Associations between sensor based behavioural parameters and transition disease in dairy cows

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Abstract

The objective of this single cohort observational study was to analyse and determine the predominant periparturient associations of behaviour during the transition period and the onset of hypocalcemia and ketosis in dairy cows based on sensor data and blood tests. For this study data of 490 mainly Holstein Friesian cows from 8 commercial dairy farms in The Netherlands was used. The cows were all equipped with a neck and a leg sensor, attached to the neck collar and one front leg respectively, to measure the following behaviour variables: minutes of eating, -ruminating, -inactivity, -lying and the leg activity (number of steps) per cow per day. The farms were visited weekly and blood samples were taken to determine the calcium concentration (<48h postpartum) and BHBA concentration in the first week (6±3 days in milk) and second week (13±3 days in milk) of lactation. Generalised linear models with a stepwise backward procedure was used to analyse the datasets. Based on this study multiple associations were found. First of all, the average eating, ruminating and inactive time in de three weeks and last two days antepartum had a strong negative association with the calcium concentration. The first two days postpartum the calcium concentration was associated with all behavioural variables. The negative association between de calcium concentration and BHBA concentration, indicates a higher risk of developing ketosis after (sub)clinical hypocalcemia. On average the BHBA concentration in the first week postpartum was 0.034 higher for each 0.1 mmol decline of calcium concentration. For the BHBA concentration in the second week postpartum this is 0.045 higher for each 0.1 mmol decline of calcium. The negative association between the highest measured BHBA concentration within the first two weeks postpartum and the calcium concentration was a rise of 0.047 BHBA for each 0.1 mmol decline of calcium concentration. Average lying time in 6-3 weeks antepartum and 2 days antepartum and the standard deviation in the two days postpartum show a strong association with BHBA concentrations two weeks postpartum. The analysis of the behavioural variables postpartum showed significantly that the behaviour postpartum was associated with antepartum. All of the behavioural variables are associated with BHBA levels in the first week. We can conclude that behavioural sensor data output during the dry period as early as 6 weeks antepartum is associated with the calcium- and BHBA concentrations postpartum.

Keywords: dairy cow, sensor data, transition period, hypocalcemia, ketosis

1. Introduction

The relevance of (sub)clinical metabolic diseases is recognized by the dairy sector. High-yielding dairy cows are on the verge of abnormal homeostasis and the incidence of metabolic diseases increases as the milk yield accelerates (Constable et al., 2017). Therefore, more knowledge of the periparturient predictive variables for the development of (sub)clinical hypocalcemia and (sub)clinical ketosis is of high importance. Different health and activity monitoring systems were developed to support optimal farm management (Rutten et al., 2014). Sensor technology has made it possible to monitor cattle 24/7 and is a tool to help the farmer detect unhealthy cows. Being able to detect the onset of disease in an early stage could help prevent it in the future. To keep track of the calcium- and ketone body concentration may become increasingly important in herd health and nutrition monitoring programs (Oetzel 2004, Rutten et al., 2013).

1.1 Hypocalcemia

The inability of the cow to match the rise in demand of calcium at the end of gestation and the onset of lactation with an adequate mobilisation causes hypocalcemia. This typically occurs within the first 48 hours postpartum (Constable et al., 2017, Oetzel, 2004). The reduction of serum calcium levels usually determines the severity of the clinical signs. The calcium concentration in the blood during hypocalcemia is <2,0 mmol/L, yet can even plummet to 0.5 mmol/L. Subclinical hypocalcemia is defined as total plasma Ca between 1.4 and 2.0 mmol/L without clinical symptoms (Constable et al., 2017).

Besides the primary clinical problems, it is reported that hypocalcemia increases the risk of other postpartum disorders, for example retained placenta, and metritis, which additionally stresses the significance of preventing hypocalcemia to advance cattle health and welfare (Caixeta et al., 2017; Rodríguez et al., 2017; Neves et al., 2018). Also, there seems to be an association between the development of hypocalcemia and ketosis. Hypocalcemia is said to be a predisposing disorder for ketosis/hepatic lipidosis, which is 5.5 times more likely in subclinical hypocalcemic cows than in normocalcemic cows (Rodríguez et al., 2017). Subclinical cases of hypocalcemia and ketosis are quite frequent with respectively 19.9% and 16% prevalence. A combination of the two was even found in 4.5% of the cases (Reinhardt et al., 2011, Sepúlveda-Varas et al., 2015). It is suggested that subclinical hypocalcemia affects the energy balance by compromising appetite and alternating metabolism (Sepúlveda-Varas et al., 2015).

1.2 Negative Energy Balance and Ketosis

There is no other period that affects the performance of the following lactation as much as the dry period (Constable et al., 2017). In the transition period, an increase in energy demand arises in dairy cows caused by foetal growth and lactogenesis, which cannot be met by a sufficient increase of energy intake (Constable et al., 2017). The energy demand is much larger and therefore causes a negative energy balance (NEB). Consequently, the NEB forces the cow to address her energy reserves. Through several compensation mechanisms, the ketone body concentration in the blood will increase due to increased lipolysis. The ketone bodies used as an energy resource are acetoacetate, non-esterified fatty acids and β-hydroxybutyrate (BHBA). The presence of ketones in the body fluid of lactating cows is regarded as a physiological occurrence (Bauman et al., 1980, McArt et al., 2012, Sepúlveda-Varas et al., 2015).

Ketosis is one of the metabolic disorders which develops as a result of a poor adaptive response to the NEB. Ketosis is also known as acetonemia, ketoacidosis or hyperketonaemia and is primarily divided in clinical ketosis (CK) and subclinical ketosis (SCK) (Constable et al., 2017). The symptoms of CK consist of a selective decrease in appetite, milk yield decline, abnormal weight loss, an apathetic impression and the smell of acetone in saliva, milk and/or urine. Ketosis can be diagnosed based on a combination of the clinical manifestations and an increased concentration of ketone bodies in blood, urine or milk (Constable et al., 2017, Iwersen et al., 2009, Oetzel et al., 2004). Ketosis is often a gateway condition for other health problems by increasing the odds of developing for example displaced abomasum, metritis, decreased milk yield and compromised reproductive performance (Duffield et al., 2000, McArt et al. 2012). It is usually associated with problems during the cows' transition from late gestation to early lactation, especially in the first six weeks postpartum (McArt et al., 2012, Najm et al., 2020).

When there is an increase in BHBA concentration in the blood without evident clinical manifestations, it is called subclinical ketosis (SCK). The peak risk of SCK occurs at 5 days in milk (DIM) (McArt et al., 2012). The prevalence of SCK in the first 2 weeks after calving in 528 dairy herds from 10 European countries averaged 22% and ranged from 11-37% based on blood BHBA ≥1.2 mmol/L. However, there are large variations between individual cows regarding the daily BHBA levels and the severity of the (S)CK (Suthar et al., 2013).

1.3 Behavioural parameters in relation to metabolic status

To prevent clinical metabolic disorders, at risk dairy cows should be identified in an early stage. Calcium could be prophylactically administered orally if the at risk cows could be identified (Goff et al. 2008). In order to identify potential hypocalcemic cows, the association between behaviour and the calcium concentration is studied.

Eating and ruminating time is affected by more factors than metabolic status. The eating time reported in different studies is highly variable (Beauchemin, 2018). White et al. (2017) reported a mean eating time of 284 minutes per day (n=182), ranging from 141 to 507 minutes per day, with data collected from 60 published, peer-reviewed papers. It proved to be difficult to compare eating time between studies, since the definition may differ and is highly affected by feed management, DMI, composition of the diet and variability among animals (Beauchemin, 2018). To objectively assess behaviour, automated monitoring systems are used (Barraclough et al., 2020).

Since calcium is an essential mineral for multiple cell functions, including skeletal and smooth muscle contraction, it was theorized that hypocalcemia would affect cow behaviour (Wilkes et al., 2020). However, behaviour directly peripartum is closely connected to pain and exhaustion (Miedema et al., 2011) and subtle changes may therefore not be linked to a reduced calcium concentration (Barrier et al., 2012). A more recent study has found that lying time is elongated by illness, including hypocalcemia, in the days post-partum compared to healthy cows. Moreover, associations were found between subclinical hypocalcemia and behaviour within the first three weeks postpartum (Sepúlveda-Varas et al., 2015). This included fewer eating bouts per day, suggesting a more efficient feed intake per bout. Other studies have identified that there is no association between serum calcium concentration and lying or leg activity (Hendriks et al., 2020).

Multiple studies were done to research periparturient behavioural variables in association with ketosis. Ante- and postpartum dry matter intake are related to the length and severity of the NEB (Butler, 2000). An association between the development of ketosis and standing up longer than average was found (Itle et al., 2015). Measuring steps with an activity sensor attached to the leg was proven not to be very accurate contrary to standing and lying time (Nielsen et al., 2018). Müller et al. (2018) reported increased eating- and ruminating time in grazing cows. Moreover, standing time increased while walking time decreased and no significant effect on lying time was reported. Despite that, others have found that cows which walk more antepartum are less likely to develop ketosis. Sepúlveda-Varas (2015) has found that lying behaviour is affected by illness in general. Grazing dairy cows that developed more than one clinical disease, excluding lameness, spent more time lying and tended to have longer lying bouts in the days post-partum compared to healthy cows. A three year study in Florida found that cows with ketosis were detected 5-6 days earlier based on walking activity and milk yield (Edwards et al., 2004). Despite the fact that they also found that sick cows generally had lower walking activity, cows with ketosis had higher than average activity 8 days before diagnosis. On the other hand a longitudinal pilot study found declined ruminating time and activity levels before subclinical ketosis diagnosis with no significant change in lying behaviour (King et al., 2017). More recent studies showed associations between less motion activity (0-70 DIM) and ketosis (Najm et al., 2020). Moreover, Najm et al. (2020) uses higher activity levels during the dry period as a possible explanation for better metabolization of larger amounts of ketone bodies. Therefore, despite the numerous studies periparturient behaviour remains an uncertain aspect during the onset of ketosis.

1.4 Aim of the study

The objective of this study is to analyse and to determine the associations between predominant periparturient variables regarding the development of hypocalcemia and ketosis in dairy cows on commercial dairy farms based on sensor data and blood tests. It is expected that cows which develop hypocalcemia and ketosis already behave differently during the dry period. Moreover, an association is expected to be found between the calcium- and the BHBA concentration in the blood.

2. Materials and methods

2.1 Farms and animals

For the “Sense of Sensors in Transition Management" study, eight commercial dairy farms across The Netherlands monitor cows all year round. Data collection took place from November 2016 to May 2018, leading to a multi seasonal study period. Every cow at each farm is equipped with two sensors. For this specific sub-study, the sensor data during the transition period was used and the farms were visited weekly. These farms have different breeds, but are mostly Holstein Friesians. Table 1 contains the details of the farms. The individual farms have different managements, feeding techniques, milking techniques and housing. No alterations were made for this study, so the farms represent field conditions. The rations fed consisted of grass- and corn silage with additional concentrates. Moreover, cows of different parity were used and divided in the following groups: first (n=121), second (n=148), third (n=91), fourth (n=60), fifth (n=40) and sixth and more (n=30).

Table 1. Details of the 8 farms used in this study. Per farm the total number of dairy cows are shown, followed by the exact number of cows used in the datasets. The type of breed and the average production in kilograms per cow per year is shown as well.

Farm no.	No. dairy cows	No. cows used in datasets	Breed	Production (kg milk/year)
1	169	57	Holstein Friesian	10299
2	193	68	Fleckvieh	8916
3	124	45	Holstein Friesian	9449
4	135	100	Holstein Friesian	11174
5	131	57	Holstein Friesian	9482
6	104	23	Mixed breed	9060
7	122	100	Red Holstein	9152
8	148	40	Holstein Friesian	8731

2.2 Blood analyses

The calcium concentration was measured in blood serum(n=490). The blood samples were taken from each cow within 48 hours after calving. This was done from the tail vein with a vacutainer into a heparin tube. The collected samples are centrifuged in an Eppendorf Centrifuge 5804 R for 10 minutes on 4500 RPM. The serum was pipetted, put into a small vial, numbered and stored in the freezer at -20 degrees until analysis at the laboratory for the calcium concentration in mmol/L. A quantitative determination of calcium concentration of the serum was performed by Olympus AU680 (Beckman Coulter, Brea A), method Calcium Arsenazo (Limit of Quantitation = 1 mmol/L) with an end point determination of 660 nm (Leary, Pembroke et al., 1992).

The BHBA concentration in the blood was determined with the Precicion Xtra system of Abbott. Monitoring blood BHBA is superior to monitoring urine acetoacetate in urine. Using the Precision Xtra meter to measure BHBA concentration in dairy cows is a commonly used method. This way of cow side testing has very good test characteristics, especially when blood samples are drawn from the tail vessels (Mahrt et al., 2014). According to several studies the sensitivity is 91% and the specificity is 94% for diagnosing ketosis, however, it is not sufficiently accurate to evaluate small changes in blood BHBA that fall below the ketosis threshold (Bach, Heuwieser et al., 2016, Iwersen, Falkenberg et al., 2009). A small blood sample was taken from the tail vein with a 3 ml syringe and a green 21G needle. This was done in the first week post-partum (days 3-9) and in the second week post-partum (days 10-16), hereafter referred to as respectively BHBA1 and BHBA2. One drop (±1,5 microliters) was administered on the Blood Ketone test strips in the Precision Xtra and the BHBA concentration is given in millimol/milliliter within 10 seconds.

2.3 Sensors and variables

The sensors which were used in this research are the Smarttag Neck and the Smarttag Leg produced by Nedap Livestock Management (Nedap, Groenlo, The Netherlands). The Smarttag Neck is a collar-mounted activity sensor which registers eating, ruminating and inactive time per individual cow. The Smarttag Leg is an activity meter attached to one of the front legs, which registers: standing- and lying time and number of steps and stand-ups per cow per day. The recording of activities by the tags is based on accelerometers in a three-dimensional space along x-, y-, and z-axis. The Smarttags were validated by HAS Univeristy of Applied Sciences in Den Bosch, The Netherlands (Van Erp-Van der Kooij, E., Van de Brug et al., 2016). The behaviour variables which were included in this sub-study are the minutes of eating, ruminating and inactivity per cow per day, measured by the neck sensor. The minutes of lying per day and the leg activity (number of steps) per day per cow measured by the leg sensor.

As shown in figure 1 the sensor data is used from 42 days antepartum up till and including 28 days postpartum, with 0 being the calving date. Based on the raw data and the average behaviour, it was decided to divide the transition period in 6 periods of unequal length. Periods A, B and C are antepartum and periods D, E and F are postpartum. The cows' behaviour is clearly changing in the last two days antepartum, whilst a week before it could still be normal. The same goes for the first two days postpartum. These periods were made after a rough analysis of the averages shown in figure 8 in the results. The average number of minutes (or steps) per period per cow was calculated for every behaviour variable. Moreover, the standard deviation per period per cow was calculated for every behaviour. All of the statistics were performed on the averages and standard deviations per period per behaviour per cow. Previously published article included the slope, however, since there was no association this has not been repeated in this study (Hut et al., 2019).

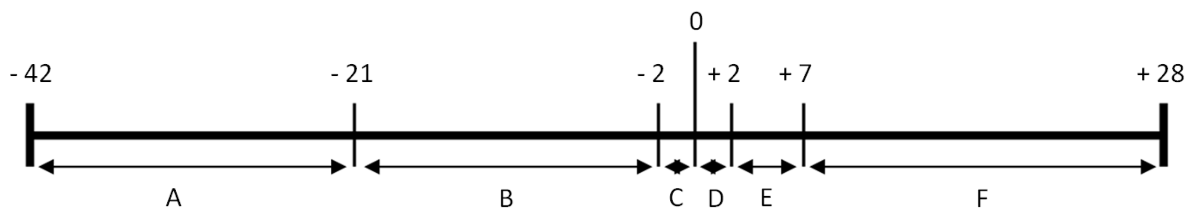

Fig. 1. Division of transition period into six periods A, B, and C are antepartum, 0 is the calving day, D, E and F are postpartum

2.4 Statistical Analysis

All analyses and data manipulations were carried out using the data the program RStudio (RStudio Desktop 1.1.423). The five datasets containing the sensor data for each specific behaviour variable also include the life number, parity and farm number of each individual cow. In order to create a more normal distribution, a log transformation was performed on both the calcium– and BHBA concentrations. The normal distribution after the log transformation is shown in figures 2-4. These concentrations with log transformation were used in the analysis, no cut-off points were included. These datasets were merged to create five datasets containing one behaviour type and the corresponding calcium- and BHBA log transformations.

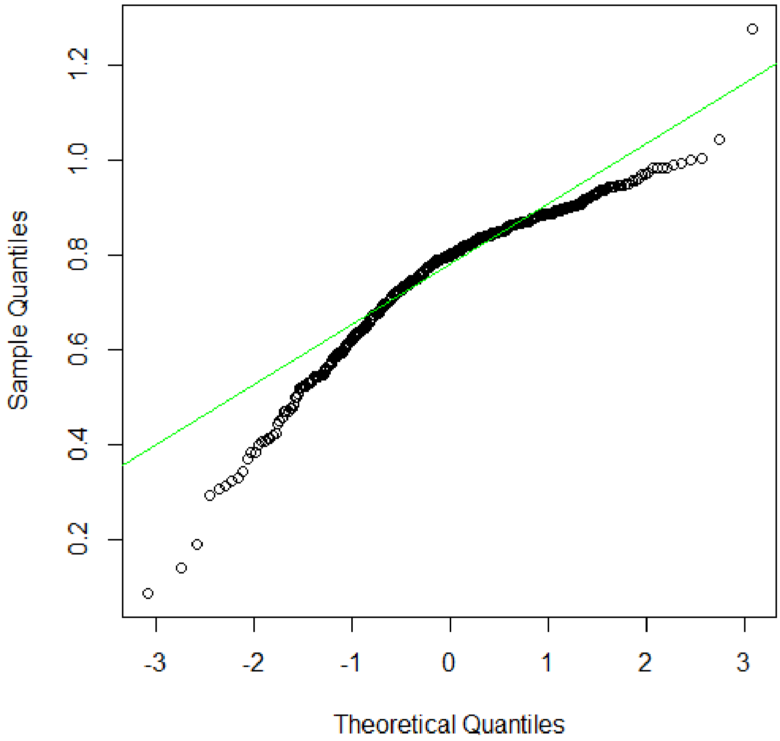

Fig 2. Q-Q plot of the log transformation of calcium concentration in (mmol/L) in blood samples <48 hours postpartum of 8 dairy farms in The Netherlands (n=490)

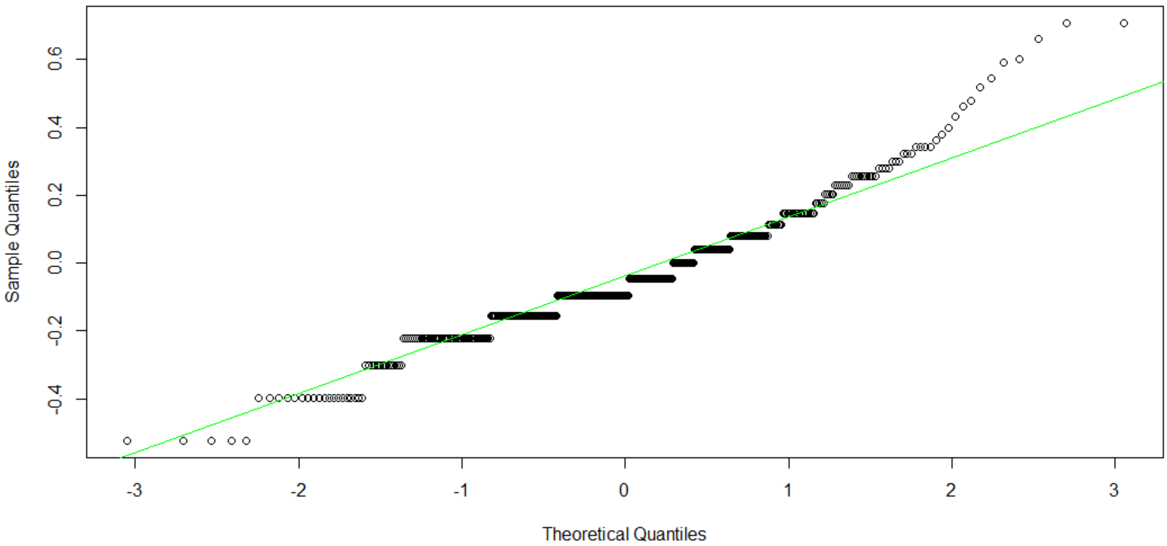

Fig. 3 Q-Q plot of the log transformation of BHBA concentration in (mmol/L) in blood samples 3-9 days postpartum of 8 dairy farms in The Netherlands (n=490)

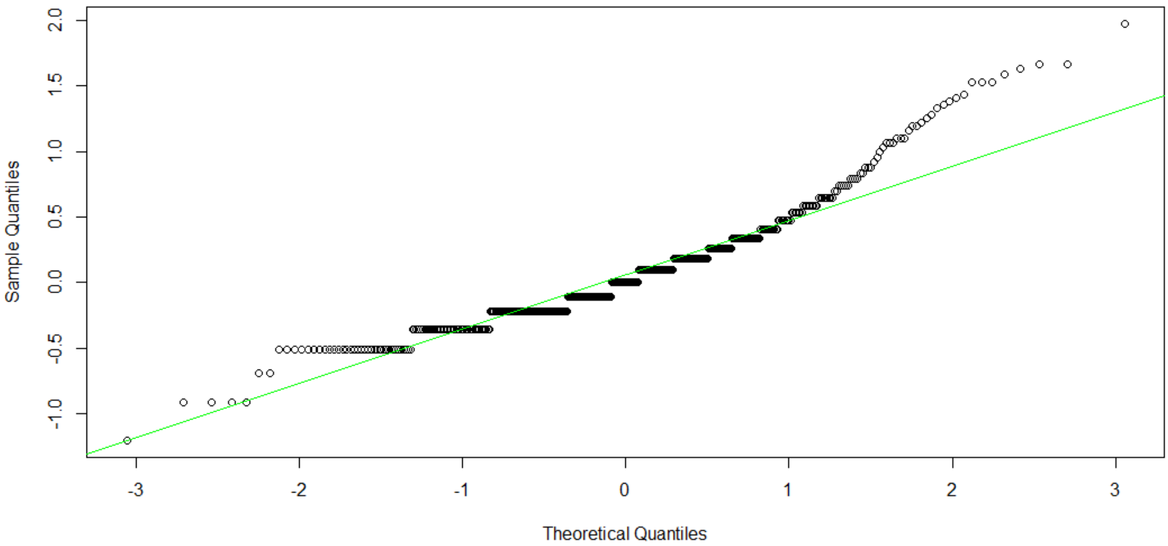

Fig 4. Q-Q plot of the log transformation of BHBA concentration in (mmol/L) in blood samples 10-16 days postpartum of 8 dairy farms in The Netherlands (n=490)

Cow identification was included to account for repeated behavioural measurements per cow. Of the 490 in total, only the cows which had complete datasets to combine with calcium and BHBA concentrations were used in the statistical analysis. Animals that developed clinical disease were not excluded from the study as long as the data was complete. All cows with incomplete cases were excluded, regardless of the reason. Because of this the datasets from the neck sensor contain 373 cows and the datasets of the leg sensor contain 403 cows.

Generalised linear models (GLM) were performed on each of the five datasets. Per dataset seven GLMs were made for the dependent variables of: calcium concentration, BHBA1, BHBA2, BHBA H and the average behaviour postpartum in periods: D, E and F. Moreover, a GLM was made for the associations between just BHBA concentrations and calcium concentration. In order to keep these GLMs correct in comparison to the clinical manifestations, only variables which were obtained before the dependent variable were included. Therefore, not every variable is included in every GLM as shown in table 2. Besides these variables the farm and parity of the cows were included as fixed factors in the GLMs.

Table 2. Division of factors included for every generalised linear model. Only factors obtained before the clinical manifestation of the dependent variable were included in the GLM.

	Calcium	**BHBA 1**	**BHBA 2**	**BHBA H**	**A**	**B**	**C**	**D**	**E**	**F**
Calcium	×	×	×	×	✓	✓	✓	×	×	×
BHBA 1	✓	×	×	×	✓	✓	✓	×	×	×
BHBA 2	✓	×	×	×	✓	✓	✓	✓	×	×
BHBA H	✓	×	×	×	✓	✓	✓	×	×	×
D	✓	✓	✓	✓	✓	✓	✓	×	×	×
E	✓	✓	✓	✓	✓	✓	✓	✓	×	×
F	✓	✓	✓	✓	✓	✓	✓	✓	✓	×

A stepwise backward procedure was performed on each GLM to find which variables had the strongest associations with the formula. Variables with the most significant effect on the formula were selected by the stepwise backward procedure by looking at 'Akaike's Information Criterion' (AIC). The AIC is an extension of the method of maximum likelihood to the multimodel situation. In this model it is assumed that all variables are mutually independent (Akaike, 1987). The stepwise algorithm selects a formula-based model, which resulted in a final multivariable GLM per behaviour per formula. Through this test of significance, the number of variables is stopped when the data does not add a significant improvement fit of the model. Non-explanatory variables were dropped. These final GLMs, with only the significant variables included, form the base of the results. The variables which were not included in the final multivariable GLMs do not show an association with the calcium or BHBA concentration. In order to show these results, a confidence interval for the GLM parameters was computed. The "confint" function was used to show the lower and upper limits of the 95% interval, which was considered significant if it did not include 1. This depicts the reliability of the difference in the averages in log concentrations. Statistical significance was taken as $P \leq 0.05$, trends as $P>0.05$ and nonsignificant as $P>0.1$.

3. Results

3.1 Calcium concentration

The calcium concentration in mmol/L in the serum of 490 blood samples <48 hours postpartum were sorted per parity which is shown in figure 5. The average calcium concentration is higher in the first parity cows with a smaller standard deviation compared to the higher parities.

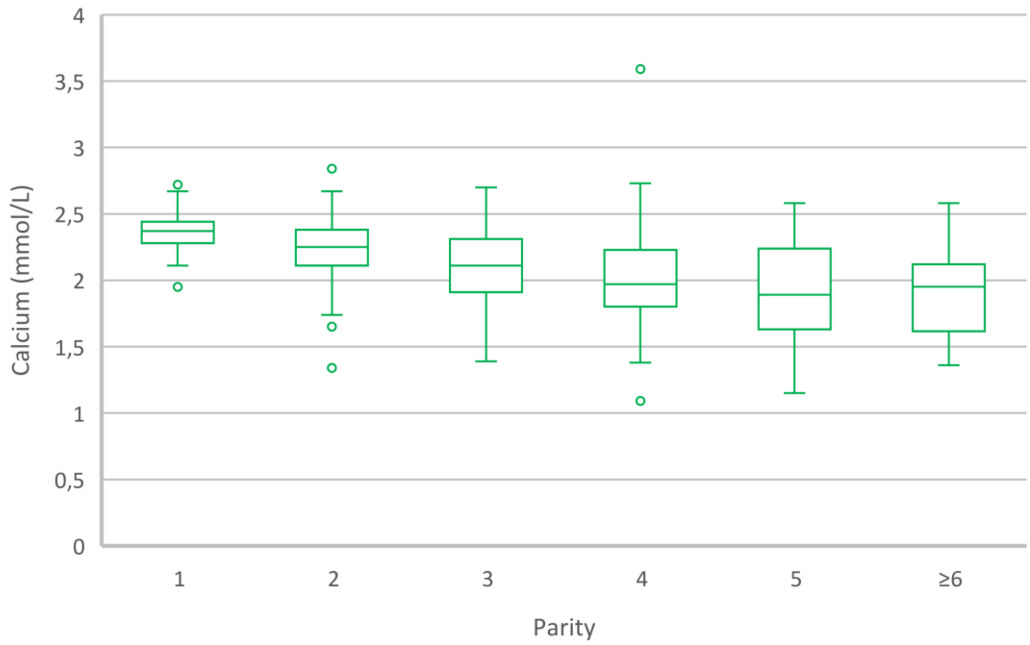

Fig. 5. The calcium concentration (mmol/L) in blood samples <48 hours postpartum sorted per parity of 8 farms in The Netherlands. Parities: first (n=121), second (n=148), third (n=91), fourth (n=60), fifth (n=40) and sixth and more (n=30).

3.2 Calcium related to antepartum behavioural variables

The GLMs with the calcium concentration as the dependent variable provided multiple significant associations. First, a higher parity was associated with a lower calcium concentration. Behaviour averages in the last week antepartum (period B) and the last two days antepartum (period C) were most associated with the postpartum calcium concentration. A higher calcium concentration was associated with a lower average eating time in period B and a higher average eating time in period C. A higher average ruminating time in period C was associated with a higher calcium concentration, and a lower average ruminating time in period B showed a trend. A higher calcium concentration was associated with a higher average inactive time in period B and a lower inactive time in period C. The strength of these associations based on the confidence intervals is shown in table 4 in the appendix. No significant associations were found between calcium concentration and lying time or leg activity in any antepartum period. Moreover, no significant association was found for the different farms for any behaviour.

3.3 BHBA concentration

The BHBA concentration in mmol/L of 490 blood samples <9 days postpartum were sorted per parity which is shown in figure 6. The average BHBA concentration in the first week postpartum is lower in the first parity cows with a smaller standard deviation compared to the higher parities. The BHBA concentration 10-16 days postpartum have also were sorted per parity which is shown in figure 7. The average BHBA concentration in the second week postpartum is lower in the first parity cows with a smaller standard deviation compared to the higher parities.

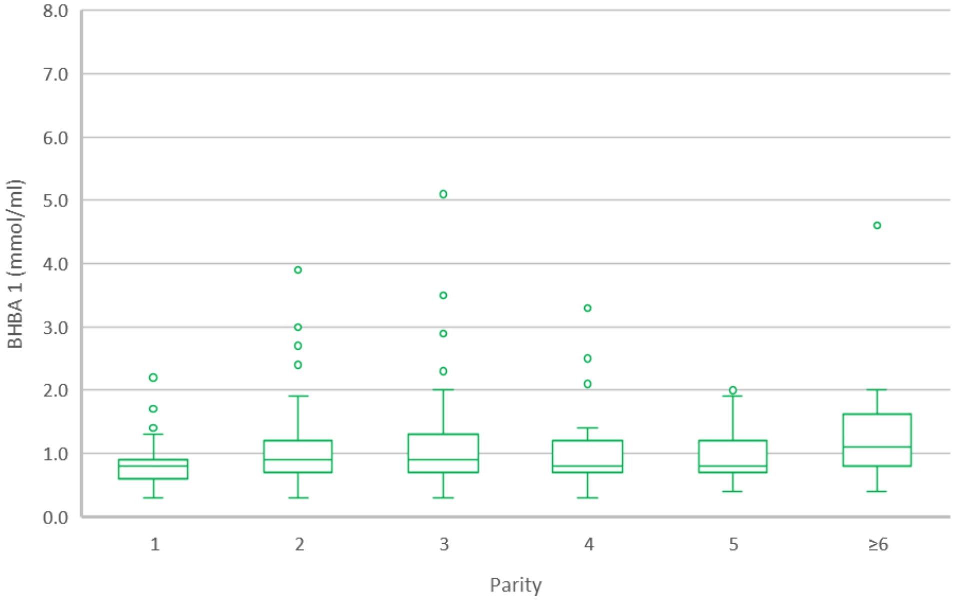

Fig. 6. BHBA concentration (mmol/L) in blood samples 2-9 days postpartum of dairy cows per parity (n=490) of 8 farms in The Netherlands. Parities: first (n=121), second (n=148), third (n=91), fourth (n=60), fifth (n=40) and sixth and more (n=30).

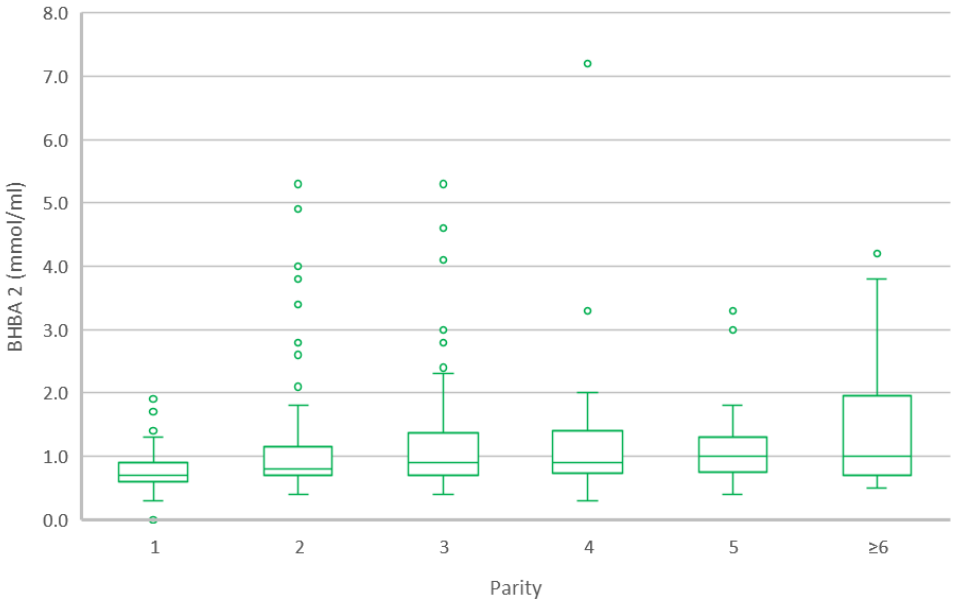

Fig. 7. BHBA concentration (mmol/L) in blood samples 10-16 days postpartum in the blood of dairy cows per parity (n=490) of 8 farms in The Netherlands. Parities: first (n=121), second (n=148), third (n=91), fourth (n=60), fifth (n=40) and sixth and more (n=30).

3.3 BHBA related to calcium concentration

A higher BHBA concentration was associated with a lower calcium concentration. Table 9 shows the values which were found for the association between the BHBA concentration as a result of calcium concentration. The column "Real values" shows the amount of mmol the BHBA concentration will decline when the calcium concentration will rise with 1.0 mmol. On average the BHBA concentration in the first week postpartum is 0,034 higher for each 0.1 mmol decline of calcium concentration with a standard deviation of 0.0156 and a significance of $p<0.01$. For the BHBA concentration in the second week postpartum this is 0,045 mmol higher for each 0.1 mmol decline of calcium with a standard deviation of 0.016 and a significance of $p<0.01$. The association between the highest measured BHBA concentration within the first two weeks postpartum and the calcium concentration is a rise of 0.047 mmol BHBA for each 0.1 mmol decline of calcium concentration with a standard deviation of 0.016 and a significance of $p<0.01$. This association is positive during the first two lactations and becomes a negative association after the third lactation.

Table 3. Association between BHBA- and calcium concentrations of blood samples of dairy cows (n=490) of 8 farms in The Netherlands

	Estimate	**Standard Error**	**Real values (mmol)**
BHBA 1	-0.54	0.19	-0.34
BHBA 2	-0.66	0.21	-0.45
Highest BHBA	-0.67	0.20	-0.47

3.4 BHBA concentration and behavioural variables

The statistical analysis of the BHBA1 levels showed a strong association with the calcium concentration, which returns in every GLM. For the BHBA concentrations there is an association with the farm regarding all behavioural variables. A higher BHBA concentration was associated with a lower average eating time in period A and showed a trend with a higher standard deviation in period C. Moreover, a higher BHBA was associated with higher average ruminating time in period A, a lower average in period B and a trend with a lower standard deviation of ruminating time in period C. Also a higher average inactive time in period B was associated with a higher BHBA concentration and a lower standard deviation in period A showed a trend. Furthermore, there is an association between a higher average lying time in period B and a lower standard deviation in period A shows a trend. A higher BHBA was associated with a lower number of steps in period A and a higher number of steps in period D. A trend was shown between higher BHBA with a higher average of steps in period C and a lower standard deviation of steps in period C. The strength of these associations is shown in table 5 of the appendix.

The statistical analysis with BHBA 2 as the dependent variable showed different results. A less significant association was found with the calcium concentration than with BHBA1. For BHBA 2 there is an association between the concentration and the farm where the cow is at for all behaviours. A higher BHBA 2 was associated with a lower standard deviation of eating time in period A and showed a trend with a lower average eating time in period B. A higher BHBA 2 was associated with lower average ruminating time in period C. Moreover, a higher BHBA2 was associated with a higher inactive time in period B and trends were found with the averages of periods A and C and the standard deviation in period B. Furthermore, the higher BHBA2 was associated with a higher average lying time in period A and lower in C. A trend was shown between BHBA2 and the average lying time in period C. The lower average number of steps in period A, the higher in average in period C and the higher standard deviation of steps in period D were associated with a higher BHBA2. The strength of these associations is shown in table 6 of the appendix.

From the GLMs with the highest BHBA as the dependent variable the following results were found. Firstly, all GLMs have the farm included as fixed factor. The parity factor was only included in inactive time, lying time and leg activity. An association was found with a lower calcium concentration. The association between BHBA 1 and 2 was not included in the analysis. The lower average and lower standard deviation of eating time in period A show an association with a higher BHBA. The standard deviation of eating time in period C shows a trend. A higher average ruminating time in period A and a lower average in period B are associated with a higher BHBA and a trend is shown with the standard deviation of ruminating in period C. Furthermore, associations were shown between a higher BHBA and a higher average inactive time in period B, and trends for the average in period E and the standard deviation in period C. A higher average lying time in period A and lower in period E were associated with a higher BHBA, as well as the higher standard deviation in period A. A trend is visible with the standard deviation of lying in period E. A higher average number of steps in period A, a lower average in period C and a higher standard deviation of steps in period D show an association with a higher BHBA.The strength of these associations is shown in table 7 of the appendix.

3.5 Postpartum behaviour related to antepartum behaviour

The behaviour variables which were included in this sub-study are the minutes of eating, ruminating and inactivity per cow per day, measured by the neck sensor. The minutes of lying per day and the leg activity (number of steps) per day per cow measured by the leg sensor. The averages per day of all the cows combined are shown in figure 8. This figure shows that the antepartum cows behaviour is overall quite constant during the dry period, followed by a radical change at day -2. Generally the behaviour variables remain abnormal the first 2 days postpartum, after which it gradually normalises to the antepartum level in the period from +7 to +28 days.

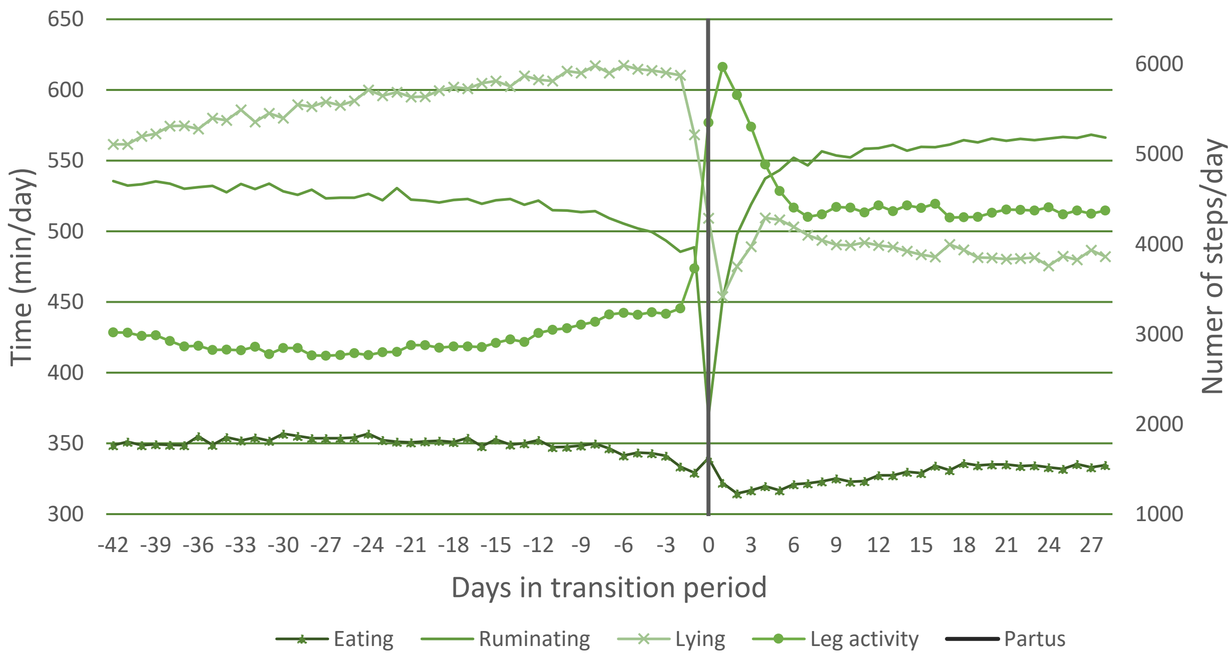

Fig. 8. The course of behaviour in average minutes or steps per day during the transition period of dairy cows (n=1002) in which 0 is day of calving of 8 farms in The Netherlands

As shown in figure 8 the antepartum eating time averaged around 355 minutes per day, reduced to 315 at calving and stabilized around 330 minutes three weeks postpartum. Average ruminating time was 525 minutes per day antepartum, 375 at calving and rising to around 570 minutes per day on average three weeks postpartum. Lying time is a steady rise of antepartum lying time from around 9 hours to more than 10 hours a day. After a serious decrease to 7,5 hours a day at calving it stabilizes around 8 hours per day three weeks postpartum. The number of steps are around 3000 steps per day in the antepartum period, which doubles directly after calving and stabilizes around 4400 steps per day after the first week postpartum.

The statistical analysis of the behavioural variables postpartum (periods D-F) showed significantly that the behaviour postpartum was linked to antepartum. In period D the calcium concentration is associated with all behavioural variables. In period E most of the variables are associated with BHBA levels in the first week and in period F with the highest BHBA concentration. The strength of these associations is shown per behaviour in tables 8-12 of the appendix.

4. Discussion

The aim of this study was to analyse the associations between sensor based behavioural parameters (feeding-, ruminating- and lying behaviour and number of steps) and transition disease in dairy cows. Associations were found between calcium concentration in the blood and behaviour during the dry period. Moreover, an association between calcium- and BHBA concentration was analysed. Associations were found between BHBA concentration in the blood and behaviour during the dry period. Lastly, associations were found between calcium- and BHBA concentrations and behaviour postpartum.

4.1 Calcium related to behaviour antepartum

In our research a higher calcium concentration was associated with the average eating, ruminating and inactive time in the last week antepartum. Similar results have previously been reported by Jawor et al. (2012), in which cows (n=15) with subclinical hypocalcemia (serum calcium concentration ≤1.8 mmol/L), had a higher dry matter intake during the last two weeks antepartum in comparison to control cows. The increase of dry matter intake and average eating time in the weeks antepartum cannot be explained.

No significant associations were found between calcium concentration and average lying time or leg activity in any antepartum period. These results are in line with previous research which found no association between calcium concentration (<24 hours postpartum) and lying time or step count in the week antepartum (Barraclough et al., 2020, Jawor et al., 2012). Other research found similar differences in antepartum behaviour related to calcium concentration, however, only regarding lying and steps on the last day antepartum (-24 h to 0 h) (Hendriks et al. 2020). Jawor et al. (2012) found cows with subclinical hypocalcemia stood 2,6 hours more on the day before calving compared with control cows.

4.2 Calcium associated with ketosis

An association between a calcium concentration and a BHBA concentration was shown, especially in the first week postpartum. This could be explained by a decrease in dry matter intake due to hypocalcemia, leading to a bigger NEB and secondary (sub)clinical ketosis. Interestingly, association with the lower calcium concentration is with a higher BHBA during the first two lactations and turns to an association with lower BHBA after the third lactation. To our knowledge this association has not been found in previous research. This association could either indicate that cows with better resilience become older or that older cows are more resilient, however, based on this study no differentiation can be made between these theories.

4.3 Ketosis related to antepartum behaviour

Our results indicate that less eating time antepartum leads to higher BHBA levels postpartum, which is consistent with another study where less eating time before calving is associated with the development of subclinical ketosis (Goldhawk et al., 2009, Schirmann et al., 2016). Moreover, our research showed associations between higher BHBA concentrations and less ruminating time from the beginning of the dry period up to and including the last days antepartum. The associations with the standard deviations of ruminating time suggest more variety in behaviour on cow level is related to the onset of ketosis. These results are in line with other studies, indicating less ruminating time antepartum is associated with the development of subclinical ketosis postpartum (Schirmann et al., 2016) King et al. (2017) reported a decrease in ruminating time starting 6 days before the development on subclinical ketosis. Feeding- and ruminating time and -number of bouts are related to the actual amount of food intake or DMI (Halachmi et al., 2016). However, there is an individual difference between cows ranging from 150-230 g/min DMI. Including feeding behaviour in DMI models, makes the model more accurate. Reduced DMI is a well-recognized sign of the development of clinical ketosis in dairy cattle, 10kg/d less for sick animals and 3kg/d for SCK during the week before diagnosis (Duffield et al., 2000, Goldhawk et al., 2009).

Higher BHBA concentrations were associated with a higher average lying time in the last week antepartum. These results are in line with Itle et al. (2014), who reported a longer total daily standing time for 15 clinically ketotic cows (at least 3 consecutive samples of BHBA ≥1.2 mmol/L and at least one BHBA >2.9 mmol/L) during the last week antepartum compared to non-ketotic cows. It is concluded that the BHBA concentration in the first week postpartum shows associations with multiple behavioural variables as early as six weeks antepartum. This is in contrast with the results of King et al. (2017), who did not find an association between lying behaviour and subclinical ketosis. This could be explained by the smaller sample size, more cow-level variability and less external validity due to the conditions at the research facility instead of commercial farms.

The study by Najm et al. (2020) presented associations between ketosis and less motion activity before diagnosis (4-70 DIM) on a 75-cow dairy farm. Both this study and Edwards et al. (2004) showed a generally lower walking activity in sick cows compared to a healthy group of fresh cows. Moreover, Najm et al. (2020) uses higher activity levels during the dry period as an explanation for better metabolization of larger amounts of ketone bodies. This explanation is in line with the associations found in our study between higher BHBA and a lower number of steps per day as early as six weeks antepartum. Our results concerning the BHBA2 indicate that the BHBA concentration in the blood in the second week postpartum is more related to postpartum management than the BHBA concentration in the first week postpartum. This explanation is underlined by the fact that the association with farm factor is stronger for the second week postpartum than for the first week.

4.4 Postpartum behaviour related to antepartum behaviour

The statistical analysis of the behavioural variables in the four weeks postpartum showed that the behaviour postpartum is associated with behaviour antepartum. The eating time reported in different studies is highly variable (Beauchemin, 2018). White et al. (2017) reported a mean eating time of 284 minutes per day (n=182), ranging from 141 to 507 minutes per day which is in line with our averages. A similar average ruminating time was reported by White et al. (2017) with 436 minutes per day on average (n=179), ranging from 236 to 610 minutes per day. Zebeli et al. (2006) reported 434 minutes per day (n=99), ranging from 151 to 630 minutes per day, in a quantitative study with an independent dataset generated from 33 experiments.

A complementarity between eating- and ruminating time was reported for dairy cows with unrestricted feed access (Beauchemin, 2018). Cows which spend less time eating tend to ruminate longer (Dado et al., 1994). In our study this variation of eating and ruminating between cows, is captivated by incorporating the inactive time as the no-chewing time. However, the results show stronger associations between eating- or ruminating time individually than to inactive time.

The use of the periods is unconventional when looking at this research. Others have used more standardised timeframes. However, based on the course of the behaviour in the transition period it was decided that these periods make more sense than manmade timeframes such as weeks. The cows' behaviour is clearly changing in the last two days antepartum, whilst a week before it could still be normal. The same goes for the first two days postpartum. Including those days in the same period, like others do, makes less sense.

4.5 Postpartum behaviour related to metabolic status

In our study the calcium concentration is associated with all behavioural variables in the first two days postpartum. This is similar to the report of Barraclough et al. (2020) who found that cows with clinical hypocalcemia spent fewer steps and more time lying down than subclinical hypocalcemic or normocalcemic cows, respectively 88 minutes/day and 125 minutes/day. Moreover, cows with subclinical hypocalcemia were reported to eat less (Jawor et al., 2012). Hendriks et al. (2020) found that in the postpartum period (d1-d21) multiparous cows with clinical hypocalcemia spend more time lying than cows with subclinical hypocalcemia or normocalcemia. The explanation given for these findings is a display of sickness, for sick cows are reported to alter daily behaviour (Dittrich et al., 2019). Our study showed this association with the calcium concentration shortly after calving in period D. Eating and ruminating time in period D showed a positive association and inactive time had a negative association.

In period E (day 2-7 postpartum) most of the behavioural variables are associated with BHBA1 and in period F with the highest BHBA concentration. A higher BHBA1 concentration is associated with a lower average lying time in the first week postpartum. These are in line with a previous study which found that SCK cows (BHBA ≥1.2mmol/L) continued to eat less in the 14 days postpartum (Schirmann et al., 2016). These results can be interpreted a display of sickness (Dittrich et al., 2019), yet more analysis is needed to find out what the exact association is. BHBA concentrations show associations up and until the end of our research period F, especially lying time in period F and BHBA2 concentration. Most other studies do not continue monitoring the cows this long postpartum, which makes it difficult to compare these findings.

The dataset used for this study is much bigger than just the data used for this sub-study. Therefore it is possible that there are more associations between other variables, such as lying bouts or disease or temperature, which could be added in future studies to create an even more reliable result. Moreover, the different behavioural datasets could be combined to create a set of variables that can predict the onset of metabolic disease. Which aspects of the behaviour in the dry period are most dominant could be shown through such research. Additional analysis is needed and should also focus on ways to incorporate these variables into practical on-farm disease-monitoring systems (Goldhawk et al., 2009). More research is needed to create a mathematical model to be able to predict the onset of hypocalcemia and ketosis in cattle.

5. Conclusion

Overall in this study it is shown that behaviour, especially eating, ruminating and inactive time, during the dry period is strongly associated with the calcium concentration postpartum. As early as three weeks antepartum, the behaviour of dairy cows is associated with the calcium concentration in the blood of dairy cows postpartum. The cows with lower a calcium concentration spend less time eating and ruminating and more time being inactive, in the weeks before calving.

It is concluded that the BHBA concentration in the first week postpartum shows associations with multiple behavioural variables as early as six weeks antepartum. A relatively higher number of steps per day during the dry period showed a negative association with the BHBA concentration postpartum. Whilst the few days before the onset of ketosis a lower number of steps was associated with a higher BHBA concentration. A negative association was found between the BHBA concentration and the calcium concentration. This indicates a higher risk of developing ketosis after suffering hypocalcemia.

Postpartum behaviour is associated with behaviour antepartum and with the calcium and BHBA concentrations. Dairy cows with lower calcium concentration spend less time eating and ruminating and more time lying down. Cows with higher BHBA concentrations show altered behaviour not only during the first two weeks, this continues until 4 weeks postpartum. This illustrates that postpartum behaviour is affected significantly by the peripartum metabolic status and underlines the importance of preventative measures.

These findings are crucial to continue the development of predictive algorithms, so that sensors can not only detect when an individual cow has developed an illness, but to actually help with early detection of at risk cows so that preventative measures can be taken. The development and improvement of sensor technology will lead to more effective preventative herd health management.

REFERENCES

Akaike H. (1987) Factor Analysis and AIC. In: Parzen E., Tanabe K., Kitagawa G. (eds) Selected Papers of Hirotugu Akaike. Springer Series in Statistics (Perspectives in Statistics). Springer, New York, NY. https://doi-org.proxy.library.uu.nl/10.1007/978-1-4612-1694-0_29

Bach, K.D., W. Heuwieser, W., McArt, J.A.A. (2016) Technical note: Comparison of 4 electronic handheld meters for diagnosing hyperketonemia in dairy cows, Journal of Dairy Science, Volume 99, issue 11, 2016, Pages 9136-9142, ISSN 0022-0302, https://doi.org/10.3168/jds.2016-11077.

Barraclough, R.A.C., Shaw, D.J., Thorup, V.M., Haskell, M.J., Lee, W. Macrae, A.I. (2020). The behavior of dairy cattle in the transition period: Effects of blood calcium status, Journal of Dairy Science, Volume 103, Issue 11, 2020, Pages 10604-10613, ISSN 0022-0302, https://doi.org/10.3168/jds.2020-18238.

Barrier, A.C., M.J. Haskell, A.I. Macrae, C.M. Dwyer, (2012). Parturition progress and behaviours in dairy cows with calving difficulty, Applied Animal Behaviour Science, Volume 139, Issues 3–4, 2012, Pages 209-217,ISSN 0168-1591, https://doi.org/10.1016/j.applanim.2012.03.003.

Bauman, D.E., Bruce Currie, W. (1980). Partitioning of Nutrients During Pregnancy and Lactation: A Review of Mechanisms Involving Homeostasis and Homeorhesis, Journal of Dairy Science, Volume 63, Issue 9, 1980, Pages 1514-1529, ISSN 0022-0302, https://doi.org/10.3168/jds.S0022-0302(80)83111-0.

Beauchemin K. A. (2018). Invited review: Current perspectives on eating and rumination activity in dairy cows. Journal of dairy science, 101(6), 4762–4784. https://doi.org/10.3168/jds.2017-13706

Butler, W.R. (2000) Nutritional interactions with reproductive performance in dairy cattle, Animal Reproduction Science, Volumes 60–61, 2000, Pages 449-457, ISSN 0378-4320, https://doi.org/10.1016/S0378-4320(00)00076-2.

Caixeta, L. S., P. Ospina, M. Capel, D. Nydam. (2017). Association between subclinical hypocalcemia in the first 3 days of lactation and reproductive performance of dairy cows. Theriogenology 94:1–7. https://doi.org/10.1016/j.theriogenology.2017.01.039.

Constable, P.D., Hinchcliff, K.W., Done, S.H. and Gruenberg, W. (2017). Metabolic and Endocrine Diseases. A textbook of the diseases of cattle, horse, sheep, pigs and goats. 11 edn. Elsevier, pp. 1662.

Dittrich, I., Gertz, M., & Krieter, J. (2019). Alterations in sick dairy cows' daily behavioural patterns. Heliyon, 5(11), e02902. https://doi.org/10.1016/j.heliyon.2019.e02902

Duffield, T. (2000). Subclinical Ketosis in Lactating Dairy Cattle, Veterinary Clinics of North America: Food Animal Practice, Volume 16, Issue 2, 2000, Pages 231-253, ISSN 0749-0720, https://doi.org/10.1016/S0749-0720(15)30103-1.

Edwards, J.L., Tozer, P.R. (2004). Using Activity and Milk Yield as Predictors of Fresh Cow Disorders, Journal of Dairy Science, Volume 87, Issue 2,2004, Pages 524-531, ISSN 0022-0302, https://doi.org/10.3168/jds.S0022-0302(04)73192-6.

Goff J. P. (2008). The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. Veterinary journal (London, England : 1997), 176(1), 50–57. https://doi.org/10.1016/j.tvjl.2007.12.020

Goff J. P. (2014). Calcium and magnesium disorders. The Veterinary clinics of North America. Food animal practice, 30(2), 359–vi. https://doi.org/10.1016/j.cvfa.2014.04.003

Goldhawk, C., N. Chapinal, D.M. Veira, D.M. Weary, Von Keyserlingk, M.A.G. (2009). Antepartum feeding behavior is an early indicator of subclinical ketosis, Journal of Dairy Science, Volume 92, Issue 10,2009, Pages 4971-4977, ISSN 0022-0302, https://doi.org/10.3168/jds.2009-2242.

Halachmi, I.,Ben Meir, Y., Miron, J., Maltz, E. (2016). Feeding behavior improves prediction of dairy cow voluntary feed intake but cannot serve as the sole indicator, Animal, Volume 10, Issue 9, Pages 1501-1506, ISSN 1751-7311, https://doi.org/10.1017/S1751731115001809.

Hendriks, S.J., J.M. Huzzey, B. Kuhn-Sherlock, S.-A. Turner, K.R. Mueller, C.V.C. Phyn, D.J. Donaghy, Roche, J.R. (2020). Associations between lying behavior and activity and hypocalcemia in grazing dairy cows during the transition period, Journal of Dairy Science, Volume 103, Issue 11,2020,Pages 10530-10546, ISSN 0022-0302, https://doi.org/10.3168/jds.2019-18111.

Hut, P.R., A. Mulder, J. van den Broek, J.H.J.L. Hulsen, G.A. Hooijer, E.N. Stassen, F.J.C.M. van Eerdenburg, Nielen, M. (2019). Sensor based eating time variables of dairy cows in the transition period related to the time to first service, Preventive Veterinary Medicine, Volume 169, 2019, 104694, ISSN 0167-5877, https://doi.org/10.1016/j.prevetmed.2019.104694.

Itle, A.J., J.M. Huzzey, D.M. Weary, M.A.G. von Keyserlingk, (2015). Clinical ketosis and standing behavior in transition cows, Journal of Dairy Science, Volume 98, Issue 1, 2015, Pages 128-134,ISSN 0022-0302, https://doi.org/10.3168/jds.2014-7932.

Iwersen, M., U. Falkenberg, R. Voigtsberger, D. Forderung, Heuwieser, W. (2009). Evaluation of an electronic cowside test to detect subclinical ketosis in dairy cows, Journal of Dairy Science, Volume 92, Issue 6, 2009, Pages 2618-2624, ISSN 0022-0302, https://doi.org/10.3168/jds.2008-1795.

King, M.T.M., K.M. Dancy, S.J. LeBlanc, E.A. Pajor, DeVries, T.J. (2017). Deviations in behavior and productivity data before diagnosis of health disorders in cows milked with an automated system, Journal of Dairy Science, Volume 100, Issue 10, 2017, Pages 8358-8371, ISSN 0022-0302, https://doi.org/10.3168/jds.2017-12723.

Leary, N.O., Pembroke A., Duggan P.F., (1992). Measuring albumin and calcium in serum in a dual test with the Hitachi 704. Clin Chem. 1992 Jul;38(7):1342-5. PMID: 1623602.

Mahrt, A., O. Burfeind, W. Heuwieser, W. (2014). Effects of time and sampling location on concentrations of β-hydroxybutyric acid in dairy cows, Journal of Dairy Science, Volume 97, Issue 1, 2014, Pages 291-298, ISSN 0022-0302, https://doi.org/10.3168/jds.2013-7099.

McArt, J.A.A., D.V. Nydam, Oetzel, G.R. (2012). Epidemiology of subclinical ketosis in early lactation dairy cattle, Journal of Dairy Science, Volume 95, Issue 9, 2012, Pages 5056-5066, ISSN 0022-0302, https://doi.org/10.3168/jds.2012-5443.

Miedema, H.M., Cockram, M.S.,Dwyer ,C.M., Macrae, A.I. (2011). Changes in the behaviour of dairy cows during the 24h before normal calving compared with behaviour during late pregnancy, Applied Animal Behaviour Science, Volume 131, Issues 1–2,2011, Pages 8-14, ISSN 0168-1591, https://doi.org/10.1016/j.applanim.2011.01.012.

Müller, E., Münger, A., Mandel, R., Eggerschwiler, L., Schwinn, A. C., Gross, J. J., Bruckmaier, R. M., Hess, H. D., & Dohme-Meier, F. (2018). Physiological and behavioural responses of grazing dairy cows to an acute metabolic challenge. Journal of animal physiology and animal nutrition, 102(5), 1120–1130. https://doi.org/10.1111/jpn.12931

Neves, R.C., Leno, B., Bach, K., McArt, J. (2018). Epidemiology of subclinical hypocalcemia in early-lactation Holstein dairy cows: The temporal associations of plasma calcium concentration in the first 4 days in milk with disease and milk production, J. Dairy Sci., 101 (2018), pp. 9321-9331, https://doi-org.proxy.library.uu.nl/10.3168/jds.2018-14587

Najm, N.A., L. Zimmermann, O. Dietrich, A. Rieger, R. Martin, Zerbe, H. (2020). Associations between motion activity, ketosis risk and estrus behavior in dairy cattle, Preventive Veterinary Medicine, Volume 175, 2020, 104857, ISSN 0167-5877, https://doi.org/10.1016/j.prevetmed.2019.104857.

Nielsen, P. P., Fontana, I., Sloth, K. H., Guarino, M., Blokhuis, H. (2018). Technical note: Validation and comparison of 2 commercially available activity loggers. Journal of Dairy Science, 101(6), 5449–5453. https://doi.org/10.3168/jds.2017-13784

Oetzel, G.R. (2004). Monitoring and testing dairy herds for metabolic disease, Veterinary Clinics of North America: Food Animal Practice, Volume 20, Issue 3, 2004, Pages 651-674, ISSN 0749-0720, https://doi.org/10.1016/j.cvfa.2004.06.006.

Reinhardt, T.A., J.D. Lippolis, B.J. McCluskey, J.P. Goff, Horst, R.L. (2011). Prevalence of subclinical hypocalcemia in dairy herds, The Veterinary Journal, Volume 188, Issue 1, 2011, Pages 122-124, ISSN 1090-0233, https://doi.org/10.1016/j.tvjl.2010.03.025.

Rodríguez, E. M., Arís, A., Bach, A. (2017). Associations between subclinical hypocalcemia and postparturient diseases in dairy cows. Journal of Dairy Sciences. 100:7427–7434. https://doi.org/10.3168/jds .2016-12210.

Rutten, C.J., Velthuis, A.G.J., Steeneveld, W. and Hogeveen, H., (2013). Invited review: Sensors to support health management on dairy farms. Journal of Dairy Science, ISSN: 0022-0302, Vol: 96, Issue: 4, Page: 1928-1952, https://doi.org/10.3168/jds.2012-6107

Schirmann, K., Weary, D. M., Heuwieser, W., Chapinal, N., Cerri, R., Von Keyserlingk, M. (2016). Short communication: Rumination and feeding behaviors differ between healthy and sick dairy cows during the transition period. Journal of dairy science, 99(12), 9917–9924. https://doi.org/10.3168/jds.2015-10548

Sepúlveda-Varas P, Weary DM, Noro M, von Keyserlingk MAG (2015). Transition Diseases in Grazing Dairy Cows Are Related to Serum Cholesterol and Other Analytes. PLoS ONE 10(3): e0122317. https://doi.org/10.1371/journal.pone.0122317

Suthar, Vishal & Canelas-Raposo, J & Deniz, Abdülkerim & Heuwieser, Wolfgang. (2013). Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows. Journal of Dairy Science. 96. 10.3168/jds.2012-6035.

Van Erp-Van der Kooij, E., Van de Brug, M., Roelofs, J.B., (2016). Validation of Nedap Smarttag Leg and Neck to assess behavioural activity level in dairy cattle. Precision Dairy Farming: HAS University of Applied Sciences,.

White, R. R., Hall, M. B., Firkins, J. L., & Kononoff, P. J. (2017). Physically adjusted neutral detergent fiber system for lactating dairy cow rations. I: Deriving equations that identify factors that influence effectiveness of fiber. Journal of dairy science, 100(12), 9551–9568. https://doi.org/10.3168/jds.2017-12765

Wilkens, M.R., C.D. Nelson, L.L. Hernandez, J.A.A. McArt, J.A.A. (2020). Symposium review: Transition cow calcium homeostasis—Health effects of hypocalcemia and strategies for prevention, Journal of Dairy Science, Volume 103, Issue 3, 2020, Pages 2909-2927,ISSN 0022-0302,https://doi.org/10.3168/jds.2019-17268.

Zebeli, Q., Tafaj, M., Steingass, H., Metzler, B., & Drochner, W. (2006). Effects of physically effective fiber on digestive processes and milk fat content in early lactating dairy cows fed total mixed rations. Journal of dairy science, 89(2), 651–668. https://doi.org/10.3168/jds.S0022-0302(06)72129-4

APPENDIX

Table 4. Confidence interval of the GLM between calcium and eating-, ruminating-, inactive-, lying tim e and number of steps

	2,5%	97,5%
(Intercept Eat)	0.780	0.968
Av. Eat B	<-0.001	<-0.001
Av. Eat C	<0.001	<0.001
(Intercept Rum)	7.38e-01	0.977
Av. Rum B	<-0.001	<0.001
Av. Rum C	<0.001	<0.001
(Intercept Ina)	7.88e-01	9.81e-01
Av. Ina B	<0.001	<0.001
Av. Ina C	<-0.001	<-0.001
SD Ina C	<-0.001	<0.001
(Intercept Ly)	7.85e-01	0.877
SD Ly C	<0.001	<0.001
(Intercept Step)	7.87e-01	8.78e-01
SD Step C	<0.001	<0.001

Table 5. Confidence interval of the GLM between BHBA 1 and eating-, ruminating-, inactive-, lying tim e and number of steps

	2,5%	97,5%
(Intercept Eat)	0.437	1.316
Calcium	-0.877	-0.169
Av. Eat A	-0.002	-0.001
SD Eat A	-0.007	-0.001
SD Eat C	-0.000	0.003
(Intercept Rum)	9.257e-02	1.211
Calcium	-9.392e-01	-0.216
Av. Rum A	<0.001	0.003
Av. Rum B	<-0.001	-0.001
SD Rum C	<-0.001	<0.001
(Intercept Ina)	-0.655	0.178

Calcium	-0.847	-0.138
Av. Ina B	0.001	0.002
SD Ina A	-0.003	<0.001
(Intercept Ly)	-0.210	0.548
Calcium	-1.029	-0.238
Av. Ly B	<0.001	<0.001
SD Ly A	-0.004	<0.001
(Intercept Step)	-4.391e-01	4.660e-01
Calcium	-1.059e+00	-1.598e-01
Av. Step A	<-0.001	<-0.001
Av. Step C	<-0.001	<0.001
Av. Step D	<0.001	<0.001
SD Step C	<-0.001	<0.001

Table 6. Confidence interval of the GLM between BHBA 2 and eating-, ruminating-, inactive-, lying tim e and number of steps

	2,5%	**97,5%**
(Intercept Eat)	0.636	1.514
Calcium	-1.026	-0.275
Av. Eat B	-0.002	<-0.001
SD Eat A	-0.008	-0.002
(Intercept Rum)	-0.204	0.813
Calcium	-0.806	0.0792
Av. Rum C	-0.00164	<-0.001
(Intercept Ina)	-1.336	-0.207
Calcium	-0.834	0.051
Av. Ina A	-0.002	<0.001
Av. Ina B	4.134e-04	0.003
Av. Ina C	-1.362e-04	0.001
SD Ina B	-0.003	<0.001
(Intercept Ly)	-0.591	0.345
Calcium	-0.968	-0.036
Av. Ly A	<0.001	0.001

Av. Ly C	-0.001	<-0.001
SD Ly C	-0.002	<0.001
(Intercept Step)	-5.130e-01	4.002e-01
Calcium	-1.007e+00	-1.053e-01
Av. Steps A	<-0.001	<-0.001
Av. Steps C	<0.001	<0.001
Av. Steps E	<-0.001	<0.001
SD Steps D	<0.001	<0.001

Table 7. Confidence interval of the GLM between highest BHBA and eating-, ruminating-, inactive-, lyi ng time and number of steps

	2,5%	**97,5%**
(Intercept Eat)	0.918	1.798
Calcium	-1.026	-0.312
Av. Eat A	-0.002	<-0.001
SD Eat A	-0.007	-0.002
(Intercept Rum)	0.233	1.362
Calcium	-0.988	-0.241
Av.Rum A	5.582e-04	0.003
Av. Rum B	-0.003	<-0.001
Av. Rum C	-0.002	<-0.001
(Intercept Ina)	-1.221	-0.125
Calcium	-0.944	-0.010
Av. Ina B	<0.001	0.002
Av. Ina E	<-0.001	<0.001
SD Ina C	<-0.001	0.001
(Intercept Ly)	-3.406e-01	5.544e-01
Calcium	-1.065e+00	-1.755e-01
Av. Ly A	2.747e-04	1.159e-03
Av. Ly E	<-0.001	<-0.001
SD Ly A	<-0.001	<-0.001
SD Ly E	<-0.001	<0.001
(Intercept Step)	-3.022e-01	0.587

Calcium	-1.078e+00	-0.193
Av. Step A	<-0.001	<-0.001
Av. Step C	<0.001	<0.001
SD Step D	<0.001	<0.001

Table 8. Confidence interval postpartum eating time in periods D, E and F (n=373)

	2,5%	**97,5%**
(Intercept Eat D)	-88.732	44.056
Calcium	53.354	170.249
Av. Eat A	0.183	0.583
Av. Eat B	-0.562	-0.102
Av. Eat C	0.688	0.961
(Intercept Eat E)	-11.094	76.609
Av. Eat A	-0.004	0.310
Av. Eat B	0.001	0.357
Av. Eat C	-0.225	0.036
Av. Eat D	0.547	0.733
(Intercept Eat F)	-28.324	47.541
Av. Eat A	0.194	0.377
Av. Eat E	0.528	0.673
SD Eat E	0.149	0.634
BHBA 2	-23.115	0.383

Table 9. Confidence interval postpartum ruminating time in periods D, E and F (n=373)

	2,5%	**97,5%**
(Intercept Rum D)	-167.018	45.397
Calcium	65.499	187.481
Av. Rum A	0.163	0.500
Av. Rum C	0.392	0.500
SD Rum A	0.526	1.375
SD Rum C	-0.624	-0.266
(Intercept Rum E)	126.779	246.923
Av. Rum B	0.214	0.465
Av. Rum D	0.270	0.438
SD Rum D	0.159	0.450
BHBA 1	-31.845	3.368
(Intercept Rum F)	-38.350	96.892
Av. Rum A	0.179	0.441
Av. Rum C	0.006	0.219
Av. Rum D	-0.274	-0.075
Av. Rum E	0.591	0.792
SD Rum A	0.025	0.645
SD Rum C	-0.258	0.027
SD Rum E	0.123	0.527

Table 10. Confidence interval postpartum inactive time periods D, E and F (n=373)

	2,5%	**97,5%**
(Intercept Ina D)	86.752	330.682
Calcium	-319.330	-127.903
Av. Ina A	0.122	0.502
Av. Ina C	0.429	0.714

SD Ina A	-1.052	-0.011
SD Ina C	0.396	0.849
(Intercept Ina E)	45.614	193.888
Av. Ina A	0.094	0.420
Av. Ina C	-0.033	0.232
Av. Ina D	0.419	0.625
SD Ina A	-0.764	0.113
SD Ina C	-0.572	-0.155
SD Ina D	-0.337	0.013
BHBA 1	-3.071	49.410
(Intercept Ina F)	1.617	150.861
Av. Ina A	0.215	0.486
Av. Ina D	-0.246	-0.054
Av. Ina E	0.634	0.859
SD Ina A	-0.885	-0.088
SD Ina B	-0.530	0.080
SD Ina C	0.047	0.387
SD Ina E	-0.722	-0.281

Table 11. Confidence interval postpartum lying time in periods D, E, and F (n=403)

	2,5%	**97,5%**
(Intercept Ly D)	2.420	156.983
Calcium	-188.117	4.669
Av. Ly A	0.367	0.779
Av. Ly B	-0.853	-0.339
Av. Ly C	0.693	0.974
SD Ly A	-0.051	0.950
(Intercept Ly E)	-85.061	7.559
Av. Ly B	0.331	0.528
Av. Ly C	-0.017	0.245
Av. Ly D	0.296	0.475
SD Ly B	-0.116	0.668
SD Ly D	-0.432	-0.124
BHBA 1	-41.959	1.879
(Intercept Ly F)	-41.674	47.087
Av. Ly A	0.166	0.322
Av. Ly C	0.045	0.237
Av. Ly D	-0.152	0.0135
Av. Ly E	0.486	0.686
Highest BHBA	-45.012	-6.307

Table 12. Confidence interval postpartum number of steps in periods D, E and F (n=403)

	2,5%	**97,5%**
(Intercept Step D)	-8.534e+02	1442.025
Calcium	-1.393e+02	2353.989
Av. Step A	9.173e-02	0.536
Av. Step C	4.404e-01	0.826
SD Step C	5.316e-01	1.070
(Intercept Step E)	545.106	1.628e+03
Av. Step A	0.318	6.058e-01
Av. Step D	0.373	5.617e-01
SD Step A	-0.699	7.814e-02
SD Step C	-0.395	-9.090e-03
SD Step D	0.1293	4.515e-01
(Intercept Step F)	397.280	1202.623
Av. Step A	0.279	0.451

Av. Step E	0.470	0.645
SD Step D	0.109	0.340
SD Step E	-0.511	-0.147
Highest BHBA	-41.555	353.111