BRIEF COMMUNICATION: The effect of milking frequency in early lactation on milk yield and milk protein gene expression in the bovine mammary gland

R Murney[a]*, K Stelwagen[b] and K Singh[a]

[a]*AgResearch Ruakura, Private Bag 3123, Hamilton 3240, New Zealand;* [b]*SciLactis, Waikato Innovation Park, Ruakura Road, Hamilton 3240, New Zealand*
**Corresponding author. Email: regan.murney@agresearch.co.nz*

Keywords: milking frequency; early lactation; carry-over effects; milk yield; dairy cow

Introduction

In dairy cows, increased milking frequency (MF) has a positive effect on daily milk yield (MY), providing dietary energy intake is sufficient to maintain the increase in production (Phillips *et al.* 1980). Short-term increases in MF during early lactation may have both an acute effect and a carry-over effect on MY once the animals have returned to less frequent milking (Hale *et al.* 2003). The critical period for increased MF to generate a carry-over effect on MY is within the first three weeks of lactation and may only need to be applied for as little as 14 days (Hale et al. 2003; Wall & McFadden 2007). In contrast, decreased MF for the first three weeks of lactation does not result in a negative carry-over in MY, which can be obtained if the treatment is extended for six weeks (Rémond et al. 1999). Furthermore, unilateral milking experiments where opposing udder halves are milked differentially have demonstrated that both the acute changes in MY and the carry-over effect are regulated, at least in part, locally within the mammary gland (Knight et al. 1992; Stelwagen & Knight 1997; Wall & McFadden 2007). The mechanisms underlying this MY response are not well understood, but may be due to either an increase in the number of secretory mammary epithelial cells and/or the activity of these cells (Stelwagen 2001). The objective of this study was to establish a MF model to investigate molecular mechanisms that regulate changes MY within the mammary gland.

Materials and methods

Animals and manipulations

Seventeen multiparous Holstein-Friesian and Holstein-Friesian x Jersey cows were milked twice daily (2x) until the experiment commenced (2–7 days in milk). Udders halves were randomly assigned to milking once a day (1x) or four times a day (4x) for 14 days, with 1x at 1100 h and 4x at 0500, 1100, 1700 and 2300 h. For the entire treatment period cows were grazed on pasture *ad libitum* and supplemented with 2 kg of a commercial concentrate (12.9 MJ/kg ME, 12.0% CP based on DM) per day. On Day 14, 3–5 hours after the 1100 h milking both rear quarters of 10 animals were biopsied as described by Farr et al. (1996). Cows were then returned to normal farm practice of 2x milking. Half-udder MY data were collected daily during the treatment period. Following treatment period, monthly half-udder MY data and milk samples were collected until Day 200 post-treatment. Milk samples were analysed by infrared spectrometry for fat, protein and lactose (Fossomatic equipment, Livestock Improvement Corporation Herd Testing Station, Hamilton, New Zealand).

RNA extraction and real-time polymerase chain reaction

Mammary biopsy tissue samples were snap frozen in liquid nitrogen and stored at -80°C until processing. Total RNA was extracted, purified and converted to cDNA as described by Singh et al. (2005). Abundance of milk protein mRNA for αS_1-casein, β-casein, α-lactalbumin, and lactoferrin were quantified by real-time polymerase chain reaction (RT-PCR) using the comparative quantification method, with SYBR Green master mix in the Rotogene 6000 system (Qiagen, Hilden, Germany) as described by Smith et al. (2007). The geomean of ubiquitin and β2-microglobulin mRNA abundance was used as an internal control.

Statistical analysis

Differences between udder half MY, calculated as the mean of two adjacent days, and $\log_{10}$-transformed normalised mRNA abundance were analyzed using ANOVA (Minitab 2006) by treatment with cow as a random effect. Differences were considered to be significant at $P < 0.05$.

Results and discussion

The pre-treatment MY for 1x and 4x udder halves were not statistically different at 8.4 kg/d and 8.5 kg/d respectively. By Day 2 of treatment, the MY of the 4x udder halves was 5.2 kg/d ($P < 0.001$) higher than that of the 1x udder halves (Fig. 1). Following this initial increase, the difference between 4x and 1x udder halves steadily increased by 0.3 kg/d per day through the treatment period, culminating in a maximal difference of 7.6 kg/d ($P < 0.001$) by Day 14. During the 2x milking post-treatment period from Day 50 to Day 200, the MY of the 4x udder halves was 1.4 kg/d ($P < 0.001$) greater than the 1x udder halves. There was no difference in milk composition during the post treatment period (R Murney, Unpublished data). The results were consistent with similar unilateral differential milking experiments

Figure 1 Mean milk yield of udder halves for cows differentially milked for 14 days in early lactation. Cows were milked twice daily (2x) (Day -1) and then udder halves were milked either once a day (1x) (dotted line) or four times a day (4x) (solid line) (Days 1–14), and post-treatment 2x milking period (Days 50–200). Bars indicate standard error of the difference.

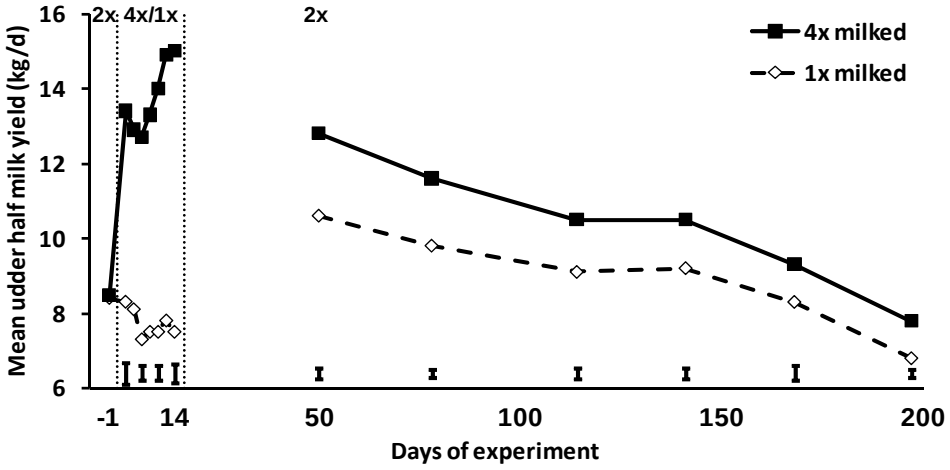
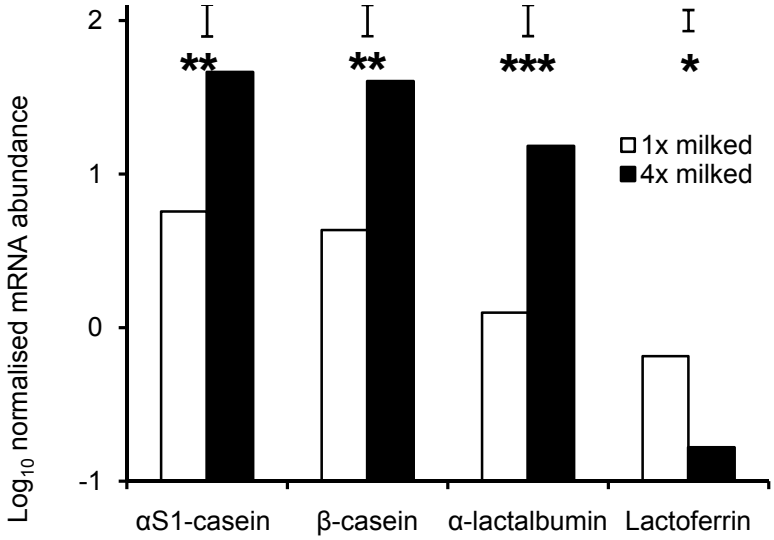


Figure 2 Relative mRNA abundance of milk protein genes in tissue samples collected from udder halves differentially milked once a day (1x) (open bars) and four times a day (4x) (solid bars) for 14 days during early lactation, expressed as log_{10}-transformed means. Bars indicate standard error of the difference and asterisks indicate levels of significance.



and demonstrate that pasture-fed cows have the ability to respond to increases in MF similarly to more intensively managed cows (Wall & McFadden 2007).

The initial response to MF occurred rapidly and by Day 2 the differential MY response between the udder halves was 61%, suggesting that cellular activity increased in 4x halves and decreased in 1x halves, rather than cell number was driving the MY response (Stelwagen 2001).

The milk protein mRNA levels were greater ($P < 0.01$) in 4x milked tissue samples for αS_1-casein by eight-fold, β-casein by nine-fold and α-lactalbumin by 12-fold than in 1x milked tissue samples (Fig. 2). In contrast, the level of lactoferrin mRNA was decreased four-fold ($P < 0.01$) in the 4x milked glands compared with 1x milked glands (Fig. 2). Molenaar et al. (1996) observed that mammary epithelial cells switch between expressing αS_1-casein and lactoferrin suggesting that these genes are reciprocally controlled. The increase in mRNA abundance of αS_1-casein, β-casein and α-lactalbumin, and the decrease in mRNA abundance of lactorferrin in the 4x milked tissue samples may indicate a switch of non-secretory quiescent mammary epithelial cells to a secretory state, as postulated by Vetharaniam et al. (2003).

In conclusion, a MF model has been established that clearly demonstrates a treatment effect, which

can now be utilised to further explore the molecular mechanisms within the mammary gland of dairy cows that respond to changes in MF.

Acknowledgements

The authors gratefully acknowledge the help of the farm staff at Tokanui Dairy and the statistical advice of Harold Henderson. This research was funded by the Ministry of Science and Innovation.

References

Farr VC, Stelwagen K, Cate LR, Molenaar AJ, McFadden TB, Davis SR 1996. An improved method for the routine biopsy of bovine mammary tissue. Journal of Dairy Science 79: 543–549.

Hale SA, Capuco AV, Erdman RA 2003. Milk yield and mammary growth effects due to increased milking frequency during early lactation. Journal of Dairy Science 86: 2061–2071.

Knight CH, Hillerton JE, Kerr MA, Teverson RM, Turvey A, Wilde CJ 1992. Separate and additive stimulation of bovine milk yield by the local and systemic galactopoietic stimuli of frequent milking and growth hormone. Journal of Dairy Research 59: 243–252.

Minitab 2006. Minitab 15 Statistical software. Minitab Inc., Pennsylvania State University, PA, USA.

Molenaar AJ, Kuys YM, Davis SR, Wilkins RJ, Mead PE, Tweedie JW 1996. Elevation of lactoferrin gene expression in developing, ductal, resting regressing parenchymal epithelium of the ruminant mammary gland. Journal of Dairy Science 79:1198–1208.

Phillips DSM, Woolford MW, Copeman PJA 1980. The implications of milking management strategies involving variations of milking frequency in the immediate post-partum period. Proceedings of the New Zealand Society of Animal Production 40: 166–174.

Rémond B, Coulon JB, Nicloux M, Levieux D 1999. Effect of temporary once-daily milking in early lactation on milk production and nutritional status of dairy cows. Annales de Zootechnie 48:341–352.

Singh K, Dobson J, Cooper C, Davis S, Farr V, Molenaar A, Stelwagen K 2005. Milk accumulation decreases expression of genes involved in cell-extracellular matrix communication and initiates apoptosis in the bovine mammary gland. Livestock Production Science 98: 67–78.

Smith C, Berg D, Beaumont S, Standley NT, Wells DN, Pfeffer PL 2007. Simultaneous gene quantitation of multiple genes in individual bovine nuclear transfer blastocysts. Reproduction 133: 231–242.

Stelwagen K, Knight CH 1997. Effect of unilateral once or twice daily milking of cows on milk yield and udder characteristics in early and late lactation. Journal of Dairy Research 64: 487–494.

Stelwagen K 2001. Effect of milking frequency on mammary functioning and shape of the lactation curve. Journal of Dairy Science 84(E. Suppl.): E204–E211.

Vetharaniam I, Davis SR, Soboleva TK, Shorten PR, Wake GC 2003. Modeling the interaction of milking frequency and nutrition on mammary gland growth and lactation. Journal of Dairy Science 86: 1987–1996.

Wall EH, McFadden TB 2007. Optimal timing and duration of unilateral frequent milking during early lactation of dairy cows. Journal of Dairy Science 90: 5042–5048.