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Author/s:

Bani Hassan, E;Nouri, M;Vogrin, S;Pyman, M

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Corresponding author

Name: Ebrahim Bani Hassan

Email: bani@unimelb.edu.au

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Can neutral dietary cation–anion difference (DCAD) decrease occurrence of clinical periparturient hypocalcaemia in dairy cattle?

E Bani Hassan,^{a,b,*} M Nouri,^c S Vogrin^a and M Pyman^b

*Corresponding author

^aAustralian Institute for Musculoskeletal Science and Department of Medicine – Western Health, Melbourne Medical School, The University of Melbourne, Australia

^bFaculty of Veterinary and Agricultural Sciences, The University of Melbourne, 250 Princes Hwy, Werribee, Victoria 3030, Australia; bani@unimelb.edu.au

^cDepartment of Clinical Sciences, School of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran

Background Adjusting the dietary cation–anion difference (DCAD) is one of the most efficient ways to stimulate calcium homeostasis in periparturient dairy cattle. However, adjusting DCAD to the recommended negative values (–100 to –150 mEq/kg) is associated with decreased food intake and metabolic acidosis. The critical conditions of the animals at peripartum (i.e. drastic hormonal changes, decreased appetite and negative energy balance) can be detrimental to the health, productivity and welfare of the animals if combined with decreased feed intake caused by unpalatable acidogenic salts.

Methods In a cross-sectional study, we analysed the ration of eight small to large dairy herds with intensive husbandry systems, including 6949 dry cows. Sodium, potassium, chlorine and sulfur concentrations in the feed were determined and DCAD was calculated.

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The DCAD of the ration of the farms ranged from –33.5 to +24.7 mEq/kg. Parturient paresis (PP, or milk fever) prevalence was investigated and correlated to DCAD values.

Results Clinical PP occurrence in the dairies of this investigation on average declined by 87% (ranging from a 97% decline to 5% increase). This indicates that adjusting DCAD at neutral values (0 ± 30 mEq/kg range) may both lower PP prevalence and increase ration palatability by lowering acidogenic salts in the ration.

Conclusions Further research is recommended to investigate the effects of neutral DCAD on subclinical hypocalcaemia and food intake of the cattle.

Keywords calcium homeostasis; dairy cattle; dietary cation–anion difference; hypocalcaemia; parturient paresis; milk fever

Abbreviations DCAD, dietary cation–anion difference; PP, parturient paresis; PTH, parathyroid hormone; RR, relative risk; TMR, total mixed rations

Parturient paresis (PP; parturient hypocalcaemia or milk fever) is a clinical disease of domestic ruminants, especially dairy cows, which is caused by an inability to maintain calcium homeostasis, because of a sudden increase in calcium demand at parturition.^{1,2} Its significant consequences can range from malaise and a severe drop in milk production to downer syndrome and death, if it not treated, or, in cases of no response to treatment, because of other periparturient complications.^{1,2} Additionally, subclinical hypocalcaemia reduces feed intake, which can further complicate the already complex periparturient metabolism and reduce milk yield.^{1,3}

Negative energy balance combined with reduced calcium available for cellular activity can predispose cattle to other metabolic and infectious disorders. A causal relationship has been drawn between subclinical hypocalcaemia and ketosis, abomasal displacement, dystocia, uterine prolapse, udder oedema and decreased immune function, leading to infections such as mastitis and metritis.^{4–9} Subclinical hypocalcaemia, compared with normocalcaemia, is associated with increased dystocia, retained placenta, ketosis and mastitis by odds ratios of 6.5, 3.2, 8.9, and 8.1, respectively,¹⁰ in addition to impaired neuroendocrine and metabolic systems, impaired immune cell function, immunosuppression, decline in plasma zinc concentration (an essential cofactor for many metabolic, signal transduction and transcription factors) and even neonatal hyperthyroidism and hypoinsulinemia.^{2,11–14}

Direct and indirect costs of every clinical case of PP can add up to US\$334.¹⁵ However, losses from subclinical hypocalcaemia are estimated to be several times more than clinical

cases in a herd.^{16,17} Therefore, prevention of hypocalcaemia in periparturient animals has significant financial and welfare importance.

Although only 5–10% of periparturient cows may exhibit clinical signs of PP, without intervention up to 54% of all mature cows can be subclinically hypocalcaemic at parturition.^{17–19} However, there are reports of clinical PP prevalences greater than 30%, with high proportions of cases of animals being unresponsive to calcium therapy (16.7%) and significant numbers of deaths (8.6%).²⁰

An effective and less labour-intensive method to control clinical and subclinical hypocalcaemia is the adjustment of so called ‘strong’ or ‘fixed’ ion concentrations. These are cations and anions that exist as charged particles dissociated from their partner ions in physiological milieus (e.g. extracellular fluids) and drive the pH of the medium. Adjustment of such ions in the ration in such a way that the dietary cation–anion difference (DCAD) favours the amounts of anions (e.g. Cl and S) can activate calcium metabolism pathways. The calculation of DCAD involves adding together the milliequivalents of dietary cations (Na + K) and subtracting the sum of the milliequivalents of dietary anions (Cl + S). With higher levels of strong anions, DCAD will tend to be more negative, whereas higher levels of strong cations will deliver more positive DCAD values.

A negative DCAD has been shown to have an effect on calcium metabolism. It can induce a more acidic intestinal pH for calcium absorption and facilitate recruitment of bone reserves, increase the excretion of calcium in urine and consequently activate calcium reabsorption and recruitment processes, increase production and/or receptor receptiveness to 1,25(OH)₂ D₃ and parathyroid hormone (PTH).^{11,21–25} Another proposed mechanism is increased release of calcium from endoplasmic reticulum reserves in response to cellular acidosis.²⁶ A non-acid/base role has also been suggested for sulfur and chlorine in periparturient calcium homeostasis.²⁷

Several controlled experiments on DCAD and its effects on blood and urine elements and the metabolic status of cattle using small sample sizes (usually < 40) are available.^{28–32} However, there are few applied studies of the on-farm usefulness of the method in preventing hypocalcaemia, especially in intensive dairy farms.

Adjusting DCAD to the recommended negative values (e.g. at –100 to –150 mEq/kg) makes the feed unpalatable, leading to decreased feed intake,^{2,33} with serious consequences related to negative energy balance. Additionally, diets high in magnesium sulfate (which is

widely used as an anionic salt to acidify rations) have a mild laxative effect and can interfere with digestion and absorption of the feed.³⁴ However, the benefits of using magnesium salts include the induction of mild acidosis, as well as providing magnesium, both of which are critical in calcium metabolism in the transition period^{2,35} and unpalatability can be minimised by feeding bitter anionic salts (e.g. ammonium chloride) with grains, total mixed rations (TMR) and flavourings. Empirically, feeding anionic salts with molasses and pulps has proven to be well-tolerated by cattle. Using lesser amounts of bitter acidogenic salts can make the feed more palatable and decrease the costs.

We hypothesised that adjusting DCAD to a value near zero would be sufficient for lowering PP prevalence to levels comparable to those with more negative DCAD values.

Materials and methods

In this observational cross-sectional study, the study population consisted of 6949 dry Holstein dairy cows (9–4166 on each farm) from eight intensive dairy farms (a total milking cow population of 15,967; range 39–10,466 milking cows on each farm), located in Azerbaijan–Iran.

The inclusion criteria for the farms were: having a recording system for diseases, having ration adjustment by a specialist nutritionist or veterinarian according to the US-NRC protocols³⁶ and having used the same prepartum diet for at least 6 months. A brief questionnaire was designed and filled in at sampling visits to acquire farm information, the feeding system used and recent changes in management.

The diets fed on the eight farms were sampled. Five farms used a TMR feeding system and the rest fed the silage and/or concentrate separately (non-TMR) for production as well as transition rations. Non-TMR rations were collected at farms and mixed in the laboratory according to the nutritional program of each farm.

The investigators were blinded to the origin of the feed during the study period. Over a 4-month period, 2–3 samplings were carried out. The samples were submitted to a certified commercial laboratory for analysis of sodium (Na), potassium (K), chloride (Cl) and sulfur (S) concentrations. The Na and K contents of rations were measured using atomic absorption (Konic 210, VGP, Spain); Cl was measured in the presence of other halides with the precision of 2 ppm; and S was determined using sodium nitroprusside with the precision of 1 ppm.

The DCAD was defined as the strong ion difference in milliequivalents. We used the most commonly used 4-element equation:^{1,21,28,33,35,37–40}

$$\text{DCAD (mEq/kg)} = (\% \text{Na}^+ / 0.023 + \% \text{K}^+ / 0.039) - (\% \text{S}^{2-} / 0.016 + \% \text{Cl}^- / 0.0355).$$

Cows that displayed clinical signs of hypocalcaemia and completely (non-complicated cases) or partially (complicated or recumbent cases) responded to calcium therapy as given by a veterinarian or animal attendant were included as PP cases. The most common formulations used were a combination of calcium, magnesium and phosphate salts and calcium borogluconate (25%; 250 mL, IV; repeated IV/SC, up to three times, if there was no or incomplete response), under various proprietary names.

The mean prevalence of PP was acquired for the study period (4 months) and the relative risks (RR) of the condition for each farm were calculated and compared with the pre-adjustment prevalence, as reported by the farm's veterinarian or animal attendants. Because the pre-adjustment prevalence data were not accurate for some farms (**Table 1**), the post-adjustment prevalence was also compared to low (4%), mean (6.5%), and high (9%) prevalence percentages as reported previously.^{2,19,35}

Statistical analysis

The relationships between PP prevalence and DCAD value, concentrations of chemical elements (Na, K, Cl and S) and farm size were evaluated using Spearman's correlation. Differences in PP prevalence between farms with positive and negative DCAD and also semi-automated large farms versus small non-automated farms were assessed using the Student's t-test. Estimates of $P < 0.05$ were considered statistically significant. SPSS v.22 and MS Excel 2010 were used for analyses and calculations.

Results

The diets of the farms were adjusted by four different nutritionists or specialist veterinarians. All farms had used DCAD-adjusted diets for less than 5 years.

Based on the responses to our questionnaire, most of the farmers, animal attendants and veterinarians considered that DCAD adjustment had effectively reduced the clinical hypocalcaemia incidence to a non-common condition. Also, according to their subjective assessment, responsiveness of the afflicted animals to treatment had improved significantly and cases of relapsing uncomplicated PP was rare. All farms used ammonium chloride to lower DCAD to the adjusted level.

Negative DCAD diets were fed to cattle in a period starting 28–45 days prepartum. All farms' dietary DCAD values had been adjusted close to neutral within 0 ± 30 mEq/kg (**Table 1**). For 2 of the 8 of farms, the prevalence of PP from the pre-adjustment period was not available. On 5 of the 8 farms PP decreased more than 5-fold and 1 farm (farm 3) did not show any change and was therefore designated an outlier (**Table 1**). On average, after DCAD adjustment the risk of developing clinical PP was 72% lower compared with the pre-adjustment period, ranging from a decline of 97% (farm 8) to an increase of 5% (farm 3). Excluding farm 3 from the analysis resulted in an 87% lower risk of developing clinical PP. The RR of the condition compared with the reported low (4%), medium (6.5%) and high (9%) prevalences^{2,19,35} was 0.61, 0.37 and 0.27, respectively, and 0.59, 0.52 and 0.39 when including the outlier farm (**Table 1**).

The prevalence of PP was not correlated with Na ($\rho = -0.11$, $P = 0.82$), K ($\rho = -0.14$, $P = 0.74$), Cl ($\rho = -0.12$, $P = 0.77$) or S ($\rho = -0.52$, $P = 0.18$) concentration or the DCAD value ($\rho = -0.02$, $P = 0.99$). No difference was detected in PP prevalence in farms with positive ($3.9 \pm 4.8\%$) and negative ($2.9 \pm 1\%$) DCAD (**Table 1**; $P = 0.69$).

Also, no relationship was found between farm size and management system (semi-automated large farms vs small non-automated farms) and PP occurrence (data not shown).

Discussion

Our results suggested that DCAD in the 0 ± 30 mEq/kg range effectively decreased PP incidence in this population and within this range, the DCAD value did not correlate with clinical hypocalcaemia.

Others¹⁹ have also shown that cattle receiving diets with DCAD adjusted to -7 mEq/kg perform better after parturition. Others³⁸ also report that heifers maintained calcium homeostasis at calving, but ate less when fed the -15 DCAD diet. Roche et al.⁴¹ showed that a DCAD of -12 mEq/kg is capable of inducing mild non-respiratory systemic acidosis that theoretically favours calcium recruitment from reserves. The same investigators report that a DCAD of $+16$ mEq/kg increased the urine calcium/creatinine ratio in periparturient cattle, which can be caused by increased serum calcium or increased excretion. A mathematical model predicted that adjusting DCAD at 0 mEq/kg can effectively lower PP incidence and decrease urinal pH.⁴² These studies further support the effectiveness of DCAD adjusted to approximately zero.

Others^{22,43} have found a strong negative correlation between DCAD and peripartum serum calcium concentration, but we did not find such a correlation. Those previous studies included only about 20 animals and used a very broad range of DCAD (+50 to +400 mEq/kg), which is not comparable to our field-oriented study design and DCAD range. Adjusting DCAD to as close as possible to zero appears to be a reasonable approach because it may both increase the palatability of the ration and decrease the risk of hypocalcaemia. However, we recommend further studies to examine the repeatability of this study in other populations and to establish the effects of neutral DCAD on subclinical hypocalcaemia by measuring blood calcium concentrations and the pH of both serum and urine.

To the best of our knowledge, there are no comparable studies of DCAD in a large number of peripartum cows. Even meta-analyses that included several studies had much smaller numbers of cases than in the present study.^{44,45} Additionally, this study was designed to investigate the efficacy of DCAD adjustment over longer periods and under farm conditions rather than being a controlled study of induced hypocalcaemia.

Highly positive DCAD values (e.g. via K supplementation) are important during the production period for rumen health, increased productivity and milk fat, particularly in closed intensive systems.^{46,47} Specific attention should be paid to DCAD measurement and adjustment at all stages of the reproductive cycle and not only during the prepartum period. Some feedstuffs, including quality pastures and handfed roughage, usually provide enough cations for a highly positive DCAD and adding sodium bicarbonate to counter ruminal acidosis in cows receiving high concentrate diets may also prove useful in increasing DCAD during production.

Despite having a completely clinical orientation and being the largest study of its kind, our study is subject to some limitations. Because of the large scale of the study, we could not accurately measure feed intake. However, if the addition of approximately 30% less anionic salts is required for neutral DCAD compared with a DCAD of -100 mEq/kg, theoretically such diets should be more palatable, on the basis that much less bitter salts are added to the ration, and consequently, feed intake should increase. Because the study was retrospective, no control over the conditions of the farms was possible and there were no farms with high or very low DCAD values for comparison. Therefore, we used PP prevalence

data of the same farms prior to the application of DCAD and also the prevalences reported by other studies^{2,19,35} to calculate the RR.

Also, we could not explain the lack of response on farm 3 to DCAD adjustment. However, as this farm had all the selection criteria, we included it and present the results both including and excluding farm 3. Multiple factors could interfere with the efficacy of neutral DCAD on this farm. We did not measure the urinary or serum pH and calcium concentrations, and data were based on clinical PP prevalence only, which was consistent with the clinical design and aims of the study and the original purpose of DCAD adjustment. We also did not measure the concentrations of calcium, phosphorus and magnesium in the transition diets, but we applied the most commonly used formula for DCAD adjustment, which does not account for these elements. However, as the diets were balanced according to the US-NRC protocols,³⁶ we are confident that the calcium, phosphorus and magnesium concentrations were within the recommended ranges. Nevertheless, considering the important role of magnesium and phosphorus in calcium homeostasis and the response of hypocalcaemic cattle to therapy,⁴⁸ future studies can benefit from measuring calcium, phosphorus and magnesium concentrations.

Some experienced animal attendants can identify the change in the behaviour of an animal and treat it with calcium-containing solutions before hypocalcaemia is fully clinically evident. This could interfere with the estimation of the PP prevalence; however, given the similar results from the different farms of various sizes and system practices, this confounding factor does not appear to be of significance to our study. The disparity in the sizes of farms and their dry cow population (9–4166) was beyond our control. However, almost all farms, irrespective of their system and size (from 10s, through 100s to 1000s), uniformly showed the capability of neutral DCAD to lower PP prevalence, which should be a plus for this study. In conclusion, neutral DCAD seemed to be as effective as highly negative DCAD in decreasing milk fever prevalence. Further studies are required to (a) test the repeatability of our results, (b) determine the effects of neutral DCAD on subclinical hypocalcaemia by measuring serum calcium and urinary pH, and (c) test the feed intake and appetite of the periparturient cattle using diets with neutral DCAD compared with those containing high concentrations of anionic salts.

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Conflicts of interest and sources of funding

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Table 1: The concentration of major cations and anions in the ration, and the resultant DCAD for each farm: Relative risks (RR) have been calculated at 4 levels by comparing post DCAD adjustment prevalence to: pre-adjustment prevalence and low, medium and high prevalences reported in other studies.

Farm no.	Av. Production (range) L/day	Ration				DC AD	Clinical PP prevalence after DCA D adjustment (%)	Clinical PP prevalence before DCA D adjustment (%)	RR vs period before DCA D adjustment (%)	RR vs reported low prevalence (i.e. 4%)	RR vs reported medium prevalence (i.e. 6.5%)	RR vs reported high prevalence (i.e. 9%)
		Na (%)	K (%)	Cl (%)	S (%)							
1	21.7 [§]	0.155	0.795	0.53	0.28	-14.6	2.9	16	0.18	0.73	0.45	0.32
2	27.5 (15–55) [‡]	0.123	0.783	0.48	0.32	-33.5	1.8	> 10*	0.18	0.45	0.28	0.2
3 [†]	32 [§]	0.204	1.192	0.64	0.45	14.5	10.5	> 10*	1.05	0.5	1.6	1.2
4	25.5 (12–37.5)	0.356	0.635	0.57	0.27	9.4	0.5	7.5	0.07	0.13	0.08	0.06
5	18.5 [§]	0.171	0.77	0.57	0.29	0.4	4.5	–	–	1.13	0.69	0.5
6	22 [§]	0.225	0.896	0.57	0.37	-29.4	2.7	14.5	0.19	0.68	0.42	0.3
7	24.5 [§]	0.223	0.068	0.54	0.26	-10.6	4.2	–	–	1.05	0.65	0.47
8	21 (8–34)	0.195	1.242	0.59	0.25	24.7	0.3	> 10*	0.03	0.08	0.05	0.03
Av. including farm no. 3	24.1	–	–	–	–	-4.9	3.4	11.3	0.28	0.59	0.52	0.39
Av.	23	–	–	–	–	-7.	2.4	11.6	0.13	0.61	0.37	0.27

exclu ding farm no.3						7						
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*Estimated by the veterinarians, animal carers and farm owners. In RR calculation it was considered =10. [†]The outlier farm in which DCAD adjustment seemingly did not affect PP prevalence. [‡] Heat stress decreased production from >31. [§] No reliable data available