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 NH_4Cl, 36 g $(NH_4)_2SO_4$ and 68 g $MgSO_4$ (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 *versus* 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.

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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 metabolical changes that occur during this period[15,19,28,34]. The signs of an inadequate transition programme are well-known and include cows with a poor appetite after calving, a greater incidence of post-parturient disorders, excessive loss of body condition after caving, variable milk production peaks and persistence and decreased reproductive performance[9,10,19].

Cows with hypocalcaemia are more likely to contract peripartal problems such as retained placenta, metritis, endometritis, udder oedema, ketosis, milk fever and displaced abomasum[9,10,19]. 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[5,15,22,27,29,37]. However, lowering calcium in the diet often does not work well in practice[15,26,37].

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 dry cow nutrition and diet formulation[2,3,6,11,12,15,20,25,27,37].

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[19,28]. 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[24,34].

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, *Eragrostis 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 %[24]. 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

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

	Control diet					Experimental diet				
	Na	**K**	**Cl**	**S**	**Final DCAD[c]**	**Na**	**K**	**Cl**	**S**	**Final DCAD[c]**
Mineral(%)[a]	0.098	1.312	0.504	0.162		0.096	1.290	1.087	0.293	
DCAD[b]	+4.26	+33.64	–14.20	–10.13	+13.57	+4.17	+33.08	–30.62	–18.31	–11.68

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

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 on a DM basis according to the ration formulation were calculated for the control and experimental transition diets (Table 1). The DCAD of the respective diets was calculated and reported as milliequivalent per 100 g dietary dry matter (mEq/100 g DM). One equivalent weight is equal to the molecular weight divided by the valence or ion charge (one milliequivalent equals 1/1000th of an equivalent)[2–4,6,27]. The equation used for calculating the DCAD was:

[(% Na/0.023) + (%K/0.039)] – [(%Cl/0.0355) + (%S/0.016)] per 100 g dietary dry matter (Table 1).

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 added to the complete feed, containing 118 g NH_4Cl, 36 g $(NH_4)_2SO_4$ and 68 g $MgSO_4$ (total 222 g) per animal per day to provide an excess of chloride and sulphur relative to sodium and potassium. This reduced the DCAD to –11.68 mEq/100 dietary dry matter compared to +13.57 for the control diet. Any experimental animal that calved before consuming the anionic diet for at least 11 days or that calved more than 10 days after her due date was removed from the trial. The animals were weighed and condition scored on day 21 *pre-partum,* day of calving and 30 days *post-partum.* Health and reproductive data for all animals on trial were collected by either the farmer or during the routine fortnightly veterinary visits to the farm. General clinical conditions such as peripartal udder oedema, dystocia (veterinary assisted), prolapsed uterus, retained placenta (>24 hours), parturient paresis and mastitis were noted and treated when they occurred. During the routine veterinary examinations, conditions such as displaced abomasum, metritis and endometritis were diagnosed and treated, the reproductive tract was evaluated for uterine involution and ovarian activity, and other routine procedures such as pregnancy diagnosis were performed.

Milk production and quality data (protein, butterfat and lactose percentages and cow somatic cell counts) were obtained from the National Milk Recording Scheme and reported as 4 % fat-corrected milk (FCM) for a 305-day lactation. Between 60 and 90 days in milk, the projected 305 days fat-corrected milk was calculated using the following formula:

Production total up to date + [(average milk production of the last two milk recordings × 0.85) × (305 –DIM)]

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 (Nova Biomedical). 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 SM4-0161D91 modification of Gitelman[16]. Serum inorganic phosphate was determined according to the modified Fiske and Subbarow phospho-molybdate method: Technicon method SM-4-0144D91 modification of Amador and Urban[1].

Total serum creatinine was determined according to the kinetic modification of the Jaffe alkaline picrate reaction: Technicon method SM4-0141D91 modification of Rossignol *et al.*[30].

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.*[8]

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

FC%Ca = (urinary Ca/serum Ca) × (serum creatinine/urinary creatinine) × 100[13,25].

Relevant data from each cow in the trial were transferred to the Lotus programme

Table 2: **Laboratory results on day of partus.**

Parameters[b]	Experimental diet[a]			Control diet			
	n	$\bar{X}$	SEM	*n*	$\bar{X}$	SEM	*P*
Primiparous							
Serum Ca	8	1.93	0.09	7	2.16	0.06	0.1044
Serum iCa	8	1.19	0.02	7	1.15	0.004	0.0849
Serum P	8	1.53	0.12	7	1.80	0.12	0.1740
Serum creatinine	8	180	41	7	146	8.43	0.8140
Urine creatinine	8	5583	1033	7	8394	2473	0.7564
Urine Ca	8	1.07	0.28	7	0.43	0.13	0.0438
FC%Ca	8	1.75	0.49	7	0.45	0.17	0.0088
Multiparous							
Serum Ca	7	2.07	0.06	7	1.60	0.15	0.0023
Serum iCa	7	1.12	0.02	7	1.02	0.008	0.0003
Serum P	7	1.58	0.14	7	1.61	0.17	0.9029
Serum creatinine	7	134	6.20	7	137	5.52	0.7846
Urine creatinine	7	3864	1036	7	11091	2147	0.7651
Urine Ca	7	0.92	0.20	7	0.10	0.04	0.0006
FC%Ca	7	1.88	0.55	7	0.09	0.04	0.0001

[a] *n* = sample size, $\bar{X}$ = mean, SEM = standard error of the mean, *P* = probability value.

[b] serum iCa = serum ionised calcium in mmol/ℓ; serum P = serum phosphate in mmol/ℓ; serum creatinine = serum creatinine in µmol/ℓ; urine creatinine = urine creatinine in µmol/ℓ; serum Ca = serum calcium in mmol/ℓ; urine calcium = urine calcium in mmol/ℓ; FC%Ca = % of fractional clearance of Ca.

(Lotus 123 release 5 for Windows, Lotus Development Corporation) for summary and analysis. Statistical calculations were done by the Statistical Analysis System[31], an integrated software system with full control over data management, analysis, and presentation at the computer centre of the University of Pretoria. All data were subjected to descriptive statistics. Ordinal observations or data that were not normally distributed, such as condition and mass, blood parameters, somatic cell counts, production data and fertility parameters were statistically evaluated by the Wilcoxon rank test. The non-parametric analysis of Kruskal-Wallis was applied to samples larger than 2. Where the distribution of the data was suitable, the above tests were replaced with the Student's *t*-test or ordinary ANOVA. All incidence data were evaluated by the Fisher exact test or by McNemar's procedure. Within groups the binomial procedure was used.

RESULTS

The calculated mineral concentration and the DCAD of the control and experimental diets are presented in Table I. Major differences between the 2 diets were content of chloride and sulphur owing to the added anionic salts, which reduced the DCAD of the experimental diet to –11.68 mEq/100 g dietary dry matter, compared to +13.57 for the control diet.

There were no significant differences between the treatment groups, within parity, in body condition, body mass, and incidence of peripartal conditions, reproductive performance and milk production. These results were therefore excluded from the report.

The total serum calcium (2.07 *versus* 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 (Table 2). In the primiparous cows there were no significant differences in serum calcium concentrations, 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 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[28]. 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[19,28,34]. 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[34].

The calculated dietary calcium of 0.8 % in this study is higher than the recommended NRC standards of 0.39 %[24], but corresponds with the recommended higher values for the transition diet[34]. The calculated dietary phosphorus of 0.3 % corresponds closely with recommended standards[24]. 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[5,22]. 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[22,27,29]. 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[2,14,26,27,37].

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)[37]. 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[36–38]. Sodium, potassium and chloride (strong or fixed ions) are thought to exert the strongest ionic effects on acid-base balance in biological fluids[25]. However, many researchers include sulphur in the DCAD calculation, because although sulphur is not a fixed ion,

sulphates directly acidify biological fluids and can alter acid-base balance strongly if included at high dietary concentrations[26,33,37].

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[2,27,37]. 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[2,27,37]. 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 animals on the cationic 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[5,29]. Calcium can be mobilised from the skeleton more easily in younger cows. The low serum calcium level of the multiparous control cows at calving 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[5,29]. 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)[2,2,27,37,38]. The use of dietary anions such as chlorides and sulphur in controlling hypocalcaemia has been studied extensively[3,4,11,17,27,33]. 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[27]. 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[14]. Practical guidelines for balancing for DCAD have been extensively described[2,6,7,21,27]. 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[7,21].

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 a negative DCAD diet (excessive anions in relation to cations) can produce mild metabolic acidosis, which increases serum calcium levels and urinary excretion of calcium[2–4,14,23,26,32]. 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[2,4,14,17–19,22,25,35].

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[3,9,19,27]. 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 overemphasised. 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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