Journal of Dairy Research

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Research Article

Cite this article: Müller U, Hefter LM, Wedeking SD, Büscher W and Barth K (2023). Incomplete milking before drying off does not impair the udder health of cows infected with minor pathogens. *Journal of Dairy Research* **90**, 393–397. https://doi.org/10.1017/S0022029923000699

Received: 30 March 2023
Revised: 7 October 2023
Accepted: 16 October 2023
First published online: 15 January 2024

Keywords:
Gradual dry-off; residual milk; somatic cell count

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Incomplete milking before drying off does not impair the udder health of cows infected with minor pathogens

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Abstract

When implementing the transition from regular milk production to the dry period, drying off is mostly conducted simply by abrupt cessation of milking. Efforts to reduce milk synthesis before cessation of milking aim to reduce stress in cows as well as to lower the risk of mastitis. A previous study demonstrated that incomplete milking during the last ten days of lactation gradually reduced the milk yield of healthy, high-yielding cows. However, a reduction period of ten days might be too long for cows with lower yields. Therefore, a follow-up study was conducted on an organic dairy research farm with a lower average milk yield. We investigated whether automated incomplete milking can reduce milk synthesis within one week without impairing the health status of udder quarters infected with minor pathogens. Before drying off, 15 German-Holstein cows with 58 lactating quarters, 21 of which were infected with minor pathogens (coagulase-negative *Staphylococci* (CNS) and *Corynebacterium bovis*), were milked twice daily using the software module AutoDry (GEA Farm Technologies) with the 5%-step-down-per-day-program. The level of udder emptying was gradually reduced over 8–10 milkings beginning at a mean milk yield of $17.2 \pm 4.4\ \text{kg d}^{-1}$. During the last three milkings before drying off, milking clusters were automatically removed when a milk flow rate of $0.3\ \text{kg min}^{-1}$ was reached. Quarter fore-milk samples were collected at two time points (14 d before and at the date of the last milking, ie drying off) and were analysed for mastitis pathogens and somatic cell count. The gradual reduced emptying of the udder induced a clear decline in milk yield by $0.8 \pm 0.3\ \text{kg d}^{-1}$. Within the reduction period, the somatic cell count of quarter foremilk did not change regardless of the infection status. Furthermore, no cow suffered from clinical mastitis. Thus, a reduction in milk synthesis could be achieved within less than one week before drying off without any impairment in udder health of cows, even when infected with minor mastitis pathogens.

Abrupt cessation of milking at the end of the lactation causes an increase in udder pressure (Bertulat *et al*., 2013) and, partly as a result, a shift in the mammary gland from milk synthesis to involution. Especially high yielding cows suffer from the strain that is caused by large amounts of milk left in the udder when dried off abruptly. Therefore, various approaches aim to lower the milk synthesis prior to drying off (eg Vilar and Rajala-Schultz, 2020, Cattaneo *et al*., 2024). Most of these studies have focused on the effects of changes in milking frequency and feed composition (e.g. Bushe and Oliver, 1987; Gott *et al*., 2016). Incomplete milking also efficiently reduces milk yield (Penry *et al*., 2017; Albaaj *et al*., 2018). When milking frequency was reduced from twice to once per day, an average milk yield reduction of approximately 22% was achieved in the study by Stelwagen *et al.* (2013). Martin *et al.* (2020) successfully tested a procedure of automatic milk reduction before drying off in cows free of udder infections. Over a period of 10 d the milk production of 29 cows was lowered by 33% relative to daily milk yield before treatment. However, from an economic and management point of view, it might be more reasonable to achieve a lower yield within a shorter time period.

With regard to udder health, some studies have observed slightly increased somatic cell counts (SCC) in milk from quarters that were emptied to different degrees. In the half-udder study done by Penry *et al.* (2017) approximately 30% of the milk was left behind in one udder half of 12 cows due to incomplete milking over 6 weeks starting five days after calving. The authors observed a significant effect of this treatment on SCC. However, the average SCC of the incomplete milked halves was still lower than 50.000 cells/ml. Albaaj *et al.* (2018) reported the effects that different levels of udder emptying (100, 70, 40, and 0%) in one milking can have on subsequent milkings of cows in their second month of lactation. If the udders were not milked at all or only 40% emptied, the somatic cell score remained elevated in the following milkings. Consequently, incomplete milking can be expected to compromise udder health. In contrast, Martin *et al.* (2020) demonstrated that a reduction in milk yield due to incomplete milking at the end of the lactation did not negatively affect the SCC of healthy cows that were free of infection with mastitis pathogens.

In infected udder quarters, residual milk is considered a suitable substrate for multiplication of microorganisms in the mammary gland (Bruckmaier and Wellnitz, 2008). However, Clarke *et al.* (2008) showed that incomplete milking due to different settings of the automatic cluster removal did not lead to a significant increase of SCC in udder quarters either infected by major (*Streptococcus uberis*, *Staphylococcus aureus*, *Streptococcus dysgalactiae*) or minor mastitis pathogens, such as *Corynebacterium bovis* (*C. bovis*). Therefore, we aimed to test (a) whether milk yield reduction can be achieved by automated incomplete milking within one week and (b) whether incomplete milking impairs the status of already infected udder quarters.

Material and methods

The experimental procedures were approved by the relevant authority (Ministry of Energy, Agriculture, the Environment, Nature and Digitalization; No V244-28210/2019) and were in strict accordance with the German Animal Protection Law.

Animals

The study was conducted in June/July 2019 at the research farm of the Thünen-Institute of Organic Farming in North Germany. The dairy herd consisted of 90 German Holstein cows and was managed according to the EU Regulation, 2018/848 on organic production. In 2019, the average annual milk yield of the herd given by the milk recording organization was of 6,491 kg/ cow. However, it must be considered that calf rearing was practiced as a whole-day dam–calf contact (see definitions by Sirovnik *et al.*, 2020) over the first three months of lactation causing an underestimation of the cows' performance (Barth, 2020). All cows were kept on pasture between milking times, and a total mixed ration was provided after milking.

Calving took place almost evenly throughout the year, so animals were also regularly dried off. Fifteen cows were included in the study. Two of them had only three lactating teats, therefore only 58 quarters could be sampled. Parity of the experimental cows varied from one to six (mean ± standard deviation (SD): 3.0 ± 1.6) and cows were dried off when 336 ± 43 DIM. The planned dry period length was 60 d.

Cows were milked twice daily at 5.00 a.m. and 3.30 p.m. in an auto tandem milking parlour (GEA Farm Technologies GmbH, Bönen, Germany) with a system vacuum level set at 38 kPa, pulsation rate of 60 min^{-1} and a pulsation ratio of 60:40. Forestripping and udder cleaning was followed by mechanical prestimulation (300 pulses min^{-1} for the first 40 s). Milk yield (kg) and milk flow rate (g min^{-1}) were recorded during each milking using the milkmeter Metatron C21 (GEA Farm Technologies GmbH). The automated reduction period excluded, the cluster take-off level was set to a milk flow rate of 0.3 kg min^{-1} until drying off. Before and during the reduction phase, of each milk flow curve the last milk flow value before cluster removal was recorded.

Experimental treatment and sampling

Starting 4–5 d before drying off, the experimental cows were milked using the software module AutoDry (Schmidt *et al.*, 2017; German patent DE 10 2017 120 656; International patent WO 2019/048,521 A1) that gradually reduced the level of udder emptying. Due to technical reasons the number of incomplete milkings varied between 8 and 10 (mean ± SD: 9.4 ± 1.0 milkings). As the day of drying off was set to a fixed day of the week, it was not possible to correct this by simply increasing the number of milkings from 8 to 10 for the cows concerned. Following the procedure applied by Martin *et al.* (2020), the cow's milk yield at the beginning was calculated as the average of the last seven days before the reduction started. The individual pre-trial yield was reduced by 5% each day to achieve a gradual reduction in the individual's milk yield. The milk yield on the last day of this period was defined as the target final milk yield (kg/day). This procedure was selected based on the average reduction that was achieved by Martin *et al.* (2020), in which milk yield decreased from 20.6 to 10.4 kg d^{-1} within 10.4 d, corresponding to a daily reduction of 5% . For the last three milkings before drying off, the automatic cluster take-off level was reset on a milk flow rate of 0.3 kg min^{-1}, and cows were milked completely to remove the milk accumulated in the udder during the reduction period as well as to estimate the adaptation of milk synthesis to the short time incomplete milking (the 'achieved milk yield' after reduction).

On the experimental farm, quarter fore-milk samples were routinely collected aseptically fourteen days (day −14) before and on the day of the last milking (ie dry-off: day 0) to assess udder health. Bacteriological analyses were conducted at the laboratory of the Max-Rubner-Institute (Federal Research Institute for Nutrition and Food, Department of Safety and Quality of Milk and Fish Products, Kiel, Germany) according to the guidelines of the German Veterinary Society about isolation and identification of mastitis pathogens (DVG, 2009). All cows were examined for clinical symptoms at quarter level at each milking.

Statistical analyses

All statistical analyses were done with the SPSS Statistics program (Version 27.0, SPSS Inc., Chicago, IL) using linear mixed models. SCC were $\log_{10}$-transformed to obtain a normal distribution. The $\log_{10}$SCC model included infection status of the quarter (bacteriological negative, infected by coagulase-negative *Staphylococci* (CNS) or *C. bovis*, time point before and after automated reduction of udder emptying (day −14 and day 0 relative to the drying off date), and their interaction as fixed effects. Parity (1–6) and quarter nested in cow were included as random effects. In the milk yield model, only time point included as fixed affect and cow as well as parity were included as random effects. The best model was selected according to the lowest Akaike's information criterion and the normal distribution of the residuals. Finally, the $\log_{10}$SCC at day −14 and day 0 per bacterial group were compared using paired t-tests. Results were considered as significant at $P < 0.05$. Values are given as means ± SD.

Results

None of the 15 cows had to be excluded from the study. All of them were dried off after the experiment according to the research

Table 1. Average milk yield at the beginning (mean of the last 7 d), and at the last day of milk reduction as well as the reduction achieved by incomplete milking over 8–10 milkings ($n = 15$ cows)

		Mean	SD	Min	Max
Duration of incomplete milking	Milkings	9.4	1.0	8	10
Milk yield					
At beginning of reduction	kg d^{-1}	17.2	4.4	6.9	24.4
Targeted	kg d^{-1}	12.9	3.7	4.6	19.2
Achieved[a]	kg d^{-1}	13.4	4.6	2.8	22.6
Reduction in milk yield					
Targeted	kg	4.3	1.0	2.3	5.8
Achieved	kg	3.7	1.6	1.8	8.0
Reduction per day[b]	kg d^{-1}	0.8	0.3	0.4	1.6

[a]Physiological milk production after period of milk yield reduction, calculated on last milkings before drying off following conventional cluster take-off level (0.3 kg min^{-1})
[b]Total achieved milk yield reduction (kg) per duration of milk yield reduction (d)

station's routine of quarter selective dry cow therapy based on the outcome of the bacteriological examination of the fore-milk samples that were collected two weeks before.

Milk yield and milk flow

Earlier cluster removal over 8–10 milkings (9.4 ± 1.0 milkings) effectively reduced the total amount of milk obtained by machine milking at the end of the treatment phase by 3.7 ± 1.6 kg (22% of the milk yield at beginning: Table 1). Thus, the average reduction was 0.8 ± 0.3 kg per day. The average milk flow at the time of the earlier cluster removal was 2.9 ± 1.0 kg min^{-1} (ranging from 0.4 to 5.8 kg min^{-1}).

Udder health

During the whole experiment no case of clinical mastitis occurred. Before the start of the reduction period (d −14), 21 of the 58 udder quarters were infected with minor mastitis pathogens (CNS, $n = 18$ or *C. bovis*, $n = 3$). Only one quarter was infected with a major pathogen (*Streptococcus uberis*) before and after the reduction period and could not be included in the statistical analyses since it was a single case. The 36 remaining quarters were not infected. However, one of them had a positive test result (CNS) on day 0, whereas one of the 17 quarters infected with CNS, and two of the three quarters infected with *C. bovis* at day −14 were bacteriologically negative after the reduction period. The estimated $\log_{10}$SCC did not differ significantly between the timepoints before and after the reduction phase (Fig. 1). Also, the pairwise comparison of $\log_{10}$SCC values within each bacterial group revealed no significant differences (paired *t*-test, negative: $t = -1.74$, df = 35, $P = 0.09$; CNS: $t = -1.16$, df = 17, $P = 0.26$; *C. bovis*: $t = 0.88$, df = 2, $P = 0.47$).

Discussion

The outcomes of our study can be directly compared with those of Martin *et al.* (2020) as the present experimental design was based on their study. However, with a mean milk yield per cow of 17.2 + 4.4 kg d^{-1}, the initial yield was lower than in the study done by Martin *et al.* (2020), where it was 20.6 + 3.8 kg d^{-1}. Nevertheless, the milk yield achieved after the reduction period was nearly the same (current 13.4 ± 4.6 vs. published 13.8 ± 4.5 kg d^{-1}). Since the mean effective reduction in daily milk yield was also comparable (current 0.8 ± 0.3 vs. published 0.7 ± 0.2 kg d^{-1}). Only the duration of application determines the achievable milk yield before drying off (current 9.4 ± 1.0 vs. published 20.8 ± 3.6 milkings). Consequently, the algorithm used to determine the timing of automatic cluster removal at

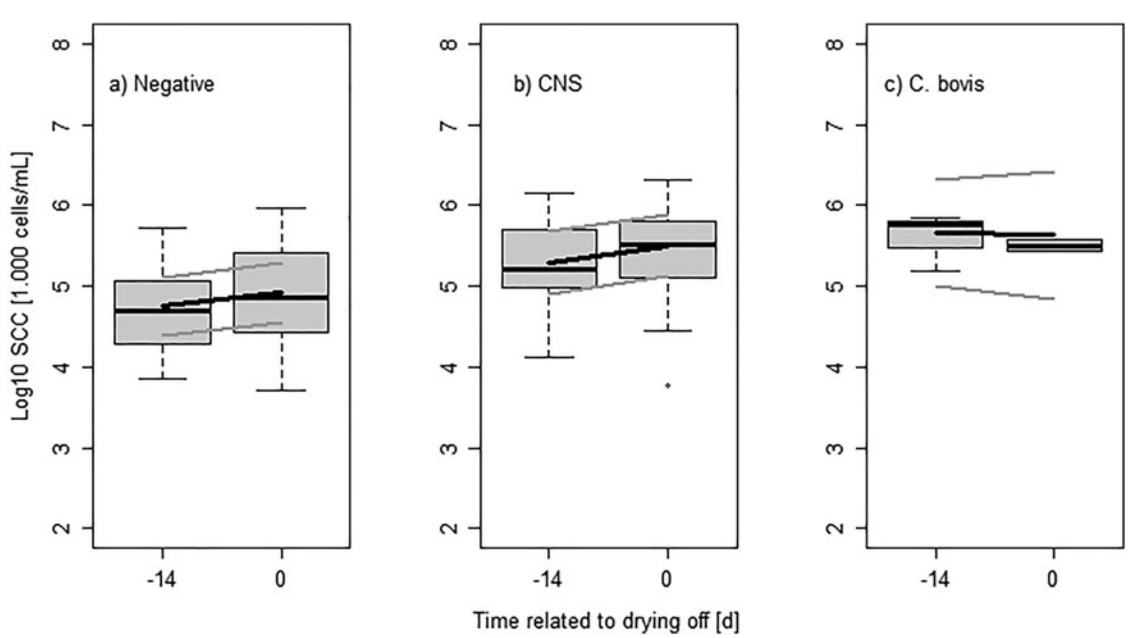

Figure 1. Boxplots of $\log_{10}$SCC in foremilk quarter samples depending of infection status ((a) bacteriological negative ($n = 36$); (b) coagulase-negative Staphylococci (CNS) positive ($n = 18$); (c) *Corynebacterium bovis* positive ($n = 3$)) before (d −14 before drying off) and after the reduction period (d 0 of drying off)). The black line represents the slope of the estimated mean values with the 95% confidence interval (grey lines). No significant differences ($P > 0.05$) between the timepoints.

https://doi.org/10.1017/S0022029923000699 Published online by Cambridge University Press

each milking is independent of the initial milk quantity and thus equally applicable in herds with different milk production levels.

The reduction of milk synthesis of 22% in our study was within the range of 13–23% given by Phyn *et al.* (2010), who compared once-daily milking to twice-daily milking in mid-lactation in short-term studies. Vilar and Rajala-Schultz (2020) recommended a target value of 15 kg d^{-1} of milk or less at drying off and suggested a 5–7 d intervention during which cows should be milked only once per day. In our study, even less time was necessary, and the target value was reached without the cows having to adapt their common daily routines. As changes in daily routines can cause stress in cows (Munksgaard and Simonsen, 1996; Zobel *et al.*, 2013), it can be beneficial to curb milk secretion through earlier cluster removal while maintaining regular milking times.

Martin *et al.* (2020) used the last milk flow recording at the time of cluster removal as an indirect indicator of the degree of udder filling. In the present study, the mean last milk flow before cluster removal were approximately 1 kg min^{-1} lower than in the study by Martin *et al.* (2020). This difference (current 2.9 ± 1.0 vs. published 3.7 ± 1.2 kg min^{-1}) can be explained mathematically by the fact that the initial levels of milk yield varied and the decrease in the amount of milk obtained was not linear but degressive (5% per day) and lasted for different periods of time. However, both values are well above 0.8 kg min^{-1} which is commonly used as cluster take-off levels for practical or scientific purposes (Edwards *et al.*, 2013; Ferneborg *et al.*, 2016; Krawczel *et al.*, 2017).

In the past, residual milk has been considered a substrate for the multiplication of pathogens in udder tissue (Bruckmaier and Wellnitz, 2008). For this reason, Martin *et al.* (2020) emphasised the application of the software module for incomplete milking only in cows with healthy udders. By following these recommendations, even cows infected with minor mastitis pathogens and showing no signs of clinical mastitis could not benefit from milk yield reduction before drying off. This is despite the fact that a lower intramammary pressure reduces the risk of milk leakage after cessation of milking, and is also connected to the defense mechanisms of the teat canal against the invasion of potential pathogens (Rovai *et al.*, 2007). Contrary to the concerns expressed, the results of our study indicated that the SCC of the infected udder quarters did not change by reason of increased residual milk remaining in the udder. This supports the conclusions of Clarke *et al.* (2008) that incomplete milking due to different settings for automatic cluster removal (0.3 kg min^{-1}, 0.7 kg min^{-1} and 0.8 kg min^{-1}) does not lead to a significant increase of SCC in uninfected udder quarters, but also not in quarters infected with minor mastitis pathogens.

In conclusion, reducing the amount of milk obtained by machine milking by 5% per day relative to the previous day for 8–10 milkings effectively reduced milk yield in cows before drying off without affecting udder health, even when udder quarters were infected by minor mastitis pathogens. Consequently, with the presented method, udders can be prepared for the dry period in an animal friendly manner without increasing the risk of existing infections with minor pathogens developing into clinical mastitis.

Acknowledgements. The authors gratefully acknowledge the assistance of the staff of the research station of the Thünen-Institute of Organic Farming. We also thank the members of the Center of Integrated Dairy Research (CIDRe, University of Bonn). Special thanks go to GEA Farm Technologies GmbH (Bönen, Germany) for the support of the developed software module AutoDry.

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