Effect of unilateral once or twice daily milking of cows on milk yield and udder characteristics in early and late lactation

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Summary. Twelve multiparous British Friesian cows in early (40 ± 23 d in milk; $n = 6$) or late (216 ± 17 d in milk; $n = 6$) lactation were used to study the effects of milking frequency on yield, udder volume and milk storage within the udder. After a 2 week control period of twice daily milking, diagonally opposed udder halves within a cow were milked once or twice daily for 3 weeks. Milk yield was 28–38% lower from the halves that were milked once daily than from halves that were milked twice daily. The loss of milk yield, expressed as a decrease in the relative milk yield quotient (an index that accounts for pretreatment differences), was greater for cows in early than in late lactation (0·59 *v.* 0·68). Empty udder-half volume was not decreased by once daily milking, suggesting that no cell loss occurred. Instead, once daily milking reduced the secretion efficiency (units of milk per unit of empty udder-half volume) by 46 and 27% respectively in early and late lactation; thus, at least part of the loss was due to reduced metabolic activity of the mammary epithelium. There were positive correlations between the relative milk yield quotient and the proportion ($r = 0{\cdot}804$) or volume ($r = 0{\cdot}644$) of cisternal milk in the glands that were milked once daily. These results confirm that, during extended milking intervals, milk loss was smallest for cows that stored a larger proportion of milk in the gland cistern.

For dairy industries, such as those in New Zealand, that have low input and are not subsidized, once daily milking (ODM), as opposed to twice daily milking (TDM), during part of the lactation cycle may be a viable management tool to reduce costs. Previous studies, however, demonstrated a reduction in milk yield varying from as little as 7% to as much as 50% as a result of ODM (Claesson *et al*. 1959; Holmes *et al*. 1992; Carruthers *et al*. 1993; Knight & Dewhurst, 1994; Stelwagen *et al*. 1994*a*). The mechanisms through which milk yields are reduced remain elusive. However, in studies in which cows were subjected to unilateral TDM or milking three times daily, an increase in milk yield was observed only in the more frequently milked glands (Cash & Yapp, 1950; Knight *et al*. 1994). Moreover, in studies with sheep (Morag, 1968) and goats (Wilde & Knight, 1990) that were unilaterally milked once or twice daily, milk yield was only compromised from the glands that were milked once daily. These results suggest that milk secretion is a function of local intramammary control. The first objective of the present study was to examine whether in cows exposure to simultaneous ODM and TDM of diagonally opposed

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udder halves caused a reduction in yield only from the glands that were milked once daily, and to determine whether such a reduction in yield depended on the stage of lactation (i.e. early or late lactation).

Recent studies suggested that udder anatomy, in relation to milk storage characteristics, might be an important factor in determining reduced yield associated with ODM. Stelwagen *et al.* (1996) showed that, for cows, continuous unilateral draining of cisternal milk during a 24 h period increased milk yield over that of the opposite glands that were drained only once after 24 h. These data indicate that cisternal capacity might be limiting during ODM. Moreover, Knight & Dewhurst (1994) showed that cows that store a bigger proportion of milk in the gland cistern are better able to tolerate ODM than those that store a relatively larger proportion of milk in the alveolar compartment. A second objective of the study was to investigate the relationship between the reduction in milk yield and cisternal milk as a proportion of total yield.

For goats, milk yield prior to peak yield is predominantly a function of the metabolic activity of secretory cells, but post-peak yield is mainly a function of the number of secretory cells (Knight & Wilde, 1987). Moreover, the number of mammary secretory cells increases in goats as a long-term response to a unilateral increase in milking frequency from TDM to milking three times daily (Wilde *et al.* 1987). The final objective of this study, therefore, was to use empty udder-half volume (EUHV) to assess tissue loss and gross secretion efficiency.

MATERIALS AND METHODS

Cows and management

Twelve multiparous (second to eighth parity) British Friesian cows from the Hannah Research Institute herd (Ayr, Scotland) were used. Cows were either in early (40 ± 23 d in milk; $n = 6$) or in late (216 ± 17 d in milk; $n = 6$) lactation at the start of the experiment. Cows were milked at 05.00 and 15.00. Consistent with normal summer management practices, cows were allowed to graze day and night on rye grass pasture, supplemented with concentrates (180 g crude protein/kg) at an amount dictated by pretreatment milk yield. Cows were allowed *ad lib.* access to fresh water.

Milking of half udders

Both early and late lactation groups were subjected to the same experimental protocol; the only difference between the groups was stage of lactation. The cows were routinely milked in a milking parlour equipped to collect milk separately from half udders (i.e. diagonally opposed glands) and to weigh the milk to the nearest 100 g. During the first 2 weeks of the experiment, the control period, mammary glands of all cows were milked twice daily. Then, during the following 3 weeks (treatment period), the right rear and left front quarters of the udder were switched to ODM at 05.00; the left rear and right front quarters remained on TDM throughout the experiment.

Measurement of udder-half volume

Measurements of EUHV were made 4–5 h after the morning milking on day 8 for cows in late lactation and on day 9 for cows in early lactation during the control period and at the end of the treatment period (day 35 for late and day 36 for early lactation cows). To ensure the udders were empty at the time of measurement, each cow was milked with the aid of oxytocin (10 i.u., i.m. in the hip area; Oxytocin-S;

Intervet, Cambridge CB4 4FP, UK) immediately before each EUHV measurement. Volume measurements were based on a previously described method (Knight & Dewhurst, 1994). Briefly, the cows were placed in a cattle crush, and following mild general sedation (30 mg xylazine, i.m.; Rompun; Bayer, Bury St Edmunds IP32 7AH, UK), the hind legs were tied apart. Udder cream was applied all over the udder. The udder was then covered with a quick-setting (~ 2 min), spray-on foam (Froth-Pak; Foampax Scotland, Newmilns KA16 9HG, UK), which produced a cast of the udder. In the present study, this method for measuring udder volume was adapted to measure the volumes of separate quarters, by fitting cardboard partitions along the position of the median and lateral ligaments. The median ligament causes a clear indentation between the left and right udder halves, which shows up as a definite ridge in the casts, thus providing for easy separation of the two halves. Differences between the front and rear halves are much less pronounced. However, in a side view the front of the udder could be clearly distinguished from the rear half. Immediately before casting a line was painted between the front and rear halves, and after removal of the casts this line had copied on to the inside of the cast, indicating where to position the cardboard partitions. The volume of each quarter was measured by filling the cast with barley (specific gravity of 0·62). The final volume used for each quarter was the mean of three separate volume measurements. By adding the volumes of diagonally opposed quarters it was possible to determine treatment effects on EUHV.

Measurement of cisternal milk

Cisternal and alveolar milk were measured 8 h after the morning milking in the rear glands on two occasions, 2 d before the udder volume measurements (i.e. on days 6 and 33 for cows in late lactation and on days 7 and 34 for cows in early lactation). Cisternal milk was drained from the glands using cannulas that were normally used to drain milk from glands with damaged teat sphincters. Cannulas had been inserted into the rear teats after the morning milking and were closed with plugs. Milk was drained 8 h after the morning milking by carefully removing the plugs so that no milk ejection reflex was induced. Knight *et al.* (1994) previously determined that 8 h after morning milking is the most effective time to measure the cisternal milk fraction. Alveolar milk was obtained immediately following drainage, when the cows were machine milked, using a quarter milker. To ensure removal of milk, each cow received 10 i.u. oxytocin i.m. (Oxytocin-S; Intervet) immediately before the removal of alveolar milk.

Statistical analysis

Differences between udder halves or differences within gland between the control and treatment periods were evaluated by paired t test, and effects of stage of lactation were assessed by ANOVA. Differences were considered to be significant at $P < 0{\cdot}05$. The relationship between reduced milk yield and the proportion of cisternal milk was assessed by simple linear regression analysis. SEM are presented for the ANOVA test and SED for the paired t test. All statistical analyses were carried out using Minitab (1991).

During the control period, yield from the glands of one cow in late lactation that was milked twice daily was less than half of that from the opposite glands. This anomaly persisted through the treatment period. Therefore, all results pertaining to this cow were excluded from analyses and results reported for the late lactation group were based on five cows.

Results and discussion

The overall objective of the present experiment was to study udder characteristics in relation to a reduction in milk yield associated with ODM, using a within-udder model for comparing ODM with TDM. The milk yield from the glands that were milked once daily was significantly lower during both stages of lactation compared with the yield from glands that were subjected to TDM (Fig. 1). For cows in late lactation, milk yield was reduced by 28% when TDM glands were compared with ODM. However, when the reduction attributable to ODM was expressed as a percentage of the yield during the control period (i.e. comparisons within an udder half), milk yield was reduced by as much as 37% (Fig. 1*b*). For cows in early lactation, reduction in yield as a result of ODM was 38% (i.e. within or between udder halves) (Fig. 1*a*). However, during the first week of the treatment period, there was a small (6%) but significant ($P < 0{\cdot}05$) compensatory increase in the yield of the glands on TDM. Linzell & Peaker (1971) introduced the relative milk yield quotient ($RMYQ = (t_2/t_1)(c_1/c_2)$, where t_2 is yield of the test glands in the treatment period, t_1 the average yield of the test glands in the control period, c_1 the average yield of the control glands in the control period and c_2 the yield of the control glands in the treatment period). The *RMYQ* accounts for compensatory increases or decreases in yield of the control glands during the treatment period and, as such, presents the net treatment effect. Thus, an *RMYQ* of < 1, 1 or > 1 indicates, respectively, a decrease, no effect or an increase in yield due to treatment. Table 1 lists the *RMYQ* for cows in early and late lactation for each week of the treatment period; the means in the control period were used for c_1 and t_1. In all cases, ODM decreased net yield. The milk yield reduction attributable to ODM was consistent with that observed in other recent studies involving cows (Carruthers *et al.* 1993; Knight & Dewhurst, 1994; Stelwagen *et al.* 1994*a*, 1996), sheep (Fernandez *et al.* 1995), or goats (Wilde & Knight, 1990). Furthermore, the *RMYQ* for cows in early lactation was lower ($P < 0{\cdot}05$) than that for cows in late lactation during weeks 3 and 4, but not during week 5 ($P > 0{\cdot}05$). Nevertheless, the mean *RMYQ* was lowest ($P < 0{\cdot}05$) for early lactation cows, indicating that the greatest reduction in milk yield occurred for cows in early lactation. This experiment was the first that directly compared the reduction in milk yield attributable to ODM during different stages of lactation and showed that this reduction was greatest during early lactation. This notion would seem to be supported by the combined data of several experiments (Carruthers *et al.* 1993).

The EUHV for early and late lactation cows is shown in Table 2. The 3 week period of ODM did not cause a decrease in EUHV, suggesting that no cell loss occurred. In fact, in late lactation cows, there was a small but significant increase in EUHV, perhaps as an artifact of the technique. The cisternal space increased during lactation (Dewhurst & Knight, 1993), and the skin around the udder was looser in late lactation, so perhaps the flaccidness of glands might have been exacerbated with ODM.

It is important to note that EUHV is a gross measurement, and the lack of cell loss with ODM could not be substantiated with detailed tissue analysis. However, these results are in agreement with those in goats, showing no cell loss attributable to short-term ODM (Wilde & Knight, 1990). Instead, Knight *et al.* (1988) suggested that brief periods of ODM, such as those in the present study, lead to reduced metabolic activity of secretory cells, as measured by reduced activity of key mammary enzymes, and that cell loss only occurred after ODM over a longer period. Consistent with a loss of cellular activity is a significant decrease in gross secretion

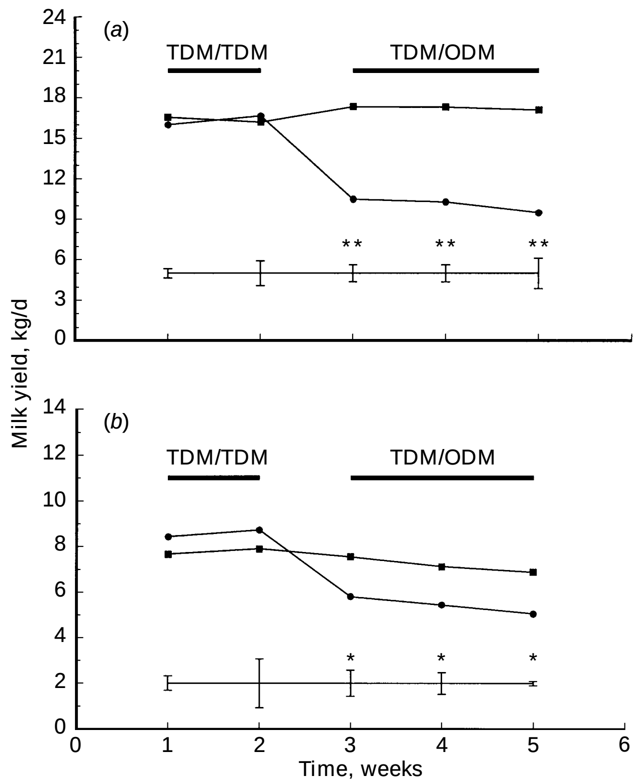

Fig. 1. Effect of unilateral ●, once (ODM) and ■, twice (TDM) daily milking of diagonally opposed mammary glands of cows during (*a*) early and (*b*) late lactation on milk yield. Vertical bars represent SED. Values for ODM and TDM glands were significantly different: $*P < 0{\cdot}05$, $**P < 0{\cdot}01$.

Table 1. *Relative milk yield quotients† in each treatment week for groups of cows in early and late lactation*

(Values are means ± SED)

Experimental period	Early lactation ($n = 6$)	Late lactation ($n = 5$)
Week 3	0·61 ± 0·02***	0·69 ± 0·04***
Week 4	0·60 ± 0·02***	0·68 ± 0·03***
Week 5	0·56 ± 0·04***	0·56 ± 0·05***

† The relative milk yield quotient was calculated as $(t_2/t_1)(c_1/c_2)$, where t_2 is yield of the test glands in the treatment period, t_1 the average yield of the test glands in the control period, c_1 the average yield of the control glands in the control period and c_2 the yield of the control glands in the treatment period. Each mean differed from unity: $***P < 0{\cdot}001$.

efficiency (i.e. milk yield to EUHV ratio; the mean milk yield of the same week that the EUHV was measured was used to calculate the ratio; Table 2). Moreover, in a recent study in which cows were subjected to unilateral ODM or TDM (Farr *et al.* 1995*b*), the activities of several key mammary enzymes were lowest in the glands exposed to the longest milking interval, suggesting that ODM implemented over a brief period resulted in reduced metabolic activity of mammary secretory cells in cows, as in goats (Wilde & Knight, 1990). Moreover, the gross secretion efficiency was lower ($P < 0{\cdot}05$) during late lactation than during early lactation (Table 2).

Knight *et al.* (1994) showed that the cisternal milk as a proportion of total yield

Table 2. *Changes in empty udder-half volume and milk secretion efficiency between twice (TDM) and once (ODM) daily milking, after 3 weeks of ODM for cows in early and late lactation*

(Values are means with SED)

	Early lactation ($n = 6$)			Late lactation ($n = 5$)		
	TDM	ODM	SED	TDM	ODM	SED
Empty udder-half volume, l	11·2	10·7	0·8	7·0	8·8	0·3*
Milk secretion efficiency, l/l†	2·76	1·48	0·18**	1·43	1·04	0·13*

† Milk secretion efficiency was calculated as the ratio of milk yield to empty udder-half volume (the mean milk yield of the same week that the empty udder-half volume was measured was used to calculate the ratio).
$*P < 0·05$, $**P < 0·01$.

at 8 h was highly correlated with that after an extended milking interval (20 h). Based on those data, cisternal measurements in the present study were made 8 h after the morning milking. Neither the amount (196·6 g for cows in late lactation *v.* 121·5 g for cows in early lactation, SED 84·4 g) nor the proportion (17·0% for cows in late lactation *v.* 7·3% for cows in early lactation, SED 6·6%) of cisternal milk was significantly different for the two stages of lactation. This would seem to be at odds with data on multiparous cows from an earlier study (Dewhurst & Knight, 1993) in which cisternal volume declined and the proportion of cisternal milk increased as lactation progressed. The reason for this discrepancy is not fully understood. However, in the previous study (Dewhurst & Knight, 1993) measurements were made throughout lactation on the same cows while in the present study measurements were made on small groups of different cows.

Earlier results showed a strong inverse correlation between the reduction in milk yield attributable to ODM and cisternal milk as a proportion of total milk (Knight & Dewhurst, 1994). The present study, using the half udder model and combining the values for early and late lactating cows, substantiated those results. The proportion of cisternal milk, as well as cisternal milk volume of the glands that were milked once daily, were positively correlated with the *RMYQ*.

$$RMYQ = 0·583(\pm 0·018) + 0·004(\pm 0·001) \text{ cisternal proportion}$$
$$r = 0·804, P < 0·01$$

$$RMYQ = 0·589(\pm 0·014) + 0·001(\pm 0·000) \text{ cisternal volume}$$
$$r = 0·644, P < 0·05$$

Thus, cows that store a smaller proportion of milk as cisternal milk are more susceptible to a reduction in milk yield with ODM. The importance of cisternal milk storage has been further emphasized by a recent study (Stelwagen *et al.* 1996) in which milk yield from continuously drained (i.e. unlimited cisternal space) glands increased unilaterally compared with the yield of the opposite glands that were drained once after 24 h. The view that cisternal capacity is the only limiting factor may be too simplistic, however. A recent study by Farr *et al.* (1995*a*) suggested that the flow of milk from the alveolar compartment to the cistern might also be important. That hypothesis was supported by the work of Grosvenor & Findlay (1968), which showed that in rats denervation of the mammary gland affected intramammary fluid flow, and also by that of Labussière *et al.* (1978) using sheep with denervated udders.

Finally, although it is understood that the control of milk secretion occurs locally within the mammary gland, the exact mechanism by which reduced milking frequency exerts its effects on milk secretion is much less clear. Milk storage and movement within the gland is one level of control that presumably precedes control by the secretory cell. Consistent with this notion is the presence of a small protein in milk that seems to act as a feedback inhibitor of secretion (Rennison *et al*. 1993; Wilde *et al*. 1995), and that tight junctions of the mammary epithelium become leaky with ODM, which in turn is associated with a reduction in milk yield (Stelwagen *et al*. 1994*b*, 1995). Thus, the regulation of milk secretion is the result of a complex interaction of mechanisms operating at different levels within the gland (e.g. anatomy of the gland and cellular processes).

In conclusion, in cows a unilateral decrease in milking frequency from TDM to ODM reduced milk yield only from the glands that had been milked once daily, indicating local control of secretion. The EUHV did not decrease with ODM, suggesting that no cell loss occurred but that ODM caused a decrease in cell activity, as evidenced by a reduction in gross secretion efficiency. The positive correlations between proportion or volume of cisternal milk and *RMYQ* in the glands that had been milked once daily demonstrated the importance of milk storage in the gland during ODM.

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