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URINE CALCIUM BUT NOT PLASMA CALCIUM OR URINE HYDROXYPROLINE IS INCREASED BY A SYSTEMIC ACIDOSIS IN THE DAIRY COW

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Abstract

Eight non-lactating, pregnant Holstein-Friesian cows were allocated to two treatments and individually offered diets differing in dietary cation-anion difference. Decreasing the dietary cation-anion difference reduced the urine pH within hours of anionic salt supplementation. Plasma calcium concentration was unaffected by dietary cation-anion difference but urine calcium concentration was significantly increased within 10 days of including anionic salts in the diet. Faecal calcium concentration was significantly reduced, indicating increased calcium absorption. Dietary calcium concentration or dietary cation-anion difference did not significantly affect urinary hydroxyproline.

Keywords: Dairy cows, non-lactating, pasture, dietary cation-anion difference.

Introduction

Some researchers have measured an increase in plasma and urine calcium concentration at calving when a reduced dietary cation-anion difference (DCAD) was fed (Block, 1984). In contrast Roche (1999) found no increase in plasma calcium during a decrease in DCAD, although pre-calving urinary calcium concentration increased

exponentially ($r^2 = 0.95$). Schonewille *et al.* (1994) found that increased absorption of calcium only accounted for 60% of the increased excretion of calcium. The source of the additional calcium in urine remains unclear.

Some researchers (Block, 1984; Goff *et al.,* 1991) have measured an increase in urinary hydroxyproline as a result of feeding a low DCAD and have concluded that the reduction in blood pH increased bone resorption. However, Schonewille *et al.* (1994) and Roche (1999) found no increase in urinary hydroxyproline when blood pH was reduced and Van Mosel *et al.* (1994) claimed that the extra calcium excreted was due to decreased bone accretion.

The objective of the work reported here was to test the effects of a reduction in systemic pH on the excretion of calcium and hydroxyproline by the cow.

Materials and Methods

Eight non-lactating, pregnant, multiparous, rumen-fistulated Holstein-Friesian cows were allocated, in a randomized block design, to two treatments on the basis of age (5.75 ± 0.44 years) and live weight (584 ± 52 kg). Treatments differed in DCAD; the high treatment (High) had a DCAD of +24 mEq $100g^{-1}$ and the low treatment (Low) had a DCAD of –20 mEq $100g^{-1}$, achieved by the addition of magnesium chloride ($MgCl_2.6H_20$) and ammonium chloride (NH_4Cl). DCAD was calculated using the equation of Tucker *et al.* (1992).

Management and feeds

Animal measurements taken for four days (days -3 to 0) prior to the beginning of the experiment were used as a covariate. Cows in both treatments were fed the same base feed of 7kg DM of hay and 3 kg DM of barley. Cows on Low were supplemented with $MgCl_2.6H_20$ and NH_4Cl, through the rumen fistula, at 09.00 and 15.00 h. Following the adaptation period

(Days 1-14) cows were individually fed indoors for seven days (days 15-21) during which individual DM intake and total urine and faecal measurements were made. Barley (1.5 kg cow^{-1}) was offered for 20 minutes at 09.00 and 15.00 h after which the cows had access to hay until 21.30 h. On day 16 the cows were fitted with faecal and urine separation equipment as described by Grainger (1982). The calcium balance period extended from day 17 to 21.

Measurements

A midstream urine sample was collected from each cow following manual stimulation on day –3 to 16. Urine pH was determined and two sub-samples were frozen awaiting calcium and creatinine analysis.

Samples of all feeds were analyzed for macro minerals using x-ray spectroscopy (Norrish and Hutton, 1977) and DCAD calculated.

During the Ca balance period total faecal and urine outputs were taken for faecal DM and calcium concentration, and urinary pH and calcium, hydroxyproline and creatinine concentration. Blood was collected from each cow daily, centrifuged for 10 minutes at 1120 g and the plasma removed.

Faecal, urine and plasma calcium concentrations were det□rmined on a Perkin Elmer 372 atomic absorption spectrophotometer at 422.7 nm against a series of calcium standards. Urine hydroxyproline was determined by a method developed by Parekh and Jung (1970) using a micro plate reader (Biorad 550, USA). Urine creatinine was determined by a method modified from Bartels *et al.* (1972), using an autoanalyser (Boehringer Manheim Hitachi 911).

Statistical analysis

All data were analyzed by analysis of variance for a randomized block design using the statistical procedures of Genstat V (1997), with cows as the experimental unit.

Results and Discussion

The lower DCAD caused a significant reduction ($P < 0.001$) in the pH of excreted urine within a couple of hours of anionic salt supplementation (Figure 1). Urine pH on the Low treatment continued to decline for 2 days, reached a plateau and on day 6 increased from 5.98 to 7.83. On day 9, urine pH began to decline again to a trough of approximately 5.5 at day 19. Figure 1 also shows a concomitant rise in the corrected urinary calcium concentration (CUCa) as urine pH declined after day 9, which is consistent with the results of Vagg and Payne (1970) and Roche (1999).

There was no significant difference between treatments in calcium intake or plasma calcium concentrations (Table 1). The reduced DCAD caused a decrease in urine pH, an increase in urinary calcium excretion ($P < 0.001$) and a reduction in faecal calcium excretion ($P < 0.05$) suggesting calcium absorption was increased.

Hydroxyproline excretion in urine, an indicator of bone resorption (Robins, 1994), was not affected by treatment (Table 1). These results may indicate that when DCAD was reduced bone resorption was not responsible for the additional calcium excretion unaccounted for by increased absorption. Alternatively, they question the effectiveness of urinary hydroxyproline as a marker, specific to bone resorption, when used in the periparturient period. As hydroxyproline indicates any catabolism of collagen including the release of collagen by the uterus its use as a specific marker for collagen released during bone resorption is probably not accurate. The direct measure of bone vicissitude by Van Mosel *et al.* (1994) who reported a decrease in bone accretion during systemic acidosis but found no effect on bone resorption supports this theory.

A DCAD of –20 mEq $100g^{-1}$ caused a significant depression in urine pH and an increase in urine calcium: creatinine ratio. Plasma calcium and urine hydroxyproline were unaffected by DCAD. Urine hydroxyproline is probably not a reliable indicator of bone resorption in the periparturient period.

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Table 1 - Effect of High or Low DCAD on the net calcium balance of the non-lactating dairy cow.

	High	Low	SEM
Calcium Intake (g day^{-1})	27.6	28.4	1.66
Urine Calcium (g day^{-1})	0.8[a]	4.7[b]	0.59
Faecal Calcium (g day^{-1})	46.0 [b]	32.8 [a]	3.7
Plasma Calcium (mg l^{-1})	100.2	97.0	1.56
Urinary Hydroxyproline (mg day^{-1})	0.0087	0.0077	0.00081

[a, b] Means in a row with different superscripts differ significantly

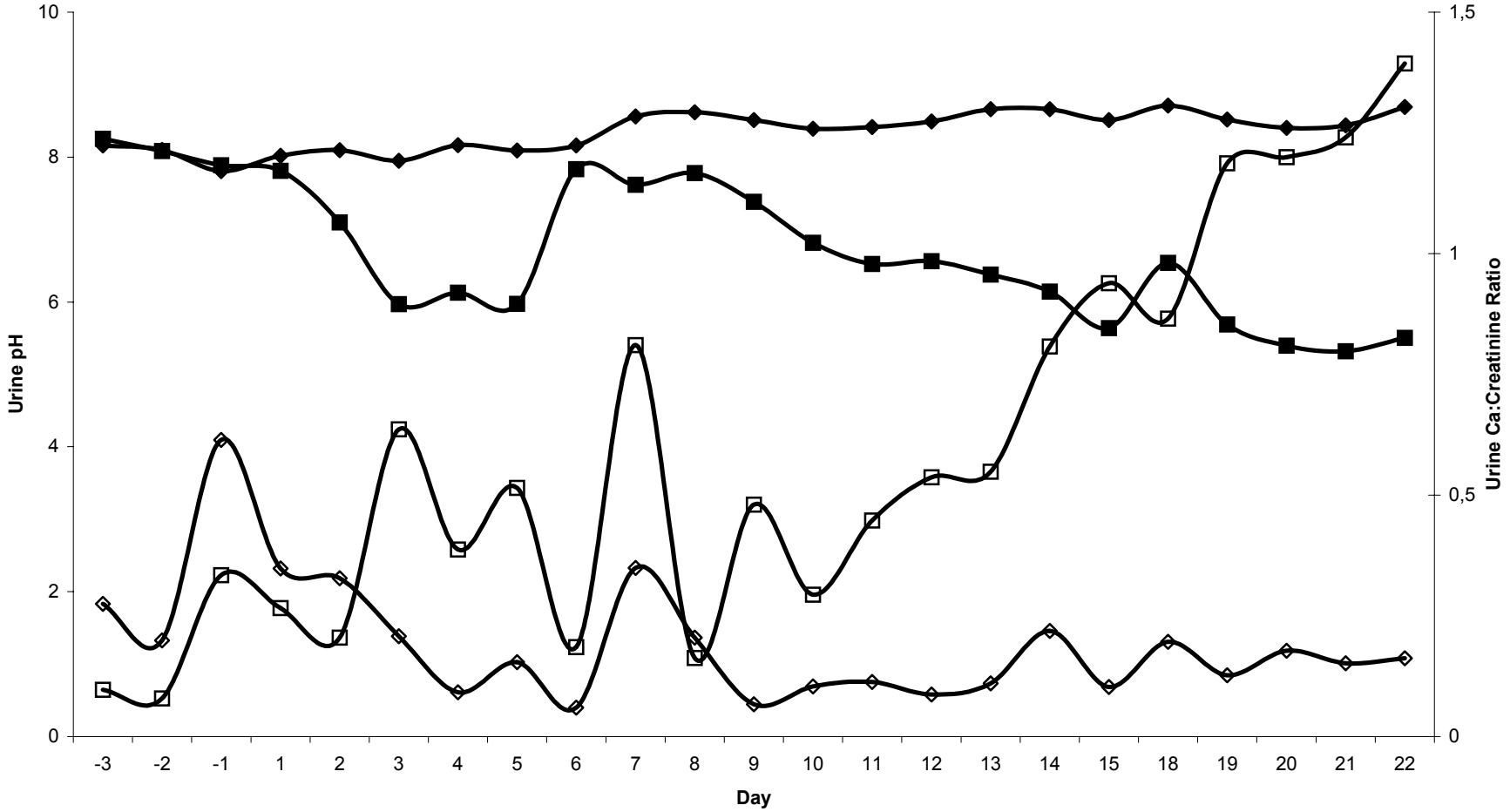

Figure 1 - Effect of dietary cation-anion difference on the urine pH (◆ and ■) and urine Ca: creatinine ratio (◇ and □) of High and Low DCAD, respectively