



Associations between days in the close-up group and milk production, transition cow diseases, reproductive performance, culling, and behavior around calving of Holstein dairy cows

P. L. Venjakob,^{1,2*} W. Heuwieser,¹ and S. Borchardt¹

¹Clinic for Animal Reproduction, Faculty of Veterinary Medicine, Freie Universität Berlin, Königsplatz 65, 14163 Berlin, Germany

²Clinic for Ruminants, Faculty of Veterinary Medicine, Justus-Liebig-Universität Gießen, Frankfurter Str. 104, 35392 Gießen, Germany

ABSTRACT

The objective of experiment I was to evaluate the association between days in the close-up group (DINCUCU) and milk production, early lactation diseases, reproductive performance, and culling. In experiment II behavioral changes associated with DINCUCU were evaluated using a neck-mounted sensor (Smarttag neck, Nedap Livestock Management, Groenlo, the Netherlands). Cow-lactations of 28,813 animals from 14,155 individual cows of 2 farms in northern Germany and western Slovakia, calving between January 2015 and December 2020, were included in the study. After exclusion of cows with a gestation length <262 and >292 d and cows with >42 DINCUCU data from 8,794 and 19,598 nulliparous and parous cows, respectively, were available for final statistical analyses. To analyze the association between DINCUCU and second test-d 305-d mature-equivalent milk projection, linear mixed models were calculated. Binary data (i.e., clinical hypocalcemia, hyperketonemia, retained placenta [RP], acute puerperal metritis [APM], mastitis, left displaced abomasum [LDA], first service pregnancy risk) were analyzed using logistic regression models. To analyze the association between DINCUCU and culling or death during the first 300 DIM Cox proportional hazards were used. To analyze the association between DINCUCU and behavior 7 d before to 7 d after calving (i.e., activity, inactivity, eating, ruminating time), linear mixed models were calculated. Nulliparous cows with a short (<10 DINCUCU) and a long stay (>30 DINCUCU) in the close-up group had a lower milk production an increased risk for hyperketonemia, RP, and APM compared with nulliparous cows with DINCUCU between 21 to 28 d. Parous cows with a short (<10 DINCUCU) and a long stay (>30 DINCUCU) in the close-up group had a lower milk production, an increased risk for RP and mastitis, a reduced first service

pregnancy risk, and an increased culling risk, compared with parous cows with DINCUCU between 21 to 28 d. Furthermore, the risk for clinical hypocalcemia and LDA was increased in parous cows with >30 DINCUCU compared with parous cows with <30 DINCUCU. The risk for APM was increased in parous cows with <10 DINCUCU compared with parous cows with >10 DINCUCU. In nulliparous cows no association was found between DINCUCU and the risk for left displaced abomasum and mastitis. In experiment II, cows with 7 and 35 DINCUCU had an impaired behavior around calving compared with cows with 14, 21, and 28 DINCUCU. During the last 7 d before parturition, these cows were more inactive and had a reduced eating and ruminating time. After calving, cows with 7 DINCUCU spent less time eating. In conclusion, cows with <10 DINCUCU and cows with >30 DINCUCU had a lower milk production, a higher risk to incur diseases and an impaired behavior, especially before calving.

Key words: days in close-up group, 305-d mature-equivalent milk projection, early lactation diseases, rumination

INTRODUCTION

The transition from late gestation to early lactation is subject to hormonal, behavioral and metabolic changes (Chebel, 2021). In the transition period, typically defined as the last 3 wk of gestation and the first 3 wk of lactation (Grummer, 1995), more than 90% of production diseases occur (Ingvarsen et al., 2003). To adjust for reduced DMI during the last days of gestation and to reduce susceptibility to diseases, dry cows are fed with different diets during the dry period (Grummer, 1995; Drackley, 1999; Van Saun and Sniffen, 2014). Whereas far-off diets are typically fed for the first 4 to 6 wk after dry off, close-up diets are provided starting around d 255 of gestation (Vieira-Neto et al., 2021). Far-off diets are prepared to feed high forage and high NDF content in combination with low energy density to avoid gain of adipose tissue (Drackley, 1999). During

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*Corresponding author: peter.venjakob@vetmed.uni-giessen.de

the last weeks of gestation DMI decreases, reaching a nadir on the day of calving (Soriani et al., 2012; Pérez-Báez et al., 2019a). This is accompanied by a reduction in rumination time (Soriani et al., 2012). To account for the change in DMI, close-up diets have a higher energy density compared with far-off diets. Cows in the close-up period need more energy and protein in response to synthesis of mammary tissue and colostrum as well as for fetal and uterine growth (Van Saun and Sniffen, 2014). Furthermore, a 2-phase dry period offers the possibility of feeding a more aggressive negative dietary anion cation difference (**DCAD**) to cows in the close-up pen to reduce the risk for subclinical and clinical hypocalcemia (**CH**; Block, 1984). The ideal duration of feeding such a diet, however, is still being discussed. Recent studies provide evidence that a certain duration of days in the close-up group (**DINCU**) should be achieved to improve milk production and reduce susceptibility to early lactation diseases. There is a quadratic association between DINCU and milk production of parous cows (Chebel, 2021; Vieira-Neto et al., 2021; Venjakob et al., 2022) with a maximum milk production when cows stayed 21 DINCU (Venjakob et al., 2022). Vieira-Neto et al. (2021) also evaluated the association between DINCU and disease incidence until 60 DIM. In parous cows, there was a quadratic association between morbidity and DINCU. Cows with a short (i.e., <10 d) or a long (i.e., >30 d) stay in the close-up group had a greater risk for diseases until 60 DIM. Also, Chebel (2021) found an increased risk for uterine diseases such as retained placenta (**RP**), metritis and acute puerperal metritis (**APM**) in cows with a short or long stay in the close-up group. One reason for the detrimental effects of a short and long stay in the close-up group might be attributed to behavioral changes due to the need to establish a new social hierarchy close to calving (Nordlund et al., 2006) and an altered feeding behavior (Grant and Albright, 2001). On the contrary, a long stay in the close-up group may increase the risk for metabolic diseases caused by an increased risk for excessive body condition and reduced DMI (Olagaray et al., 2020).

The objective of experiment I was to evaluate the association between DINCU and milk production, early lactation diseases, reproductive performance, and culling. The objective of experiment II was to evaluate the association between DINCU and time spent eating, ruminating, active, and inactive. Our hypotheses were that cows with a short and a long stay in the close-up group have a reduced milk production, a greater risk for diseases after calving, a decreased reproductive performance, and ultimately an increased culling risk. We also hypothesized that cows with a short stay in the close-up group have an altered behavior.

MATERIALS AND METHODS

The current study is a retrospective data analysis. No human or animal subjects were used, so this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board.

Study Design

This observational retrospective cohort study used the data from 2 farms of 6 calendar years between January 2015 and December 2021. In experiment I, we used health and performance data from both farms from January 2015 to December 2020. In experiment II, we collected data on time spent eating, ruminating, active, and inactive 7 d before calving to 7 d after calving. The data were generated by a commercial sensor system (Smarttag neck, Nedap Livestock Management, Groenlo, the Netherlands; Borchers et al., 2021) in farm 2 between January 2020 and December 2021.

Farm 1 was located in northern Germany and farm 2 was located in western Slovakia. Both farms kept exclusively Holstein Friesian cows. During the study period, farm 1 and farm 2 had an average of 2,872 (± 110 ; SD) and 2,823 (± 216 ; SD) calvings per year, respectively. Inclusion criteria were participation in a DHIA testing system and a 2-phase dry cow period consisting of a far-off and a close-up period with feeding of a negative DCAD diet (<0 mEq/100g of DM). Furthermore, farms were required to record cow movements from the far-off to the close-up group in their herd management software (Dairy Comp 305, Valley Ag Software, Tulare, CA).

Close-up cows of both farms were fed a TMR once daily to meet or exceed minimum nutritional requirements (Table 1; NRC, 2001) and feed was pushed up 10 times a day. The TMR samples from the close-up diets were analyzed in certified commercial laboratories (farm 1: Landwirtschaftliche Kommunikations- und Servicegesellschaft mbH, Lichtenwalde, Germany; farm 2: Rock River Laboratory Europe, Heiddorf, Germany).

Transition Cow Management

In farm 1 and 2, nulliparous cows were moved once weekly to the close-up pen at $d 255.7 \pm 4.5$ (SD) and 256.5 ± 5.2 (SD) of gestation, respectively. Parous cows were dried-off approximately 55 d before expected parturition and moved to the far-off group. Transfer of parous cows from the far-off to the close-up group was conducted on a weekly basis at $d 257.0 \pm 4.5$ (SD) and 254.6 ± 5.2 (SD) of gestation on farm 1 and 2, respectively. On farm 1, nulliparous and parous cows were kept separately in sand-bedded freestall pens during the

Table 1. Chemical composition (% unless otherwise noted) of the close-up diets from both farms enrolled¹

Nutrient composition (DM basis)	Farm 1				Farm 2			
	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
DM	40.8	3.4	33.8	46.1	42.8	3.4	37.9	47.6
CP	14.1	1.0	12.1	17.3	14.3	0.9	13.0	15.6
Ether extract	3.0	0.5	1.7	4.3	2.8	0.2	2.5	3.2
NDF	45.6	3.6	40.4	56.2	40.7	4.1	36.0	49.2
NFC ²	29.9	8.4	19.6	34.9	33.3	4.0	27.8	38.6
Starch	16.5	2.6	9.9	22.9	17.1	3.6	11.1	24.2
Ash	8.6	0.7	6.8	9.7	6.3	0.3	6.1	6.8
DCAD ³ meq/kg of DM	-108.0	45.5	-212.0	-6.0	-88.0	47.1	-152.0	-40.0

¹Chemical compositions were determined based on at least 4 composites per year during the study period.

²Calculated as 100 – CP – ether extract – NDF – ash.

³DCAD = [(Na% of DM/0.023) + (K% of DM/0.039)] – [(S% of DM/0.016) + (Cl% of DM/0.0355)].

close-up period. On farm 2 nulliparous and parous cows were housed together in pens with deep-straw bedding. Prepartum nulliparous and parous cows were monitored every hour to detect signs of imminent parturition (i.e., restlessness, vaginal discharge with bloody traces, lying lateral with abdominal contractions, a visible or broken amniotic sac, or feet of the emerging calf outside the vulva). Animals were moved into an individual maternity pen (3.5 × 3.5 m on farm 1 and 8 × 8 m on farm 2) bedded with fresh straw when the amniotic sac was visible or broken outside the vulva, or appearance of feet of the emerging calf were detected outside the vulva. If the cow had not delivered the calf within 1 h calving assistance was provided (Schuenemann et al., 2011). All parous cows received prophylactic calcium supplementation (Bovikalc, Boehringer Ingelheim, Ingelheim am Rhein, Germany) at parturition and 12 to 24 h later. After parturition, cows on both farms were milked twice daily. Postpartum cows were examined daily by the farm personnel until 10 DIM following standard operating procedures created by the herd manager. These examinations have been described in detail, previously (Venjakob et al., 2021). Between 11 and 30 DIM cows were screened for health disorders based on visual observations performed by the farm personnel every morning at 0800 h and on deviations in their daily milk production.

Disease Definitions

For the purpose of this study, the following diseases were recorded during the first 30 DIM: CH, RP, APM, hyperketonemia (**KET**), left displaced abomasum (**LDA**) and mastitis. Because both of the farms had a long-term history in collaborating with the Clinic for Animal Reproduction, disease definitions were consistent between both farms: when cows were recumbent during one of the first 5 DIM and rose in response to

an intravenous Ca infusion, cows were considered as having CH; hyperketonemia was defined as serum BHB concentrations ≥1.2 mmol/L using a cow-side BHB test (Precision Xtra, Abbott Laboratories, Abbott Park, IL; on farm 1 cows between 2 and 14 DIM were checked for ketosis every Monday and Friday; on farm 2 all cows were tested on 5 and 10 DIM); when percussion of the left flank resulted in tympanic resonance auscultated with a stethoscope, cows were considered to suffer from LDA; when fetal membranes were not expelled by 24 h after calving, cows were diagnosed with RP; signs of systemic illness (e.g., decreased milk production, dullness, or other signs of toxemia) in combination with an animal with an abnormally enlarged uterus, fetid watery red-brown uterine discharge and fever >39.5°C was considered as APM (Sheldon et al., 2006); clinical mastitis was defined as visible signs of inflammation in an affected mammary gland (i.e., redness, swelling, pain, or heat) and alterations such as clots, flakes, discoloration, or abnormal consistency of secretions (Vasquez et al., 2017). Udder health was evaluated 2 times daily by farm personnel during regular milking.

Reproductive Management

Farm 1. From January 2015 until February 2016, cows were inseminated for first service using a combination of AI after estrus detection or timed AI (**TAI**) with the Presynch-Ovsynch protocol (PGF_{2α}, 14 d later PGF_{2α}, 12 d later GnRH, 7 d later PGF_{2α}, 56 h later GnRH, and 16 to 18 h later TAI; Moreira et al., 2001). Cows underwent AI after estrus detection if detected in estrus after the second PGF_{2α} treatment of the Presynch portion of the protocol given at 50 ± 3 DIM. Cows not detected in estrus completed the Ovsynch portion of the protocol and received TAI at 72 ± 3 DIM. Estrus detection was conducted by farm personnel, using a combination of visual observation and tail

chalking. Cows detected in estrus based on tail chalk removal conducted daily after the second PGF_{2α} injection of the presynchronization protocol were inseminated. Cows received second and subsequent AI services after detected estrus any time after a previous insemination. Cows not re-inseminated at detected estrus underwent pregnancy diagnosis through transrectal ultrasound (Easi-Scan:GO, IMV Imaging, Bellshill, Scotland) 32 ± 3 d after AI. Open cows received a Resynch protocol (GnRH, 7 d later PGF_{2α}, 56 h later GnRH, 16 to 18 h later TAI, Lopes et al., 2013) for resynchronization of ovulation and TAI 42 ± 3 d after their previous insemination. From March 2016 until December 2020, cows were inseminated for first service using only TAI after implementing a Double-Ovsynch protocol (GnRH, 7 d later PGF_{2α}, 3 d later GnRH, 7 d later GnRH, 7 d later PGF_{2α}, 24 h later PGF_{2α}, 32 h later GnRH, and 16 to 18 h later TAI at 72 ± 3 DIM; Souza et al., 2008). At 25 ± 3 d after AI, all cows received a GnRH treatment. Seven days later at 32 ± 3 d after AI, cows underwent pregnancy diagnosis through transrectal ultrasound. Open cows with at least one corpus luteum (CL) ≥15 mm received a PGF_{2α} treatment, a second PGF_{2α} 24 h later, GnRH 32 h after the second PGF_{2α}, and TAI 16 to 18 h later. Cows that did not meet the criteria to be included in the CL group received a modified Ovsynch protocol (i.e., 2 PGF_{2α} treatments) with progesterone (P4) supplementation through an intravaginal P4-releasing device (CIDR; 1.38 g of progesterone, Eazi-Breed CIDR, Zoetis, Florham Park, NJ) from the time of the first GnRH to the first PGF_{2α} treatment of the protocol (GnRH and CIDR inserted, 7 d later CIDR removal and PGF_{2α}, 24 h later PGF_{2α}, 32 h later GnRH, and 16 to 18 h later TAI; Pérez et al., 2020). All pregnancy diagnoses were conducted by trained farm personnel. A second pregnancy diagnosis was performed 80 d after AI.

Farm 2. Cows were inseminated after estrus detection according to the alert of the automated activity monitoring system (i.e., 2015 to 2019: CowManager SensOor, Agis, Harmelen, the Netherlands; 2020 and 2021: Nedap Livestock Management, Groenlo, the Netherlands) that was confirmed by a technician via transrectal palpation of a highly contractile uterus or visualization of clear, stringy vaginal discharge or received timed AI after hormonal intervention. Voluntary waiting period was 50 DIM (January 2015 to June 2020) and 60 DIM (July 2020 to December 2021), respectively. The sensor system generated a list of cows eligible for breeding, based on the activity value. If cows were confirmed being in estrus, insemination was conducted on the same day. Cows that were not bred until 80 ± 3 DIM received timed AI using a modified 7-d Ovsynch protocol including a second PGF_{2α} treat-

ment on d 8 (GnRH, 7 d later PGF_{2α}, 24 h later PGF_{2α}, 32 h later GnRH, and 16 to 18 h later TAI; Wiltbank et al., 2015). Cows received second and subsequent AI services after detected estrus any time after a previous insemination. Cows not re-inseminated at detected estrus underwent pregnancy diagnosis through transrectal ultrasound (Easi-Scan:GO, IMV Imaging, Bellshill, Scotland) 39 ± 3 d after AI. At 32 ± 3 d after AI, all cows received a GnRH treatment. Open cows with at least one CL ≥15 mm received 2 PGF_{2α} treatments 24 h apart, GnRH 32 h after the second PGF_{2α}, and TAI 16 to 18 h later. Cows that did not meet the criteria to be included in the CL group received a modified Ovsynch protocol (i.e., 2 PGF_{2α} treatments; GnRH, 7 d later PGF_{2α}, 24 h later PGF_{2α}, 32 h later GnRH, and 16 to 18 h later TAI). All pregnancy diagnoses were conducted by trained farm personnel. A second pregnancy diagnosis was performed 80 d after AI.

Behavioral Data

Time spent eating, ruminating, active, and inactive was recorded with a 3-dimensional accelerometer (Nedap Smarttag Neck, Nedap, city) validated for behavioral monitoring previously (Borchers et al., 2021). The sensor distinguishes between forward and backward, left and right and up and down movements. Rumination measurements are based on the periodic movement of the jaw during regurgitation and subsequent chewing of the bolus in both lying and standing positions. Eating measurements are based on the specific head movements of the cow during the intake of feed. Inactive time measurement occurs when the cow is not actively moving and can occur in either standing or lying positions (Borchers et al., 2021). The sensor classifies specific behavior-associated movements performed for the majority of each minute. Data were obtained in records containing observations for each cow in 1-min periods and summed into 24 h periods starting 7 d before parturition until 7 d after parturition using a Python script (Plenio et al., 2021). Only complete data sets were considered for this analysis.

Statistical Analyses

Experiment 1. Individual cow data of cows that calved between January 2015 and December 2020 were transferred from Dairy Comp 305 (Valley Ag Software, Tulare, CA) to Microsoft Excel (Office 2013, Microsoft Deutschland Ltd., Munich, Germany). Statistical analyses were performed using SPSS for Windows (version 25.0, SPSS Inc., Chicago, IL).

During the study period 34,170 calvings occurred. After exclusion of records with missing information

about DINCUCU, 28,856 lactations from 14,173 cows were remaining. As shown by Vieira-Neto et al. (2017) gestation length (**GL**) is associated with health and milk production in early lactation. Because GL is highly correlated with DINCUCU ($r = 0.565$; $P = 0.001$), we initially considered GL in all models as a potential confounder; however, both farms consistently moved cows once weekly based on their GL from the far-off to the close-up group, leading to potential collinearity among the predictor variables in the statistical models. Therefore, we recalculated all models excluding GL. As the results changed marginally, GL was not included in the final models to avoid autocorrelation. We excluded, however, cows with a GL longer (>292 d, $n = 64$) and shorter (<262 d, $n = 239$) than 3 standard deviations (SD) from the mean. In addition, cows with DINCUCU >42 DINCUCU ($n = 118$) were excluded from the study.

To analyze the association between DINCUCU and second test-d 305-d mature-equivalent milk projection (**2nd305ME**; mean \pm SD DIM at second test was 56.5 ± 14.5 d) linear mixed models were calculated using the GENLINUX procedure of SPSS. This outcome was chosen as it allows to include cows that were culled after the second test day. Furthermore, waiting for a complete 305-d milk production record would bias the estimate due to missing data of cows that did not complete the period of 305 d (McCarthy and Overton, 2018). Cow within year was the experimental unit. To account for the fact that observations from cows that were enrolled repeatedly in different years were not independent from each other, parity was used as a repeated measure in the models of parous cows. Separate models were built for nulliparous and parous cows. As described by Dohoo et al. (2009) each variable considered for the mixed model was analyzed in a univariate model, including the variable as a fixed factor (i.e., categorical variable) or covariate (i.e., continuous variable). The final mixed model included all variables resulting in univariate models with $P \leq 0.100$. We included the year of calving (2015 to 2020), farm (farm 1 vs. farm 2), season of calving (winter from December 1 to February 28, spring from March 1 to May 31, summer from June 1 to August 31, and autumn from September 1 to November 30), sex of the calf (female vs. male calf), calving ease (0 = not observed, 1 = unassisted calving, 2 = calving assisted by 1 person, 3 = calving assisted by more than 1 person), stillbirth (yes vs. no), twins (yes vs. no), DINCUCU and the interaction DINCUCU by DINCUCU (**DINCUCU** \times **DINCUCU**) as explanatory variables. In addition, the model for nulliparous cows contained age at first calving (continuous) and the model of parous cows contained parity (second vs. third vs. \geq fourth lactation) and 305-d milk production of the previous lactation (continuous).

Binary data on diseases (CH, RP, APM, KET, LDA, mastitis) and first service pregnancy risk (**FSPR**; yes vs. no) were analyzed using logistic regression models using the GENLINUX procedure of SPSS. As in nulliparous cows, only 5 of 8,798 cows were suffering from CH, no model was calculated to investigate the relation between DINCUCU and CH in these cows. Continuous variables were analyzed as covariates and categorical variables were analyzed as fixed factors in univariate models. Variables with $P \leq 0.100$ were considered for the mixed model. In the model of FSPR, farm was forced to remain in the model as there have been considerable differences in reproductive management. As described above, cow within year was the experimental unit. To account for the fact that observations from cows that were enrolled repeatedly in different years were not independent from each other, parity was used as a repeated measure in the model of parous cows. Separate models were built for nulliparous and parous cows. The initial models for nulliparous and parous cows contained the same variables as described above for the linear mixed models. To account for multiple comparisons, the P -value was adjusted using a Bonferroni correction. Variables were declared significant when $P < 0.050$.

Collinearity among variables was explored for all GENLINUX models developed using the collinearity diagnostics in SPSS that provide the variance inflation factor (**VIF**) and tolerance. In each model, a correlation matrix is produced for all predictor variables. The VIF indicates whether a predictor has a strong linear relationship with the other predictor(s) and the tolerance statistics, which is its reciprocal ($1/\text{VIF}$). If more than one strongly collinear variable qualified for entry into a model, one variable was chosen based on biological significance. Collinearity was not detected based on the VIF (<1.5).

Using the SURVIVAL procedure of SPSS, 2 different Cox proportional hazards were calculated to model the time to event outcomes for culling or death during the first 300 DIM of nulliparous and parous cows, respectively. The variables year of calving, season of calving, farm, GL, DINCUCU and DINCUCU \times DINCUCU were tested as risk factors. In addition, the model for nulliparous cows contained age at first calving and the model for parous cows contained parity and milk yield in previous lactation. The proportional hazard assumption was checked using Schoenfeld residuals.

Experiment II. Data on time spent eating, ruminating, active, and inactive were obtained 7 d before calving until 7 d after calving from the Nedap system between January 2020 and December 2021. In total, 4,977 observations from 723 cows of farm 2 were included in the analyzes. Data were processed using a Python

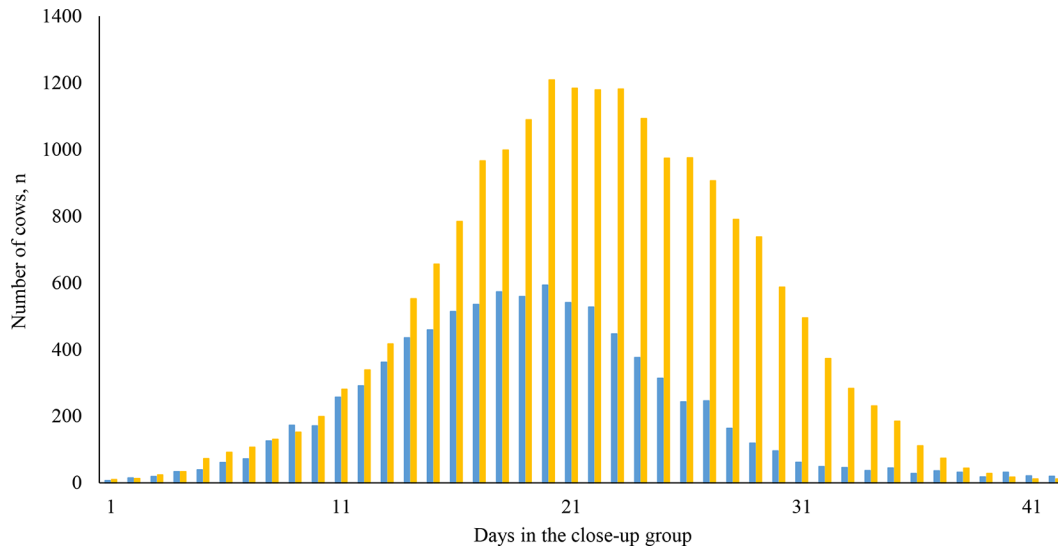


Figure 1. Frequency distribution of days in the close-up group of 8,798 nulliparous (blue) and 19,641 parous cows (yellow).

script and transferred to Microsoft Excel (Office 2013, Microsoft Deutschland Ltd., Munich, Germany) and analyzed using R (Version 4.0, R Core Team).

The GAMLj procedure of R was used to analyze the association between DINCUs and time spent eating, ruminating, active, and inactive around calving, linear mixed models were calculated separately for the time period before (-7 to -1 d) and after ($+1$ to $+7$ d) calving. For both periods, separate models were built for time spent eating, ruminating, active, and inactive. To evaluate the effect of DINCUs, only cows were considered that spent exactly 7, 14, 21, 28, and 35 DINCUs. Each variable considered for the mixed model was analyzed in a univariate model, including the variable as a fixed factor (i.e., categorical variable) or covariate (i.e., continuous variable; Dohoo et al., 2009). All variables resulting in univariate models with $P \leq 0.10$ were included in the final mixed model. We included parity (nulliparous vs. parous), time (-7 , -6 , -5 , -4 , -3 , -2 , -1 or 1 , 2 , 3 , 4 , 5 , 6 , 7 d relative to calving), DINCUs (7, 14, 21, 28, or 35 d), and the interaction of time by DINCUs. To account for multiple comparisons, the P -value was adjusted using a Bonferroni correction.

RESULTS

Experiment 1

Of the 34,170 records obtained, records were excluded due to missing data on DINCUs ($n = 5,314$), cows with GL longer than 292 d ($n = 64$), cows with GL shorter than 262 d ($n = 239$), and cows with DINCUs longer than 42 d ($n = 114$). Overall, 28,439 records

from 14,161 cows were available for final statistical analyses. Of those, 8,798 and 19,641 were entering their first and \geq second lactation, respectively. The frequency distribution for DINCUs of nulliparous and parous cows is displayed in Figure 1. In nulliparous cows, 546 (6.2%) and 426 (4.8%) cows stayed shorter than 10 d and longer than 30 d in the close-up group, respectively. In parous cows, 636 (3.2%) and 1,865 (9.5%) cows stayed shorter than 10 d and longer than 30 d in the close-up group, respectively. For farm 1, the average 305-d milk production, GL, and DINCUs were 10,088 ($\pm 2,239$; SD) kg, 276.7 (± 4.8 ; SD) d and 20.0 (± 6.0 ; SD) d, respectively. For farm 2 the average 305-d milk production, GL, and DINCUs were 11,454 ($\pm 1,977$; SD) kg, and 277.4 (± 4.7 ; SD) d, and 22.2 (± 7.2 ; SD) d, respectively.

Association with Milk Production. In both nulliparous ($P = 0.001$) and parous cows ($P < 0.001$) we detected an association between DINCUs and 2nd305ME (Figure 2; Supplemental Tables S1 and S2, <http://dx.doi.org/10.22029/jlupub-15669>, Venjakob, 2023). Nulliparous cows with 7, 14, 21, 28, and 35 DINCUs had a 2nd305ME of 10,488 (± 80), 10,844 (± 33), 11,155 (± 29), 11,188 (± 53), and 10,628 (± 102) kg milk, respectively (Table 2). In nulliparous cows, we also detected an association between the interaction of DINCUs \times DINCUs and 2nd305ME ($P = 0.049$). Parous cows with 7, 14, 21, 28, and 35 DINCUs had a 2nd305ME of 10,754 (± 107), 11,044 (± 47), 11,110 (± 32), 10,917 (± 39), and 10,746 (± 81) kg milk, respectively (Table 2). The milk production of nulliparous and parous cows was greatest, when they stayed 25 and 22 DINCUs, respectively.

Association with Diseases. Incidence rates for diseases of nulliparous and parous cows monitored in

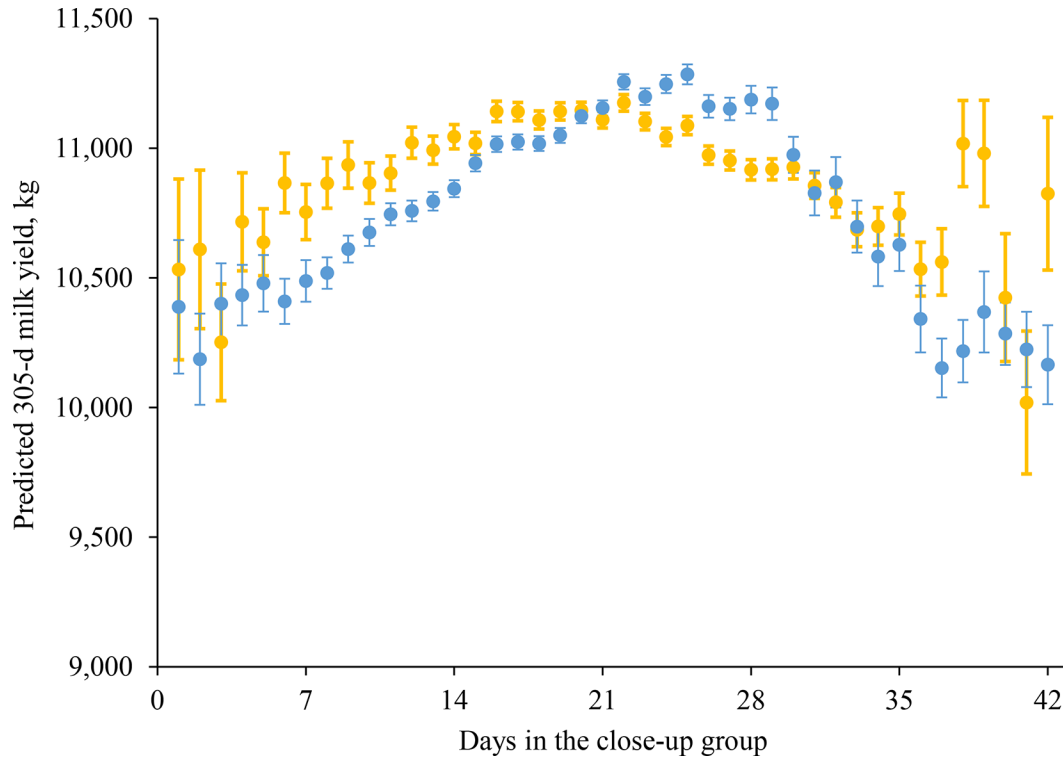


Figure 2. Association between days in the close-up group (DINCUs) and predicted 305-d milk projection based on second test day milk production using least square estimates (mean \pm SEM) from the generalized linear mixed model. Milk production of nulliparous cows ($n = 7,985$; blue) was associated with the year of calving ($P < 0.001$), farm ($P < 0.001$), age at first calving ($P < 0.001$), season ($P < 0.001$), calving ease ($P = 0.003$), stillbirth ($P = 0.002$), DINCUs ($P = 0.001$) and DINCUs \times DINCUs ($P = 0.049$). In parous cows ($n = 17,483$; yellow), the year of calving ($P < 0.001$), farm ($P < 0.001$), season ($P < 0.001$), parity ($P < 0.001$), calving ease ($P < 0.001$), stillbirth ($P < 0.001$), milk production in previous lactation ($P < 0.001$), and DINCUs ($P < 0.001$) were associated with milk production.

this study are displayed in Table 3. In parous cows, DINCUs were associated with the risk of CH ($P < 0.001$; Figure 3; Supplemental Table S3, <http://dx.doi.org/10.22029/jlupub-15669>, Venjakob, 2023). Parous cows with 7, 14, 21, 28, and 35 DINCUs had a predicted probability for CH of 2.7 (± 0.4), 2.6 (± 0.2), 3.5 (± 0.1), 4.1 (± 0.2), and 6.0 (± 0.3) %, respectively (Table 2). In nulliparous and parous cows, DINCUs were associated with the risk for KET ($P < 0.001$; Figure 4; Supplemental Tables S4 and S5, <http://dx.doi.org/10.22029/jlupub-15669>, Venjakob, 2023). Nulliparous cows with 7, 14, 21, 28, and 35 DINCUs had a predicted probability for KET of 14.7 (± 1.4), 11.9 (± 0.6), 11.9 (± 0.5), 13.4 (± 0.9), and 13.9 (± 1.7) %, respectively (Table 2). Parous cows with 7, 14, 21, 28, and 35 DINCUs had a predicted probability for KET of 30.8 (± 1.2), 28.3 (± 0.5), 29.1 (± 0.4), 29.7 (± 0.4), and 33.8 (± 0.9) %, respectively (Table 2). Moreover, in parous cows DINCUs were associated with LDA ($P = 0.027$; Figure 5; Supplemental Table S6, <http://dx.doi.org/10.22029/jlupub-15669>, Venjakob, 2023). Parous cows with 7, 14, 21, 28, and 35 DINCUs had a predicted probability for LDA of 2.0 (± 0.2), 2.1 (± 0.1), 2.3 (± 0.1), 2.2

(± 0.1), and 2.5 (± 0.1) %, respectively (Table 2). In nulliparous cows no association between DINCUs and LDA was observed ($P = 0.806$). In nulliparous ($P = 0.001$) and parous ($P = 0.001$) cows, we detected an association between DINCUs and RP and the interaction of DINCUs \times DINCUs and RP (Figure 6; Supplemental Tables S7 and S8, <http://dx.doi.org/10.22029/jlupub-15669>, Venjakob, 2023). Nulliparous cows with 7, 14, 21, 28, and 35 DINCUs had a predicted probability for RP of 9.3 (± 0.4), 4.5 (± 0.2), 3.1 (± 0.1), 3.2 (± 0.3), and 3.7 (± 0.5) %, respectively (Table 2). Parous cows with 7, 14, 21, 28, and 35 DINCUs had a predicted probability for RP of 14.7 (± 0.6), 8.1 (± 0.3), 6.7 (± 0.2), 7.1 (± 0.2), and 8.6 (± 0.4) %, respectively (Table 2). In nulliparous ($P = 0.020$) and parous cows ($P < 0.001$), we detected an association between DINCUs and APM (Figure 7; Supplemental Tables S9 and S10, <http://dx.doi.org/10.22029/jlupub-15669>, Venjakob, 2023). Nulliparous cows with 7, 14, 21, 28, and 35 DINCUs had a predicted probability for APM of 19.1 (± 1.0), 15.1 (± 0.4), 13.2 (± 0.4), 12.4 (± 0.7), and 13.4% (± 1.3), respectively (Table 2). Parous cows with 7, 14, 21, 28, and 35 DINCUs had a predicted probability for APM

Table 2. Multiple comparison among cows that stayed 7, 14, 21, 28, or 35 d in the close-up group and the association with 305-d milk projection (2nd305ME), retained placenta (RP), acute puerperal metritis (APM), hyperketonemia (KET), clinical hypocalcemia (CH), left displaced abomasum (LDA), mastitis, first service pregnancy risk (FSPR), and survival during the first 300 DIM (CULL300)¹

Item	DINCU					P-value	
	7 d (95% CI)	14 d (95% CI)	21 d (95% CI)	28 d (95% CI)	35 d (95% CI)	DINCU	DINCU × DINCU
Nulliparous							
2nd305ME, ² kg	10,488 (10,331–10,646) ^a	10,844 (10,780–10,908) ^b	11,155 (11,098–11,212) ^c	11,188 (11,083–11,292) ^c	10,628 (10,429–10,827) ^{a,b}	<0.001	0.049
KET, ³ %	14.7 (12.0–17.4) ^a	11.9 (10.8–13.0) ^a	11.9 (10.9–12.9) ^a	13.4 (11.6–15.2) ^a	13.9 (10.5–17.3) ^a	<0.001	NS
RP, ⁴ %	9.3 (8.5–10.0) ^a	4.5 (4.2–4.9) ^b	3.1 (2.8–3.4) ^c	3.2 (2.7–3.7) ^c	3.7 (2.7–4.6) ^{b,c}	<0.001	<0.001
APM, ⁵ %	19.1 (17.1–21.1) ^a	15.1 (14.3–16.0) ^b	13.2 (12.5–13.9) ^c	12.4 (11.1–13.8) ^c	13.4 (10.8–16.0) ^{b,c}	0.020	NS
CULL300, %	84.0 (82.8–85.1) ^a	86.1 (85.6–86.5) ^b	85.8 (85.4–86.3) ^b	83.7 (83.0–84.5) ^a	83.7 (82.2–85.2) ^a	<0.001	<0.001
Parous							
2nd305ME, kg	10,754 (10,545–10,963) ^{a,b}	11,045 (10,952–11,137) ^{b,c}	11,110 (11,047–11,173) ^c	10,917 (10,840–10,994) ^{a,b}	10,746 (10,588–10,904) ^a	<0.001	NS
CH, ⁶ %	2.7 (1.9–3.5) ^{a,b}	2.6 (2.3–3.0) ^a	3.5 (3.2–3.7) ^{b,c}	4.1 (3.8–4.4) ^d	5.9 (5.4–6.6) ^c	<0.001	NS
KET, %	30.8 (28.4–33.1) ^{a,b,c}	28.3 (27.3–29.3) ^a	29.1 (28.4–29.8) ^{a,b}	29.7 (28.8–30.6) ^{a,b}	33.8 (32.0–35.6) ^c	<0.001	NS
LDA, ⁷ %	2.0 (1.7–2.4)	2.1 (2.0–2.3)	2.3 (2.2–2.4)	2.2 (2.1–2.3)	2.5 (2.2–2.7)	<0.001	NS
RP, %	14.7 (13.5–15.8) ^a	8.1 (7.6–8.6) ^b	6.7 (6.4–7.1) ^c	7.1 (6.7–7.5) ^c	8.6 (7.7–9.5) ^b	<0.001	<0.001
APM, %	19.1 (17.1–21.1) ^a	15.1 (14.3–16.0) ^b	13.2 (12.5–13.9) ^c	12.4 (11.1–13.8) ^c	13.4 (10.8–16.0) ^{b,c}	<0.001	NS
Mastitis, %	19.7 (18.3–21.0) ^a	11.2 (10.6–11.8) ^b	9.8 (9.4–10.2) ^c	10.4 (10.0–10.9) ^{b,c,d}	11.8 (10.8–12.8) ^d	<0.001	<0.001
FSPR, %	33.7 (32.1–35.3) ^a	41.1 (40.5–41.7) ^b	42.3 (41.9–42.7) ^c	40.8 (40.3–41.4) ^b	37.6 (36.3–38.8) ^a	0.003	0.001
CULL300, %	73.8 (71.9–75.7) ^{a,b}	75.4 (74.5–76.3) ^a	77.1 (76.5–77.7) ^c	75.7 (75.0–76.5) ^{a,c}	72.4 (70.9–73.9) ^b	<0.001	<0.001

^{a–c} Letters with different superscripts differ as the 95% CI did not overlap.

¹ All comparisons are based on estimates from the GENLIMMIXED model or the Cox proportional hazard model. Outcomes were only included in the table when associated ($P < 0.050$) with days in the close-up group (DINCU) or the interaction of DINCU × DINCU.

² 305-d milk projection based on second test day milk production.

³ Hyperketonemia was defined as BHB ≥ 1.2 mmol/L using a cow-side blood BHB test.

⁴ Retained placenta was defined as failure to expel fetal membranes by 24 h after calving.

⁵ Acute puerperal metritis was defined as sign of systemic illness (e.g., decreased milk production, dullness, or other signs of toxemia) in combination with an animal with an abnormally enlarged uterus, fetid watery red-brown uterine discharge, and fever $>39.5^{\circ}\text{C}$.

⁶ Clinical hypocalcemia was defined as a recumbent cow during one of the first 5 DIM that rose in response to an intravenous Ca infusion.

⁷ Left displaced abomasum was defined as tympanic resonance in response to percussion of the left flank during auscultation with a stethoscope.

Table 3. Incidence rate (n) of diseases in nulliparous (n = 8,798) and parous (n = 19,641) cows within the first 30 DIM and culling within 300 DIM

Disease	Nulliparous, % (n)	Parous, % (n)
Clinical hypocalcemia ¹	0.01 (5)	3.55 (696)
Retained placenta ²	4.14 (364)	7.66 (1,504)
Acute puerperal metritis ³	13.75 (1,210)	10.60 (2,081)
Hyperketonemia ⁴	11.83 (1,041)	29.54 (5,802)
Left displaced abomasum ⁵	0.42 (37)	2.28 (447)
Mastitis ⁶	5.25 (462)	6.48 (1,272)
Culling	8.23 (724)	9.88 (1,942)

¹Clinical hypocalcemia was defined as a recumbent cow during one of the first 5 DIM that rose in response to an intravenous Ca infusion.

²Retained placenta was defined as failure to expel fetal membranes by 24 h after calving.

³Acute puerperal metritis was defined as sign of systemic illness (e.g., decreased milk production, dullness, or other signs of toxemia) in combination with an animal with an abnormally enlarged uterus, fetid watery red-brown uterine discharge and fever >39.5°C.

⁴Hyperketonemia was defined as BHB ≥ 1.2 mmol/L using a cow-side blood BHB test.

⁵Left displaced abomasum was defined as tympanic resonance in response to percussion of the left flank during auscultation with a stethoscope.

⁶Mastitis was defined as visible signs of inflammation in an affected mammary gland (i.e., redness, swelling, pain, or heat) and alterations such as clots, flakes, discoloration, or abnormal consistency of secretions.

of 16.3 (± 0.9), 10.4 (± 0.4), 10.3 (± 0.3), 10.5 (± 0.3), and 9.8 (± 0.7) %, respectively (Table 2). Whereas in nulliparous cows no association was observed between DINCUs and mastitis, DINCUs were associated with the

risk for mastitis in parous cows ($P < 0.001$; Figure 8; Supplemental Table S11, <http://dx.doi.org/10.22029/jlupub-15669>, Venjakob, 2023). Parous cows with 7, 14, 21, 28, and 35 DINCUs had a predicted probability for

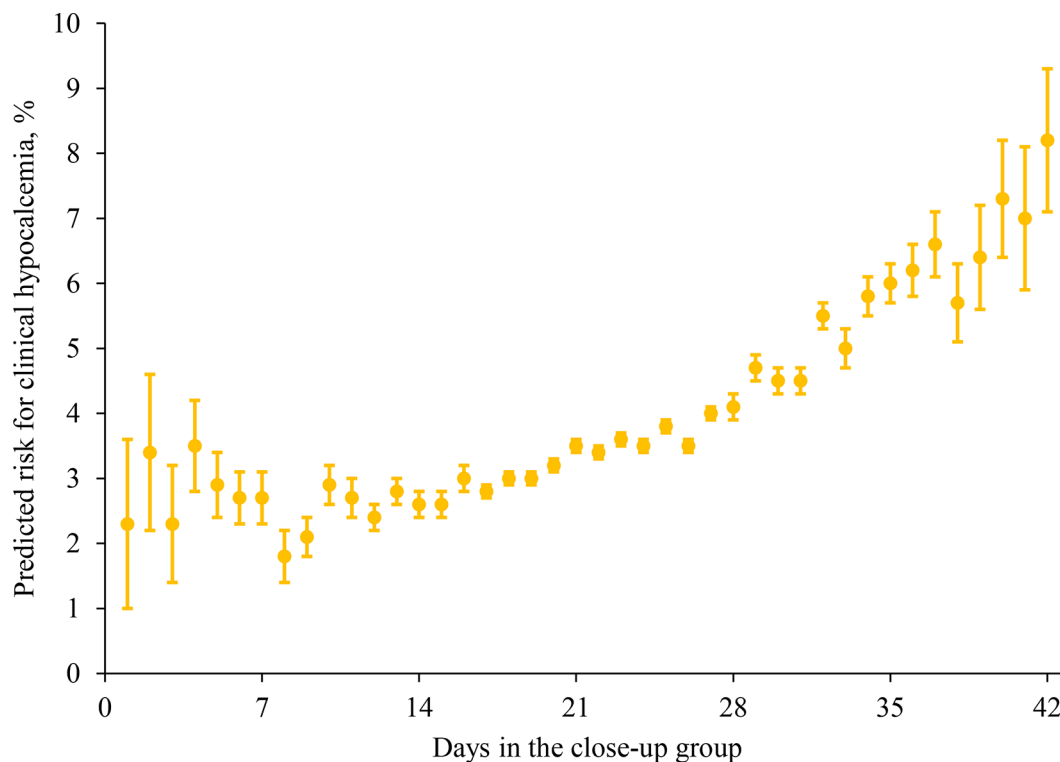


Figure 3. Association between days in the close-up group (DINCUs) and predicted risk of clinical hypocalcemia (CH) in parous cows (n = 19,641) using least square estimates (mean \pm SEM) from the generalized linear mixed model. Year of calving ($P = 0.007$), farm ($P = 0.060$) season ($P < 0.001$), parity ($P < 0.001$), and DINCUs ($P < 0.001$) were associated with the risk for clinical hypocalcemia.

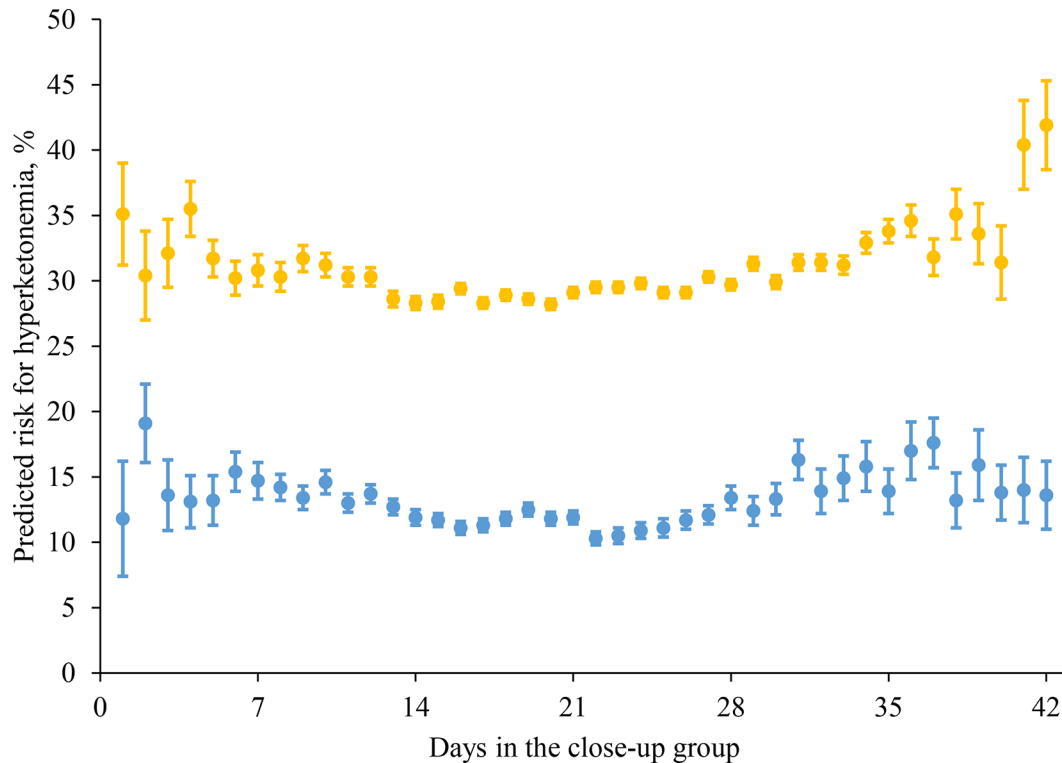


Figure 4. Association between days in the close-up group (DINCUs) and the predicted risk of hyperketonemia (BHB ≥ 1.2 mmol/L using a cow-side blood BHB test) using least square estimates (mean \pm SEM) from the generalized linear mixed model. In nulliparous cows ($n = 8,798$, blue), the risk of hyperketonemia was associated with the year of calving ($P < 0.001$), farm ($P < 0.001$), calving ease ($P < 0.001$), age at first calving ($P < 0.001$), and DINCUs ($P < 0.001$). In parous cows ($n = 19,641$; yellow), the year of calving ($P < 0.001$), farm ($P < 0.001$), season ($P < 0.001$), parity ($P < 0.001$), calving ease ($P < 0.001$), twins ($P < 0.001$), milk production in previous lactation ($P < 0.001$), and DINCUs ($P < 0.001$) were associated with hyperketonemia.

mastitis within 30 DIM of 19.7 (± 0.7), 11.2 (± 0.3), 9.8 (± 0.2), 10.4 (± 0.2), and 11.8 (± 0.5) %, respectively (Table 2). Furthermore, we detected an association between DINCUs \times DINCUs ($P < 0.001$) and the risk of mastitis.

Association with Reproduction. In nulliparous cows, no association was observed between DINCUs and FSPR ($P = 0.184$), and DINCUs \times DINCUs and FSPR ($P = 0.164$). In parous cows, FSPR was associated with DINCUs ($P = 0.003$) and DINCUs \times DINCUs ($P = 0.001$). Parous cows with 7, 14, 21, 28, and 35 DINCUs had a predicted FSPR of 33.7 (± 0.8), 41.1 (± 0.3), 42.3 (± 0.2), 40.8 (± 0.3) and 37.6 (± 0.6) %, respectively (Table 2; Figure 9; Supplemental Table S12, <http://dx.doi.org/10.22029/jlupub-15669>, Venjakob, 2023). No association was observed between farm and FSPR ($P = 0.941$).

Association with Culling Risk. In nulliparous cows and parous cows, DINCUs ($P < 0.001$) and DINCUs \times DINCUs ($P < 0.001$) were associated with the predicted probability to survive 300 DIM (Figure 10; Supplemental Tables S13 and S14, <http://dx.doi.org/>

[10.22029/jlupub-15669](http://dx.doi.org/10.22029/jlupub-15669); Venjakob, 2023). Nulliparous cows with 7, 14, 21, 28, and 35 DINCUs had a predicted probability to survive 300 DIM of 84.0 (± 0.6), 86.1 (± 0.2), 85.8 (± 0.2), 83.7 (± 0.4), and 83.7 (± 0.8) %, respectively (Table 2). Parous cows with 7, 14, 21, 28, and 35 DINCUs had a predicted probability to survive 300 DIM of 73.8 (± 1.0), 75.4 (± 0.4), 77.1 (± 0.3), 75.7 (± 0.4), and 72.4 (± 0.6) %, respectively (Table 2).

Experiment II

Association with Behavioral Changes. Evaluating behavior before calving, an association was observed between DINCUs and time spent eating ($P < 0.001$), ruminating ($P < 0.001$), and inactive ($P < 0.001$; Figure 11, Table 4). Furthermore, we observed an association between the interaction of time by DINCUs and time spent active ($P < 0.001$), time spent inactive ($P = 0.010$), and time spent eating ($P = 0.006$). Evaluating the results on rumination cows with 7 DINCUs spent less time ruminating than cows with 14 ($P = 0.011$) and 28 DINCUs ($P = 0.058$). In addition, cows with

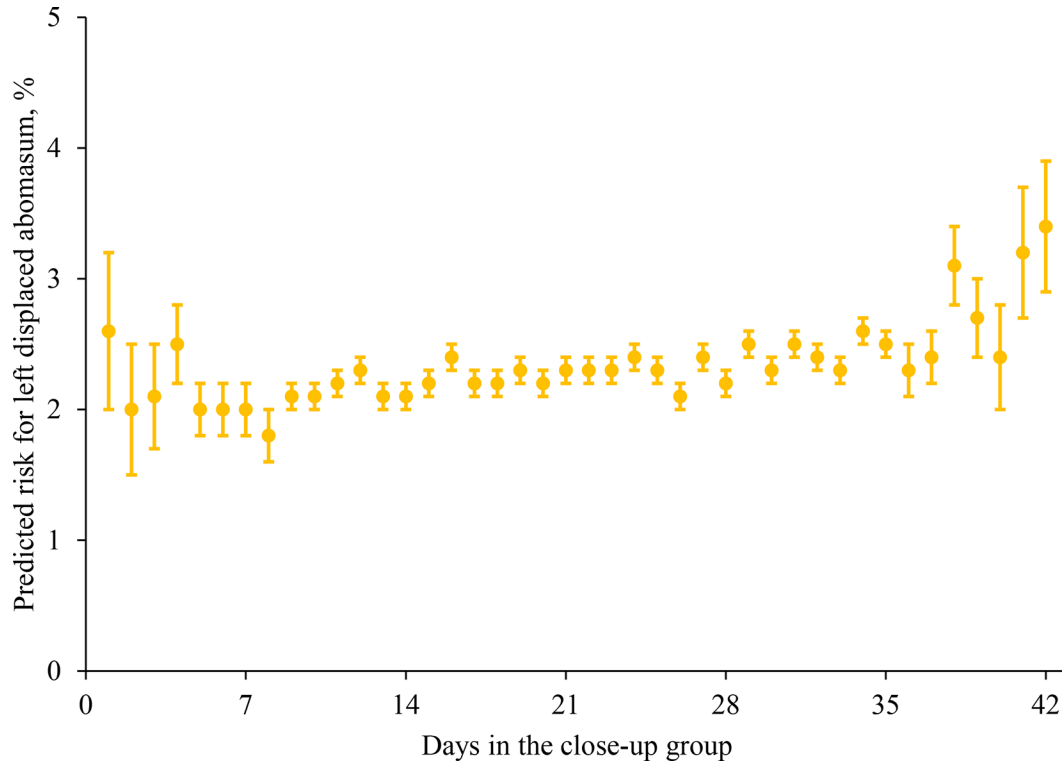


Figure 5. Association between days in the close-up group (DINCUC) and the predicted risk for left displaced abomasum using least square estimates (mean \pm SEM) from the generalized linear mixed model of parous cows ($n = 19,641$). The risk for left displaced abomasum was associated with the year of calving ($P = 0.021$), farm ($P < 0.001$), season ($P = 0.014$), parity ($P < 0.001$), calving ease ($P < 0.001$), twins ($P < 0.001$), and DINCUC ($P < 0.027$).

35 DINCUC spent less time ruminating compared with cows with 14 ($P < 0.001$), 21 ($P = 0.023$), and 28 DINCUC ($P = 0.028$).

After calving, we detected an association between DINCUC and time spent inactive ($P < 0.001$), eating ($P = 0.005$), and ruminating ($P = 0.008$). Cows with 28 DINCUC spent less time eating compared with cows with 14 ($P = 0.008$) and 21 DINCUC ($P = 0.024$). Furthermore, cows with 28 DINCUC spent less time ruminating than cows with 14 DINCUC ($P = 0.008$). Moreover, we detected an association between the interaction of time by DINCUC and time spent inactive ($P = 0.030$). Associations between behavior around calving and DINCUC, GL, time relative to calving, parity and time by DINCUC are described in Table 4.

DISCUSSION

The frequency distribution of DINCUC in nulliparous and parous cows is displayed in Figure 1. Compared with a previously published study (Venjakob et al., 2022) cows were more consistently transferred from the far-off to the close-up group on a weekly basis on both farms. As can be derived from Figure 1, this led to

a relatively small variation in DINCUC. In nulliparous and parous cows, we observed an association between DINCUC and milk production with an optimum milk production at 25 and 22 DINCUC, respectively. In nulliparous cows with a short (<10 d) and a long (>30 d) stay in the close-up group, DINCUC was associated with a higher risk for KET and RP compared with cows with DINCUC between 21 to 28 d. Nulliparous cows with a short stay in the close-up group (<10 d) had an increased risk for APM compared with nulliparous cows with a longer stay in the close-up group. In parous cows, a long stay (>30 d) in the close-up group was associated with a higher risk for CH, KET, LDA, RP, mastitis and culling and a short stay in the close-up group (<10 d) was associated with an increased risk for RP, APM, mastitis and culling in comparison to cows with DINCUC between 21 to 28 d. Whereas we detected no association between FSPR and DINCUC in nulliparous cows, FSPR of parous cows was lower when staying <10 and >30 DINCUC compared with parous cows with DINCUC between 21 to 28 d. In nulliparous cows, DINCUC was not associated with the risk for LDA. In contrast, parous cows with ≥ 35 DINCUC had an increased risk for LDA compared with parous cows

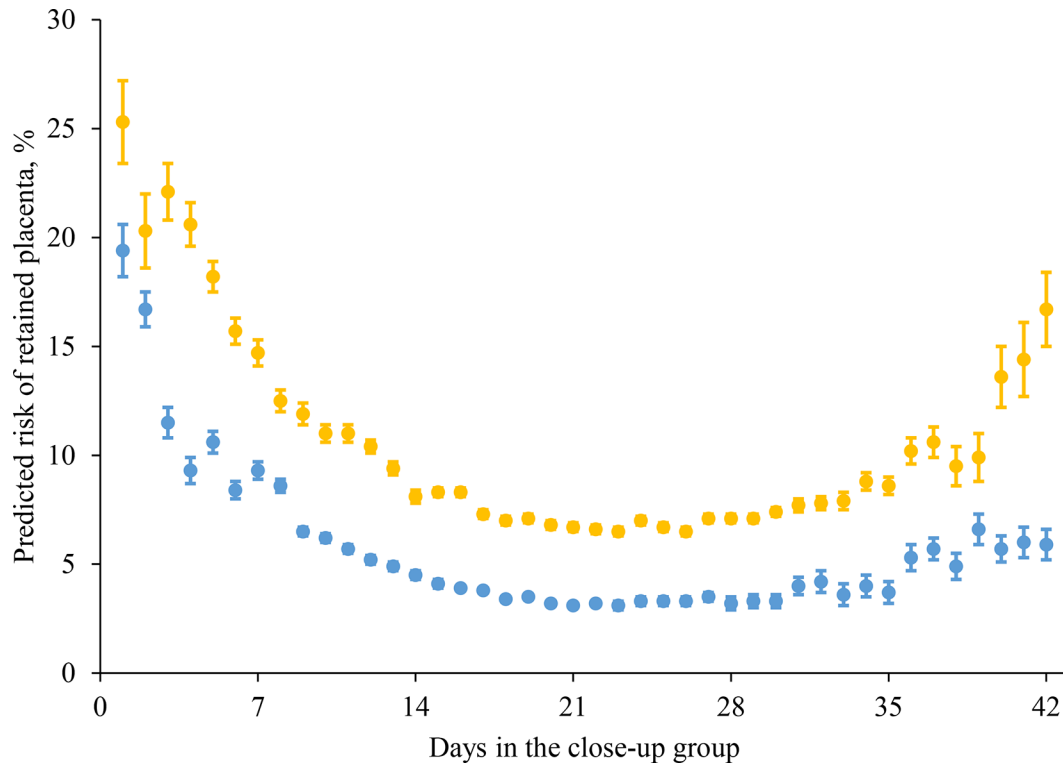


Figure 6. Association between days in the close-up group (DINCUs) and the predicted risk of retained placenta (RP) using least square estimates (mean \pm SEM) from the generalized linear mixed model. In nulliparous cows ($n = 8,798$, blue) the risk of RP was associated with the year of calving ($P < 0.001$), farm ($P < 0.147$), season ($P = 0.005$), calving ease ($P < 0.001$), stillbirth ($P < 0.001$), sex of the calf ($P < 0.001$), DINCUs ($P < 0.001$) and DINCUs \times DINCUs ($P < 0.001$). In parous cows ($n = 19,641$; yellow), the year of calving ($P < 0.001$), farm ($P < 0.001$), season ($P = 0.016$), parity ($P < 0.001$), calving ease ($P < 0.001$), stillbirth ($P < 0.001$), sex of the calf ($P < 0.001$), DINCUs ($P < 0.001$), and DINCUs \times DINCUs ($P < 0.001$) were associated with the risk of RP.

with <35 DINCUs. Moreover, DINCUs were associated with behavioral changes around calving. Before calving, cows with 7 and 35 DINCUs were more inactive and spent less time eating and ruminating than cows with 14, 21, and 28 DINCUs.

Association with Milk Production

In the present study, nulliparous and parous cows with a short (<10 DINCUs) and long (>30 DINCUs) stay in the close-up group had a reduced 2nd305ME compared with cows with 21 to 28 DINCUs (Figure 2). Evaluating the association between DINCUs and 2nd-305ME, we observed an association with an optimum milk production at 25 and 22 DINCUs for nulliparous and parous cows, respectively. A similar observation was made by Vieira-Neto et al. (2021); although nulliparous cows were not exposed to a negative DCAD diet in their study, these authors also found a quadratic association between 300-d milk production and DINCUs in nulliparous and parous cows with an optimum at 21 and 24 DINCUs, respectively. In a previous multisite

study, including 18 farms, we also observed a quadratic association between DINCUs and milk production at first test day for parous cows (Venjakob et al., 2022). In this study however, DINCUs were linearly associated with milk production of nulliparous cows. Especially, nulliparous cows exceeding 28 DINCUs had an increased milk production. A comparable association was found by Degaris et al. (2008), who investigated the association of DINCUs with milk production in grazing Holstein and Jersey \times Holstein cows. These authors did not differentiate between nulliparous and parous cows and found a plateau in milk production when DINCUs were between 17 and 35 d.

Association with Diseases

Parous cows with >30 DINCUs had an increased risk for CH (Figure 3) compared with cows with ≤ 30 DINCUs. Lean et al. (2006) speculated that feeding of acidogenic diets for a long time period might increase the risk for hypocalcemia as long-term activation of Ca metabolism could deplete Ca stores around calving. In

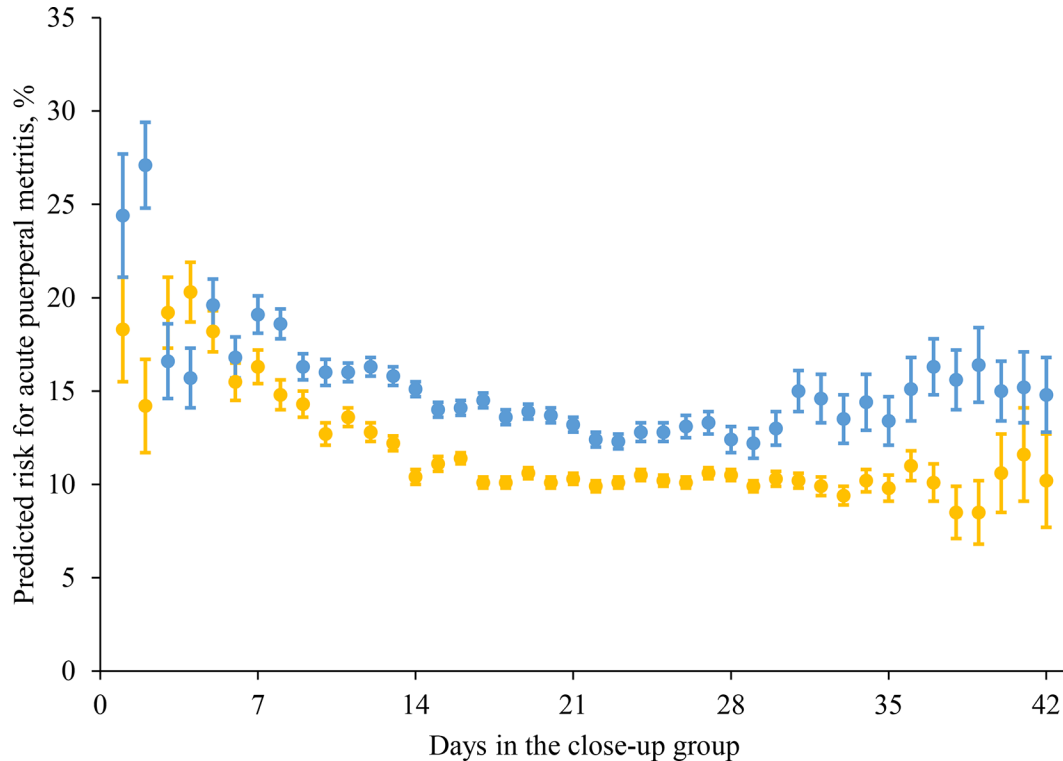


Figure 7. Association between days in the close-up group (DINCUs) and the predicted risk of acute puerperal metritis (APM) using least square estimates (mean \pm SEM) from the generalized linear mixed model of nulliparous cows and parous. In nulliparous cows ($n = 8,798$, blue), the risk for APM was associated with the year of calving ($P < 0.001$), farm ($P < 0.001$), calving ease ($P < 0.001$), stillbirth ($P < 0.001$), sex of the calf ($P < 0.001$), and DINCUs ($P = 0.020$). In parous cows ($n = 19,641$; yellow), the year of calving ($P < 0.001$), farm ($P < 0.001$), season ($P = 0.003$), parity ($P < 0.057$), calving ease ($P < 0.001$), stillbirth ($P < 0.001$), sex of the calf ($P < 0.001$), milk production in previous lactation ($P = 0.066$), and DINCUs ($P < 0.001$) were associated with the risk of RP.

contrast, this effect was not observed by Weich et al. (2013) who found a higher postpartum Ca concentration in cows fed with a negative DCAD diet for 42 d, compared with cows fed with a negative DCAD diet for the last 21 d of gestation. In a previous study colostrum yield increased with increasing DINCUs (1 d increase in DINCUs was associated with 60 g more colostrum; Borchardt et al., 2022). As colostrum contains high amounts of calcium (i.e., 2.3 g/kg; Goff, 2014) an increased colostrum yield in cows with a long stay in the close-up group might increase the Ca demand after calving, which may explain a greater risk for CH in parous cows. This can, however, not be confirmed in the present study, as data on colostrum yield were not available for the whole study period. Nulliparous and parous cows with a short (<10 DINCUs) and a long stay (>30 DINCUs) in the close-up group had an increased risk for KET compared with cows with DINCUs between 21 to 28 d (Figure 4). Especially, an increased risk for KET in cows with a long stay in the close-up group seems plausible, as it might be attributed to increased gain of adipose tissue. For example,

Chebel (2021) found increased BHB and nonesterified fatty acid concentrations in cows with a long stay in the close-up group. Likewise, we observed an increased risk for LDA in parous cows with ≥ 35 DINCUs, compared with parous cows with <35 DINCUs. As KET is a risk factor for LDA (Ospina et al., 2013) it seems plausible that the risk for LDA is increased alongside KET. In nulliparous cows no association between LDA and DINCUs was observed. This might be attributed to the low incidence of LDA (0.42%; 37/8,798 cows) in nulliparous cows during the study period (Table 3).

In nulliparous and parous cows, we detected a quadratic association between DINCUs and the risk of incurring RP (Figure 5). This is in agreement with Vieira-Neto et al. (2021). In their study, the incidence of RP was highest in cows with <10 DINCUs. Similar to our study, the risk for RP was also increased when cows stayed >30 DINCUs. One might speculate that cows with a short stay in the close-up group experience more stress due to regrouping shortly before calving and therefore have a higher risk for RP. In contrast, a long stay in the close-up group may be associated with

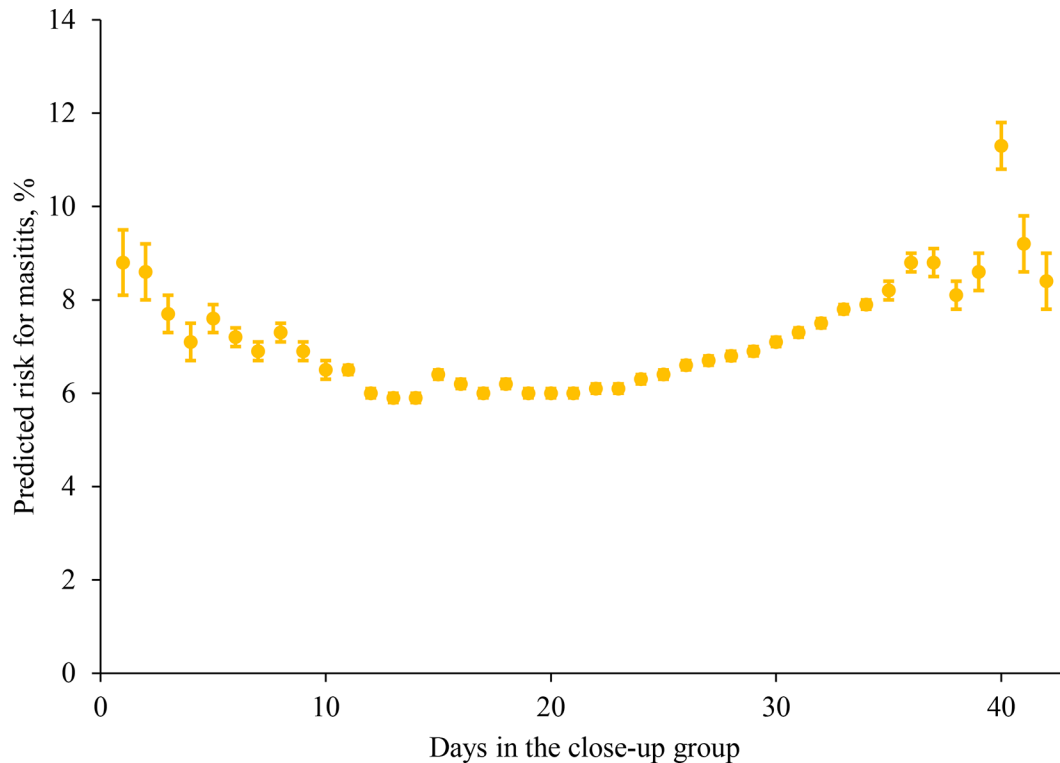


Figure 8. Association between days in the close-up group (DINCUC) and the predicted risk for mastitis within 30 DIM ($n = 19,641$) using least square estimates (mean \pm SEM) from the generalized linear mixed model. The risk for mastitis was associated with the year of calving ($P < 0.001$), farm ($P < 0.001$), parity ($P = 0.014$), calving ease ($P < 0.001$), stillbirth ($P < 0.001$), 305-d milk production in previous lactation ($P = 0.078$), DINCUC ($P < 0.001$), and DINCUC \times DINCUC ($P < 0.001$).

excessive body condition, which in turn increased the risk for dystocia, vulvovaginal laceration and uterine diseases. It is well accepted that vulvovaginal laceration is associated with postpartum uterine diseases (Vieira-Neto et al., 2016). In nulliparous and parous cows, a short stay (<10 DINCUC) in the close-up group was also associated with an increased risk for APM compared with cows with DINCUC >10 d (Figure 6). Although cows with a short GL (<262 d) were excluded from the study, it is possible that some cows had a short stay in the close-up group due to biological reasons, as for example heat stress and twin birth may lead to a shortened GL. It has been shown, that short GL is a risk factor for postpartum uterine diseases (Vieira-Neto et al., 2017). Accordingly, Olagaray et al. (2020) observed detrimental effects of shortened dry periods due to biological reasons (i.e., short GL) in comparison to shortened dry periods due to management reasons.

In parous cows, a short (<10 DINCUC) and a long stay (>30 DINCUC) in the close-up group was associated with an increased risk for mastitis until 30 DIM compared with cows with DINCUC between 21 to 28 d (Figure 7). These findings contradict results of Vieira-Neto et al. (2021). In their study, the same association

was found in nulliparous cows. In parous cows, however, an increase from 7 DINCUC to 28 DINCUC led to a 2.3% increase in mastitis incidence until 90 DIM. These authors observed the lowest risk for mastitis, when parous cows stayed in the close-up group for 1 d. As the risk for mastitis increased until 25 DINCUC and cows with 27 DINCUC had the highest milk production, the authors speculated that these cows might have had increased risk factors for mastitis, such as milk leakage and longer exposure to bacteria during the milking process. Degaris et al. (2010) also observed an increased risk for mastitis until 150 DIM with increasing DINCUC. Per day increase of DINCUC the hazard for mastitis increased by 2%. These authors also suggested an association between the risk of mastitis and higher milk production as a cause for udder infections. Vieira-Neto et al. (2021) and Degaris et al. (2010) evaluated relatively long risk periods for mastitis (i.e., 90 and 150 d, respectively). The different observation periods (i.e., 30 vs. 90 or 150 DIM) might explain the controversial findings. This assumption is supported by results of Chebel (2021) who found similar results to ours, when evaluating the association between DINCUC and mastitis by 30 and 60 DIM.

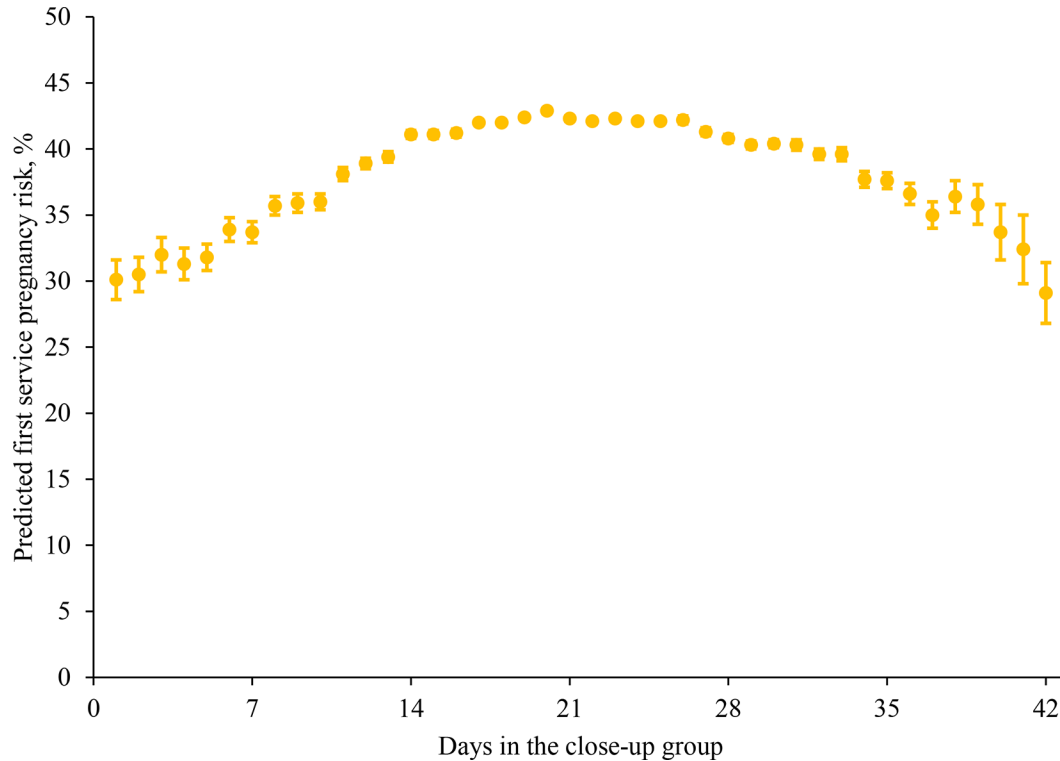


Figure 9. Association between days in the close-up group (DINCUs) and the predicted first service pregnancy risk (FSPR) using least square estimates (mean \pm SEM) from the generalized linear mixed model of parous cows ($n = 19,585$; yellow). The year of calving ($P < 0.001$), season ($P = 0.003$), parity ($P < 0.001$), stillbirth ($P = 0.007$), calving ease ($P < 0.001$), DINCUs ($P = 0.003$), and DINCUs \times DINCUs ($P = 0.001$) were associated with the predicted FSPR. To account for the different reproductive management on both farms, farm ($P = 0.941$) was forced to remain in the model.

Association with Reproduction

In parous cows we detected an association between DINCUs and FSPR and DINCUs \times DINCUs and FSPR (Figure 9). Chebel (2021) and Vieira-Neto et al. (2021) also found an associations between DINCUs and reproductive outcomes. Chebel (2021) observed an association between DINCUs and pregnancy by 305 DIM. The chance of being pregnant was highest when nulliparous and parous cows stayed 28 DINCUs. In parous cows, the probability of pregnancy was increased by 18.8% when comparing cows with ≤ 10 DINCUs to cows with 28 DINCUs. Vieira-Neto et al. (2021) observed a linear association between DINCUs and FSPR. An increase from 7 to 28 DINCUs was associated with a 4.4% reduced FSPR and cows had the least predicted FSPR when DINCUs was the greatest.

Association with Culling Risk

In nulliparous and parous cows, we observed an increased culling risk in cows with a short (< 10 DINCUs) and a long stay (> 30 DINCUs) in the close-up group, compared with cows with DINCUs between 21 to 28 d

(Figure 10). This result is plausible as nulliparous and parous cows with short and long DINCUs had an increased risk for downstream outcomes, such as disease or culling. The result on culling is in agreement with Vieira-Neto et al. (2021). In their study the same observation was made for nulliparous cows. Chebel (2021) and Venjakob et al. (2022) observed an increased culling risk, predominantly in cows with a short stay in the close-up group.

Association with Behavioral Changes

This is the first study evaluating the association between DINCUs and time spent eating, ruminating, active, and inactive around parturition. Cows with 7 DINCUs were highly active on the day of movement from the far-off to the close-up group (Figure 11A). According to Nordlund et al. (2006), a cow that is moved into a new group experiences stress, as she has to establish her rank within the social group of the pen. Compared with new arrivals, cows resident within the pen tend to maintain their social position. Kondo and Hurnik (1990) suggested that reestablishment of rank within a group takes approximately 2 d. This can

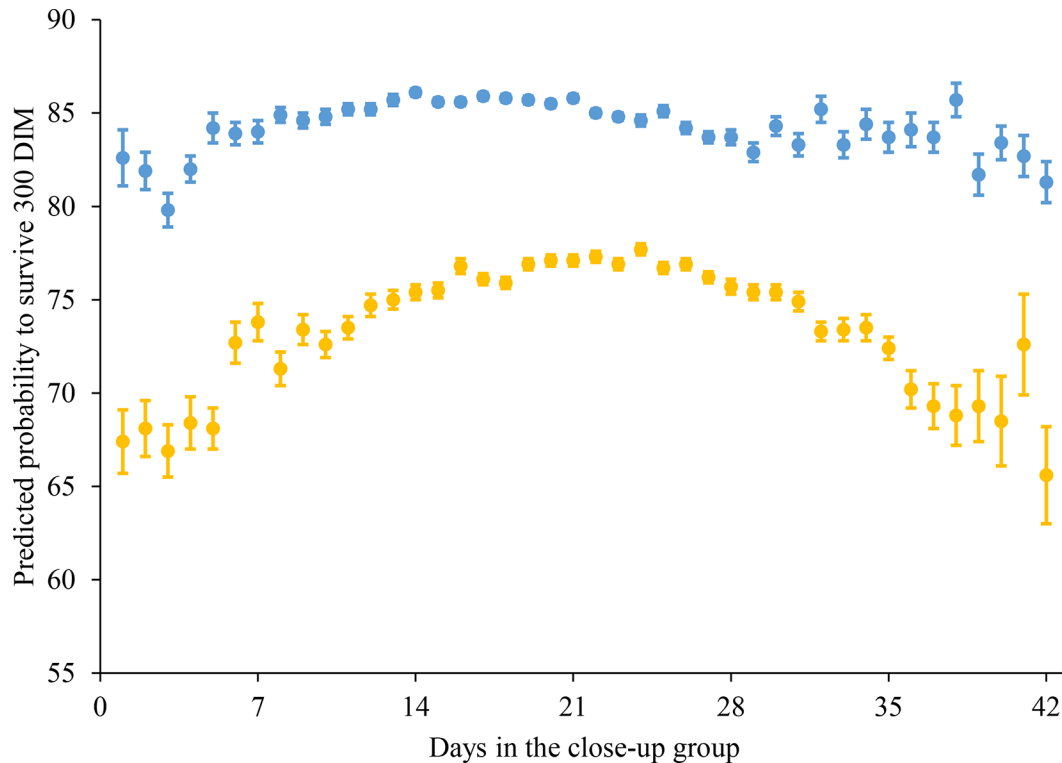


Figure 10. Association between days in the close-up group (DINCUs) and predicted probability to survive 300 DIM using least square estimates (mean \pm SEM) from the Cox proportional hazards models. In nulliparous cows ($n = 8,798$; blue) survival until 300 DIM was associated with the year of calving ($P < 0.001$), farm ($P = 0.002$), season ($P = 0.008$), age at first calving ($P = 0.025$), DINCUs ($P < 0.001$) and DINCUs \times DINCUs ($P < 0.001$). In parous cows ($n = 19,641$; yellow), the year of calving ($P < 0.001$), farm ($P < 0.001$), season ($P = 0.001$), parity ($P < 0.001$), 305-d milk yield of previous lactation ($P < 0.001$), DINCUs ($P < 0.001$) and DINCUs \times DINCUs ($P < 0.001$) were associated with survival.

be confirmed by our data on activity, as cows with 7 DINCUs also had an increased activity on d -6 (Figure 11A). Nordlund et al. (2006) suggested that pen moves close to calving do have adverse effects, as cows were involved in more interactions per hour. It has been shown that pen moves may alter feeding behavior for several days (Grant and Albright, 2001). The increase in social interactions indicative of the establishment of the rank within the hierarchy of the pen in cows with 7 DINCUs might explain, why time spent eating (Figure 11C) and ruminating (Figure 11D) were reduced and inactivity increased in these cows (Figure 11B) before calving (Cook and Nordlund, 2004). Cows with 35 DINCUs were also more inactive and had a reduced feeding and ruminating time before calving. A reduction in pre-partum feeding behavior may be associated with an increased risk for certain diseases as observed in our study, but also by others (Huzzey et al., 2007; Pérez-Báez et al., 2019a, 2019b). The relation between a reduced feeding behavior can, however, be confounded by DINCUs, as cows with a long stay (35 d) in the close-up group spent less time eating. Extended dry period length is associated with increased body condition at calving (Chebel

et al., 2018), which may lead to metabolic stress and enhanced lipolysis at calving (Weber et al., 2015) and decreased DMI (Roche et al., 2013). Because rumination time is reduced alongside feeding time (Beauchemin, 2018), it seems plausible that cows with a reduced feeding time, spend less time ruminating. It has been shown that prepartum intake is associated with diseases after calving (Huzzey et al., 2007; Pérez-Báez et al., 2019a, 2019b). Behavioral changes associated with a short and long stay in the close-up group may explain the increased risk for negative downstream outcomes in these cows.

Study Limitations

When evaluating the association of DINCUs and early lactation health and performance, it is difficult to differentiate the effect of DINCUs from the effect of GL using observational studies. In the present study, we addressed this issue by including GL into all initial models. As cows were moved very consistently to the close-up group in these 2 farms, we assumed a strong correlation among GL and DINCUs and recalculated all

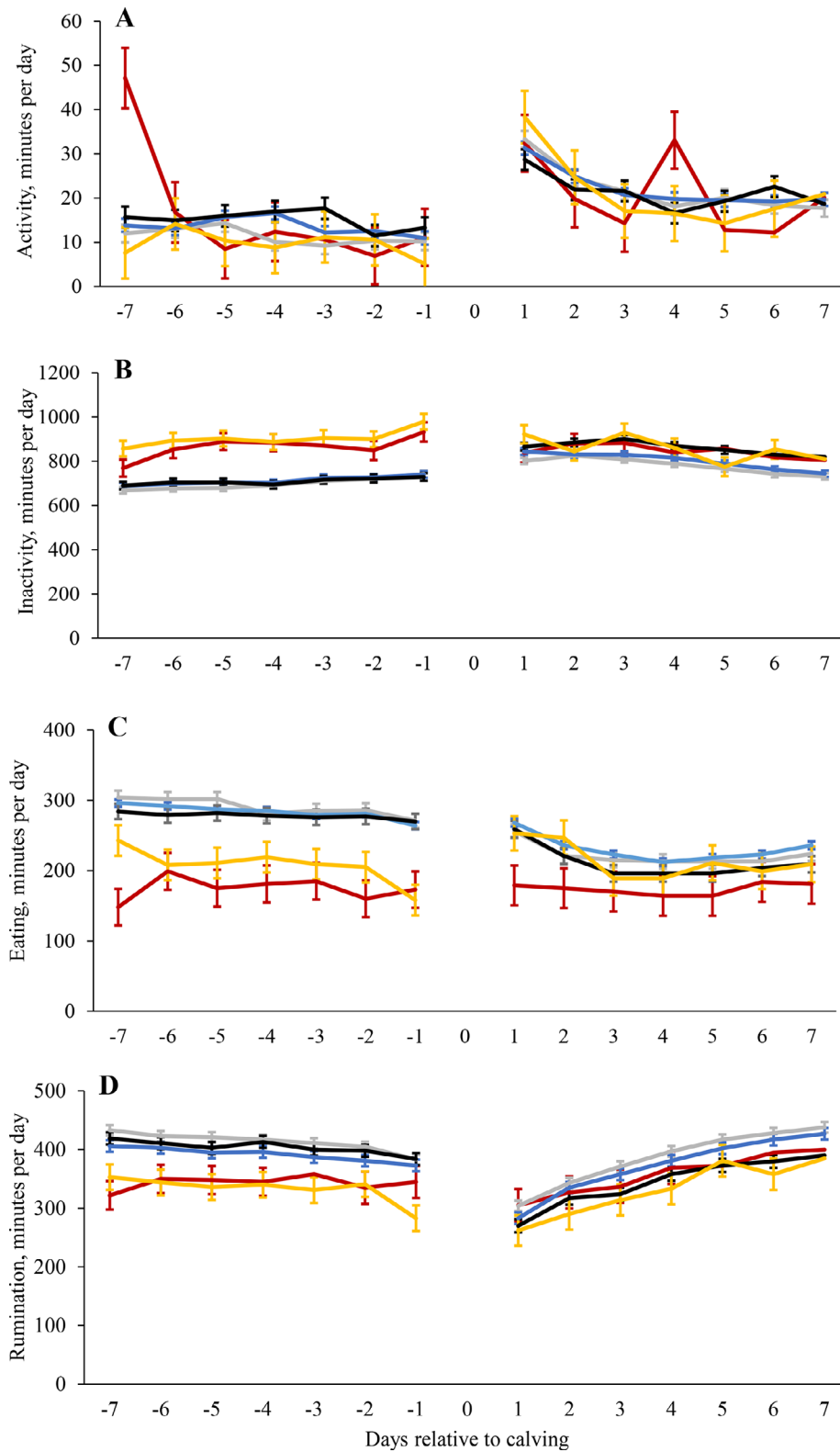


Figure 11. Association between days in the close-up group (DINCUs) and time spent eating, ruminating, active, and inactive around calving, using least square estimates (mean \pm SEM) from the linear mixed model. Data about time spent active (Panel A), inactive (Panel B), eating (Panel C), and ruminating (Panel D) were obtained from the Nedap system (Nedap Livestock Management, Groenlo, the Netherlands). Cows with 7 ($n = 16$), 14 ($n = 186$), 21 ($n = 403$), 28 ($n = 123$), and 35 DINCUs ($n = 19$) are displayed in red, gray, blue, black, and yellow, respectively. Associations with DINCUs, time, parity, and time \times DINCUs can be derived from Table 4.

Table 4. The least squares means and SEM as derived from the linear mixed model for time spent eating, ruminating, active, and inactive in minutes per day, considering days in the close-up group (DINCU)

Behavior ¹	DINCU					P-value			
	7	14	21	28	35	DINCU	Time ²	Parity	Time × DINCU
Prepartum									
Activity	16 ± 4	11 ± 1	14 ± 1	15 ± 2	10 ± 4	0.286	<0.001	0.137	<0.001
Inactivity	864 ± 33 ^a	698 ± 13 ^b	712 ± 9 ^b	708 ± 16 ^b	903 ± 31 ^a	<0.001	<0.001	0.661	0.010
Eating	194 ± 18 ^a	296 ± 7 ^b	283 ± 4 ^b	273 ± 8 ^b	200 ± 16 ^a	<0.001	<0.001	0.050	0.006
Rumination	343 ± 20 ^a	413 ± 8 ^{b,c}	391 ± 5 ^{b,c}	404 ± 9 ^b	333 ± 19 ^{a,c}	<0.001	<0.001	0.044	0.199
Postpartum									
Activity	21 ± 4	22 ± 1	22 ± 1	21 ± 2	21 ± 4	0.987	<0.001	<0.001	0.272
Inactivity	846 ± 35	780 ± 12	802 ± 8	860 ± 15	857 ± 34	<0.001	<0.001	0.146	0.030
Eating	209 ± 19	237 ± 7	229 ± 5	201 ± 8	195 ± 19	0.005	<0.001	0.005	0.436
Rumination	358 ± 22	385 ± 8	372 ± 5	345 ± 9	332 ± 21	0.008	<0.001	0.008	0.651

^{a-c}Letters with different superscripts differ.

¹Time spent eating, ruminating, active, and inactive was measured in farm 2 using a neck-mounted 3-dimensional accelerometer (Smarttag neck, Nedap Livestock, Groenlo, the Netherlands).

²Time = d -7, -6, -5, -4, -3, -2, -1 relative to calving in models on prepartum behavior; d 1, 2, 3, 4, 5, 6, 7 in models on postpartum behavior.

models excluding GL. Because exclusion of GL did not lead to major changes in the results, the results of the models without GL are presented herein. Future studies should be conducted in a randomly controlled fashion, specifically evaluating the effect of short DINCU on health and performance.

Even though it has been shown that the 3-dimensional sensor accurately monitored time spent eating, ruminating, active, and inactive (Borchers et al., 2021), we caution to extrapolate these results to actual dry matter intake, as the relationship between time spent eating and ruminating and DMI has not been sufficiently investigated. Future studies should investigate whether the behavioral changes observed in this study have a lasting effect on health and performance. Moreover, only 723 cows from one of the 2 farms were included in experiment II, limiting the validity of the results.

We are aware that disease identification on different farms for 6 years is prone to variability and bias among different observers, which might lead to false-negative or false-positive diagnoses (Kelton et al., 1998; Sannmann et al., 2012). As both farms had a long-term history of collaboration with the Clinic for Animal Reproduction and standard written operating procedures were provided by the herd manager in coordination with the researchers, we are confident that the risk of misclassification is low and that diseases have been recorded consistently over the course of this study. Evaluating the association between DINCU and APM we caution however that only severe cases of metritis were included in the statistical analyzes. A broader definition of the condition might have altered the results (McCarthy and Overton, 2018).

On the 2 participating farms, transfer from the far-off to the close-up group was conducted consistently based on the d of GL on a weekly basis. Only few cows stayed <10 d and longer than 30 DINCU. Moreover, nulliparous and parous cows both received a negative DCAD diet before calving. Therefore, the external validity of this study is limited.

CONCLUSIONS

The present study provides further evidence that DINCU were associated with productive, reproductive and disease outcomes. Moreover, DINCU were associated with time spent eating, ruminating, active, and inactive around the time of calving. Nulliparous cows with a short and long stay in the close-up group had a reduced milk production and an increased risk for RP, KET and APM compared with nulliparous cows with DINCU between 21 to 28 d. In parous cows, an increased risk for culling was observed in addition to increased disease incidences, a reduced FSPR, and a reduced milk production compared with parous cows with DINCU between 21 to 28 d. The results of our study suggest that pen moves during the last 10 d of gestation should be avoided. The reduced time spent eating and ruminating might lead to lower DMI increasing the risk for subsequent diseases. A similar effect was observed for cows that stayed for a longer period (30 DINCU) in the close-up group. Based on the observed GL (277 ± 4.8 d) we recommend to move cows from the far-off to the close-up group on d 254 of gestation to achieve an average stay in the close-up group of 3 wk. Randomized controlled trials are needed to further elucidate the importance of DINCU

as a risk factor for health and performance in transition dairy cows.

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ORCID

- P. L. Venjakob  <https://orcid.org/0000-0001-7541-5508>
 W. Heuwieser  <https://orcid.org/0000-0003-1434-7083>
 S. Borchardt  <https://orcid.org/0000-0003-3937-5777>