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Original article

Combined effects of once-daily milking and feeding level in the first three weeks of lactation on milk production and enzyme activities, and nutritional status, in Holstein cows

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Abstract — Twenty-four multiparous cows were divided into 4 groups immediately after calving according to a 2 × 2 factorial design: 2 milking frequencies (1 daily [morning only] milking for the first three weeks of lactation, then 2 daily milkings vs. 2 daily milkings throughout) under two diet energy concentrations (normal or low). The trial lasted 30 weeks, on average, and ended in the pasture on 28 May 2000. During the winter period, the cows were housed together in a free-stall barn, and were given complete rations ad libitum. The cows that were not milked were significantly more vocal than the controls, close to the omitted milking, and in a large number of them, milk leaked from the udder. Once-daily milking gradually reduced the amount of milk secreted in relation to that produced by cows milked twice a day, with differences of 8.5 kg·d^{-1} ($P < 0.01$) at week 3. As early as the first week of resumption of twice-daily milking, that difference ceased to be statistically significant but amounted to 1.3 kg·d^{-1} ($P > 0.10$) throughout the rest of the trial. Once-daily milking did not affect the contents of milk fat, true protein, serum albumin and lactose contents or somatic cell count, plasmin activity and plasminogen-derived activity. That treatment also increased the G1 immunoglobulin concentration ($P = 0.02$; measured at week 3) and reduced milk lipoprotein lipase activity. Once-daily milking improved the nutritional status at the beginning of lactation (reduced live weight and body condition losses, and increased the computed energy balance) and had no effect on the ration intake. The normal- energy diet produced expected results, in relation to the low-energy diet: a significant increase in intake (1.2 kg DM·d^{-1}), milk yield (3.1 kg·d^{-1}), milk protein content (2.0 g·kg^{-1}), and nutritional indices, in particular. The decrease in milk yield induced by once-daily milking during the

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first three weeks of lactation was 5.5 kg·d^{-1} in the low-energy group and 8.5 kg·d^{-1} for the normal energy group (non- significant interaction; $P > 0.10$).

once-daily milking / level of feeding / milk production / enzyme activities / nutritional status

Résumé — **Effets de la traite une fois par jour combinée au niveau d'alimentation pendant les trois premières semaines de la lactation sur la production et les activités enzymatiques du lait, et l'état nutritionnel, chez des vaches Holstein.** Vingt-quatre vaches multipares ont été réparties dès le vêlage en 4 lots, selon un schéma factoriel 2 × 2 : 2 fréquences de traite (1 traite par jour pendant les 3 premières semaines de lactation, puis 2 traites par jour vs. 2 traites par jour en permanence) × 2 concentrations énergétiques de la ration (normale ou basse). L'essai a duré en moyenne 30 semaines et s'est terminé au pâturage, le 28 mai 2000. Pendant la période hivernale, les vaches étaient logées ensemble dans une stabulation libre à logettes et recevaient à volonté une ration complète. La traite omise était celle du soir. Les vaches non traites ont émis significativement plus de vocalisations que les témoins autour de la traite omise, et elles ont été plus nombreuses à perdre du lait. La traite 1 fois par jour a progressivement réduit la quantité de lait sécrétée par rapport à celle produite par les vaches traites 2 fois par jour, l'écart atteignant 8,5 kg·j^{-1} ($P < 0{,}01$) à la semaine 3. Dès la 1re semaine de la reprise de la traite 2 fois par jour, cet écart n'a plus été significatif. Il a été de 1,3 kg·j^{-1} ($P > 0{,}10$) pour l'ensemble de l'essai. La traite 1 fois par jour n'a pas affecté la teneur du lait en matières grasses, en protéines (vraies), en albumine sérique, et en lactose, ni le nombre de cellules somatiques ou l'activité de la plasmine et celle induite par l'activation du plasminogène. Elle a accru la teneur en immunoglobulines G1 ($P = 0{,}02$; mesure à la semaine 3) et a diminué l'activité de la lipoprotéine lipase, ce qui n'avait pas encore été rapporté. Elle a amélioré l'état nutritionnel en début de lactation (pertes de poids vif et d'état corporel réduites, bilan énergétique calculé accru) et n'a eu aucun effet sur la quantité de ration ingérée. La ration de concentration énergétique normale a entraîné, par rapport à celle de concentration énergétique basse, les effets attendus : accroissement significatif des quantités ingérées (1,2 kg MS·j^{-1}), de la quantité de lait produite (3,1 kg·j^{-1}), de son taux protéique (2,0 g·kg^{-1}) et des index nutritionnels, notamment. La réduction de quantité de lait produite au cours des 3 premières semaines de la lactation entraînée par la traite 1 fois par jour a été de 5,5 kg·j^{-1} et 8,5 kg·j^{-1} respectivement pour les lots recevant les rations de concentration énergétique faible ou normale (interaction non significative ; $P > 0{,}10$).

traite une fois par jour / niveau d'alimentation / production laitière / activités enzymatiques / état nutritionnel

1. INTRODUCTION

For a number of reasons, feeding of dairy cows may evolve towards diets containing more pasture or preserved grass than is currently the case. These include milk quotas and the decrease in milk prices paid to farmers, both of which lead breeders to reduce their milk production costs to preserve their income. One implication is an increase in the proportion of forage in the diet promoting consistency between the consumers' perception of the ruminant, eater of forage, and breeding practices. Finally, there is the society's wishes for a more comprehensive use of rural land by a more environment-friendly farming industry, all being easier to implement on permanent grassland than on annual crop fields.

However, increasing the proportion of forage in the diet often decreases energy intake, which lowers milk yield and protein content, generally reducing animal fertility, and increasing the incidence of health risks related to energy metabolism [5, 12, 14, 16, 27]. These effects are especially high in the early stages of lactation and are broadly attributable to the animals' lower energy status. To palliate these effects, a number of methods are available: (i) increasing the energy input level by forage (earlier crop, hay drying…); (ii) managing increased underfeeding by husbandry methods that are yet to be explored (body condition control at calving, mastering diet composition, etc.) and (iii) reducing, preferably temporarily, milk secretion through innovative breeding methods, for example by shortening the duration of the dry period [35] or reducing milking frequency in early lactation [33, 34, 36]. In the latter study [36], milking the cows once daily for the first three weeks of lactation, then twice daily, halved the cows' energy deficit during the first three months of lactation and improved milk protein content by 2.3 $g \cdot kg^{-1}$, whereas the loss in milk yield during the twice-daily milking period did not exceed 2.4 $kg \cdot d^{-1}$.

The interest of once-daily milking during the first three weeks of lactation has continued to be explored. In this trial, we tested the possibility of combining this technique with a nutritional situation where it could be the most interesting, i.e. when the animals' feeding level is low. Moreover, unlike in the previous trial, special attention was paid to the animals' behaviour, to elicit the possible stress or pain induced by such a milking management, and detailed milk composition, including lipase and protease activities, for which results are scarce or missing [13].

2. MATERIALS AND METHODS

The beginning and the end of the experimental treatments, dates of measures and samplings, and changes in management of milking or feeding in the course of the trial, occurred either at precise stages of lactation (calving or nth week of lactation) or at fixed dates of the calendar, thus at different weeks of lactation among cows (see Tab. I). Nevertheless, in these last cases (that occurred from week 16 of lactation on), the average week of lactation (calculated on the 24 experimental cows) will generally be given in the place of calendar dates in order to make the presentation of the trial and the results clearer.

2.1. Animals and the experimental design

Twenty-four multiparous Holstein cows (mean number of lactations = 2.7), were included. These were distributed upon calving (between 29 September and 22 November, 1999) into four groups, to study, according to a 2×2 factorial design, the effect of two milking regimes and two feeding levels. Milking was performed either twice-daily for the entire trial (until 28 May [week 30 of lactation]; 2M groups) or once-daily during the first three weeks of lactation, then twice daily (1M groups). The two feeding levels were induced by two rations of different energy concentration, one low and one normal (see composition in Tab. II; the values were expected to be 0.84 and 0.90 $UFL \cdot kg^{-1}$ DM respectively) given ad libitum (rations L and N). The four groups (1MN, 1ML, 2MN, 2ML) were formed as previously described [36]. The cows, which were in the pasture over the summer period, were returned to free stabling with cubicles at least two weeks before the expected calving date and not later than the 1st of November. At the end of the indoor period (weeks 22 to 27), the four groups, which were so far milked with a milking robot (see below), were subdivided into two similar subgroups destined to be milked either twice or thrice a day. That treatment was aimed at studying the effect of the reduced milking frequency in early lactation on the ability of the udder to respond to an increased milking frequency during the declining phase of lactation. Cows were turned out to pasture on 4 May.

Table I. Physiological dates (week [w] after calving) and calendar dates (day/month) at which the main modifications in milking and feeding occurred during the trial.

Physiological or calendar dates		Calving (29/9 to 22/10)	w 3	w 4	14/2	15/2	28/2	29/2	7/3	8/3	27/3	28/3	3/5	4/5	28/5
Mean week* after calving corresponding to the calendar dates					16	16	18	18	19	19	22	22	27	27	30
Milking	frequency	1 vs. 2		2								2 vs. 3		2	
	equipment	milking parlour						milking robot						milking parlour	
Feeding	levels (n)	2								1					
	basal diet	grass silage + hay				grass silage + maize sil. + hay				maize silage + hay				pasture	

* Calculated for the 24 experimental cows.

Table II. Composition of the feeds and the rations.

Basic feed used	Grass silage	Maize silage	Hay (regrowth)	Concentrates	Soybean cakes
Chemical composition of the feeds (% DM):					
Organic matter	86.7	95.9	86.9	91.7	93.0
Crude protein (N × 6.25)	16.4	7.8	17.3	15.9	48.6
Crude fibre	27.4	18.8	25.1	8.0	6.9
Composition of the rations (% DM):					
Rations fed from calving to 14/02 (week 16)					
High energy level	60	-	10	30	-
Low energy level	71	-	12	17	-
Ration fed from 08/03 (week 19) to pasture turnout	-	68	18	4	10

2.2. Feeding

Once in stables, the pregnant cows were fed, until calving, the same complete ration as the L ration fed to half the lactating cows until 14 February (week 16) (Tab. II). After calving, the ration was maintained for the two L groups whereas the two N groups were given a ration containing more concentrates (Tab. II). These two complete rations, based on grass silage and hay, should, according to the Inration feeding software [19] based on expected feed nutritional values [18], satisfy the maintenance and production requirements of 26 kg and 35 kg of milk per day, respectively. The silage grass stocks were insufficient to complete the winter period and maize silage replaced grass silage, partially for the weeks 16 to 19 (transition period), then totally until turn out to pasture (weeks 19 to 27). During this last period, a single diet was fed to all cows. The chemical composition of the feeds used and of the diets is shown in Table II.

Complete rations were prepared with a desiling-mixing-distributing machine fitted with strain gauges. These rations were given ad libitum between 8:00 and 9:00 a.m., in individual troughs fitted with electronically controlled gates.

2.3. Milking

For the first 18 weeks after calving, and from turn out to pasture to the end of the trial (weeks 27 to 30), the cows were milked at 6:00 h and at 17:00 h in a 2 × 6 station milking parlour (Europarallèle, Alpha Laval) fitted with automatic cluster removers and milk meters (Alpro) (Tab. I). The omitted milking was the evening one. These cows that were to be milked were sorted a few minutes before milking. From week 18 and until turn out to pasture (week 27), the cows were milked using a milking robot (AMS-Prolion); they crossed the robot's stalls when going from their cubicles to their troughs. In the first month of use of the robot, they were only milked if the time interval since the previous milking exceeded 8 hours (twice daily target). Thereafter, the 8-hour time interval between two milkings was maintained (twice daily target) for half of the cows or was reduced to 5 hours (thrice daily target) for the other half.

2.4. Measurements and dosages

2.4.1. Animal behaviour

Some of the cows' activities and attitudes (lying down, lying down and ruminating, standing immobile, standing and ruminating, feed intake, etc.) were recorded every 10 minutes for 10 cows from each of the two milking groups (1M and 2M) at the Wednesday evening milking and the Thursday morning milkings, between half an hour before the beginning of milking (as defined by cow sorting before going to the milking parlour) and half an hour after the end (return of the last cows from the milking parlour). Some activities were recorded every time they occurred (miction, defecation, vocalisation, leakage of milk from the udder). Observations were carried out during weeks 1 to 3, and 5 and 6 of lactation.

2.4.2. Milk yield and composition

Milk yield was measured at each milking with milk meters. Milk specimens were sampled from each Tuesday and Wednesday milkings for protein, fat and lactose assays by infrared spectrophotometry (Milkoscan 4000; Foss Electric; Hillerod, Denmark), and for somatic cell count (automatic counter Fossmatic 5000; Foss Electric; Hillerod, Denmark). During the 3rd and 9th weeks of lactation, total protein and whey protein contents (after precipitation of protein by rennet) were measured on a Tuesday milk specimen (1 or 2 milkings). G1 immunoglobulins and blood albumin were assayed by radial immunodiffusion assays [28, 31, and lipoprotein lipase (LPL) activity using a radioactive substrate [32 modified by 15]. Plasmin activity and plasminogen- derived activity were determined according to the Bugaud et al's technique [4].

2.4.3. Udder condition

The udder stretching caused by milk accumulation was assessed from the difference in the distance between the end of the teats (front right and rear left) and the ground, as measured before and after the evening milking. This measurement was taken in the course of weeks 3 and 4 of lactation, in the milking parlour. Microbiological tests were carried out on milk specimens from each of the four quarters sampled during weeks 3 and 9, upon return to the pasture and during mastitis occurrence (data to be published elsewhere).

2.4.4. Feed intake, bodyweight, body condition, energy balance

Feed intake was measured on four days, weekly, during the stalling period. The cows were weighed between 13:00 and 14:00, twice during the first week following calving, then once every other week. Their body condition was assessed and rated 0 to 5 [3] at calving, during week 8 and every four weeks henceforth. Their energy balance was assessed as the difference between ration inputs and maintenance and production requirements [18, 37].

2.5. Data analysis and figure presentation

Milk yield and composition and food intake analyses involved three periods: (i) the first three weeks of lactation, during which half of the cows were milked once daily; (ii) the period from lactation weeks 4 to 16, when all cows were milked twice daily and were given the first N or L rations (see Tab. II); (iii) the entire experimental lactation period (30 weeks, on average). Data were subjected to analysis of variance and covariance using Statgraphics Plus software [38]. The number of milkings (1 or 2) during the first three weeks of lactation and the feeding level (L or N) were systematically taken into account in the analyses, as well as their interactions. Various covariates (live weight at calving, milk yield during the previous lactation…) were added to the model, depending on the analyses carried out (as documented in the result analyses or in the tables). In analysing the results of the entire trial, the duration of each cow's lactation was taken into account (as the covariate).

The milking robot discards the first 400 mL of milk (a prior measure of 250 milkings) without taking this quantity into account in the calculation of milk yield. That amount was added to the milk yield of each milking when the cows were milked by the robot.

Behavioural events were expressed as the mean number of occurrences per hour and per cow (comparison of data by the Mann-Whitney test) and as the number of cows manifesting such behaviour (values analysed by the independence test between the two characters).

In each of the 3 figures, data (milk yield, live weight change, body condition score change) are presented in 2 separate parts: (i) on the left part (first 16 weeks of lactation) they are plotted against the number of weeks of lactation (all cows are at the same stage); (ii) on the right part (from 21 February to 28 May; weeks 17 to 30, on average) data are plotted against the weeks of the calendar, in order to show more clearly the changes related to feeds, feeding, housing… Nevertheless, in the abcissa of the 3 figures, we substituted weeks of lactation for calendar weeks, for more clarity.

3. RESULTS

3.1. Cows' behaviour

Among the various behaviours, attitudes and events noted, only the results involving vocalisation and teat leakage exhibited differences between the 1M and 2M groups and therefore they alone will be presented. During the first three weeks of lactation, the number of vocalisations and the number of cows that expressed them were higher in the 1M than in the 2M groups. These differences were often significant ($P < 0.10$; Tab. III), at

Table III. Effect of milking once or twice a day during the first three weeks of lactation on vocalisations and milk losses from the udder.

Number of daily milkings for the first 21 days of lactation (number of cows)	1 (n=10)				2 (n=10)		
Concerned milking (1)	evening			morning	evening		morning
Period concerned (2)	before	during	after	before	before	after	before
Weeks 1 to 3							
Vocalisations·cow^{-1}·hour^{-1}	3.2^{a}	8.7	1.6^{A}	3.6	0.2^{b}	0.1^{B}	1.3
Number of vocalising cows	5	7	8^{A}	7^{a}	2	1^{B}	3^{b}
Number of cows losing milk	-	-	-	9^{a}	-	-	5^{b}
Weeks 5 and 6							
Vocalisations·cow^{-1}·hour^{-1}	1.7	-	0.7	4.9	0.3	0.1	0.5
Number of vocalising cows	3	-	1	4	3	1	1
Number of cows losing milk	-	-	-	4	-	-	5

(1) For the cows milked once daily, the evening milking was omitted.
(2) The period "before" was the half-an-hour preceding the departure of the cows that had to be milked to the milking parlour. The period "during" followed the "before" one and ended when the first milked animal came back from the milking parlour. The period "after" followed the "during" one and finished half an hour after the coming back of the last animal from the milking parlour.
^{aA} Significance of the differences was calculated between data from the same milking time ("morning" or "evening") and the same period ("before" or "after"). Means with different capital letters differ at the 0.05 level. Means with different lower case letters differ at the 0.10 level.

the evening milking in particular. It was during the milkings of the 2M groups that the number of vocalisations by cows in the 1M group was the highest. Nevertheless, separating cows milked once daily from the others, before milking, did not pose any problem to the handlers.

Individual leakages of milk from the udder could only be reliably counted before the morning milking because the cows' activities were more reduced than before the evening milking. During the first three weeks of lactation, the number of cows whose teats exhibited leakage of milk was higher ($P < 0.10$) in the 1M than in the 2M groups. The differences in vocalisation and milk leakage disappeared in weeks 5 and 6.

3.2. Milk yield

Cows from the 2M groups secreted more milk than those from the 1M groups during the first three weeks of lactation, when milking frequencies were different (Fig. 1 and Tab. IV). The difference in milk yield changed from 4.3 kg·d^{-1} in week 1 (P = 0.026) to 8.5 kg·d^{-1} in week 3 (P = 0.0001). The decrease in milk yield averaged over three weeks was proportional to the cows' production potential, as assessed from their yield during the previous lactation. As early as the 4th week, when all cows were milked twice a day, the difference in milk yield between the 2M and 1M groups was no longer significant. It changed from 2.3 kg·d^{-1} in week 4 (P = 0.18) to 1.1 kg·d^{-1} in the last week of the trial (P = 0.35).

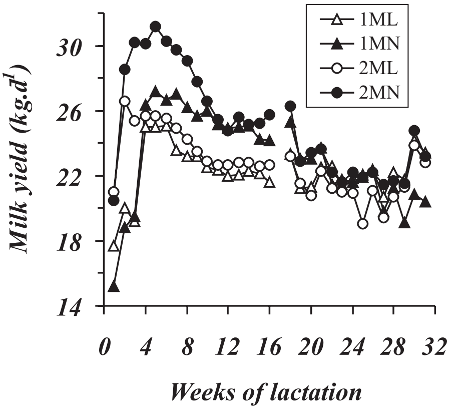

Figure 1. Effect of once or twice daily milking in the first three weeks of lactation and level of feeding on milk yield. Legend: 1M, 2M = once or twice daily milking; L, N = diet of low or normal energy concentration. For the explanation of the two parts (left and right) of the figure, see Materials and methods, paragraph "2.5. Data analysis and figure presentation".

Feeding N or L rations did not induce any difference in milk yield during the first three weeks of lactation. From week 4 to week 16, the N groups produced 3.1 kg·d^{-1} (P = 0.036) more milk than the L groups. That difference decreased to 0.75 kg·d^{-1} (P = 0.51) when the same diet was given to all cows (from week 19) and to 1.3 kg·d^{-1} (P = 0.23) when the cows were at pasture. No significant interaction between the milking frequency and feeding level of the ration was noted during the various experimental periods. However, the decrease in milk yield during weeks 1 to 3, as induced by once-daily milking, was 23% and 32% for feeding levels L and N.

During weeks 22 to 26, when the minimum interval between two milkings was 5 or 8 hours (see Materials and Methods and Tab. I), the mean number of milkings per day were 2.7 and 2.1, respectively (instead of 3 and 2 as expected). The amount of milk produced was 1.5 kg·d^{-1} greater ($P < 0.01$) in the cows milked 2.7 times a day. Milking frequency in early lactation had no effect on this response.

3.3. Milk composition

3.3.1. Fat, total protein, lactose and somatic cell count

The number of milkings at the beginning of lactation had no effect on protein, fat or lactose contents or on somatic cell count throughout the experiment (Tab. IV). With the N ration, protein and fat contents were

Table IV. Effect of milking once or twice a day during the first three weeks of lactation and level of feeding on milk yield and composition.

Dependent variable	Mean				Significance (2)			
	Group (1)				Number of milkings		Level of feeding	
	1ML	1MN	2ML	2MN	2M-1M	*P*	N-L	*P*
Milk ($kg \cdot d^{-1}$) (3)								
weeks 1 to 3	18.9[a]	17.8[a]	24.4[b]	26.3[b]	+ 7.0	< 0.05	+ 0.4	ns
weeks 4 to 16	23.0	25.7	23.9	27.4	+ 1.3	ns	+ 3.1	< 0.05
whole trial (4)	22.0	23.1	22.8	24.9	+ 1.3	ns	+ 1.6	ns
Fat ($g \cdot kg^{-1}$)								
weeks 2 and 3	43.5	49.8	41.9	46.5	– 2.5	ns	+ 5.5	< 0.10
weeks 4 to 16	36.0	40.0	38.6	40.6	+ 1.6	ns	+ 3.0	0.05
whole trial (4)	39.8	41.7	41.1	42.5	+ 1.1	ns	+ 1.6	ns
Proteins ($g \cdot kg^{-1}$)								
weeks 2 and 3	31.1	33.6	31.5	32.3	– 0.4	ns	+ 1.7	< 0.05
weeks 4 to 16	26.8	29.8	27.9	28.9	+ 0.1	ns	+ 2.0	< 0.05
whole trial (4)	29.4	31.1	30.4	31.0	+ 0.4	ns	+ 1.1	ns
Lactose ($g \cdot kg^{-1}$)								
weeks 2 and 3	47.6	48.0	48.3	49.6	+ 1.2	ns	+ 0.9	ns
week 4 to 16	46.3	48.2	47.2	47.8	+ 0.2	ns	+ 1.2	0.10
whole trial (4)	47.1	48.3	47.8	48.4	+ 0.4	ns	+ 0.9	ns
Somatic cell count ($log \cdot mL^{-1}$)								
weeks 2 and 3	4.65	4.77	4.69	4.63	– 0.05	ns	+ 0.03	ns
weeks 4 to 16	4.79	4.50	4.57	4.70	– 0.01	ns	– 0.08	ns
whole trial (4)	4.89	4.67	4.71	4.75	– 0.05	ns	– 0.09	ns

Means with different letters are different at the 0.05 level of significance.
ns = $P > 0.10$
(1) 1M = once-daily milking; 2M = twice-daily milking; N = normal level of feeding; L = low level of feeding.
(2) The interaction between the milking frequency and the level of feeding was never significant ($P > 0.10$).
(3) The peak yield of the previous lactation was introduced as a covariate and was always significant ($P < 0.05$).
(4) Thirty weeks of lactation, on average. The number of weeks of lactation of each cow was introduced as a covariate in statistical analysis.

significantly higher at the beginning of lactation than with the L ration (2.0 $g \cdot kg^{-1}$; $P = 0.030$, and 3.0 $g \cdot kg^{-1}$; $P = 0.054$, respectively). These differences disappeared when all cows were fed the same diet indoors (from week 19 on), as well as during the grazing period. Lactose levels and somatic cell counts were not significantly different between the N and L rations.

3.3.2. Caseins, whey proteins and enzyme activities

The milking frequency during the first three weeks of lactation had no effect on casein contents during weeks 3 and 9 (Tab. V). The whey protein content of the 1MN group was higher than that of the other groups in week 9, and consequently the casein/protein ratio in the milk was lower. The milk from that group was also richer in IgG1 in week 3. Serum albumin was the same in the 1M and 2M groups. LPL activity was lower in the milk of cows milked once daily than in that of cows milked twice daily, in week 3. That difference disappeared on week 9. The plasmin activity and plasminogen-derived activity exhibited no difference between the 1M and 2M groups.

Table V. Effect of once or twice daily milking in the first three weeks of lactation and the level of feeding on some proteins and enzymes in the milk.

Dependent variable	Mean				Significance				
	Group (1)				Number of milkings		Level of feeding		Inter-action (2)
	1ML	1MN	2ML	2MN	2M-1M	*P*	N-L	*P*	
Casein (g·kg^{-1})									
week 3	23.7	24.7	23.9	25.6	+ 0.6	ns	+ 1.4	< 0.05	ns
week 9	21.4	23.7	23.0	23.5	+ 0.7	ns	+ 1.4	ns	ns
Whey Protein (g·kg^{-1})									
week 3	5.9	7.2	6.0	5.8	– 0.6	ns	+ 0.6	ns	0.10
week 9	5.1^{a}	6.5^{b}	5.3^{a}	4.8^{a}	– 0.7	< 0.10	+ 0.4	ns	< 0.05
Casein / proteins (%)									
week 3	80.3	77.5	80.0	81.5	+ 1.9	ns	– 0.6	ns	= 0.10
week 9	80.8^{ab}	78.7^{a}	81.1^{ab}	82.9^{b}	+ 2.3	< 0.05	– 0.2	ns	< 0.05
Serum Albumin (mg·L^{-1})									
week 3	213	195	156	179	– 36	ns	+ 3	ns	ns
week 9	144	145	141	163	+ 8	ns	+ 12	ns	ns
Ig G1 (mg·L^{-1})									
week 3	752^{ab}	822^{b}	690^{ab}	542^{a}	– 171	< 0.05	– 39	ns	ns
week 9	450	437	412	412	– 32	ns	– 6	ns	ns
LPL (nmol·mn^{-1}·mL^{-1})									
week 3	653	971	1 022	1 394	+ 398	< 0.10	+ 347	< 0.10	ns
week 9	1 281	1 707	1 136	1 662	– 95	ns	+ 476	0.10	ns
Plasmin (3) (ΔDO $\times 10^4$)									
week 3	1.99	2.51	2.48	2.51	– 0.24	ns	– 0.21	ns	ns
week 9	2.83	2.47	3.16	2.37	+ 0.11	ns	– 0.58	ns	ns
Plasminogen (3) (ΔDO $\times 10^4$)									
week 3	20.80	17.78	20.42	21.07	+ 1.45	ns	– 1.18	ns	ns
week 9	25.66	26.83	23.56	29.71	+ 0.39	ns	+ 3.67	ns	ns
Plasmin/plasminogen (%)									
week 3	9.7	16.5	12.6	8.1	– 2.7	ns	+ 1.1	ns	ns
week 9	11.3	10.7	16.4	8.8	+ 1.6	ns	– 4.1	ns	ns

Means with different letters are different at the 0.05 level of significance.
ns = $P > 0.10$
(1) Definition of the treatments: see Table IV.
(2) Interaction between the number of milkings and the level of feeding.
(3) Activity is expressed in variation of optical density. Statistical analysis was performed on logarithmically transformed data.

In week 3, the N ration was associated with significantly higher milk casein contents and increased LPL activity ($P < 0.10$). Feeding level had no effect on the other parameters analysed.

3.4. Intake

Milking frequency had no effect on the feed intake amount throughout the trial (0.1 kg·d^{-1} difference between the 1M and 2M groups during weeks 1 to 16; $P = 0.86$). More N ration was ingested than the L ration (1.2 kg·d^{-1} during weeks 4 to 16; $P = 0.018$). That difference between the N and L groups disappeared during the single period ration (week 19 to 27): 0.20 kg·d^{-1}; $P = 0.83$.

3.5. Nutritional index

3.5.1. Live weight

The mean live weight after calving (677 kg on average) did not differ between the groups. The cows that were milked twice daily for the first three weeks of lactation lost 36 kg more live weight ($P < 0.01$) than those that were milked once a day (Tab. VI and Fig. 2). Throughout the rest of the trial, the weight of the cows in the 1M and 2M groups did not evolve in any significantly different manner.

The L ration induced a live weight loss of 35 kg more than did the N ration, between calving and week 16 of lactation. In contrast, between week 17 and return to the pasture, the cows of the L groups (which received the same ration as the cows of the N groups, from week 19 onwards) regained 29 kg more live weight than the cows previously fed the N ration ($P = 0.005$). During the grazing period, live weight changes were not different.

3.5.2. Body condition

Body condition scores at calving were 2.58, 2.83, 2.33, 3.17 for the 1ML, 1MN, 2ML, and 2MN groups, respectively. They were, on average, 3.00 and 2.46 in the N and L groups respectively ($P = 0.071$; Tab. VI and Fig. 3). Once-daily milking in early lactation had effects, comparatively to twice-daily milking, on body score and live weight evolution, which were similar to those of a high energy concentration diet, comparatively to a low energy concentration diet.

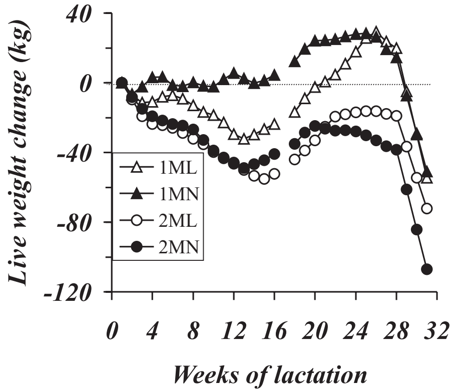

Figure 2. Effect of once or twice daily milking in the first three weeks of lactation and level of feeding on live weight change relative to live weight at calving. Legend and explanation of the two parts (left and right) of the figure: see Figure 1.

3.5.3. Energy balance

Cows from the 1M group exhibited a less negative or more positive energy balance than those from the 2M group during weeks 2 and 3 post-partum (Tab. VI) and also during weeks 4 to 16 (+ 3.4 UFL·d^{-1}; [$P = 0.0001$] and + 0.70 UFL·d^{-1}; [$P = 0.08$], respectively). Cows from the N groups presented a less negative energy balance than those from the L group during weeks 2 to 3 and 4 to 16 (+ 1.91 UFL·d^{-1} [$P = 0.02$] and + 1.46 UFL·d^{-1} [$P = 0.002$], respectively).

Table VI. Effect of once or twice daily milking in the first three weeks of lactation and level of feeding on live weight, body condition score and energy balance evolutions.

Dependent variable	Mean				Significance			
	Group (1)				Number of milkings		Level of feeding	
	1ML	1MN	2ML	2MN	2M-1M	P	N-L	P
Live weight change (kg) (2)								
Week 1 to 16	-27.8^{ab}	7.5^{a}	-63.4^{b}	-28.5^{ab}	–35.8	< 0.05	+35.1	< 0.05
Week 17 to 27	36.8^{a}	3.4^{b}	23.4^{ab}	-0.1^{b}	–8.5	ns	–28.7	< 0.05
Whole trial (3)	–60.5	–46.6	–88.8	–88.6	–35.2	< 0.05	+7.1	ns
Body condition score evolution (2)								
Week 1 to 16	-0.95^{a}	-0.24^{b}	-1.57^{c}	-0.79^{a}	–0.58	< 0.05	–0.75	< 0.05
Week 17 to 27	0.58	0.15	0.27	0.19	–0.14	ns	–0.27	< 0.10
Whole trial (3)	-0.64^{a}	0.33^{a}	-1.38^{b}	-1.03^{ab}	–1.05	< 0.05	+0.66	ns
Energy balance ($UFL \cdot d^{-1}$) (2)								
Weeks 2 and 3	-1.86^{b}	0.45^{c}	-4.82^{a}	-3.32^{ab}	–3.37	< 0.05	+1.91	< 0.05
Week 4 to 16	-1.28^{a}	0.37^{b}	-1.78^{a}	-0.53^{ab}	–0.70	< 0.10	+1.46	< 0.05

Means with different letters are different at the 0.05 level of significance.
ns = $P > 0.10$
(1) Definition of the treatments: see Table IV.
(2) The body condition score at calving was introduced as the covariate for the statistical analysis and was always significant at the 0.01 level. The interaction between the milking frequency and the level of feeding was never significant ($P > 0.10$).
(3) From calving to 28 May (30 weeks of lactation on average).

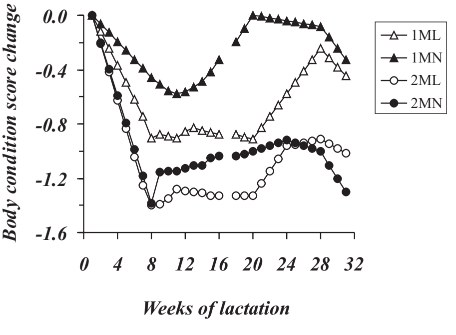

Figure 3. Effect of once or twice daily milking on the first three weeks of lactation and level of feeding on body condition score change relative to body condition score at calving. Legend and explanation of the two parts (left and right) of the figure: see Figure 1.

3.6. Health condition and udder condition

The somatic cell count of milk was not affected according to the number of milkings or the energy concentration of the ration throughout the experiment (Tab. IV). One cow from each of the 1MN, 2ML, and 2MN groups suffered a case of mastitis (twice in one cow from the 2MN group). Three cases of milk fever were noted in the 2ML group, one case each in the 1MN and 2MN groups and none in the 1ML group.

The variation of teat to ground clearance before the beginning and end of milking, as measured in week 3, was 1.8 cm in the 1M groups and 0.9 cm ($P = 0.04$) in the 2M groups. The difference between the two milking frequencies remained significant

(1.6 cm and 0.7 cm; $P = 0.003$) during week 4. Feeding level had no influence.

4. DISCUSSION

The performance of this trial was disrupted by the shortage and poor quality of grass silage, caused by vole (*Arvicola terrestris*) proliferation in the meadows, which induced a decrease in grass production and its contamination by dirt (high ash content in hay and silage). So the forage was changed in the course of the trial, intake level was lower than in the previous trial by approximately 2 kg DM (N ration) and milk yield was lower than expected, by about 5 $kg \cdot d^{-1}$ (cows from the 2MN group).

Despite these events, once daily milking for the first three weeks of lactation produced results in this trial similar to those previously observed [36]: a marked decrease in the amount of milk produced during the once-daily milking period without any significant carry-over effect after the twice-daily milking was restored; a notable improvement of nutritional status of cows at the beginning of lactation (live weight, body condition, energy balance) and sustained intake capacity. These aspects have already been discussed [36] and they will not be discussed any further here, in the absence of any new literature data.

This trial also confirmed:

– the apparently low impact of this management on the cows' behaviour, considered as an indicator of their well-being. This is in accordance with cow handlers expertise [36]. The behavioural changes that were measured here, were restricted to a significantly higher, albeit limited, number of vocalisations. Their cause has yet to be identified: pain induced by milk accumulation in the udder, or social stress linked to the transient dissociation of the group during milking? The low number of vocalisations out of these milking periods and their very sharp decrease (by a factor of 5) induced in just a few minutes by the return from the milking parlour of the cows milked twice indicates that the change of social context likely accounts for a great part of the cows' behaviour during the milking session that they were not a part of;

– the absence of an effect of once-daily milking on somatic cell count. There is conflicting literature on this aspect: an increase in somatic cell count [17, 22, 30, 40], no effect [25, 26, 41], increase or not depending on whether the baseline cell count was high or low [21]. Trials overall nonetheless showed the absence of an impact on mastitis incidence [11, 17, 30]. However, the greater stretching of udders observed here under the effect of a larger quantity of milk built up after 24-hours of secretion could, in the long run, lead to a higher incidence of collapsed udders. Also, milk leakage from the udder during the omitted milking could promote germ proliferation around the teat orifice and risk of mastitis. The lack of a large-scale application of this management method precludes any proper risk assessment.

On the contrary to the previous trial, milk fat and above all protein contents were not higher in the milk from the cows from the 1M group after the twice-a-day milking frequency was resumed, despite the more favourable nutritional status of these cows (lesser weight loss, etc.). It has previously been established that milk protein content is rather closely linked to energy balance [12]. We do not have any explanation for this result.

In week 3, the significant increase in IgG content and the trend towards an increase in albumin level during once-a-day milking were consistent with the literature results [1, 25]. But these variations were limited (0.2 $g \cdot kg^{-1}$ for the sum of these two proteins) and were not associated with an increased whey protein content, as often noted [1, 17, 26]. Also, somatic cell count was unchanged (re. Above). These various indices of the opening of mammary epithelium tight junctions in cows from the 1M groups were therefore less clear and not as consistent as in many literature reports. It should be noted that the protocol of this trial differed in several important aspects from most trials described in the literature: (i) protein fractions (IgG, blood albumin) were assayed 2.5 weeks on average following the implementation of once-daily milking, which excluded transient changes that occurred when switching from twice-daily to once-daily milking [39, 40, 42]; (ii) once-daily milking was applied immediately after calving, which may have helped the udder to gradually adapt to that milking frequency and thus restrict the effects noted with a brutal switch from twice to once-daily milking in the course of lactation; (iii) this trial was conducted at a very early lactation, when somatic cell count and IgA and BSA concentrations are at their lowest, because milk yield is the highest at that time (dilution effect) and also perhaps for physiological reasons (mammary cells in the differentiation process [23]; non gravid cows); (iv) in the statistical analysis, the cows could not be their own controls because the milking frequencies were applied just from calving, reducing the significance of certain differences. These various specific conditions may explain the lack of negative effects on milk composition of the once-daily milking, noted in this trial, in comparison with many literature reports.

The lower LPL activity in the milk of cows milked once a day may come as a surprise, but the literature on the subject is particularly scarce (see review by Davis et al. [13]). Milk LPL may originate from two sources of unknown respective importance: passage of LPL from non-mammary tissue to the blood, then from the blood to the milk, or the escape into the milk of some LPL synthesised by mammary epithelial cells, with the other part migrating towards blood capillaries where it would act upon circulating lipoproteins [6, 8]. The latter hypothesis would be consistent with a negative effect of increased intramammary pressure (or the accumulation of a Feedback Inhibitor of Lactation [43]) on mammary LPL synthesis and/or passage into milk since the milking frequency is decreased. Our findings are also consistent with the results of Azzara and Dimick [2] who showed that changing from two to 24 milkings a day induced a 5-fold increase in milk LPL activity in three of five experimental goats. These authors considered their results consistent with those of Linzell and Peaker ([29]; cited by Azzara & Dimick [2]) who observed that hourly milkings through the physiological administration of ocytocin activated paracellular passage of blood components through the mammary epithelium. These latest observations would be compatible with the former hypothesis, although it would need to be confirmed in cows because goat's milk LPL is secreted through different mechanisms than cow's milk LPL [6, 9]. The absence of an effect of once-daily milking on plasmin activity and plasminogen-derived activity has already been noted by Knutson et al. [24] (plasmin only) and Lacy-Hulbert et al. [26], but not by Kelly et al. [22] or Stelwagen et al. [41], who noted an increased activity. In these 4 literature cases, animals were near the end of lactation whereas our measurements were performed on milk samples taken at the beginning of lactation.

The feeding level had expected effects on milk yield and protein content [12]. Conversely, the increase in butter fat content in the milk from the best-fed cows is surprising [20] and we cannot explain it. These results need not be discussed any further since only the interaction between milking frequency and the level of feeding was of interest to us. Despite the different decreases in milk yield entailed by once-daily milking, whether the feeding level was normal or low (33% and 23%, respectively), the interaction was not significant. The low number of cows and the surprisingly fast drop in milk yield in the 2MN group probably limited the significance of the interaction. Auldist and Prosser [1] noted that once-daily milking reduced milk secretion more in well-fed cows than in underfed ones at the beginning of lactation, whereas in late lactation and in low-producing cows, Lacy-Hulbert et al. [25] did not observe any interaction. The trend ($P < 0.10$) towards a stimulating effect of feeding level on LPL activity is consistent with the inhibition of goat [7] and cow [10] milk LPL activity by underfeeding and its high stimulation by resumption of feeding.

5. CONCLUSION AND PROSPECTS

This trial broadly confirmed and completed the results from the previous one [36]. Once-daily milking in early lactation greatly improves the nutritional status of cows at this stage with only a limited impact on milk yield after the twice-daily routine has been restored, and on milk composition and enzyme activities.

This management technique could be of interest in herds chronically or circumstantially underfed and presenting a high frequency of health and/or reproduction problems. First behavioural observations indicate no major disturbance of the well being of cows. However, it should complicate breeders' tasks, except in large herds which make it possible to form a group of cows to be milked once daily for the first few weeks of lactation. The milking robot, which provides for simultaneous management of two milking intervals for individual cows, should ensure easy application of this management providing that the fitting of the teats on the once-daily cows is effective. Early observations indicate that such is not yet the case.

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