The effects of incomplete milking and increased milking frequency on milk production rate and milk composition¹

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ABSTRACT: Increased milking frequency and incomplete milking have differential effects on milk yield and mammary gland physiology that are important for optimization of milking practices in dairy herds. The objectives of this experiment were to determine the effects of increased milking frequency and incomplete milking on milk production rate (MPR) and milk composition and to determine if milking 3 times daily (3×) could rescue the negative production effects of incomplete milking. Twenty-two multiparous cows were enrolled onto this experiment beginning at 5 days in milk (DIM) and continuing through 47 DIM. A split-plot design was used to randomize the 2 treatments, which were milking frequency and incomplete milking. Eleven cows were randomly assigned to be milked 2 times (2×) daily and 11 cows were randomly assigned to be milked 3×. Within each cow, a contralateral half-udder was randomly assigned to be incompletely milked (30% milk remaining in the gland; IM), and the other half-udder was randomly assigned to be milked completely (CM). Quarter-level milk yields were recorded at each milking session. Milk samples from all quarters were collected twice weekly at the beginning of the morning milking for analysis. Cows milked 2× tended to have reduced MPR compared with

3× milked cows (1.81 ± 0.06 vs. 1.97 ± 0.06 kg milk/h; $P = 0.06$). Half-udders that were CM and IM produced 1.09 ± 0.03 and 0.80 ± 0.03 kg milk/h, respectively. There was an interaction between incomplete milking treatment and week of lactation ($P = 0.04$). No interaction was detected between milking frequency and incomplete milking for MPR or milk components. Cows milked 3× had increased milk fat percent ($1.93 \pm 0.09\%$ vs. $1.65 \pm 0.09\%$, $P = 0.047$), decreased milk lactose percent ($4.80 \pm 0.04\%$ vs. $4.93 \pm 0.04\%$, $P = 0.04$), and exhibited no differences in milk protein percent or milk somatic cell count (SCC) compared with cows milked 2×. Half-udders that were IM had increased milk fat percent ($2.15 \pm 0.07\%$ vs. $1.43 \pm 0.07\%$, $P < 0.0001$), decreased lactose percent ($4.75 \pm 0.03\%$ vs. $4.99 \pm 0.03\%$, $P < 0.0001$), increased milk $\log_{10}$ SCC (4.22 ± 0.05 vs. 4.41 ± 0.05 , $P = 0.0004$), and no differences in milk protein percent compared with CM half-udders. These results indicate that a 3× milking frequency in IM half-udders was not able to improve milk production compared with IM half-udders milked 2×. Our results indicate that 30% milk remaining in the gland had an irreversible impact on milk yield as increased milking frequency was not able to reverse the milk yield lost.

Key words: incomplete milking, increased milking frequency, milk composition, milk production rate

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INTRODUCTION

In the past, cows have traditionally been considered sufficiently milked when the milk flow rate decreases below the threshold of 0.2 kg/min (Rasmussen, 1993). Along with a potential decrease in milk yield, another consequence of incomplete milking could be an increased rate of clinical mastitis. However, results from recent experiments have challenged these ideas, as an increase in flow rate threshold up to 0.8 kg/min resulted in no negative effects on milk production (Edwards et al., 2013a, 2013b) or clinical mastitis rates (Magliaro and Kensinger, 2005). Furthermore, the average machine-on time per cow has been shown to be decreased by up to 30 s or more in situations where an increased milk flow rate threshold was incorporated into the milking routine (Rasmussen, 1993; Magliaro and Kensinger, 2005).

An alternative strategy to decrease the time that the cow spends attached to the milking unit, rather than increasing the milk flow rate threshold, could be detachment of the milking unit after a designated volume of milk is harvested. The effects of this incomplete milking method, which would increase the amount of milk remaining in the gland (MRG) after a milking event, have been largely uncharacterized in terms of the effect on milk yield or milk production rate (MPR). A recent experiment revealed no long-term negative effect on milk yield of incomplete milking applied in the first 5 DIM, after which cows resumed complete milking (Carbonneau et al., 2012). In this study, incomplete milking was achieved by removing 6 liters of milk on day 1, 8 liters on day 2, 10 liters on day 3, 12 liters on day 4, and 14 liters on day 5 at the udder level. Another recent experiment observed a 25% reduction in MPR after leaving approximately 23% MRG in half-udders milked twice-daily (2×) from 5 to 47 DIM (Penry et al., 2017). In this study, MPR decreased almost immediately after application of the incomplete milking treatment and persisted throughout the experimental period. The half-udder design, also known as a unilateral milking model (UMM) is considered a powerful tool as it reduces variation due to environmental, genetic, and nutritional factors (Wall and McFadden, 2012). Furthermore, the UMM allows researchers to study the autocrine–paracrine modulation of lactation by designating a treatment

to one contralateral half-udder while holding the other as a control (Weaver and Hernandez, 2016).

Cows in conventional milking system (CMS) herds are typically milked either 2× or thrice-daily (3×) with a relatively constant milking interval over time. In Penry et al. (2017), all cows were milked 2× for the duration of the experiment. Experiments have consistently observed increased milk production as a result of increased milking frequency (IMF), especially when applied during early lactation. For cows milked 3× during the first 20 wk of lactation, a 19% increase in milk production was observed compared with cows milked 2× (Poole, 1982). In Hale et al. (2003), cows milked 4 times daily (4×) from day 1 to day 21 or from day 4 to day 21 of lactation experienced a 26% or 14% increase in milk production, respectively, compared with cows milked 2×. Results from an experiment performed by Wall and McFadden (2007) are in general agreement with Hale et al. (2003), with half-udders milked 4× for the first 21 d of lactation producing 3.5 kg/d more milk than half-udders milked 2×. Another experiment testing the effects of 2× and 4× milking frequencies, this time for 4 wk beginning in week 11 of lactation, found a 14% increase in milk production for glands milked 4× compared with 2× (Hillerton et al., 1990). There is no research reporting on the interaction of milking frequency and incomplete milk removal from the gland.

The primary objective of this experiment was to determine the effects of increased milking frequency and incomplete milking on MPR and milk composition. Furthermore, it was sought to determine whether utilizing a 3× milking frequency could rescue the negative effect of incomplete milking on MPR when compared with a 2× milking frequency. As significant filling of the gland cistern does not occur until after 6 h postmilking (Knight et al., 1994), cows milked 3× may be able to adapt better to a regimen of incomplete milking compared with cows milked 2×. Our hypothesis was that a 3× milking frequency would be able to partially compensate for the milk yield loss due to the incomplete milking treatment of 30% MRG in cows milked 2×.

MATERIALS AND METHODS

This experiment was approved by the Institutional Animal Care and Use Committee protocol A005178. Cows were housed at the Dairy

Cattle Center at the University of Wisconsin-Madison. A total of 22 multiparous Holstein cows, with an average lactation number of 2.73 ± 0.18 , were enrolled in the study. Baseline quarter milk yields were established by completely milking cows $2\times$ on 3 and 4 DIM. Beginning on 5 DIM and continuing until 47 DIM, a split-plot design was used to apply 2 different treatments: one at the cow level and the second at the quarter-level within each animal. Daily milking frequency was applied at the cow level (main plot), and each cow was randomly assigned to a $2\times$ or $3\times$ milking frequency, resulting in an $N = 11$ per milking frequency. Incomplete milking (30% MRG; **IM**) was randomly applied to a contralateral half of the udder within each cow (subplot). This experimental design resulted in 4 treatment groups in total, which were half-udders milked $2\times$ completely (**CM**), $3\times$ CM, $2\times$ IM, and $3\times$ IM.

For this experiment, both milk yield and components were measured at the quarter-level. The milk yield of each quarter was recorded using a quarter milking device, which consisted of teat cups attached to individual buckets on digital scales. The objective of the incomplete milking treatment was to leave approximately 30% of milk unharvested in a designated half-udder of the cow to achieve either a partial or full cisternal milk fraction after each milking event. To estimate the amount of milk necessary to leave 30% MRG, all quarters were completely milked for the first 4 DIM and quarter MPR was calculated during this baseline period (Penry et al., 2017). Weekly recalibrations of MPR were performed of the IM quarters. These quarters were milked completely on 2 consecutive milkings with the difference in yield between the first and second milking used to calculate quarter MPR, and the difference between the first complete milking and preceding IM used to calculate MRG. The total amount of milk in an IM quarter prior to subsequent milkings was calculated as the MRG plus the milk produced during the intermilking period. The target milk removal of IM quarters was 70% of the estimated quarter total, leaving 30% MRG. Control quarters were completely milked out during each milking, which was determined by visual inspection and appearance of no further milk flow.

Milk samples were collected twice weekly at the beginning of the morning milking on the third and seventh day of each week, and were analyzed for milk fat, protein, lactose, solids nonfat, milk urea nitrogen (MUN), and SCC by a commercial laboratory (AgSource, Verona, WI). As milk samples were collected at the beginning of a milking event,

the data for each component is representative of the cisternal milk fraction rather than for an entire milking event. Fat and lactose have been shown to be affected by the time of sample collection. Milk fat percent increases over the course of milking with early-milking cisternal milk having 4-fold lower milk fat percent than alveolar milk harvested at the end of milking. Milk lactose percent increases slightly from the cisternal fraction to the 25%, 50%, and 75% milk fractions and then decreases slightly at the 100% milk fraction to the level observed in the cisternal fraction (Ontsouka et al., 2003).

Statistical analyses were performed using SAS (version 9.4; SAS Institute Inc., Cary, NC). The experimental unit was the half-udder, while the observational unit was each individual quarter (Bello et al., 2016). Half-udder MPR was calculated as the sum of the MPR for the 2 contralateral quarters within each half-udder. Milk component and SCC values were averaged for each half-udder. Values for SCC were log-transformed prior to analysis. A Bonferroni outlier test was performed for each milk component within each week to identify and exclude any potential outliers within the data set.

The SAS MIXED procedure was utilized to evaluate the effects of milking frequency, incomplete milking, week, and all 2- and 3-way interactions on MPR, milk components, and $\log_{10}\text{SCC}$ using the following model:

$$Y_{ijkl} = \mu + my0_{ijl} + F_i + I_j + (F \times I)_{ij} + W_k + (F \times W)_{ik} + (I \times W)_{jk} + (F \times I \times W)_{ijk} + (ID \times F)_{il} + (ID \times F \times I)_{ijl} + \varepsilon_{ijkl}$$

where Y_{ijkl} = tested response variable of MPR, milk components, or $\log_{10}\text{SCC}$; μ = overall mean; $my0$ = covariate for each response variable; F = milking frequency treatment; i = control or treatment (fixed effect); I = incomplete milking treatment; j = control or treatment (fixed effect); W = week; $k = 1-6$ wk (fixed effect); ID = cow; $l = 1-22$ ID (random effect); ε_{ijkl} = residual error. The terms $(ID \times F)_{il}$ and $(ID \times F \times I)_{ijl}$ were utilized to estimate the main plot error and subplot error, respectively. The Kenward-Roger approximation was used to compute the denominator degrees of freedom for the model. The autoregressive AR(1) error structure was applied to the model to account for correlation between repeated measures within each cow over time. Pairwise comparisons of the 4 treatment groups within each week utilized the difference of least squares mean (LSM). Significance for calculated LSM was declared at $P < 0.05$.

Table 1. Summary of effects of incomplete milking and milking frequency on milk production rate (MPR), milk fat percentage, milk protein percentage, milk lactose percentage, and Log₁₀SCC¹

Parameter ²	MPR	Fat %	Protein %	Lactose %	Log ₁₀ SCC
My0	<0.0001	0.07	0.002	0.16	0.11
F	0.06	0.047	0.43	0.04	0.33
I	<0.0001	<0.0001	0.053	<0.0001	0.0004
F × I	0.72	0.25	0.67	0.32	0.97
W	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
F × W	0.13	0.045	0.016	0.19	0.93
I × W	0.02	0.84	0.92	0.99	0.59
F × I × W	0.95	0.81	0.99	0.70	0.43

¹*P* values for the covariate, main effect of each measurement, 2-way, and 3-way interaction for MPR, Milk Fat%, Milk Protein %, Milk Lactose %, and Log₁₀SCC.

²F = milking frequency treatment; I = incomplete milking treatment; my0 = covariate for each response variable; W = week.

RESULTS

The *P* values of the covariate, main effects, 2-way interactions, and the 3-way interaction in the model for MPR, milk components, and log₁₀SCC are presented in Table 1.

In the first 5 DIM at the initiation of the experiment, there was no difference in MPR between treatment groups as detected by baseline milk production measurements. The significant covariates in the MPR model were initial MPR (*P* < 0.0001) and week (*P* < 0.0001). The main effect of incomplete milking was significant (*P* < 0.0001) with IM half-udders having 27% lower MPR than CM half-udders. The interaction between week and incomplete milking was also significant (*P* = 0.016). The main effect of milking frequency tended to be significant (*P* = 0.06) with the least squares mean MPR of 1.81 ± 0.06 kg milk/h for 2× and 1.97 ± 0.06 kg milk/h for 3× cows milked. No 2-way interactions were detected between milking frequency and incomplete milking or milking frequency and week. The 3-way interaction of milking frequency, incomplete milking, and week was also not significant.

Pairwise comparisons of MPR between the 4 treatment groups within each week revealed that the CM half-udders had increased MPR compared with IM half-udders in the same milking frequency group (i.e., 2× CM vs. 2× IM) throughout the entire treatment period (*P* < 0.01). Half-udders in the 3× CM group displayed increased MPR compared with 2× CM half-udders during only week 2 of the experiment (Fig. 1; *P* = 0.01). There was no significant difference in MPR between 3× IM and 2× IM half-udders throughout the entire experimental period or during any individual week of the experiment.

Milk fat percent was not different between treatment groups at the beginning of the experiment.

Figure 1. Average weekly milk production rate (kg/h) in 2× control (●; complete milking), 2× treatment (○; incomplete milking), 3× control (■; complete milking), and 3× treatment (□; incomplete milking) half-udders. Vertical bars indicate SEM.

Overall, IM half-udders produced milk with increased milk fat compared with CM half-udders ($2.15 \pm 0.07\%$ vs. $1.43 \pm 0.07\%$, respectively) (Fig. 2; *P* < 0.0001). Half-udders that were IM produced milk with a higher milk fat percent than the CM half-udders within the same milking frequency (i.e., 2× CM vs. 2× IM) during each week of the treatment period (*P* < 0.0001). The interaction between milking frequency and week was found to be significant (*P* = 0.045). Milk fat percent was different between 2× and 3× CM half-udders only during week 1 (*P* = 0.046; Fig. 1).

Milk protein percent was not different between treatment groups at the beginning of the experiment. The interaction between milking frequency and week of lactation was found to be significant (Fig. 3; *P* = 0.02). No significant difference between treatment groups were observed for milk protein percent.

Milk lactose percent was significantly different at the beginning of the experiment. Half-udders

designated to the 3× IM treatment group produced milk with decreased milk lactose percent compared with 2× CM (4.46 ± 0.06% vs. 4.63 ± 0.06%, respectively; $P = 0.03$) and 2× IM half-udders (4.46 ± 0.06% vs. 4.67 ± 0.06%, respectively, $P = 0.01$). The 3× CM half-udders produced milk with decreased lactose percent compared with 2× IM half-udders (4.50 ± 0.06 vs. 4.67 ± 0.06%, respectively; $P = 0.03$).

Both milking frequency and incomplete milking affected milk lactose percent. Throughout the experimental period, 2× cows produced milk with an increased lactose percent compared with 3× cows (4.93 ± 0.04% vs. 4.80 ± 0.04%, respectively) (Fig. 4; $P = 0.04$). Additionally, CM half-udders produced milk with an increased lactose percent compared with IM half-udders (4.99 ± 0.03% vs. 4.75 ± 0.03%, respectively,

respectively) (Fig. 4; $P < 0.0001$). The effect of week of lactation on milk lactose percent was significant ($P < 0.0001$), with half-udders producing milk with the least amount of milk lactose during week 1 (4.73 ± 0.03%) and the highest amount of milk lactose during weeks 3 and 6 (4.91 ± 0.03%).

Log₁₀-transformed SCC were not different between treatment groups at the start of the experiment, nor during weeks 1 and 5. The effect of incomplete milking was significant on milk SCC (Fig. 5; $P = 0.0004$), whereas milking frequency was not. Throughout the experimental period, IM half-udders had increased log₁₀SCC compared with CM half-udders (4.41 ± 0.05 vs. 4.22 ± 0.05, respectively). During weeks 2 through 4, 2× half-udders had increased milk SCC compared with 2× CM half-udders (Fig. 5; $P < 0.05$). During weeks 2, 3, 5, and 6, 3× IM half-udders

Figure 2. Average weekly milk fat (%) in 2× control (●; complete milking), 2× treatment (○; incomplete milking), 3× control (■; complete milking), and 3× treatment (□; incomplete milking) half-udders. Vertical bars indicate SEM.

Figure 3. Average weekly milk protein (%) in 2× control (●; complete milking), 2× treatment (○; incomplete milking), 3× control (■; complete milking), and 3× treatment (□; incomplete milking) half-udders. Vertical bars indicate SEM.

Figure 4. Average weekly milk lactose (%) in 2× control (●; complete milking), 2× treatment (○; incomplete milking), 3× control (■; complete milking), and 3× treatment (□; incomplete milking) half-udders. Vertical bars indicate SEM.

Figure 5. Average weekly milk SCC (log-transformed) in 2× control (●; complete milking), 2× treatment (○; incomplete milking), 3× control (■; complete milking), and 3× treatment (□; incomplete milking) half-udders. Vertical bars indicate SEM.

had elevated milk SCC compared with 3× CM half-udders (Fig. 5; $P < 0.05$). There was only 1 case of clinical mastitis during the experimental period, which occurred in a 3× IM quarter during week 6. After transformation of $\log_{10}$ SCC to raw SCC, milk SCC was numerically increased in the IM half-udders (25,700) compared with CM half-udders (16,600).

DISCUSSION

The positive effect of IMF on milk production is a widely established phenomenon in dairy cows (Bar-Peled et al., 1995; Erdman and Varner, 1995). In contrast, the effect of incomplete milking on the dairy cow has been studied very sporadically, with the bulk of the literature being published in either the middle of the 20th century (Schmidt et al., 1964; Wheelock et al., 1965) or within the last 10 yr (Carbonneau et al., 2012; Penry et al., 2017; Krug et al., 2018). To the best of our knowledge, this experiment is the first of its kind examining both the effects of milking frequency and incomplete milking concurrently to characterize both the separate effects and whether a biological interaction is present.

In this experiment, 3× cows had tended to have 9% higher MPR than 2× cows. The milking frequency treatment utilized in this experiment was applied across cows, and as such may have been limited in its ability to detect a significant difference compared with the incomplete milking treatment. This increase in milk production is lower than the value observed during a previous experiment, which found a 19% increase in milk production in cows milked 3× during the first 20 wk of lactation compared with cows milked 2× (Poole, 1982). A fixed increase of 3.5 kg/d in milk production in response to an IMF of 3× compared with 2× has been noted previously (Erdman and Varner, 1995). This fixed value is in general agreement with the increase in average MPR of 3.84 kg/d from 2× to 3× cows observed in this experiment.

Very little research effort has been directed towards examining the effect of incomplete milking on long-term milk production. A significant decrease in milk production due to incomplete milking has been observed previously, with the severity and irreversibility of the decrease being directly related to the volume of milk left behind (Schmidt et al., 1964; Wheelock et al., 1965). One inconsistency between incomplete milking experiments from the 1960s is the stage of lactation tested, as it was not reported in Schmidt et al. (1964) and ranged from

early to end of middle lactation in Wheelock et al. (1965).

The main objective of this experiment was to determine whether a 3× milking frequency would be able to rescue the negative effects of incomplete milking on MPR in early lactation. This hypothesis is based on the notion that substantial filling of the cisternal fraction does not occur until 6 h postmilking (Knight et al., 1994) and that cows milked 3× would potentially be able to better manage increased milk remaining in the gland due to incomplete milking. Our results demonstrate that 3× milking of IM udders is not able to recover a significant volume of the milk yield decrease observed due to the incomplete milking treatment. The incomplete milking methodology of our experiment resulted in high levels of milk left behind in the gland and for an extensive period of time, which could explain the inability to recover milk yield through IMF. A large variability in the proportion of total milk volume stored in the cisternal fraction has been documented previously (Dewhurst and Knight, 1994). This level of variance suggests that some animals would be better suited to mitigating milk production loss due to incomplete milking practices. Furthermore, stage of lactation (Stelwagen and Knight, 1997), breed (Carruthers et al., 1993), and parity (Clark et al., 2006) would seemingly also play a major role in how well a cow deals with incomplete milking, as these factors have been implicated in milk production losses during once-daily milking, whereby cows are subjected to 24-h milking intervals. Future research should focus on different amounts of milk left behind as well as for different periods of time.

In this experiment, incomplete milking of 2× half-udders resulted in a 27% decrease in MPR, similar to the 25% decrease found by Penry et al. (2017). Both experiments observed the effect of incomplete milking over a period of 6 wk in early lactation. One aspect of milk production which was not investigated during these experiments is whether a negative carryover effect of incomplete milking on milk production is present after cows resume a complete milking practice. Although a flattened lactation curve appeared for half-udders subjected to IM, we did not examine the effects of incomplete milking after we terminated our treatment protocol at 47 DIM. A positive carryover effect of IMF during early lactation on milk production has been observed previously (Bar-Peled et al., 1995; Soberon et al., 2011; Shoshani et al., 2017). Because IMF involves more frequent removal of milk from the mammary gland and incomplete milking

involves removing a smaller volume of milk, it can be reasoned that incomplete milking could have a negative carryover effect on milk production. Based on previous observations of irreversible milk production loss due to incomplete milking, it can be speculated that the lactation performance of the cow during the current lactation will remain impacted until the dry period, whereby remodeling of the udder can occur. However, this needs to be investigated in future studies. There appears to be an undiscovered amount of time during early lactation, particularly right after parturition, where incomplete milking can be performed without a sustained decrease in milk production thereafter. Further testing of incomplete milking directly after parturition will be necessary to evaluate whether 5 d is the maximum amount of time before negative effects on milk production are observed (Carbonneau et al., 2012).

To the best of our knowledge, there is only one experiment that has examined the temporal effects of incomplete milking on milk components (Penry et al., 2017). No difference in milk fat and milk protein was observed in this experiment when incomplete milking was applied during a 2× milking frequency. In the present experiment, it was determined that IM half-udders within each milking frequency had consistently elevated milk fat throughout the treatment period. This could possibly be attributed to the sampling method utilized in this experiment, as milk samples were collected from the cisternal milk fraction only. In this experiment, milk harvested from IM half-udders is theoretically comprised of the cisternal milk fraction and milk ejected from the alveoli up to approximately the 70% milk fraction. Therefore, milk left in the cistern after a milking event would be from the later (75% and 100%) milk fractions, which have been shown to have a much higher milk fat percent than the earlier milk fractions (Ontsouka et al., 2003). As the cisternal portion of the mammary gland accumulates milk between milking events, due to milk shifting from the alveoli to the cistern, these later milk fractions left behind by the previous milking are diluted by milk destined for the cisternal milk fraction for the subsequent milking event, which have a much lower milk fat percent. Dilution of the later milk fractions from a previous milking event with the cisternal milk fraction of a subsequent milking event likely explains why increased milk fat percent was observed with the incomplete milking treatment regardless of milking frequency.

The milk fat percentages observed in this experiment were much lower than typically reported in

the literature. Composite milk samples, depending on several factors, yield a milk fat percentage normally around 3.5–5%, whereas cisternal milk samples are closer to 2%, as seen in our experiment (Ontsouka et al., 2003). Milk sampling method is assumed to only affect the milk fat and milk lactose percentages observed in this experiment as samples were collected at the beginning of milking. A previous experiment found minimal and variable effects in milk protein percent and milk SCC between cisternal and alveolar milk fractions studied (Ontsouka et al., 2003). The values analyzed for milk protein percentage in the present experiment agree with Penry et al. (2017), with both studies observing no difference between CM and IM.

Milk lactose percentage was decreased in the IM half-udders in each milking frequency group throughout the experiment. Furthermore, cows milked 3× produced milk with a lower milk lactose percentage compared with cows milked 2×. A decrease in milk lactose percentage in IM half-udders may indicate a compromise of tight junction integrity. Loss of tight junction integrity has been previously observed in situations where accumulation of milk in the gland cistern occurred for a period greater than 18 h (Stelwagen et al., 2013). Another potential explanation for decreased milk lactose percentage in IM half-udders could be a decrease in secretory activity. In this experiment, an immediate decrease of both MPR and milk lactose percentage was observed in IM half-udders. Additionally, decreased MPR and milk lactose percentage of IM half-udders persisted throughout the entire experimental period. Milk yield is known to be influenced by the production of lactose, which is regarded as the main osmoregulator of milk in dairy cows (Bleck et al., 2009). As lactose is synthesized in the Golgi apparatus of the mammary epithelial cell, water is moved from the cytoplasm of the cell into secretory vesicles and subsequently ends up in milk (Kuhn et al., 1980).

There was no effect of milking frequency on SCC in this experiment, which is in general agreement with other experiments (Hale et al., 2003; Soberon et al., 2011; Murney et al., 2015). Increased SCC of IM half-udders observed in this experiment is consistent with the results of a previous incomplete milking experiment (Penry et al., 2017). In the previous incomplete milking experiment, IM half-udders displayed a geometric mean SCC of 48,300 when compared with 26,300 in CM half-udders. Even lower SCC were observed in the present experiment, with IM half-udders yielding an average SCC of 25,700 when compared with

16,600 for CM half-udders. Although SCC was elevated in IM half-udders, this difference proved to be inconsequential. The widely accepted SCC threshold for quarters infected with mastitis pathogens is 200,000 (Harmon, 1994; Bradley and Green, 2004), with more recent experiments establishing an even lower threshold of 100,000 for uninfected quarters (Bobbo et al., 2017). These extremely low SCC values are attributed to the high level of equipment maintenance and the teat preparation routine utilized in the experiment. Only 1 case of clinical mastitis was documented during the experimental period, which occurred in a 3× IM quarter.

In conclusion, we did not observe a rescue of MPR in IM half-udders by IMF. These data suggest that prolonged and extensive incomplete milking has irreversible effects on the ability of the mammary gland to recover milk synthesis. Furthermore, we observed that incomplete milking, regardless of milking frequency, resulted in extensive reductions in MPR.

LITERATURE CITED

- Bar-Peled, U., E. Maltz, I. Buckental, Y. Folman, Y. Kali, H. Gacitua, and A. R. Lehrer. 1995. Relationship between frequent milking or suckling in early lactation and milk production of high producing dairy cows. *J. Dairy Sci.* 78:2726–2736. doi:10.3168/jds.S0022-0302(95)76903-X
- Bleck, G. T., M. B. Wheeler, L. B. Hansen, H. Chester-Jones, and D. J. Miller. 2009. Lactose synthase components in milk: concentrations of alpha-lactalbumin and beta1,4-galactosyltransferase in milk of cows from several breeds at various stages of lactation. *Reprod. Domest. Anim.* 44:241–247. doi:10.1111/j.1439-0531.2007.01047.x
- Bradley, A. J., and M. J. Green. 2004. The importance of the nonlactating period in the epidemiology of intramammary infection and strategies for prevention. *Vet. Clin. North Am. Food Anim. Pract.* 20:547–568. doi:10.1016/j.cvfa.2004.06.010
- Bello, N. M., M. Kramer, R. J. Tempelman, W. W. Stroup, N. R. St-Pierre, B. A. Craig, L. J. Young, and E. E. Gbur. 2016. Short communication: on recognizing the proper experimental unit in animal studies in the dairy sciences. *J. Dairy Sci.* 99:8871–8879. doi:10.3168/jds.2016-11516
- Bobbo, T., P. L. Ruegg, G. Stocco, E. Fiore, M. Giancesella, M. Morgante, D. Pasotto, G. Bittante, and A. Cecchinato. 2017. Associations between pathogen-specific cases of subclinical mastitis and milk yield, quality, protein composition, and cheese-making traits in dairy cows. *J. Dairy Sci.* 100(6):4868–4883. doi:10.3168/jds.2016-12353
- Carbonneau, E., A. M. de Passillé, J. Rushen, B. G. Talbot, and P. Lacasse. 2012. The effect of incomplete milking or nursing on milk production, blood metabolites, and immune functions of dairy cows. *J. Dairy Sci.* 95:6503–6512. doi:10.3168/jds.2012-5643
- Carruthers, V. R., S. R. Davis, A. M. Bryant, H. V. Henderson, C. A. Morris, and P. J. A. Copeman. 1993. Response of Jersey and Friesian cows to once a day milking and prediction of response based on udder characteristics and milk composition. *J. Dairy Res.* 60:1–11. doi:10.1017/S0022029900027291
- Clark, D. A., C. V. Phyn, M. J. Tong, S. J. Collis, and D. E. Dalley. 2006. A systems comparison of once- versus twice-daily milking of pastured dairy cows. *J. Dairy Sci.* 89:1854–1862. doi:10.3168/jds.S0022-0302(06)72254-8
- Dewhurst, R. J., and C. H. Knight. 1994. Relationship between milk storage characteristics and the short-term response of dairy cows to thrice-daily milking. *Anim. Prod.* 58:181–187. doi:10.1017/S135772980004248X
- Edwards, J. P., J. G. Jago, and N. Lopez-Villalobos. 2013a. Milking efficiency for grazing dairy cows can be improved by increasing automatic cluster remover thresholds without applying premilking stimulation. *J. Dairy Sci.* 96:3766–3773. doi:10.3168/jds.2012-6394
- Edwards, J. P., J. G. Jago, and N. Lopez-Villalobos. 2013b. Short-term application of pre-stimulation and increased automatic cluster remover threshold affect milking characteristics of grazing dairy cows in late lactation. *J. Dairy Sci.* 96:1886–1893. doi:10.3168/jds.2012-6191
- Erdman, R. A., and M. Varner. 1995. Fixed yield responses to increased milking frequency. *J. Dairy Sci.* 78:1199–1203. doi:10.1016/j.cvfa.2004.06.010
- Hale, S. A., A. V. Capuco, and R. A. Erdman. 2003. Milk yield and mammary growth effects due to increased milking frequency during early lactation. *J. Dairy Sci.* 86:2061–2071. doi:10.3168/jds.S0022-0302(03)73795-3
- Harmon, R. J. 1994. Physiology of mastitis and factors affecting somatic cell counts. *J. Dairy Sci.* 77:2103–2112. doi:10.3168/jds.S0022-0302(94)77153-8
- Hillerton, J. E., C. H. Knight, A. Turvey, S. D. Wheatly, and C. J. Wilde. 1990. Milk yield and mammary function in dairy cows milked four times daily. *J. Dairy Res.* 57:285–294. doi:10.1017/S0022029900026935
- Krug, C., P. Morin, J. Roy, J. Dubuc, P. Lacasse, S. Dufour. 2018. Incomplete milking in early lactation increases the odds of eliminating intramammary infections: Results from a randomized controlled trial. *NMC Ann. Meet. Proc.* 152–153.
- Knight, C. H., D. Hirst, and R. J. Dewhurst. 1994. Milk accumulation and distribution in the bovine udder during the interval between milkings. *J. Dairy Res.* 61:167–177. doi:10.1017/S0022029900028181
- Kuhn, N. J., D. T. Carrick, and C. J. Wilde. 1980. Lactose synthesis: the possibilities of regulation. *J. Dairy Sci.* 63:328–336. doi:10.3168/jds.S0022-0302(80)82934-1
- Magliaro, A. L., and R. S. Kensinger. 2005. Automatic cluster remover setting affects milk yield and machine-on time in dairy cows. *J. Dairy Sci.* 88:148–153. doi:10.3168/jds.S0022-0302(05)72672-2
- Murney, R., K. Stelwagen, T. T. Wheeler, J. K. Margerison, and K. Singh. 2015. The effects of milking frequency in early lactation on milk yield, mammary cell turnover, and secretory activity in grazing dairy cows. *J. Dairy Sci.* 98(1):305–311. doi:10.3168/jds.2014-8745
- Ontsouka, C. E., R. M. Bruckmaier, and J. W. Blum. 2003. Fractionized milk composition during removal of colostrum and mature milk. *J. Dairy Sci.* 86:2005–2011. doi:10.3168/jds.S0022-0302(03)73789-8
- Penry, J. F., E. L. Endres, B. de Bruijn, A. Kleinhans, P. M. Crump, D. J. Reinemann, and L. L. Hernandez. 2017. Effect of incomplete milking on milk production rate and composition with 2 daily milkings. *J. Dairy Sci.* 100(2):1535–1540. doi:10.3168/jds.2016-11935

- Poole, D. A. 1982. The effects of milking cows three times daily. *Anim. Prod.* 34:197–201. doi:10.1017/S0003356100000672
- Rasmussen, M. D. 1993. Influence of switch level of automatic cluster removers on milking performance and udder health. *J. Dairy. Res.* 60:287–297. doi:10.1017/S0022029900027631
- Schmidt, G. H., R. S. Guthrie, and R. W. Guest. 1964. Effect of incomplete milking on the incidence of udder irritation and subsequent milk yield of dairy cows. *J. Dairy Sci.* 47(2):152–155. doi:10.3168/jds.S0022-0302(64)88608-2
- Shoshani, E., M. Cohen, and J. J. Doekes. 2017. Short- and long-term effects of high milking frequency during the first 21 days of lactation on production and reproductive performance in high-lactating cows. *Animal* 11:91–100. doi:10.1017/S1751731116001324
- Soberon, F., C. M. Ryan, D. V. Nydam, D. M. Galton, and T. R. Overton. 2011. The effects of increased milking frequency during early lactation on milk yield and milk composition on commercial dairy farms. *J. Dairy Sci.* 94:4398–4405. doi:10.3168/jds.2010–3640
- Stelwagen, K., and C. H. Knight. 1997. Effect of unilateral once or twice daily milking of cows on milk yield and udder characteristics in early and late lactation. *J. Dairy Res.* 64:487–494. doi:10.1017/S0022029997002458
- Stelwagen, K., C. V. C. Phyn, S. R. Davis, J. Guinard-Flament, D. Pomiès, J. R. Roche, and J. K. Kay. 2013. Invited review: Reduced milking frequency: Milk production and management implications. *J. Dairy Sci.* 96:3401–3413. doi:10.3168/jds.2012-6074
- Wall, E. H., and T. B. McFadden. 2007. The milk yield response to frequent milking in early lactation of dairy cows is locally regulated. *J. Dairy Sci.* 90:716–720. doi:10.3168/jds.S0022-0302(07)71555-2
- Wall, E. H., and T. B. McFadden. 2012. Triennial lactation symposium: A local affair: How the mammary gland adapts to changes in milking frequency. *J. Anim. Sci.* 90:1695–1707. doi:10.2527/jas.2011-4790
- Weaver, S. R., and L. L. Hernandez. 2016. Autocrine-paracrine regulation of the mammary gland. *J. Dairy Sci.* 99:842–853. doi:10.3168/jds.2015-9828
- Wheelock, J. V., J. A. F. Rook, and F. H. Dodd. 1965. The effect of incomplete milking or of an extended milking interval on the yield and composition of cow's milk. *J. Dairy Res.* 32:237–248. doi:10.1017/S0022029900018604