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The impact of automated, constant incomplete milking on energy balance, udder health, and subsequent performance in early lactation of dairy cows

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ABSTRACT

Incomplete milking (IM) is one way of mitigating the negative energy balance (NEB) that is characteristic for early lactation and may increase the risk for disease. Our objectives were to test the effects of IM in early lactation on energy balance (EB), metabolic status, udder health, and subsequent performance. To facilitate the practical application, an automated system was used to remove the milking clusters once a predefined amount of milk is withdrawn. Forty-six Holstein cows were equally allocated to either the treatment (TRT, starting on 8 d in milk) or the control group (CON; conventional cluster removal at milk flow rate <0.3 kg/min). Milk removal in the TRT group was limited to the individual cow's milk yield 1 d before IM started and held constant for 14 d. Thereafter, all cows were conventionally milked and records related to EB, performance, and udder health were continued up to 15 wk of lactation. During the 14 d of IM, on average 11.1% less milk was obtained from the TRT cows than from the CON cows. Thereafter, milk yield increased in the TRT group, eliminating the group difference throughout the remaining observation period until wk 15 of lactation. The TRT cows tended to have less dry matter intake and also water intake than the CON cows. The extent of the NEB and the circulating concentrations of fatty acids, β-hydroxybutyrate, insulin-like growth factor-1, and leptin mostly did not differ between the groups. The IM did not affect body condition. Udder health was maintained over the entire observation period in all cows. Our results demonstrate the applicability of the automated cluster removal for limiting milk withdrawal to a defined amount in early lactation. However, it remains to be determined whether the absent effect on energy metabolism was due to the relatively stable energy status of the cows or to the relatively mild IM setting used herein.

Key words: incomplete milk removal, energy status, management strategy

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INTRODUCTION

The negative energy balance (**NEB**) during early lactation results from the gap between the energy needed for maintenance and milk secretion, and the energy supply provided by feed intake that is not covering the needs at that time. For sustaining the increasing milk yields during the first weeks of lactation, dairy cows have to mobilize body reserves, mainly fat. The release of fatty acids (**FA**) from the adipose depots is reflected by increased circulating FA concentrations. During the NEB, the capacity of the liver for FA oxidation is limited, and incomplete FA oxidation can result in metabolic diseases such as ketosis and fatty liver (Drackley et al., 2005; Esposito et al., 2014). For mitigating the NEB and thus the risk for such health disturbances, the main variables affecting the energy balance (**EB**) can be tackled: energy intake can be modified to some extent by dietary measures (e.g., by providing palatable, energy-dense, and adequately structured diets). Supplements that support rumen stability, supply additional energy, and improve intermediary metabolism can be helpful in mitigating the NEB (Jouany, 2006; McGuffey, 2017). However, voluntary feed intake is negatively associated with the increased pro-inflammatory cytokine levels arising from subacute inflammation in various peripheral organs shortly before or after calving; these cytokines reach the hypothalamus where they signal reduction in feed intake (Kuhla, 2020), a phenomenon that cannot be overcome by dietary intervention. The other significant variable in the early-lactation EB concerns the energy output via milk. Several approaches for temporarily reducing milk yield were suggested: inhibiting the secretion of the galactopoetic hormone prolactin (**PRL**) by administering dopamine agonists (Lacasse et al., 2019), by omitting or shortening the dry period (Kok et al., 2019) and thus lowering the peak milk yield, by reducing the milking frequency to once daily as reviewed by Stelwagen et al. (2013), or by incomplete milking (**IM**) at each milking. The latter was achieved by removing only a defined amount of milk from the mammary gland (e.g., Carbonneau et al., 2012; Krug et al., 2018a,b). Lacasse et al. (2018) assumed that IM is the most promising approach to reduce metabolic stress without compromising the productivity of high-yielding dairy cows. Studies aiming at improving the NEB and the concomitant metabolic consequences in early lactation by IM were largely limited to the colostrum phase (Carbonneau et al., 2012; Morin et al., 2018; Valldecabres et al., 2022). In addition, the knowledge about potentially sustained effects of IM beyond the time of IM treatment is limited. The IM approaches targeting the amount of milk rather than the milk flow as a steering criterion for cluster removal were impeded by the need for manual intervention. Manual cluster removal enhances the potential for errors in accurate cluster removal after reaching the target milk yield and implies a substantial workload (Morin et al., 2018). Moreover, the onset of IM during the first days of lactation, when colostrum and the following milkings are not considered as salable milk, requires more effort outside of the normal milking routine. With this background, we aimed at starting IM not before the second week after parturition when the milk is no longer separated. Moreover, for targeting cluster removal when a defined amount of milk is withdrawn rather than using milk flow rate, we aimed at applying a software module that was initially developed for drying cows off by reducing the amount of milk withdrawn per milking (Martin et al., 2020; Schmidt et al., 2020). Our objective was to delay the increase in milk yield during the first weeks of lactation by limiting the amount of milk withdrawn to the volume milked at the beginning of the second week in milk over the 2 following weeks. We hypothesized that a 2-wk automated and constant IM would mitigate the NEB, without compromising udder health and performance thereafter.

MATERIALS AND METHODS

The animal experimental procedures (July 2020 to April 2022) were approved by the relevant authority (State Office for Nature, Environment, and Consumer Protection [LANUV], North Rhine-Westphalia, Recklinghausen, Germany; 81–02.04.2019.A404) and were carried out at the Campus Frankenforst, Faculty of Agriculture, University of Bonn.

Animals and Housing

Forty-six pluriparous German Holstein cows were selected from the 60-cow herd, and randomly allocated to either the control group (**CON**, n = 23; average lactation number 3.5 ± 1.6) or the treatment group (**TRT**, n = 23; average lactation number 3.1 ± 1.5; mean ± SD). All cows were housed in a freestall barn with slatted concrete floor and cubicles. During the first 15 wk of lactation, all lactating cows were fed a partial mixed ration with an average energy content of 6.2 MJ of NE_L/kg of DM (Supplemental Table S1; https://doi.org/10.6084/m9.figshare.24533176.v1; Meyer et al., 2023). The diet was provided for ad libitum intake after each of the 2 milking times (0530 and 1630 h). In addition, a dairy compound feed was provided through separate feeding stations (GEA Farm Technologies GmbH, Bönen, Germany) via transponder access according to DIM and milk yield. Feed intake was recorded daily via weighing troughs (Hokofarm Group B.V., Marknesse, the Netherlands). The cows had ad libitum access to fresh water and water intake was recorded by water weighing bowls (Hokofarm Group B.V.). After each milking, BW were recorded via an electronic scale (Hokofarm Group B.V.). A double-4 in-line milking parlor was used (Classic 300 cluster and ClassicPro 21–27 liners; GEA Farm Technologies GmbH) with 62 pulses/min, 64:36 pulsation ratio, and a vacuum level of 40 kPa. The threshold for automatic, milk flow–dependent cluster removal was set at 0.3 kg/min. The pre-milking routines of cleaning with a wet paper towel (UdderoClean; Albert Kerbl GmbH, Buchbach, Germany) and forestripping were followed by mechanical pre-stimulation (300 pulses/min during the first 30 s). Milk yield, milking duration, and last milk flow in the measuring unit at the moment of cluster removal were recorded automatically at the udder level for each cow during each milking (Metatron P21; GEA Farm Technologies GmbH). The value obtained for the last milk flow in the measuring unit at the moment of triggering cluster removal is composed of the set threshold for milk flow–dependent cluster removal and additional milk that accumulates in the milk draining components of the milking unit preceding the measuring unit due to the delay time. The lactation preceding the current investigation was terminated by drying off the cows at 52 ± 9 d before expected calving and applying an intramammary antimicrobial treatment (Orbenin Extra, Zoetis Deutschland GmbH, Berlin, Germany) and an internal teat sealer (Orbeseal, Zoetis Deutschland GmbH) after the last milking.

Experimental Design

During the first 8 ± 1.1 DIM, all cows were milked in the same manner (i.e., with cluster removal at milk flow rate <0.3 kg/min). The cows were allocated to either the CON or TRT group by stratifying for udder health in the preceding lactation, calving date, and lactation number. The TRT cows were milked using the software module (Schmidt et al., 2020) by which the milking clusters are removed after obtaining a defined amount of milk (kg) until d 21 ± 1.1 of lactation. We hypothesized that the TRT cows would adapt their milk production to the lower demand during the 14-d treatment period and would thus have a less negative EB, without udder health restrictions. By setting a constant milk quantity for the IM according to the animal-specific performance level 1 d before IM was started, we could take the individual's milk yield into account, and thus exert the same relative impact on all cows. The software module was originally developed for facilitating dry-off (Martin et al., 2020) and was integrated into the milking computer (Metatron C21; GEA Farm Technologies GmbH) on the farm. The constant amount of milk (kg) used during the 2-wk IM period corresponded to the cow's individual milk yield 1 d before the IM started. At the end of IM, the conventional automatic cluster removal at <0.3 kg/min was reactivated and records of milk yield and feed intake were continued and evaluated for this study until wk 15 of lactation.

Milk Composition and Energy-Corrected Milk

At the udder level, milk samples (proportionate total composite milking) were collected weekly (equivalent to the routine monthly milk recording samples) at consecutive evening and morning milkings, and were analyzed for milk fat, protein, and lactose (Landeskontrollverband Nordrhein-Westfalen e.V., Krefeld, Germany).

The ECM (kg; 4% fat and 3.4% protein) was calculated individually for each week according to the equation of Tyrrell and Reid (1965): ECM = milk (kg) × [(0.38 × fat (%) + 0.21 × protein (%) + 1.05)/3.28].

Udder Health and Indicators of Udder Filling

For udder health control, the SCC was assessed weekly in quarter foremilk samples (DCC cell counter; DeLaval GmbH, Glinde, Germany) in the first 7 wk of lactation. During wk 1 and 4, foremilk samples from both groups were subjected to cytobacteriological examination according to the recommendations of the German Veterinary Society (DVG, 2009) at a commercial laboratory (Milchtierherden-Betreuungs- und Forschungsgesellschaft mbH [MBFG], Wunstorf, Germany).

During the first 4 wk of lactation, both groups were observed twice per week for milk leakage based on the protocol by Gott et al. (2016): one investigator stood in the milking parlor and visually observed each study animal standing in the milking parlor for 10 to 15 s before fore-milking, after fore-milking before attaching the milking clusters, and after cluster removal. Milk leakage was recorded as "yes" or "no" at the quarter level. The cow was scored as "yes" if at least one leaking teat was observed.

The udder firmness was assessed for estimating the filling state of the udders. The measurements were performed twice per week before attaching the milking clusters by using a digital dynamometer (FMI-B30B5, 50 N, 0.15% accuracy; Alluris GmbH & Co. KG, Freiberg, Germany). The device was equipped and the measurement was performed considering the standard operation procedure implemented by Bertulat et al. (2012). Herein one representative measurement point located on the border between the lower third and middle third of the rear udder quarter was used. The measurement was performed and readouts were transferred in real time to the computer using an advice-related software (FMI_Connect 3.02, Alluris GmbH & Co. KG). The output value represented the arithmetic mean (N) and the coefficient of variation (%) of 2 consecutive measurement series. Values with a coefficient of variation exceeding 10% were discarded and the measurement repeated.

Feed Analyses and Estimation of Energy Balance

For calculating the EB, the energy content of the feed was estimated first: samples of the partial mixed ration were taken twice per week over the entire experimental period. The DM content was determined by oven-drying (Memmert GmbH & Co. KG, Schwabach, Germany) at 105°C overnight. The DM was corrected (**DMcor**) for the loss of volatiles during drying according to Weißbach and Kuhla (1995): DMcor (%) = 2.08 + 0.975 × DM (%). Concentrate samples were taken from each batch at delivery and stored at −20°C. Analysis of DM content (%; VO [EG] 152/2009, III, A) and estimation of energy concentration (MJ of NE_L/kg of DM; GfE, 2001) were done by a commercial laboratory (Landwirtschaftliche Untersuchungs- und Forschungsanstalt [LUFA] North Rhine-Westphalia, Münster, Germany).

The EB (MJ of NE_L/d) was calculated individually for each week according to the Gesellschaft für Ernährungsphysiologie (GfE, 2001): feed energy intake (MJ of NE_L/d) minus the NE requirement for maintenance [MJ/d = 0.293 × (kg of BW)$^{0.75}$], and the energy output in milk [MJ/d = (38.5 × fat (%) × 0.01 + 24.2 × protein (%) × 0.01 + 16.5 × lactose (%) × 0.01 + 0.1) × (milk amount (kg)] (Susenbeth, 2018).

Body Condition Score, and Measurements of Backfat Thickness and Muscle Diameter

The BCS (Edmonson et al., 1989), average backfat thickness (**BFT**), and measurement of the average M. longissimus dorsi diameter (**MLD**) were measured every second week starting 3 wk before the expected calving date until 6 wk thereafter. The BFT measurement was done above the M. longissimus dorsi, right side, and perpendicular to the spinal cord between the 12th and 13th rib and at the 5th lumbar vertebra (Bruckmaier et al., 1998a,b) by ultrasound (LOGIQ V2; GE Healthcare GmbH, Solingen, Germany) using an endolinear probe (5–10 MHz; LK760-RS, GE Healthcare GmbH) at 9 MHz and 7 cm depth. Ultrasound images were stored immediately for later analysis (IC Measure 2.0.0.245, The Imaging Source Europe GmbH, Bremen, Germany). Image analysis allowed for assessing not only BFT but also MLD.

Blood Sampling and Analyses

Blood was obtained from the v. jugularis in wk 3 and 2 before calving and weekly during the first 7 wk after calving. Blood was collected into tubes containing coagulation activator (9 mL; S-Monovette, Sarstedt AG & Co. KG, Nümbrecht, Germany). After collection, samples were left to rest for 30 min at room temperature. Samples were centrifuged at 1,118 × g for 15 min at 15°C (Z 366 K; HERMLE Labortechnik GmbH, Wehingen, Germany). Subsequently the serum was removed and stored at −20°C for later analyses. Nonesterified fatty acid (**NEFA**) and BHB concentrations were measured in serum by using an automatic photometric analyzing system (Eurolyser; Type VET CCA, Salzburg, Austria) at the Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI) in Braunschweig, Germany. Serum leptin was analyzed with an enzyme immunoassay validated for bovine samples (Sauerwein et al., 2004). The mean intraassay coefficient of variation (**CV**) was 7.37%, and the interassay CV was 9.97%. An enzyme immunoassay (Mediagnost GmbH, Reutlingen, Germany) was used for the quantitative determination of IGF-1 in serum. The mean intraassay CV was 3.17%, and the interassay CV was 5.15%.

Statistical Analysis

Statistical analyses for all data were performed using SPSS 28 (IBM, Armonk, NY). Before analysis, raw data on a daily basis were checked for extreme outliers. Outliers in milk yield, feed intake, water intake, BW, milk yield, and milk composition were defined as deviating more than 3 SD from the mean. In addition, the plausibility of data was checked and eventually corrected for technical failures that occurred during the trial (e.g., temporary loss of function of the weighing troughs or the scale). Data were analyzed by calculating the data within 2 or 4 periods of weeks or days (before calving and before start of the treatment; with treatment and after treatment), depending on the availability of data from before calving and data on day or week level. Data for each period were tested for normal distribution, normal distribution of the residuals of each variable, and the homogeneity of variance with the Levene's test. Using a linear mixed model and Bonferroni correction within periods for multiple comparisons, data were analyzed as repeated measures with "treatment group" (treatment) and "time" (week[s] or day[s] relative to calving) and their interaction as fixed effects. "Cow" was used as random factor. For the udder health control by the SCC of foremilk samples, "udder quarter" was considered as random factor instead of "cow." As covariance structure, the variance components covariance structure was used. Student's t-test was carried out for the variables "time of peak" and "peak yield," and the chi-squared test was performed for "milk leakage" for each week. All data are presented as means with standard error of the mean. The level of significance was set at $P \leq 0.05$ and a trend was defined at $0.05 < P \leq 0.10$.

RESULTS

Milk Production

One day before starting the IM, milk production was not different between the groups (Table 1). Figure 1a shows the time course of daily milk yields during the 2-wk IM period. The average amount of milk (kg/d) from CON cows was greater than from TRT cows, starting from d 4 of IM (Figure 1a). While milk yield remained constant in the TRT group, that of the CON group continued to increase over the 14 d of IM, resulting in 11.1% less milk in the TRT group than in the CON group during IM. Milk yields during the entire period from IM and the subsequent time until wk 15 of lactation are shown as weekly averages in Figure 1b. When IM ceased, milk production of the TRT cows immediately reached the level of the CON cows (Figure 1, Table 1), increasing by 18.6% above the yield from the preceding last day of IM. At the second day of conventional milking, daily milk yield decreased ($P \leq 0.001$) again by 3.2 kg on average to 36.5 ± 1.12 kg in the TRT cows, but remained unchanged in the CON cows ($P = 1.000$; Figure 1a). No further group differences in milk yield were observed both on a daily and a weekly basis after the IM treatment (Table 1 and Figure 1b). From wk 4 to 15 after IM, average milk yield was 39.2 ± 0.23 kg/d. Neither time of peak nor peak yield were affected by the treatment (Table 1).

Milk Composition and Energy-Corrected Milk

Milk composition as well as fat, protein, and lactose yields are summarized in Supplemental Figure S1a–f

Table 1. Milk yields (means ± SEM) related to the automated and constant incomplete milking (IM) period in control cows (CON, n = 23) and treatment cows (TRT, n = 23)

Item	TRT[1]	SEM	CON	SEM	*P*-value
Start milk yield (kg)	32.3	0.99	32.8	1.41	0.788
Average milk yield (kg/d) in wk 1 of IM	31.8	0.91	34.4	0.98	0.052
Average milk yield (kg/d) in wk 2 of IM	32.3	0.94	37.5	1.01	≤0.001
Milk yield (kg/d) 1 d after IM	39.7	1.09	37.3	1.13	0.133
Average milk yield (kg/d) in wk 1 after IM	37.4	1.22	38.9	1.23	0.376
Average milk yield (kg/d) in wk 4 to 15 after IM	38.3	0.28	40.1	0.36	0.246
Time of peak[2] (DIM)	52.3	4.06	50.8	4.32	0.804
Peak yield[3] (kg/d)	43.9	0.80	45.8	1.29	0.209

[1]Milked incompletely twice daily from d 8 ± 1.1 until d 21 ± 1.1 of lactation; the amount of milk (kg) harvested was limited to the amount of milk collected 1 d before IM was started.

[2]Day with highest milk yield achieved within the first 100 DIM.

[3]Milk yield at the time of peak yield.

(https://doi.org/10.6084/m9.figshare.24533176.v1; Meyer et al., 2023). Milk fat concentration was not affected by IM during or after the treatment (Supplemental Figure S1a). Milk fat yield during IM tended to be 1.13-fold higher in the CON than in the TRT group ($P = 0.074$). For milk protein content, no group difference was found during the IM period (Supplemental Figure S1c). After IM, there were no differences between the groups. Protein yield differed between groups during IM with 1.15-fold more protein in the CON than in the TRT group (Supplemental Figure S1d). After the IM, no further group difference was observed. The milk lactose concentrations and yields differed between groups and there was a group × time interaction during the IM phase. The group difference in lactose concentrations was limited to the second week of IM, with 0.15 ± 0.05% higher contents in CON than in TRT (Supplemental Figure S1e). Within the groups, the concentration remained constant during IM in TRT and increased from 4.73 ± 0.04% to 4.82 ± 0.04% in CON as substantiated by the time × group interaction. After IM, the lactose concentration did not further differ between the groups except a trend for a group by time interaction ($P = 0.099$) with greater values in CON as compared with TRT in wk 4 ($P = 0.085$) and wk 13 ($P = 0.077$) of lactation (Supplemental Figure S1e). Milk lactose yields tended to be and were higher in CON than in TRT in the first and the second week of IM, respectively. After IM, no differences were noted (Supplemental Figure S1f).

Figure 2 shows the time course of ECM yields (kg/d) until wk 15 of lactation. Approximately 12% less ECM was milked from TRT than from CON cows during the 2 wk of IM (32.8 ± 0.91 kg/d vs. 37.3 ± 0.87 kg/d). Time was significant during IM only for the CON animals ($P = 0.014$) increasing their ECM yield from 36.3 ± 1.35 kg/d to 38.5 ± 1.25 kg/d. After switching back to conventional milking, ECM did not differ between groups and averaged 38.3 ± 0.20 kg/d (Figure 2).

Udder Health, Indicators of Udder Filling, and Milking Duration

Neither elevated SCC nor mastitis-relevant bacteria were detected in the foremilk quarter samples of both groups. Figure 3 shows the time course of SCC in the quarter milk samples from both groups. At any time, the values were well below the threshold for mastitis (SCC $\log_{10}$/mL <2.00) as defined by the German Veterinary Association (DVG, 2009). Cases of milk leakage occurred in both groups before and after fore-milking (Supplemental Table S2; https://doi.org/10.6084/m9.figshare.24533176.v1; Meyer et al., 2023). Before fore-milking, at the second assessment in the first week of IM, more cases of milk leakage occurred in TRT (7 cases) than in CON (1 case). After fore-milking, at both assessments in the second week with IM, also more cases of milk leakage occurred in TRT (6 and 5 cases) than in the CON cows (1 and 0 cases), respectively (Supplemental Table S2). After milking, milk leakage was not observed in either group.

Udder firmness before milking did not differ between the groups during the entire observation period (Supplemental Figure S2a; https://doi.org/10.6084/m9.figshare.24533176.v1; Meyer et al., 2023). The last milk flow in the measuring unit at the moment of triggering cluster removal was 2.87-fold higher in the TRT than in the CON cows (Figure 4a). After switching back to conventional milking, no further differences were observed (Figure 4a). Before the start of IM, milking took the same time in both groups (Figure 4b). During IM, the CON cows had 1.37-fold longer ($P = 0.001$) milking times than the TRT cows (7.53 ± 0.13 min vs. 5.49 ± 0.10 min). In the week after IM, milking time was again

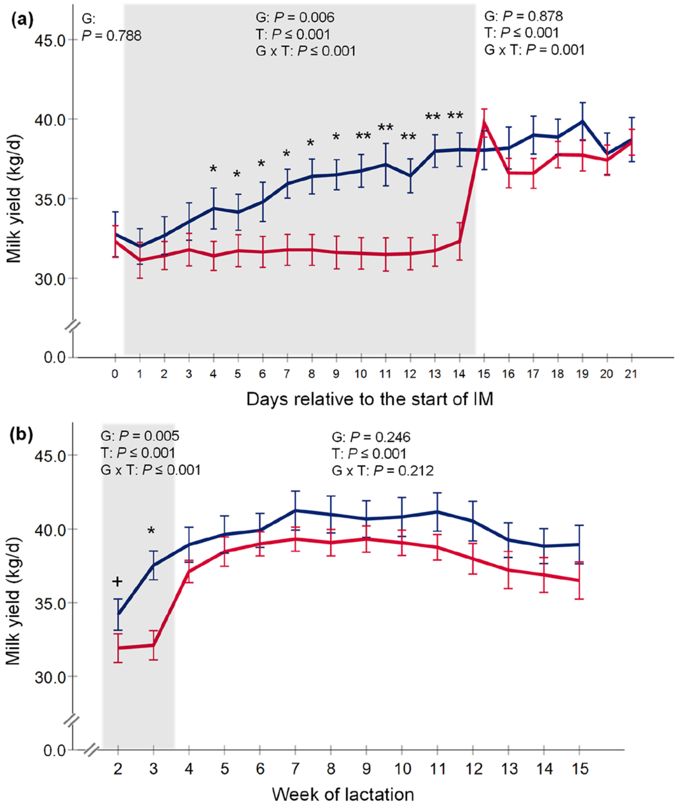

Figure 1. Effect of automated incomplete milking (IM) in wk 2 and 3 of lactation as compared with conventional milking on (a) daily milk yield over the first 21 d relative to the start of IM and (b) weekly average of milk yield over the first 15 wk of lactation. Two groups (G): blue line = control cows (CON, n = 23); red line = treatment cows (TRT, n = 23). In TRT, the amount of milk (kg) harvested was limited to the amount of milk collected 1 d before IM was started. The treatment period is highlighted with a gray background. Data are presented as means ± SEM. Differences between the groups are indicated with asterisks (*$P \leq 0.05$ or **$P < 0.001$) at a given time point (T). Trends ($P \leq 0.10$) for differences between the groups at a given time point are indicated by +. Interactions between group (G) and time point (T) are indicated with G × T.

the same in both groups (average of both groups: 7.74 ± 0.14 min; Figure 4b).

Indicators of the Energetic Status

The DMI in the CON cows tended to be higher than in the TRT group during the IM period (Figure 5a). In the first IM week, both groups had similar DMI (P = 0.286; CON 21.8 ± 0.34 kg/d; TRT 21.3 ± 0.32 kg/d). Both groups increased their DMI in the subsequent week, without further group differences and averaging 25.9 ± 0.11 kg/d (Figure 5a). For water intake, there was a group × time interaction (P = 0.006) during IM due to increased water intake by the CON cows in contrast to constant intakes in the TRT cows. Distinct group differences were observed in the second week of

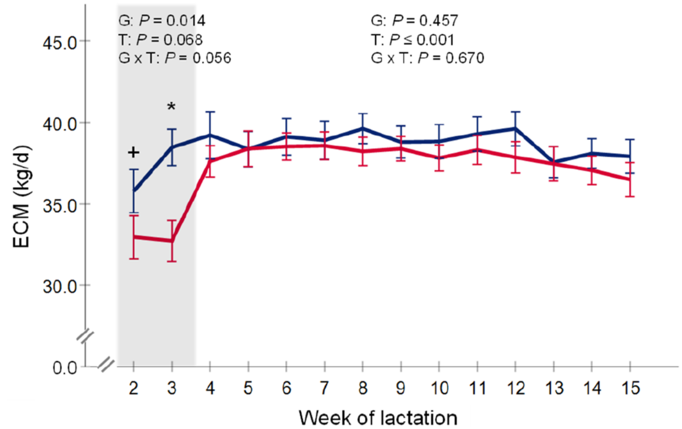

Figure 2. Effect of automated incomplete milking (IM) in wk 2 and 3 of lactation on ECM yield over the first 15 wk of lactation. Two groups (G): blue line = control cows (CON, n = 23); red line = treatment cows (TRT, n = 23). In TRT, the amount of milk (kg) harvested was limited to the amount of milk collected 1 d before IM was started. The treatment period is highlighted with a gray background. Data are presented as means ± SEM. Differences between the groups are indicated with asterisks (*$P \leq 0.05$) at a given time point (T). Trends ($P \leq 0.10$) for differences between the groups at a given time point are indicated by +. Interactions between group (G) and time point (T) are indicated with G × T.

IM: the CON cows drank more water than the TRT cows (CON 88.4 ± 1.96 L/d; TRT 79.6 ± 1.78 L/d). In the subsequent period, no further differences between groups were observed (average of both groups: 85.0 ± 0.40 L/d; Figure 5b).

Throughout each period of the experiment, BW was not different between groups (CON: 662 ± 3.9 kg/d; TRT: 660 ± 3.9 kg/d; Figure 5 c). When all time points were considered in the statistical model, time was significant for BCS, BFT, and MLD (Supplemental Figure S3a–c; https://doi.org/10.6084/m9.figshare.24533176.v1; Meyer et al., 2023), but treatment was not significant; for BFT only, a trend (P = 0.055) for greater values in CON cows was noted. When considering BFT in the period before calving, CON cows had approximately 0.32 cm thicker backfat than TRT cows (P = 0.043; 1.44 ± 0.11 cm vs. 1.12 ± 0.08 cm), whereas no difference between groups occurred in the period after calving (average of both groups: 1.10 ± 0.039 cm; Supplemental Figure S3b). The CON cows exhibited the most pronounced decrease from wk 3 before calving (1.32 ± 0.14 cm) to wk 6 after calving (1.11 ± 0.10 cm) by 0.21 cm, whereas BFT in TRT cows declined by only 0.03 cm (1.02 ± 0.10 cm to 0.99 ± 0.10 cm). The BCS declined from wk 3 before to wk 6 after calving by about 0.27 in both groups (3.38 ± 0.05 to 3.11 ± 0.05; Supplemental Figure S3a). In both groups MLD declined by about 0.51 cm from the period before to after calving (average of both groups: 5.63 ± 0.09 cm to 5.12 ± 0.08 cm; Supplemental Figure S3c).

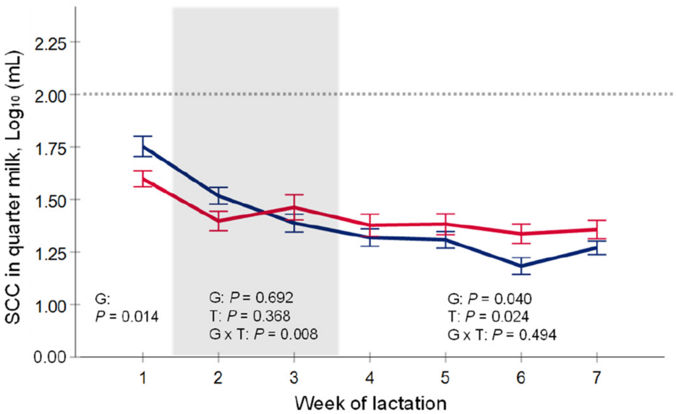

Figure 3. Effect of automated incomplete milking (IM) in wk 2 and 3 of lactation on the time course of SCC ($\log_{10}$/mL) in foremilk samples of each quarter (n = 184 samples/time point) over the first 7 wk of lactation. Two groups (G): blue line = control cows (CON, n = 23); red line = treatment cows (TRT, n = 23). In TRT, the amount of milk (kg) harvested was limited to the amount of milk collected 1 d before IM was started. The IM period is highlighted with a gray background. The horizontal dashed line designates the upper SCC threshold for healthy quarters (i.e., 100,000 cells/mL; DVG, 2009). Data are presented as means ± SEM. Interactions between group (G) and time point (T) are indicated with G × T.

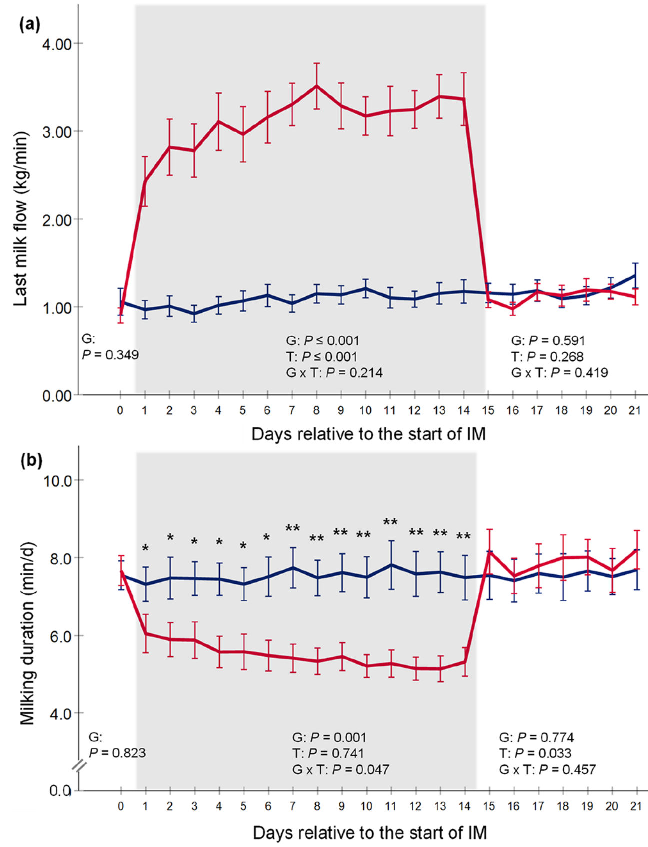

Figure 4. Effect of automated and incomplete milking (IM) in wk 2 and 3 of lactation on (a) last milk flow per day, and (b) daily milking duration over the first 21 d relative to the start of IM. Day 0 is the day before starting the treatment. Two groups (G): blue line = control cows (CON, n = 23); red line = treatment cows (TRT, n = 23). In TRT, the amount of milk (kg) harvested was limited to the amount of milk collected 1 d before IM was started. The treatment period is highlighted with a gray background. Data are presented as means ± SEM. Differences between the groups are indicated with $^*P \leq 0.05$ and $^{**}P < 0.001$ at a given time point (T). Interactions between group (G) and time point (T) are indicated with G × T.

The calculated EB during the IM period was not influenced by group but by time (Figure 5 d). Altogether, the 2 wk of constant IM led to 14.4 MJ/d less energy output with milk in TRT as compared with CON cows (120.9 MJ/d). The DMI in TRT cows tended to increase less than in the CON cows during IM, and the energy intake of TRT cows was approximately 5.4 MJ of NE_L/d below that of CON cows (150.7 MJ of NE_L/d). Considering that the NE requirement for maintenance did not differ between groups (average of both groups: 38.0 MJ/d), around 9.0 MJ of NE_L/d could be saved in TRT cows during the entire period of IM. In general, the EB during that time was hardly negative, with a nadir in the second week of CON cows that was not undercut in the entire observation period. The EB increased with time in both groups without group differences throughout the observation time ($P = 0.664$); the average EB of both groups between wk 4 and 15 of lactation was 8.75 ± 0.71 MJ of NE_L/d.

The circulating concentrations of NEFA, BHB, IGF-1, and leptin did not differ between the groups in each of the periods recorded (Figure 6a–d).

DISCUSSION

To mitigate the energy deficit in early lactation of pluriparous cows, we used the IM approach, thus modifying the energy output with milk as one of the main variables affecting EB. We selected 8 DIM for the start of IM because we aimed at integrating it into the normal milking routine when cows do not have to be milked separately anymore. According to the legislation in most countries, the mammary secretions during the colostrum phase are excluded in the definition of salable milk, for example by stressing that milk has to be "practically free from colostrum" as in the United States (Food and Drug 21 CFR § 131.110[a]) or by explicitly banning colostrum and transition milk from the first DIM from sale (e.g., 5 d in Germany; §18, [2] Milchverordnung). Moreover, depending on the length of the preceding dry period, the maximal residue concentration of antibiotics applied for dry cow therapy may still be exceeded during the first day of milking (Bachmann et al., 2018), thus requiring separation of the milk. However, the greater practicability of starting the IM only after the first week of lactation is offset by the loss of salable milk. To minimize the latter as well

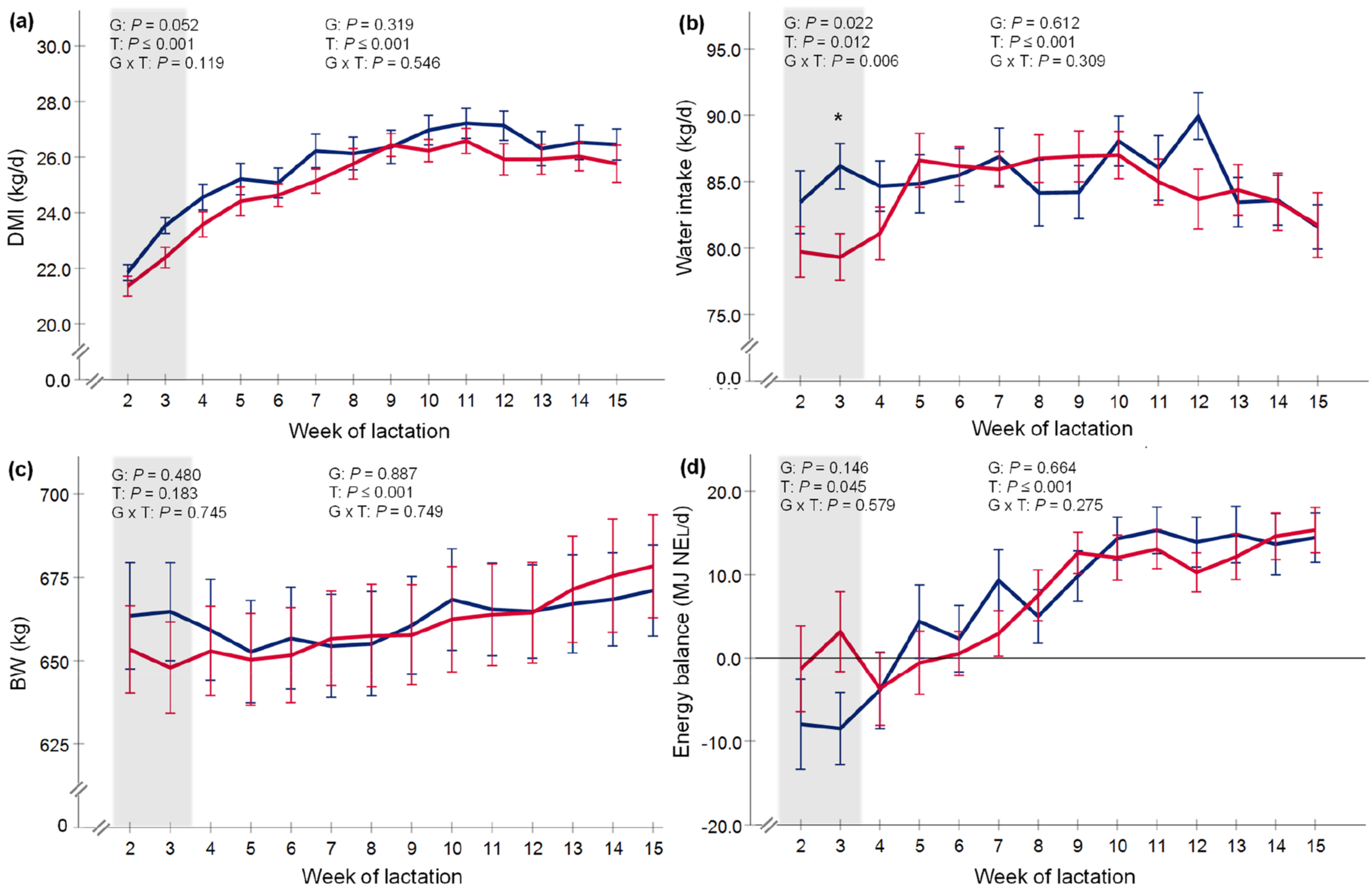

Figure 5. Effect of automated incomplete milking (IM) in wk 2 and 3 of lactation on (a) DMI, (b) water intake, (c) BW over the first 15 wk of lactation, and (d) energy balance (EB) over the first 15 wk of lactation. Two groups (G): blue line = control cows (CON, n = 23); red line = treatment cows (TRT, n = 23). In TRT, the amount of milk (kg) harvested was limited to the amount of milk collected 1 d before IM was started. The treatment period is highlighted with a gray background. Data are presented as means ± SEM. Differences between the groups are indicated with $*P \leq 0.05$ at a given time point (T). Interactions between group (G) and time point (T) are indicated with G × T.

as the risk for possible adverse effects that may result from IM, such as increased intramammary pressure (Krug et al., 2017; Blau et al., 2019), increasing SCC (Penry et al., 2017; Albaaj et al., 2018; Kuehnl et al., 2019), and irreversible decline in productivity known from once-daily milking (**ODM**; Stelwagen et al., 2013; Lacasse et al., 2018), we performed a 2-wk constant IM to achieve a smooth delay in the increase in milk yield during the first weeks of lactation. For practical realization, a software module, first developed as a tool for dry-off (Martin et al., 2020; Schmidt et al., 2020), was used that allows individual automatic cluster removal after reaching a defined amount of milk instead of a defined milk flow.

Milk Production

In the present study, in addition to daily milk yield, the last milk flow rate in the measuring unit at the moment of triggering milk yield–dependent cluster removal was recorded to reflect IM and indicate the possible subsequent adaptation of the mammary gland. During the IM period, the average last milk flow was elevated to about 3.1 kg/min in TRT cows, reflecting the remaining udder filling due to the IM and was well above common cluster removal thresholds as applied for practical or scientific purposes (Krawczel et al., 2017; Wieland et al., 2020). Given that the value of the last milk flow remained relatively constant during the period of IM in TRT cows, we assumed that milk production was largely maintained and not adapted to the lesser milk removal. So far, only 2 studies (Penry et al., 2017; Kuehnl et al., 2019) have captured the development of milk production during IM in early-lactating cows. In both studies, from DIM 5 to 47, a constant percentage of milk was removed (targeting approximately 30% of milk remaining in the gland) using a half-udder design. The milk production rate was

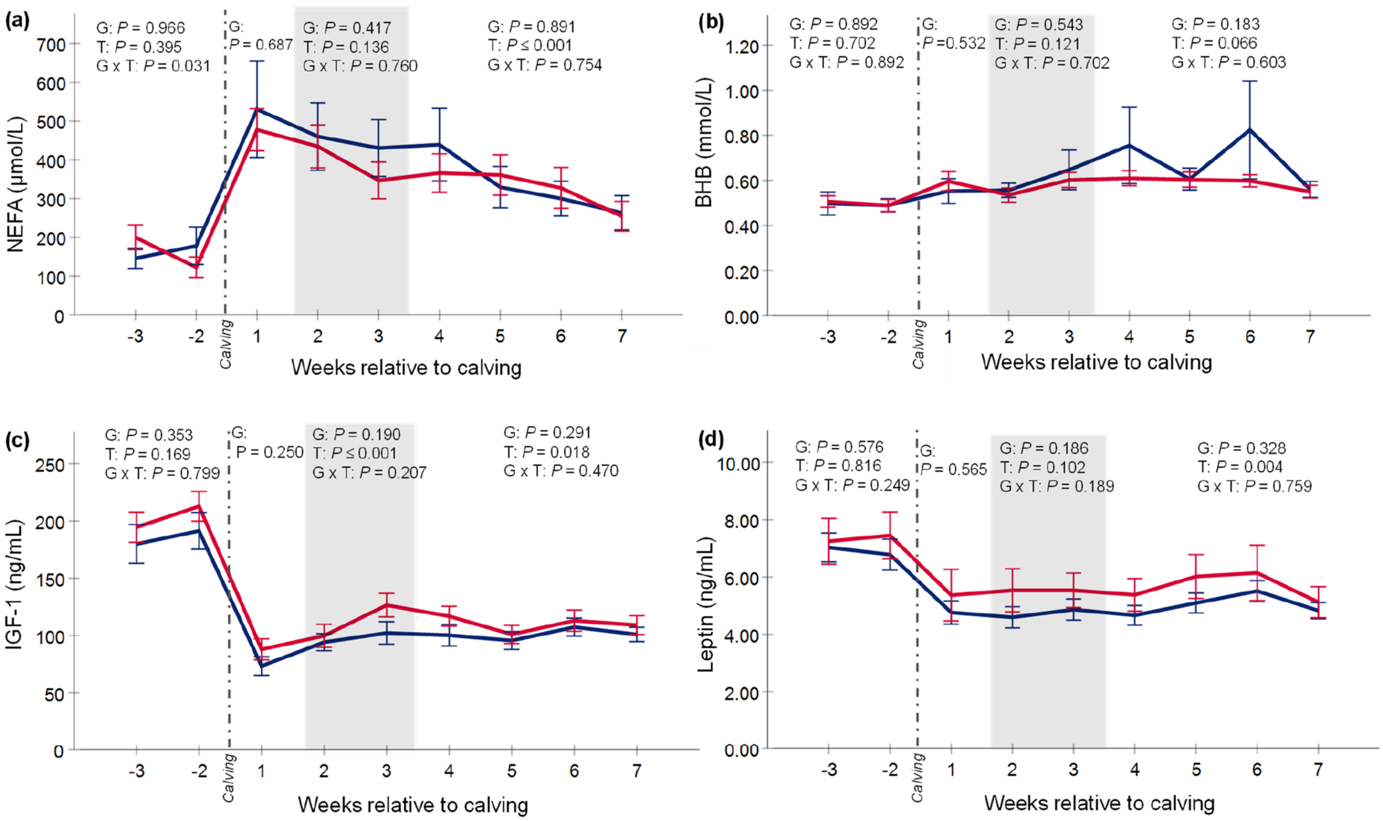

Figure 6. Effect of automated incomplete milking (IM) in wk 2 and 3 of lactation on serum concentrations of (a) nonesterified fatty acids (NEFA), (b) BHB, (c) IGF-1, and (d) leptin over the first 15 wk of lactation. Two groups (G): blue line = control cows (CON, n = 23); red line = treatment cows (TRT, n = 23). In TRT, the amount of milk (kg) harvested was limited to the amount of milk collected 1 d before IM was started. The treatment period is highlighted with a gray background. The vertical dashed line designates calving. Data are presented as means ± SEM. Interactions between group (G) and time point (T) are indicated with G × T.

calculated once per week when the TRT udder half was milked completely to recalibrate the estimate of the target milk yield. Contrary to our findings, a decrease in milk production rate in TRT udder halves of approximately 25% (Penry et al., 2017) and 27% (Kuehnl et al., 2019) was observed during the period of IM. Moreover, Deacon et al. (2023) recently applied the IM concept of Penry et al. (2017) for 4 wk in mid-lactating cows. Although they observed a decline in milk production, the mammary gland adapted quickly to IM, with milk production reaching a new level within a few days (Deacon et al., 2023).

After switching back to conventional milking with milk flow–dependent automatic cluster removal, we observed that milk production of the TRT cows reached the level of the CON cows immediately, increasing by 18.6% above the yield from the preceding last day of IM. The increase in milk yield on the first day of conventional milking could be attributed to an accumulation of residual milk that was retained in the mammary gland due to IM, an effect that has also been observed in previous studies (Albaaj et al., 2018; Martin et al., 2020). In addition to the accumulation effect, an additional feature of milk stasis caused by IM (Albaaj et al., 2018; Deacon et al., 2023) or extended milking intervals (Stelwagen et al., 2013; Lacasse et al., 2018) can be reduced productivity beyond treatment. However, we observed no further group differences in daily and weekly milk yield from the second day of conventional milking onward. Moreover, we could not find a difference in the day of reaching peak yield and peak yield between groups. Therefore, milk losses due to IM were limited solely to the treatment period, suggesting that a 2-wk constant IM while maintaining the overall milking routine may be used without compromising subsequent performance.

Investigations of potentially adverse carryover effects on performance after IM during the colostrum phase showed either no (Carbonneau et al., 2012; Valldecabres et al., 2022) or only negligible (Krug et al., 2018b) effects on subsequent milk production. In studies applying IM for several weeks in early lactation (Penry et al., 2017; Kuehnl et al., 2019), the consequences for the posttreatment milk yields were not reported. However, Deacon et al. (2023) determined the mammary functional capacity of TRT udder halves after terminating a 4-wk IM in mid-lactation cows (targeting approximately 30% of milk remaining in the gland). They observed a greater loss of mammary functional capacity in the TRT udder half, suggesting an irreversible loss of mammary tissue due to IM (Deacon et al., 2023).

The cause of the variation in findings between our and previous studies may be due to the differences in experimental design concerning the targeted amount of milk remaining in the gland, the treatment duration, and the phase of lactation in which the IM was tested. In future studies, various degrees of IM should be combined with different treatment lengths to identify the range initiating the sustained modulation of milk production. Moreover, a better understanding of the underlying adaptation processes on the functional level remains to be determined. Since a body of evidence was generated for the galactopoietic role of PRL in dairy cows (Lacasse et al., 2016), Deacon et al. (2023) were the first to determine the abundance and activation levels of proteins related to PRL signaling in mammary gland biopsies obtained in the week after completing IM. As only negligible effects of IM on PRL signaling were observed, these results constrain the assumption of a modulatory effect of IM on mammary gland responsiveness to the galactopoietic signal of PRL (Deacon et al., 2023). Aside from PRL, other autocrine-paracrine mechanisms (e.g., parathyroid hormone-related protein, transforming growth factor, IGF-1, epidermal growth factor, and serotonin) that are involved in the functional adaptation of the mammary gland to IM (Weaver and Hernandez, 2016) remain to be investigated.

Milk Composition and Energy-Corrected Milk

In general, ECM and milk yield from the TRT cows differed by a similar extent (by 12 and 11%, respectively) from the CON cows during the IM period. After switching back to conventional milking, group differences in ECM were leveling off, thus supporting that milk production including composition was not decreased beyond the treatment period. For lactose, a largely consistent decrease in the concentration was reported during IM in our and in previous studies (Penry et al., 2017; Kuehnl et al., 2019; Deacon et al., 2023). The milk lactose content after IM has only been reported in the study by Carbonneau et al. (2012) and was not different between the groups from wk 2 to 9 of lactation. We also observed no group differences in lactose content after IM. In most studies (Penry et al., 2017; Kuehnl et al., 2019; Deacon et al., 2023), the decrease in milk lactose content was mainly related to the loss of tight junction integrity of the mammary gland and the related efflux of lactose from milk into blood, which may be caused by increasing intramammary pressure as a consequence of IM (Stelwagen and Singh, 2014). The decreased milk lactose content during the period of IM observed in our study may also be explained by reduced lactose synthesis (Grala et al., 2011) and lower mammary uptake of glucose (Guinard-Flament et al., 2007) as already described in the context of extended milking intervals (Stelwagen et al., 2013), though we did not assess blood lactose concentrations.

The causes for differences in milk composition between studies could be, in addition to differences in the degree and duration of IM, a varying sampling procedure. Kuehnl et al. (2019) pointed to the importance of milking fraction to explain the differences between their and another study using the same experimental design (Penry et al., 2017). Likewise, the varying milk sampling frequency between studies ranged from once (Carbonneau et al., 2012; Deacon et al., 2023) or twice per week (Penry et al., 2017; Kuehnl et al., 2019) to once per month (Krug et al., 2018b), which could account for the different outcomes.

Udder Health, Indicators of Udder Filling, and Milking Duration

During the period of IM, we found neither signs of impaired udder health nor increased SCC in TRT cows. After IM, the SCC in TRT cows increased but did not exceed the threshold of 100,000 cells/mL milk per quarter as an indicator of inflammation (DVG, 2009). Furthermore, no positive bacteriological findings were detected in any of the cows during the first 7 wk of lactation, thus supporting that a 2-wk constant milk withdrawal does not impede udder health. Similar results were obtained in studies in which IM was applied during the first days of lactation (Carbonneau et al., 2012; Krug et al., 2018a; Valldecabres et al., 2022). All these studies consistently reported no increase of the incidence of clinical mastitis due to IM, but the impact on SCC differed between studies. Conducting IM for a single milking interval (Albaaj et al., 2018) or several weeks (Penry et al., 2017; Kuehnl et al., 2019) led to a slight increase in SCC during IM. Penry et al. (2017) assumed that the differences in SCC between groups could be due to the increased dilution of SCC in the completely milked udder half. In contrast, Albaaj et al. (2018) presumed an inflammatory reaction in cows as a response to increased milk accumulation. However, the differences could also be due to the aforementioned variation in IM settings across studies.

Milk leakage is a symptom of compromised teat sphincter function that may occur during all stages of lactation and is associated with an increased risk of mastitis (Rovai et al., 2007; Gott et al., 2016). The occurrence of milk leakage is farm specific and ranged between 0% and 36% of cows within a farm (Schukken et al., 1990). We decided to control milk leakage before and after fore-milking when the amount of milk stored in the udder due to IM and the resulting increased pressure on the teat sphincter was assumed to be greatest, and after milking to indicate whether rising milk accumulation could reinforce milk leakage even though the milking process has already been finished. The greater portion of cows with milk leakage in the TRT group, both before (30%) and after fore-milking (24%), is in line with Gleeson et al. (2007) who reported that 30% of ODM cows showed milk leakage in early lactation. Milk leakage can be caused by multiple factors, such as stage of lactation (Bruckmaier and Hilger, 2001; Klaas et al., 2005), rising internal milk pressure (Persson Waller et al., 2003; Bruckmaier and Wellnitz, 2008), anatomical characteristics (e.g., udder storage capacity; Bruckmaier and Hilger, 2001), and functional traits (e.g., milk flow profiles; Klaas et al., 2005; Rovai et al., 2007). However, no cases of milk leakage were observed in either group after completing milking, indicating that the milk left in the mammary gland immediately after milking due to constant milk withdrawal did not exceed the closing forces of the teat sphincter of cows.

For addressing intramammary pressure that is associated with discomfort and pain for the cow (Tucker et al., 2007), we also assessed udder firmness as a proxy for udder filling and intramammary pressure. We observed no group differences in udder firmness before milking, suggesting that a 2-wk IM does not cause discomfort for the cows. Krug et al. (2017) investigated the impact of IM on lying behavior as an indicator of discomfort due to increased intramammary pressure. During the IM period, they collected a maximum of 10 to 14 L of milk in the first 5 DIM, and lying behavior was recorded via acceleration data loggers attached to the hind leg of cows. Neither lying time was shortened nor were adverse effects observed on lying bouts or lying bout duration during the IM period (Krug et al., 2017).

Labor costs are a key factor in the decision-making process on commercial farms concerning changes in the milking strategy (Stockdale, 2006). Thus, reducing the duration of milking can improve labor efficiency and also reduces the mechanical load of the teat tissue due to shortened cluster attachment time (Martin et al., 2020; Wieland et al., 2020). During IM, milking time was about 2 min shorter for TRT than for CON cows, and increased to the same level as in CON cows after IM in our study. Edwards et al. (2013) observed that approximately 1.33 min per cow could be saved when changing the milk flow rate thresholds from 0.2 to 0.8 kg/min. Even though reducing the milking frequency to ODM halves the required milking time, it is accompanied by an irreversible loss of productivity thereafter (Stelwagen et al., 2013). In addition to the time savings for the milking process, we could diminish extra effort outside of the regular milking routine through the software-induced automation of cluster removal after obtaining a defined amount of milk. In contrast, milk-flow–dependent cluster removal requires manual intervention by the producer and enhances the potential for errors in accurate cluster removal after reaching the target milk yield (Morin et al., 2018). Accordingly, our findings confirm the applicability of the automated cluster removal that is further associated with less milking time needed during IM.

Indicators of the Energetic Status

In the present study, DMI was hardly compromised, with approximately 21.8 kg of DM/d already in the second lactation week of CON cows, thus exceeding the values typical for this period (Ingvartsen and Andersen, 2000; Grummer et al., 2004). However, DMI in TRT cows did not increase as rapidly as in CON cows; similarly, water intake was less in the TRT cows, thus confirming the association between DMI and water intake (Kramer et al., 2009; Kume et al., 2010). After IM all group differences were diminished. In contrast, ODM studies with accurate DMI recordings have reported both reduced DMI in ODM cows or no differences compared with twice-daily milked cows (Stelwagen et al., 2013); for example, McNamara et al. (2008) suggested that the reduced milk withdrawal during IM periods induces an adaptation of feed intake. In future studies, DMI should be recorded during the IM phase to determine whether this phenomenon of decreased feed intake recurred. Moreover, the underlying mechanisms involved in the DMI regulation of IM cows remain to be investigated.

To maintain milk synthesis even during a NEB, body fat and protein reserves are mobilized (Kuhla et al., 2011) what in turn leads to losses of BW and body condition (Pires et al., 2013). When reducing the milking frequency to once daily, an improvement of the energetic status was reflected in an attenuated loss of BW and BCS in ODM when compared with twice-daily milked cows, as summarized by Stelwagen et al. (2013). Similar to Carbonneau et al. (2012), who harvested 6 to 14 L of milk/d in the first 5 DIM, we observed in our study no group differences in BW. Moreover, the IM treatment did not affect BCS, BFT, and MLD. Likewise, Morin et al. (2018) observed no differences in

BCS between the groups after removing only a maximum of 10 to 14 L of milk in the first 5 DIM of cows. It is known that fatter cows are mobilizing more body resevers when entering lactation (Pires et al., 2013); however, the trend for greater BFT in the CON cows ante partum was not related to greater BFT losses after calving in the present study.

In a review, Lacasse et al. (2018) summarized the efficacy of milking management strategies (e.g., the use of dopamine agonists to inhibit PRL release, prepartum milking, ODM, or IM) for reducing the susceptibility to metabolic and infectious diseases. The strategy of IM turned out as the most promising approach for reducing metabolic stress without compromising the productivity of high-yielding dairy cows (Lacasse et al., 2018). So far, studies aiming at improving the metabolic situation in early lactation by IM were largely limited to the colostrum phase (Carbonneau et al., 2012; Morin et al., 2018; Valldecabres et al., 2022). In contrast, we decided to start the 2-wk IM in the second week after parturition when the milk is no longer separated and limited the energy output with milk by clamping the amount of milk withdrawn to a constant level. In general, we observed that the EB during that time was hardly negative, with a nadir of only −8.5 MJ of NE_L/d in the second lactation week of CON cows and thus was above the values that are typical for early-lactating cows (e.g., de Vries and Veerkamp, 2000; Weber et al., 2013). However, we are the first providing data about the calculated EB in the context of IM treatment, whereas previous IM studies have focused solely on the analysis of blood metabolites to reflect the energy status of cows due to IM (Carbonneau et al., 2012; Morin et al., 2018; Valldecabres et al., 2022).

In general, the metabolic condition of both groups from our study hardly changed during the observation period. During the first 7 wk of lactation, the average blood concentrations of NEFA and BHB remained within the range considered as healthy. Only 3 cases of hyperketonemia (blood BHB >1.2 mmol/L) in CON cows and 0 cases in TRT cows were observed in the period after calving. We also observed no group differences in the circulating concentrations of NEFA, BHB, IGF-1, and leptin in each of the periods recorded. The energy status of both groups seemed hardly challenged by the onset of lactation and thus the effects of IM might have been more pronounced in a situation of a more NEB. Likewise, Valldecabres et al. (2022) found no group difference in blood concentration of BHB when removing only 3 L of milk/milking on the first 2 DIM compared with twice-daily milked cows. However, a small numerical difference in the proportion of cows with subclinical ketosis (blood BHB >1.2 mmol/L) was observed between CON (42%) and TRT (18%) cows at 3 and 11 DIM (Valldecabres et al., 2022). They suggested that the findings further elucidate the relevance of the degree and duration of IM (Valldecabres et al., 2022). In contrast, the preceding study of Carbonneau et al. (2012), who harvested a maximum of 6 to 14 L of milk in the first 5 DIM of cows (n = 47), observed that within the treatment period and lasting until 21 DIM, the glucose concentration increased while the BHB concentration decreased in the TRT compared with the CON group. The NEFA concentration was also lower in the TRT compared with the CON group but showed no carryover effect beyond the IM period. Furthermore, both Carbonneau et al. (2012) and Morin et al. (2018), reported the number of cows with subclinical and clinical ketosis was smaller in cows that underwent IM. Morin et al. (2018) suggested that the treatment effect would be greater in herds with more NEB and interpreted the low prevalence of subclinical ketosis was due to the well-functioning transition management of the enrolled commercial farms (Morin et al., 2018). Nevertheless, they and also Carbonneau et al. (2012) concluded that IM in the colostrum period is an effective strategy to reduce the prevalence of hyperketonemia in the early postpartum period. Altogether, the degree of milk accumulation and the duration of milk stasis seem to influence the findings and stronger effects can be expected in herds with a more extended NEB.

CONCLUSIONS

Our findings confirm the applicability of the milking software module used herein for delaying the increase in milk yield during the first weeks of lactation through a 2-wk automated, constant milk withdrawal. When IM ceased, milk production of the TRT cows immediately reached the level of the CON cows, and udder health was not compromised over the entire observation period in all cows. The EB, as well as body condition and several blood metabolites, remained largely unaffected, as the TRT cows tended to eat less than the CON cows during the period of IM. It remains to be determined whether the absent effect on energy metabolism was due to the relatively stable energy status of the cows or to the relatively mild IM setting used here. Further research is needed to understand the link between the degree and duration of IM and the adaptive mechanism of the organism in terms of milk synthesis, feed intake, and energy metabolism.

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