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The Effect of Subclinical Hypocalcaemia Induced by Na_2EDTA on the Feed Intake and Chewing Activity of Dairy Cows

S.S. Hansen[1]*, P. Nørgaard[2], C. Pedersen[2], R.J. Jørgensen[1], L.S.B. Mellau[1] and J.D. Enemark[1]

[1]*The Cattle Production Medicine Research Group, Department of Clinical Studies, Dyrlaegevej 88, The Royal Veterinary and Agricultural University, DK-1870 Frederiksberg C, Denmark;* [2]*Department of Animal Science and Animal Health, The Royal Veterinary and Agricultural University, Frederiksberg C, Denmark*

**Correspondence: E-mail: S_Stige_Hansen@hotmail.com*

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ABSTRACT

The effects of induced subclinical hypocalcaemia (SCHC) on feed intake and chewing activity during eating and rumination were studied in dairy cows. Two non-lactating and non-pregnant cows were subjected to three different treatments, with one test per day, such that the plasma free (ionized) calcium ($_F$Ca) concentration was maintained at the eucalcaemic level or at one of two constant SCHC levels. The cows and test days followed a 2×3 crossover design. SCHC was maintained for 7 h by repeatedly infusing 5% Na_2EDTA so that constant $_F$Ca concentrations of 0.8 mmol/L or 0.6 mmol/L in plasma were achieved. Control conditions were achieved by infusing isotonic saline. Feed intake and the number of the rumination periods were recorded during test days. The proportion of feed eaten during each test meal (EatPro) was related to the mean plasma $_F$Ca. An almost linear decrease in EatPro was observed when the plasma $_F$Ca was 0.6–0.9 mmol/L. The cows showed no other clinical signs of hypocalcaemia during Na_2EDTA-infusion. The time spent chewing during eating and rumination, and the number of rumination periods during a test day, decreased with a decline in plasma $_F$Ca concentration.

It was concluded that induced SCHC depresses the feed intake and ruminative activity of dairy cows.

Keywords: chewing activity, dairy cows, feed intake, induced hypocalcaemia, rumination, subclinical hypocalcaemia

Abbreviations: ADF, acid detergent fibre; DM, dry matter; DMI, dry matter intake; EatPro, the proportion of feed eaten during each test meal; $Eptime_{eat}$, the effective time spent chewing during eating; $Eptime_{rum}$, the effective time spent chewing during rumination; $_eJM_{eat}$, the effective rate of JM_{eat}; $_eJM_{rum}$, the effective rate of JM_{rum} during rumination; $_F$Ca, free (ionized) calcium; JM_{eat}, total number of jaw movements recorded during eating; JM_{rum}, total number of jaw movements during rumination; KVL, Royal Veterinary and Agricultural University, Copenhagen; NDF, neutral detergent fibre; N_{rum}, number of rumination periods during a test day; OM, organic matter; PO, pressure oscillation; $Ptime_{eat}$, the time spent eating during TM; $Ptime_{rum}$, the time spent ruminating during a test day; SCHC, subclinical hypocalcaemia; T1, infusion with saline; T2, treatment aimed at 0.8 mmol/L $_F$Ca; T3, treatment aimed at 0.6 mmol/L $_F$Ca; $_T$Ca, total calcium; TM, test meal

INTRODUCTION

All the calcium in plasma or serum is present as ionized calcium. It can be divided into three groups, either associated with ionic binding to small anions or proteins, or not bound, hence the term 'free calcium' (Endres and Rude, 1996). The normal total blood calcium concentration ($_{T}$Ca) for dairy cows lies between 2.19 and 2.83 mmol/L (Kvart and Larsson, 1978), whereas the reference interval for free (ionized) calcium ($_{F}$Ca) in serum is 1.06–1.26 mmol/L (Kvart *et al.*, 1980; Lincoln and Lane, 1990). Cows with calcium values below these ranges may therefore be considered to be hypocalcaemic (Kvart *et al.*, 1982).

In the initial stages of hypocalcaemia, at $_{T}$Ca below 2.0 mmol/L (McKay, 1994; Jonsson *et al.*, 1999) or 1.87 mmol/L (Kamgarpour *et al.*, 1999), cows are asymptomatic, and the condition is therefore referred to as subclinical hypocalcaemia (SCHC). A further decrease in blood calcium will result in signs of hypocalcaemia, for example a depressed appetite, reduced ruminal function and a reduced rate of defecation (Larsson *et al.*, 1983).

The signs observed with naturally occurring hypocalcaemia can be satisfactorily reproduced by intravenous infusion with disodium ethylenediamine tetraacetic acid (Na_2EDTA) (Fenwick and Daniel, 1990; Jørgensen *et al.*, 1999). Experiments conducted on cattle have shown a direct relationship between the concentration of calcium in the blood and smooth muscle contraction in the rumen (Daniel, 1983; Desmecht *et al.*, 1995; Jørgensen *et al.*, 1998). In other words, severe cases of induced hypocalcaemia result in greatly depressed muscle function.

In dairy cows, a gradual depression in dry matter intake (DMI) usually occurs shortly before calving (Coppock *et al.*, 1972; Bertics *et al.*, 1992). This depression is usually of the order of a 30% reduction in DMI, but returns to normal within 2 days of calving (Goff and Horst, 1997). This prepartum reduction in feed intake may be due to physical limitations related to pregnancy, as mentioned by Oetzel and Berger (1985), to endocrine status (Grummer *et al.*, 1990) or to the energy value of the feed (Studer *et al.*, 1993; Vasquez-Anon, 1994).

Moreover, impairment of rumen motility due to hypocalcaemia may exacerbate the prepartum reduction in feed intake, as suggested by Jørgensen and colleagues (1999). Indeed, Marquardt and colleagues (1977) showed that the DMI was lower in cows developing milk fever than that of cows experiencing a normal parturition and postparturient period, whilst Oetzel (1996) concluded that the higher feed intake found in cows supplemented with anions could be indirectly due to an observed reduction in milk fever cases.

In short, these results suggest that parturient SCHC affects feed intake in dairy cows by impairing the function of smooth and striated muscle. However, the periparturient reduction in feed intake, and the observed decrease in rumination around calving (Brydl, 1995), have yet to be directly related to the drop in $_{F}$Ca or $_{T}$Ca known to occur in a large proportion of dairy cows in third parity and above (Hove, 1986; Ballantine and Herbein, 1991).

It was therefore hypothesized that reducing the $_{F}$Ca in blood, leading to different levels of SCHC, would depress the act of eating in cows, as monitored by recordings of

feed intake, rumination behaviour and chewing activity. The objective of this experiment was to study the effect of SCHC induced with Na_2EDTA on these parameters.

SCHC was induced using intravenous Na_2EDTA so as to keep the plasma $_FCa$ depressed but constant over a period of 4 h in two dairy cows (Hansen *et al.*, 2001), thereby allowing comparisons of feed intake and chewing activity between periods characterized by different blood calcium levels. The cows were therefore kept, as near as possible, at a constant plasma $_FCa$ concentration of 0.8 mmol/L or 0.6 mmol/L in order to reduce ruminal contractions by 25% and 50%, respectively (Jørgensen *et al.*, 1998).

MATERIALS AND METHODS

Experimental design

Three different test days, on which one of three treatments was applied, were administered to two cows. The distribution of cows and test days followed a 2×3 crossover design (Christensen, 1996), with three test meals of 1 h duration on each test day. Each cow thereby served as its own control. There was approximately one week between each test day.

Animals

Two non-pregnant, non-lactating dairy cows of similar body weight during the study (691 ± 19 kg one week before the first test day, and 690 ± 1 kg after completion of the experiment) were used. Cow 287 was a Holstein-Friesian (48 months old and at the end of her second lactation), and cow 817 was a Danish Red (66 months old and at the end of her third lactation). The animals had been stabled for more than 3 months prior to the study and were well accustomed to the stables, daily routine and personnel at KVL.

Test days

The treatments on the test days consisted of a continuous intravenous infusion with either saline or a Na_2EDTA solution, from 08:30 to 15:30.

During the control test day, the cows were infused with physiological saline for 7 h (T1). The first 30 min were at an infusion rate of 13 ± 1 ml/min, which was then lowered to a constant infusion rate of 6.5 ± 0.5 ml/min for the remaining 6.5 h.

Two different levels of SCHC (T2 and T3) were induced by infusion of 5% (w/v) Na_2EDTA. A plasma $_FCa$ of either 0.8 mmol/L (T2) or 0.6 mmol/L (T3) was achieved by continuously regulated infusion with Na_2EDTA (Hansen *et al.*, 2001). The first 30 min of infusion was given at a constant predetermined rate of 13 ± 1 ml/min Na_2EDTA. Thereafter, the rate of infusion with Na_2EDTA solution was manually regulated according to the simultaneous $_FCa$ monitoring, using a digital console drive

(MasterFlex, Buch & Holm A/S, 2730 Herlev, Denmark) in order to maintain the desired constant level of $_{F}Ca$ in the plasma, as previously described (Hansen *et al.*, 2001).

Blood sampling

Venous blood samples were obtained at 30 min intervals throughout T1, and at least every 20 min during T2 and T3. The blood was collected through central vein catheters (Secalon® Seldy, Ohmeda, Swindon, UK) and stored in 10 ml sodium-heparin vacutainer tubes (Becton-Dickinson, DK-2605 Brøndby, Denmark).

Blood analysis

The blood samples were analysed in duplicate on a Stat Profile 5 Analyser (Nova Biomedical, Waltham, MA, USA), the intra-assay variation being less than 1.5%, when the range of $_{F}Ca$ was 1.21–1.43 mmol/L, as previously described (Hansen *et al.*, 2000). The time between withdrawal of blood and determination of $_{F}Ca$, as shown on the display of the analyser, was less than 5 min. The infusion rate of Na_2EDTA was subsequently adjusted, if necessary, according to the $_{F}Ca$ analysis.

Feeding

Pre-experimental days. The cows were fed wrapped grass silage for 18 days (day –18 to –1) before the first test day. The bales, which had been stored in a freezer, were defrosted at 5°C before being fed to the cows. Samples of 50 g from each bale of silage used were frozen and later analysed, after 20 h of drying at 80°C, for DM, ash, crude protein (AOAC, 1990), NDF, ADF, lignin (Van Soest, 1963), crude fibre (Tecator, 1978), calcium (King, 1984) and phosphorus (Stuffins, 1967). The *in vitro* digestibility was determined as described by Tilley and Terry (1963). The dry matter content of silage that was refused during the test meals was determined after 20 h of drying at 80°C. The cows had free access to water during the experiment.

From day –18 to –10, the cows were fed *ad libitum* twice daily. During the period from day –10 to day –5, the cows were fed 120% of their previous *ad libitum* intake. This pre-trial period was used to observe the amounts of *ad libitum* intake (Adlib, kg DM) for each cow.

Four days before each test day, the cows were fed, restrictively, 84% of their Adlib intake. On each day from day –4 to day –2, the allocated feed was divided into two meals.

Test days. On both day –1 and on the test day, the feed given was from the same bale. The cows were given three test meals, 14% of their anticipated DMI being offered at the start of each test meal. At 15:30, an additional 42% of DMI was given to the cows.

Test meals. Each test meal was of 1 h duration. The three periods commenced at 09:30, 11:30 and 13:30, respectively. The allotted amount of grass silage was given at the start of each test meal. The residual silage was removed and recorded after each test meal. The percentage eaten compared to the allocated amount (EatPro, %) was calculated as the amount of silage eaten (kg DM) divided by the allocated amount (kg DM).

Between test days. From the day after the test day until 4 days before next test day, the cows were fed *ad libitum* twice daily.

Recording of chewing

A specially designed 'chewing halter' (Schleisner *et al.*, 1999), which was connected to a computer, was placed on the cow 24 h before the start of each test day. This monitored the individual jaw movements before and during the test day. The same chewing halter was always used on the same cow.

The jaw movements were recorded continuously from 09:00 to 15:00. Jaw movements produced pressure oscillations (PO) in a water filled tube placed around the mouth. A pressure transducer recorded the PO. The signals were digitized and sampled at 20 Hz to identify the time and amplitude of each jaw movement (Schleisner *et al.*, 1999).

The time spent eating during each test meal ($Ptime_{eat}$) (min) was observed visually. The actual effective time spent chewing while eating ($Eptime_{eat}$) (min) was corrected for breaks of more than 4 s without any jaw movement. These values were then related to the actual DM intake (kg) at the respective test meal.

The daily total number of jaw movements recorded while eating (JM_{eat}) was used to estimate the effective rate of JM_{eat} ($_eJM_{eat}$) as $JM_{eat}/Eptime_{eat}$ (JM/min).

The number of rumination periods during a test day (N_{rum}), the time spent ruminating ($Ptime_{rum}$), and the daily total number of jaw movements while ruminating (JM_{rum}) were identified on the basis of plots of the pattern of jaw movements, as described by Schleisner and colleagues (1999). The effective time spent chewing during rumination ($Eptime_{rum}$) was calculated as $Ptime_{rum}$ minus the sum of the intercycle times, this being the pause without jaw movements from swallowing a bolus until the next bolus was regurgitated. The effective rate of JM_{rum} ($_eJM_{rum}$) (jaw movement/min) during rumination was estimated as $JM_{rum}/Eptime_{rum}$.

Because only two cows were used in this experiment, the results are presented graphically (GraphPad Prism®, GraphPad Software, Inc., San Diego, CA, USA). They are expressed as the overall mean values $\pm$ SD, unless otherwise stated.

RESULTS

Free calcium

The average values of $_FCa$ obtained from the two cows are shown in Figure 1. A steady state of hypocalcaemia was reached approximately 3 h after the beginning of infusion with Na_2EDTA. Because of this, the desired $_FCa$ levels were not present during the first test meal on treatments T2 and T3.

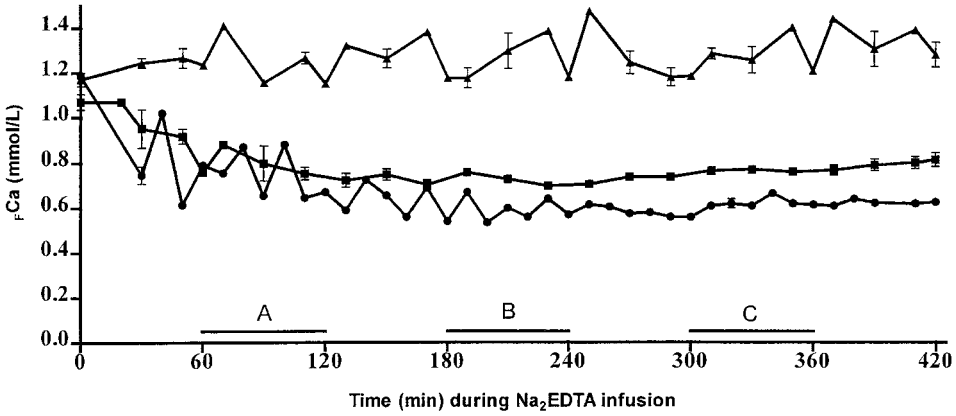

Figure 1. Plasma $_{F}$Ca in two cows during intravenous infusion with either physiological saline (▲, treatment T1), or 5% Na_2EDTA, to achieve a plasma $_{F}$Ca value of 0.8 (■, treatment T2) or 0.6 mmol/L (●, treatment T3), respectively. A: first test meal (from 09:30 to 10:30); B: second test meal (from 11:30 to 12:30); C: third test meal (from 13:30 to 14:30). Each point represents the overall mean of all duplicate measurements taken at that minute. Vertical bars are SEM

Feed intake

Both cows showed normal eating behaviour and ate their allocated silage on the day before each test day. The chemical composition of the grass silage is shown in Table I.

The EatPro values in relation to the overall mean of $_{F}$Ca during the individual test meal are shown in Figure 2. The EatPro value decreased almost linearly with decreasing $_{F}$Ca between 0.9 and 0.6 mmol/L. On treatment T3, only cow 287 ate during the first TM (EatPro = 73%). The relationship between $Ptime_{eat}$ and $EPtime_{eat}$ per kg DM intake was related to T1 and T2 (Figure 3). The observed $Ptime_{eat}$ and $EPtime_{eat}$ on T3 were 17 and 15 min/kg DM, respectively.

Chewing activity

The mean values for $_{e}JM_{eat}$ were 74 ± 3.3 on treatment T1 and 66 ± 8.6 on treatment T2 for both cows. On treatment T3, cow 287 had an $_{e}JM_{eat}$ of 62.

During T1, cows 287 and 817 were seen to ruminate 4 and 3 times, respectively. On treatment T2, cow 287 ruminated three times but cow 817 ruminated only once. Only cow 287 ruminated once on treatment T3.

The average $Ptime_{rum}$ value for T1 was 21 ± 9 min. For T2, the average $Ptime_{rum}$ was 10 ± 6 min, while for T3 the only rumination period observed had a $Ptime_{rum}$ of 6 min.

$Eptime_{rum}$ averaged 18 ± 8 min on the control test days and 9 ± 4 min during T2; cow 287 had an $Eptime_{rum}$ of 6 min during T3.

The effective rates of jaw movements during rumination period, $_{e}JM_{rum}$, are shown in Figure 4.

TABLE I
Chemical composition, fermentation characteristics and *in vitro* OM digestibility (IVOMD) of grass silage

Dry matter (%)	41
DM basis:	
OM (%)	90
Crude protein (N × 6.25) (%)	19.9
Crude fat (%)	3.6
Crude fibre (%)	23.1
NDF (%)	38.4
ADF (%)	25.5
Ca (%)	0.73
P (%)	0.43
IVOMD (%)	72

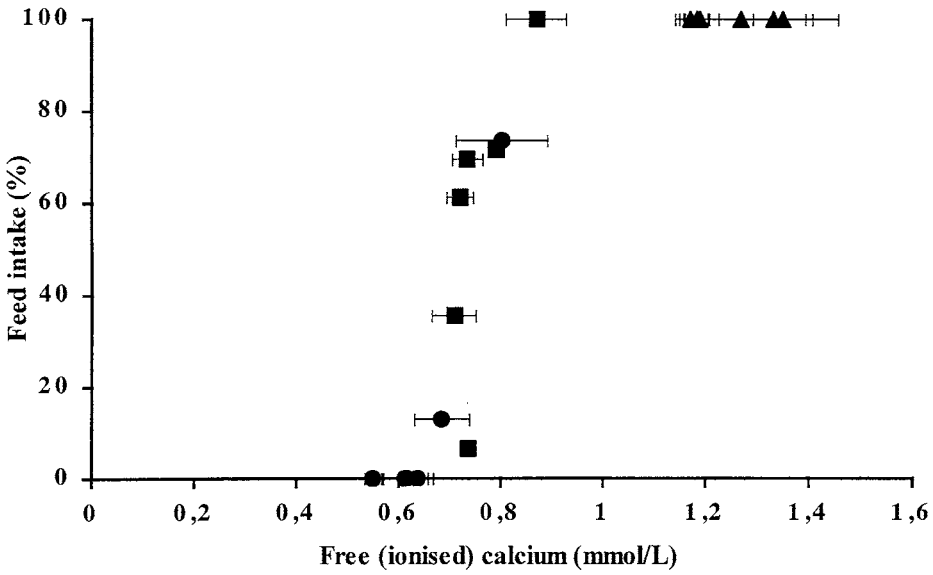

Figure 2. The impact of $_{F}$Ca on feed intake, expressed as the percentage intake of the allocated amount fed (EatPro) in two dairy cows. Free calcium is the average of duplicates (mmol/L) obtained during the specific test meal (TM) shown; horizontal bars are SEM. ■, treatment T1 (the control test day, TM = 6). ▲, treatment T2 (TM = 6). ●, treatment T3 (TM = 6)

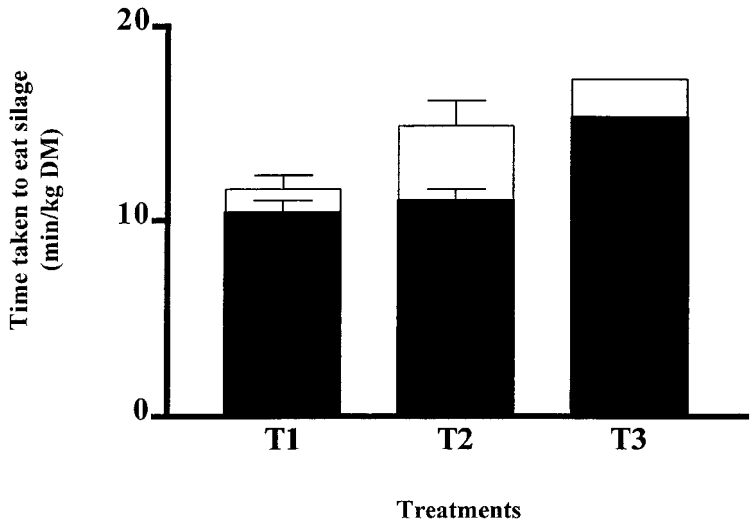

Figure 3. White columns: eating time ($Ptime_{eat}$) (min/kg DM). Black columns: effective eating time ($EPtime_{eat}$) (min/kg DM). T1, infusion with saline during 7 h to mimic normocalcaemia; T2, infusion with Na_2EDTA (attempted $_{F}Ca$ = 0.8 mmol/L) during 7 h; T3, infusion with Na_2EDTA (attempted $_{F}Ca$ = 0.6 mmol/L) during 7 h. Regular JM_{eat} dates were only recorded for cow 287 during treatment T3. Vertical bars are SEM

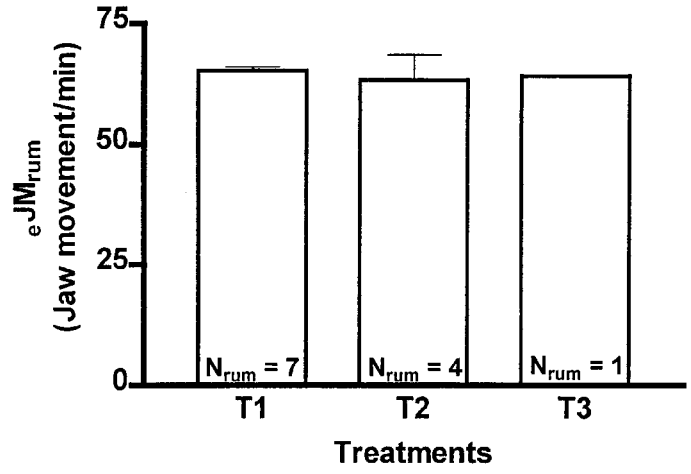

Figure 4. Frequency of jaw movements during the effective rumination time ($_{e}JM_{rum}$) and the number of rumination periods (N_{rum}) during different test days. Vertical bars are SEM

DISCUSSION

Induced SCHC

Inducing hypocalcaemia by intravenous infusions of Na_2EDTA in dairy cows is a well known and accepted method for use in research (Fenwick and Daniel, 1990; Jørgensen *et al.*, 1999). Desmecht and colleagues (1995) observed that the sodium salt used in combination with EDTA can create a slight change in the acid–base balance towards acidosis in calves. In this study, the possible effect of the sodium salt was not considered important, because any slight acidosis was thought to be counteracted by the use of frequent determination of blood plasma $_{F}Ca$ as the overall regulator of the infusion of Na_2EDTA.

Two animals completed all the test days. Similar results were achieved with a third cow but, owing to non-specific illness and reduced feed intake on its control test day, this cow was excluded. Despite this drawback, the use of only two cows may be justified by reason of the similar responses during treatments. Intensive surveillance as well as frequent $_{F}Ca$ determinations during test days excluded the use of many experimental animals in this study.

Feed intake

A reduced feed intake in dairy cows might predispose them to production diseases, such as fatty liver (Bertics *et al.*, 1992). A diminished feed intake should therefore be avoided in the calving dairy cow, if possible.

In this study, the cows ate the allocated silage before each test day as well as during the control test day T1 (EatPro = 100%), even though they were bled three times during each test meal on those days.

The pronounced reduction in feed intake, following the lowering of plasma $_{F}Ca$ concentrations by treatments T2 and T3, implied that feed intake was sensitive to the decline in plasma $_{F}Ca$ concentration, thereby supporting the findings of Marquardt and colleagues (1977). The reduced feed intake was recognizable at $_{F}Ca$ concentrations below 0.9 mmol/L, before the cows had developed other signs of hypocalcaemia. The cows remained without signs of hypocalcaemia during treatment T2, when a reduction in EatPro values of about 25% was recorded.

A marked decrease in feed intake occurred between 0.9 and 0.75 mmol/L $_{F}Ca$ (Figure 2). Indeed, feed intake (EatPro) approached zero when $_{F}Ca$ came close to 0.6 mmol/L. These observations indicate that hypocalcaemia may contribute to the reduction in feed intake *pre partum* observed by Bertics and colleagues (1992) and others. In our study, the cows did not show other signs of hypocalcaemia, apart from the decrease in feed intake and an apparent general depression on treatment T3.

The observed decrease in feed intake in our study, when the $_{F}Ca$ of the cows was in the range 0.7–0.9 mmol/L, may be compared to the first signs in clinically hypocalcaemic cows with similar calcium concentrations, as observed by Larsson and colleagues (1983). As observed in this study, the abrupt cessation in feed intake at an $_{F}Ca$ of about

0.6 mmol/L could mimic the description of severe hypocalcaemia, in which appetite, ruminal contractions and defecation are all absent (Larsson *et al.*, 1983). Their tests were, however, performed on dairy cows with spontaneous, clinically diagnosed milk fever, whereas hypocalcaemia was confirmed in only 84% of these cases (Larsson *et al.*, 1983).

It may be questioned whether a reduction in feed intake of 'only' 25% will be spotted by herdsmen, when cows appear otherwise clinically unaffected. A more pronounced reduction in feed intake of, for example, 50% is more likely to be noticed, even in the standing, non-paretic cow.

The observations from this study showed that, with a reduced concentration of $_{F}$Ca, the mean eating time ($Ptime_{eat}$) (min/kg DMI) was prolonged, but the mean effective time spent chewing during eating ($EPtime_{eat}$) (min/kg DMI) did not differ between eucalcaemia (treatment T1) and an induced SCHC of approximately 0.8 mmol/L $_{F}$Ca (treatment T2). In other words, the $EPtime_{eat}$ values appeared to be independent of the $_{F}$Ca concentration above 0.7 mmol/L. However, the single observation during feed intake on treatment T3 indicated a prolonged $Ptime_{eat}$, as well as the prolongation of $EPtime_{eat}$ compared to the other test days ($_{F}$Ca = 0.88–0.67 mmol/L. This observation may indicate that SCHC below 0.7 mmol/L $_{F}$Ca, which occurred after 40 min during this test meal, was able to produce a prolonged $EPtime_{eat}$, but further studies on this topic seem mandatory.

Rumination

The regurgitation process is highly dependent on precise and coordinated movements between the rumen and the oesophagus (Herdt, 1997). This complicated process is likely to be vulnerable to calcium disturbances because of the effect of hypocalcaemia on ruminal contractions (Jørgensen *et al.*, 1999).

The induction of two levels of hypocalcaemia resulted in a decrease in the number of periods during which the cows ruminated (from 4 periods to 0), with a tendency towards a shorter duration in both $Ptime_{rum}$ and $Eptime_{rum}$ for the rumination periods observed. These findings are supported by the observations of Brydl (1995), who noted a reduction in rumination in dairy cows around calving, but did not relate this to the blood calcium concentration. Hypothetically, hypocalcaemia could reduce the anti-peristaltic oesophageal movements during regurgitation, but the rumination observed appeared normal. The rate of jaw movements during rumination ($_{e}JM_{rum}$) supported this observation.

These results strongly suggest that SCHC depressed the rumination process. This finding is in accordance with the observations of Jørgensen and colleagues (1998), who found that a gradual lowering of plasma $_{F}$Ca caused an initial depression in ruminal contractions, followed by cessation of rumination and finally anorexia. However, our clinical observations during the test days showed that the observed rumination appeared normal. This was consistent with the observation of $_{e}JM_{rum}$, which remained the same during all rumination periods, irrespective of the plasma $_{F}$Ca values during these periods.

Chewing activity

The decrease in chewing movements during eating and rumination may be explained as a result of the general depression of the cows caused by the level of induced hypocalcaemia (Fenwick and Daniel, 1990). However, both cows showed normal behaviour at 0.8 mmol/L Ca^{2+}, and a general depression was not detected in the cows on treatment T2. In contrast, a general state of depression was evident in the cows on treatment T3, making this explanation more applicable.

Desmecht and colleagues (1995) found that the general depression seen in induced hypocalcaemia was not directly due to the infusion with Na_2EDTA but to the stage of hypocalcaemia produced. Therefore, the observed depression in chewing during T2 may be linked to the influence of the induced SCHC. Hypothetically, this could be an effect of SCHC on the appetite regulation centre, or an effect of Na_2EDTA on the same centre, but this cannot be deduced or denied from our experiment.

As the $_{F}$Ca declined from 0.9 mmol/L towards 0.6 mmol/L, the number of recorded jaw movements declined towards zero. Whether this effect on chewing movements was caused directly by the SCHC, or indirectly from the general depression of the cow, cannot be decided from this experiment. It may well have been due to all factors in combination.

The reduction in the activity of both smooth (reduced number of rumination periods, N_{rum}) and skeletal musculature (measured as the number of jaw movements) suggests a high degree of correlation between $_{F}$Ca and muscle contraction. These findings are in agreement with Bowen and colleagues (1970), who demonstrated a reduction in skeletal neuromuscular transmission due to hypocalcaemia in dairy cows. Our results suggest that the smooth musculature in the gastrointestinal system, which participates in the rumination process, is more vulnerable to SCHC than the skeletal musculature, measured as jaw movements.

The changes observed in this study suggested a linear relation between the different test days and chewing variables during $_{F}$Ca levels of 0.9–0.6 mmol/L. For that reason, it seems likely that a parturient reduction in feed intake and chewing activity might be explained by a concomitant SCHC in the dairy cow.

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