and 59% , respectively, to reach a mean of 556 kg and 1.9 U by d 42 of lactation.

Supplementation with K had no effect on subsequent milk production (mean yield for the period d 0 to 42 : control = 33.4 L, KS = 34.3 L; SED = 1.76 ; $P =$

Table 3. Rate of change in liveweight and BCS for the cows in the control (no K supplementation) versus K-supplemented (KS) groups for key periods relative to the expected date of parturition¹

Item	Treatment		SED ²	P-value
	Control	KS		
Liveweight change (kg/d)				
d -140 to -70	0.81	1.08	0.158	0.109
d -70 to -14	1.14	1.05	0.141	0.502
d -14 to 0	-5.40	-5.50	0.595	0.226
d 0 to 2.5 ³	-2.08	-2.46	0.813	0.639
d 2.5 to 42	-1.34	-1.14	0.527	0.716
BCS change ⁴ (scores/d)				
d -140 to -70	0.0074	0.0082	0.00065	0.245
d -70 to -14	0.013	0.015	0.00116	0.452
d -14 to 0	-0.0464	-0.0407	0.00408	0.656
d 0 to 2.5 ³	-0.0039	-0.0046	0.00011	0.240
d 2.5 to 42	-0.0290	-0.0324	0.00541	0.321

¹Periods of K supplementation for the KS cows were from d -140 to -70 (late lactation) and d -70 to -14 (early dry).

²Standard error of the difference.

³Day 2.5 values were estimated from d 0 and 7 measures of liveweight and BCS.

⁴On a scale from 1 (poor) to 5 (good).

0.719), fat percentage (control = 4.5; KS = 4.7; SED = 0.36; $P = 0.891$), or protein percentage (control = 3.2, KS = 3.3; SED = 0.14; $P = 0.911$). No incidence of milk fever or any other health issues were encountered at parturition or any other stage of the experiment.

Measures of BMD, bone mineral concentrations, and bone turnover were unaffected by K supplementation ($P > 0.05$; Table 4 and 5). However, all showed pat-

Table 4. Bone mineral density and concentration means for biopsy samples taken from the 12th or 13th ribs of the cows in the control (no K supplementation) versus K-supplemented (KS) groups relative to the expected date of parturition¹

Item	Treatment		SED ²	P-value
	Control	KS		
Bone mineral density (g/cm ³)				
d -140	1.68	1.71	0.137	0.834
d -14	1.90	1.91	0.098	0.916
d 42	1.98	2.00	0.109	0.815
Bone Ca (mmol/g)				
d -140	3.68	3.58	0.402	0.133
d -14	3.65	3.84	0.363	0.130
d 42	4.14	4.51	0.258	0.160
Bone P (mmol/g)				
d -140	1.23	1.21	0.102	0.110
d -14	1.32	1.35	0.088	0.120
d 42	1.58	1.31	0.080	0.130
Bone Mg (mmol/g)				
d -140	0.10	0.11	0.009	0.165
d -14	0.09	0.09	0.003	0.301
d 42	0.11	0.11	0.007	0.380

¹Period of K supplementation for the KS cows was from d -140 to -14.

²Standard error of the difference.

terns of change over time. The density of bone in the rib biopsies increased by 12% from 140 d to 14 d before the expected date of parturition (d -140 = 1.70, d -14 = 1.91; SED = 0.118 g/cm³; $P = 0.002$), but there was no detectable change thereafter (d -14 = 1.91, d 42 = 1.99; SED = 0.056 g/cm³; $P = 0.776$; Table 4). Concentrations of Ca, P, and Mg in the rib biopsies increased by 19, 19, and 8%, respectively, between 140 d before the expected date of parturition and 42 d after (Ca: d -140 = 3.64, d 42 = 4.33, SED = 0.115 mmol/g, $P = 0.002$; P: d -140 = 1.22, d 42 = 1.45, SED = 0.036 mmol/g, $P < 0.001$; Mg: d -140 = 0.10, d 42 = 0.11, SED = 0.003 mmol/g, $P = 0.002$; Table 4). Both plasma osteocalcin and urinary deoxypyridinoline excretion ratios declined as the cow approached parturition (Table 5). The decline was particularly precipitous for osteocalcin for the 14 d to expected parturition and was lowest on the day of parturition. Both osteocalcin and deoxypyridinoline measures increased at similar rates thereafter. By 42 d after parturition, plasma osteocalcin had returned to levels similar to those recorded at 140 d before expected parturition. Over the same timeframe, deoxypyridinoline excretion ratio finished 72% higher (urinary deoxypyridinoline:creatinine ratio: d -140 = 10.72; d 42 = 18.40; SED = 1.812; $P < 0.001$).

Of the plasma minerals, only Mg was affected by K supplementation, and the effect was delayed. A time lag of at least 11 d occurred between the cessation of K supplementation prepartum and a sustained depression

Table 5. Mean concentrations of biochemical markers of bone turnover for the cows in the control (no K supplementation) versus K-supplemented (KS) groups relative to the expected date of parturition¹

Item	Treatment		SED ²	P-value
	Control	KS		
Plasma osteocalcin (ng/mL)				
d -140	12.20	12.00	2.734	0.960
d -14	9.71	9.31	1.561	0.743
d 0	3.27	2.34	0.526	0.094
d 0.5	2.31	1.68	0.290	0.060
d 2	5.50	4.64	1.638	0.610
d 2.5	4.81	5.50	1.591	0.670
d 42	10.42	10.61	1.728	0.914
Urinary DPD ³ :creatinine				
d -140	10.56	10.87	1.276	0.810
d -14	9.88	10.10	0.836	0.785
d 0	10.86	8.65	1.161	0.080
d 0.5	10.19	10.61	1.018	0.688
d 2	13.38	13.10	1.215	0.827
d 2.5	14.72	14.22	3.383	0.884
d 42	17.02	19.78	2.564	0.296

¹Period of K supplementation for the KS cows was from d -140 to -14.

²Standard error of the difference.

³Urinary deoxypyridinoline crosslinks.

Table 6. Mean concentrations of plasma minerals for the cows in the control (no K supplementation) versus K-supplemented (KS) groups relative to the expected date of parturition¹

Item	Treatment		SED ²	P-value
	Control	KS		
Ca (mmol/L)				
d -140	2.20	2.21	0.103	0.130
d -14	2.11	1.97	0.102	0.072
d 0	1.56	1.59	0.093	0.741
d 0.5	1.72	1.62	0.138	0.078
d 2	1.80	1.84	0.111	0.882
d 2.5	1.84	1.82	0.184	0.543
d 42	2.08	2.01	0.136	0.522
P (mmol/L)				
d -140	1.28	1.27	0.221	0.320
d -14	1.18	1.17	0.106	0.920
d 0	0.75	0.88	0.123	0.080
d 0.5	0.87	1.00	0.132	0.310
d 2	1.01	0.97	0.129	0.752
d 2.5	1.08	0.98	0.131	0.432
d 42	1.28	1.33	0.113	0.530
Mg (mmol/L)				
d -140	0.75	0.75	0.043	0.990
d -14	0.75	0.71	0.036	0.137
d 0	0.86	0.82	0.062	0.551
d 0.5	0.88	0.69	0.075	0.021
d 2	0.75	0.67	0.063	0.042
d 2.5	0.67	0.57	0.054	0.031
d 42	0.77	0.75	0.049	0.556
K (mmol/L)				
d -140	3.92	3.90	0.227	0.501
d -14	3.95	3.97	0.158	0.788
d 0	3.86	3.85	0.220	0.975
d 0.5	4.04	3.93	0.149	0.467
d 2	3.85	3.98	0.182	0.475
d 2.5	3.90	3.94	0.195	0.780
d 42	3.76	3.75	0.136	0.418

¹Period of K supplementation for the KS cows was from d -140 to -14.

²Standard error of the difference.

in the plasma Mg of the KS cows relative to the control cows that started on the day of parturition (Table 6). The depression was deepest on the day of parturition and lasted for at least the next 2.5 d ($P < 0.05$). On the day of parturition, the KS cows had concentrations that were 22% lower than the control cows (control = 0.88, KS = 0.69; SED = 0.075 mmol/L; $P = 0.021$). None of the KS cows had plasma Mg concentrations <0.4 mmol/L, but all the KS cows had plasma Mg concentrations within the range of 0.6 to 0.8 mmol/L 12 h after parturition; this decreased to a range of 0.55 to 0.65 mmol/L by 2.5 d after parturition. For both treatment groups there was a general trend for plasma Mg concentrations to increase over the 14 d prepartum, decrease for the 2 to 2.5 d after parturition, and then increase to prepartum levels by 42 d postpartum. By contrast, plasma Ca and P concentrations declined toward parturition and increased thereafter to reach prepartum levels by 42 d postpartum (Table 6). Mean

Table 7. Mean urinary excretion of Ca, P, Mg, and K for the cows in the control (no K supplementation) versus K-supplemented (KS) groups relative to the expected date of parturition¹

Item	Treatment		SED ²	P-value
	Control	KS		
Urinary Ca:creatinine				
d -140	0.075	0.075	0.0156	0.223
d -14	0.077	0.074	0.0328	0.199
d 0	0.082	0.046	0.0242	0.039
d 0.5	0.064	0.057	0.0140	0.629
d 2	0.074	0.046	0.0170	0.027
d 2.5	0.075	0.052	0.0211	0.035
d 42	0.105	0.136	0.0454	0.511
Urinary P:creatinine				
d -140	0.037	0.038	0.0430	0.393
d -14	0.022	0.024	0.0068	0.408
d 0	0.024	0.034	0.0146	0.041
d 0.5	0.018	0.058	0.0267	0.017
d 2	0.059	0.032	0.0309	0.392
d 2.5	0.032	0.035	0.0242	0.877
d 42	0.014	0.022	0.0044	0.661
Urinary Mg:creatinine				
d -140	1.77	1.87	0.236	0.665
d -14	1.51	0.74	0.219	0.029
d 0	0.95	0.42	0.269	0.042
d 0.5	0.92	0.55	0.278	0.021
d 2	0.91	0.49	0.369	0.028
d 2.5	0.53	0.81	0.399	0.029
d 42	1.78	1.86	0.734	0.661
Urinary K:creatinine				
d -140	20.54	19.18	4.310	0.756
d -14	21.44	37.90	6.071	0.014
d 0	14.88	11.59	4.476	0.472
d 0.5	15.15	12.44	4.014	0.508
d 2	16.88	13.12	3.585	0.307
d 2.5	12.70	15.09	3.909	0.547
d 42	17.18	17.44	3.374	0.941

¹Period of K supplementation for the KS cows was from d -140 to -14.

²Standard error of the difference.

concentrations of K in the plasma of both treatment groups remained steady throughout the trial (Table 6).

Excretion of K in the urine increased in the KS group during the period of K supplementation (d -14: $P = 0.014$), returned to similar levels as in the control cows by parturition (d 0: $P = 0.472$), and stayed similar thereafter (Table 7). Urinary excretion of Mg also declined during the period of K supplementation in the KS cows compared with the control cows (d -14: $P = 0.029$). However, as for plasma Mg, the depression in urinary Mg excretion was still apparent at parturition (d 0: $P = 0.042$) and persisted for 2 d after ($P < 0.05$). A similarly delayed effect of K supplementation on the urinary excretion of Ca was also apparent, starting at parturition (d 0: $P = 0.039$) and persisting for 2.5 d.

A delayed effect of K supplementation on the urinary excretion of P was also defined (Table 7). Compared with the control cows, P excretion in the KS cows in-

Table 8. Mean concentrations (pg/mL) of plasma 1,25 dihydroxyvitamin D₃ for the cows in the control (no K supplementation) versus K-supplemented (KS) groups relative to the expected date of parturition¹

Plasma 1,25 dihydroxyvitamin D ₃ concentration (pg/mL)	Treatment		SED ²	P-value
	Control	KS		
d -140	14.7	13.5	2.23	0.490
d -14	20.1	19.0	3.11	0.470
d 0	93.1	62.5	13.90	0.027
d 0.5	140.9	106.9	14.25	0.031
d 2	88.0	69.1	13.22	0.040
d 2.5	58.0	55.5	10.74	0.764
d 42	30.2	29.6	2.56	0.496

¹Period of K supplementation for the KS cows was from d -140 to -14.

²Standard error of the difference.

creased to peak on the day of parturition (d 0.5: $P = 0.017$) and then declined, reaching control levels 2 d after parturition.

Similarly, a delayed effect of K supplementation was defined on the concentration of 1,25(OH)₂D₃ in the plasma (Table 8). Both treatment groups showed similarly low concentrations before and during the period of K supplementation, increasing more than 5-fold to peak on the day of parturition. However, concentrations in the control cows peaked 32% higher than in the KS cows (d 0.5: control = 140.9, KS = 106.9; SED = 14.25 pg/mL; $P = 0.031$) and remained greater for the 2 d postpartum ($P < 0.05$) before both decreased to prepartum levels by 42 d postpartum.

DISCUSSION

The purpose of this study was to investigate the potential for positive effects of a high K diet through late lactation and into the dry period. The proposed mechanism was that alkalinizing diets promote an improvement in BMD, which allows a greater mobilization of bone mineral at parturition. We successfully alkalinized the urine of the KS cows relative to the controls for 18 wk, up until on average 11 d prepartum, when supplementation ceased. Yet, no improvement in any of the measures of bone was detected as a result of treatment. Nor was there a beneficial carryover effect on Ca homeostasis at parturition, subsequent milk production, or patterns of liveweight and body condition change. To the contrary, alkalinizing the diet negatively affected Ca homeostasis, a carryover effect possibly mediated by a mild hypomagnesemia that began at parturition.

Potassium supplementation did not improve BMD or turnover. Despite achieving an extended alkalosis abruptly starting and finishing within the 18-wk period of supplementation, all measures of bone remained un-

affected by treatment. As expected, urinary pH stayed higher (the difference averaging 0.25 U) in the KS cows compared with the control cows for the entire period of supplementation. Consequently, although it was an effective means of alkalinizing metabolism, using a K₂CO₃ supplement to promote BMD and turnover cannot be recommended on the basis of the present results. However, a qualification to this should be noted based on the observation that, on average, BMD improved by 12% for both treatment groups through late lactation and the dry period until 11 d prepartum. The lack of effect of the K may simply have been a consequence of the control cows already being in a sufficiently mild alkalotic state to promote BMD and turnover. The DCAD for each diet being well into the positive supports this (control = +19 mEq/100 g of DM, KS = +62 mEq/100 g of DM).

A control diet with a DCAD closer to zero could have been more helpful in allowing a more definite conclusion as to the benefits of an alkalosis to bone tissue. However, it proved difficult to find natural ingredients (excluding anionic products) of sufficient quality that were any lower in DCAD than those used.

Nonetheless, our data confirm the general finding of others that cows replenish bone mineral throughout late lactation and into the dry period (Zetterholm, 1978; Holmberg et al., 1985; van Mosel et al., 1990). However, we were surprised to see a tendency for the trend to continue through early lactation. By contrast, others have noted a generalized resorption of bone that may start as early as parturition but increases through early lactation. Several studies have suggested that BMD increases during late lactation and the dry period and then decreases from 90 d onwards after the onset of lactation (Zetterholm, 1978; Holmberg et al., 1985; van Mosel et al., 1990). Similarly, Liesegang (2003) used peripheral quantitative computed tomography to measure BMD and bone mineral concentration together with several biochemical markers of bone turnover in live pregnant and lactating sheep and goats and reported a decrease in bone mass during lactation and an increase in bone resorption at the beginning of lactation. Similar to the present study, Beighle (1999) noted an increase in the Ca and Mg content of the cortical portion of rib bone biopsies of dairy cows during late gestation, which continued for 30 d after parturition.

We are unsure why BMD and bone mineral concentration did not decrease from 11 d prepartum to 42 d into early lactation. A decrease was expected and was indicated by the bone marker results, but may simply have been too small to detect. Concentrations of the marker for bone formation, plasma osteocalcin, showed an obvious decline relative to the marker for resorption, urinary DPD. The decline started at parturition

and the difference was still evident 42 d into lactation. Moreover, the concentrations of both markers were clearly higher 42 d into lactation; because rate of mineral turnover should positively relate to availability of mineral to extracellular fluid, this was indicative that the importance of bone as a source of mineral may be more important in early lactation than immediately at parturition.

Whereas bone tissue remained unaffected, an intriguing carryover effect of K supplementation occurred for Ca, P, and Mg in plasma and urine. At least 11 d after supplementation ceased, plasma concentrations of Mg and urinary excretion rates of Mg and Ca were depressed in the KS cows compared with the control cows. Concentrations of Mg commonly increase immediately after parturition (Goff, 2006). However, in the case of the KS cows, the increase was delayed by at least 1 d compared with the control cows. The negative effects of supplementation were first detected on the day of parturition and persisted for the next 2 d. Similarly, concentrations of 1,25 OH vit D₃ stayed depressed for the same period. By contrast, urinary excretion of P increased in the KS cows, but only on the day of parturition.

In explaining the carryover effect of K, plasma Mg could be the mediating link. Several recent meta-analyses list low dietary Mg as a major risk factor for periparturient hypocalcemia (Enevoldsen, 1993; Charbonneau et al., 2006; Lean et al., 2006; DeGaris and Lean, 2008). The reasoning used to explain this is that Mg is essential for the functioning of the second messenger system that amplifies the message of PTH, itself induced by a decrease in plasma Ca. Parathyroid hormone, via this second messenger system, stimulates the kidney to synthesize more 1,25 OH vit D₃ and hence improve Ca flows into the blood stream (Goff, 2006). Our data are consistent with the above reasoning; the normally observed increase in plasma Mg at parturition was delayed in the KS cows, presumably leading to a dampening of the PTH message and hence our observed reduction in the concentration of 1,25 OH vit D₃ in plasma. An intriguing question here is whether a dampening of the PTH message could stimulate an increased concentration of PTH in the plasma of the KS cows to compensate. Unfortunately, PTH was not measured in the present study. However, it has been reported that PTH stimulates a reduction in rate of excretion of Ca and Mg in the urine and increases the excretion of P (Goff, 2006), all of which were responses noted in the KS cows.

If hypomagnesemia is the mediating link, then the solution to reducing the risk of hypocalcemia could be as simple as supplementing the cows with Mg immediately before and for the few days after parturition. For example, in studies with cows on a predominantly

pasture diet in New Zealand, where DCAD levels can easily approach those fed to the KS cows in the present study, Roche (2003) and Roche et al. (2003) observed that about 70% of the cows were hypomagnesemic on the day of parturition and that supplementation with Mg alleviated problems of both hypomagnesemia and hypocalcemia.

A further question is how an effect of K could persist for 11 d after supplementation ceased. Immediate effects of K on Mg metabolism are well documented, but delayed effects are not. It is well accepted that there is an immediate link among high ruminal levels of K, poor ruminal absorption of Mg, and depressed concentrations of Mg in plasma (Reinhardt et al., 1988; Hungerford, 1990; van Mosel et al., 1991; Leonhard-Marek and Martens, 1996; Ram et al., 1998; Schonewille et al., 1999; Underwood and Suttle, 1999; Goff, 2000). Two mechanisms exist for the transport of Mg across the rumen wall (Schonewille et al., 1999). The first is driven by the magnitude of the difference in electric charge across the apical membrane of the ruminal mucosa. High concentrations of K increase this difference in potential, reducing the rate of diffusion of Mg from the rumen to the blood stream. The second mechanism is a carrier-mediated transport mechanism. However, there was no indication that K concentrations, ruminal or otherwise, remained increased in the KS cows at parturition. Urinary excretion of K and urinary pH converged to similar levels in the KS cows compared with the control cows from parturition onwards, so we discount the possibility that the hypomagnesemia was a consequence of a relatively immediate effect of high K.

Nor was there any indication of an effect of treatment on feed intake at parturition. A depressed intake in the KS cows could have reduced their intake of Mg, and daily intake of Mg is a key driver of Mg absorption. It was not possible to measure feed intake at parturition because by this time the cows had been returned to the commercial herd. Liveweight patterns and subsequent milk and milk component yields were the only indicators of feed intake perturbations, and no treatment effects on these were detected.

Alternatively, the carryover phenomenon may be an indication of damage or impairment to the second type of Mg transport, the carrier-mediated system (Martens and Blume, 1986; Schonewille et al., 1999; Martens and Schweigel, 2003). Constant exposure to a high K diet may have depressed the basal level of activity of the carrier-mediated system. We speculate that once the cows were returned to the lower K diet it took several weeks for the absorptive capacity of the ruminal epithelium to be restored. Presumably, the demand for Mg in late pregnancy was still low enough for the impaired

system to cope, but the system became overloaded once the extra demands for lactogenesis and lactation were incurred. Magnesium would have been the most affected of all the bone minerals by a persistent perturbation to the ruminal mucosa. This is because in the adult the rumen remains the principal site of absorption for Mg, whereas for Ca and P, the principal site of absorption is the small intestine (Underwood and Suttle, 1999).

To conclude, a highly alkalinizing diet fed through late lactation and into the dry period conferred no detectable benefit to BMD and turnover. It is possible, however, that such a diet may impair the metabolism of Mg, P, and Ca at parturition, even if DCAD is reduced for at least 11 d prepartum and Mg intakes are apparently adequate. Where K intakes are high it may be necessary to provide additional Mg to any generalized strategy to reduce DCAD prepartum.

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