

Final Report on DCAD Project

“Determining the effect of a reduced dietary cation-anion difference (DCAD) on the incidence of milk fever in a milk fever prone dairy herd”

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Introduction

The DCAD Project was conducted at Elliott Research and Demonstration Station during July and August, 2007. The project aimed to determine the effect of altering the dietary anion-cation difference (DCAD) on the incidence of milk fever in a milk fever prone dairy herd.

Trial Conduct

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Thanks to: Scott Carlson, Karen Christie, Ross Corkrey, Danny Donaghy, Wynyard Veterinary Clinic and staff at Burnie Hospital Lab.

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Milk Fever and DCAD

Milk fever is a metabolic disorder that occurs in cattle, most commonly within several days pre- or post-calving. Cows suffer from milk fever when they are unable to meet the sudden increase in requirement for calcium that occurs at the start of lactation. Because calcium is needed in the body for muscle contraction and relaxation, symptoms of milk fever include staggered gait and lateral recumbency. While treatment of milk fever is quite simple (intravenous or subcutaneous injection of calcium borogluconate) and recovery can be rapid, if the cow is not treated quickly enough she can die or become a 'downer' cow which requires a large effort to care for. In a survey conducted in 2007, Tasmanian dairy farmers ranked milk fever as the second biggest animal health problem (after mastitis) that they have to deal with.

Figure 1 shows a model of the tissues and hormones associated with calcium homeostasis (maintaining calcium at the required level within the body). A cow that isn't lactating is normally able to meet her calcium requirements from her diet. At the onset of lactation, the calcium requirement increases dramatically and the cow is unable to meet this requirement with the readily available calcium in the blood (about 3 grams) or the readily mobilisable stores (10-20 grams). However, the cow does have enough calcium within her body to meet these demands - about 99% of calcium is stored in the bones – but it can take 3-4 days to mobilise this calcium (McNeill *et al.*, 2002).

The regulation of calcium is controlled mainly by the hormones calcitonin, parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D₃. Most milk fever prevention strategies aim to manipulate these hormonal controls in order to start the mobilisation of the calcium from the bones prior to the onset of lactation so that the cow has a greater store of readily mobilisable calcium. Commonly used milk fever prevention strategies are:

- Reducing calcium intake prior to calving
- Injecting with vitamin D₃ prior to calving
- Supplementing with magnesium prior to calving
- Reducing the dietary cation-anion difference prior to calving

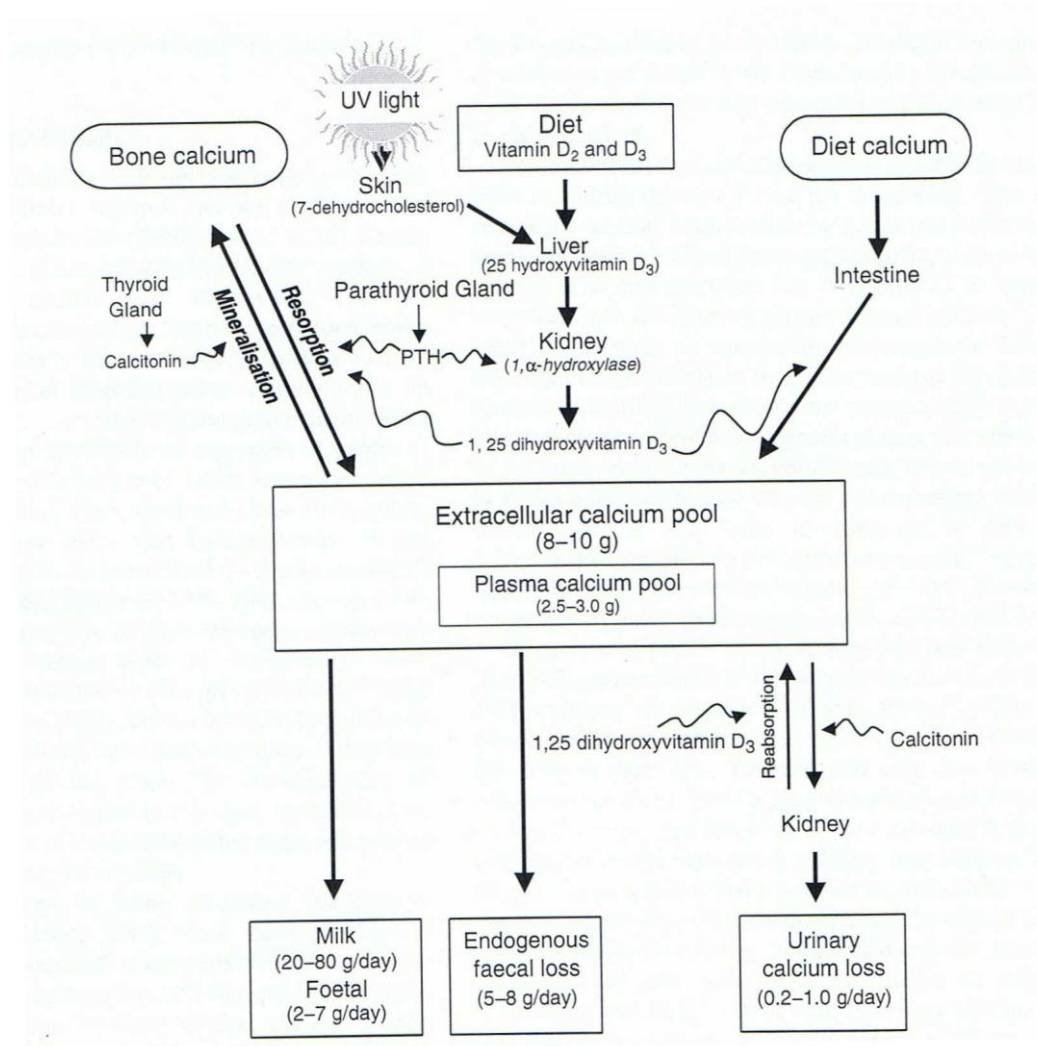


Figure 1 Model of the tissues and hormones associated with calcium homeostasis in a 500 kg cow (McNeill *et al.*, 2002)

Reduce calcium intake prior to calving

This is normally done by restricting intake to pasture and feeding hay as a supplement which has lower levels of calcium. The effect of restricting dietary calcium is to stimulate the synthesis of 1,25-dihydroxyvitamin D₃ which promotes the absorption of calcium from the small intestine and stimulates bone degradation (releases calcium). This means that the cow can respond more quickly to the drain on

calcium caused by the onset of lactation. However, in pasture based systems, even with restricting the intake of fresh pasture, dietary intakes of calcium may still be sufficient to meet cow requirements and if so, this method of preventing milk fever will not work.

Inject with vitamin D₃ about 1 week prior to calving

When a cow is injected with vitamin D₃, she converts the Vitamin D₃ into its active form, 1,25-dihydroxyvitamin D₃, which may take 2 days to occur. Once converted to 1,25-dihydroxyvitamin D₃, the amount of readily available calcium is increased, through increased absorption of calcium from the intestine and bone mobilisation. The difficulty with this prevention strategy is to predict the individual cow calving dates accurately enough so the injection can be given to the cow at least 2 days prior to calving but no more than 8 days prior to calving.

Supplementing magnesium prior to calving

If magnesium levels are low, it reduces the secretion and effect of PTH and 1,25-dihydroxyvitamin D₃ which are two of the hormones involved in mobilisation of calcium. It is recommended that dietary magnesium intakes are within the range of 0.2-0.4% of the total drymatter intake. Supplementation of magnesium is usually done by spreading magnesium on pasture or hay, adding magnesium to the water supply or including in a lead feeding mix.

Reducing DCAD

Within her body, a cow contains a range of cations (positively charged ions) and anions (negatively charged ions). While all of the cations and anions have a role to play, two cations, sodium (Na) and potassium (K) and two anions, chlorine (Cl) and sulphur (S) have been identified as the primary strong ions in the body that have an effect on blood pH. If the difference in concentration between these strong cations and anions increases, the blood pH will increase and if the difference between them decreases, the blood pH will decrease. Reducing the blood pH, in effect creating a state of metabolic acidosis, increases the absorption of calcium from the small intestine and mobilises calcium from the bones. The difference in concentration between the strong cations and anions can be manipulated by the feeding strategies. Just as the cow contains a range of cations and anions, so too do feeds. The Cation-Anion Difference (CAD) of feeds can be calculated by analysing the quantity of each of the strong ions. The DCAD of the cows diet can then be calculated by using the following equation:

$$\text{DCAD (mEq/100 g DM)} = [(\text{Na}\% \div 0.023) + (\text{K}\% \div 0.039) - (\text{Cl}\% \div 0.0355) + (\text{S}\% \div 0.016)]$$

For DCAD to have an effect on blood pH and hence milk fever, it must be at 0 mEq/100 g DM or below (Roche *et al.*, 2003). A study conducted at Ellinbank Research Station in Victoria during 1996-97 found that CAD of pasture ranged from 0 to +80 mEq/100 g DM and with including the supplements fed, the DCAD ranged from 0 to + 95 mEq/100g DM (Roche *et al.* 2000). Studies of indoor feeding systems in other countries have measured ranges of DCAD from -20 to +32 mEq/100 g DM. This lower DCAD is due to the type of feed (grains have a lower CAD than pasture) and also the use of anionic salts. Examples of anionic salts are magnesium chloride, magnesium sulphate, calcium chloride and calcium sulphate.

These salts add extra sulphate or chloride to the diet which decreases the difference in concentration between the anions and cations (lowered DCAD) leading to a lower blood pH. Urine pH, because it is linked to blood pH, can be used to monitor the success of feeding strategies designed to lower DCAD. A urine pH in the order of 6.0-6.5 is necessary.

Due to the naturally high CAD of pastures, it is difficult in pasture based systems to reduce the DCAD to below 0 mEq/100g DM. This experiment aimed to determine if the feeding of anionic salts in a pasture-based system, and in a milk fever prone herd, resulted in a reduction in the incidence of milk fever.

Method

The experiment was conducted at Elliott Research and Demonstration Station (41°05' S, 145°46' E) on the north-west coast of Tasmania, Australia during July and August, 2007. All procedures in this study were approved by the University of Tasmania Animal Ethics Committee.

Experimental Design and Treatments

The project began on the 9th July, 2007. Fifty-two multiparous cows were allocated to 4 different treatment groups (13 cows/treatment) in a completely randomised design, ensuring that milk production in the first 100 days of lactation, age, and breed were similar across all treatments. Groups consisted of an untreated control (CONTROL), a LOW DCAD, a HIGH DCAD, and a treatment receiving both the HIGH and LOW treatment mixtures (BOTH). The cows were (mean \pm SD) 8 ± 3 years old and had produced 2614 ± 394 kg of milk in the first 100 days of the previous lactation.

Feeds

All cows remained in the one herd and were offered a daily allocation of 8 kg DM of perennial ryegrass/cocksfoot pasture and hay (4 kg DM of each).

Dietary Cation-Anion Difference

The base pasture/hay diet of the cows had an average DCAD of +38 mEq/100g. The four treatment groups, LOW, HIGH, BOTH and CONTROL, had a target DCAD of -10, 86, 38 and 38 mEq/100g respectively. The salts fed to reach the target DCAD are listed in Table 1.

Table 1 The amount of each salt required (g/cow/day) to reach target DCAD

Treatment Group	Req. DCAD (mEq/100g)	SALTS (g/cow/day)						
		NaHCO ₃	MgCl ₂	MgSO ₄	CaCl ₂	CaSO ₄	MgO	CaCO ₃
LOW	-10	0	146	120	106	80	0	0
HIGH	+86	404	0	0	0	0	50	126
BOTH	+38	404	146	120	106	80	25	63
CONTROL	+38	0	0	0	0	0	0	0

The salts for each treatment group (15 cows) were mixed with 13.5 litres of water and 1.5 litres of molasses to make the salts more palatable. The cows were drenched twice daily at 0800 and 1500 h (Photo 1) with the salt solution. The cows in the CONTROL group were drenched with molasses dissolved in water. Drenching of the cows began on 10th July, nineteen days prior to the planned calving start date (29th July).

Milk production

Individual cow milk yield was recorded at each milking post-calving. A milk sample was collected from each cow at an a.m. and p.m. milking on a weekly basis from week 2 to week 6 post-calving. Milk samples were analysed for fat and protein percentage using Milkoscan.

Pasture measurement and quality

Pre- and post-grazing measurements of pasture were taken using a rising plate meter to determine herd intakes. Pasture samples were collected by cutting an 8x8 cm area of pasture to a height of 5 cm. Pasture samples were collected every 15-20 m in a transect across the area to be grazed, prior to grazing. Samples were bulked and a sub-sample sent for analysis of metabolisable energy, crude protein, neutral detergent fibre, digestibility, chloride, sodium, magnesium, phosphorus, sulphur, potassium, calcium, manganese, iron, copper, zinc and boron.

Blood sampling

A blood sample was taken from the cows prior to the start of the trial. The cows were then blood sampled on a weekly basis prior to calving and then on Day 0, 1, 2, 3, 4, 7, and 14 post-calving. The blood sample was collected from the tail vein into a 9 ml sodium heparin evacuated tube. All blood samples were centrifuged immediately post-sampling for 10 minutes at 1120 x g, and the plasma pipetted off and frozen awaiting analysis.

Plasma was analysed for calcium, magnesium, phosphate, β -hydroxybutyrate (BHB), sodium, potassium, chlorine, and non-esterified fatty acids (NEFA).



Photo 1 Drenching a cow



Photo 2 Taking a blood sample from the tail vein

Urine sampling

A urine sample was collected from each cow at the start of the trial and then on a weekly basis until the cow calved. The sample was collected at approximately 0800 h prior to the cows being drenched (approx 16 hours after previous drench). Cows were manually stimulated to urinate and a sample of urine was collected into a 100 ml container. The pH of the urine was measured and the sample frozen for later analysis of creatinine, calcium and magnesium.

Results

Feeds

The average daily intakes of the cows during the pre-calving period are shown in Table 2. The average quality of the pasture and hay during this period is shown in Figure 22 and the change in pasture quality is shown in Figure 33.

Table 2 Pre-calving cow intakes

Start Date	Finish Date	Pasture intake (kg DM/cow)	Hay intake (kg DM/cow)	Total intake (kg DM/cow)
11/07/2007	12/07/2007	4.0	4.2	8.2
13/07/2007	16/07/2007	6.3	2.1	8.4
17/07/2007	20/07/2007	4.7	2.2	6.8
21/07/2007	25/07/2007	4.4	1.7	6.1
26/07/2007	08/08/2007	8.5	0.0	8.5

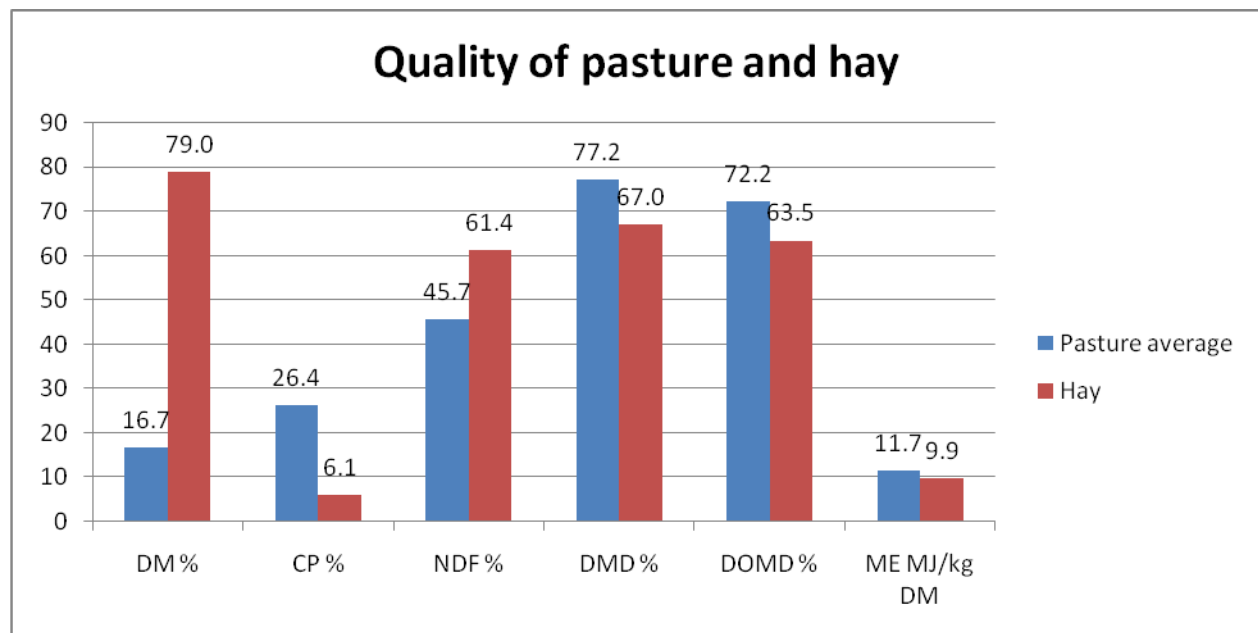


Figure 2 Analysis of quality of pasture and hay during the pre-calving period – averaged

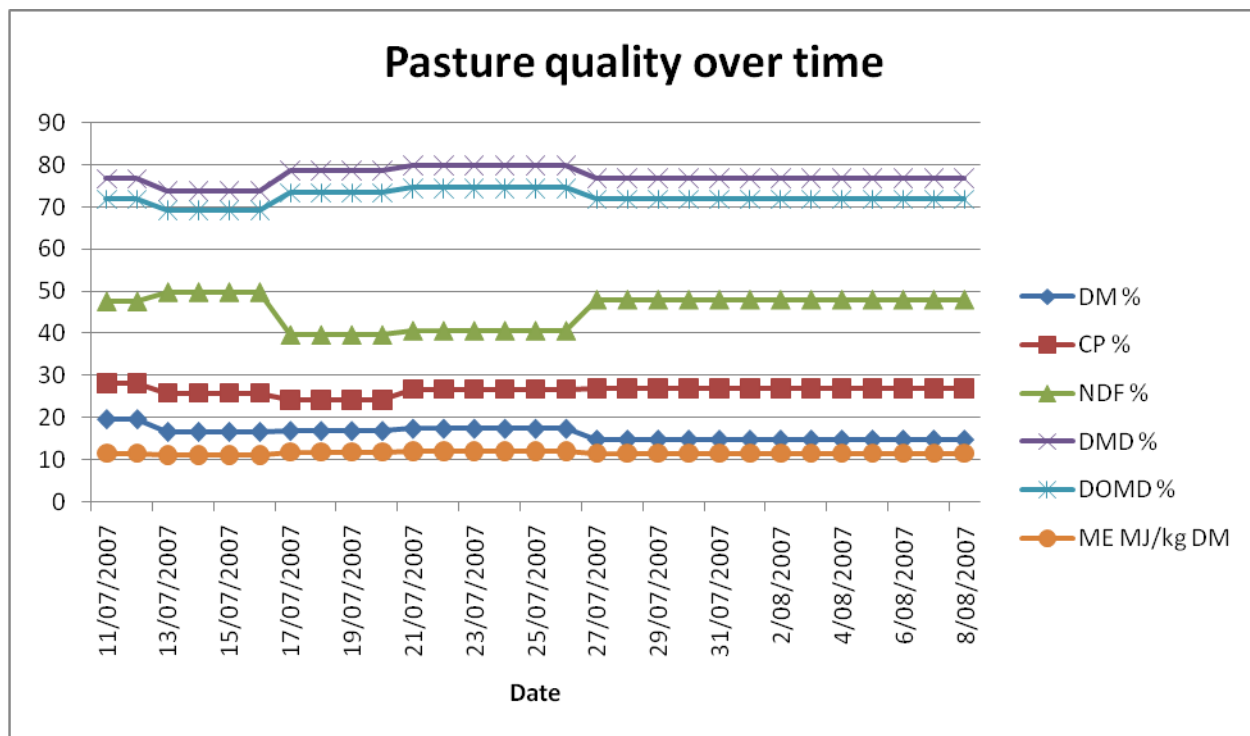


Figure 3 Changes in pasture quality during the pre-calving period

Pasture and hay samples were analysed for mineral content. Figure 4 shows the percentage of the strong ions contained in the pasture and Figure 7 shows the resulting CAD of the pasture and hay and DCAD for the pre-calving period. The mineral content of the pasture, and hence the CAD, varied considerably between the different paddocks grazed as part of the trial. As it takes about 1 week for pasture to be analysed for mineral content, the variability in mineral content makes it difficult to calculate the amount of anionic salts required to have a certain DCAD.

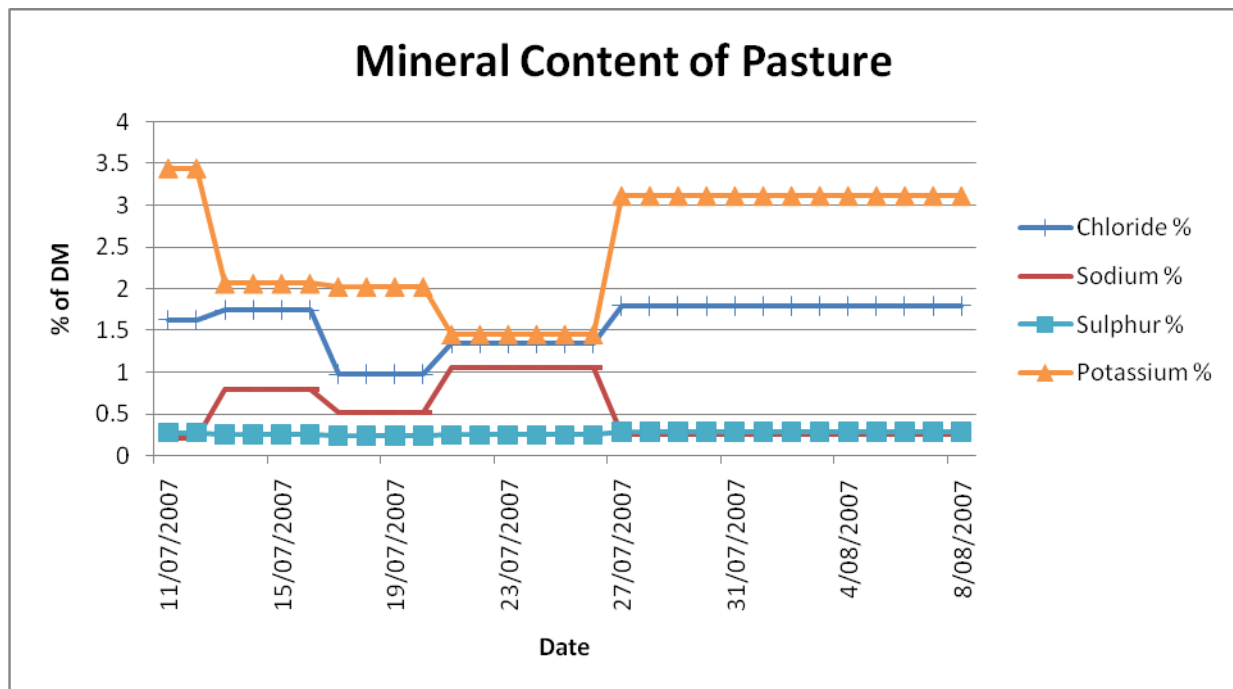


Figure 4 Mineral content of pasture during the pre-calving period

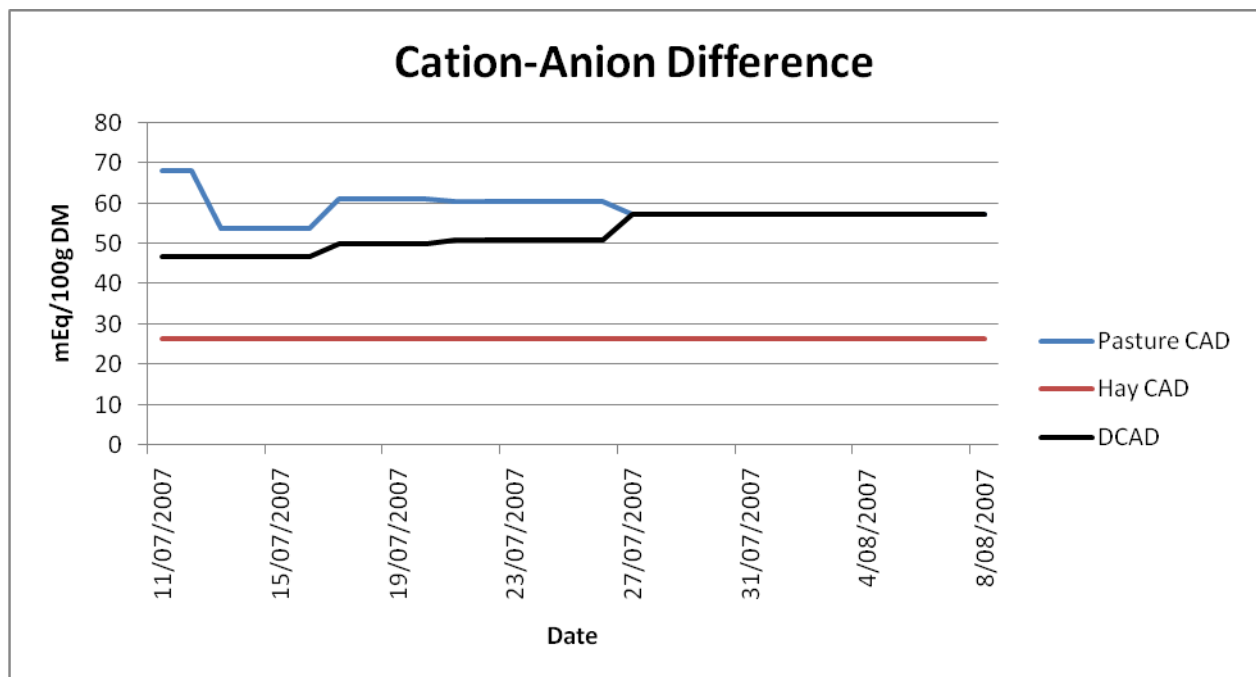


Figure 5 Cation-anion difference of pasture and hay and the dietary cation-anion difference in the precalving period of the trial

Urine

Urine pH

The aim of feeding anionic salts is to cause a mild metabolic acidosis (reduction in blood pH) which increases the mobilisation of calcium from the bones. Urine pH is an effective indicator of blood pH (Roche *et al.*, 2003). The relationship between blood and urine pH in dairy cows has been determined as $\text{blood pH} = 0.02 \times \text{urine pH} + 7.263$ ($r^2 = 0.76$; $P < 0.01$) (Spanghero, 2004).

Table 3 Urine pH pre-trial and pre-calving

	CONTROL	LOW	HIGH	BOTH	SED	P
Urine pH pre-trial	8.14	8.34	8.34	8.40		
Urine pH pre-calving	8.25	7.57	8.29	8.11	0.131	<0.001

From Table 3 it can be seen that while urine pH was similar for all groups prior to the start of the trial, after feeding the salts for 2 weeks, the pH of the LOW treatment group had significantly decreased compared to the other groups.

Urine Calcium

The concentration of calcium in the blood is very tightly controlled by hormones to stay within normal limits. However, in the short-term, an excess of calcium (or any other minerals) will be excreted in urine. For this reason, an increase in urinary calcium levels indicates that the absorption of calcium has been increased even if there is no change in blood calcium levels. Figure 6 shows that the cows on the LOW DCAD diet did have an increase in urinary calcium output (it is measured as a ratio to creatinine to take into account differences in urine volume) which indicates that there was increased absorption of calcium. There was also an increase in urinary calcium output in the BOTH treatment group even though the cows had the same DCAD as the control group.

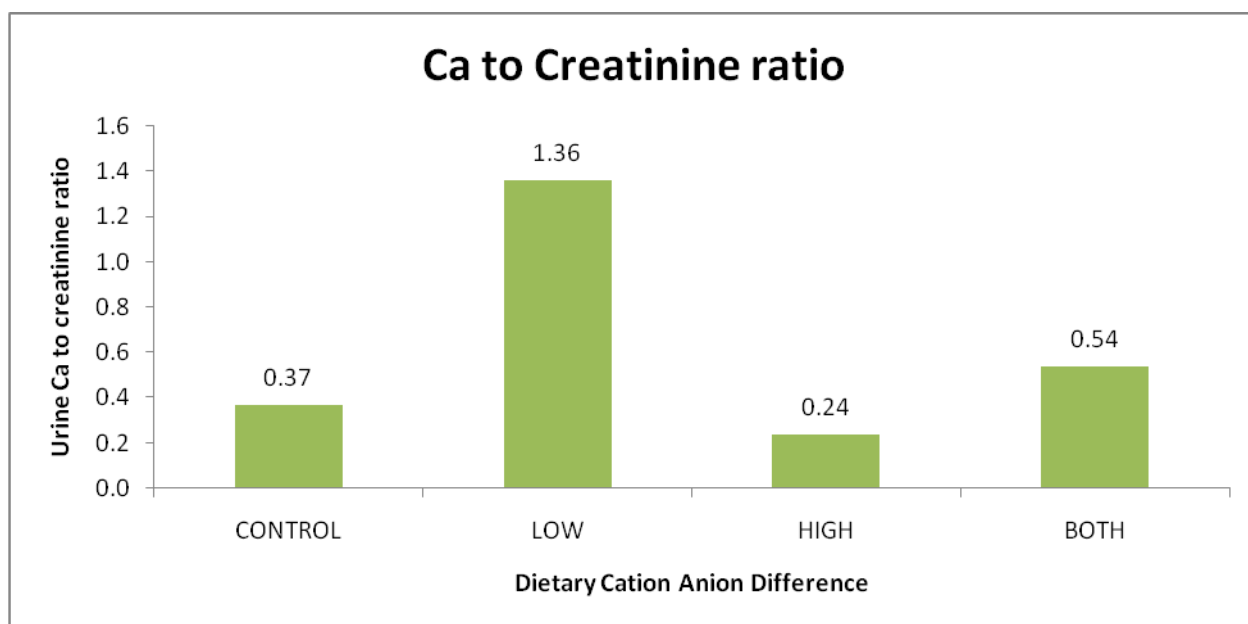


Figure 6 Urine calcium to creatinine ratio in the urine of cows receiving different precalving DCAD

Blood

Calcium

Prior to calving, the calcium requirements of the cow are generally met from the diet. After calving, the calcium requirements of the cow increase dramatically and cannot be met by the readily available calcium in the blood and readily mobilisable stores. Calcium stores are available in the bones but it may take 3-4 days to increase the calcium mobilisation to sufficient levels to meet cow requirements (McNeill *et al.*, 2002). Cows with hypocalcaemia will have total plasma calcium levels in range of 1.0-1.4 mmol/L while cows suffering from subclinical hypocalcaemia (no visible symptoms of milk fever) will have total plasma calcium levels in the range of 1.25-2.0 mmol/L.

Table 4 shows the average and range of total blood calcium levels at key times of the cows used in this DCAD trial.

Table 4 Average and range of total plasma calcium levels in cows used in the trial

Day	Average	Range
Pre-trial	2.5	1.9-2.8
Day 0 (day of calving)	1.8	0.95-2.7
Day 14 (post-calving)	2.1	1.7-2.4

Figure 7 shows the total plasma calcium levels for cows in each of the treatments. There was no significant difference between the treatments.

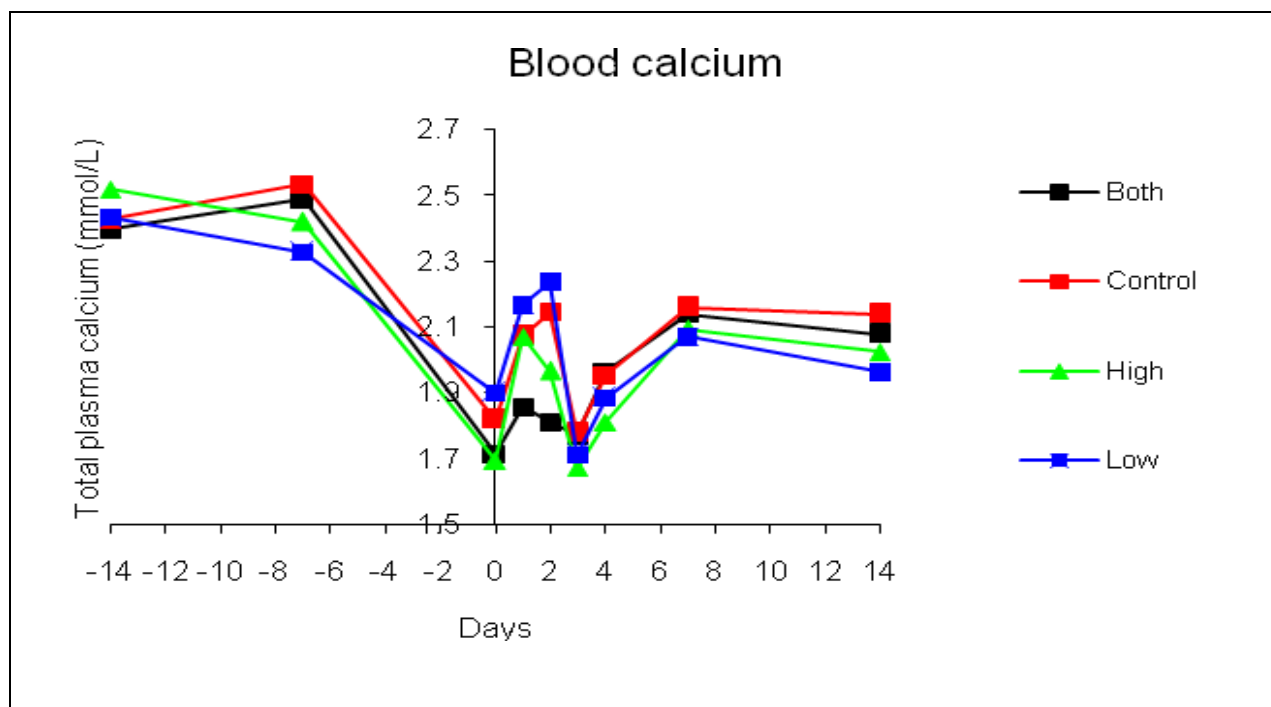


Figure 7 Total plasma calcium (mmol/L) from 14 days pre-calving to 14 days post-calving

Magnesium

The level of magnesium influences the calcium homeostasis of the cow. Cows that are low in magnesium are less able to mobilize calcium when they have increased requirements. The normal level of magnesium is around 1.0 mmol/L. Diets that are high in potassium can reduce the concentration of plasma magnesium. However, plasma magnesium levels can be easily lifted through supplementation of magnesium in the diet even when the diet is also high in potassium.

Table 5 Average and range of total plasma magnesium

Day	Average	Range
Pre-trial	0.81	0.51-1.0
Day 0 (day of calving)	0.90	0.52-1.7
Day 14 (post-calving)	0.87	0.62-1.2

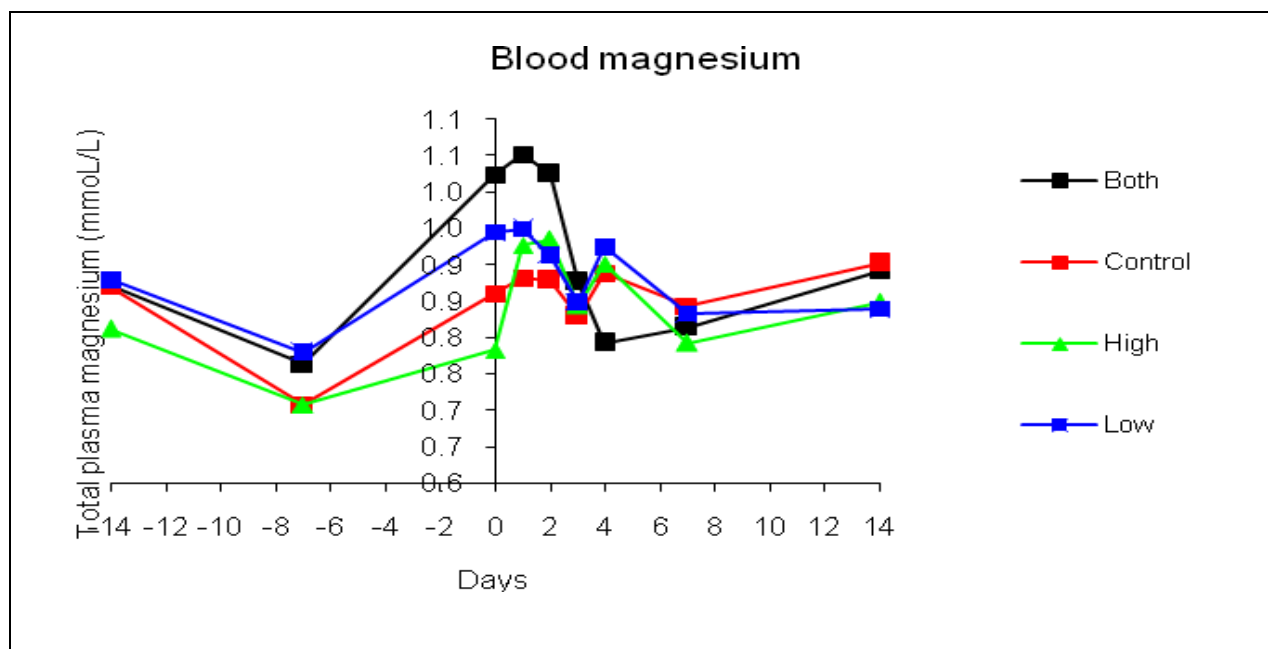


Figure 8 Total plasma magnesium (mmol/L) from 14 days prior to calving to 14 days post-calving

Milk Production

There was no significant difference in milk production between the different treatment groups (Table 6).

Table 6 Average daily milk production of cows receiving different precalving DCAD

	CONTROL	LOW	HIGH	BOTH	SED	P
Milk yield (L)	29.4	28.4	27.8	29.6	1.69	0.59
Fat %	4.32	4.15	4.35	4.30	0.210	0.78
Protein %	3.23	3.24	3.22	3.21	0.071	0.99

Discussion

For the use of anionic salts to be successful as a milk fever prevention strategy, the DCAD must be below 0 mEq/100g DM in order to reduce the blood pH. Perennial ryegrass/cocksfoot pastures have a high CAD in late winter/early spring, in this experiment it ranged from 57 to 68 mEq/100g with the pasture hay at 27 mEq/100g DM. The amount of anionic salts required to reduce the DCAD to the necessary level make this an impractical solution when pasture (and hay) make up the bulk of the diet.

However, there was an increase in the calcium to creatinine ratio in both the LOW and BOTH treatment groups which indicates that there was an increase in calcium absorption. As this was not due to the reduction in DCAD and blood pH, it indicates that the magnesium or the associated anions are having an impact on calcium absorption. This is supported by the meta-analyses carried out by Lean et al. (2006) and Charbonneau et al. (2006) which found that while there was a linear relationship between DCAD and the incidence of milk fever, the relationship between DCAD and urine (and blood) pH is curvilinear.

Conclusions

1. The use of anionic salts to reduce DCAD as a milk fever prevention strategy does not work on pasture based systems. The high CAD of pasture and its variable nature make this an ineffective, at best, and dangerous, at worst, solution to the prevention of milk fever.
2. The use of magnesium salts, magnesium sulphate and magnesium chloride, do increase calcium absorption and cows should be supplemented with these salts from 2 weeks prior to calving.

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