Pre- and postpartum behaviour in relation to calcium status after calving in dairy cattle

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Abstract

Little is known about relations between cow's behaviour in the transition period and calcium status postpartum. Hypocalcaemia is associated with developing diseases in the first months after parturtion. The objectives of the present study were 1) to determine the distribution of plasma calcium concentrations among dairy cows within 48 hours after calving, 2) to investigate if Ca concentrations in blood within 48 hours after calving are related to, and can be estimated by, the number of steps, eating-, idle-, lying-, and rumination time in the transition period, 3) to determine relative influence of observed factors to [Ca], and 4) to investigate if Ca concentrations in blood within 48 hours after calving are related to developing illness. Blood samples of 247 dairy cattle from eight farms were collected within 48 hours after calving and calcium concentration was determined. Nedap Smarttag Neck sensor and Smarttag pedometer collected data of 166 animals from day -42 to +28 relative to day of calving (day 0), and relations were analyzed by Pearson correlation and multiple linear regressions with parity included. Parity, breed, farm and disease registrations were analysed by Levene's test and Tukey. The mean calcium concentration was 2.19 mmol/L (SD = 0.297). Parity influenced postpartum [Ca] ($p < 0.000$), and breed showed a trend ($p = 0.056$). Farm 7 and 8 differed significant ($p = 0.030$), which was explained by farm 7 having a higher number of cows in the first lactation. Correlations were found between all behavioural elements and plasma [Ca], during the pre-, and the postpartum period, and the strongest correlations were found between Ca concentration and rumination during day -2 to 0 ($r = 0.309$, $p < 0.000$) and day +1 to +4 ($r = -0.427$, $p < 0.000$), idle time during day 0 to +5 ($r = 0.374$, $p < 0.000$), and number of steps of day -42 to -10 ($r = 0.302$, $p < 0.000$). Based on only eating or number of steps, no significant estimation can be made of plasma [Ca]. Lying time on day +5 to +28 and parity can estimate [Ca] for 29.3%. Rumination of day -42 to -3, day +1 to +4, and parity can estimate Ca concentration, and idle time of day -42 to -4, 0 to +28, and parity can estimate plasma [Ca] as well by explaining the variance of [Ca] for 44.7% and 49.6%, respectively. Ninety-three animals had a complete behaviour profile and taking those sensor data into a multiple regression gave that parity (ß = -0.087, $p < 0.000$), rumination on day +1 to +4 (ß = -0.003, $p < 0.000$), idle time on day -42 to -4 (ß = 0.002, $p < 0.000$) and +6 to +28 (ß = -0.001, $p = 0.001$), lying time on day +5 to +28 (ß = 0.001, $p = 0.008$), and number of steps of day -42 to -10 (ß = 9.640E-5, $p = 0.035$) influenced the plasma [Ca] after parturition the most, and explaining the variance of [Ca] by 53,9%. Developing a tool for detecting herd problems caused by hypocalcaemia can be designed based on those behaviours. Rumination and idle time were the most important estimators for calcium status after parity, which might be explained by smooth muscle relaxation and decreased rumen activity because of hypocalcaemia.
Among disease registration, only milk fever had a significant ($p = 0.005$) lower mean calcium concentration of 1.90 (CI: 1.69, 2.10) compared to the group without disease registration with a mean calcium concentration of 2.26 (CI: 2.17, 2.35). Relations between plasma [Ca] and developing another illness, besides milk fever, were not proven, but a lower mean [Ca] was observed in cattle which had cystic ovarium, abomasum displacement, developed lameness, ketosis, endometritis and mastitis. This study confirmed previous reports about the distribution of calcium levels after calving and proofs relations between postpartum calcium

concentration and animal behaviour, measured by sensors, which can predict individual cows calcium status postpartum and indicate herd problems caused by postpartum hypocalcaemia. Those indicated herd problems can be solved by change in management strategies and contributes in this way to improved animal health.
Keywords: hypocalcaemia, calcium, animal behaviour, eating, rumination, lying, steps, idle time, milk fever, dairy cows

Introduction

Major outflow of calcium (**Ca**) equivalent to 7 to 10 times the amount of Ca present in blood are imposed on the first day of lactation, which is due to the synthesis and secretion of colostrum (Horst, Goff et al. 2005). It is assumed that normal serum calcium concentrations in cows range from 2.1 to 2.8 mmol/L during lactation, and from 1.6 to 2.6 mmol/L during the week after calving among cows without clinical disease (Quiroz-Rocha, LeBlanc et al. 2009). Cut off values in the classification of hypocalcaemia (**HC**) vary from <1.9 to ≤2.14 mmol/L at different moments after calving in previous studies. For example, <2.0 mmol/L is used to determine HC in the period of 12 to 24 hours after calving (Reinhardt, Lippolis et al. 2011), <1.9 mmol/L for within 24 hours after calving (Goff, Horst et al. 1996), <2.14 mmol/L for the period of 24 to 48 hours postpartum (Rodríguez, Arís et al. 2017), and <2.0 mmol/L for within <48 hours (Horst, Goff et al. 2005, Neves, Leno et al. 2017). The study of Goff et al. (1996) reported 10-50% of Jersey cows developing HC within 24 hours after parturition, and Reinhardt et al. (2011) found approximately 50% of older cows in the US dairy herds have a blood Ca concentration of <2.0 mmol/L between 12 and 24 h after calving. Because of different cut off values, it is unclear what normal distribution of [Ca] after calving is.

Calcium concentration in blood is tightly regulated by the parathyroid gland. It secretes parathyroid hormone (PTH) which directly increases renal tubular reabsorption of Ca from glomerular filtrate, and triggers osteoclastic resorption of bone Ca to make up the deficit on midterm. Besides these effects, PTH activates production of 1,25-dihydroxyvitamin D (also 1,25-dihydroxycholecalciferol or calcitriol). Hypomagnesemia has a negative effect on PTH function, firstly by reducing PTH secretion in response to HC, and secondly by reducing tissue sensitivity to PTH. Diet cation-anion difference can also have a negative effect on tissue sensitivity to PTH, in case of metabolic alkalosis by a diet that supplies more cations (Goff 2008). Calcitriol and prolactin have similar effects on calcium as PTH (Cross, Hillman et al. 1995), where calcitonin inhibits the effects of PTH by inhibiting bone resorption. With increasing age, the Ca metabolism becomes impaired, because bone and intestinal vitamin D receptors are declining over the years (Neves, Leno et al. 2017, Horst, Goff et al. 1990). Metabolic adaption mechanisms for calcium after calving are sometimes not fast enough to maximize calcium inflow from the gastrointestinal tract and bone to the mammary gland (Ramberg, Mayer et al. 1970). The, in this way induced, HC in blood can lead to hyperexcitability of the nervous system, causing muscle fasciculations. The nadir of calcium concentration is approximately between 12 to 24 hours after calving and [Ca] stabilizes around day 5 postpartum (Grünberg, Donkin et al. 2011, Goff 2008, Kimura, Reinhardt et al. 2006).

Milk fever is a severe form of HC in dairy cattle, which is diagnosed based on clinical signs of disease, which are, for example, muscle paresis, recumbency, dry muzzle, and a lower body temperature (cold ears). The costs of each case of clinical milk fever are estimated at $300 dollar (Oetzel 2013). The overall incidence of milk fever is reported 5-7% (DeGaris, Lean 2008, Goff 2008, Mulligan, Doherty 2008). Acute milk fever occurs typically in multiparous cattle within two days postpartum (Smith 2015). Cows with milk fever have a

great decline in feed intake, resulting in an exacerbated negative energy balance, decreased Ca resorption from the gut, and a greater risk of displacement of the abomasum by reduced rumen and abomasal motility leading to gas accumulation (Chapinal, Carson et al. 2011, Goff, Horst 1997). Besides milk fever, HC is known to be a risk factor for developing several diseases by suppressing smooth muscle contractions due to the effects of low ionized Ca, and contributes in this way to metritis, mastitis, and retained placenta by affecting uterine and teat sphincter contractility (Smith 2015, Martinez, Risco et al. 2012, Goff 2008). The ability of immune cells to respond to stimuli are decreased in HC cattle (Kimura, Reinhardt et al. 2006) which increases the number of infections like mastitis and lameness. Lower [Ca] in plasma were found in cows with retained fetal membranes (Melendez, Donovan et al. 2004) and in dystocic cows (Benzaquen, Galvão et al. 2015).

Measurements of cow behaviour can be used to identify cows which might have health problems (Rutten, Velthuis et al. 2013). It is, therefore, interesting that during the last decades, sensors are developed to track activity of dairy cattle for oestrus detection. Now, improved sensors are available with accelerometers for neck and legs and they are recording not only the number of steps, but also the time cattle spend lying, standing, and moving. This information is used to develop algorithms for automatic detection of cow behaviour and lameness (Trenel, Jensen et al. 2009, Nielsen, Pedersen et al. 2010). Cattle in the middle of their lactation period have a daily time budget as follows: feeding 3-5 h/d, lying 12-14 h/d, and ruminating 7-10 h/d (Grant 2004). Cows in the transition period have a slightly different time budget. Daily lying time is, for example, decreased by 1.1 h/d from the pre-fresh period (11.7 h/d) to the post-fresh period (10.6 h/d) (Huzzey, Von Keyserlingk et al. 2005) and feeding time of healthy dry cows are declining from 214 min/d in wk -2 relative to calving to 192 min/d in wk -1 (Huzzey, Veira et al. 2007). In the transition period, dairy cattle are vulnerable to metabolic and infectious diseases, that induce changes in their time budget (Goff, Horst 1997). Cows with severe and mild forms of metritis have decreased eating times and food intakes relative to healthy cows, which could be detected already 2 weeks before calving (Huzzey, Veira et al. 2007). Additionally, Kaufman (2016) found a correlation between decreased rumination time and subclinical hyperketonaemia from 14 days before calving until 28 days after calving.

About eating time different results were published. Jawor et al. (2012) found that cows with subclinical HC (≤1.8 mmol/L) visited the feed bins fewer times during the first and third week after calving, however, no differences had been found in the number of visits during the weeks leading up to calving and the second week after calving. Calculated costs of subclinical HC ($125) by Oetzel (2013) was accounted on reduced milk yield, and direct costs due to increased incidence of ketosis and displaced abomasum. In contrast, subclinical HC and milk fever were associated with increased milk yield (Østergaard, Gröhn 1999, Rajala-Schultz, Gröhn et al. 1999, Jawor, Huzzey et al. 2012), which is expected to be associated with increased dry matter intake (Bargo, Muller et al. 2003). Cows with an induced subclinical HC had reduced dry matter intake on the day of infusion (Martinez, Sinedino et al. 2014), which resulted in a decreased total feed intake and rumination (Hansen, Norgaard et al. 2003). Liboreiro et al. (2015) reported less daily rumination time on day 16, 13, and 11 prepartum compared to healthy cows too but they found no significant difference over the whole pre- and postpartum period. To our knowledge, no reports were published whether multiple cow behaviour measured by sensors can estimate HC. Our study will contribute to more knowledge about relations between behavioural elements, measured by sensors, and plasma [Ca] after calving, and will be supportive in recognising herd problems caused by HC postpartum.

Our objectives were 1) to determine the distribution of Ca concentrations in blood among dairy cows within 48 hours after calving, 2) to investigate if Ca concentrations in

blood within 48 hours after calving are related to, and can be estimated by the number of steps, eating-, idle-, lying-, and rumination time in the transition period, 3) to determine relative influence of observed factors, and 4) to investigate if Ca concentrations in blood within 48 hours after calving are related to developing illness. We expected that the distribution of [Ca] within 48 hours after calving would be between 1.6 to 2.6 mmol/L as described by Quirez-Rocha et al. (2009), and that eating time and rumination time were positively correlated with plasma [Ca] postpartum. We hypothesized, that postpartum disease, resulting in prolonged lying and idle time, and reduced number of steps would affect [Ca] within 48 hours postpartum.

Materials and Methods

Enrolment

This case-control and retrospective study was conducted on 8 dairy herds located in The Netherlands, from November, 2016 until July, 2017, and was part of the Sense of Sensors in Transition Management project in collaboration with NEDAP, Vetvice, Utrecht University, Wageningen University, CRV, and Boehringer Ingelheim. The study will continue until at least 2018. The amount of cattle in total on the farms was around 1090 heads. Herd size ranged from 110 to 210 milking cows. One farm had Fleckvieh cattle, all others Holstein-Friesian or mixed breeds. Five of eight farms had a milking parlour, and two had an automatic milking system. One farm worked with both milking systems. Three farms were using mattresses, the other farms were using deep litter as bedding material. Each herd was visited at the same day and time each week. In the period of November, 2016 and July, 2017, all cows which were within 48 hours after parturition at the weekly visiting were enrolled, which were 247 cattle. Number of cows and parity per farm are described in table 1 and 4.

Calcium concentration

Blood samples were collected from the coccygeal vein within 48 hours after parturition via evacuated blood collection tubes containing heparin as anticoagulant (Vacutainer; Becton Dickinson). Blood samples were centrifuged (Centrifuge 5804 R; Eppendorf, Germany) for 10 minutes at 4500 rpm. Serum was pipetted in Eppendorf cup by hand-held pipette and stored at -20 Celsius until further analysis. Quantitative determination of calcium concentration of the serum was performed by Olympus AU680 (Beckman Coulter, Brea, CA), method Calcium Arsenazo (Limit of Quantitation = 1 mmol/L) with an end point determination of 660 nm (Leary, Pembroke et al. 1992). Because of different ranges described in previous studies, it was decided not to use a cut off value to determine HC.

Sensors

Each cow carried two sensors: a Nedap Smarttag pedometer (NEDAP, Groenlo, The Netherlands), and a Nedap Smarttag Neck sensor (Nedap, Groenlo, The Netherlands), which were attached at a front limb and to the neck collar, respectively. These Smarttag sensors are G-sensors, which are three-axis accelerometers and measured movements in three dimensions. Neck sensor collected the eating, rumination and idle time, where the pedometer collected the number of steps and lying time, from day 42 before until day 28 after calving. Idle time, measured by sensors of Nedap, is time without sensor activity, which means not eating or rumination or other active behaviour (like drinking, ranking behaviour, and other social behaviour) and differs in this way from inactive time as described in other reports. It does include sleeping, lying, and waiting. Due to different patterns of behaviour between

eating, rumination, idle, lying and amount of steps, it is decided to divide each behaviour into its unique periods. Periods are described in table 2, based on figure 5. Number of cows used for eating-, rumination-, idle- and lying time, and number of steps were 138, 107, 107, 136, and 129, respectively. Different number of cows were explained by cattle not wearing neck- and/or pedosensor and old neck sensor not measuring rumination and idle time. The difference in number of cows between lying and number of steps, measured both by pedometer, is explained by lost data which cause is unclear. Data was sent every 15 minutes to the receiver or was saved on the sensor for a maximum of 24 hours when out of range (cows in pasture). Data was transformed to number of steps per day, and lying-, eating-, rumination, and idle time in minutes per day. 93 cows had a complete behaviour profile: animals from the 1st (n = 9), 2nd (n = 33), 3rd (n =2 4), 4th (n = 12), 5th (n = 8), 6th (n = 3), 7th (n = 2), 8th (n = 1), and 10th (n = 1) lactations and from farms 1 (n = 9), 2 (n = 16), 3 (n = 24), 4 (n = 23), and 8 (n = 21). Four farms started during the research with applying grazing of the lactating cows, and one farm started with applying grazing of the dry cattle as well, which resulted in great impact on behaviour. It was decided to exclude the data of the grazing period. Factory data on the first days of wearing sensor was erased, as well as data on days that had not collected 96 quarters.

Postpartum disease

Producers reported details about calving on a form (figure 1). On the form, producers could also describe details about the cow (short registration number and parity) and observed diseases like lameness, milk fever, ketosis, abomasum replacement and their treatment. Registration number was extracted from the herd management software Velos (Nedap, Groenlo, The Netherlands).

Disease was assigned based on disease criteria. Dystocia was determined by calving score 2 or higher (Lombard, Garry et al. 2007). As soon as the placenta retained beyond 24 hours, retentio secundarium was classified (LeBlanc 2008). Lameness was defined by increased score to ≥3 after calving on scale 1 to 5 (Thomsen, Munksgaard et al. 2008). Displacement of abomasum was diagnosed by a veterinarian. Ketone concentration in blood was measured between day 2 and 9 postpartum with a Precision Xtra handheld ketone meter (Abott Diabetes Care Canada, Mississauga, ON, Canada). Sensitivity of the test had been reported to be 98.6%, and specifity of 98.2% (Panousis, Brozos et al. 2012). The test was repeated seven days after the first ketone concentration measurement. Ketosis was determined as soon as one of two measurements were ≥1.2 mmol beta-hydroxybutyrate per Litre as recommended universal threshold for herd evaluations (Suthar, Canelas-Raposo et al. 2013).

Metritis was diagnosed within 21 days postpartum, with purulent uterine discharge detectable in the vagina and fetid discharge (Sheldon, Lewis et al. 2006). Endometritis was diagnosed by manual palpation of the uterus for involution and the use of ultrasound (Tringa Linear, Esaote-Pie Medical) for uterine discharge after 21 days postpartum (Sheldon, Lewis et al. 2006). Manual palpation and linear scanning was executed by a veterinarian, who checked cows which were in the fourth and eighth week after calving. Animals with one or more cystic ovaries were detected in this way too.

Still births, milk fever and mastitis were reported by producers. Disease registration continued until two months after parturition for each cow. In total, 166 animals were enrolled for disease registration, which were the same animals as for sensor data.

Statistical analysis

Data input and management were completed in Excel 2016 (Microsoft Corp.). Outliers were included to find sensor pattern of ill animals., and descriptive statistics included the 5% trimmed mean to show results without the outliers.

Data of all cattle was centred to periods which were based on specific found patterns of behaviour elements around calving (described in table 2 and figure 5), combined with calculated highest R^2. Based on progress of behaviour, for each period per cow was calculated a mean and standard deviation (SD), or a slope and standard error of residuals (SEres), which were used for statistical analyses. Slope and standard error of residuals were calculated to estimate the degree of change of behaviour, which indicates the level of deterioration or recovery.

All statistical analyses were completed in SPSS software (IBM Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp. Released 2015), using calcium concentration as the experimental unit. A $p < 0.05$ was considered significant. To report distribution of [Ca], descriptive statistics including minimum, maximum, median, mean, and 5% trimmed mean were employed. Pearson correlation and multi-level logistic regression models including parity were used to determine relations between serum calcium concentration (as continuous variable) and the continuous variables of calculated periods per cow of eating-, rumination-, idle- and lying time, and number of steps, using plasma [Ca] as the experimental unit. First all behaviour were analyzed in separated analyses and then all behaviours together were analyzed by Pearson correlation and multi-level logistic regression models to determine which behaviour per period were the most important estimators. Missing data was excluded listwise, entry of probability of F was determined at 0.05 and removal at 0.10. Levene's test and Tukey were constructed among the categorical variables of farms, lactation numbers and disease registration, to find significant differences in plasma [Ca] among groups. For disease registration, no cut off value was used.

Results

Descriptive statistics Ca

In total, 247 cows calved in the period of November 20^{th,} 2016 and July 4th, 2017, and were enrolled. Minimum (1.34), maximum (3.59), median (2.23), mean (2.194; SD = 0.297, SE = 0.019), and 5% trimmed mean (2.20) were determined and calculated, and shown in figure 1, and table 3 and 4. Seven extreme outliers were detected:1.34, 1.36, 1.37, 1.41, 1.47, 1.47, and 3.59 mmol/L. Descriptive statistics about farms are shown in table 5 and figure 3. A significant difference among farms was found ($p = 0.011$), which resulted in a dissimilarity between farm 7 and 8 ($p = 0.030$) after completing Tukey. A trend was observed between breeds ($p = 0.056$). Statistics about parity are described in table 3 and 4, and figure 4. There were several significant differences observed between parities. In general between the first 2 with the older ones. The group of 166 cows which had sensor data available, had a mean calcium concentration of 2.19 (SD = 0.05) mmol/L. Descriptive statistics of sensor data per period are shown in table 7. Three extreme outliers of [Ca] were observed in this group: 1.34, 1.36 and 1.37 mmol/L. Sensor data of outliers were included. No statistical difference was found among farms ($p = 0.235$), where a trend is observed between breeds ($p = 0.098$).

Eating, rumination and idle time

Statistical analyses were performed on data extracted from the neck sensor, and described in table 7. Significant correlations between plasma [Ca] within 48 hours after parturition and eating time in the period of +1 to +28 days, rumination time on day -2 to +4, and idle time of day 0 to +5 were found. Correlation between [Ca] and idle time in period of day -3 to -1 showed a trend ($r = -0.138$, $p = 0.079$).

No significant regression coefficients were found besides parity for eating time. For rumination, a significant regression equation was found ($F(3,103) = 27.739$, $p < 0.000$) with an R^2 of 0.447. Cows' estimated calcium concentration based on rumination time is equal to 3.315 – 0.100 x parity – 0.003 x RSlope3 – 0.001 x RMean1, where RSlope3 is the calculated slope of rumination time during day 1 to 4 after calving in minutes per day and RMean1 is calculated mean rumination time in minutes per day during day 42 to 3 before calving. The regression coefficients of standard deviation of day -42 to -3 (ß = 0.145, p = 0.054) and mean rumination of day +5 to +28 (ß = 0.148, p = 0.091) showed both a trend.
Regression analysis of idle time indicated four estimators explaining the variance (R^2 =0.496, $F(4,102) = 25.058$, $p < 0.000$). It was found that mean idle time of day -42 to -4 , slope of idle time of day 0 to +5 , mean idle time of day +5 to +28, and parity estimate calcium concentration.

Lying time and amount of steps
Results of data of the pedometer are described in table 6. Significant correlations between plasma [Ca] and lying of day +2 to +28, number of steps of day -42 to -10 and day +2 to +28 were found. Trends were observed between [Ca] and lying time of day -2 to 0, and number of steps of day -9 to -2. Besides parity, no significant regression coefficients were found for number of steps. A multiple linear regression was used to test if lying time significantly estimated cow's calcium concentration within 48 hours after calving. The mean lying time of day 5 to day 28 postpartum (Lmean4) and parity were estimators of calcium concentration ($F(2,133)=27.543$, $p < 0.000$) with a R^2 of 0.293. The estimate of calcium concentration based on lying time is calculated 2.185 + 0.000447 x Lmean4 – 0.100 x Parity.

Relative influence of observed factors
Results of multiple linear regression of all observed factors of sensor data and parity are described in table 8. A significant regression equation was found ($F(6,68) = 16.755$, $p < 0.000$) with a R^2 of 0.539. Cow's estimated calcium concentration is equal to 1.563 – 0.087 x parity – 0.003 x Rslope3 + 0.002 x Imean1 – 0.001 x Imean4 + 0.001 x Lmean4 + 9.640E-5 x Smean1, where Rslope3 is the slope of rumination time during the first until the fourth day after parturition in minutes per day, IMean1 is the mean idle time in minutes per day from day 42 to day 4 before calving, IMean4 is the mean idle time during day 6 to 28 after calving, Lmean4 is mean lying time in minutes per day in the period of day 5 to 28 after parturition, and SMean1 is the mean number of steps per day in the period of -42 to -10.

Disease registration
Milk fever (prevalence of 9.6%), cystic ovarium (2.4%), abomasal dislocation (0.6%), developed lameness (25.3%), ketosis (21.1%), endometritis (19.9%), mastitis (7.2%), dystocia (22.3%), stillbirth (4.8%), metritis (6.0%), and retention secundarium (7.2%) were compared to no disease registration (27.7%) of in total 166 cattle during the first two months after parturition. Results are described in table 9. Cattle which were reported by farmers having a milk fever had a significant lower calcium concentration within 48 hours postpartum ($p = 0.005$). Only slightly lower mean calcium concentrations were observed in cattle with cystic ovarium, abomasum dislocation, developed lameness, ketosis, endometritis or mastitis compared to the group with no disease registration.

Discussion

The first objective of the present study was to determine the distribution of [Ca] within 48 hours after parturition. We decided not to use a cut-off value for separating cattle in groups with or without HC because there is no clear line drawn from literature. Goff et al. (1996) defined HC when plasma [Ca] was below 1.9 mmol/L. They found 10-50% of cattle had HC, where in our study 15.9% (n = 42) had a plasma [Ca] below 1.9 mmol/L. Reinhardt et al. (2011) found subclinical HC (<2.0 mMol/L) in 25% in the 1st, 41% in the 2nd, 49% in the 3rd, 51% in the 4th, 54% in the 5th, and 42% in the 6th lactation in the first 48 hours after calving. In the study of Neves et al. (2017), the prevalence of subclinical HC (≤2.1 mmol/L) was 2, 40, and 66% for first, second, third and greater parities, respectively. Our findings are in agreement and complement existing research (Goff, Horst et al. 1996, Reinhardt, Lippolis et al. 2011) by having HC defined when plasma [Ca] is below 2.0 mmol/L, which was 3% in the first lactation (n = 2), 18% in the second lactation (n = 13), 20% in the third lactation (n = 9), 46% in the fourth lactation (n = 13), 40% in the fifth lactation (n = 8) and 58% in the sixth and higher lactation (n = 11).

Differences among breeds showed a trend ($p = 0.056$) between Holstein Friesian and Fleckvieh, further research with a higher number of animals is thus needed. We have found a significant difference in mean calcium concentration between farm 7 and 8 ($p = 0.030$), which might be explained by having 44% blood samples of the first lactation on farm 7 compared to 14% on farm 8. The main limitation of this work was sampling frequency. Due to labour and financial constraint, blood sampling could be done only once a week, which resulted in missing animals at enrolment.

Second and third objective of the present study were to investigate if Ca concentration is related to, and can be estimated by eating-, ruminating-, idle-, lying time, and number of steps in the transition period around calving, and determine relative influence of observed factors to [Ca]. Missing data was explained by heifers not wearing sensors or applying grazing in April and May. Erased days were days of which sensors measured less than 96 quarters caused for example by change of clock times or malfunction of the receiver, or sensors more than 24 hours out of range of receiver.

Data of eating time on day 0 was not included due to neck sensor measuring licking of the calf as eating time. Compared to Huzzey (2007), who found decreased eating times before calving on cows which developed metritis postpartum, our study did not find any such associations. We found a weak correlation ($r = 0.186$, $p = 0.014$) in mean eating time during day 2 until day 28 after parturition which does not support the finding of Jawor et al. (2012), who found a tendency for increased dry matter intake during week 2 postpartum in cattle who developed HC. The weak correlation is caused by dissimilarity among parities, which could, for example, be explained by introducing heifers into the herd after calving. Other, unpublished, results had shown heifers having a higher eating time compared to older cows. Besides, it suggests that feeding time might not be as important as feed composition for cattle, such as diet cation-anion balance, which is known to have great influence on calcium status (Goff 2008, Moore, VandeHaar et al. 2000).

Rumination time on day -2 to 0 ($r = 0.309$, $p = 0.001$) and day 1 to 4 ($r = -0.429$, $p < 0.000$) were correlated with calcium concentration. Rumination time on day 1 to 4 ($ß = -0.003$, $p < 0.000$) and on day -42 to -3 ($ß = -0.001$, $p = 0.014$), in combination with parity ($ß = -0.100$, $p < 0.000$), can estimate calcium concentration. The regression found of prepartum rumination behaviour is contrary to findings of Liboreiro et al. (2015), who found no significant difference between subclinical HC and healthy cows in the prepartum and postpartum period. Findings of rumination on day 1 to 4, however, are aligning with their statement that cows with HC have shorter daily rumination time than normocalcemic cows on

day 1 and 3 postpartum (Liboreiro, Machado et al. 2015), which would result in a greater slope of rumination time at recovery. Smooth muscle and skeletal muscle contractions depend on availability of calcium. An induced HC leads to decreased rumen activity (Martinez, Sinedino et al. 2014), which is likely to lead to decreased rumination time.

Idle time correlated with plasma [Ca] with on day -3 to -1, and day 0 to +5. Correlations of plasma [Ca] with idle time are found on practically the same days as with rumination time. Regression analysis showed four estimators of calcium concentration: parity, idle time in period of day -42 to -4, 0 to +5, and +6 to +28. The explanation of the estimative aspect of mean idle time in the prepartum period based on our data is explained by heifers having a lower idle time as multiparous cattle as described in previous study in which high-producing dairy cows spending less time eating and ruminating per unit of intake (Grant, Albright 1995), which would result in higher idle time measured by Nedap sensors. Schirmann et al. (2012) found no correlation between feeding time and rumination time, which both are influencing Nedap's measured idle time. Cattle which have a decreased rumination time due to HC, would in this way show higher idle time. We expect higher idle time after calving compared to other cows as an indicator of illness, which could explain the estimative aspect of idle time postpartum (day +6 to +28), further research, however, is needed to confirm these results. Slope of day 0 to +5 of idle time is highly correlated, which could be explained by decreased rumen activity at HC, as discussed above.

Lying time and amount of steps were both weakly correlated and little estimative or not estimative, respectively, and correlations found were between [Ca] and those behaviour after calving. Postpartum correlations between [Ca] and lying time on day +2 to +4, +5 to +28, and number of steps on day +2 to +7 and +8 to +28, could be explained by behavioural differences between parities, which is proven having great influence on [Ca]. Prepartum number of steps in the period of day -42 to -10 are positively correlated with [Ca] but not estimative, which indicates a confounder bias which could be caused by parity. Previous unpublished results indicated higher number of steps in primiparous cattle before calving.

Relative influence of observed factors where shown by the results of the multiple linear regression which included all calculated behaviour elements per period and parity. Only 93 of 166 cattle had a complete behaviour profile, because not all farms were enrolled at the start of this study and some primiparous cattle received sensors at moment of calving which resulted in missing data in prepartum period. Those missing animals contributed to a nondirectional misclassification bias, however, to create a complete view of whole period only cattle with complete pre- and postpartum data were included. Based on the R^2, variance of postpartum [Ca] can be estimated for 53.9% by parity, slope of rumination time on day +1 to +4, mean idle time in the periods of day -42 to -4 and day +6 to +28, mean lying time on day +5 to +28 and mean number of steps on day -42 to -10. It should be taken in mind that rumination on its own had a R^2 of 0.447 and idle time a R^2 of 0.496, resulting in both highly estimating the variance of calcium concentration on their own with a slightly bigger sample size of both 107 instead of 93 animals. Based on the R^2 of this study, it can be stated that animal behaviour on its own does not estimate and predict plasma [Ca] after parturition completely. Correcting for the exact moment of taking the blood sample (Neves, Leno et al. 2017) will make estimation more precise and will give a better explanation of the variance of [Ca]. Number of steps in the prepartum period were a surprisingly significant estimator for [Ca], however, the regression coefficient was very low and regression of number of steps on its own did not have significant regression coefficients. To use the behaviour in the postpartum period as an predictor of plasma [Ca] within 48 hours after calving is not practically relevant to prevent disease in an individual cow because it is based on consequences and causes of [Ca] rather than causation on its own. It can, however, be used as a basis to develop tools to detect herd problems caused by HC. By focusing on the prepartum

period in an enlarged sample size with complete behaviour profiles, prediction might be possible. This is the very first study in which all behaviour elements have been combined in one model, which is the next step detecting herd problems caused by HC.

The last objective of the study was to investigate if [Ca] is related to developing illness postpartum. Cattle which developed milk fever had a lower mean [Ca] within 48 hours after calving of 1.90 (CI: 1.69, 2.10) compared to the mean of 2.26 (CI: 2.17, 2.35) of the group of no disease registration ($p = 0.005$). The prevalence of milk fever (9.6%) was similar with previous reports (5-10%) (DeGaris, Lean 2008, Goff 2008, Mulligan, Doherty 2008). Cattle with the clinical signs of milk fever did not have the lowest plasma [Ca], which suggests that the clinical signs of milk fever are not related to the plasma calcium concentration alone, but for example to hypomagnesemia too (Goff 2008). In several studies it is mentioned HC cattle have a higher odds ratio for developing postpartum diseases (Smith 2015, Martinez, Risco et al. 2012, Goff 2008, Chapinal, Carson et al. 2011, Kimura, Reinhardt et al. 2006), but our data do not confirm those findings. By calculating odds ratio, Rodrigez et al. (2017) choose a cut off value and used mixed-effects logistic regression model with calcemia as a binary variable, followed by a receiver-operating characteristic analysis. Other studies have comparable statistical analysis. The choice of cut-off point has a great influence on calculated odds ratio in previous studies, so we decided not to use this analysis but Levene's test and Tukey to compare [Ca] means per registered disease. In this way comparison between our report and previous studies is not possible. We do see slightly lower mean calcium concentrations postpartum in cattle with cystic ovarium, abomasum dislocation, developed lameness, ketosis, endometritis and mastitis compared to the group without disease registration, thus indicates that low calcium concentration might be associated with those diseases and in this way explains the increased odds for developing illness in HC cattle in previous reports. Cattle with a retentio secundarinum had surprisingly even a higher mean [Ca] which is contrary to Martinez et al. (2012). Our report is in agreement with distribution of [Ca] of other studies, as discussed above, thus there might be some errors in disease definition or registration which explain the found differences. The number of cows that developed a disease postpartum in the present study was low, the continuation of this study, therefore, might provide a larger number of animals and other results in the field of relations between plasma [Ca] and developing illness. In the future, analysing data in the same way as the previous reports might give comparable results.

Conclusions

This study contributes to improved animal health by proven relations between postpartum plasma calcium concentration and aspects of animal behaviour (rumination, idle, lying and amount of steps) in the pre- and postpartum period, which can be used to develop a tool to detect herd problems caused by HC. It confirms previous reports about the distribution of calcium concentrations after calving and complement them. Especially rumination and idle time could be estimators for calcium concentrations which might be explained by decreased smooth muscle contractions and decreased rumen activity caused by HC. Additionally, parity and observed factors of rumination, idle time, number of steps and lying can estimate the calcium concentration within 48 hours after calving, explaining the variance by 50%. Relations between plasma [Ca] and developing illness are not proven, except for milk fever, but we did observe slightly lower mean [Ca] in cattle which had cystic ovarium, abomasum displacement, developed lameness, ketosis, endometritis and mastitis, which might be associated. It is the first time, relative influence of observed behaviour to plasma [Ca] are described. Further research should examine if HC can be predicted by only prepartum behaviour.

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List of Tables

		PARITY						
	N	1	2	3	4	5	6<	BREED
1	22	10	7	2	1	1	1	HOLSTEIN FRIESIAN
2	18	1	6	4	3	2	2	HOLSTEIN FRIESIAN
3	38	12	7	8	3	6	2	FLECKVIEH
4	29	4	11	6	4	1	3	HOLSTEIN FRIESIAN
5	26	6	11	3	4	2	0	HOLSTEIN FRIESIAN
7	1	1	0	0	0	0	0	MIXED
8	32	11	5	6	6	3	1	HOLSTEIN FRIESIAN
TOTAL	166	45	47	29	21	15	9	

Table 1: Number of cows per farm, used for analysis of sensor data in combination with plasma calcium concentration within 48 hours after calving. *166 animals, which calved in the period of November, 2016 to May, 2017 and had sensor data available, were included and sorted by parity.*

	PERIOD 1	PERIOD 2	PERIOD 3	PERIOD 4	PERIOD 5
EATING	-42 to -20	-19 to -1	+1 to +28		
RUMINATION	-42 to -3	-2 to 0	+1 to +4	+5 to +28	
IDLE	-42 to -4	-3 to -1	0 to +5	+6 to +28	
LYING	-42 to -3	-2 to +1	+2 to +4	+5 to +28	
STEPS	-42 to -10	-9 to -2	-1 to +1	+2 to +7	+8 to +28

Table 2: Periods of behaviour elements, based on patterns shown in figure 5. *Periods are relative to day of calving (day 0).*

PARITY	N	MEAN	5% TRIMMED MEAN	MEDIAN	MINIMUM	MAXIMUM
1	68	2.36 (2.33;2.40)	2.37	2.38	1.84	2.64
2	71	2.23 (2.17;2.29)	2.24	2.24	1.34	2.84
3	44	2.16 (2.09;2.24)	2.17	2.16	1.47	2.70
4	28	2.08 (1.93;2.23)	2.04	2.02	1.60	3.59
5	20	2.01 (1.86;2.15)	2.01	2.04	1.41	2.58
6<	16	1.84 (1.65;2.03)	1.83	1.82	1.36	2.58

Table 3: Calcium concentration within 48 hours after calving per parity. *247 animals were included.*

PARITY		**1**	**2**	**3**	**4**	**5**	**6<**
1	DIFF.	x	0.13	0.20	0.28	0.35	0.52
	SIGN.		*0.044***	*0.002****	*0.000****	*0.000*****	*0.000****
2	DIFF.	-0.13	x	0.07	0.15	0.22	0.39
	SIGN.	*0.044***		*0.750*	*0.095**	*0.011***	*0.000****
3	DIFF.	-0.20	-0.07	x	0.09	0.16	0.32
	SIGN.	*0.002****	*0.750*		*0.761*	*0.241*	*0.000****
4	DIFF.	-0.28	-0.15	-0.09	x	0.07	0.24
	SIGN.	*0.000****	*0.095**	*0.761*		*0.941*	*0.052**
5	DIFF.	-0.35	-0.22	-0.16	-0.07	x	0.16
	SIGN.	*0.000****	*0.011***	*0.241*	*0.941*		*0.423*
6<	DIFF.	-0.52	-0.39	-0.32	-0.24	-0.16	x
	SIGN.	*0.000****	*0.000****	*0.000****	*0.052**	*0.423*	

***sign<0.10 **sign<0.05 ***sign<0.01**

Table 4: Differences in mean [Ca] within 48 hours after calving between parities. *247 animals were included. Mean plasma [Ca] are described in table 3.*

	N	PARITY 1	2	3	4	5	6<	BREED	MEAN	5% TRIMMED MEAN	MEDIAN	MINIMUM	MAXIMUM
1	33	11	12	4	2	1	3	HOLSTEIN FRIESIAN	2.12 (2.01;2.23)	2.15	2.24	1.34	2.48
2	26	3	6	7	4	3	3	HOLSTEIN FRIESIAN	2.18 (2.05;2.31)	2.20	2.26	1.36	2.64
3	43	14	8	10	3	6	2	FLECK VIEH	2.27 (2.18;2.36)	2.29	2.37	1.47	2.65
4	34	5	11	8	4	1	5	HOLSTEIN FRIESIAN	2.12 (2.01;2.22)	2.12	2.15	1.37	2.69
5	49	17	18	6	6	2	0	HOLSTEIN FRIESIAN	2.30 (2.21;2.39)	2.29	2.33	1.60	3.59
6	2	0	2	0	0	0	0	HOLSTEIN FRIESIAN	2.35 (1.14;3.55)	-	2.35	2.25	2.44
7	9	4	1	2	1	1	0	MIXED	2.20 (2.14;2.26)	2.20	2.17	2.11	2.32
8	51	14	13	7	8	6	3	HOLSTEIN FRIESIAN	2.12 (2.04;2.19)	2.13	2.15	1.41	2.50
	247	**68**	**71**	**44**	**28**	**20**	**16**		**2.19 (1.89;2.49)**	**2.20**	**2.23**	**1.34**	**2.20**

Table 5: Calcium concentration within 48 hours postpartum per farm. *247 animals were included. Farm 7 and farm 8 differed significantly in mean calcium concentration (p = 0.030).*

	N	PEARSON COR.	SIGN. (1-TAILED)	ß	SE	T	SIG.	F	DF	P	R^2
LYING	136							27.543	2,133	0.000***	0.293
LMEAN1		-0.010	0.456	-0.046		-0.560	0.577				
LSD1		0.017	0.422	0.095		1.292	0.199				
LSLOPE2		-0.134	0.060*	-0.074		-1.013	0.313				
LSERES2		-0.102	0.119	-0.014		-0.190	0.850				
LSLOPE3		0.072	0.203	0.048		0.641	0.523				
LSERES3		-0.160	0.032**	-0.096		-1.286	0.201				
LMEAN4		0.176	0.020**	0.000447	0.000	2.263	0.025**				
LSD4		-0.066	0.224	-0.034		-0.449	0.654				
PARITY		-0.515	0.000***	-0.100	0.014	-7.017	0.000***				
(CONSTANT)				2.185	0.133	16.411	0.000***				
STEPS	129							41.847	1,127	0.000***	0.248
SMEAN1		0.302	0.000***	0.124		1.481	0.141				
SSD1		0.198	0.012**	0.060		0.747	0.456				
SMEAN2		0.077	0.191	-0.029		-0.364	0.717				
SSD2		0.021	0.406	0.002		0.030	0.976				
SSLOPE2		-0.129	0.073*	-0.106		-1.375	0.172				
SSERES2		-0.001	0.494	-0.020		-0.265	0.792				
SSLOPE3		0.127	0.076	0.006		0.081	0.935				
SSERES3		-0.005	0.478	-0.006		-0.075	0.940				
SSLOPE4		-0.158	0.037**	-0.077		-0.987	0.326				
SSERES4		0.103	0.122	0.016		0.203	0.839				
SMEAN5		0.254	0.002**	0.078		0.936	0.351				
SSD5		0.164	0.031**	0.040		0.500	0.618				
PARITY		-0.498	0.000***	-0.098	0.015	-6.469	0.000***				
(CONSTANT)				2.457	0.051	48.262	0.000***				

****sign<0.10 **sign<0.05 ***sign<0.01***

Table 6: Statistical analysis of behaviour extracted from the pedometer and parity. *Periods of observed factors of behaviour are described in table 2, where Mean1 is calculated mean of specific behaviour in period 1, SD1 is standard deviation of period 1, Slope2 is calculated slope of behaviour in period 2, and SEres2 is standard error of residuals of behaviour in period 2 . Pearson correlations and multiple linear regression with [Ca] were conducted. Only standard error of significant regression coefficients were given. No regression coefficients were found next to parity for amount of steps. Cows' estimated calcium concentration based on lying time is equal to 2.185 + 0.000447 x LMean4 – 0.100 x parity.*

	N	PEARSON COR.	SIG. (1-TAILED)	ß	SE	T	SIG.	F	DF	P	R^2
EATING	138							55.443	1,136	0.000***	0.290
FMEAN1		0.100	0.121	-0.111		-1.430	0.155				
FSD1		0.026	0.380	0.061		0.841	0.402				
FSLOPE2		0.054	0.266	0.082		1.131	0.260				
FSERES2		-0.012	0.444	0.004		0.056	0.955				
FMEAN3		0.186	0.014**	0.047		0.629	0.530				
FSD3		0.186	0.194	-0.093		-1.296	0.197				
PARITY		-0.538	0.000***	-0.102	0.014	-7.446	0.000***				
(CONSTANT)				2.471	0.046	53.641	0.000***				
RUMINATION	107							27.739	3,103	0.000***	0.447
RMEAN1		0.007	0.470	-0.001	0.001	-2.506	0.014**				
RSD1		0.078	0.211	0.145		1.953	0.054*				
RSLOPE2		0.309	0.001**	0.093		1.147	0.113				
RSERES2		-0.071	0.235	-0.062		-0.842	0.402				
RSLOPE3		-0.427	0.000***	-0.003	0.001	-4.724	0.000***				
RSERES3		-0.272	0.000***	0.033		0.391	0.696				
RMEAN4		0.015	0.437	0.148		1.709	0.091*				
RSD4		-0.085	0.192	-0.053		-0.709	0.480				
PARITY		-0.548	0.000***	-0.100	0.014	-6.994	0.000***				
(CONSTANT)				3.315	0.324	10.226	0.000***				
IDLE TIME	107							25.058	4,102	0.000***	0.496
IMEAN1		0.008	0.466	0.002	0.000	4.482	0.000***				
ISD1		0.034	0.363	0.039		0.448	0.655				
ISLOPE2		-0.138	0.079*	0.012		0.155	0.877				
ISERES2		0.065	0.254	0.043		0.599	0.550				
ISLOPE3		0.374	0.000***	0.003	0.001	4.488	0.000***				
ISERES3		-0.325	0.000***	-0.068		-0.849	0.398				
IMEAN4		-0.116	0.116	-0.001	0.000	-3.591	0.001**				
ISD4		-0.052	0.297	0.089		0.778	0.439				
PARITY		-0.560	0.000***	-0.109	0.014	-7.747	0.000***				
(CONSTANT)				2.294	0.143	16.019	0.000***				

sign<0.10 **sign<0.05 ***sign<0.01

Table 7: Statistical analysis of behaviour extracted from the neck sensor and parity. *Periods of observed factors of behaviour are described in table 2, where Mean1 is calculated mean of specific behaviour in period 1, SD1 is standard deviation of period 1, Slope2 is calculated slope of behaviour in period 2, and SEres2 is standard error of residuals of behaviour in period 2. Pearson correlations and multiple linear regression were conducted. Only standard errors of significant regression coefficients were given. No regression coefficients were found besides parity for eating time. Cows' estimated calcium concentration based on rumination time is equal to 3.315 – 0.100 x parity – 0.003 RSlope3 – 0.001x RMean1. Cows' estimated plasma [Ca] based on idle time is equal to 2.294 + 0.002 x IMean1 + 0.003 x ISlope3 – 0.001 x IMean4 - 0.109 x parity.*

		N	MINIMUM	MAXIMUM	MEAN	SD MEAN	ß	SE	T	SIG.
	Parity	166	1	10	2.70	1.653	-0.087	0.016	-5.505	0.000***
EATING	**FMean1**	145	193.877	507.631	364.728	68.564	0.027		0.158	0.874
	FSD1	145	8.505	105.560	47.799	12.936	0.067		0.895	0.373
	FSlope2	153	-47.000	12.096	-2.568	5.590	0.042		0.557	0.579
	FSEres2	153	17.146	108.176	46.485	14.009	0.027		0.346	0.730
	FMean3	152	131.700	511.704	324.568	76.221	-0.064		-0.469	0.640
	FSD3	151	17.037	116.607	52.056	18.008	0.000		-0.003	0.997
RUMINATION	**RMean1**	127	389.000	623.825	538.078	45.945	-0.039		-0.330	0.742
	RSD1	125	27.165	145.119	60.749	21.362	0.043		0.481	0.632
	RSlope2	124	-254.500	239.000	-68.363	65.337	0.089		1.106	0.272
	RSEres2	120	0.408	308.636	75.060	53.281	-0.054		-0.708	0.481
	RSlope3	123	-67.200	158.400	28.905	35.825	-0.003	0.001	-4.402	0.000***
	RSEres3	122	9.816	196.042	49.066	28.209	0.028		0.333	0.740
	RMean4	129	387.900	712.792	575.948	57.528	0.039		0.395	0.694
	RSD4	128	17.521	216.701	48.614	24.387	0.118		0.226	0.822
IDLE	**IMean1**	125	365.515	768.636	541.810	91.483	0.002	0.000	4.520	0.000***
	ISD1	124	27.333	168.903	76.042	26.901	0.003		0.028	0.978
	ISlope2	122	-191.000	424.000	28.713	81.993	-0.062		-0.819	0.415
	ISEres2	120	0.816	269.444	62.340	49.266	-0.052		-0.689	0.493
	ISlope3	127	-137.143	55.200	-33.622	36.850	0.169		1.797	0.076*
	ISEres3	124	14.786	272.604	79.457	47.690	-0.114		-1.378	0.172
	IMean4	128	333.826	974.333	522.786	102.270	-0.001	0.000	-3.312	0.001***
	ISD4	128	23.919	300.687	64.186	35.024	0.027		0.226	0.822
LYING	**LMean1**	144	506.500	1,147.525	789.929	105.306	-0.027		-0.297	0.767
	LSD1	144	23.335	214.651	95.349	33.132	0.057		0.742	0.460
	LSlope2	142	-342.900	190.300	-81.051	80.853	-0.081		-1.029	0.306
	LSEres2	142	7.273	318.751	107.587	68.287	-0.003		-0.035	0.972
	LSlope3	145	-668.000	237.500	26.910	105.075	0.019		0.244	0.808
	LSEres3	143	2.449	323.741	69.214	59.354	-0.065		-0.847	0.399
	LMean4	144	368.458	974.875	624.253	114.264	0.001	0.000	2.730	0.008***
	LSD4	144	35.198	307.486	92.541	35.185	-0.003		-0.036	0.971
STEPS	**SMean1**	142	1,342.545	5,731.957	2,393.925	662.895	0.000	0.000	2.137	0.035**
	SSD1	142	161.429	2,422.905	482.200	285.701	0.043		-0.813	0.419
	SMean2	143	1,343.000	5,075.500	2,464.063	678.397	-0.092		-0.821	0.414
	SSD2	143	148.585	1,825.860	442.921	265.855	0.043		0.536	0.593
	SSlope2	143	-306.536	538.000	26.208	116.179	-0.034		-0.434	0.666
	SSEres2	143	103.534	1,881.158	408.096	244.990	0.014		0.170	0.865
	SSlope3	143	-5,615.000	10,746.000	1,079.633	1,708.352	-0.053		-0.677	0.500
	SSEres3	137	6.532	5,435.009	895.873	849.331	0.033		0.440	0.661
	SSlope4	142	-3,429.143	1,397.743	-288.363	480.842	-0.029		-0.366	0.715
	SSEres4	142	113.570	3,293.020	665.540	541.224	0.030		0.388	0.699
	SMean5	142	1,545.647	6,840.952	3,310.430	917.511	-0.040		-0.383	0.703
	SSD5	142	70.618	4,093.364	781.285	705.899	0.010		0.120	0.905
	(constant)						1.563	0.238	6.555	0.000***

sign<0.10 **sign<0.05 ***sign<0.01

Table 8: Relative influence of observed behaviour elements and parity. *Descriptive statistics of all 247 animals are shown per periodical of behaviour element, which are described in table 2. 93 animals had a complete behaviour profile and were included for multiple linear regression. A significant regression equation was found ($F(6,68) = 16.755$, $p < 0.000$) with a R^2 of 0.539. Cows' calculated calcium concentration within 48 hours after parturition is equal to 1.563 – 0.087 x parity – 0.003 x Rslope3 + 0.002 x Imean1 – 0.001 x Imean4 + 0.001 x Lmean4 + 9.640E-5 x Smean1.*

	N	MEAN	SE	MEAN DIFFERENCE	STD. ERROR	SIGN.
NO DISEASE REGISTRATION	46	2.26 (2.17;2.35)	0.045	x	x	x
MILK FEVER	16	1.90 (1.69;2.10)	0.077	0.363	0.090	0.005**
CYSTIC OVARIUM	4	2.01 (1.38;2.64)	0.153	0.246	0.161	0.949
ABOMASUM DISLOCATION	1	2.12	0.306	x	x	x
LAMENESS	42	2.14 (2.03;2.25)	0.053	0.118	0.066	0.857
KETOSIS	35	2.15 (2.04;2.26)	0.052	0.108	0.069	0.942
ENDOMETRITIS	33	2.16 (2.04;2.27)	0.053	0.100	0.070	0.970
MASTITIS	12	2.17 (2.03;2.31)	0.088	0.091	0.100	0.999
DYSTOCIA	37	2.22 (2.13;2.31)	0.050	0.039	0.068	1.000
STILLBIRTH	8	2.22 (2.09;2.35)	0.108	0.036	0.118	1.000
METRITIS	10	2.23 (2.00;2.45)	0.097	0.035	0.108	1.000
RETENTIO SECUNDARINUM	12	2.30 (2.16;2.43)	0.088	-0.039	0.100	1.000

***sign<0.10 **sign<0.05 ***sign<0.01*

Table 9: Calcium concentration within 48 hours after parturition per registered disease. *166 animals were included. Diseases were compared with the group without disease registration. Milk fever is reported by farmer, cystic ovarium by ultrasound in the fourth or eight week postpartum, abomasum dislocation by a veterinarian, lameness is increased locomotion score after parturition, ketosis is a detected ketone concentration >1.2 mmol/L BHB, endometritis by ultrasound and manual palpation, mastitis by report of farmer, dystocia by calving score ≥2, stillbirth is reported by producer, metritis within 21 days postpartum with purulent, fetid discharge and retentio secundarinum longer than 24 hours.*

Figures

Number of farm	Cow	Parity	Date of parturition	Point of time parturition	
					exact[1]
					+/-
Calving type → Circle or tick					
1. Without help	2. Small amount of traction	3. Several traction (2 persons are pulling)	4. Severely traction/ calving jack	5. Section caesarean	
Calf taken away from cow → Circle or tick					
1. Immediately after birth	2. Within 1 hour	3. Within 4 hours	4. Within 12 hours	5. Within 24h	6. After 24 hours

Calving details → Circle or tick								
Twin	**Calf dead**		**Afterbirth off**				**Treatment afterbirth**	
	Immediately	Within 24h	Within 6u	6-12h	12-24h	After 24h	Capsules	Oxytetracycline flush

Diseases	**Date:**					
Mastitis			**Cow lame**		**Abomasum displacement**	
Ill cow "coli"	Aberrant milk		Moderate lame	Severely lame	Left	Right
Milk fever			**Treatment milk fever**			
Cow is standing	Cow can not stand		Infusion, amount of bottles:	Subcutaneous	Oral	
Ketosis diagnosis			**Treatment ketosis**			
Veterinarian	Smell of acetone	Stick	Injection	Propylene glycol	Drenching	
Comments:						

Registration of treatment of other cattle			
Cow	**Date**	**Diagnosis**	**Treatment (what, how much, how long)**

[1]Exact = + or – 10 minutes

Figure 1: Parturition form. *Producers filled in parturition form for each cow individually after calving.*

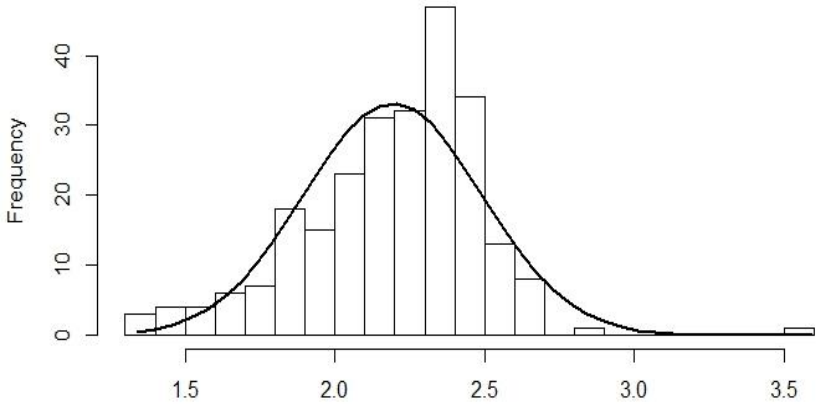

Figure 2: Histogram presenting serum calcium concentration within 48 hours after parturition and normal distribution. *Blood samples of 247 cattle were included. Minimum (1.34 mmol/L), maximum (3.59), median (2.23), mean (2.194; SD=0.297, SE=0.019), and 5% trimmed mean (2.20) were determined and calculated.*

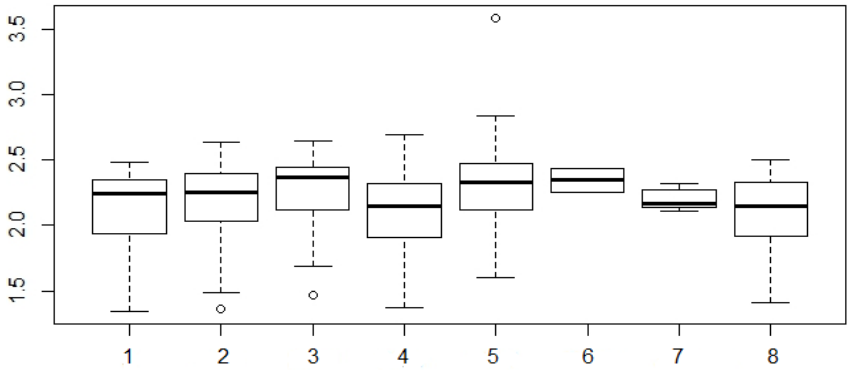

Figure 3: Boxplot presenting serum calcium concentration within 48 hours after parturition per farm. *Blood samples of 247 cattle were included. Samples were collected by farm: 1 (n = 33), 2 (n = 26), 3 (n = 43), 4 (n = 34), 5 (n = 49), 6 (n = 2), 7 (n = 9) and 8 (n = 51). Statistical difference is found between farm 7 and 8 in mean calcium concentration (p = 0.030). Three extreme outliers are detected: 1.36 (milk fever), 1.47 (milk fever), and 3.59 (milk fever, after intravenous calciumsalts).*

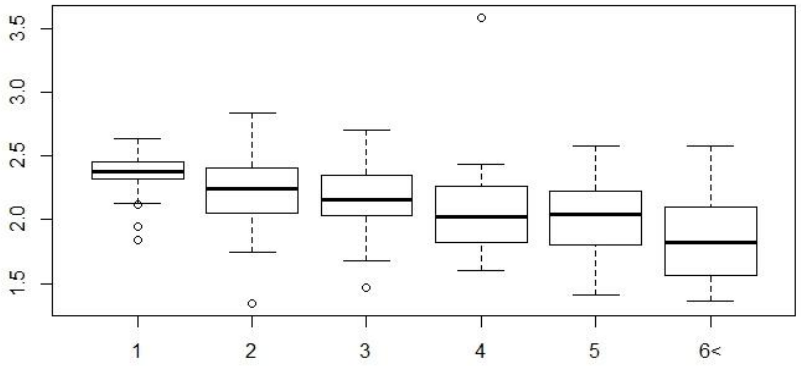

Figure 4: Boxplot presenting postpartal serum calcium concentration per parity. *Blood samples of 247 cattle were included and analysed. Parity 1 (n = 68), 2 (n = 71), 3 (n = 44), 4 (n = 28), 5 (n = 20), 6 (n = 7), 7 (n = 5), 8 (n = 2), 10 (n = 1) and 13 (n = 1) were included. Parity had a significant influence on calcium concentration (p < 0.000). Extreme outliers were represented by 1.84, 1.95, and 2.12 in the first lactation, 1.34 in the second lactation, 1.47 in the third lactation, and 3.59 in the fourth lactation. Significant differences were found between parity 1 to 2 (p = 0.044), parity 1 to 3 (p = 0.002), parity 1 to 4 (p < 0.000), parity 1 to 5 (p < 0.000), parity 1 to >6 (p < 0.000), parity 2 to 5 (p = 0.011), parity 2 to >6 (p < 0.000), and parity 3 to >6 (p = 0.001).*

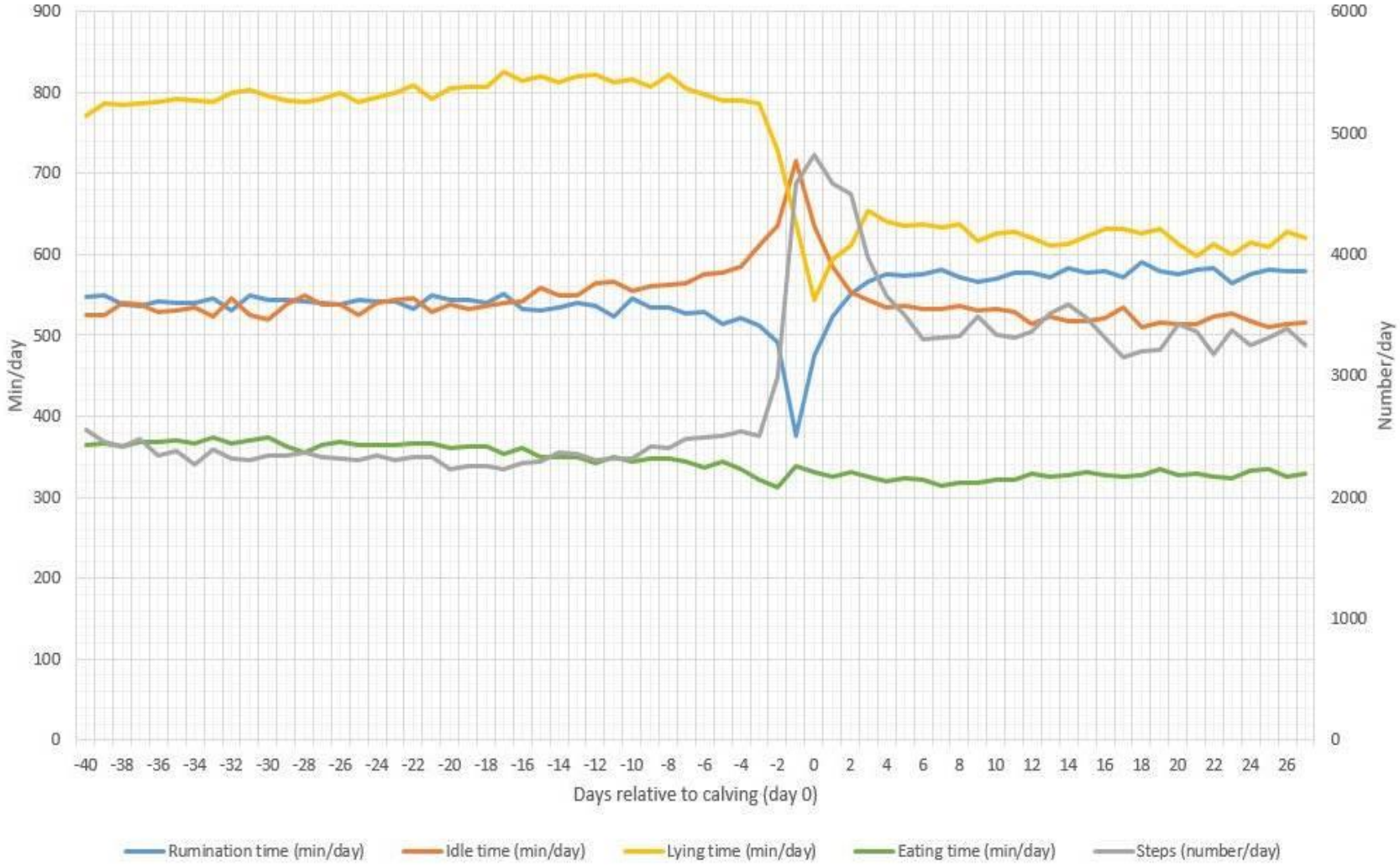

Figure 5: Pattern of mean rumination, idle, eating, lying and number of steps per day in the transition period and the first four weeks after calving of 166 cows. *Based on patterns found, feeding time is separated into period -42 to -20, -19 to -1, and +1 to +28 relative to calving. Rumination is separated into -42 to -3, -2 to 0, 1 to 4, and 5 to 28, idle time into -42 to -4, -3 to -1, 0 to 5, 6 to 28, lying into -42 to -3, -2 to 1, 2 to 4, and 5 to 28, and number of steps into -42 to -10, -9 to -2, -1 to 1, 2 to 7, and 8 to 28.*