Effects of anionic salts in a *pre-partum* dairy ration on calcium metabolism

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**ABSTRACT**

The effects of anionic salts in the transition diet on serum and urine calcium at calving and on peripartal health, subsequent milk production and fertility performance were studied in a well-managed, high-producing Friesland dairy herd. Over a period of a year, approximately 21 days before the expected date of calving, 28 pre-partum heifers and 44 multiparous dry cows were randomly allocated within parity to 1 of 2 transition diets, designated control and experimental anionic diets. The anionic diet contained the same quantities of the basic transition ration fed to the control group as well as a standard anionic salt mixture containing 118 g NH₄Cl, 36 g (NH₄)₂SO₄ and 68 g MgSO₄ (total 222 g) per animal per day. This reduced the DCAD to -11.68 mEq/100 g dietary dry matter compared to +13.57 for the control diet. Blood and urine were randomly sampled from 7 to 8 animals within each category within 3 hours post-partum. Serum calcium (total and ionised) and creatinine, urine calcium and creatinine and the fractional clearance of calcium were assessed. Relevant clinical, milk production, and fertility data were collected. The total serum calcium (2.07 vs 1.60 mmol/l), serum ionised calcium (1.12 vs 1.02 mmol/l), urine calcium (0.92 vs 0.10 mmol/l) and the fractional clearance of calcium (1.88 vs 0.09 %) were significantly higher (*P < 0.01*) at calving for multiparous cows fed the anionic diet compared to those fed the control diet. In the primiparous cows there were no significant differences in serum calcium levels. However, the urine calcium (1.07 vs 0.43 mmol/l) and the fractional clearance of calcium was higher (1.75 vs 0.45 %) in cows fed the anionic diet (*P < 0.05* and 0.01 respectively). These results illustrated that there were benefits, although no differences were demonstrated with respect to health, milk production or fertility. The supplementation of diets with anionic salts in the last 2–3 weeks before calving has the potential to significantly improve parturient calcium homeostasis.

**Key words:** dairy cow, dietary cation-anion difference (DCAD), *pre-partum* transition diet, serum and urine calcium levels.


**INTRODUCTION**

Dairy cows experience many transitions during their production cycle. Probably the most important phase is the transition from late pregnancy through early lactation. Feeding the dairy cow during the last 21-28 days of the dry period is a challenge, owing to the unique set of nutritional, physiological and metabolic changes that occur during this period. Cows with hypocalcaemia are more likely to contract peripartal problems such as retained placenta, metritis, endometritis, udder oedema, ketosis, milk fever and displaced abomasum. In the past, the traditional method of preventing hypocalcaemia in dairy cattle has been to restrict the dietary calcium intake during the pre-partum period. However, lowering calcium in the diet often does not work well in practice.

The dietary cation-anion difference (DCAD), also termed dietary cation-anion balance (DCAB), dietary electrolyte balance (DEB), strong ion balance etc., rather than high dietary calcium, seems to be a major risk for the development of hypocalcaemia. Manipulating the DCAD of the transition diet of dry cows has been shown to prevent hypocalcaemia and its associated diseases in dairy cows, and has attracted a lot of attention recently in dairy cow nutrition and diet formulation.

The dry period should be regarded as a preparatory period and as an investment for optimal health and performance in the subsequent lactation. The rumen must be adapted gradually to the higher energy diet that will be fed in early lactation. Normal serum calcium levels must be maintained to reduce the risk of diseases associated with hypocalcaemia. The maintenance of a strong immune system and a positive energy balance up to the time of calving is critical. The close-up diet should be formulated as accurately as possible to provide the required nutrients and have a DCAD of approximately -15 mEq/100 g dietary dry matter.

The objectives of this study were to study the effects of anionic salts in the transition or close-up dry cow ration on serum and urine calcium levels at calving, and on peripartal health, subsequent milk production and fertility performance in a well-managed, high-producing Friesland dairy herd.

**MATERIALS AND METHODS**

The animals used in this study were from a Friesland herd totalling 150 cows, characterised by high milk yields averaging 38 kg/cow in milk per day at the time of the study. The herd had an all-year-round calving pattern and breeding was effected by artificial insemination. Routine reproductive, health and milk production data were recorded using an on-farm computer programme (Agrimilk Software Farm). The herd followed a reproductive health programme with scheduled 2-weekly veterinary visits.

The feeding system for the lactating cows was a well-managed total mixed ration (TMR) group-feeding system. The ration for cows newly in milk contained a commercial dairy concentrate, lucerne hay, whole cottonseed, molasses meal, Ergosteris curvula hay, and a trace mineral vitamin premix. The ration was formulated to meet NRC nutrient requirements for an average cow of 600 kg producing 35 kg of milk with a butterfat percentage of 3.5 %. Dietary components were mixed and offered as a complete ration twice daily and animals were fed ad libitum.

During Phase 1 of the dry period, in the first approximately 30 days after drying off, the basic diet fed to dry cows and...
pregnant heifers consisted of Eragrostis curvula hay ad libitum. From approximately day 21 before the expected calving date until calving the cows and heifers received a close-up ration consisting of the diet for cows newly in milk (2.5 kg twice daily per cow/heifer) and ad libitum consumption of Eragrostis curvula hay. It was assumed that each animal consumed 5 kg complete feed and 8 kg hay per day based on a dry matter intake of 2% body mass. The calculated nutrient composition on a dry matter basis was 12% crude protein, 10 MJME/kg, 30% crude fibre, 25% ADF, 40% NDF, 0.8% Ca and 0.3% P. Animals received this ration until calving when they were switched to the normal feeding programme of the lactating cows.

Feed samples were analysed at the beginning of the trial for dry matter, ash, total crude protein, ether extract, and crude fibre by standard procedures (Weende analysis). Samples were also analysed for calcium, phosphate, sodium, potassium, chloride and sulphur by atomic absorption spectrophotometry (OTK Lab, Bethal). From these results the nutrient composition of the diets during the transition phase and for the cows newly in milk were calculated.

From the laboratory mineral analysis on the feeds and the relative percentage values of chloride and sulphur in the anionic salts, the percentages of sodium, potassium, chloride and sulphur by atomic absorption spectrophotometry (OTK Lab, Bethal). From these results the nutrient composition of the diets during the transition phase and for the cows newly in milk were calculated.

Table 1: Calculated mineral concentrations and DCAD in the control and experimental diets.

<table>
<thead>
<tr>
<th>Mineral(%)</th>
<th>Control diet</th>
<th>Experimental diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>0.098</td>
<td>0.096</td>
</tr>
<tr>
<td>K</td>
<td>1.312</td>
<td>1.290</td>
</tr>
<tr>
<td>Cl</td>
<td>0.504</td>
<td>1.087</td>
</tr>
<tr>
<td>S</td>
<td>0.162</td>
<td>0.293</td>
</tr>
<tr>
<td>DCAD</td>
<td>+4.26</td>
<td>+14.71</td>
</tr>
<tr>
<td>Final DCAD</td>
<td>-14.20</td>
<td>-30.62</td>
</tr>
</tbody>
</table>

*a Calculated mineral concentration of the respective diets on a dry matter basis.
*b Meq/100 g dietary dry matter.
*[\((%Na/0.023) + (%K/0.039) – (%Cl/0.0355) + (%S/0.016)\] per 100 g dietary dry matter.

Blood and urine were randomly sampled from 7 or 8 animals within each category within 3 hours post-partum. Venous blood from the coccygeal vein was obtained in two vacutainers, one with Anderson’s fluoride buffer for total serum phosphate and one tube with no additive (serum) for determination of total serum calcium, serum ionised calcium and serum creatinine. The samples were allowed to clot at room temperature for 1 hour, centrifuged, and the serum was separated immediately and stored at -18 °C until assayed by the laboratory.

Urine was obtained by manual stimulation of the vulva or via a urethral catheter (Abelein catheter) and collection of a midstream sample of approximately 30 ml from each cow and stored at -18 °C until assayed.

All the laboratory chemical analysis was performed by the Section of Clinical Pathology, Faculty of Veterinary Science, University of Pretoria.

Ionised calcium was determined by a Nova 8 ionised calcium/pH analyser (NovaBiomedical). All the other chemical analyses were performed by the Technicon RA1000 automated chemical analyser (Technicon Instruments Corporation).

Total serum calcium was determined by the Technicon method SM 4-0161D91 modification of Gitelman. Serum inorganic phosphate was determined according to the modified Fiske and Subbarow phospho-molybdate method: Technicon method SM 4-014D91 modification of Amador and Urban.

Total serum creatinine was determined according to the kinetic modification of the Jaffe alkaline picrate reaction: Technicon method SM 4-0341D91 modification of Rossignol et al.

Urine was analysed for total Ca, total P and creatinine using the previously described automated chemistry analyser utilising a diluent as described by Christopher et al.

Fractional clearance of Ca (FC%Ca) was calculated using the following formula:

\[FC%Ca = \frac{\text{serum Ca × (urinary creatinine/serum creatinine) × 100}}{\text{urinary creatinine}}\]

Relevant data from each cow in the trial were transferred to the Lotus programme.
Results
The total serum calcium (2.07 versus 1.60 mmol/l), serum ionised calcium (1.12 versus 1.02 mmol/l), urine calcium (0.92 versus 0.10 mmol/l) and the fractional clearance of calcium (1.68 versus 0.09 %) were significantly higher (P < 0.01) at calving for multiparous cows fed the anionic diet compared to those fed the control diet (Table 2). In the primiparous cows there were no significant differences in serum calcium concentrations, however, the urine calcium (1.07 versus 0.43 mmol/l) and the fractional clearance of calcium was higher (1.75 vs 0.45 %) in heifers fed the anionic diet (P < 0.05 and 0.01 respectively).

Discussion
The estimated dry matter intake (DMI) and the calculated diet specifications in this study are debatable. Feeding a totally mixed lactation diet and forages separately to dry cows is undesirable because cows will be able to select what they eat. Each cow will formulate her own diet based on preference of feed ingredients and/or social standing in their group. There is a gradual decline in DMI during the transition period, followed by a rapid drop 3-5 days before calving. The most severe depression in feed consumption takes place just before or on the day of calving, varying from 28-40 %. Physical rumen fill is not the sole reason for reduced DMI before calving. The cow experiences accelerated growth of the foetus and new mammary tissue growth and also undergoes a number of complex hormonal and metabolic changes associated with calving and the initiation of milk production that cause a depressed appetite and reduced rumination. The density of all nutrients should therefore be higher in the transition diet to compensate for decreasing feed intake, prevent negative energy balance and subsequent mobilisation of adipose tissue, and meet the nutrient requirements of advanced pregnancy.

The calculated dietary calcium of 0.8 % in this study is higher than the recommended NRC standards of 0.39 %, but corresponds with the recommended higher values for the transition diet. The calculated dietary phosphorus of 0.3 % corresponds closely with recommended standards. Providing adequate magnesium is an important factor that was not determined in this study. Dry cow rations that contain low levels of calcium and phosphorus have been recommended for the prevention of hypocalcaemia. Restriction of dietary calcium stimulates PTH secretion and 1,25 dihydroxy vitamin D synthesis before calving, which will increase active intestinal calcium absorption and bone calcium mobilisation. This will prevent these mechanisms from becoming quiescent and unable to respond to the sudden calcium outflow that occurs at parturition. However, low calcium diets are difficult to formulate because most commonly-used feeds are typically high in calcium and potassium. Eliminating legumes such as lucerne simply because of their high calcium and potassium content is not always possible and low calcium feeds such as maize silage are not always available.

An important determinant of calcium homeostasis is the acid-base status of the cow at the time of parturition. The potential of a diet to affect the acid-base balance (acidogenic or alkalogenic) can be estimated by calculating its dietary cation-anion difference (DCAD). Several equations for calculating the DCAD have been used, and in its most complete form this should include all the physiologically active macro-mineral cations and anions. The equation used in this study is a useful practical, relatively accurate prediction of the DCAD of a diet, although its limitations must be recognised. Because of the lower absorption rates of calcium, magnesium, sulphur and phosphates, their inclusion in practical expressions has been limited. Sodium, potassium and chloride (strong or fixed ions) are thought to exert the strongest ionic effects on acid-base balance in biological fluids.

However, many researchers include sulphur in the DCAD calculation, because although sulphur is not a fixed ion, and influences the metabolic balance. Therefore, the DCAD calculation can be used to predict the acid-base balance of biological fluids. An important consideration is that the DCAD calculation does not take into account the acid-base balance of the rumen, which is the most important organ for acid-base balance.
The calculated DCAD of +13.57 mEq/100 g dietary dry matter in this study (Table 1) of the basic diet fed to the control cows corresponds with the typical DCAD of diets fed to dry cows of about +5 to +30 mEq/100 g of dietary dry matter. All typical feed sources are high in cation content, particularly potassium. Transition diets commonly have a positive DCAD, and the dietary strong cations induce a slight metabolic alkalosis, which tends to cause hypocalcaemia. The relationship among the DCAD, calcium metabolism, and parturient hypocalcaemia have been outlined and reviewed. Although the significance was not determined it should be noted that there was a large difference in the serum calcium levels between primiparous and multiparous control on the calvate diet (Table 2). It is well known that breed, age and milk production level are important risk factors for the development of hypocalcaemia in dairy cattle. Calcium can be mobilised from the skeleton more easily in younger cows. The low serum calcium level of the multiparous control animals on the cationic diet (Table 2) illustrates that many cows experience some degree of hypocalcaemia during this period. The incidence of milk fever generally increases with parity and with higher levels of milk production and thus higher demands for calcium, regardless of breed. The onset of lactation places a sudden dramatic demand on the calcium homeostatic mechanisms of the multiparous cow. Metabolic alkalosis appears to alter the physiological activity of PTH so that bone calcium mobilisation and production of 1,25 dihydroxy vitamin D are impaired, thus reducing the animals ability to successfully adjust to increased calcium demands. Therefore, dietary Ca is less important as the causative factor in milk fever than the metabolic status of the cow at parturition, which causes a reduced or enhanced sensitivity of the target tissues to Ca-regulating hormones.

The calculated DCAD of the experimental (anionic) diet of -11.68 mEq/100 g dietary dry matter corresponds with the recommendations of various research workers. Optimal acidification generally occurs when anions are added to achieve a final DCAD between -50 to -150 mEq/kg dry matter (-5 to -15 mEq/100 g DM). The use of dietary anions such as chlorides and sulphur in controlling hypocalcaemia has been studied extensively. Addition of any single anionic salt or a combination of anionic salts can achieve a negative DCAD (anionic diet that contains more ions of chloride and sulphur than of sodium and potassium). It has been shown that methods to decrease high K or neutralise its detrimental effects are clearly important and forages with a low K such as maize silage can be very useful in achieving this. Forage management to decrease K and supplementation of diets with chloride and sulphate salts in the transitional period has the potential to significantly decrease the incidence of parturient hypocalcaemia. High potassium forages such as lucerne, when supplemented with anionic salts, are suitable for pre-partum dairy cows. Practical guidelines for balancing for DCAD have been extensively described. Monitoring urinary pH is a simple tool to monitor the DCAD efficacy. An advantage of this approach is that it accounts for inaccuracies in mineral analysis and for variable roughage mineral contents. To be effective, anionic diets should yield urinary pH levels below 6.5.

From the results (Table 2) at the initiation of lactation, it is clear that the anionic diet had a significant effect on serum calcium levels in multiparous cows with no significant effect in primiparous cows. Furthermore, the urinary calcium loss was greater in heifers and cows fed the anionic diet. Manipulating the DCAD of the diet is probably more important in controlling hypocalcaemia than the calcium intake. It is well documented that an effective DCAD diet (excessive anions in relation to cations) can produce mild metabolic acidosis, which increases serum calcium levels and urinary excretion of calcium. Urinary excretion of calcium must follow because serum concentration of total calcium is maintained within the normal range. The metabolic possibilities for increasing the entry of calcium to blood by altering the DCAD during the transition stage have been extensively researched and reviewed. The indirect participation of the ions in kidney function, buffer systems and cellular maintenance is probably responsible for the effects seen when the balance of these ions is altered. The mild metabolic acidosis probably increases the receptivity and responsiveness to PTH, and increases the amount of 1,25 dihydroxy vitamin D, followed by an increase of bone calcium mobilisation and intestinal calcium absorption. Calcium is mobilised from bone as a result of the physiological need to maintain electrical neutrality in the body. This occurs because bone acts as a buffer against excessive systemic acidity by exchanging calcium ions for hydrogen ions from the bloodstream.

Further evidence suggests that a better response to a low DCAD diet occurs when the dietary concentration of calcium is increased. Therefore, high dietary calcium with a low DCAD may be necessary to prevent hypocalcaemia.

In this study, feeding an anionic ration during the transition period did not show any advantages with respect to health, milk production or fertility. Several studies have documented benefits. The lack of response in this study may have been attributable to the small sample size.

CONCLUSIONS

These results illustrate that supplementation of transition diets with anionic salts before calving can significantly improve parturient calcium homeostasis. The importance of proper dry period nutritional management cannot be over-emphasised. If the key dietary components that influence the blood calcium levels are controlled, the incidence of peripartal diseases related to hypocalcaemia can be minimised and losses from decreased milk production and fertility performance will be limited. By becoming familiar with the basic principles of dry period nutritional management, veterinarians can assist their dairy clients in preventing periparturient problems.

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