Effect of incomplete milking on milk production rate and composition with 2 daily milkings

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ABSTRACT

The primary aim of this study was to assess the effects of incomplete milking on milk secretion and milk composition at the quarter level. Twelve cows were enrolled beginning at 5 d in milk and remained on study through 47 d in milk. Half of each contralateral udder was incompletely milked (treatment), detaching the teat cup early to leave approximately 30% of the total milk yield behind. This target milk remaining in the gland was based on weekly calibration milking measurements of quarter total milk yield. Control quarters were milked completely until milk flow had decreased to 0 kg/min based on visual assessment. Harvested milk yield was measured twice daily at each milking, and milk components (fat, protein, lactose, solids non-fat, milk urea nitrogen) and somatic cell count, were measured twice weekly at the quarter level. The experimental unit in this design was the half-udder, and a mixed-model approach was used to assess the main and interactive effects of experiment week and treatment on milk production rate, milk remaining in the gland, and milk composition. The effect of treatment on milk production rate was significant, with the average control half-udder producing 0.97 kg/h and the treatment half-udder 0.73 kg/h. The effect of week on milk production rate and the interaction of week $\times$ treatment were also significant. The effect of treatment on milk remaining in the gland was significant, illustrating that an increase in milk remaining in the cisternal compartment had been achieved. We detected a significant decrease in milk lactose percentage in treatment half-udders, and a significant increase in somatic cell count ($\log_{10}$). The increase was relatively small, from a geometric mean of 26,300 cells/mL in control quarters to 48,300 cells/mL in treatment quarters. The decrease in milk production rate in treatment half-udders supports current knowledge about how mammary epithelial cell

secretion, proliferation, and apoptosis are modulated by autocrine-paracrine factors.

Key words: milk remaining, milk production rate, milk components

INTRODUCTION

Machine milking has been described as a compromise between milking gently, quickly, and completely (Reinemann, 2012). Here, the term “completely” describes the amount of strippings or milk remaining in the gland (MRG), in an individual quarter cistern and duct network after milking. Milk remaining in the gland should be distinguished from residual milk, which is the milk volume left in the alveolar compartment at the end of milking; this milk can be harvested only by administering exogenous oxytocin at supraphysiological levels (Elliot, 1961a; Peaker and Blatchford, 1988). After teat cup detachment, it is possible to harvest MRG by hand milking, or by machine milking with additional downward pressure on the teat cup to open the connection between the gland cistern and the teat sinus. This downward pressure can have the added effect of opening collapsed milk ducts in the gland parenchyma. What is unstated but potentially assumed in machine milking’s compromise between gentleness, speed, and completeness is the aim of milking in a manner that does not negatively affect the quarter milk production rate (MPR), defined as the rate of milk secretion per hour (kg/h), calculated using harvested milk yield and milking interval.

A unilateral frequent milking model (Wall and McFadden, 2008; Weaver and Hernandez, 2016) has been presented as a powerful tool for exploring the effect of milking frequency on MPR and the autocrine-paracrine modulation of lactation more generally. Since the 1980s, evidence has accumulated that points toward local control of mammary epithelial cell secretion and cell turnover at the individual gland level (Peakier and Blatchford, 1988; Davis et al., 1999; Wall and McFadden, 2008). In these experiments, modulation of mammary epithelial cell activity is demonstrated to

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be influenced by chemicals, hormones, growth factors, peptides, and proteins in milk that are in contact with mammary epithelial cells in the alveolar lumen. Such a mechanism has been explored using half-udder experiments (Nørgaard et al., 2005; Wall and McFadden, 2008), indicating that autocrine-paracrine processes, rather than systemic processes, drive mammary epithelial cell response to treatments, such as decreased milking interval. The degree of cisternal filling between milkings, or cisternal volume as a proportion of total gland capacity, has been implicated as a factor that can partially determine the concentration of milk-modulating substances in contact with mammary epithelial cells (Henderson and Peaker, 1987; Davis et al., 1998, 1999). A half-udder experimental design was used to compare the changes from twice-per-day ($2\times$) to once-per-day ($1\times$) milking with the changes from $2\times$ to 3-times-per-day ($3\times$) milking (Bernier-Dodier et al., 2010). In this study, after 8 wk of altered milking frequency, the $1\times$ half-udder had only 40% of the milk yield of the $3\times$ half-udder. The degree of cisternal filling between milkings was implicated as a cause of the reduced milk yield, but this was a function of milking frequency and not incomplete milking.

Cisternal filling is likely to be accelerated if MRG is increased at the end of milking, irrespective of milking interval. The primary aim of this study was to assess the effects of incomplete milking on milk secretion and milk components at the quarter level.

MATERIALS AND METHODS

We used a half-udder design for this experiment, which was performed under Institution Animal Care and Use Committee protocol A005178 at the Dairy Cattle Center, University of Wisconsin-Madison. We enrolled 12 cows at 5 DIM, and they remained on the experiment through 47 DIM. All cows were milked twice per day, with a 12 h interval between milkings. One half contralateral udder was the control, with complete milking of both quarters at each milking session. The other half was assigned to treatment, for which an estimated 30% of the total quarter milk yield was left behind at the end of milking as MRG. As each cow was enrolled, we alternated control and treatment udder halves.

We measured milk yield and components at the quarter level. At the first milking after enrollment, all quarters were milked out completely to determine quarter milk yield and the ratio of treatment to control quarter milk yield. We used this ratio to estimate milk production of the treatment quarters and calculate milk removal targets at each milking that would result in approximately 30% of total milk yield left behind as MRG

for the next 6 d. We measured cumulative milk yield for each quarter using a quarter milking device (individual teat cups connected to sealed collection buckets on digital scales). Control quarters were milked completely at each milking, as indicated by visual inspection of the end of milk flow from each quarter. Teat cups on treatment quarters were detached when the cumulative milk yield target for that quarter was indicated by the quarter milking device. At a.m. and p.m. milkings on d 7, 14, 21, 28, 35, and 42 after enrollment, all quarters were milked completely (designated calibration milkings 1 and 2) to recalibrate the estimate of the milk yield in the treatment quarters. Milk samples were taken twice weekly after enrollment for milk components. Testing for milk fat, protein, lactose, solids nonfat, MUN, and SCC was performed by a commercial laboratory (Ag-source, Verona, WI).

Statistical analysis was performed in SAS (version 9.4; SAS Institute Inc., Cary, NC). For all analyses, the experimental unit was the half-udder, and the observational unit was the quarter (Bello et al., 2016). We calculated half-udder MPR by taking the control or treatment half-udder milk yield at calibration milking 2 and dividing it by the milking interval. Half-udder MRG for control and treatment halves was calculated by taking the half-udder milk yield at calibration milking 1 and subtracting the corresponding milk yield at calibration milking 2. The assumption was that any available milk not harvested at the milking before calibration milking 1 was available for harvesting at calibration milking 1, in addition to the milk produced in that half-udder in the 12 h before calibration milking 1. This additional milk was presumed not to be present at calibration milking 2. Milk component percentages and SCC for each half-udder per week were the average of the 2 weekly tests performed at the quarter level. All SCC values were log 10 transformed.

We used the SAS MIXED procedure to estimate the effect of treatment and week on MPR, MRG, and milk components using the following model:

$$Y_{ijk} = \mu + T_i + \text{cowid}_j + (T \times \text{cowid})_{ij} \\ + W_k + (T \times W)_{ik} + \varepsilon_{ijk},$$

where Y_{ijk} = dependent variable: MPR or MRG or milk component; μ = overall mean; T = treatment; i = treatment or control (fixed effect); j = 1–12 cowid (random effect); W = week; k = 1–6 weeks (fixed effect); and ε = residual error. We applied the autoregressive AR(1) error structure to this model to account for correlation between repeated measurements within cow that were equidistant over time. Significance for calculated least squares means was declared at $P < 0.05$.

Figure 1. Average milk production rate (kg/h) in control half-udders (●; complete milking) and treatment half-udders (□; incomplete milking) per week. Significance: treatment, $P = 0.0002$; week, $P < 0.0001$; treatment $\times$ week, $P = 0.0002$. Vertical bars indicate SEM. Different letters (a–c) indicate significance ($P < 0.05$) for treatment $\times$ week interaction.

Figure 2. Average milk fat percentage in control half-udders (●; complete milking) and treatment half-udders (□; incomplete milking) per week. Significance: treatment, $P = 0.446$; week, $P < 0.0001$; treatment $\times$ week, $P = 0.145$. Vertical bars indicate SEM.

RESULTS

The effect of treatment on MPR was significant ($P = 0.0002$), with the average control half-udder producing 0.97 kg/h, and the average treatment half-udder producing 0.73 kg/h, a decrease of approximately 25% (Table 1). The effect of week was also significant ($P < 0.0001$), with wk 1 having the lowest MPR, at 0.69 kg/h, and wk 6 having the highest, at 0.92 kg/h. We observed no clear MPR trend between wk 2 and 5. The treatment $\times$ week interaction was significant ($P = 0.0002$). A graph of MPR by week is shown in Figure 1. With the exception of wk 1, all treatment half-udders had lower MPR than controls.

The effect of treatment on MRG is described in Table 1. The MRG for the average control half-udder was 0.75 kg, significantly less than the 2.86 kg for the average treatment half-udder. The treatment $\times$ week interaction was not significant. The average control half-udder milk yield per milking was 11.7 kg over the experimental period. We did not use hand- or machine-stripping

to assess MRG in control quarters in this design, so we calculated the MRG estimate using the same methodology as for treatment quarters. Adding the control half-udder MRG estimate of 0.75 kg to the 11.7 kg milk yield gives a total average half-udder yield of 12.45 kg. Hence, the average treatment half-udder MRG (2.86 kg) was approximately 23% of total half-udder volume ($2.86/12.45 = 0.23$).

The effect of week was significant for all milk components, to be expected with animals in the first 50 d of lactation (Figures 2, 3, 4, and 5). We observed a significant effect of treatment only for lactose percentage (Figure 4) and SCC log₁₀ (Figure 5). For lactose, we detected a significant decrease ($P = 0.030$) in average treatment half-udder percentage compared with controls (4.67 vs. 4.86%, respectively). For SCC log₁₀, we detected a significant increase ($P = 0.006$) for treatment half-udders compared with controls (4.69 vs. 4.42, respectively). This represented an increase in quarter geometric mean SCC from 26,300 cells/mL in control half-udders to 48,300 cells/mL in treatment half-udders.

Table 1. Effect of incomplete milking on milk production rate and milk remaining in the gland, per half-udder¹

Group	Milk production rate, estimate (kg/h)	Milk remaining in the gland, estimate (kg)
Control (complete milking)	0.97 ^a (0.06)	0.75 ^a (0.06)
Treatment (incomplete milking)	0.73 ^b (0.06)	2.86 ^b (0.06)

^{a,b}Values within a column with different superscript letters differ significantly; $P = 0.0002$.

¹SEM in parentheses.

Figure 3. Average milk protein percentage in control half-udders (●; complete milking) and treatment half-udders (□; incomplete milking) per week. Significance: treatment, $P = 0.755$; week, $P < 0.0001$; treatment $\times$ week, $P = 0.912$. Vertical bars indicate SEM.

DISCUSSION

Our experimental design used known MPR to estimate MRG of 30% per treatment quarter after teat cup detachment to result in a full (or mostly full) cistern at detachment for the treatment quarters. The estimated actual treatment half-udder MRG was approximately 23% of the total half-udder volume, below the experimental design target of 30%. We postulate that the reason for the difference between the target

Figure 4. Average milk lactose percentage in control half-udders (●; complete milking) and treatment half-udders (□; incomplete milking) per week. Significance: treatment, $P = 0.030$; week, $P = 0.002$; treatment $\times$ week, $P = 0.275$. Vertical bars indicate SEM. Different letters (a, b) indicate significant ($P < 0.05$) for treatment $\times$ week interaction.

Figure 5. Average milk SCC (log₁₀ transformed) in control half-udders (●; complete milking) and treatment half-udders (□; incomplete milking) week. Significance: treatment, $P = 0.006$; week, $P = 0.007$; treatment $\times$ week, $P = 0.199$. Vertical bars indicate SEM. Different letters (a, b) indicate significant ($P < 0.05$) for treatment by week interaction.

and actual MRG was that we could not predict the precise effect size of MPR in the treatment quarters, and our calculation method assumed that MPR would not change in the 1-week period between calibration milkings. Although the 23% estimated MRG was lower than the 30% target, we still considered the treatment to have been applied successfully, in that a considerable amount of milk was left in the cistern after milking.

Knight et al. (1994) determined that cisternal percentage of total milk ranged from 6 to 56% at 8-h milking intervals. Bruckmaier et al. (1994), using ultrasound, frozen sections, and corrosion casting techniques, measured the cisternal percentage at 2 to 25% for a milking interval of 10 h. Finally, Davis et al. (1998) measured the cisternal percentage at $46\% \pm 2$ for a 24-h milking interval. All of these groups reported significant variability in cisternal volume between animals and poor correlation of udder milk yield to cisternal capacity as a fraction of total udder capacity.

Cows enrolled in the present experiment were prepared for milking with hand stripping of foremilk to check for mastitis, teat disinfection, and cloth drying before teat cup attachment. Based on Bruckmaier and Hilger (2001), we assumed that adequate milk ejection occurred before attachment, given that all cows were in early lactation with a 12-h milking interval. For this reason, the risk of inadequate milk ejection before the start of milk flow in treatment quarters was very low.

The effect of this level of incomplete milking was a significant reduction in MPR of 25% in treatment quarters compared with completely milked control quarters. We believe MPR was the appropriate metric

to report, because it can be used to measure the combined output generated by mammary epithelial cell secretion, mammary epithelial cell number, proliferation, and apoptosis, all of which predict milk yield (Knight, 2000). There are no published reports of experiments manipulating MRG using a half-udder model. As such, the most appropriate group of experiments to compare our MPR results with are those that compare 2× milking to 1× milking. A review of 1× milking studies by Stelwagen et al. (2013) reported a reduction in daily udder milk yield of 7 to 40% compared with 2× milking. A significant correlation between cistern capacity as a percentage of total milk yield and milk yield suppression associated with 1× milking has also been reported (Davis et al., 1998, 1999), implying that cows with the ability to store a larger fraction of total milk yield in their cistern experience less milk yield suppression during 1× milking.

Cisternal filling occurs in 2 stages: a small volume enters the cistern soon after milking, but at about 5 h, the cistern starts to fill more rapidly (Zaks, 1962; Knight et al., 1994). After approximately 5 h, pressure in the alveoli reaches a point at which flow through the ducts supplying the cistern increases. As far back as the 1960s, investigators have postulated the role of milk accumulation in modifying mammary epithelial cell activity (Elliot, 1961b). One potential mechanism was an increase in intramammary pressure and a concurrent mechano-physical action on mammary epithelial cells. However, this was disproven as the sole mechanism of decreased mammary epithelial cell secretion by a milking frequency experiment on goats in which an isotonic solution was infused into the cistern, leading to an increase in secretory rate (Henderson and Peaker, 1987). The authors concluded that removal of milk from the duct system was not enough to increase secretion rate, but that removal of milk from the alveoli was necessary, supporting the hypothesis that a chemical modulator in milk was in direct contact with the mammary epithelial cells. However, the idea of a single chemical modulator directing all mammary epithelial cell modulation has now been abandoned in favor of a wider array of growth factors, hormones, proteins, and other factors that may be involved in increasing or decreasing mammary epithelial cell secretion, proliferation, and apoptosis. These factors include IGF-1, transforming growth factor β , IGF-binding protein 5, and serotonin (Weaver and Hernandez, 2016). Combining the results of previous work on milk yield depression with 1× milking and the presence of modulating chemicals in alveolar milk, our result of a 25% decrease in MPR in treatment half-udders supports these findings.

Accumulation of milk in the alveolar compartment can be rapid, filling this space to 90% at 16 h (Davis et

al., 1998), and the ability to exceed the alveolar capacity can begin as early as 6 to 8 h after milking (Davis et al., 1999). If the treatment amount of available milk in most quarters resulted in a partially or completely filled cisternal compartment immediately after milking, alveolar capacity was likely to be exceeded earlier than when detachment occurs in the low milk flow period. Our treatment might also have resulted in changes in mammary epithelial cell tight junction integrity. This concept was supported by the significant decrease in lactose percentage in treatment half-udders. Stelwagen and Singh (2014) reported that tight junctions become leaky after 18 to 21 h after milking, when milkout is complete. Decreased milk lactose is a known effect of increased tight junction permeability (Stelwagen and Singh, 2014). These alterations in tight junction integrity are positively associated with a decline in mammary gland blood flow and milk secretion rate.

Our experiment found no significant differences due to treatment in percentages of milk fat, protein, solids nonfat, or MUN over the 42 experimental days. Previous 1× studies have found a trend toward higher milk fat concentration (Carruthers et al., 1991; Stelwagen et al., 1994), although another study found no change (Holmes et al., 1992). Similarly, 1× milking has resulted in significantly higher milk protein percentage (Stelwagen et al., 1994; Stelwagen and Knight, 1997), although Holmes et al. (1992) detected no difference. It is possible that the duration of our experiment was not long enough to affect milk fat or protein concentration in the treatment quarters, or that the degree of milk accumulation was not sufficient at the cellular level to modify fat or protein synthesis. We did detect a significant decrease in milk lactose percentage, and combined with MPR, the difference in lactose yield between the treatment and control half-udders was, on average, 0.82 kg/d compared with 1.13 kg/d, a decrease of 27%. This finding was in general agreement with decreases in lactose concentration reported by Wheelock et al. (1965), Holmes et al. (1992), and Stelwagen et al. (1994). With the decrease in milk lactose percentage, we might have expected to see an increase in milk fat percentage, protein percentage, or both, but this was not the case. In the present experiment, we did not take mammary parenchyma tissue samples, so we do not know what the mechanism of lactose production modulation was. However, we have planned mammary gland biopsies and associated molecular tests on parenchyma samples for a future follow-up experiment.

An increase in SCC with 1× milking has been described previously (Holmes et al., 1992; Stelwagen and Lacy-Hulbert, 1996). Our experiment also showed a significant increase in SCC ($\log_{10}$) due to treatment, but the increase in geometric mean from 26,300 to

48,300 cells/mL was much smaller than the increase of approximately 100,000 cells/mL found by Stelwagen and Lacy-Hulbert (1996). This difference was also influenced by increased dilution of somatic cells in the control half-udder, because the calculated increase in absolute SCC in the treatment half-udder was approximately 38%. It is difficult to draw firm conclusions from our findings because the number of previous studies was limited, and they involved 1× milking in the experimental design, which was different from our design of incomplete milking and 2× milking.

CONCLUSIONS

The primary finding of this experiment was an almost immediate and significant decrease in MPR in incompletely milked quarters (23% MRG) compared with completely milked quarters in the same udder. This finding supports current understanding of the influence of autocrine and paracrine factors on milk secretion rate.

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