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## Original article

# Effects of milking three times in 2 days for 3 weeks in early lactation or in the declining phase on milk production in primiparous and multiparous dairy cows

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**Summary** — Fifty-two Holstein cows (26 primiparous) were assigned at calving to two milking frequencies: either twice daily (6 h and 17 h; control) or three times/2 days (6 h, 20 h and 14 h of the following day) during the first 3 weeks of lactation and then twice daily for at least 5 weeks. Nine weeks after the last calving (at the end of the 3rd month of lactation, on average) each of the four groups (primiparous or multiparous cows  $\times$  twice daily milking/day or three times milking/2 days in early lactation) that were being milked twice daily, was divided into two sub-groups: one continued to be milked twice daily whereas the other was milked three times/2 days for 3 weeks and then twice daily. All cows were fed ad libitum the same complete diet based on grass silage. In early lactation, milking three times/2 days decreased the milk yield (3.4 kg/day; 16%), increased the fat (5.0 g/kg) and protein (2.2 g/kg) contents and decreased the lactose content (1.5 g/kg) in primiparous cows. In multiparous cows, the milk changes ( $-0.7$  kg,  $+0.9$  g fat/kg,  $+0.6$  g proteins/kg) were not significant. The energy balance was not changed. During the declining phase of lactation, milking three times/2 days reduced secretion of milk, fat, proteins and lactose by about 10% in both categories of cows. The milk composition remained unchanged. Food intake was not affected but the energy balance was improved.

**dairy cow / milk production / milking frequency / stage of lactation / lactation rank**

**Résumé** — Effet de la traite 3 fois en 2 j pendant les 3 premières semaines de la lactation ou pendant la phase descendante, sur la production laitière de vaches primipares ou multipares. Cinquante-deux vaches Holstein (26 primipares) ont été réparties au vêlage entre deux fréquences de traite : soit deux fois/jour (6 et 17 h ; témoin), soit trois fois/2 j (6, 20 et 14 h le jour suivant) pendant les 3 premières semaines de la lactation, puis deux fois/jour pendant au moins 5 semaines. Neuf semaines après le dernier vêlage (à la fin du 3<sup>e</sup> mois de lactation, en moyenne), chacun des quatre lots

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(vaches primipares ou multipares  $\times$  traite deux fois/jour ou trois fois/2 j en début de lactation), qui était alors trait deux fois/jour, a été subdivisé en deux sous-lots : l'un a continué à être trait deux fois/jour alors que l'autre a été trait trois fois/2 j pendant 3 semaines, puis de nouveau deux fois par jour. Tous les animaux ont été alimentés à volonté avec la même ration complète à base d'ensilage d'herbe. La traite trois fois/2 j en début de lactation a, chez les primipares, diminué la quantité de lait sécrétée de 3,4 kg/jour (16 %), augmenté les teneurs du lait en matières grasses (5,0 g/kg) et en protéines (2,2 g/kg), et diminué la teneur en lactose (1,5 g/kg). Chez les multipares, les modifications de sécrétion laitière ( $-0,7$  kg lait,  $+0,9$  g matières grasses et  $+0,6$  g de protéines/kg) n'ont pas été significatives. Le bilan énergétique n'a pas été modifié. Pendant la phase descendante de la lactation (début du 4<sup>e</sup> mois de lactation en moyenne), la traite trois fois/2 j a réduit les quantités de lait, de matières grasses, de protéines et de lactose d'environ 10 % chez les deux catégories de vaches. La composition du lait n'a pas changé pendant cette période. Les quantités d'aliments ingérées n'ont pas été affectées mais le bilan énergétique a été plus positif.

#### **fréquence de traite / vache laitière / production laitière / stade de lactation / rang de lactation**

## **INTRODUCTION**

The main issues (feeding, health, reproduction, social environment) in dairy herd management are concentrated in the peripartum period and the 2 following months. They are largely the result of the nutritional deficit experienced by the cows during this period, which is due to their incapacity to increase their nutrient intake as rapidly as their milk secretion. The relative failure of nutrition in alleviating the metabolic load in early lactation and the milk quota policy (which determines the quantity of milk each farmer may sell, and which decreases the interest in maximizing the milk secretion of the cows) make it possible to try to adjust the milk secretion to the nutrient intake. This strategy, which is opposite to that implemented so far, could be achieved through a temporary lower milking frequency in early lactation.

Effect of milking frequency (ie, interval between milkings) on milk secretion rate has been studied for a long time. In analytical studies of short duration where the effects of previous milking intervals and residual milk were eliminated, physiologists have shown that milk secretion rate decreases curvilinearly with the duration of the milking interval (Wheelock et al, 1966) or is virtually constant over the first 16 h

after milking (cf Elliott, 1959; Labussière and Richard, 1965), which would correspond to about 13 h under conventional milking conditions (Elliott et al, 1960). Loss in milk yield resulting from milking omissions depends on the characteristics of these omissions: frequency (from 35% and 50% for 1 omission/day (Claesson et al, 1959; Holmes et al, 1992) to only slightly for 1 omission/week (Labussière et Coindet, 1968; Radcliffe et al, 1973)), stage of lactation at which omissions are imposed (Eldridge and Clark, 1978), and duration of implementation (Claesson et al, 1959). Milk loss also depends on the cows' characteristics (rank of lactation (Woolford et al, 1985), breed (Carruthers et al, 1993), anatomy of the udder (Knight and Dewhurst, 1994)).

In a previous experiment (Rémond et al, 1992), milking primiparous cows three times/2 days in the first three weeks of lactation resulted in a large reduction in the loss of live weight ( $-34$  kg vs  $-67$  kg) despite a moderate decrease (12%) in milk yield. Moreover, the cows did not recover the control level of milk secretion once the twice daily milking frequency was resumed. To complete this experiment done with a few (12 in total) primiparous cows, we studied the three milkings/2 days again with a larger number of primiparous and multiparous cows and at two different lactation stages.

## MATERIALS AND METHODS

### Animals and experimental design

Twenty-six primiparous lactating cows (31 months old at calving) and 26 multiparous cows (11 in 2nd lactation), of the Holstein-Friesian breed, were assigned at calving to two milking frequency regimes: a control frequency (two times/day at 6 h and 17 h) or a reduced frequency (three times/2 days at 6 h, 20 h and 14 h of the following day) for the first 3 weeks of lactation (experimental period 1) and then the control frequency. Nine weeks after the last cow had calved (after 13.3 weeks of lactation on average for all cows), each of these four groups (control or reduced milking frequency regime  $\times$  primiparous or multiparous cows), which were then being milked twice daily, was divided at the same time into two similar sub-groups. One of the groups continued to be milked twice daily whereas the other was milked three times/2 days for 3 weeks (experimental period 2, from 5 to 25 February 1996), and then twice daily for at least 6 weeks. For both assignments of cows to experimental groups, the expected calving date, age, milk yield and composition (expected from the performance in the preceding lactation (multiparous cows) or from genetic index (primiparous cows), for period 1) and live weight were taken into account. The cows calved between 28 September and 6 December 1995.

### Husbandry

Cows grazed in the pasture during the summer preceding calving. They were brought into the barn 3 weeks at least before their expected calving date or on 10 November. They were then housed together in a free-stall barn equipped with electronic gates. During the dry period indoors, they were fed grass silage *ad libitum* and an increasing quantity of concentrate (1, 2, 3 kg during the last 3 weeks before calving). From their calving date to 25 February, all cows received once a day, *ad libitum*, at about 9 h, a complete diet composed, on a dry matter basis, of 54% grass silage, 13% hay and 33% concentrate (0.88 feed unit for milk, 180 g crude protein, per kg dry matter). From 26 February (ie, from the week 1 of post-experimental period 2) onwards, maize silage was included in the complete diet which was then composed (DM basis) of 46% grass silage, 17% maize silage, 12% hay and

25% concentrate (0.85 feed unit and 165 g crude protein per kg dry matter). The cows were milked in a milking parlour equipped with automatic cluster removers.

### Measurements and analyses

Milk was weighed at each milking and sampled at each milking over 2 consecutive days for its fat, protein and lactose contents, and somatic cell count (infra-red spectrophotometry and automatic counting, respectively). In samples taken at the morning milking at weeks 3 and 12.3 (on average) of lactation, the soluble proteins (after clotting of caseins with rennet), serum albumin (radioimmunoassay technique; Levieux, 1991) and free fatty acids (Chazal and Chilliard, 1986) were also analysed. Food intake was measured 4 days weekly and live weight in the week after calving and then every other week. Blood was sampled from the caudal vein, between 8.30 h and 9.00 h (before diet distribution), in the 3rd week of lactation (last week of experimental period 1) for glucose, non-esterified fatty acids and 3-hydroxybutyrate analysis (enzymatic determination).

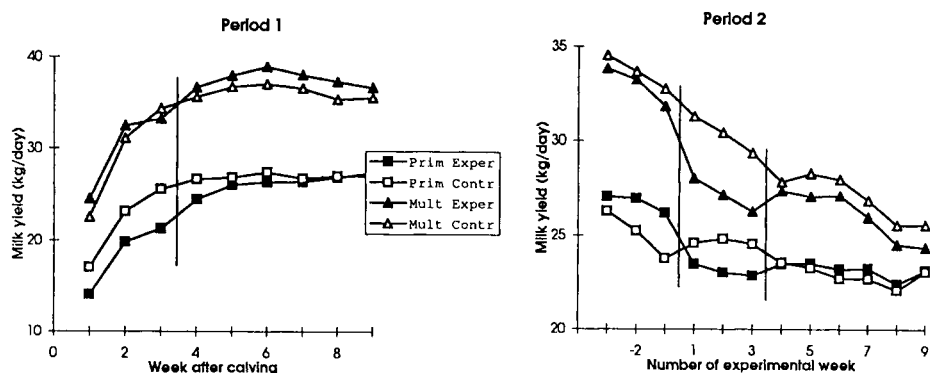
### Data analysis

Data were subjected to variance-covariance analysis (GLM procedure, SAS, 1987). Variates included were milking frequency, lactation rank, and their interaction. Parameters of milk production (yield, composition) recorded in period 1 were subjected to two analyses: without covariate (data not presented) or with the data recorded in weeks 7 and 8 of lactation (weeks 4 and 5 of the post-experimental period) as a covariate. Three cows were dropped from the trial or the data analyses.

## RESULTS

### Milk yield

In experimental period 1, low milking frequency resulted in a reduction in milk yield that was higher in primiparous cows than in multiparous (fig 1 and table I). The depressive effect increased with the num-



**Fig 1.** Effect of three milkings/2 days, compared to two milkings/day, applied for 3 weeks (1 to 3) in early lactation (period 1) or in the declining phase (period 2) on the milk yield by primiparous or multiparous cows.

**Table I.** Effect of three milkings/2 days compared to two milkings/day on milk yield and composition (adjusted means).

Lactation rank	Primiparous cows		Multiparous cows		Statistical effects of ( $P < $ )		Residual
Milking frequency	3/2 days	2/day	3/2 days	2/day	Milk freq	Freq $\times$ rank	SD
<i>Period 1</i>							
Milk (kg/d)	18.5 A	21.9 B	29.3 C	30.0 C	0.01	0.10	2.5
Fat (g/kg)	47.1 ab	42.1 c	45.8 ad	44.9 ac	0.05	NS	4.9
Proteins (g/kg)	35.3 a	33.1 b	36.2 a	35.6 a	NS	NS	3.0
Lactose (g/kg)	45.9 A	47.4 B	45.2 A	46.2 A	0.01	NS	1.5
Somatic cells ( $\times 1\,000/\text{mL}$ ) <sup>1</sup>	290	395	74	63	NS	NS	
Fat (g/day)	878 A	938 A	1329 B	1335 B	NS	NS	144
Proteins (g/day)	642 A	721 A	1048 B	1053 B	NS	NS	127
Lactose (g/day)	863 A	1060 B	1350 C	1393 C	0.01	0.05	114
<i>Period 2</i>							
Milk (kg/day)	22.6 A	25.1 B	27.4 C	30.1 D	0.01	NS	2.0
Fat (g/kg)	39.7 a	39.9 ab	41.0 ab	41.5 b	NS	NS	2.4
Proteins (g/kg)	32.8 a	32.6 ab	32.2 b	32.2 ab	NS	NS	0.9
Lactose (g/kg)	50.0 A	50.3 A	47.8 B	48.3 B	NS	NS	1.0
Fat (g/day)	898 A	1000 B	1123 C	1233 D	0.01	NS	87
Proteins (g/day)	734 A	813 B	878 C	961 D	0.01	NS	64
Lactose (g/day)	1130 A	1265 B	1315 B	1454 C	0.01	NS	111

Means with different capital letters are different at 0.01 or 0.05 level of significance; means with different lower case letters are different at 0.10 level; NS, not significant. <sup>1</sup>Data calculated on logarithms of the number of cells.

ber of the weeks the cows experienced the reduced milking frequency (RMF), being significant for each of the 3 weeks in primiparous cows ( $-2.8$  kg/day,  $P < 0.1$ ;  $-3.1$  kg/day,  $P < 0.01$ ;  $-4.1$  kg/day,  $P < 0.01$ , for weeks 1, 2 and 3, respectively) but only for week 3 in multiparous cows ( $+0.7$  kg/day,  $P > 0.1$ ;  $0$  kg/day,  $P > 0.1$ ;  $-2.7$  kg/day,  $P < 0.1$ , respectively). Consequently, the average milk yield over the 3 weeks of RMF only decreased in primiparous cows. During period 2, the depressive effect was similar for both categories of cows ( $\sim 10\%$ ) and for the 3 experimental weeks. In both post-experimental periods, differences in milk yield and composition between control cows and cows milked less frequently ceased to be significant (when they were) after 1 or 2 weeks of twice daily milking regime.

Decreases in milk yield due to RMF (as observed by the milk yield augmentation between week 3 and 4 for period 1 and by the milk yield decrease between pre-experimental weeks  $-1 + -2$  (mean) and experimental week 3 for period 2) were not related to the milk yield of the cows ( $P = 0.739$  and  $P = 0.622$ , respectively). For the 11 cows that were milked three times/2 days during both experimental periods, the relationship between both milk yield decreases (same definitions as earlier) was not significant.

### Milk composition

During period 1, RMF significantly decreased the lactose content (g/kg), increased the fat and protein content and did not change somatic cell number, in primiparous cows (table I). No significant change in milk composition was observed in multiparous cows. Consequently, RMF only decreased lactose secretion (g/day) in primiparous cows. During period 2, the lactose, fat and protein contents remained unchanged in both categories of cows. Secretions of

milk, lactose, fat and proteins decreased by about 10% in both categories of cows.

At week 3 of lactation, RMF significantly increased the soluble protein content, in primiparous cows (table II). Because the content in renneted proteins was not significantly affected, the casein/protein ratio decreased. The serum albumin content tended ( $P < 0.10$ ) to increase in multiparous cows submitted to RMF. These parameters did not change between milking frequencies in the post-experimental period (week 12.3 on average).

### Nutritional indices

RMF did not significantly change food intake during either experimental period, but improved the energy balance during period 2 (significant effect for multiparous cows). Live weights in week 1 of lactation were 596 kg for primiparous cows and 662 kg for multiparous. They slightly decreased during period 1 (12 kg for primiparous cows between calving and week 3 of lactation, 13 kg for multiparous cows between calving and week 8 of lactation), but were not different between both milking frequency regimes. During period 2, liveweight augmentations were not significantly different.

Plasma concentrations (measured during week 3 of lactation) in glucose, non-esterified fatty acids and 3-hydroxybutyrate were not modified by RMF in primiparous cows (0.64 g/L, 0.29 mmol/L; 0.85 mmol/L respectively, on average for both milking frequency regimes). In multiparous cows, RMF tended to decrease the glucose concentration (0.53 vs 0.58 g/L;  $P = 0.101$ ) and increased the 3-hydroxybutyrate (1.74 mmol/L vs 1.00 mmol/L;  $P < 0.01$ ) concentration. The concentration in non-esterified fatty acids was unaffected.

**Table II.** Effect of three milkings/2days compared to two milkings/day on the concentration of some protein fractions and of the free fatty acids in the milk secreted during the experimental period 1 (week 3 of lactation) and during the post-experimental period 1 (week 12.3 of lactation, on average).

	<i>Primiparous cows</i>		<i>Multiparous cows</i>		<i>Statistical effects of Milk freq<sup>2</sup></i>	<i>Residual SD</i>
<i>Milking frequency</i>	<i>3/2 days</i>	<i>2/day</i>	<i>3/2 days</i>	<i>2/day</i>		
<i>Experimental period*</i>						
Renneted proteins (g/kg)	26.3	25.9	26.7	26.4	NS	2.1
Soluble proteins (g/kg)	6.1 a	5.3 b	5.4 b	5.3 b	0.05	1.0
Renneted proteins (% total proteins)	81.3 A	83.1 B	83.3 B	83.3 B	0.05	2.2
Serum albumin (mg/L)	138 ab	133 ab	150 a	116 b	NS	41
Free fatty acids (mg C16/100 g fat)	139	133	107	151	NS	72
<i>Post-experimental period<sup>1</sup></i>						
Renneted proteins (g/kg)	26.5	27.6	26.5	26.9	NS	1.9
Soluble proteins (g/kg)	5.5	5.7	5.3	5.5	NS	0.9
Renneted proteins (% total proteins)	82.8	82.8	83.5	83.0	NS	2.1
Serum albumin (mg/L)	152	148	162	135	NS	40
Free fatty acids (mg C16/100 g fat)	145 ab	146 b	176 c	160 abc	NS	41

<sup>1</sup> Adjusted means calculated with the variates: lactation rank, milking frequency, their interaction and, for period 1, data recorded in the post-experimental period.

<sup>2</sup> Interaction "lactation rank (primiparous or multiparous cows) × milking frequency" was never significant. Means with different capital letters are different at 0.01 or 0.05 level of significance; means with different lower case letters are different at 0.10 level; NS, not significant.

## DISCUSSION

### Statistical analysis

The effects of RMF during period 1 that are presented are those provided by statistical analyses done with data recorded at the end of post-experimental period as a covariate. These analyses are not statistically "correct" since they imply that the effects of RMF, when present, have entirely disappeared at the end of the post-experimental period. Nevertheless this decision seems well grounded for several reasons: i) differences

between treatments, when present, were observed to fade rapidly once control milking frequency was implemented (see figure 1 for milk yield); ii) statistical analyses without covariate provided weekly evolutions of the effect of RMF similar to those provided by analyses with covariate, but sometimes different estimated effects, probably because they did not take into account intrinsic differences between groups of cows. For instance, experimental multiparous cows produced 1.8 kg milk more than controls in late post-experimental period, which is likely due to their higher

**Table III.** Effect of three milkings/2 days compared to two milkings/day on feed intake, live weight and energy balance (adjusted means).

<i>Lactation rank</i>	<i>Primiparous cows</i>		<i>Multiparous cows</i>		<i>Statistical effects of (P &lt; )</i>		<i>Residual</i>
<i>Milking frequency</i>	<i>3/2 days</i>	<i>2/day</i>	<i>3/2 days</i>	<i>2/day</i>	<i>Milk freq</i>	<i>Freq × rank</i>	<i>SD</i>
<i>Period 1</i>							
Dry matter intake (kg/day) <sup>1</sup>	13.9 A	13.2 A	16.9 B	17.6 B	NS	NS	1.9
Live weight (kg) <sup>2</sup>	587 A	588 A	656 B	660 B	NS	NS	23
Energy balance (FU/day) <sup>1</sup>	− 1.7 A	− 2.9 AC	− 4.4 B	− 3.7 BC	NS	0.10	1.9
<i>Period 2</i>							
Dry matter intake (kg/day) <sup>1</sup>	20.4 A	21.1 A	23.7 B	23.4 B	NS	0.05	0.9
Live weight (kg) <sup>3</sup>	625 A	630 A	669 B	670 B	NS	NS	15
Energy balance (FU/day) <sup>1</sup>	3.1 AB	2.3 BC	3.2 A	2.0 C	0.01	NS	1.0

Means with different capital letters are different at 0.01 or 0.05 level of significance; NS, not significant.

<sup>1</sup> Means for the three experimental weeks. <sup>2</sup> Live weight in week 4 calculated with live weight at week 1 as a covariate. <sup>3</sup> Live weight at week 1 of the post-experimental period, calculated with the live weight in the pre-experimental period 2 as a covariate.

capacity for milk secretion, despite the fact that the assignment of multiparous cows to experimental groups for period 1 was based on milk yield in the previous lactation (see *Materials and methods*); and iii) a more severe restriction of milking frequency than was used in this trial (1/day) had no carry-over effect on milk yield (Knight and Dewhurst, 1994) or an apparently low effect (Stelwagen et al, 1994). Recovery of control milk composition is even more rapid than that of milk yield (Wheelock et al, 1965) once control milking frequency was resumed.

## Performance

The very low effects of an omission rate of one milking/2 days in multiparous cows in

early lactation are consistent with the data of Eldridge and Clark (1978), which showed an effect of milking omission that is all the more lower as it is implemented sooner in the lactation. High levels of growth hormone (GH) secretion during this period (Bines and Hart, 1982) could maintain high levels of milk secretion despite the lengthening milking interval. Stelwagen et al (1994) showed that the milk yield increase resulting from the administration of exogenous GH in late lactation can offset more than the loss of milk resulting from the change from twice-daily to once-daily milking. Nevertheless, in primiparous cows, milk yield loss was relatively high (16%) and of the same order as that observed in a previous trial (-12%; Rémond et al, 1992). This particular sensitivity of the udder of primiparous cows might be related to their incom-



plete development in early lactation, contrary to multiparous cows, as shown in goats (Knight and Wilde, 1993). Moreover, possible effects due to the fact that primiparous cows are not habituated to milking cannot be discarded.

During the declining phase of lactation, losses in milk yield resulting from milking three times/2 days were moderate and similar for both primiparous (– 10%) and multiparous (– 9%) cows, in contrast with our observations for early lactation and with the data recorded by Woolford et al (1985) over an entire lactation (– 18% for primiparous cows; – 7% for multiparous). Little data are available that compare the udder capacity of primiparous and multiparous cows. Dewhurst and Knight (1994) showed that cistern milk as a proportion of total milk yield tended to be lower for primiparous than for multiparous cows, which would be consistent with the higher sensitivity of primiparous cows to milking omissions. Nevertheless, Davis and Hughson (1988) have shown that the functional capacity of the udder (liters of milk contained in the udder after 49 h without milking/previous hourly rate of milk secretion in a twice-daily milking regime, expressed in hours) is not different ( $P > 0.10$ ) between primiparous cows (27 h) and multiparous (33 h). Moreover, Walsh (1976) observed that omission of the first four or eight consecutive milkings after calving has a larger deleterious effect on milk yield in multiparous cows than in primiparous.

A lack of RMF carry-over effect in the declining phase of lactation, once the control milking frequency regime was resumed, was expected (see above). It was more surprising in early lactation, mostly in primiparous cows whose udder continues its development during this period (cf, Knight and Wilde, 1993). Knight and Dewhurst (1994) wondered if the lack of carry-over effect in mid-lactation in their trial should also have been observed in early lactation. In a pre-

vious trial involving 12 primiparous cows we did not observe milk yield recovery once twice daily milking was applied (Rémond et al, 1992). In the present trial, despite milk yield reduction (in primiparous cows), RMF did not reduce the long-term milk secretion capacity of the udder which suggested that neither the number of secretory cells nor their potential activity were affected. During this period, cows display a particular hormonal pattern in their blood, which has an high GH content. This may make it possible to preserve the normal development of secretory parenchyma despite the reduction (moderate and of relatively short duration in our trial) in milking frequency, as suggested by Knight and Wilde (1993). The lack of notable change of serum albumin concentration in the milk (an index of the tight junction integrity of secretory epithelium; Stelwagen et al, 1994) agrees with the observation of an increase in the amount of this protein for milking intervals higher than 18 h (Stelwagen et al, 1995). The decrease in milk yield due to RMF, then seemed attributable to local regulation by a protein secreted in milk (feedback inhibitor of lactation; Wilde et al, 1995) rather than to a disruption of secretory cell tight junctions as suggested by Stelwagen et al (1994).

The changes in milk composition recorded in primiparous cows during period 1 agreed with those commonly observed with RMF (Claesson et al, 1959; Holmes et al, 1992; Carruthers et al, 1993). The increase in soluble proteins, only observed in primiparous cows during period 1, was already recorded by Claesson et al (1959). Surprisingly, it was in multiparous cows that the serum albumin content tended to increase.

During period 1, the lower energy balance (not significant) in multiparous cows milked less frequently was consistent with the increase of plasma 3-hydroxybutyrate. It was probably due to their slightly effective higher milk yield (1.3 kg fat-corrected

milk/day) and their slightly lower food intake (0.7 kg dry matter/day). We previously observed a reduction of 33 kg in the live weight loss of primiparous cows milked three times/2 days in early lactation (Rémond et al, 1992). The fact that the nutritive requirements were met more satisfactorily in the present trial (low decrease in live-weight) was perhaps a reason for the discrepancy between our two trials. During period 2, the lack of effect of RMF on food intake, and the depressive effect on milk yield improved the overall energy balance. With RMF, Claesson et al (1959), Woolford et al (1985) and Holmes et al (1992) recorded higher live-weight gains during the declining phase of lactation.

In conclusion, omission of one milking/2 days accompanied by a rearrangement of milking hours resulting in approximately similar milking intervals did not result in a decrease in milk yield that was high and rapid enough to improve the nutritional balance of cows in early lactation. A more severe reduction of milking frequency will have to be tested.

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