



## Associations between time in the close-up group and milk yield, milk components, reproductive performance, and culling of Holstein dairy cows fed acidogenic diets: A multisite study

P. L. Venjakob,\*†  L. K. Neuhaus,  W. Heuwieser,  and S. Borchardt 

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

### ABSTRACT

The objective of this prospective cohort study was to evaluate the association between the prepartum days in the close-up group (DINCU) and milk yield, milk components, reproductive performance, and culling risk in the subsequent lactation for Holstein dairy cows. Dry cow feeding management of 20 farms was evaluated during 2 farm visits. All farms were feeding an acidogenic diet in the close-up group. Data from 14,843 cows were collected for 365 d following the second farm visit. Data sets of 13,314 cows were available for final statistical analysis after exclusion of cows with missing information about gestation length, cows with a gestation length shorter than 262 d or longer than 292, cows with 0 DINCU, and cows with >42 DINCU. At enrollment, 3,871 and 9,443 of those animals were nulliparous and parous cows, respectively. Continuous data such as energy corrected milk (ECM), the ratio of fat and protein, and somatic cell score (SCS) at first test day were analyzed using linear mixed models. Binary data such as stillbirth, culling within 60 DIM, and pregnancy within 150 DIM were analyzed using logistic regression models. Based on their different physiology, separate models were built for nulliparous and parous cows. All results displayed are the predicted least squares means from the multivariable analyses. A significant association between DINCU and milk yield at first test day was observed for nulliparous and parous cows. Nulliparous cows with 7, 21, or 35 DINCU had a first test day ECM of 31.8, 33.3, and 35.5 kg, respectively. Parous cows with 7, 21, or 35 DINCU had a first test day ECM of 42.8, 45.6, and 44.6 kg of ECM, respectively. In nulliparous cows, there was a tendency for an association between DINCU and the ratio of fat and protein at first test day. In parous cows, however, a

significant association was observed. Parous cows with 7, 21, or 35 DINCU had a ratio of fat and protein of 1.31, 1.35, and 1.37, respectively. There was a significant association between DINCU and SCS at first test day in nulliparous and parous cows. In nulliparous cows with 7, 21, or 35 DINCU, SCS was 2.39, 2.49, and 2.85, respectively. In parous cows with 7, 21, or 35 DINCU, SCS was 2.46, 2.53, and 2.78, respectively. No associations were observed between DINCU and occurrence of stillbirth and DINCU and the risk of pregnancy within 150 DIM. The multivariable model predicted a tendency for an association between DINCU and the risk of being culled within 60 DIM in parous cows. Particularly, 0 to 6 DINCU were associated with a substantially increased risk of being culled. In conclusion, a short stay in the close-up group should be avoided to improve milk yield at first test day and to minimize culling risk for parous cows. A long stay in the close-up group (>30 d) was associated with reduced milk production and an increased ratio of fat and protein in milk of parous cows and increased SCS of nulliparous and parous cows.

**Key words:** days in close-up, gestation length, dietary cation anion difference, dairy cow

### INTRODUCTION

In dairy cows, the transition from late gestation to early lactation is associated with dramatic changes in endocrine function and metabolism (Drackley, 1999; DeGaris et al., 2008). One of the metabolic challenges is the sudden increase of calcium demand for colostrumogenesis and milk production before calving (Goff, 2014). In the recent past, periparturient hypocalcemia has been a focus of research in dairy science (Couto Serrenho et al., 2021). A common practice to prevent clinical and subclinical hypocalcemia is the induction of a compensated metabolic acidosis by feeding a negative DCAD diet in the close-up group (Martín-Tereso and Martens, 2014; USDA, 2016), which increases urinary Ca excretion, leading to an earlier release of parathyroid hormone from the parathyroid gland. Moreover, Goff (2014) suggested that the decrease in blood pH

Received November 4, 2021.

Accepted April 18, 2022.

\*Current affiliation: Clinic for Ruminants, Faculty of Veterinary Medicine, Justus-Liebig-Universität Gießen, 35392 Gießen, Germany.

†Corresponding author: [peter.venjakob@vetmed.uni-giessen.de](mailto:peter.venjakob@vetmed.uni-giessen.de)

leads to an improved parathyroid hormone receptor function, which in turn allows a rapid Ca release via osteocytic osteolysis from the bone fluid pool. The USDA report from 2014 found that 20.7 and 27.6% of the participating herds were feeding acidogenic salts to their prepartum nulliparous and parous and cows, respectively, to decrease the DCAD of the ration and to prevent hypocalcemia. In larger operations (>500 cows), however, 54.5% of parous cows were fed acidogenic salts prepartum (USDA, 2016). These data indicate that a large proportion of cows in the United States were exposed to an acidogenic close-up ration. Two recent meta-analyses showed that feeding a negative DCAD diet in the close-up group reduced morbidity and increased milk production in the subsequent lactation (Lean et al., 2019; Santos et al., 2019). Although the concept of feeding a negative DCAD ration prepartum is well established, few studies investigated the optimum duration of feeding such a diet. Typically, the dry cow period comprises the last 60 d of gestation, consisting of the far-off (first 4–6 wk after dry off) and the close-up period, starting around d 255 of gestation (Vieira-Neto et al., 2021). Due to the biological variation of gestation length (**GL**) and variation in management (i.e., frequency of pen moves) the close-up period is also subject to variation.

DeGaris et al. (2008) found that ECM, milk protein, and milk fat yield increased with increasing days in the close-up group (**DINCU**) in grazing Holstein and Holstein × Jersey crossbred cows. Particularly, cows with >17 DINCU had improved milk production. The association between DINCU and subsequent health and performance has been supported by a recent study using Holstein cows (Vieira-Neto et al., 2021). In that study, nulliparous cows were not exposed to a negative DCAD diet. A quadratic association of DINCU and milk yield, however, was observed in both, nulliparous and parous cows. Milk yield increased, however, to a lesser extent in nulliparous than in parous cows. Independent of parity, optimum milk production was observed when cows stayed 28 DINCU. In addition, these authors also observed a quadratic association between DINCU and pregnancy by 300 DIM, with an optimum of 19 DINCU. There was also a quadratic association between DINCU and culling within 300 DIM. Increasing DINCU were associated with a decrease of culling risk in nulliparous and parous cows and reached the nadir at 20 DINCU. When DINCU were greater than 30, culling risk increased independent from parity. A similar study has been conducted with parous Jersey cows (Chebel, 2021) which showed a linear association between DINCU and first test day milk yield. For every 1 d increase of DINCU, ECM increased by 0.43 kg. Also, in parous Jersey cows there was a quadratic as-

sociation between DINCU and pregnancy by 305 DIM. The highest probability for pregnancy was observed when cows stayed 28 DINCU. In parous Jersey cows, DINCU were significantly associated with the risk of culling until 305 DIM. A shorter stay in the close-up group was associated with a substantial increase of culling risk.

All of the aforementioned studies (DeGaris et al., 2008; Chebel, 2021; Vieira-Neto et al., 2021) were observational studies. Despite the large sample size, however, these studies have limited external validity as they have been conducted in one (Chebel, 2021), 2 (Vieira-Neto et al., 2021), and 3 herds (DeGaris et al., 2008). Therefore, the objective of the present study was to evaluate the association between DINCU and milk yield, milk components, reproductive performance, and culling risk in the subsequent lactation of nulliparous and parous cows on commercial dairy farms using a multisite study design. We hypothesized that nulliparous and parous cows with a short stay in the close-up group (<14 d) have a greater culling risk and a lower milk yield in early lactation. Furthermore, we expected cows with >35 DINCU to also have an increased culling risk and an increased risk for stillbirth, and therefore an extended time to pregnancy.

## MATERIALS AND METHODS

### Study Population and Herd Management

This prospective cohort study was conducted on 20 commercial dairy farms. All herds were located in the Northeast of Germany and kept exclusively Holstein Friesian cows. The experimental procedures reported were conducted with the approval of the federal authorities. This convenience sample was chosen for logistical reasons. The average herd size was 1,116 ( $\pm 303$ , SD; range: 595–1,808) with an average 305-d milk production of 9,758 kg ( $\pm 718$  kg, SD; range: 8,091–10,808 kg). Farms were visited twice in a 14-d interval between May 2019 and December 2019 for enrollment and management of the transition cows was evaluated. The observation period started at the second visit and ended 365 d later. Inclusion criteria were a 2-phase dry cow period consisting of a far-off and a close-up period with feeding of negative DCAD diets to nulliparous and parous cows ( $\leq 0$  mEq/kg of DM). Farms participated in a DHIA system and were required to use a herd management software such as Dairy Comp 305 (Valley Ag Software), HerdeW, or Herde Plus (dsp-Agrosoft Ltd.) to record cow movement from far-off to the close-up pen. All herds enrolled transferred cows from the far-off to the close-up group on a weekly basis based on their GL.

**Table 1.** Chemical composition (% unless otherwise noted) of the close-up diets of the 18 farms enrolled in the study

Farm	Nutrient composition (DM basis)						
	CP	Ether extract	NDF	NFC <sup>1</sup>	Starch	Ash	DCAD, <sup>2</sup> mEq/kg of DM
1	12.9	2.5	45.2	32.5	22.0	6.9	-84
2	14.8	2.8	47.5	28.1	19.7	6.8	-25
3	15.6	3.4	46.7	26.6	21.3	7.7	-51
4	13.5	3.8	44.4	31.6	18.3	6.7	-45
5	13.1	3.1	38.1	38.2	21.8	7.5	-96
6	14.4	2.8	36.6	39.4	26.1	6.8	-148
7	15.6	3.8	37.5	34.9	17.3	8.2	-101
8	15.4	3.6	42.6	30.4	13.0	8.0	-60
9	16.4	3.1	37.4	30.8	15.5	9.3	-26
10	15.3	2.6	40.7	34.5	22.9	6.9	-18
11	13.6	2.6	41.7	33.7	10.0	8.4	-15
12	15.6	3.0	40.2	34.0	21.7	7.2	-26
13	16.2	2.1	38.3	35.3	18.2	8.1	-96
14	15.5	4.0	37.0	35.3	19.3	8.2	-32
15	12.9	3.9	45.8	30.6	14.9	6.8	-4
16	13.1	2.7	45.1	31.9	14.5	7.2	-115
17	15.2	2.8	41.9	32.2	12.4	7.9	-86
18	13.9	3.1	32.6	42.1	26.6	8.3	-199

<sup>1</sup>Calculated as 100 - CP - ether extract - NDF - ash.

<sup>2</sup>DCAD = [(Na% of DM/0.023) + (K% of DM/0.039)] - [(S% of DM/0.016) + (Cl% of DM/0.0355)].

Cows were milked 2 or 3 times a day in rotary or parallel milking parlors. Feed was delivered 1 to 2 times a day, and feed was pushed up multiple times daily. The TMR from far-off, close-up, and fresh cows was formulated to meet or exceed minimum nutritional requirements for high-producing dairy cows (NRC, 2001).

### Farm Visits at Enrollment

During the 2 farm visits at the beginning of the study, participating herds were carefully characterized. To control for a negative DCAD diet in the close-up group, urinary pH was measured using test strips in a subsample of at least 12 cows (4 nulliparous and 8 parous cows; Pehanon, Macherey-Nagel GmbH Co. KG) per herd. Cows had to be exposed to the close-up diet for at least 7 d before the farm visit. In the laboratory of the Ruminant Clinic (Freie Universität Berlin) urine samples were pooled for each farm and net-acid-base excretion was measured as follows. At first, each sample was swirled. Then 10 mL of the urine pool sample was pipetted into an Erlenmeyer flask. The urine was titrated to pH 3.5 with a 1 molar HCL solution and then boiled for 30 s. The cooled sample was then titrated with 0.1 molar NaOH solution to pH 7.4. Afterward, 10 mL of 20% formaldehyde solution was added to the sample and the liquid was titrated again with 0.1 n NaOH to pH 7.4. The titrated amounts of HCl ( $V_{\text{HCl}}$ ) and of NaOH ( $V_{\text{NaOH1}}$ ,  $V_{\text{NaOH2}}$ ) and the pH were documented. Net acid-base excretion (mmol/L) was calculated using the equation  $[(V_{\text{HCl}} \times 10) - (V_{\text{NaOH1}} + V_{\text{NaOH2}})] \times 10$ .

In addition, urinary calcium excretion was measured in a commercial laboratory (Synlab Services GmbH, Augsburg, Germany; accreditation number D-PL-14016-01-00 according to the European regulation no. 765/2008) using spectrophotometry (AU680, Beckman Coulter).

Total mixed ration samples from the far-off, close-up, and fresh cow diet were collected and analyzed in a commercial laboratory (Landwirtschaftliche Kommunikations und Servicegesellschaft mbH, Lichtenwalde, Germany; Table 1).

### Statistical Analyses

Individual cow data were transferred from the management software to Microsoft Excel (Office 2013, Microsoft Deutschland Ltd.). Statistical analyses were performed using SPSS for Windows (version 25.0, SPSS Inc.).

During the observation period of 365 d, 20,372 cows calved. Data on cow movement from far-off to the close-up group were available from 14,843 cows. It has been shown previously that GL was associated with health and production in the subsequent lactation (Vieira-Neto et al., 2017). Because GL is correlated with DINCUCU, we considered GL as a potential confounder. Therefore, GL was kept in all models, regardless of its significance level. To evaluate the association between DINCUCU and health and production in early lactation considering GL, the following steps have been conducted to account for collinearity between these 2

variables. Initially we excluded all cows with GL longer ( $>292$  d,  $n = 104$ ) or shorter ( $<262$ ,  $n = 168$ ) than 3 standard deviations from the mean. Moreover, cows with zero DINCUCU ( $n = 165$ ) and more than 42 DINCUCU ( $n = 504$ ) were excluded. Subsequently, we transformed GL into a categorical variable. Cows with a GL of 262 to 271 d (mean GL minus 2 to 3 SD from the mean) were considered as having a short GL. Cows with a GL of 272 to 282 d ( $GL \pm$  one SD from the mean) were categorized as having normal GL. Cows with a GL of 283 to 292 d (mean GL plus 2 to 3 SD from the mean) were considered as having a long GL. Transformation of GL into a categorical variable led to a Spearman coefficient of correlation to 0.13 between GL and DINCUCU.

Continuous data such as ECM, the ratio of fat and protein, and SCS at first test day were analyzed by linear mixed models using the GENLIMIXED procedure of SPSS. Cow was the experimental unit, and herd and the interaction of herd  $\times$  DINCUCU were considered as random effects. The models accounted for the cluster effect of cows within herd. Based on their different physiology, separate models were built for nulliparous and parous cows. According to the model-building strategies described by Dohoo et al. (2009), each variable considered for the mixed model should be separately analyzed in a univariate model, including the variable as a fixed factor. Only variables resulting in univariate models with  $P \leq 0.10$  were included in the final mixed model. The initial model for nulliparous cows contained 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), age at first calving (AFC; continuous), GL (short vs. normal vs. long), DINCUCU and DINCUCU  $\times$  DINCUCU as explanatory variables. The models evaluating the association between DINCUCU and ECM at first test day and DINCUCU and the ratio of fat and protein at first test day moreover contained SCS as explanatory variables. Somatic cell score was calculated by log-transformation  $\{\ln(\text{SCS}/100)/\ln(2)\} + 3$  as described by Ali and Shook (Ali and Shook, 1980). The model to evaluate the association between DINCUCU and SCS also contained ECM at first test day (continuous) as an explanatory variable. For parous cows, the same models were built. These models also included, however, 305-d milk yield of the previous lactation (continuous) and parity (second vs. third vs. fourth or greater lactation). Furthermore, AFC was not considered in the models for parous cows.

Binary data such as stillbirth, culling within 60 DIM, and pregnancy within 150 DIM were analyzed by logistic regression models using the GENLIMIXED procedure of SPSS. Variables with  $P \leq 0.10$  were considered for the mixed model. Cow within herd was the experimental unit. The initial models contained season of calving, sex of the calf, calving ease, stillbirth, twins, GL, DINCUCU, DINCUCU  $\times$  DINCUCU and AFC for nulliparous cows. For parous cows the model included: season of calving, sex of the calf, calving ease, stillbirth, twins, GL, DINCUCU, DINCUCU  $\times$  DINCUCU, 305-d milk yield in previous lactation, and parity.

In all models, the  $P$ -value was adjusted using a Bonferroni correction, to account for multiple comparisons. Variables were declared significant when  $P < 0.05$ . A statistical tendency was declared when  $P \geq 0.05$  and  $P \leq 0.10$ .

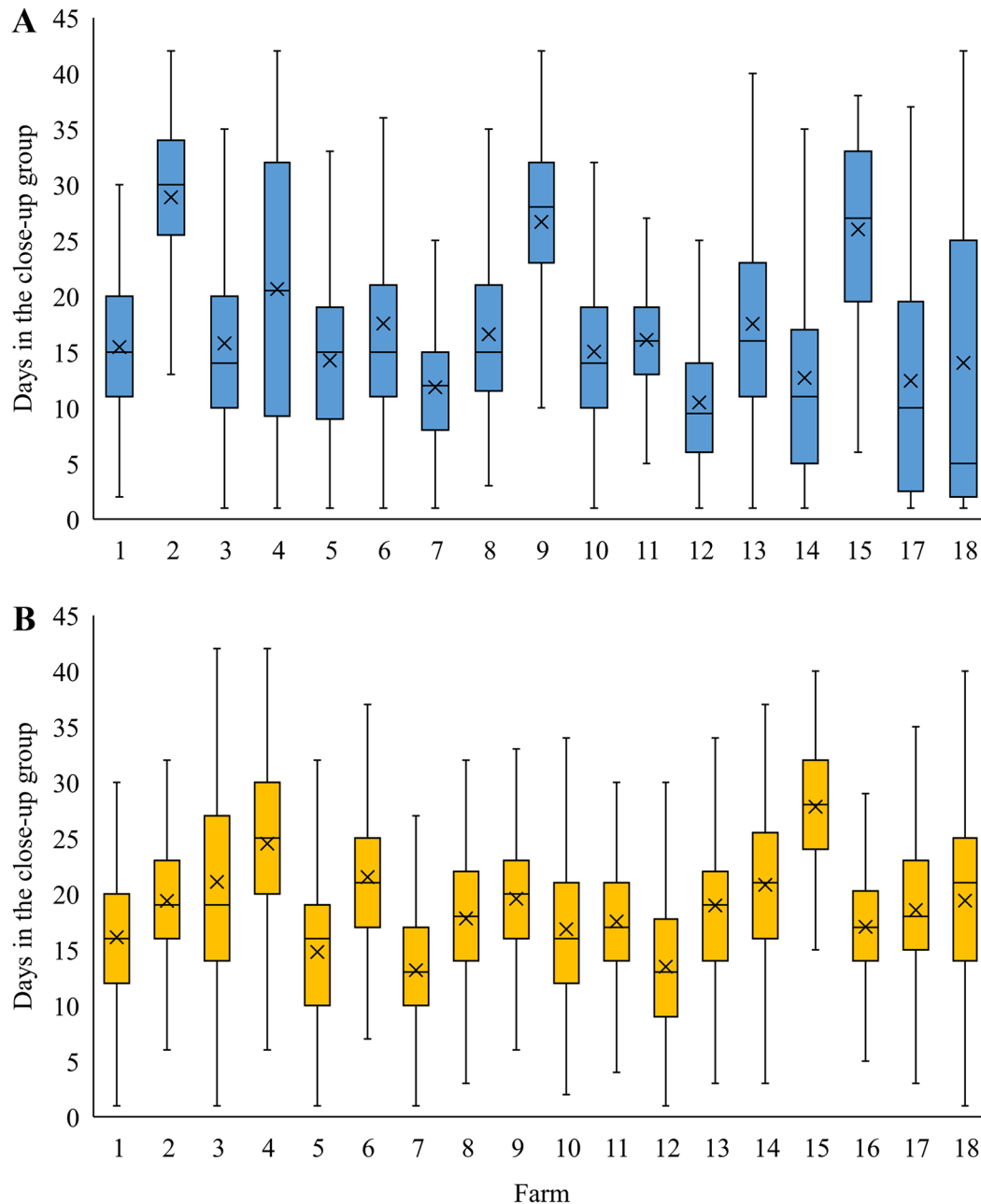
## RESULTS

Two of the 20 farms enrolled in this study had to be excluded, as movement from the far-off to the close-up group was not carefully documented. After exclusion of those 2 herds, 14,843 cows were available. Of those, 588 cows had to be excluded due to missing information about GL. Another 941 cows were excluded because of GL shorter than 262 d, GL longer than 292, zero DINCUCU and more than 42 DINCUCU. Therefore, data sets of 13,314 cows were available for final statistical analyses. At enrollment, 3,871 and 9,443 of those animals were nulliparous and parous cows, respectively. Figure 1 shows the variation of DINCUCU among different farms for nulliparous (Panel A) and parous cows (Panel B). In parous cows, 1,150 (12.2%) stayed  $\leq 10$  d in the close-up group and 591 (6.3%) stayed 30 to 42 d in the close-up group. Of the 3,871 nulliparous cows enrolled in the statistical analyses, 911 (23.5%) and 597 (15.4%) stayed  $\leq 10$  d and 30 to 42 d in the close-up group, respectively. Frequency distribution of DINCUCU of these cows is displayed in Figure 2. Mean urinary pH, mean net-acid-base excretion and mean urinary Ca excretion were  $6.6 \pm 1.05$  ( $n = 223$ ),  $15.4 \pm 27.2$  ( $n = 18$ ), and  $10.3 \text{ mmol/L} \pm 3.3$  ( $n = 18$ ), respectively.

Average GL was  $277 \pm 4.7$  d and  $278 \pm 4.8$  d for nulliparous and parous cows, respectively. Parous cows with twins ( $n = 411$ ; 4.5%) had a GL of  $274 \pm 4.4$  d.

### Milk Yield and Milk Components

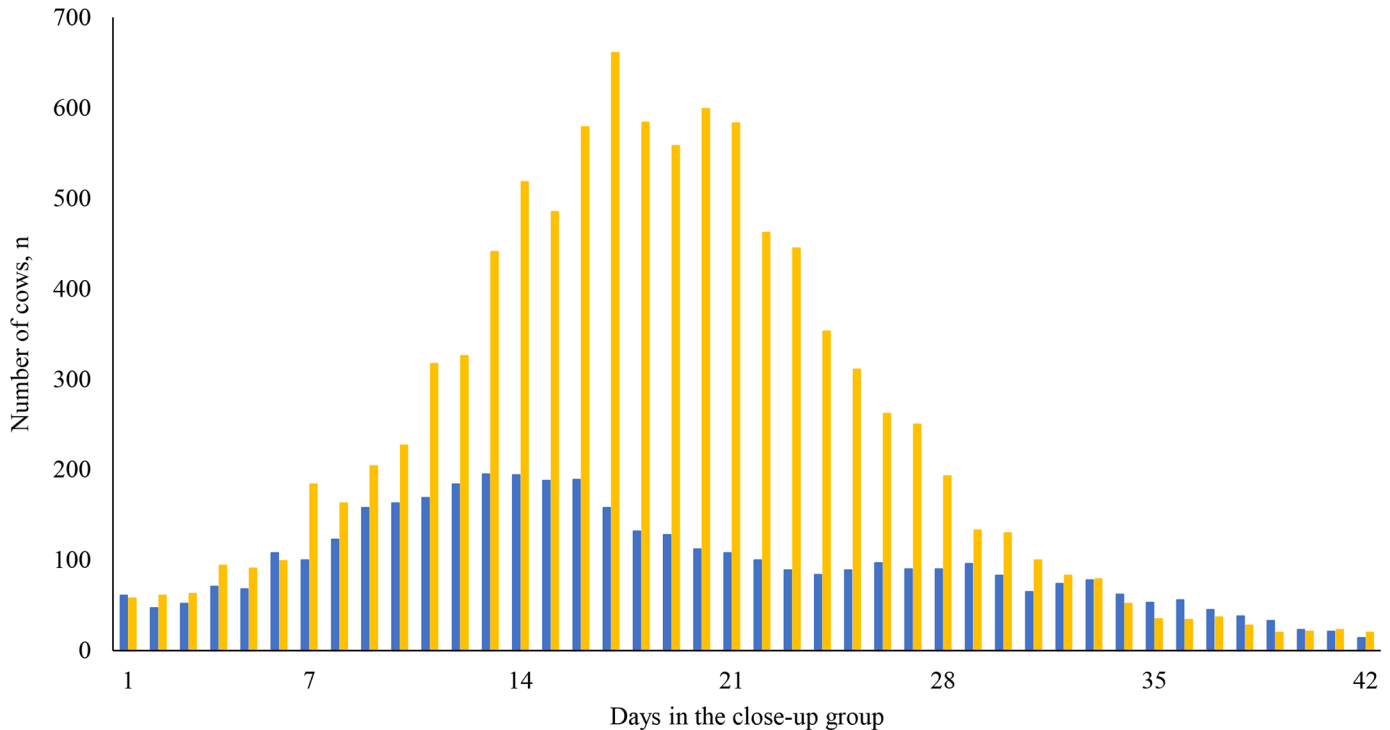
There was a significant linear association between DINCUCU and ECM at first test day in nulliparous cows ( $P < 0.001$ ; Figure 3). Increasing DINCUCU were associated with a greater milk yield at first test day (Figure 3). Multivariable analyses predicted that nulliparous



**Figure 1.** Variation of days in the close-up group (DINCUCU) between the different farms. (A) Variation of DINCUCU is shown for 3,871 nulliparous cows from 17 farms (data from farm 16 were not available, as the farm did not document transfer to the close-up group for nulliparous cows). (B) Variation of DINCUCU is shown for 9,443 parous cows from 18 farms. In the box-and-whisker plot, the central box represents the values from the lower to upper quartile (25th to 75th percentile). The middle line represents the median, whereas the x inside the box displays the mean. The vertical line (i.e., whisker) extends from the minimum to the maximum value, excluding values that are smaller than the lower quartile minus 1.5 times the interquartile range, or larger than the upper quartile plus 1.5 times the interquartile range.

cows with 7, 21, or 35 DINCUCU had a first test day milk yield of 31.8 ( $\pm 0.3$ ), 33.3 ( $\pm 0.3$ ) and 35.6 ( $\pm 0.5$ ) kg ECM, respectively. Furthermore, season ( $P = 0.001$ ), calving ease ( $P = 0.088$ ), stillbirth ( $P = 0.014$ ), SCS at first test day ( $P < 0.001$ ), and AFC ( $P < 0.001$ ) were associated with milk production at first test day. In

addition, GL was kept in the model as a potential confounder ( $P = 0.320$ ). In contrast, in parous cows there was a quadratic association between DINCUCU and milk yield at first test day (Figure 3;  $P = 0.012$ ). According to multivariable analyses, parous cows with 7, 21, or 35 DINCUCU had a first test day milk yield of 42.8 ( $\pm 0.4$ ),



**Figure 2.** Frequency distribution of days in the close-up group of 3,871 nulliparous (blue bars) and 9,443 parous cows (yellow bars).

45.6 ( $\pm 0.2$ ) and 44.6 ( $\pm 1.0$ ) kg ECM, respectively. In parous cows, parity ( $P < 0.001$ ), season ( $P < 0.001$ ), calving ease ( $P < 0.001$ ), stillbirth ( $P < 0.001$ ), twins ( $P < 0.001$ ), SCS at first test day ( $P < 0.001$ ), 305 d milk yield in previous lactation ( $P < 0.001$ ), GL ( $P < 0.001$ ) were also associated with first test day milk yield.

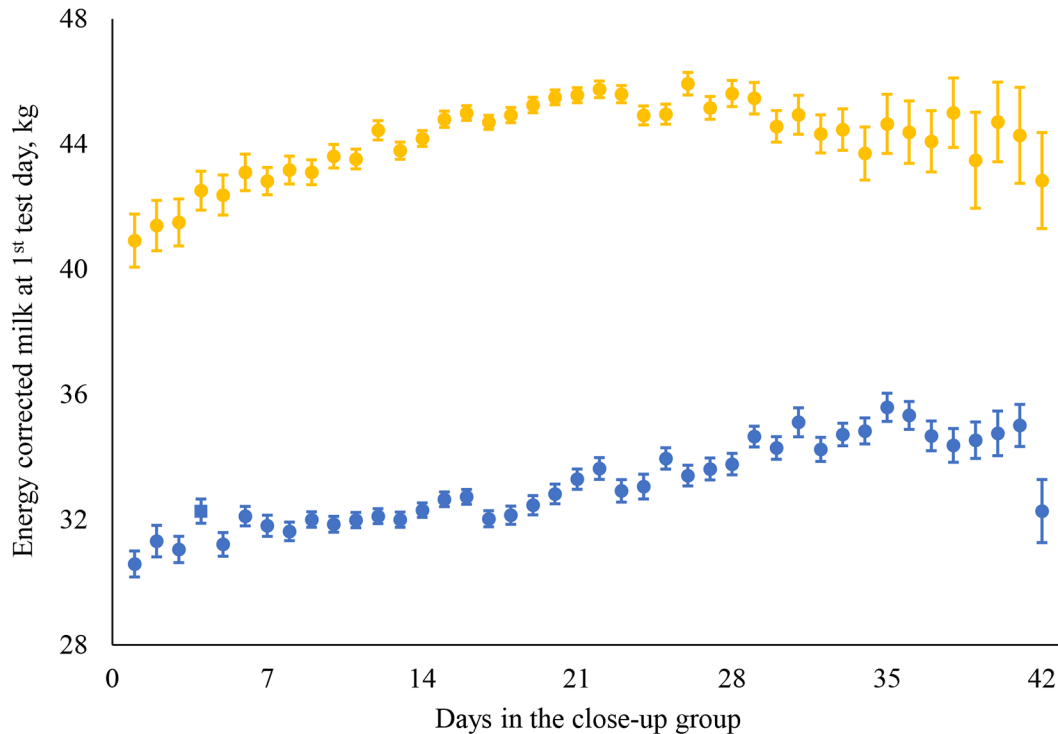
In nulliparous cows, there was a tendency for an association between DINCU and the ratio of fat and protein at first test day ( $P = 0.055$ ; Figure 4). Moreover, significant associations were found between season ( $P = 0.001$ ), AFC ( $P = 0.001$ ), and SCS at first test day ( $P < 0.001$ ) and the ratio of fat and protein at first test day in nulliparous cows. Also, GL was kept in the model as a potential confounder ( $P = 0.649$ ). In parous cows, there was a significant association between the ratio of fat and protein at first test day and DINCU ( $P < 0.001$ ; Figure 4). Multivariable analysis predicted that parous cows with 7, 21, or 35 DINCU had a ratio of fat and protein of 1.31 ( $\pm 0.006$ ), 1.35 ( $\pm 0.003$ ), and 1.37 ( $\pm 0.013$ ), respectively. Also, there was an association between parity ( $P < 0.001$ ), season ( $P < 0.001$ ), 305-d milk yield of previous lactation ( $P < 0.001$ ), SCS at first test day ( $P < 0.001$ ), and the ratio of fat and protein at first test day. As a potential confounder, GL was kept in the model ( $P = 0.283$ ).

There was a significant association between DINCU and SCS at first test day in nulliparous ( $P = 0.005$ )

and parous cows ( $P = 0.011$ ). The longer cows stayed in the close-up group, the greater was the SCS at first test (Figure 5). According to the multivariable analysis, in nulliparous cows with 7, 21, or 35 DINCU, SCS was 2.39 ( $\pm 0.04$ ), 2.49 ( $\pm 0.04$ ), and 2.85 ( $\pm 0.05$ ), respectively. In nulliparous cows, also milk yield at first test day ( $P < 0.001$ ) was associated with SCS. Furthermore, GL was kept in the model as a potential confounder ( $P = 0.450$ ). Multivariable analysis of parous cows predicted that cows with 7, 21, or 35 DINCU, had a SCS of 2.46 ( $\pm 0.06$ ), 2.53 ( $\pm 0.03$ ), and 2.78 ( $\pm 0.12$ ), respectively. Parity ( $P < 0.001$ ), season ( $P = 0.001$ ), calving ease ( $P = 0.037$ ), 305-d milk yield in previous lactation ( $P < 0.001$ ), and ECM yield at first test day ( $P < 0.001$ ) were also associated with SCS at first test day. Furthermore, GL was kept in the model as a potential confounder ( $P = 0.544$ ).

### Reproductive Performance

In nulliparous and parous cows, 313/3,871 (8.1%) and 316/9,443 (3.4%) had a stillbirth, respectively. Neither in nulliparous ( $P = 0.832$ ) nor in parous cows ( $P = 0.318$ ) a significant association between DINCU and the risk of stillbirth was observed. In nulliparous cows, calving ease ( $P < 0.001$ ) and sex of the calf were significantly associated with the risk of stillbirth. Furthermore, GL



**Figure 3.** Association between days in the close-up group (DINCUs) and ECM yield at first test day in nulliparous (blue;  $n = 3,081$ ) and parous cows (yellow;  $n = 7,731$ ) using least square estimates (mean  $\pm$  SEM) from the 2 generalized linear mixed models. In nulliparous cows, season ( $P = 0.001$ ), calving ease ( $P = 0.088$ ), stillbirth ( $P = 0.014$ ), SCS ( $P < 0.001$ ), age at first calving ( $P < 0.001$ ), and DINCUs ( $P < 0.001$ ) were associated with milk production. Furthermore, gestation length ( $P = 0.320$ ) was kept in the model as a potential confounder. In parous cows, parity ( $P < 0.001$ ), season ( $P < 0.001$ ), calving ease ( $P < 0.001$ ), stillbirth ( $P < 0.001$ ), twins ( $P < 0.001$ ), SCS ( $P < 0.001$ ), 305-d milk yield in previous lactation ( $P < 0.001$ ), gestation length ( $P < 0.001$ ), DINCUs ( $P < 0.001$ ), and DINCUs  $\times$  DINCUs ( $P < 0.017$ ) were associated with milk production.

was kept in the model as a potential confounder ( $P = 0.126$ ). In parous cows, calving ease ( $P < 0.001$ ), sex of the calf ( $P < 0.001$ ), and GL ( $P < 0.001$ ) were associated with the risk of stillbirth. Furthermore, there was a tendency for an association between parity and the risk of stillbirth ( $P = 0.074$ ). In nulliparous ( $P = 0.313$ ) and parous ( $P = 0.998$ ) cows, there was no association between DINCUs and risk of pregnancy within 150 DIM. Season of calving ( $P < 0.001$ ) and stillbirth ( $P < 0.001$ ) were associated with the risk of pregnancy within 150 DIM in nulliparous cows. In addition, GL was kept in the model as a potential confounder ( $P = 0.388$ ). In parous cows, parity ( $P < 0.001$ ), season of calving ( $P < 0.001$ ), calving ease ( $P = 0.009$ ), twin birth ( $P = 0.007$ ), and 305-d milk yield in previous lactation ( $P < 0.001$ ) were associated with the risk of pregnancy within 150 DIM significantly. Moreover, GL ( $P = 0.712$ ) was kept in the model as a potential confounder.

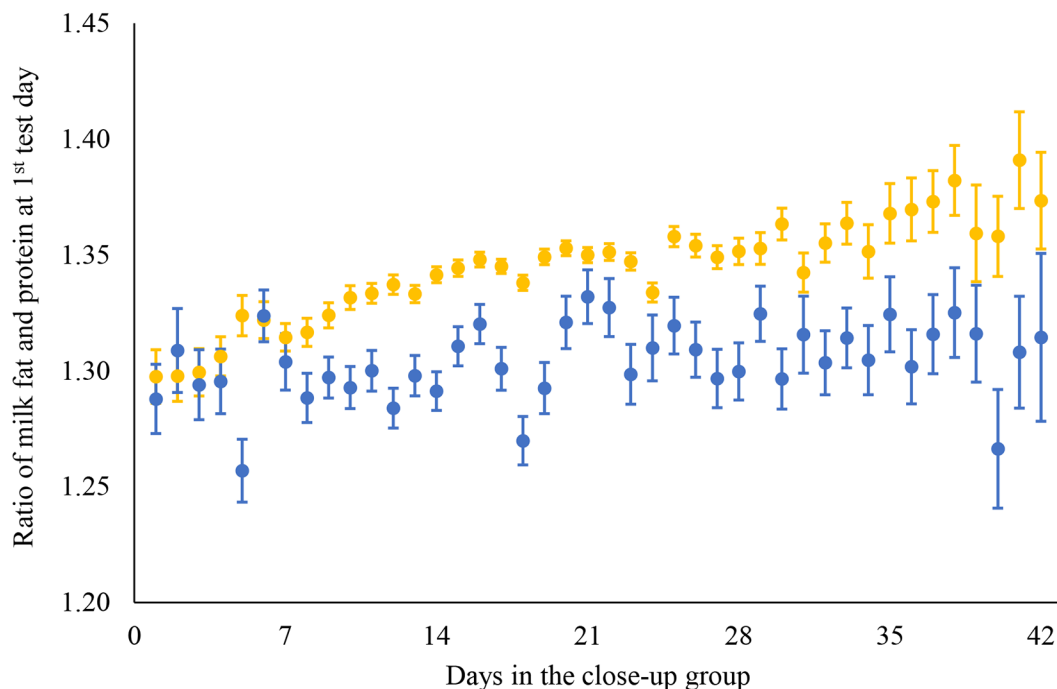
### Culling

In nulliparous and parous cows, 146/3,046 (4.8%) and 705/7,718 (9.1%) were culled within the first 60

DIM, respectively. In nulliparous cows, there was an association between GL and the risk of being culled within 60 DIM ( $P = 0.034$ ). No association was observed for DINCUs and the risk of culling ( $P = 0.139$ ). In parous cows, there was a tendency for an association between DINCUs and the risk of being culled within 60 DIM ( $P = 0.090$ ; Figure 6). Particularly, 0 to 6 DINCUs were associated with a substantially increased risk of being culled. Moreover, parity ( $P < 0.001$ ), season ( $P = 0.007$ ), calving ease ( $P = 0.003$ ), stillbirth ( $P < 0.001$ ), and 305-d milk yield in previous lactation ( $P < 0.001$ ) were associated with culling risk. Gestation length was kept in the model as a potential confounder ( $P = 0.249$ ).

### DISCUSSION

Our hypotheses were that nulliparous and parous cows with a short stay in the close-up group ( $<14$  d) have reduced milk production and a greater culling risk. Furthermore, we expected cows with  $>35$  DINCUs to also have an increased culling risk and an increased risk for stillbirth, and therefore an extended time to



**Figure 4.** Association between days in the close-up group (DINCUs) and the ratio of fat and protein at first test day in nulliparous ( $n = 3,087$ ) and parous cows ( $n = 7,733$ ) using least square estimates (mean  $\pm$  SEM) from the generalized linear mixed model. In nulliparous cows, the SCS at first test day ( $P < 0.001$ ), the age at first calving ( $P = 0.001$ ) and season ( $P = 0.001$ ) were associated with the ratio of fat and protein at first test day. Although there was a tendency for an association between DINCUs and milk components, gestation length was kept in the model as a potential confounder ( $P = 0.649$ ). In parous cows, parity ( $P < 0.001$ ), season ( $P < 0.001$ ), 305-d milk yield of previous lactation ( $P < 0.001$ ), SCS ( $P < 0.001$ ), and DINCUs ( $P < 0.001$ ) were associated with the fat-to-protein ratio. Gestation length was kept in the model as a potential confounder ( $P = 0.283$ ).

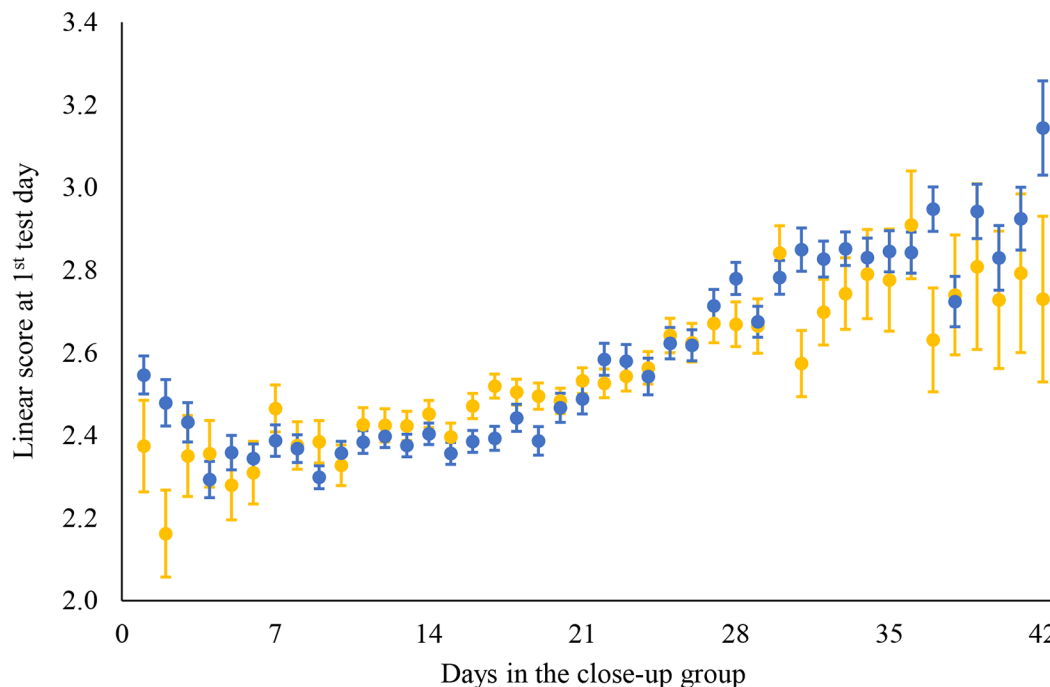
pregnancy. Our findings confirmed that nulliparous and parous cows with a short stay in the close-up group have a reduced milk production. Although there was a quadratic association between first test day ECM and DINCUs in parous cows, there was linear response for milk yield in nulliparous cows with increasing DINCUs. Furthermore, we observed a linear increase in the ratio of fat and protein and SCS in parous cows and a linear increase in SCS of nulliparous cows with increasing DINCUs. As hypothesized, parous cows with a short stay in the close-up group had a tendency for an increased culling risk. This association was, however, not observed for nulliparous cows. Our hypothesis that nulliparous and parous cows with a long stay in the close-up group have an increased culling risk, was not confirmed. In addition, we did not observe an association between DINCUs and the risk of stillbirth, and there was no association between DINCUs and reproductive performance.

Figure 1 displays a considerable variation of DINCUs between farms. Although some farms aimed for a long stay in the close-up group for nulliparous cows (farm 2, 9, and 15) other farms intended a shorter stay of nulliparous cows in the close-up group (farm 7, and 12;

Figure 1A). In parous cows, the transfer from far-off to the close-up group was also heterogeneous among farms. Strong variations within farms (farm 3, 4, and 18) showed that cows were not transferred to the close-up group systematically.

In the present study there was an association between DINCUs and milk yield of nulliparous (linear) and parous cows (quadratic) at first test day. In an older study, DeGaris et al. (2008) found that ECM increased with increasing DINCUs and plateaued between 17 and 35 DINCUs. These authors evaluated the association in Holstein and Holstein  $\times$  Jersey crossbred cows in a seasonal calving system and did not differentiate between parous and nulliparous cows. The authors speculated that improved milk production associated with a longer stay in the close-up group was caused by additional access to nutrients such as MP. According to Van Saun and Sniffen (2014), prepartum dairy cows have an increased need for energy and protein in response to synthesis of mammary tissue and colostrum as well as requirements for uterine and fetal development. An additional 120 and 130 g/d of MP are suggested by Bell et al. (2000) and the NRC (2001), respectively, to meet AA needs for mammary tissue accretion in pre-





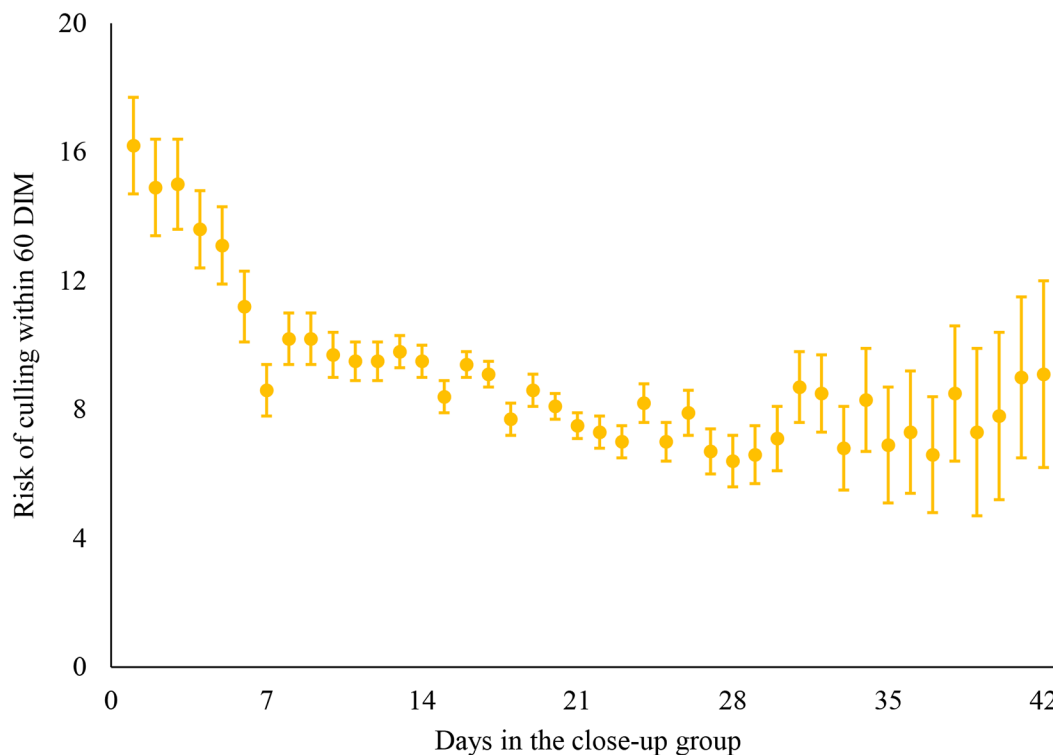
**Figure 5.** Association between days in the close-up group (DINCUs) and SCS in nulliparous (blue;  $n = 3,082$ ) and parous cows (yellow;  $n = 7,733$ ) using least square estimates (mean  $\pm$  SEM) from the 2 generalized linear mixed models. In nulliparous cow, milk yield at first test day ( $P < 0.001$ ) and DINCUs ( $P = 0.005$ ) were associated with SCS. Furthermore, gestation length ( $P = 0.450$ ) was kept in the model as a potential confounder. In parous cows, parity ( $P = 0.001$ ), season ( $P < 0.001$ ), calving ease ( $P = 0.037$ ), 305-d milk yield in previous lactation ( $P < 0.001$ ), ECM yield at first test day ( $P < 0.001$ ) and DINCUs ( $P = 0.011$ ) were associated with SCS. Furthermore, gestation length ( $P = 0.544$ ) was kept in the model as a potential confounder.

partum dairy cows. Vieira-Neto et al. (2021) observed a quadratic association between DINCUs and daily milk yield and DINCUs and cumulative milk yield by 300 DIM in nulliparous and multiparous Holstein cows. As growth of the mammary gland takes place during the last weeks of gestation and requires additional AA (Capuco et al., 1997), Vieira-Neto et al. (2021) speculated that the longer stay in the close-up group might explain improved milk yield. These authors, however, observed a considerable decrease of milk yield when DINCUs were greater than 5 wk. Parous cows with 28 DINCUs produced 1.8 kg/d more milk than cows with 42 DINCUs. This is consistent with our results, as parous cows with 28 and 42 DINCUs had an ECM of 45.6 and 44.6 kg/d at first test, respectively.

In parous cows, increased DINCUs were associated with a significant increase of the ratio of fat and protein. Although cows with 7 DINCUs had a fat and protein ratio of 1.31, the ratio increased to 1.37 when cows stayed 35 DINCUs. Our results agree with Chebel (2021) who observed an increase in postpartum nonesterified fatty acids and BHB in cows with a longer stay in the close-up group. The reason for this is unknown. Cows with an extended stay in the close-up group might consume more energy and therefore

might be more prone to lipomobilization. In contrast, DeGaris et al. (2008) found a significant decrease in milk fat percentage when DINCUs were increased but no association with milk fat yield. In this study, no association between milk protein percentage and DINCUs was observed, whereas milk protein yield reached an optimum with 22 DINCUs.

Increasing DINCUs led to an increase of SCS in both nulliparous and parous cows. Although there was a moderate increase when DINCUs were increased from 7 to 21 d (2.39 to 2.49 and 2.46 to 2.53 for nulliparous and parous cows, respectively), extension from 21 to 35 DINCUs led to more pronounced increase of the SCS (2.49 to 2.85 and 2.53 to 2.78 for nulliparous and parous cows, respectively). In nulliparous Jersey cows, an increased stay in the close-up group was associated with slightly increased odds of SCC  $>200,000$  cells/mL between 5 and 34 DIM. In parous cows, there was a quadratic association between DINCUs and SCC with the smallest odds for  $>200,000$  cells/mL at first test when DINCUs were 28 d. A short stay in the close-up group ( $<10$  d) was associated with the greatest odds of  $>200,000$  cells/mL at first test (Chebel, 2021). In addition, Vieira-Neto et al. (2021) observed a positive association with prolonged DINCUs and the risk



**Figure 6.** Association between days in the close-up group (DINCUC) and culling within 60 DIM of parous cows ( $n = 7,718$ ) using least square estimates (mean  $\pm$  SEM) from the logistic regression model. Culling within the first 60 DIM was significantly associated with parity ( $P < 0.001$ ), season ( $P = 0.007$ ), calving ease ( $P = 0.003$ ), stillbirth ( $P < 0.001$ ), 305-d milk yield in previous lactation ( $P < 0.001$ ), and DINCUC ( $P = 0.090$ ). Gestation length was kept in the model as a potential confounder ( $P = 0.249$ ).

for mastitis. The reason for this remains speculative. One aspect for this observation might be an association between increased milk yield in early lactation as a risk factor for mastitis. Schukken et al. (1991) showed an association between increased fat- and protein-corrected milk yields and the risk of mastitis caused by *Escherichia coli* and *Staphylococcus aureus*. Also, Fleischer et al. (2001) observed a positive association between milk yield and the risk for mastitis.

In Germany, it has been recommended for a long time that nulliparous cows should stay less than 10 d in the close-up group (Staufenbiel, 1999), as farmers and consultants were concerned about an increasing risk for stillbirth when DINCUC were  $>10$  d. We found no association between DINCUC and the risk for stillbirth in nulliparous or parous cows. In contrast to a previous study with Holstein and Holstein  $\times$  Jersey crossbred cows (DeGaris et al., 2010), we did not observe an association between DINCUC and the risk of pregnancy within 150 DIM. Other authors reported quadratic associations with first service conception risk (Chebel, 2021) and pregnancy by 300 DIM (Chebel, 2021; Vieira-Neto et al., 2021). In the study by Vieira-Neto et al. (2021), an increase from 7 to 28 DINCUC

slightly increased the proportion of pregnant cows by 300 DIM, whereas increasing DINCUC from 28 to 42 d led to a dramatic decrease of 14.7 percentage points in the proportion of pregnant cows. In parous Jersey cows, the probability of pregnancy by 305 DIM decreased by 0.188 when DINCUC decreased from  $28 \pm 3$  to  $<10$  DINCUC (Chebel, 2021). Different findings between our study and the aforementioned studies might be attributable to our multisite study approach. Due to farm-individual reproductive management (e.g., voluntary waiting period, heat detection and breeding strategy), there is variation in reproductive performance among herds. Therefore, the association between DINCUC and time to pregnancy may have been biased by the non-standardized approach to reproductive management.

No association was observed between DINCUC and culling risk of nulliparous cows. In parous cows; however, there was a tendency for an association between a short stay in the close-up group ( $<7$  d) and culling risk. This observation is consistent with DeGaris et al. (2010) and Chebel (2021), but contradicts the findings of Vieira-Neto et al. (2021), who demonstrated a pronounced increase of culling risk in cows with  $>30$  DINCUC.

Results from the present study provide evidence for transition cow management to improve health and performance. In contrast to the studies by Vieira-Neto et al. (2021) and Chebel (2021), in our study also nulliparous cows were exposed to diets negative in DCAD. Due to the multisite approach, the findings of the present study have a high external validity, as the results were obtained from 18 commercial dairy farms. Reducing variation in DINCUCU among farms aiming for a minimum DINCUCU of 14 d in the close-up group, represents an opportunity to improve milk yield of nulliparous and parous cows without detrimental effects on stillbirth or udder health. Based on the variation in GL ( $278 \pm 4.8$  d) we recommend to move cows from the far-off into the close-up group at d 254 [ $278 \text{ d} - 14 \text{ d}$  (desired DINCUCU)  $- 10 \text{ d}$  ( $2 \times \text{SD}$ )]. Cows bearing twins had reduced GL ( $274 \pm 4.4$  d). This is in accordance with Vieira-Neto et al. (2017). Therefore, these cows should be moved to the close-up group on d 250 of gestation.

### Study Limitations

At the beginning of the study, each farm enrolled had been characterized during 2 farm visits. At the first visit TMR samples of the close-up diet were collected and analyzed. Cows that calved during the following 365 d after the second farm visit were enrolled. As we explored the optimal DINCUCU in herds feeding diets with negative DCAD, it would have been valuable to collect TMR samples on a regular basis (i.e., monthly). Due to the long distances between the farms and costs for chemical analysis, this had to be omitted. All participating farms, however, did not initiate major changes in the close-up diet during the observation period. As our results were obtained from farms that were feeding diets with negative DCAD, it is questionable whether the results on the association between DINCUCU and health and performance can be applied to farms with a positive DCAD ration in the close-up period.

We relied on valid documentation of the transfer from far-off to the close-up group by the herd management. At the beginning of statistical analyses transfer from far-off to close-up group has been critically evaluated for each farm. As a result, 2 farms had to be excluded, as statistical analyses revealed that movement from far-off to close-up group was not carefully documented. Furthermore, disease data were not considered for statistical analysis, as disease definitions were not consistent between farms.

### CONCLUSIONS

The present study shows that DINCUCU were associated with milk production and milk components of

nulliparous and parous cows at first test day. Moreover, parous cows with a short stay in the close-up group had a tendency for an increased culling risk in early lactation. A short stay in the close-up group should be avoided to improve milk yield at first test day and to minimize culling risk for parous cows. In addition,  $>30$  DINCUCU were associated with detrimental effects on milk production and milk components in parous cows and linear score of nulliparous and parous cows. Based on the variation in GL ( $278 \pm 4.8$  d) we recommend to move cows from the far-off to the close-up group at d 254. Cows bearing twins should be moved on d 250 of gestation. Multisite, randomized controlled studies should be conducted to strengthen our observations.

### ACKNOWLEDGMENTS

The authors acknowledge the financial support of ForFarmers (Lochem, the Netherlands) and Tiergyn e.V. (Berlin, Germany). In particular, the authors thank Fred de Koning and the team of ForFarmers Beelitz GmbH (Beelitz, Germany) for their help in organizing the study. In addition, the authors are grateful to the owners and employees of the collaborating dairy farms for granting access to their facilities, cows, and herd records and their kind support. The authors have not stated any conflicts of interest.

### REFERENCES

- Ali, A., and G. E. Shook. 1980. An optimum transformation for somatic cell concentration in milk. *J. Dairy Sci.* 63:487–490. [https://doi.org/10.3168/jds.S0022-0302\(80\)82959-6](https://doi.org/10.3168/jds.S0022-0302(80)82959-6).
- Bell, A. W., W. S. Burhans, and T. R. Overton. 2000. Protein nutrition in late pregnancy, maternal protein reserves and lactation performance in dairy cows. *Proc. Nutr. Soc.* 59:119–126. <https://doi.org/10.1017/S0029665100000148>.
- Capuco, A. V., R. M. Akers, and J. J. Smith. 1997. Mammary growth in Holstein cows during the dry period: Quantification of nucleic acids and histology. *J. Dairy Sci.* 80:477–487. [https://doi.org/10.3168/jds.S0022-0302\(97\)75960-5](https://doi.org/10.3168/jds.S0022-0302(97)75960-5).
- Chebel, R. C. 2021. Associations between days on close-up diets and immune responses prepartum, metabolites peripartum, and risk of postpartum diseases in Jersey cows. *J. Dairy Sci.* 104:7135–7153. <https://doi.org/10.3168/jds.2020-19700>.
- Couto Serrenho, R., T. J. DeVries, T. F. Duffield, and S. J. LeBlanc. 2021. Graduate Student Literature Review: What do we know about the effects of clinical and subclinical hypocalcemia on health and performance of dairy cows? *J. Dairy Sci.* 104:6304–6326. <https://doi.org/10.3168/jds.2020-19371>.
- DeGaris, P. J., I. J. Lean, A. R. Rabiee, and C. Heuer. 2008. Effects of increasing days of exposure to prepartum transition diets on milk production and milk composition in dairy cows. *Aust. Vet. J.* 86:341–351. <https://doi.org/10.1111/j.1751-0813.2008.00335.x>.
- DeGaris, P. J., I. J. Lean, A. R. Rabiee, and C. Heuer. 2010. Effects of increasing days of exposure to prepartum transition diets on reproduction and health in dairy cows. *Aust. Vet. J.* 88:84–92. <https://doi.org/10.1111/j.1751-0813.2009.00529.x>.
- Dohoo, I. R., S. W. Martin, and H. Stryhn. 2009. *Veterinary Epidemiologic Research*. 2nd ed. VER Inc.

- Drackley, J. K. 1999. Biology of dairy cows during the transition period: The final frontier? *J. Dairy Sci.* 82:2259–2273. [https://doi.org/10.3168/jds.S0022-0302\(99\)75474-3](https://doi.org/10.3168/jds.S0022-0302(99)75474-3).
- Fleischer, P., M. Metzner, M. Beyerbach, M. Hoedemaker, and W. Klee. 2001. The relationship between milk yield and the incidence of some diseases in dairy cows. *J. Dairy Sci.* 84:2025–2035. [https://doi.org/10.3168/jds.S0022-0302\(01\)74646-2](https://doi.org/10.3168/jds.S0022-0302(01)74646-2).
- Goff, J. P. 2014. Calcium and magnesium disorders. *Vet. Clin. North Am. Food Anim. Pract.* 30:359–381. <https://doi.org/10.1016/j.cvfa.2014.04.003>.
- Lean, I. J., J. E. P. Santos, E. Block, and H. M. Golder. 2019. Effects of prepartum dietary cation-anion difference intake on production and health of dairy cows: A meta-analysis. *J. Dairy Sci.* 102:2103–2133. <https://doi.org/10.3168/jds.2018-14769>.
- Martín-Tereso, J., and H. Martens. 2014. Calcium and magnesium physiology and nutrition in relation to the prevention of milk fever and tetany (dietary management of macrominerals in preventing disease). *Vet. Clin. North Am. Food Anim. Pract.* 30:643–670. <https://doi.org/10.1016/j.cvfa.2014.07.007>.
- NRC. 2001. *Nutrient Requirements of Dairy Cattle*. 7th ed. Natl. Acad. Sci.
- Santos, J. E. P., I. J. Lean, H. Golder, and E. Block. 2019. Meta-analysis of the effects of prepartum dietary cation-anion difference on performance and health of dairy cows. *J. Dairy Sci.* 102:2134–2154. <https://doi.org/10.3168/jds.2018-14628>.
- Schukken, Y. H., F. J. Grommers, D. van de Geer, H. N. Erb, and A. Brand. 1991. Risk factors for clinical mastitis in herds with a low bulk milk somatic cell count. 2. Risk factors for *Escherichia coli* and *Staphylococcus aureus*. *J. Dairy Sci.* 74:826–832. [https://doi.org/10.3168/jds.S0022-0302\(91\)78231-3](https://doi.org/10.3168/jds.S0022-0302(91)78231-3).
- Staufenbiel, R. 1999. Metabolic monitoring in dairy herds in order to stabilize performance and health. 3rd Symposium on Feeding and Managing High Producing Dairy Cows. Neuruppin, Germany.
- USDA. 2016. *Dairy 2014: Dairy Cattle Management Practices in the United States*. USDA, Animal and Plant Health Inspection Service, Veterinary Services, National Animal Health and Monitoring System.
- Van Saun, R. J., and C. J. Sniffen. 2014. Transition cow nutrition and feeding management for disease prevention. *Vet. Clin. North Am. Food Anim. Pract.* 30:689–719. <https://doi.org/10.1016/j.cvfa.2014.07.009>.
- Vieira-Neto, A., K. N. Galvão, W. W. Thatcher, and J. E. P. Santos. 2017. Association among gestation length and health, production, and reproduction in Holstein cows and implications for their offspring. *J. Dairy Sci.* 100:3166–3181. <https://doi.org/10.3168/jds.2016-11867>.
- Vieira-Neto, A., G. A. Duarte, R. Zimpel, W. W. Thatcher, and J. E. P. Santos. 2021. Days in the prepartum group are associated with subsequent performance in Holstein cows. *J. Dairy Sci.* 104:5964–5978. <https://doi.org/10.3168/jds.2020-18889>.

## ORCID

- P. L. Venjakob  <https://orcid.org/0000-0001-7541-5508>  
L. K. Neuhaus  <https://orcid.org/0000-0003-3471-6843>  
W. Heuwieser  <https://orcid.org/0000-0003-1434-7083>  
S. Borchardt  <https://orcid.org/0000-0003-3937-5777>