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BRIEF COMMUNICATION

Effect of once daily milking (ODM) on enzyme activities in the bovine mammary gland

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ODM is well established among NZ dairy farmers as a management tool. However, the transition from twice to ODM is associated with a loss of milk production (Carruthers *et al.*, 1990, Holmes *et al.*, 1992, Stelwagen *et al.*, 1994, Knight and Dewhurst, 1994). To devise strategies to prevent such milk yield losses it is important to understand the causative mechanisms. It has been shown in cows and goats, that variation in milking frequency was associated with changes in the activities of several mammary enzymes (Wilde and Knight, 1990; Knight *et al.*, 1992). The aim of the present study was to investigate the relationship between key mammary enzyme activities and milk production during ODM and twice daily milking (TDM) in dairy cows.

Ten Jersey cows, in their second lactation, were used. During the first 3 weeks all quarters of all animals were subjected to TDM, intervals being 9 and 15 h. For the remaining 3 weeks of the trial one side of the udder only was subjected to ODM (am), while the opposite side remained on TDM. Separate half udder yields were measured on 2 d of each week.

Bilateral biopsies were taken from each of the hind quarters on three occasions. Initially, during the pretreatment period, while both sides of the udder were on TDM (B1), tissue samples were taken immediately before the pm milking, after 8 h of milk accumulation. The two other biopsy times (B2 and B3) were 13 d apart, and after at least 7 d of unilateral ODM. In order that each cow was biopsied before the pm milking, (after 8 h of milk accumulation for both sides of the udder), and before the am milking (after 15 h of accumulation for the TDM side and 23 h for the ODM side), cows were divided into 2 groups of 5 cows each so that the first group which was sampled before the pm milking at B2 was then biopsied before the am milking at B3, and vice versa.

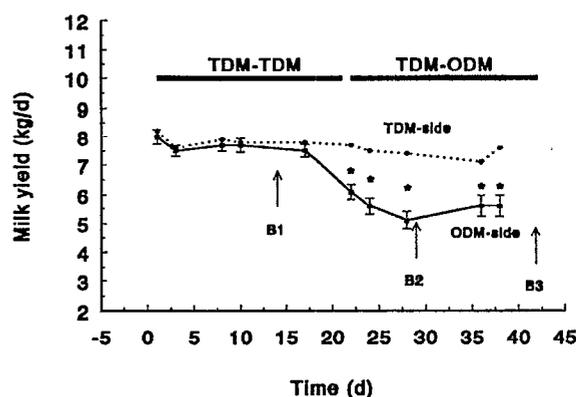
Biopsy samples were obtained while cows were lightly sedated and using a local anaesthetic. A core of mammary

tissue (approximately 1g and 7.5 cm by 0.5 cm diameter) was taken as described by Farr *et al.* (1994).

Tissue samples were snap-frozen and stored in liquid nitrogen and analysed for acetyl-CoA carboxylase (ACC), fatty acid synthetase (FAS), and galactosyltransferase (GT) activity and DNA concentration, as described by Wilde and Knight (1990). Activity was linearly related to the amount of sample and incubation time, and expressed per mg of tissue DNA.

Differences in enzyme activities and milk yield (MY) between udder halves were evaluated by paired t-test.

FIGURE 1: Half udder milk yields during bilateral twice daily milking (TDM) and unilateral once daily milking (ODM). (means \pm SED) B = biopsy, * P<0.01.



MY results are shown in Figure 1. MY of the ODM side of the udder decreased significantly below that of the TDM side (5.41 vs 7.34 ± 0.33 kg/d, $P < 0.001$) by an average of 26% with unilateral ODM. MY was unaffected by the biopsy procedure.

TABLE 1: Mammary enzyme activities for glands during bilateral twice daily milking (TDM) (pre treatment) and unilateral once daily milking (ODM) of cows after different periods of milk accumulation. Enzyme activities ($\mu\text{mol}/\text{min} \cdot \text{mg DNA}$) (means \pm SED) #P<0.1, *P<0.05, **P<0.01

Contrasts	Acetyl-CoA Carboxylase	Galactosyltransferase	Fatty Acid Synthetase
Pre Treatment			
TDM 8h vs ODM 8h	0.27 vs 0.28 \pm 0.05	0.43 vs 0.42 \pm 0.05	0.97 vs 0.76 \pm 0.12
Unilateral ODM			
TDM 8h vs ODM 8h	0.35 vs 0.27 \pm 0.05	0.51 vs 0.42 \pm 0.07	0.90 vs 0.61 \pm 0.15#
ODM 8h vs ODM 23h	0.27 vs 0.25 \pm 0.04	0.42 vs 0.34 \pm 0.62	0.61 vs 0.56 \pm 0.13
TDM 8h vs ODM 23h	0.35 vs 0.25 \pm 0.03*	0.51 vs 0.34 \pm 0.04**	0.90 vs 0.56 \pm 0.09**

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Mammary enzyme activities are shown in Table 1. Enzyme activities in tissue from the hind glands in the pretreatment period did not differ. Following 8 h of milk accumulation enzyme activities were consistently, albeit not significantly, lower on the ODM side, with differences in activity of FAS approaching statistical significance ($P < 0.1$). Activities in tissue from ODM glands after 23h of milk accumulation tended to be lower but were not significantly different from those after 8h. However, when considering the most extreme treatments of the udder halves, i.e. comparing tissue from the ODM side after 23h of accumulation and that from the TDM side after 8h of accumulation, all the mammary enzymes measured had significantly ($p < 0.05$) lower activity in the ODM tissue. This suggests an additive effect of long-term ODM and 23h of milk accumulation in inhibiting the synthetic potential of the secretory tissue.

The decrease in MY with ODM is consistent with effects seen by Carruthers *et al.* (1991), Holmes *et al.* (1992), and Stelwagen *et al.* (1994). In the present work this decrease was a unilateral effect, in the ODM side of the udder only, as found previously in cows (Claesson *et al.* 1959), indicating that the reduced yield is due to a local mechanism. Wilde and Peaker (1990) suggest that this effect is due to the degree of feedback inhibition exerted by a constituent of the whey proteins which is secreted by the mammary epithelial cells. The impaired milk secretion may also be due to the loss in tight junction integrity (Stelwagen *et al.*, 1994).

The unilateral 26% decrease in MY with ODM was associated with a local decrease in activity of key mammary enzymes (averaging 33%), indicative of a decrease in syn-

thetic potential in the bovine udder during ODM. This decrease in mammary enzyme activity may have been due to a decrease in activity in all cells or, alternatively, a proportion of the cells becoming quiescent. This, and the timing of the decrease in activity require further investigation.

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