Suspension of milking in dairy cows produces a transient increase in milk lactoferrin concentration and yield after resumption of milking

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ABSTRACT

Lactoferrin is a multifunctional glycoprotein with a range of antimicrobial and immune-related properties that is found at >10-fold higher concentration in human milk (~1.7 g/L) relative to bovine milk (~0.15 g/L). Consumer demand is increasing for bovine lactoferrin through a wide range of nutritional and cosmetic consumer products. Increasing lactoferrin yield and concentration in bovine milk could assist in satisfying this increasing demand and may also help in increasing resistance to bovine mammary infection. Two experiments with cows in mid and late lactation were carried out to examine milking strategies to increase milk lactoferrin concentration and yield. Milking was suspended in cows normally milked twice daily, for periods of 2, 4, or 7 d (mid lactation) or 2 or 4 d (late lactation) after which cows were milked out and twice-daily milking resumed for 4 d. In all groups, lactoferrin concentration was significantly increased during the remilking period, approaching concentrations similar to those found in human milk (~1 g/L). Lactoferrin yields were significantly higher in all treatment groups, although increasing the nonmilking period beyond 2 d offered no advantage. Milk yield was lower initially after resumption of milking but recovered to preexperimental values by the fourth day of remilking in all groups, except the 4-d nonmilking group in late lactation. Milk somatic cell count was significantly elevated in all groups at the start of remilking but had substantially reduced by d 4 and reached a preexperimental level in the 2-d nonmilking group of mid-lactation cows. In summary, extended milking intervals can be used as a tool to produce a short-term increase in the concentration and yield of lactoferrin from bovine milk during established lactation, without any apparent long-term effects on milk yield and quality.

Key words: lactation, mammary, milk synthesis, epithelium

INTRODUCTION

Lactoferrin (LF), also sometimes referred to as lactotransferrin, is an 80-kDa glycoprotein, best known for its iron-binding and antibacterial properties (Adlerova et al., 2008). Its biological role appears, however, to be much wider than that of simply being an iron ligand, and it is implicated in playing a role in wider host defense, exerting antiviral, antioxidant, and immunomodulating activities (El-Loly and Mahfouz, 2011). In mammals, LF is present in all biological fluids, such as tears, sweat, saliva, and milk. Neutrophils are an important source of LF, with most of the LF in blood plasma coming from neutrophils (Adlerova et al., 2008). The LF in milk and colostrum is likely also, at least partly, to originate from neutrophils, but the mammary epithelium itself can also synthesize LF (Sanchez et al., 1992; Molenaar et al., 1996).

In milk, LF is associated with the whey fraction, and in human milk, it is the second most abundant whey protein (Vegarud et al., 2000) at a concentration of ~1.7 g/L. Because of the importance of LF in human milk, its role in host defense, and the fact that there is cross-reactivity and structural homology between human and bovine LF (Magnuson et al., 1990), significant interest has arisen to commercially exploit the bioactivity of bovine milk LF. Bovine LF can now be found in a range of commercial consumer products ranging from infant formulas and other nutritional products for humans and pet animals, to skin and oral-care products (Wakabayashi et al., 2006; Stelwagen et al., 2009; García-Montoya et al., 2012). Furthermore, bovine LF has also been proposed as a therapeutic for bovine mastitis and as a synergistic adjunct to penicillin treatment (Lacasse et al., 2008). Increasing consumer demand for LF for both human and animal applications may necessitate increasing the availability of LF for extraction of LF from milk, which is the context for the current study. However, a competitive approach also exists through the production of recombinant bovine and human LF for therapeutic purposes (Weinberg, 2007).

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In bovine milk, LF is considered a minor whey protein, with its concentration being much lower at 0.1 to 0.2 g/L (Vegarud et al., 2000; Farr et al., 2002) than that of human milk. However, the concentration of LF in milk varies significantly between individual cows in the same herd (Stelwagen et al., 2009) and is affected by the physiological status of the cow, specifically stage of lactation, udder health (i.e., milk SCC score), and daily milk yield (Cheng et al., 2008). In addition, reducing milking frequency from twice daily to once daily increases the concentration of LF in milk (Farr et al., 2002; Stelwagen et al., 2011), demonstrating that milking management can be a tool to increase milk LF concentration.

The objectives of the present study were to examine the effect of an extended period without milking during mid and late lactation on the concentration and yield of LF in bovine milk and to determine whether management of milking intervals could be used to increase LF yield for commercial use. The underlying hypothesis was that suspension of milking for more than 24 h would increase the concentration and yield of LF in bovine milk during recovery and that the effect would be more pronounced during late lactation.

MATERIALS AND METHODS

Experimental Protocol

Lactating dairy cows, grazing rye grass–white clover pasture, were subjected to extended periods (up to 7 d) without milking at 2 stages of lactation (mid and late). Individual milk yields were recorded at each milking before and after treatment.

In the first experiment, 18 pregnant, mid-lactation Friesian, Jersey, and Friesian $\times$ Jersey cross cows (116 ± 16 DIM; 15.4 ± 0.7 kg/d of milk) were allocated to 1 of 3 groups ($n = 6$ per group, balanced for age, breed, and milk yield). Immediately before the trial, the milk of all cows was tested bacteriologically to ensure udders were free of infection. Subsequently, cows were not milked for 2 (2-d group), 4 (4-d group), or 7 d (7-d group), after which twice-daily milking of all cows resumed (d 0) and the production of cows followed for a further 4 d.

Experiment 2 was conducted in a similar manner to experiment 1, except that 20 cows in late lactation were used (240 ± 15 DIM; 9.3 ± 0.5 kg/d of milk). Prior to the experiment the milk of all cows was tested bacteriologically to ensure udders were free of infection. Then, cows were not milked for 2 (2-d group) and 4 d (4-d group), after which twice-daily milking was resumed (d 0) and milk yield recorded at each milking for a further 4 d.

All animal procedures were carried out in compliance with the animal welfare guidelines and the approval of the Ruakura Animal Ethics Committee (Hamilton, New Zealand).

Milk Sampling and Analyses

In experiments 1 and 2, milk samples were obtained at each milking during 2 d before trial and during the first milking and the following 4 d of remilking.

Milk samples were analyzed by infrared spectrometry for fat, protein, and lactose content (Milkoscan Foss Electric A/S, Hillerød, Denmark), and SCC was determined using a fluorometric cell-counting technique (Fossomatic; Foss Electric A/S).

Lactoferrin concentrations in milk were measured by ELISA kit, following the manufacturer's instructions (Bethyl Laboratories, Montgomery, TX).

Statistical Analyses

Statistical analyses were conducted using ProStat (Version 6.5; Poly Software International, Pearl River, NY). Comparisons with preexperimental data were analyzed by ANOVA. During the remilking period, treatment differences were analyzed by multivariate ANOVA, because of the repeated measures nature of the data at different days. In case of an overall treatment effect, individual time points were then analyzed by ANOVA. Means were considered significantly different at $P < 0.05$.

RESULTS

Milk composition was normal for the breeds and stage of lactation before the suspension of milking and did not differ among any of the groups during mid or late lactation (Table 1). Milk-composition data (i.e., fat, protein, lactose) for both experiments during the remilking period are only shown when concentrations had returned to normal, i.e., when they were no longer statistically different from pretrial values (Table 1). Initial milk-composition data following remilking are not reported, because they were outside of the normal calibration range used for mid-infrared spectroscopy or an insufficient sample was obtained. Interestingly, on d 0 a marked increase in yellow color of the secretion (most notably in the 4-d and 7-d groups) was observed, giving the secretion an appearance resembling colostrum.

Experiment 1

Milk-yield response data of the cows in mid lactation are shown in Figure 1. Preexperiment milk yield

did not differ significantly among the groups, but during remilking a treatment effect was significant ($P < 0.05$), because of the higher milk yield in the 2-d group. Overall, yield of the 4-d and 7-d groups did not differ significantly. The yield at the first milking was similar to the twice-daily pretreatment yield in the 2- and 4-d groups but was reduced ($P < 0.01$) in the 7-d group, possibly indicative of significant resorption of milk in the latter. On d 1 (the first 24 h after removal of accumulated secretion) the milk yield was substantially lower ($P < 0.001$) in all 3 groups compared with their respective preexperimental yields but began to recover in all groups on d 2, and by d 4 yields no longer differed statistically from preexperimental values. The recovery appeared to be slower with the longer periods of suspension of milking.

Milk composition is shown in Table 1. Milk fat had returned to the pretreatment level by d 2 in the 2-d group and by the following day in the 4-d and 7-d groups. Milk protein content showed a very similar pattern, except that by d 2 the milk protein content of the 2-d group had also returned to the pretreatment level. Lactose content took the longest to return to pretreatment levels, by d 2 in the 2-d group and by d 4 in the other 2 groups.

The LF concentrations and yield from milk of mid-lactating cows are in Figures 2A and 2B, respectively. Both the preexperimental LF concentration and LF yield did not differ between the 3 groups. The treatment effect for LF concentration during the remilking period was significant ($P < 0.001$), with the 4-d group having the highest concentration during the

Figure 1. Average milk yield of cows that were remilked for 4 d after cessation of milking during mid lactation for 2 (●), 4 (▲), or 7 d (■). Preexperimental values represent the average of 2 d of normal milking preceding the extended milking intervals. Day 0 represents the first milking after reinitiation of milking, and the yields at subsequent days are based on p.m. and a.m. values. Error bars are SEM. Values significantly different from preexperiment controls are indicated (* $P < 0.05$; *** $P < 0.001$).

first milking (d 0) and the 2-d group having the lowest. Subsequently, whereas the 4-d and 7-d groups no longer differed significantly, the 2-d group maintained the lowest ($P < 0.05$) LF concentration. At every time point during remilking, the LF concentration in each group remained elevated ($P < 0.05$) compared with the preexperimental period, except on d 4 when in the 2-d group the concentration had returned closest to the preexperimental level.

Table 1. Gross milk composition before treatments started (Pre) and during remilking¹ following 2, 4, or 7 d of nonmilking during mid lactation and from 2 and 4 d of nonmilking during late lactation

Item	Mid lactation				Late lactation		
	2 d	4 d	7 d	SE ² (pooled)	2 d	4 d	SE ² (pooled)
Fat (%)							
Pre	5.27	5.35	5.67	0.55	6.28	6.04	0.65
After d 2	4.55	—	—	—	—	—	—
After d 3	4.78	5.27	5.43	0.60	6.33	—	—
After d 4	4.95	5.64	5.12	0.79	5.66	4.76	0.75
Protein (%)							
Pre	3.81	3.95	4.00	0.33	4.30	4.23	0.23
After d 2	3.38	3.42	—	—	4.30	—	—
After d 3	3.80	3.71	4.15	0.22	4.30	4.43	0.28
After d 4	3.93	3.99	4.37	0.25	4.49	4.49	0.24
Lactose (%)							
Pre	4.84	4.86	4.91	0.09	4.75	4.78	0.07
After d 2	—	—	—	—	—	—	—
After d 3	4.74	—	—	—	—	—	—
After d 4	4.83	4.78	4.73	0.10	4.61	—	—

¹Data shown are those when the composition had returned to pretreatment levels.

²No significant treatment effect was observed ($P > 0.05$).

Table 2. Daily lactoferrin concentration (mg/g of protein) before treatments started (Pre) and during d 3 and 4 of remilking following 2, 4, or 7 d of nonmilking during mid lactation and from 2 and 4 d of nonmilking during late lactation

Item	Mid lactation				Late lactation		
	2 d	4 d	7 d	SE ¹ (pooled)	2 d	4 d	SE ¹ (pooled)
Pre	5.0 ^a	7.4 ^a	4.5 ^a	2.0	7.7 ^a	6.7 ^a	1.8
After d 3	17.6 ^b	22.7 ^b	20.2 ^b	3.7	26.4 ^b	31.0 ^b	4.0
After d 4	13.4 ^b	14.2 ^c	11.4 ^c	2.6	20.4 ^b	27.6 ^b	5.1

^{a-c}Means within a column with different superscripts differ ($P < 0.01$).

¹No significant differences observed among treatment lengths ($P > 0.05$).

The daily LF yield (Figure 2B) reflects the milk yield and the LF concentration. No overall treatment effect of LF yield was found during remilking. However, in the

4-d and 7-d groups, the yield was substantially higher ($P < 0.01$) than it was preexperimentally and that in the 2-d group tended ($P < 0.06$) to be elevated. In all 3 groups the LF yield remained substantially elevated ($P < 0.01$) and, although declining, had not returned to preexperimental values by d 4. Milk LF content was also calculated per unit milk protein (Table 2), when milk protein concentrations had returned to pretreatment values. Lactoferrin concentration per milligram of protein was elevated in all 3 groups on d 3 and 4, compared with pretreatment, with the values starting to decline after d 4.

The SCC in the milk of the mid-lactating cows is in Figure 3. The preexperimental SCC was low ($< 37,000 \pm 5,000$ cells/mL) in all 3 groups and did not differ statistically. During the remilking period a significant ($P < 0.05$) treatment effect existed because of the higher count in the 7-d group. The SCC peaked in all groups on d 1 and then rapidly declined but had only reached the preexperimental level by d 4 in the 2-d group.

Figure 2. Average milk lactoferrin concentration (A) and yield (B) of cows that were remilked for 4 d after cessation of milking during mid lactation for 2 (●), 4 (▲), or 7 d (■). Preexperimental values represent the average of 2 d of normal milking preceding the extended milking intervals. Day 0 represents the first milking after reinitiation of milking, and the yields at subsequent days are based on p.m. and a.m. values. Error bars are SEM. All values after treatment were significantly different from control values ($P < 0.05$), except 2-d treatment at d 4 (NS).

Experiment 2

Daily milk yields immediately preexperiment and during the remilking period in late-lactation cows are shown in Figure 4. The preexperimental values of the 2 groups did not differ significantly, and although during the remilking period the 4-d group tracked below the 2-d group, the difference between the 2 groups was not significant. As in Experiment 1, the yields during the first milking (d 0) were similar in both groups and comparable to the preexperimental yield. Subsequently, and similar to the pattern in mid-lactation cows, the yield declined sharply in both groups ($P < 0.0001$) and then increased progressively during subsequent days. By d 2 the yields in the 2-d group had returned to preexperimental values, whereas those in the 4-d group were still lower ($P < 0.05$) at d 4.

The level of milk fat returned to the pretrial level by d 3 and d 4, for the 2-d and 4-d groups, respectively (Table 1). For protein levels these times were d 2 and

Figure 3. Average milk SCC of cows that were remilked for 2 d after cessation of milking during mid lactation for 2 (●), 4 (▲), or 7 d (■). Preexperimental values represent the average of 2 d of normal milking preceding the extended milking intervals. Day 0 represents the first milking after reinitiation of milking, and the yields at subsequent days are based on p.m. and a.m. values. Error bars are SEM. All values after treatment were significantly higher than control values ($P < 0.05$).

d 3, respectively. The lactose content only returned to normal by d 4 in the 2-d group, and in the 4-d group it had not returned to normal by d 4.

Figures 5A and 5B depict the LF concentration and yield in the milk of late-lactation cows. Neither LF concentration or yield differed between the 2 groups before the experiment. The LF concentration during remilking was highest ($P < 0.01$) in the 4-d group. However, in

Figure 4. Average milk yield of cows that were remilked for 4 d after cessation of milking during late lactation for 2 (●) or 4 d (▲). Preexperimental values represent the average of 2 d of normal milking preceding the extended milking intervals. Day 0 represents the first milking after reinitiation of milking, and the yields at subsequent days are based on p.m. and a.m. values. Error bars are SEM. All milk yields after treatment were significantly lower than control values ($P < 0.05$), except for the 2-d group at d 4.

Figure 5. Average milk lactoferrin (LF) concentration (A) and yield (B) of cows that were remilked for 4 d after cessation of milking during late lactation for 2 (●) or 4 d (▲). Preexperimental values represent the average of 2 d of normal milking preceding the extended milking intervals. Day 0 represents the first milking after reinitiation of milking, and the yields at subsequent days are based on summation of p.m. and a.m. values. Error bars are SEM. All values for milk LF concentration and yield after treatment were significantly different from control values ($P < 0.05$), except for LF yield on d 1 (NS; $P > 0.05$).

both groups the concentration was elevated ($P < 0.05$) compared with preexperimental values, and although beginning to decline after d 2, by d 4 the LF concentration in both groups remained relatively elevated ($P < 0.001$) and apparently higher than in the mid-lactation groups.

Similar to experiment 1, the LF yield was significantly ($P < 0.01$) elevated at d 0, compared with preexperimental values (Figure 5B), and then dropped sharply on d 1, due to the low milk yield at this time. As a result, the LF yield in both groups did not differ on d 1 from the preexperimental values. However, subsequently, LF yield increased rapidly until d 3 and at d 4 remained elevated ($P < 0.01$). As in mid-lactation

cows, the LF yield, expressed per unit milk protein, was significantly ($P < 0.01$) elevated in both groups on d 3 and 4 compared with pretreatment levels (Table 2).

The SCC for the late-lactation groups are shown in Figure 6. Both groups had low SCC ($51,000 \pm 12,000$ cells/mL) during the preexperimental period, and no statistical differences existed between the groups at this time. Whereas SCC was not statistically different between the 2 groups during remilking, both groups had elevated ($P < 0.05$) SCC compared with the preexperimental period. Although in both groups SCC values had declined by d 4, they were still higher ($P < 0.05$) than preexperimental values.

DISCUSSION

This study examined the effect of extended periods of nonmilking during mid and late lactation on milk LF concentration and yield. The results indicate that an extended milking interval lasting for 48 h, or more, increased both the concentration and yield of milk LF for several days after the resumption of milking. Furthermore, the concentration of LF achieved in the milk was approaching that found in human milk (Vegarud et al., 2000). The elevation of LF concentration observed in late lactation was sustained through 4 d after treatment and may have continued for several more days. More data are required to establish the duration of the LF response in late-lactation animals.

Little difference existed in milk LF concentration achieved during remilking in mid or in late lactation, although concentrations were sustained for longer in late lactation. However, the yield of LF was lower in the late-lactation experiment when comparing the 2-d and 4-d groups. The higher LF yield during mid lactation appears to be a reflection of the higher milk yield at that time.

The LF yield in the 7-d group (mid lactation) was significantly lower than in the 2- and 4-d groups. The bovine mammary gland undergoes significant morphological changes during the first week of nonmilking (Holst et al., 1987; Hurley, 1989), and it is conceivable that involution of the mammary gland had progressed to a point where recovery of secretion was relatively delayed. Interestingly, it was observed that some of the mammary secretions obtained at the first milking (d 0) were of yellowish, colostrum-like appearance.

A 7-d group was not included in the late-lactation experiment, largely because from the mid-lactation data it was apparent that the shorter intervals were most efficacious and, also, recovery from a 7-d interval may have been impossible in late lactation.

Increasing the nonmilking period beyond 2 d did not appear to offer any LF yield advantage. In mid-lacta-

Figure 6. Average milk SCC of cows that were remilked for 4 d after cessation of milking during late lactation for 2 (●) or 4 d (▲). Preexperimental values represent the average of 2 d of normal milking preceding the extended milking intervals. Day 0 represents the first milking after reinitiation of milking, and the yields at subsequent days are based on summation of p.m. and a.m. values. Error bars are SEM. The SCC were significantly elevated in both treatment groups ($P > 0.05$) throughout the 4-d posttreatment period.

tion cows LF yield did not differ between the 2- and 4-d groups, and in the late-lactation cows LF yield was actually lower in the 4-d group.

Although milk yield was significantly reduced upon remilking, yield in all 3 mid-lactation groups had recovered to preexperimental yields by d 4. In late-lactation groups yields of the 2-d group had also recovered, but that of the 4-d group had not quite recovered by d 4. Similarly, gross milk composition had also returned to normal levels by d 4 (except for the lactose level in the late-lactation 4-d group). Milk SCC, although only returning to pretrial levels in the mid-lactation 2-d group, had significantly reduced by d 4 in all groups, approaching pretreatment levels. Collectively, these results indicate that the bovine mammary gland has a remarkable ability to return to a lactating state after mammary involution has been initiated.

The time at which mammary involution becomes irreversible has yet to be determined exactly. In the early phase following cessation of milking, the udder becomes full of milk and secretion ceases within 24 to 36 h of the last milking (Davis et al., 1998). Also, leucocytes invade the udder, and around 3 d of mammary engorgement, tight junctions between cells break down, milk mixes with interstitial fluid, and the process of resorption of milk is initiated (Hurley, 1989). However, it appears that with reinitiation of regular milking, yields can recover fully after at least 7 d of nonmilking (Dalley and Davis, 2006). Partial recovery may even occur after 11, 14, or even 28 d of nonmilking (Noble and Hurley, 1999; Singh et al., 2012).

Consistent with its role in (innate) immune function, endogenous LF concentrations in milk are highest during mammary infection and following cessation of milking, when cows are dried off (Hurley and Rejman, 1993; Cheng et al., 2008; Stelwagen et al., 2009). Moreover, it has been shown that when normal twice-daily milking frequency is reduced to once-daily milking, LF levels in milk were increased, by approximately 2-fold (Farr et al., 2002; Stelwagen et al., 2011). In both experiments, SCC significantly increased upon remilking, but during the course of remilking, the levels declined rapidly approaching preexperiment values after 4 d. This rapid decline, and the fact that no clinical signs of mastitis were observed, suggests that the increase in SCC was not due to mammary infection but to a transient inflammatory response related to activation of the innate immune system, similar to that observed during drying off (Hurley, 1989; Singh et al., 2005; Silanikove et al., 2013). Consistent with this notion is the brief elevation of milk SCC observed with the transition from twice- to once-daily milking (Stelwagen and Lacy-Hulbert, 1996). From a practical management standpoint, a nonmilking strategy may not be suitable for cows that have an elevated milk SCC due to mastitis, before the start of an extended milking interval, because nonmilking in such a case is likely to lead to very high SCC (Kamote et al., 1994).

Lactoferrin expression in the lactating mammary gland appears to be associated mostly with engorged (stretched) alveoli and also ductal epithelium (Molenaar et al., 1996). Furthermore, LF expression in secretory alveoli is inversely related to CN and α -LA expression. (Molenaar et al., 1992). Change in cell shape has been linked to expression of LF in mammary cells in vitro (Close et al., 1997) and may be the primary driver of the changes in LF secretion observed here. However, a role for leucocytes in the regulation of LF expression in secretory and ductal epithelium cannot be discounted. Nevertheless, it does appear that in a lactating gland, recovering its lactation state following engorgement, enhanced LF synthesis and secretion can be maintained for several days. Certainly, the enhanced concentration of LF relative to milk protein suggests enhanced differential expression of LF.

Lactoferrin can be extracted, for commercial purposes, from milk or derived from transgenic organisms. Lactoferrin derived from transgenic production is mostly used in human medical applications, whereas generally, LF isolated from, predominantly bovine, milk is used for food, oral-care, and cosmetic applications (El-Loly and Mahfouz, 2011). Unlike human milk, where LF is the second most common milk protein, in bovine milk, produced by healthy cows during established lactation, LF concentrations in milk are very low (Vegarud et al.,

2000; Cheng et al., 2008). Thus, to extract quantities of LF of commercial relevance requires large volumes of milk to be processed. Enhancing the concentration of LF in milk offers scope to increase the efficiency of commercial extraction and production of LF or the production of LF-enriched whey protein fractions.

The strategy of using extended milking intervals, as described here, was effective in increasing both milk LF concentration and yield. However, a full economic analysis of such an approach is required that takes into account the reduced yield of milk and reduced milk value because of high SCC. Furthermore, the incidence of mammary infection, as a result of the extended milking interval, may limit applicability of the extended milking interval approach, although this requires evaluation.

In conclusion, it is possible to use extended milking intervals as a tool to increase the concentration of LF in bovine milk to values approaching those found in human milk. Furthermore, it is possible to increase milk LF yield during established lactation, without any apparent long-term adverse effects on milk yield and quality. The results suggest that it is possible to employ such a strategy during both mid and late lactation and that no advantage of extending the nonmilking period beyond 2 d is apparent. A milking management plan could be envisaged where 2-d milking intervals are applied repeatedly during a lactation to enhance LF production.

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REFERENCES

- Adlerova, L., A. Bartoskova, and M. Faldyna. 2008. Lactoferrin: A review. *Vet. Med.* 53:457–468.
- Cheng, J. B., J. Q. Wang, D. P. Bu, G. L. Liu, C. G. Zhang, H. Y. Wei, L. Y. Zhou, and J. Z. Wang. 2008. Factors affecting the lactoferrin concentration in bovine milk. *J. Dairy Sci.* 91:970–976.
- Close, M. J., A. R. Howlett, C. D. Roskelley, P. Y. Desprez, N. Bailey, B. Rowning, C. T. Teng, M. R. Stampfer, and P. Yaswen. 1997. Lactoferrin expression in mammary epithelial cells is mediated by changes in cell shape and actin cytoskeleton. *J. Cell Sci.* 110:2861–2871.
- Dalley, D. E., and S. R. Davis. 2006. Effect of an extended milking interval on recovery of milk yield and somatic cell count in dairy cows. *Proc. N.Z. Soc. Anim. Prod.* 66:241–243.

- Davis, S. R., V. C. Farr, P. J. A. Copeman, V. R. Carruthers, C. H. Knight, and K. Stelwagen. 1998. Partitioning of milk accumulation between cisternal and alveolar compartments of the bovine udder: Relationship to production loss on once-daily milking. *J. Dairy Res.* 65:1–8.
- El-Loly, M. M., and M. B. Mahfouz. 2011. Lactoferrin in relation to biological functions and applications: A review. *Int. J. Dairy Sci.* 6:79–111.
- Farr, V. C., C. G. Prosser, D. A. Clark, M. Tong, C. V. Cooper, D. Willix-Payne, and S. R. Davis. 2002. Lactoferrin concentration is increased in milk from cows milked once-daily. *Proc. N.Z. Soc. Anim. Prod.* 62:225–226.
- Garcia-Montoya, I. A., T. S. Cendon, S. Arevalo-Gallegos, and Q. Rascon-Cruz. 2012. Lactoferrin a multiple bioactive protein: An overview. *Biochim. Biophys. Acta* 1820:226–236.
- Holst, B. D., W. L. Hurley, and D. R. Nelson. 1987. Involution of the bovine mammary gland: Histological and ultrastructural changes. *J. Dairy Sci.* 70:935–944.
- Hurley, W. L. 1989. Mammary gland function during involution. *J. Dairy Sci.* 72:1637–1646.
- Hurley, W. L., and J. J. Rejman. 1993. Bovine lactoferrin in involuting mammary tissue. *Cell Biol. Int.* 17:283–289.
- Kamote, H. I., C. W. Holmes, D. D. S. Mackenzie, R. J. Holdaway, and B. W. Wickham. 1994. Effect of once daily milking in later lactation on cows with low or high initial somatic cell counts. *Proc. N.Z. Soc. Anim. Prod.* 54:285–287.
- Lacasse, P., K. Lauzon, M. S. Diarra, and D. Petitclerc. 2008. Utilization of lactoferrin to fight antibiotic-resistant mammary gland pathogens. *J. Anim. Sci.* 86(Suppl.):66–71.
- Magnuson, J. S., J. F. Henry, T. T. Yip, and W. T. Hutchens. 1990. Structural homology of human, bovine, and porcine milk lactoferrins: Evidence for shared antigenic determinants. *Pediatr. Res.* 28:176–181.
- Molenaar, A. J., S. R. Davis, and R. J. Wilkins. 1992. Expression of α -lactalbumin α -S1-casein and lactoferrin genes is heterogeneous in sheep and cattle mammary tissue. *J. Histochem. Cytochem.* 40:611–618.
- Molenaar, A. J., Y. M. Kuys, S. R. Davis, R. J. Wilkins, P. E. Mead, and J. W. Tweedie. 1996. Elevation of lactoferrin gene expression in developing, ductal, resting, and regressing parenchymal epithelium of the ruminant mammary gland. *J. Dairy Sci.* 79:1198–1208.
- Noble, M. S., and W. L. Hurley. 1999. Effects of secretion removal on bovine mammary gland function following an extended milk stasis. *J. Dairy Sci.* 82:1723–1730.
- Sanchez, L., L. Lujan, R. Oria, H. Castillo, D. Perez, J. M. Ena, and M. Calvo. 1992. Synthesis of lactoferrin and transport of transferrin in the lactating mammary gland of sheep. *J. Dairy Sci.* 75:1257–1262.
- Silanikove, N., U. Merin, F. Shapiro, and G. Leitner. 2013. Early mammary gland metabolic and immune responses during natural-like and forceful drying-off in high-yielding dairy cows. *J. Dairy Sci.* 96, 6400–6411.
- Singh, K., J. Dobson, C. V. C. Phyn, S. R. Davis, V. C. Farr, A. J. Molenaar, and K. Stelwagen. 2005. Milk accumulation decreases expression of genes involved in cell-extracellular matrix communication and is associated with induction of apoptosis in the bovine mammary gland. *Livest. Prod. Sci.* 98:67–78.
- Singh, K., K. Swanson, H. V. Henderson, and K. Stelwagen. 2012. The effect of re-milking following extended non-milking periods on lactation in dairy cows. *Proc. N.Z. Soc. Anim. Prod.* 72:70–72.
- Stelwagen, K., M. K. Broadhurst, K. Kim, A. J. Molenaar, D. P. Harris, and T. T. Wheeler. 2011. Reduced milking frequency increases the concentration of host-defense proteins in milk. *J. Dairy Sci.* 94(E-Suppl.):751.
- Stelwagen, K., E. Carpenter, B. Haigh, A. Hodgkinson, and T. Wheeler. 2009. Immune components of bovine milk and colostrum. *J. Anim. Sci.* 87(Suppl.):3–9.
- Stelwagen, K., and S. J. Lacy-Hulbert. 1996. Effect of milking frequency on milk somatic cell count characteristics and mammary secretory cell damage in cows. *Am. J. Vet. Res.* 57:902–905.
- Vegarud, G. E., T. Langsrud, and C. Svenning. 2000. Mineral-binding milk proteins and peptides; occurrence, biochemical and technological characteristics. *Br. J. Nutr.* 84(Suppl 1):S91–S98.
- Wakabayashi, H., K. Yamauchi, and M. Takase. 2006. Lactoferrin research, technology and applications. *Int. Dairy J.* 16:1241–1251.
- Weinberg, E. D. 2007. Antibiotic properties and applications of lactoferrin. *Curr. Pharm. Des.* 13:801–811.