CAPÍTULO 3:

Objetivos experimentales

CAPÍTULO 3: Objetivos experimentales

El objetivo principal de este trabajo es la descripción de los fenómenos fisiológicos que ocurren durante la eyección de leche en el ordeño mecánico, y la valoración de los efectos sobre la producción de leche de la frecuencia y de los intervalos entre ordeños o de las alteraciones en las rutinas de ordeño, en vacas lecheras.

Los objetivos específicos son:

1. Efectos de la supresión de un ordeño semanal sobre los cambios productivos y morfológicos de la ubre en vacas lecheras.
   1.1. Evaluación de los efectos a medio plazo de la supresión de un ordeño por semana en la producción de leche y en el tamaño cisternal de la ubre medido por ecografía (Experiencia 1.).
   1.2. Evaluación de los efectos a corto plazo de la supresión de un ordeño por mana sobre la producción, la composición, el contenido de leche en células somáticas y el estado sanitario aparente de la ubre (Experiencia 2.).

2. Efectos de distintos intervalos entre ordeños sobre la composición química de la leche cisternal y alveolar en vacas lecheras (Experiencia 3).

3. Evolución de la leche cisternal a lo largo de la lactación y estudio del reflujo cisternal posterior a la estimulación del reflejo de eyección en la ubre de vacas lecheras.
   3.1. Evolución de la leche cisternal a lo largo de la lactación, valorada mediante ecografía, en vacas lecheras (Experiencia 4).
   3.2. Retorno de la leche desde las cisternas glandulares a los alvéolos o ‘fenómeno de reflujo cisternal’ (cisternal recoil) observado mediante ecografía en vacas lecheras (Experiencia 5).

CAPÍTULO 4:

Supresión de un ordeño por semana en vacas lecheras

(Omitting one milking weekly in dairy cows)

CAPÍTULO 4: Supresión de un ordeño por semana en vacas lecheras

(*Omitting one milking weekly in dairy cows*)

Effect of omitting one milking weekly on lactational performance and morphological udder changes in dairy cows

Abstract

The response of dairy cows to omitting one milking weekly was investigated in two successive experiments conducted with Holstein cows milked twice daily. Experiment 1 compared the lactational performances and udder changes in eight cows (31.2 L/d, 201 DIM) in the 5 wk before and the 5 wk after introducing the suppression of one milking weekly. Milk yield was recorded daily and milk composition twice weekly. Milk partitioning in the udder (alveolar and cisternal milk) and cisternal size (ultrasonography), 8h after milking, were also measured at the start and the end of the experiment. Although daily milk yield decreased 32% during the experiment (10 wk), linear regression analysis revealed a loss of milk yield of 1.1 L/d (3.7%) as a consequence of the omission of one milking weekly. Milk composition, lactation persistency and somatic cell count (SCC) were unaffected by milking omission. Milk partitioning in the udder decreased by 38% in alveolar milk volume and showed a tendency to decrease in cisternal milk volume (15%) and cisternal size (7%), as a result of milking omission and advancing lactation. Loss in total milk yield was negatively related with cisternal milk volume ($r = -0.77$) and cisternal size ($r = -0.70$) indicating smaller losses in the big cisterned udders. In Experiment 2, five cows (21.0 L/d, 227 DIM) previously adapted to the milking omission schedule were used to study the daily effects of milking omission on milk yield, milk composition and udder health during 10 wk. Milk yield and milk composition were approximately constant but SCC increased with lactation stage. The omission of one milking caused an important decrease in milk yield, fat content and SCC on the omission day and a compensatory increase over the following two days, but milk protein and lactose did not vary. All variables reached the average weekly value three days after the milking omission (6 milkings). In conclusion, under the conditions used, omitting one milking weekly slightly reduced milk yield and did not affect milk composition when healthy cows were used. Milk losses by milking omission depend on udder cistern characteristics; evaluating cistern size by

ultrasonography may be a useful tool for choosing cows that are better adapted to a reduced milking frequency.

Key words: Milking frequency, cisternal size, udder health, ultrasonography

4.1. Introduction

The excess of milk production caused by a subsidized market and by continual technical progress is a permanent problem for dairy policy in the European Community (Langer, 1990). To control this situation, a policy of rents and improvement in the quality of life for farmers has been recently proposed. With this in mind, the reduction of daily milking frequency seems to be an interesting alternative for limiting milk yield, as well as an improvement in the work conditions. Moreover, the omission of one or more milkings per week (preferably on Sunday afternoon) would provide an important improvement in the quality of life of farmers, especially in small or family based dairy farms.

Throughout the world, twice-a-day milking (2x) is the most frequent milking schedule of dairy cattle. Although, in the last 30 yr, twelve-hour intervals have been widely recommended, the 12-h milking frequency is not common in practice. Due to economical and social conditions, the unequal 8-16-h, 9-15-h or 10-14-h milking interval is more suitable than the equal 12-12-h milking interval (Sahr and Ormiston, 1966; Labussière and Coindet, 1968; Knight et al., 1994a). The accumulation of milk in the udder during extended milking intervals reduces the activity of mammary cells indicating that milk secretion is under local intra mammary control (Peaker and Wilde, 1996; Stelwagen and Knight, 1997, Stelwagen, 2001). Omitting one milking weekly, which can be done with a 24- or 18-h milking interval, may reduce milk yield to a lesser extent than once daily milking.

There are few reported studies on the effect of omitting one milking weekly, compared to twice a day milking, on milk yield in dairy animals. Yield losses ranged from 3 to 10% in cows (Labussière and Coindet, 1968; Radcliffe et al., 1973), 8 to 26% in ewes (Casu and Labussière, 1972; Labussière et al., 1974; Huidobro, 1988) and 3 to 5% in goats (Mocquot, 1978; Le Mens, 1978; Le Du, 1989). Yield losses seem to be related to the level of milk yield, breed and udder morphology. Recent studies suggest that udder anatomy (size of mammary cisterns) in terms of to milk storage characteristics may be an important factor in determining reduced yield associated with extended milking intervals (Knight and Dewhurst, 1994; Stelwagen et al., 1996; Davis et al., 1998).

A new approach for the study of udder cisterns in regard to milk production in dairy cows has been reported by Ayadi et al. (2003a) by using direct udder scanning.

The objective was to evaluate the short term effects of omitting one milking weekly (Sunday afternoon) on milk yield and milk composition and the medium term effects of the reduced milking frequency on milk yield and udder cistern size measured by ultrasonography.

4.2. Materials and Methods

4.2.1. Experiment 1

4.2.1.1. Animals and feeding

Eight Holstein cows (5 multiparous and 3 primiparous) from the S1GCE (Servei de Granges i Camps Experimentals) of the Universitat Autònoma of Barcelona were used during a 10 wk period to study the milk yield and morphological udder changes induced by the omission of one milking weekly. At the start of the experiment days in milk were 201 ± 15 d and milk yield was 31.2 ± 5.8 L/d (mean ± SEM). Cows were kept in tied stalls, with a 6 h/d period of exercise in a paddock provided with group feed-bunks and a water-point and fed ad libitum a total mixed ration (1.58 Mcal NE_l/kg; 16.6% CP, dry matter basis) according to their requirements (NRC, 2001).

4.2.1.2. Routine milking

During the experiment, milking was conducted in the stalls by using a high pipeline Westfalia Landtechnik (Granollers, Barcelona, Spain) milking system at 50 kPa (Vacurex, Westfalia Landtechnik). Pulsation ratio and rate were set at 60:40 and 60 pulses/min by using a Metatron 12 (Westfalia Landtechnik). The milking routine for the regular daily milking (0800 and 1800) included teat cleaning, machine stripping and teat dipping (P3-cide plus, Henkel Hygiene S.A., Madrid, Spain). All cows were free from clinical mastitis at the start of the experiments.

4.2.1.3. Experimental procedures, calculations and milk analysis

For the first 5 wk period of the experiment, cows were milked twice a day at a 10-14-h milking interval (0800 and 1800). For the second 5 wk period, cows were milked twice a day at the 10-14-h milking interval from Monday p.m. to Saturday p.m. and on Sunday only one milking was performed at 1300. The Monday a.m. milking was brought forward by one hour to 0700 to match the long milking intervals (18 h). So, the milking pattern at the weekend was as follows: Saturday a.m., 0800; Saturday p.m., 1800; Sunday p.m., 1300; Monday a.m., 0700; and, Monday p.m., 1800.

Yield of each milking was recorded individually by using a Metatron 12 automatic milk recording and proportional milk sampling unit (Westfalia Landtechnik). Milk yield loss produced by the omission of one milking weekly was calculated as the difference between the extrapolated values from milk yield regression of each experimental period on the week in which the milking schedule was changed (wk 5). Lactation persistency was calculated on a 4 wk basis from the slope of the milk yield regressions.

Representative milk samples for milk composition analysis of each cow were taken weekly at Friday (a.m. and p.m.) and Saturday (a.m. and p.m.) milkings to avoid residual effects of the milking omission according to the procedures recommended by Labussière and Coindet (1968). The a.m. and p.m. samples were composited in a daily sample according to milk yield at each milking. Milk fat, milk protein and milk lactose content were analyzed by using CombiFoss 5000 equipment (Foss España, Barcelona, Spain) and somatic cell count (SCC) by Fossomatic equipment (Fosselectric, Hillerød, Denmark).

To evaluate the effect of milking omission on the morphological changes produced in the cow udders, cisternal scans were performed 8 h after the a.m. milking and in duplicate for each udder quarter according to the methodology proposed by Ayadi et al. (2003a). Cows were randomly selected for scanning between Tuesday and Friday at the start and at the end of the experiment by using a real time B-mode ultrasonograph (Ultra Scan 900, Ami Medical Alliance Inc., Montreal, Canada) with a 5 MHz sectoral probe (2 dB power, 80° scanning angle, 0.5 mm axial and 1.5 mm lateral resolution). Prior to udder scanning, 10 μg/kg BW of an oxytocin receptor blocking agent (Atosiban®, Ferring Lab., Malmö, Sweden) was injected into the epigastric vein to prevent the milk ejection reflex and to make possible the recording of cisternal and alveolar milk fractions separately (Knight et al., 1994a; Bruckmaier et al., 1997b; Wellnitz et al., 1999).

Afterwards, duplicated udder scans in perpendicular planes were taken and the images were transmitted in groups of four to a portable computer and stored for processing. Image treatment software (MIP4 Advanced System, Microm España, Barcelona, Spain) was used to estimate by triplicate the cisternal area in the scans, and pixels converted to cm^2 (1 cm^2 = 1,024 pixels) for calculations as indicated by Ayadi et al. (2003a). After the scans, cisternal milk was milked from each udder quarter using a quartered claw and the values recorded. Alveolar milk was machine milked by using the quartered claw after a 20.0×10^{-3} IU/kg BW i.m. injection of oxytocin (Veterin Lobulor, Lab. Andreu, Barcelona, Spain).

Milk fractions per quarter were weighed separately following collection to an accuracy of 10 g, and the weights recorded.

4.2.2. Experiment 2

4.2.2.1. Animals, feeding and routine milking

Five Holstein cows (3 multiparous and 2 primiparous) from the S1GCE of the Universitat Autònoma of Barcelona, different from the cows used in Experiment 1 and previously adapted to the omission of one milking weekly schedule from the start of the first lactation, were used during a 10 wk period to study the daily effects of the omission of one milking weekly. At the start of the experiment days in milk were 227 ± 67 d and milk yield was 21.0 ± 3.4 L/d (mean ± SEM). Cows were kept in tied stalls with a 6 h/d period of exercise in a paddock, as in Experiment 1, and fed ad libitum a total mixed ration (1.2 Mcal NE_l/kg; 16.1% CP, dry matter basis) according to their requirements (Jarrige, 1989). The same milking routine and milking equipment as described in Experiment 1 were used.

4.2.2.2. Experimental procedures and milk analysis

For the 10 wks of the experiment, cows were milked twice a day at 0800 and 1800 but on Sunday one milking only was performed at 1200. Monday morning milking was done at the regular time (0800) due to labor organization reasons, resulting in unequal long milking intervals (Saturday p.m. to Sunday, 18-h; and, Sunday to Monday a.m., 20-h). Throughout the experiment, milk yield at each milking was recorded individually using a Metatron 12 (Westfalia Landtechnik). Milk samples of each cow at each milking were taken every day to analyze daily milk composition changes. Milk fat, protein and lactose content were analyzed by using NIRS equipment (Infra Alyser 450 D, Bran + Luebbe, Norderstedt, Germany), according to the methodology described by Albanell et al. (1999). Milk somatic cell counts were analyzed by using Fossomatic equipment (Fosselectric, Hillerød, Denmark). Average milk yield and composition for Friday and Saturday were used as reference values to evaluate the effect of changing the milking frequency on Sunday.

4.2.3. Statistical analysis

Data were processed by the PROC MIXED procedure for repeated measurements of SAS (version 8.1). The model included general means and the effects of the treatment, animal, experimental week, and the respective interactions and residual error. Logarithmic transformation ($\log_{10}$) of SCC was previously carried out for statistical analysis. When the probability of the interaction term was non significant ($P > 0.20$), it was deleted from the model. Differences between Least Square Means were localized with the PDIFF test and significance was declared at $P < 0.05$.

Linear regression analysis of milk yield for each experimental period was done by using the REPEATED statement in PROC MIXED using time as a regression variable. Pearson's correlation coefficients between cisternal areas and cisternal milk were also calculated.

4.3. Results

4.3.1. Experiment 1

4.3.1.1. Milk yield and milk composition

Milk yield decreased 32% ($P < 0.05$) during the experimental period (10 wk) according to progression in lactation (Figure 4.1). Persistency coefficient of milk yield was 95.9% on average during the experiment. Linear regression analysis of weekly milk yields for each experimental period, before and after the omission of one milking weekly, revealed non significant ($P > 0.05$) differences in the slope of lactation curves from wk 1 to 5 and 6 to 10 (Table 4.1). Interaction between the suppression of one milking on Sunday afternoon and the experimental week was not significant ($P = 0.58$) and milk yield persistency before and after the omission of one milking weekly (Table 4.1) was unaffected by the treatment.

Although milk yield decrease before and after the milking omission schedule, including the effect of advancing lactation, averaged 4.21 L/d (Table 4.1), the specific loss produced by the weekly omission was lower than calculated by mean of the milk yield regressions before and after the omission of one milking. Milk yield loss due to the weekly milking omission estimated by regression was 1.08 L/d, or 3.7%. Individual analysis per cow did not show significant differences in the milk yield slope between the before and after experimental periods. Milk composition did not change significantly ($P > 0.05$) during the experimental period (Table 4.1). Moreover, the omission of one milking applied from wk 5 did not induce any important change in any milk component (Figure 4.1).

Udder health change evaluated by monitoring the SCC during the experiment is also shown in Figure 4.2. Omitting one milking weekly did not affect ($P > 0.05$) the mean value of $\log_{10}$ SCC × 1000 which was steadily constant (2.32) during the experiment. The SCC response was very variable among cows and was dependent on SCC at the start of the experiment.

To investigate this possibility, cows were divided into two equal groups of animals according to the level of SCC: low (average 134.000 ± 40.000 cells/ml) and high (average 383.000 ± 59.000 cells/ml). Milking omission did not affect ($P > 0.05$) the SCC change in cows of the SCC low group but showed a tendency to increase in the cows of the SCC high group (+24%; $P = 0.20$).

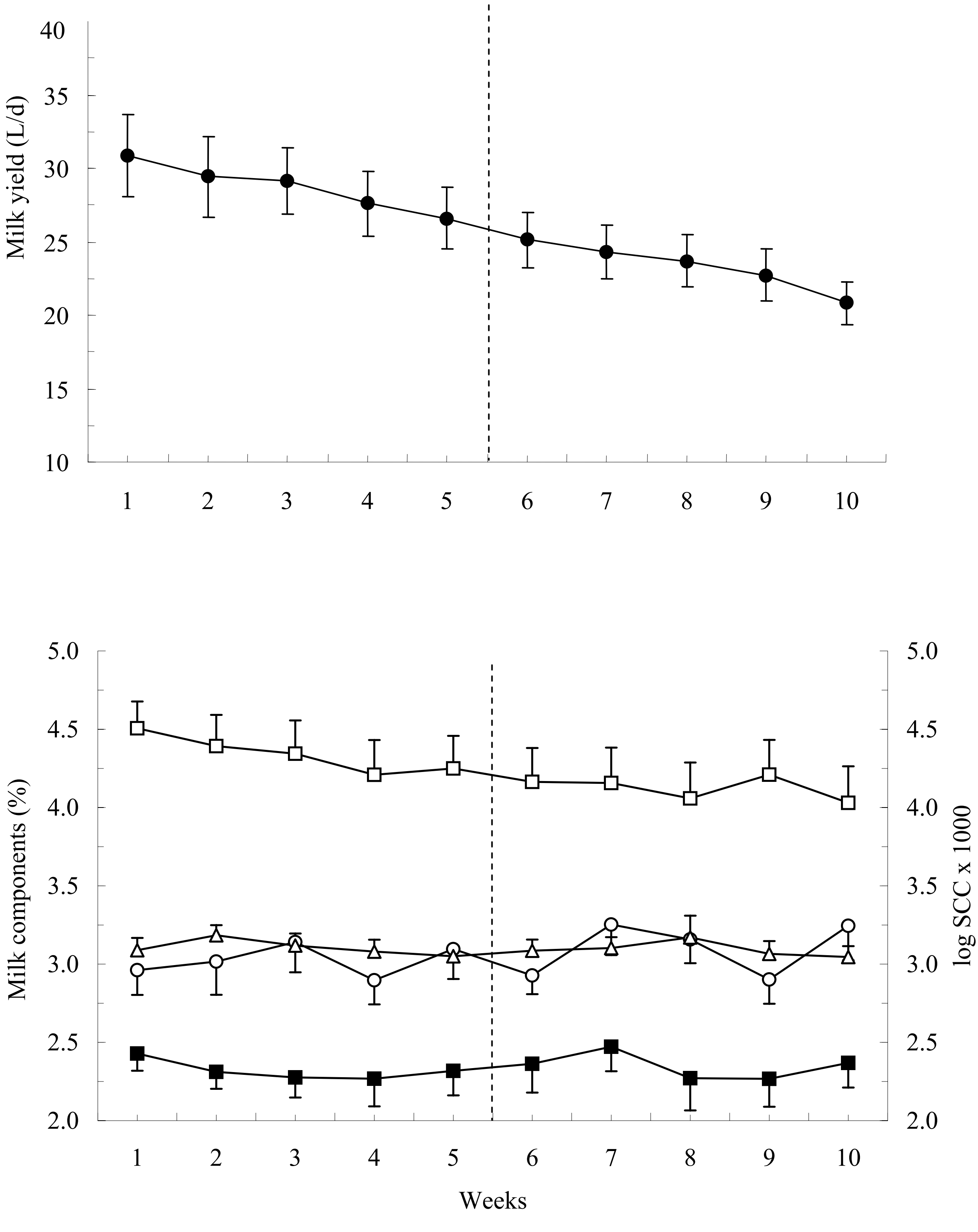

Figure 4.1. Change in milk yield (•), in percentages of milk fat (○), protein (Δ), lactose (□) and $\log_{10}$ SCC (■) during the 10 wk of the experiment. Values are the total dairy milk yield measured for 8 cows. Vertical bars represent SEM.

4.3.1.2. Relationship between udder cisternal size and yield loss

Average values of measured cisternal areas and evacuated cisternal milk volumes per quarter ranged from 4.1 to 55.0 cm^2 and from 0.21 to 1.95 L, respectively. Cisternal size did not significantly change (P > 0.05) after the alteration in milking frequency; the scanning area values were 21.7 ± 2.91cm^2 for the complete milking schedule and 19.94 ± 1.92 cm^2 for the Sunday afternoon milking omission schedule (Table 4.1).

Table 4.1. Effect of omitting one milking weekly on milk yield, milk composition, SCC and change of milk partitioning (cisternal and alveolar milk) in the udder 8 h after milking.

Item	14 milking/wk (wk 1 to 5)		13 milking/wk (wk 6 to 10)		Effect (*P*=)
	Mean	SEM	Mean	SEM	
Cows, n	8	-	8	-	-
Milk yield, L/d	28.81	2.11	24.60	2.70	0.042
Milk yield regression[1]					
a	32.06	2.18	29.76	3.19	-
b	- 1.162	0.315	- 0.912	0.315	-
R^2	0.97	-	0.94	-	-
Persistency coefficient[2], %	95.35	-	96.34	-	-
Milk composition					
Fat, %	3.11	0.21	2.91	0.21	0.771
Protein, %	3.06	0.09	3.10	0.08	0.936
Lactose, %	4.21	0.21	4.08	0.21	0.347
Log SCC × 1000	2.28	0.17	2.36	0.17	0.841
Milk partitioning[3]					
Alveolar, L	8.89	0.82	5.53	0.28	0.001
Cisternal, L	3.16	0.26	2.67	0.31	0.069
Cisternal area[4], cm^2	21.70	2.91	19.94	1.92	0.112

1: Milk yield (L/d) = a + b × (week).
2: Four wk milk yield persistency (%) = 100 – 4 × b.
3: Measured 8 h after milking.
4: Measured by ultrasonography 8 h after milking according to Ayadi *et al*. (2003a).

Milk partitioning in the udder 8 h after milking showed a decrease in alveolar milk volume (38%, P < 0.001) and a tendency to decrease in cisternal milk volume (15%, *P* < 0.07) and cisternal size (7%, *P* < 0.11) as a result of the joint effect of milking omission and advancing lactation (Table 4.1). Correlation between the scanning area and the volume of cisternal milk for each quarter 8 h after milking were significant and positive before (r = 0.82; *P* < 0.01) and after omitting one milking weekly (r = 0.80; *P* < 0.01), as well as when pooled data were used (r = 0.83; *P* < 0.01).

No significant ($P > 0.05$) correlation was found between milk yield and the loss produced by omitting one milking weekly. Moreover, loss of milk yield was not significantly correlated with alveolar milk volume at 8-h. However, there was a significant negative relationship between the cisternal area measured at 8-h and the loss of total milk yield on omitting one milking weekly which can be described by the equation,

Total milk yield loss (L/d) = 1.651 – 0.0261 × cisternal area (pixels $\times 10^{-3}$)

r = 0.701 ($P < 0.04$; residual SD = 0.44).

4.3.2. Experiment 2

4.3.2.1. Milk yield, milk composition and udder health

Average milk yield and milk composition did not significantly vary ($P > 0.05$) during the experimental weeks, but SCC increased with lactation stage. The evolution of milk yield, milk composition and SCC for the different days of the week is shown in Figure 4.2. On Sundays, milk yield (15.6 L/d), fat content (3.38%) and log SCC × 1000 (2.59) decreased by 29, 21 and 27% ($P < 0.05$), respectively, as a result of the change in milking frequency. On Mondays, milk yield (23.9 L/d), fat content (4.84%) and log SCC × 1000 (3.02) increased by 9, 14 and 100% ($P < 0.05$) respectively. The increase in SCC was dependent on the previous measurement but decreased again after four milkings. All values reached the average level by Wednesday. Milk protein (3.47%) increased by 2% and lactose (4.37%) decreased by 2% ($P < 0.05$) on average during the week. Compared with estimated for 14 milkings/week, omitting one milking weekly decreased the weekly yields of milk (3%), fat (4%), protein (5%) and lactose (5%), but milk SCC increased by 25%. Loss of milk yield varied according to the cow's yield but not to experimental wk. Clinical mastitis was not observed in any cows at any time of the experiment.

4.4. Discussion

In our experiment we obtained an overall reduction in milk yield lower than 4% when omitting one milking weekly in cows yielding around 30 L/d. Previous studies, however, demonstrated a reduction in milk yield varying from as little as 3% to as much as 10% as a result of omitting one milking weekly in comparison with twice daily milking (Radcliffe et al., 1973; Labussière and Coindet; 1968). Recent studies on the short term effects of omitting one milking weekly to reduce labor requirements on dairy farms, suggest that milk yield was not affected in late lactation (Sheehy, 2001; O'Brien et al., 2002).

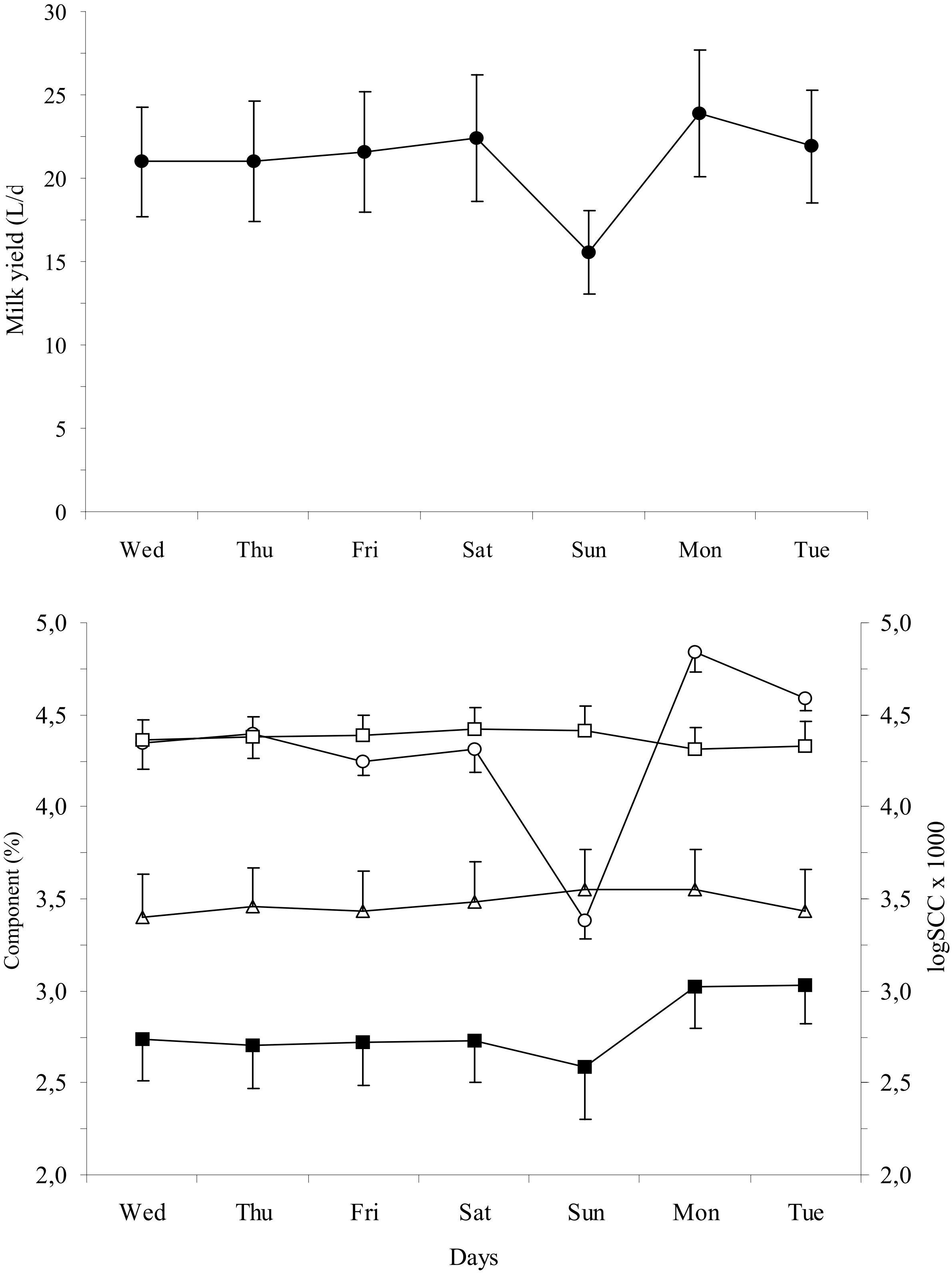

Figure 4.2. Change in milk yield (●) and percentage of milk fat (○), protein (Δ), lactose (□) and log $_{10}$ SCC (■) for different days of the week.

Moreover a reduction of 8 to 26 % in milk yield in dairy ewes (Casu and Labussière, 1972; Labussière et al., 1974; Huidobro, 1988) and a reduction of 3 to 5 % in milk yield in dairy goats (Mocquot, 1978; Le Mens, 1978; Le Du, 1989) by omitting one milking weekly has also been reported. This can be related to their respective cistern size. Pattern of milk yield change during the days of the week shown in our work agrees with the results obtained by Labussière and Coindet (1968) and Charron (1986) in which milk yield decreased during the day of the suppression and increased dramatically 24-h after. Nevertheless, milk yield values reached the average level 72-h after omitting the milking. In our experiments we observed that the loss of milk yield produced by omitting one milking weekly was not affected by milk yield (Experiment 1) or by the lactation wk of the cows (Experiment 2) although in both cases the cows were in late lactation (201 to 236 DIM in Experiment 1; and, 227 to 297 DIM in Experiment 2).

The size of the cistern plays a major role in the ability of the udder to accumulate 24-h worth of milk secretion, it is responsible for approximately half of the functional udder capacity in cows (Davis et al., 1998). However, the question arises as to whether a larger cistern is associated with increased tolerance of the extended milking interval in animals.

In relation to understanding the yield loss incurred when extended milking intervals are applied, Davis et al. (1998) and Stelwagen (2001) reported that, together with cisternal size, the freedom of alveoli to drain (actively or passively) to the cistern is an important factor to explain the loss of milk yield observed when the milking interval is extended to 24-h. Knight et al. (1994) and Ayadi et al. (2003a) showed that correlation between cisternal milk and cisternal size was higher at 8-h after milking than longer intervals. Based on those data, cisternal measurements in the present study (Experiment 1) were made 8-h after the morning milking. The average size of the cisterns observed in our work by scanning for 8-h milking intervals was similar before and after omitting one milking weekly.

Moreover, the methodology of Ayadi et al. (2003a) was adequate to obtain a repeatable image of the udder cistern, as indicated by the high correlation between cisternal milk and cisternal size in the two periods of the experiment. Correlation between the scanning area and the cisternal milk for each quarter at the 8-h milking interval were significant and positive. This result agrees with those previously reported by Bruckmaier et al. (1994) and Ayadi et al. (2003a) in cows. Moreover, Knight and Dewhurst (1994) reported a significant relationship between udder volume and milk yield. In our result (Experiment 1) we showed a strong negative correlation between the reduction in milk yield attributable to omitting one milking weekly and cisternal size measured by ultrasonography.

The same conclusion was obtained in previous studies comparing once versus twice daily milkings (Knight and Dewhurst, 1994; Stelwagen and Knight, 1997; Davis et al., 1998) with the large cisternal cows showing the smallest decrease in yield. The interpretation of these data was that the putative effect of the Feedback Inhibitor of Lactation (FIL), which is a milk protein and an autocrine inhibitor of milk secretion (Peaker and Wilde, 1996), would be concentration-dependent and inactive in the cisternal milk as reported by Knight and Dewhurst (1994) and Wilde et al. (1995). Therefore, udders with large cisterns produce more milk and are more tolerant of longer milking intervals in dairy cows (Knight and Dewhurst, 1994), dairy goats (Peaker and Blatchford, 1988; Salama et al., 2003) and dairy ewes (Labussière, 1988; Rovai et al., 2000). On the other hand, small cisterned cows, by virtue of being more affected by FIL, respond better to increased milking frequency (Knight and Dewhurst, 1994).

Omitting one milking weekly did not significantly affect the milk yield composition in Experiment 1. This result agrees with the recent short-term effects observed by Sheehy (2001) and O'Brien et al. (2002) but is not in accordance with data from all lactation. Labussière and Coindet (1968) obtained an overall reduction in milk composition of 6 to 7% when one milking was omitted. The results obtained in Experiment 2 about milk composition and SCC changes for the different days of the week were in accordance with Labussière and Coindet (1968) and all values reached the average level 72-h after omitting one milking weekly. All cows were free from clinical mastitis in both trials. Although SCC in milk after the omission of one milking weekly vary, previous studies confirmed our results in short and long term experiments in dairy cows (Labussière and Coindet, 1968; Sheehy, 2001; O'Brien et al., 2002), dairy ewes (Casu and Labussière, 1972; Labussière, 1974) and dairy goats (Mocquot, 1978; LeMens, 1978).

4.5. Conclusions

We conclude that, with our conditions, omitting one milking weekly slightly reduced milk yield and did not affect milk composition nor lactation persistency when healthy cows were used. Milk losses by milking omission depend on udder cistern characteristics; evaluating cistern size by ultrasonography may be a useful tool for choosing cows that are better adapted to a reduced milking frequency. Milk yield is reduced less in cows that store a greater proportion of their total milk production within the cistern. Also, milk recording should be conducted in the middle of the week to avoid alterations caused by the milking omission.

New research on the effects of omitting one milking weekly on high yielding cows in early lactation is required before recommending this milking schedule for general practice.

CAPÍTULO 5:

Efectos de distintas intervalos entre ordeños sobre la composición química de la leche en vacas lecheras

(The effects of different milking intervals on milk composition in dairy cows)

CAPÍTULO 5: Efectos de distintas intervalos entre ordeños sobre la composición de la leche en vacas.

(*The effects of different milking intervals on milk composition in dairy cows*)

The effect of different milking intervals on milk composition of cisternal and alveolar milk in dairy cows

Abstract

With the aim of studying the change of milk composition in the udder compartments for different milking intervals, four lactating Holstein cows were used in a 5 week trial. Cisternal and alveolar milk were measured randomly by udder quarter at 4, 8, 12, 16, 20 and 24 h intervals with a 3 d interperiod of regular milking. Cisternal milk was evacuated using a cannula after an injection of an oxytocin receptor blocking agent, followed by an injection of oxytocin to remove the alveolar fraction. Milk samples from each fraction and quarter were collected for analysis. Cisternal and alveolar milk increased with milking interval and represented on average 30 and 70% of the milk stored in the udder, respectively. Fat content in alveolar milk remained constant during the first 16 h, increasing rapidly thereafter and reaching its maximum at 24 h (6·95%). Fat content in cisternal milk decreased with milking interval and reached its minimum at 24 h (0·96%). Total fat yield tended to increase for cisternal milk with longer milking intervals, but it increased markedly for alveolar milk, showing that fat globules do not pass freely from alveoli to cistern between milkings. Milk protein content was greater in rear quarters than in front quarters for both milk fractions. Milk protein content increased in the cisternal milk fraction and tended to increase in the alveolar milk fraction with longer milking intervals, but values did not differ between cisternal and alveolar fractions or between front and rear quarters. Total protein yield increased with milking interval in both fractions, indicating that casein micelles pass more freely than fat globules from the alveolar to the cisternal compartment. In conclusion, the short term effects of milking intervals in milk composition were explained by the changes observed in alveolar and cisternal milk ratio with for dairy cows.

Key words : cisternal milk, alveolar milk, milking interval, milk composition

5.1. Introduction

Milk partitioning in the udder (i.e. milk fractions), residual milk (i.e. obtained after i.v. oxytocin injection) and milk flow during machine milking have been generally used in the different species of dairy ruminants to evaluate machine milking ability (Labussière, 1988; Le Du et al. 1994; Peris et al. 1996). Large differences between species exist with respect to the proportion of total milk that can be stored within the cistern. After a normal milking interval (8 to 16 h) dairy cattle store less than 30% of the total milk yield volume in the cistern (Bruckmaier et al. 1994*a*; Pfeilsticker et al. 1996; Ayadi et al. 2003*a*) whereas on the contrary the cisternal fraction ranges from 57 to 88% in dairy goats (Peaker & Blatchford, 1988; Knight et al. 1994*a*). In sheep, big differences have been reported for cisternal milk with values ranging from more than 50% for dairy breeds (McKusick et al. 2002; Rovai et al. 2002) to less than 30% for meat breeds (Caja et al. 1999), demonstrating the effects of selection on udder morphology.

Partitioning of milk between alveolar and cisternal compartments in the udder also differs for different species according to milking intervals (Peaker & Blatchford, 1988; Knight et al. 1994*b*; McKusick et al. 2002) and the concentration of milk components is dramatically affected by the place of milk storage in the mammary gland. There are several reports that demonstrate differences in distribution and accumulation of cisternal and alveolar milk fractions according to milking interval in dairy cows (Knight et al. 1994*a*; Stelwagen et al. 1996; Ayadi et al. 2003*a*). Davis et al. (1998) and Stelwagen (2001) reported that, together with cisternal size, the ability of alveoli to drain (actively or passively) to the cistern is an important factor in explaining the change of milk yield and milk composition observed when the milking interval is extended to 24 h. However, only Davis et al. (1998) reported the changes observed simultaneously in yield and composition of each milk compartment of the cow udder for long milking intervals (24 and 40 h).

A lower concentration of milk fat within the cistern, compared to the alveoli, has been reported in dairy cows (Labussière, 1985; Davis et al. 1998; Waldmann et al. 1999), and in dairy ewes (Labussière, 1969; McKusick et al. 2002). On the contrary, differences between alveolar and cisternal content of milk protein and lactose appear to be minimal (Labussière, 1985; Davis et al. 1998). Milk protein content is less variable than milk fat according to milking interval (Villiers & Smith, 1976) and milking frequency (DePeters et al. 1985; Holmes et al. 1992).

The increase of milking frequency from 1 to 2 times a day (Carruthers et al. 1993; Stelwagen et al. 1994b) or from 2 to 3 times a day (Szuchs et al. 1986; Erdman & Varner, 1995) decreased fat and protein content in the milk of dairy cows. These results do not agree with Holmes et al. (1992), Oltenacu et al. (1994) and Klei et al. (1997) who all found that fat and protein content was constant or increased when milking frequency increased. When milking frequency was higher than 3 times a day, milk composition in dairy cows only varied slightly (Van der Iest & Hillerton, 1989; Ipema & Benders, 1992). Moreover, Labussière (1985) reported that fat and protein contents are greater in the front than in the rear quarters of the dairy cow udder.

Knowledge of the interaction between secretion of milk components and milk storage in the udder at different milking intervals can be useful in determining appropriate management systems and milking routines for dairy animals, specially when automatic milking is adopted (Weiss et al. 2002). Moreover, variation of milk composition according to milking interval can be useful for establishing the sampling schedule at automatic milk recording with variable milking intervals (Friggens & Rasmussen, 2001; Peeters & Galesloot, 2002).

The aim of this study was to complete previous research which was carried out on the effects of different milking intervals on milk yield (Ayadi et al. 2003*a*) by studying the change in milk composition in the alveolar and cisternal compartments of the udder of dairy cows. This will help to understand the effects of milk ejection between milkings on milk composition and the implications for udder morphology.

5.2. Materials and Methods

5.2.1. Animals, feeding and routine milking

Four Holstein cows were used for a 5-week period as indicated by Ayadi et al. (2003*a*). Cows (215 ± 25 d in milk and 20 ± 3 l/d milk yield) were kept in tied stalls and fed ad libitum a total mixed ration (5·02 MJ NE_l; 16·1% CP, dry matter basis) distributed in two equal portions at 09.00 and 15.00. Cows were milked in the stalls using a high pipeline Westfalia Landtechnik (Granollers, Barcelona, Spain) milking system set at 50 kPa, 60:40 pulsation ratio and 60 pulses/min. The milking routine for the regular daily milking (08.00 and 18.00) included teat cleaning, foremilk hand milking, machine milking, machine stripping (with hand massage when milk flow was smaller than 0.25 l/min), and teat dipping. Udder health was checked weekly by using a Californian Mastitis Test reagent (Pitman-Moore España S.A., Porriño, Pontevedra, Spain) in the foremilk collected during udder preparation of a regular morning milking.

5.2.2. Experimental procedures

Experimental design consisted of an incomplete Latin square with six milking intervals at random. Cisternal and alveolar milk volumes were determined in duplicate at 4, 8, 12, 16, 20 and 24 h after a regular milking. A 3 d interperiod with regular milking was applied to prevent carry over effects between experimental milking intervals.

In order to prevent the milk ejection reflex and to make possible the recording of cisternal and alveolar milk fractions separately (Knight et al. 1994*b*; Bruckmaier et al. 1997b), 10 μg/kg BW of an oxytocin receptor blocking agent (Atosiban®, Ferring Lab., Malmö, Sweden) as reported by Wellnitz et al. (1999) was injected into the epigastric vein. Cisternal milk was drained from each udder quarter using a teat cannula (3 mm Ø × 10 cm length; VP 23 N Aesculap, Hanover, Germany) and values were recorded. Alveolar milk was obtained after an i.m. injection of oxytocin at a supraphysiological dose ($20{\cdot}0 \times 10^{-3}$ i.u./kg BW, Veterin Lobulor, Lab. Andreu, Barcelona, Spain) to remove residual Atosiban from the oxytocin receptors and to induce milk ejection. Ten min after, alveolar milk was machine milked by using a quartered claw.

Milk fractions per quarter were weighed following collection to an accuracy of 10 g, the weights were recorded and a representative sample of each fraction and quarter was collected for composition analysis. Milk fat and protein content were determined by using an NIRS analyzer (Infra Alyser 450 D, Bran + Luebbe, Norderstedt, Germany), according to the methodology described by Albanell et al. (1999).

5.2.3. Statistical analysis

Results were analysed by the PROC MIXED procedure for repeated measurements of SAS (version 8·1). The model included general mean and the effects of the animal, milking intervals, udder quarter, measuring day, their interactions and the residual error. When the interaction term was non significant ($P > 0{\cdot}20$), it was deleted from the model. No differences were observed for the left and right udder quarters and therefore their values were averaged and analysed together. Differences between least square means were determined with the PDIFF (pretty print difference between files) test of SAS and significance was declared at $P < 0{\cdot}05$. Linear regression analysis was done by using the REPEATED statement in PROC MIXED using milking interval as a regression variable.

5.3. Results

No subclinical mastitis were detected in any udder quarter during the experimental period by using the Californian Mastitis Test, and udders were considered healthy. Changes in total milk yield and milk composition according to milking interval are summarized in Table 5.1. in which the estimated yield in daily energy corrected milk at 3·5% fat (3·5% ECM) has also been included. Linear regression analysis indicates a significant relationship between 3·5% ECM daily yield and milking frequency, making the equation

$$3{\cdot}5\%\ \text{ECM (l/d)} = 1{\cdot}604 \times \text{milkings per day} + 17{\cdot}25,$$

$$r^2 = 0{\cdot}91,\ P < 0{\cdot}01,\ \text{RSD} = 0{\cdot}36.$$

Extreme differences were observed in the 3·5% ECM ranging from a reduction of 10%, for the once a day milking frequency (24 h milking interval), to an increase of 30% for the six milkings a day (4 h milking interval) when compared to 12 h milking interval.

Table 5.1. Milk yield and milk composition of dairy cows at different milking intervals (Values are means ± SEM)

Milking interval, h	Yield, l	Fat, %	Protein, %	Estimated ECM[1], l/d
4	3·8 ± 0·4[a]	4·77 ± 0·17[a]	2·93 ± 0·07[a]	26·7 ± 2·2[a] (130%)
8	7·0 ± 1·2[ab]	4·04 ± 0.11[bc]	2·97 ± 0·07[ab]	22·2 ± 3·7[b] (108%)
12	9·7 ± 2.0[bc]	4·07 ± 0.09[bcd]	3·13 ± 0.07[bc]	20·5 ± 4·0[c] (100%)
16	14·6 ± 2.8[c]	3·49 ± 0·13[d]	3·36 ± 0·07[bd]	21·3 ± 4·2[cd] (104%)
20	15·4 ± 3·1[c]	3·50 ± 0·19[cd]	3·30± 0·08[cd]	18·0 ± 3·7[d] (88%)
24	16·0 ± 2·3[c]	4·62 ± 0·13[ab]	3·29 ± 0·07[bd]	18·4 ± 2·3[d] (90%)

[a,b,..d] : Different letters in the same column indicate significant differences at $P < 0{\cdot}05$.

[1]: Energy Corrected Milk at 3·5% fat (l/d) = milk yield (l/d) × [0·162 × (% fat) + 0·432].

No differences were observed in milk yield or milk composition for the left and right udder quarters but, on the contrary, significant differences in milk yield and some tendencies in milk composition were observed between front and rear quarters. Cisternal milk increased according to milking interval and total volume ranged from 0·10 to 1·89 l per quarter (15 to 42% of the total milk in the udder) for the 4 to 24 h milking interval (Table 5.2), being 30% on average.

Table 5.2. Milk yield and milk composition in the front and rear udder quarters at different milking intervals in dairy cows (Values are means ± SEM)

		Milk, l		Fat, %		Protein, %	
Fraction	Milking interval, h	Front	Rear	Front	Rear	Front	Rear
Cisternal	4	0·10 ± 0·01[a]	0·18 ± 0·01[a]	5·97 ± 0·12[a]	5·42 ± 0·38[a]	2·49 ± 0·35[a]	2·92 ± 0·01[a]
	8	0·18 ± 0·03[a]	0·30 ± 0·03[a]	3·45 ± 0·05[b]	2·72 ± 0.43[b]	2·93 ± 0·01[a]	2·93 ± 0.05[a]
	12	0·31 ± 0·06[a]	0·49 ± 0.05[b]	2·17 ± 0.01[c]	2·10 ± 0.01[b]	3·18 ± 0.01[a]	3·27 ± 0.02[b]
	16	0·98 ± 0.22[b]	1·32 ± 0.13[c]	0·89 ± 0.05[d]	0·96 ± 0·01[c]	3·24 ± 0.03[b]	3·22 ± 0·01[b]
	20	1·41 ± 0·27[b]	1·89 ± 0·25[d]	1·01 ± 0·03[d]	1·07 ± 0·01[c]	3·31 ± 0·01[b]	3·37 ± 0·01[b]
	24	1·25 ± 0·20[b]	1·86 ± 0·02[d]	0·92 ± 0·03[d]	1·00 ± 0·02[c]	3·23 ± 0·02[b]	3·35 ± 0·11[b]
Alveolar	4	0·71 ± 0·09[a]	0·91 ± 0·07[a]	4·66 ± 0·07[ab]	4·60 ± 0·04[ab]	3·06 ± 0·01	2·88 ± 0·15
	8	1·26 ± 0·19[ab]	1·77 ± 0·17[b]	4·27 ± 0·12[ab]	4·17 ± 0.07[a]	3·28 ± 0·01	2·78 ± 0.45
	12	1·71 ± 0.24[bc]	2·32 ± 0.12[bc]	4·45 ± 0.21[a]	4·46 ± 0·07[ab]	2·89 ± 0.59	3·28 ± 0·07
	16	2·06 ± 0.27[c]	3·03 ± 0.22[c]	4·57 ± 0·06[a]	4·66 ± 0·03[ab]	3·22 ± 0·01	3·23 ± 0·01
	20	1·87 ± 0·11[c]	2·53 ± 0·03[bc]	5·51 ± 0·07[b]	5·21 ± 0·04[b]	3·22 ± 0·05	3·31 ± 0·05
	24	2·23 ± 0·44[c]	2·65 ± 0·12[c]	6·92 ± 0·02[c]	6·98 ± 0·01[c]	3·19 ± 0·03	3·36 ± 0·07

[a,b,...d] : Different letters in the same column indicate significant differences at $P < 0·05$.

There was a slight increase in cisternal milk volume until 12 h after milking (16%) but thereafter the increase was more rapid, reaching a plateau after 20 h (42%).

Rear quarters stored, on average, 34% more cisternal milk (25 to 44%) than the front quarters, and the overall ratio of front to rear cisternal milk was 40 : 60.

Alveolar milk volume also increased from 0.71 to 2.65 l per quarter (Table 5.2) according to milking interval, but its accumulation pattern differed markedly from cisternal milk. Contribution of alveolar milk to total milk in the udder decreased from 85 to 48% and represented 70% on average. Rear quarters showed 27% greater (16 to 34%) alveolar milk volume than the front quarters, and the overall ratio of front to rear alveolar milk was 42 : 58. Ratio of cisternal to alveolar milk indicates a dramatic change in the milk storage pattern in the udder between 12 h (17 : 83) and 20 h (43 : 57), the difference being significant ($P < 0{\cdot}01$).

Fat content in total milk was high and evolved quadratically ($R^2 = 0.77$; $P < 0{\cdot}05$) in a concave shape with milking interval (Table 5.1). It showed constant values for the 16 to 20 h milking intervals, but no differences ($P > 0{\cdot}05$) were observed between milk fat content at the extreme milking intervals (4 and 24 h). Milk composition of cisternal and alveolar fractions varied inversely at different milking intervals (Fig. 5.1a). Milk fat percentage decreased in cisternal milk ($P < 0{\cdot}001$) and increased in alveolar milk ($P < 0{\cdot}001$). No differences were reported between the left and right quarters ($P = 0{\cdot}64$), but percentage of fat in cisternal milk tended to be higher ($P < 0{\cdot}07$) in the front quarters at the 4 and 8 h milking intervals, compared to the rear quarters (Table 5.2). Despite this tendency in fat content of cisternal milk in the front and rear quarters, the minimum milk fat percentage was reached at the same milking interval (16 h) in both quarters. Fat percentage in alveolar milk remained constant during the first 16 h, increasing rapidly and significantly ($P < 0{\cdot}05$) thereafter (Fig. 5.1a). Final value of fat percentage in mean alveolar milk (6·95%) was higher than initial (5·62%; $P < 0{\cdot}05$) and final (0·96%; $P < 0{\cdot}001$) values of mean cisternal milk as can be calculated from Table 5.2.

As a consequence of the fractional changes in volume and composition, daily fat yield tended to increase for cisternal milk ($P = 0{\cdot}10$), and increased significantly for alveolar milk ($P < 0{\cdot}001$), with longer milking intervals.

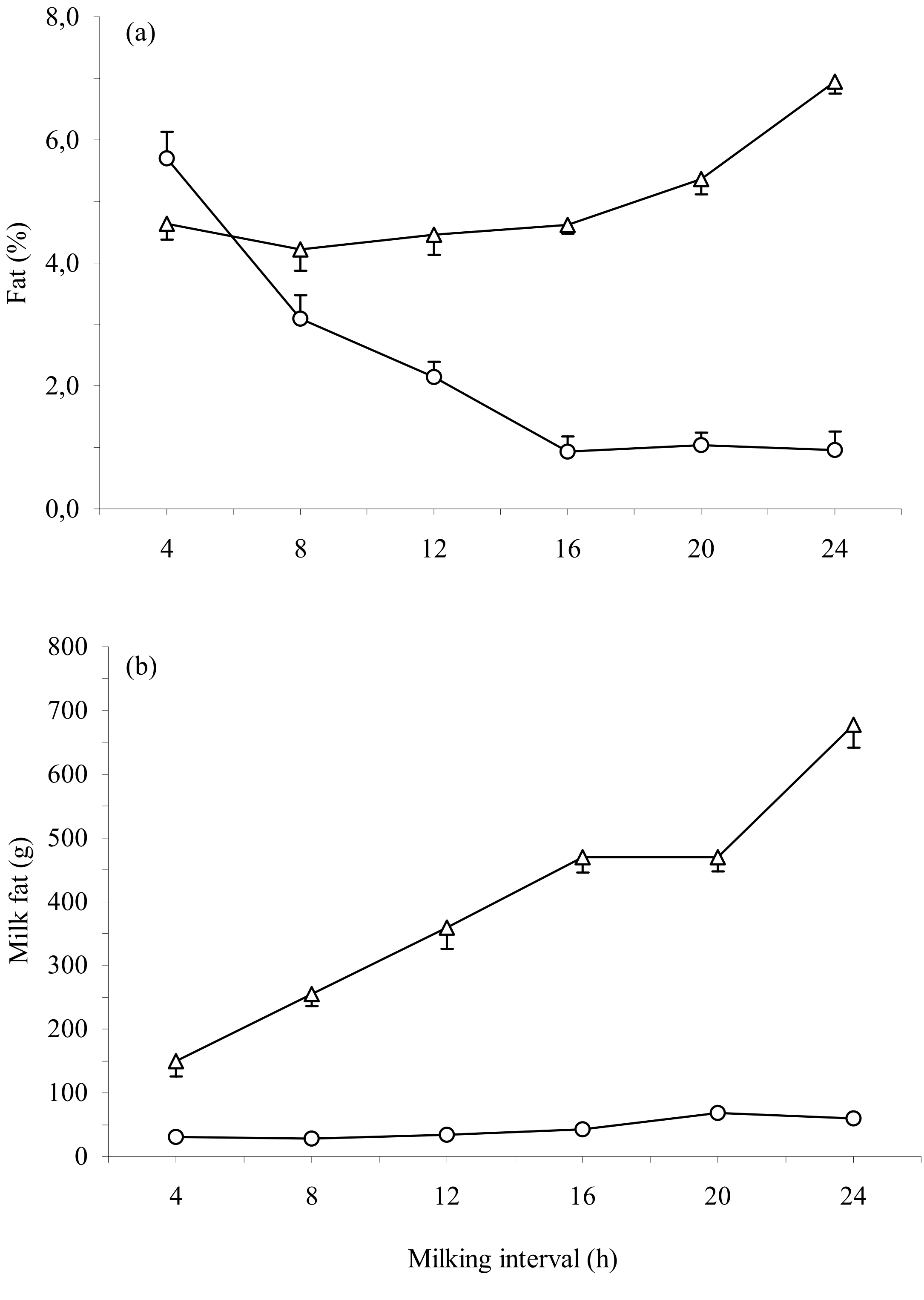

Figure 5.1. Changes in fat content (a) and fat yield (b) for cisternal (○) and alveolar (Δ) milk fractions for different milking intervals in four dairy cows. Data are means ± SEM.

Fat yield was greater ($P < 0{\cdot}001$) in both milk fractions (Fig. 5.1b) in the rear quarters than in the front quarters (data not shown). Changes in cisternal and alveolar milk protein according to milking interval are shown in Fig. 5.2a. Milk protein content was less variable than milk fat content according to milking interval.

Milk protein content increased in the cisternal milk fraction ($P < 0{\cdot}001$) and tended to increase in the alveolar milk fraction ($P = 0{\cdot}08$) with longer milking intervals. Initial and final milk protein values did not differ between cisternal and alveolar fractions or between front and rear quarters (Table 5.2.).

Protein yield increased ($P < 0{\cdot}05$) with milking interval in both fractions as shown in Fig. 5.2b and was greater in the rear quarters than in the front quarters ($P < 0{\cdot}01$) for both milk fractions (Table 5.2.).

5.4. Discussion

The change of cisternal and alveolar milk during different intervals observed in our work, is in close agreement with earlier observations (Knight et al. 1994*b*; Davis et al. 1998) and current models of milk accumulation (Stelwagen, 2001) as indicated by Ayadi et al. (2003*a*). Nevertheless the methodology used in our experiment only allowed to see the direct and short term effects of milking frequency in late lactation. This was the case of the significant linear effect of the number of milkings per day on the daily milk yield which agreed with the hypothesis of a fixed effect of the number of milkings as Erdman & Varner (1995) propose. Nevertheless, the regression coefficient calculated in our case (1·6 l/milking) was lower than the average value (3·5 l) calculated by Erdman & Varner (1995) for an increase in milking frequency from two to three milkings a day. Moreover, this is also the case of the estimated reduction in milk yield when only one milking per day was done (–10%) to much lower than previous reports in dairy cows (Holmes et al. 1992; Stelwagen et al. 1994).

An increase, or a reduction, in the rate of milk secretion is only the first of a concerted series of responses to more frequent milking, as stated by Knight & Wilde (1993), who suggest the occurrence of primary (milk yield), secondary (cell number and activity) and putative (udder anatomy) effects on the mammary gland. Whereas, Ayadi et al. (2003*b*) only report a slight reduction in the milk yield (–1·1 l/d; 3·7%) without any important change in any milk component or milk persistency when one milking is omitted weekly in dairy cows.

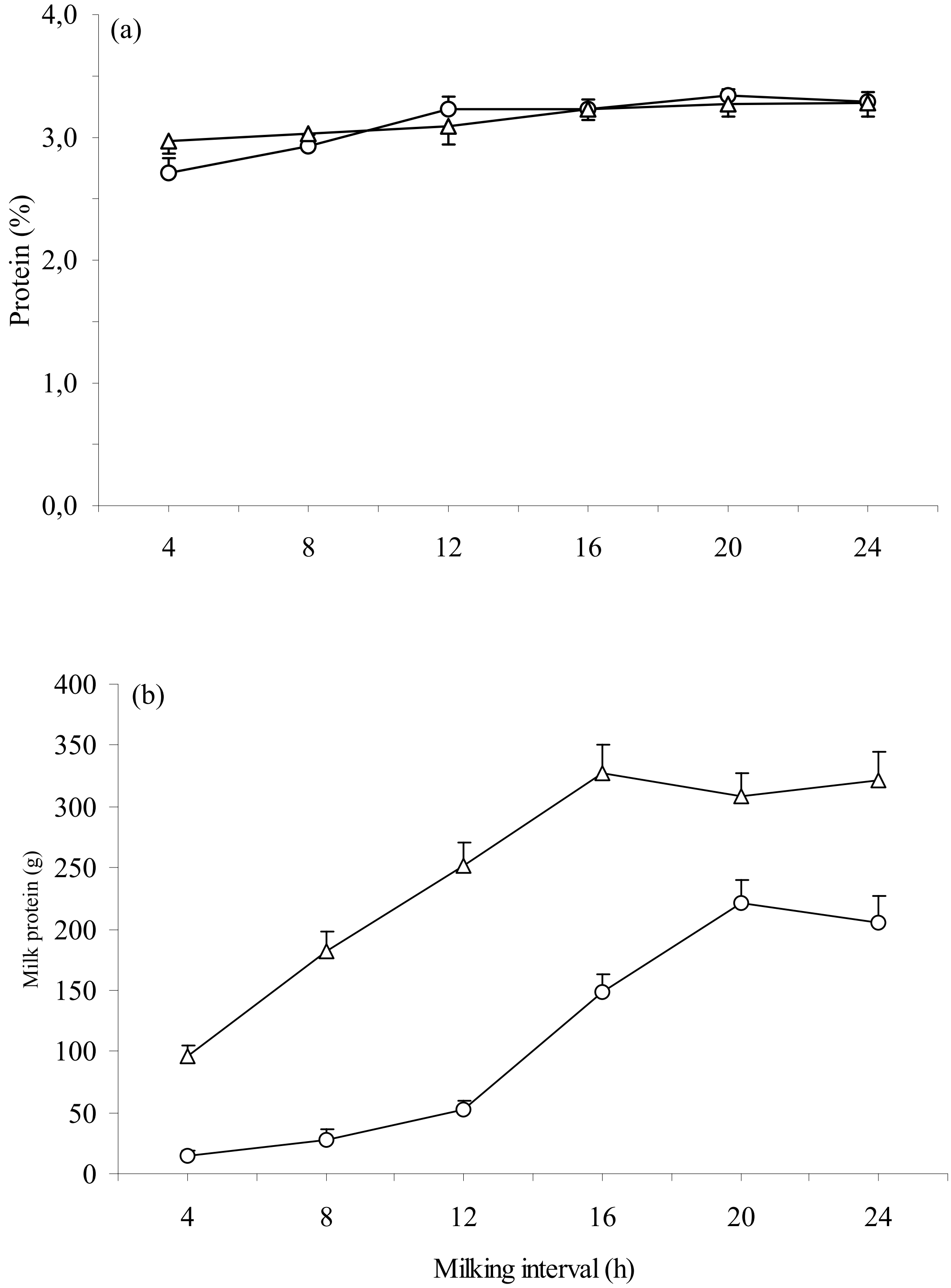

Figure 5.2. Changes in protein content (a) and protein yield (b) in cisternal (○) and alveolar (Δ) milk fractions for different milking intervals in four dairy cows. Data are means ± SEM.

Milk fat is the most variable component in the milk of ruminants. Fat content in cisternal milk was very high at the first 4 h milking interval as a consequence of the drainage of the residual milk stored in the upper part of the mammary gland by the fresh secreted milk. Thereafter, fat content in cisternal milk decreased as milking interval increased as a result of the dilution effect produced in the cisternal compartment (Labussière, 1969; 1985; Stelwagen, 2001).

On the contrary, fat content in the alveolar milk was constant during the first 16 h of milking interval and increased when the tight junction leakage should starts (17 to 18 h) according to Stelwagen et al. (1997). The same results were reported by Davis et al. (1998) when comparing 24 and 40 h milking intervals in dairy cows and McKusick et al. (2002) in dairy ewes when comparing 4 to 24 h milking interval. Moreover, Stelwagen et al. (1997) indicate that the diameter of milk fat globules is greater than the intercellular joints. The changes observed in our results agree with the milk accumulation model described by Stelwagen (2001). Also, low milk fat concentration in cisternal milk fractions may be explained by the upward movement of the globules, in the opposite direction to the downward draining and newly secreted milk (Waldmann et al. 1999; Stelwagen, 2001).

Cisternal milk fat yield tended to increase in the interval between milkings while alveolar milk fat yield increased markedly. This indicated to us that the transfer of milk fat from the alveoli to the cistern no longer took place, resulting in a back-up of milk fat in the alveolar compartment. Our results showed that up to 89% of the total fat yield resided in the alveolar compartment and is only obtainable if milk ejection occurs during milking. McKusick et al. (2002) report that alveoli can store up to 70% of total fat yield in dairy ewes in agreement with their greater cisternal milk percentage.

The decrease observed in total milk fat with milking interval was in accordance to the changes observed in total milk yield and in milk fractions for the different milking intervals, stressing the differences between the mechanisms of secretion of non-soluble (fat) and the soluble (protein and lactose) constituents of milk (Lacy-Hulbert et al. 1999). Weiss et al. (2002) report significant decrease in total milk fat according to milking interval (4 to 12 h) in early lactation but no differences were observed at mid and late lactation, as in our results with longer milking intervals (4 to 24 h). McKusick et al. (2002) also indicate a dramatic decrease in total milk fat with milking interval (4 to 24 h) in dairy sheep. Nevertheless, results are not directly comparable due to the differences in experimental procedures. Thus, McKusick et al. (2002) and Weiss et al. (2002) removed the residual milk of the udder between milking intervals, whereas we introduced a 3 d interperiod of regular milking to prevent carry over effects. Moreover, Weiss et al. (2002) estimated cisternal milk by collecting a partial sample of total cisternal milk in which no differences in milk fat content were observed when residual milk was or not was previously removed by using oxytocin.

Protein content in total milk increased for the first milking intervals (4 and 8 h) but was hardly affected by udder fraction as a consequence of its homogeneous distribution in the colloidal phase of milk. Protein content in total milk was steadily constant after 12 h.

No similar results are available for comparison in dairy cows, but McKusick et al. (2002) reported the same pattern of change in dairy sheep according to milking interval. Moreover, milk protein content do not vary (Holmes et al. 1992) or slightly increase (Klei et al. 1997) when milking frequency is decreased in dairy cows.

At the first 4 h milking interval, protein content in cisternal milk was at its lowest, but its value increased slightly until 12 h as a result of the arrival of the newly secreted milk. After 12 h of milking interval, protein content in cisternal milk was constant. This may be a consequence of the decrease in milk protein synthesis and the accumulation of the feedback inhibitors of lactation within the alveoli at longer milking intervals (Wilde et al. 1995).

Protein content in alveolar milk showed the same trend but no significant effects were detected in our work for any milking intervals, as indicated by Labussière (1985). The tendency of protein content to increase for extended milking interval in our experiment may be explained by the increased tight junction leakiness (Stelwagen et al. 1997) allowing serum protein to spill over into the milk (Stelwagen & Lacy-Hulbert, 1996).

Changes in protein yield were in accordance to the changes observed in milk yield and in milk fractions for the different milking intervals and are in accordance with the results of Stelwagen et al. (1997) and Auldist & Prosser (1998) for different milking frequencies.

When extrapolating the equivalent milking frequency at each milking interval, milk fat and protein contents did not vary significantly between 1 and 3 milkings per day. But fat increased and protein tended to decrease when milking frequency was higher of 3 milkings/d, as a consequence of deeper removal of residual milk imposed by the experimental methodology.

5.4. Conclusions

In conclusion, varying the milking interval in dairy cows will induce major changes in milk composition of fat and minor changes in milk protein in the short term. A decrease in milk fat and an increase in protein content in total milk is expected when the milking interval is extended due to the change of the contribution of alveolar and cisternal fractions.

A larger cisternal compartment in the udder could give support to lower milk yield losses and to smaller milk composition changes for lower milking frequencies.

CAPÍTULO 6:

Evolución de la leche cisternal a lo largo de la lactación y con el tiempo transcurrido desde la estimulación del reflejo de eyección en la ubre de vacas lecheras.

(Changes in udder cisterns according to lactation stage and time elapsed after milk letdown in the udder of dairy cows)

CAPÍTULO 6: Evolución de la leche cisternal a lo largo de la lactación y con el tiempo transcurrido desde la estimulación del reflejo de eyección en la ubre de vacas lecheras.

(*Changes in udder cisterns according to lactation stage and time elapsed after milk letdown in the udder of dairy cows***)**

Changes in udder cisterns according to lactation stage and time elapsed after milk letdown in the udder of dairy cows

Abstract

A total of 25 dairy cows were used in two experiments to study the udder cisterns at different lactation stages and after an oxytocin challenge. In Experiment 1, eighteen British Friesian cows grouped according to lactation stage (early, n = 6, 56 DIM, and, 27.1 L/d; mid, n = 5, 151 DIM, and, 22.0 L/d; and, late, n = 7, 311 DIM, and, 9.8 L/d) were used to study differences in cisternal and alveolar milk, and in cisternal area by ultrasonography, at a 12 h milking interval. Averaged values of fore quarters by cow ranged: cisternal milk (0.19 to 2.09 L), alveolar milk (0.23 to 3.20 L), and cisternal area (1.98 to 16.9 cm^2). A positive correlation between cisternal milk and cisternal area was observed for lactation stage grouped data ($r = 0.74$ to 0.82) and on pooled data ($r = 0.80$). As lactation stage increased, cisternal milk and alveolar milk volumes, and cisternal area significantly decreased. Proportion of cisternal milk changed according to lactation stage (early, 33.2%; mid, 23.1%; and, late, 42.6%). In experiment 2, three British Friesian and four Holstein cows were used to show the return of milk from cisternal to alveolar compartments when milk letdown was induced without milking. Cisternal area was measured by ultrasonography at the a.m. (16 h) and p.m. (8 h) milking intervals before (0 min) and after (3, 15, 30 and 60 min) an i.v. oxytocin injection (5 IU/cow). Average cistern area increased dramatically (93%) after oxytocin injection reaching its maximum distension at 3 min, and decreased slowly thereafter until 60 min. The 0 and 3 min data provide clear evidence of milk ejection and their difference is proposed as an index of cistern elasticity. The maximum value of cistern area in each cow was unaffected by the amount of milk stored for 8 and 16 h milking interval. The decrease in cistern size was significant at 15 min and later time points but not at earlier time points. This decrease in cistern size provides the first report documenting the return of milk to the alveolar

compartment following milk letdown in dairy cows. We term this ‘cisternal recoil’. We conclude that, ultrasonography is a useful method to evaluate dynamic changes in cisternal milk throughout lactation and after udder stimulation in dairy cows. There is clear evidence that udder cisterns decrease as lactation advances and that milk returns to the alveolar compartment when cows were unmilked after milk letdown.

Key words: Milk back-flush, cisternal milk, ultrasonography, lactation stage

6.1. Introduction

Milk storage in the udder can be explained using a simplistic model of two anatomical compartments (Wilde et al., 1996) corresponding to cisternal milk (milk drained from the alveoli and stored within the large ducts and the gland and teat cisterns), and alveolar milk (secreted milk stored within the lumen of alveoli). There are several reports that demonstrate the differences in distribution and accumulation of cisternal and alveolar milk fractions in dairy cows (Knight et al., 1994a; Stelwagen et al., 1996; Stelwagen, 2001). A new approach to studying milk storage in the udder cisterns of dairy cows has been recently made by Ayadi et al. (2003a) using direct udder scanning.

Several reports showed that cisternal and alveolar milk volume decreases throughout lactation in dairy cows (Dewhurst and Knight, 1993; Pfeilsticker et al., 1996; Bruckmaier and Blum, 1998). Rovai (2000) and Rovai et al. (2002) reported that cisternal milk volume and areas of cisterns measured by ultrasonography decrease in dairy ewes as lactation advanced. Nevertheless, ultrasonography has not been used before to evaluate changes in the cistern area during lactation in dairy cows.

The importance of milk ejection for an effective machine milking has been clearly demonstrated in dairy cows (Schams et al., 1984; Mayer et al., 1991) and oxytocin (OT) is mainly responsible for milk letdown (Lefcourt and Akers, 1983; Blum et al., 1989). There are close connections between OT effects and the sympathetic nervous system in the mammary gland (Lefcourt and Akers, 1983). The OT induces milk ejection by contracting the myoepithelial cells surrounding the mammary alveoli. Thus, cisternal milk fraction and milk yield are significantly greater in milkings with stimulation (Pfeilsticker et al., 1996). Therefore, alveolar-cisternal milk transfer during milking is due to OT release and milk letdown. Milk ejection causes a sudden pressure increase within the teat cistern (Mayer et al., 1991; Bruckmaier et al., 1994b) and an enlargement of the cisternal volume measured by ultrasonography (Bruckmaier and Blum, 1992; Ayadi and Caja, 2000; Ayadi et al., 2003a). If milk is not withdrawn after stimulation, intramammary pressure remains stable for at least 10 min before beginning to decrease (Bruckmaier et al., 1991).

Linzell (1955) was the first to demonstrate the back-flux of milk from ducts into alveoli of mice after milk ejection, but he found no evidence for the possibility that the elastic nature of the udder cistern results in an equivalent phenomenon in dairy cows. The return of milk to the ductal and alveolar compartments of the cow udder following the completion of milk letdown was reported by Knight (1994), but this phenomena was not confirmed by Pfeilsticker et al. (1996) at different times after stimulation in dairy cows.

A delay between activation of milk letdown reflex and milk evacuation from the udder can negatively affect milk yield. Several reports on dairy cows observed losses in milk yield of between 5 and 30% when milking was delayed after stimulation (Murray and Lightbody, 1962**;** Labussière, 1981; Mayer et al. 1984), but Phillips (1984) and Pfeilsticker et al. (1996) did not observe these losses when teats were stimulated before milking.

Knowledge of the change of cisternal compartment throughout lactation and the reasons for the reduction of milk yield when time between milk letdown and milk evacuation are prolonged may be useful in the improvement of milking routines and in the optimisation of milk production in dairy cows. The aims of this work are: 1) to evaluate the changes of udder cisterns according to lactation stage; and, 2) to study the return of milk from the cisternal to the ductal and alveolar compartments in the cow udder when milk is not evacuated after milk letdown.

6.2. Materials and Methods

6.2.1. Experiment 1: Cisternal change with lactation stage

6.2.1.1. Animals, feeding and routine milking

Eighteen Holstein-Friesian cows (4 primiparous and 14 multiparous) from the HRI (Hannah Research Institute, Ayr, UK) were used during early (n = 6; 56 ± 13 DIM; and, 27.1 ± 1.5 L/d), mid (n = 5; 151 ± 18 DIM; and, 22.0 ± 1.8 L/d) and late (n = 7; 311 ± 21 DIM; and, 9.8 ± 2.0 L/d) lactation. Cows were kept in free stalls, grazed on natural pasture for 8 h/d**,** and were fed ad libitum a total mixed ration containing 4.5 MJ EN_l and 15% CP. The cows were milked twice daily at an 8-16 h milking schedule (0700 and 1500) in a herringbone milking parlour (Gascoigne Melotte, Ayr, UK) equipped to record and collect milk separately from half udders (i.e. diagonally opposed gland). Milk yield was recorded at each milking to a precision of 100 g.

6.2.1.2. Experimental procedure

Milk partitioning in the udder and cistern area were evaluated at random for each cow on three consecutive days. With this aim, cows were moved to a barn with tied stalls to prevent spontaneous milk letdown during udder manipulation by placing the cows in unfamiliar surroundings as indicated by Bruckmaier et al. (1993). Cisternal area was measured by ultrasonography according to the methodology proposed by Ayadi et al. (2003a). Udder scans for the left and the right front udder quarters were performed 12 h after the a.m. milking in duplicate by using a real time B-mode ultrasonograph (Ultra Scan 900, Ami Medical Alliance Inc., Montreal, Canada) with a 5 MHz sectoral probe (2 dB power, 80° scanning angle, 0.5 mm axial and 1.5 mm lateral resolution). Images were transmitted to a portable computer and processed in triplicate by using image treatment software (MIP4 Advanced System, Microm España, Barcelona, Spain) and areas in pixels were converted to cm^2 (1 cm^2 = 1,024 pixels) as indicated by Ayadi et al. (2003a). Individual milk yield was recorded at each milking during the experimental period.

After the scans, cisternal milk was drained from each front udder quarter using a cannula (100 mm long, 2.77 mm-o.d., and, 1.88 mm-i.d.; Portex, Hythe, Kent, UK) and values were recorded. Alveolar milk was machine milked after a 10 IU/cow i.m. OT injection (Intervet, Cambridge, UK).

6.2.2. Experiment 2: Cisternal recoil

6.2.2.1. Animal feeding and routine milking

A total of seven multiparous Holstein-Friesian cows varying in milk yield (23.0 ± 3.2 L/d) and lactation stage (215 ± 49 DIM) were used in this experiment. Three cows were from the HRI and were kept, managed and milked as previously described in Experiment 1. The remaining four cows were from the S1GCE (‘Servei de Granges i Camps Experimentals’) of the Universitat Autònoma de Barcelona (Bellaterra, Barcelona, Spain) and were kept in tied stalls, with a 6 h/d period of exercise in a paddock, and fed ad libitum a total mixed ration (5.02 MJ NE_l; 16.1% CP, dry matter basis). Water and the total mixed ration were also available in the paddock. The S1GCE cows was milked in stalls (0800 and 1900) using a high pipeline milking system (Westfalia Surge Ibérica, Granollers, Barcelona, Spain) at 50 kPa (Vacurex, Westfalia Surge Ibérica), and at 60:40 pulsation ratio and 60 pulses/min (Metatron

12, Westfalia Surge Ibérica). The milking routine for the regular daily milking included teat cleaning, machine stripping and teat dipping (P3-cide plus, Henkel Hygiene S.A., Madrid, Spain).

6.2.2.2. Experimental procedure

Cows were randomly submitted to an OT challenge and cistern size measured by using a real time B-mode ultrasonograph as previously described (Ultra Scan 900; 5 MHz sectoral probe). Duplicated scans of the left and right front quarters were taken before (0 min) and after (3, 15, 30 and 60 min) an i.v. injection of OT (5 IU/cow, Intervet, Cambridge, UK; and, Veterin Lobulor, Lab. Andreu, Barcelona, Spain) in the jugular vein. As milking facilities were placed in tied-stalls in this case cows were injected in the epigastric vein with 10 μg/kg BW of an oxytocin receptor blocking agent (Atosiban®, Ferring Lab., Malmö, Sweden) before scanning to prevent spontaneous milk letdown. Scan images of the udder cisterns were transmitted to a portable computer and processed (MIP4 Advanced System) by triplicate as indicated by Ayadi et al. (2003a).

Cisternal measurements were randomly repeated in each cow for the long (16 h) and short (8h) milking interval.

6.2.3. Statistical analysis

Data of Experiment 1 were analyzed by the GLM procedure of SAS (version 8.1). The model included the general means and the fixed effect of the stage of lactation (early, mid and late) and the random effects of animal (1 to 18), udder side (left and right), the respective interactions, and the residual error. When the probability of the interaction term was non significant ($P > 0.20$), it was deleted from the model. Differences between Least Square Means were localized with the Newman-Keules test and significance was declared at $P < 0.05$.

Results of Experiment 2 were processed by the PROC MIXED for repeated measurements of SAS. The model included the general means and the fixed effects of the treatment time (0, 3, 15, 30 and 60 min) and milking interval (8 and 16 h), and the random effects of animal (1 to 7), front quarters (left and right), and the respective interactions and the residual error. Differences between Least Square Means were localized with the PDIFF test and significance was declared at $P < 0.05$. When the probability of the interaction term was non significant ($P > 0.20$), it was deleted from the model.

No differences were observed between left and right front udder quarters and therefore, their values were averaged and discussed jointly. Pearson's correlation coefficients between cisternal milk and cistern areas were also calculated.

6.3. Results and Discussion

6.3.1. Experiment 1

Changes in milk yield and milk partitioning in the udder according to stage of lactation are summarized in Table 6.1. Milk yield decreased significantly ($P < 0.01$) throughout lactation. Cistern area, and volumes of cisternal and alveolar milk ranged from 1.98 to 16.9 cm^2, 0.19 to 2.09 L and 0.23 to 3.20 L, respectively. On average, cisternal and alveolar milk represented 33 and 67 %, respectively, of the milk stored in the udder 12 h after milking. No differences were observed between left and right front udder quarters in cistern size ($P = 0.51$), cisternal milk volume ($P = 0.60$) and alveolar milk volume ($P = 0.53$).

Table 6.1. Milk partitioning in the udder of dairy cows according to stage of lactation[1]

Item	Stage of lactation		
	Early	Mid	Late
Cows, n	6	5	7
Milk yield, L/d	$27.10^a \pm 1.51$	$22.04^b \pm 1.49$	$9.80^c \pm 1.42$
Lactation stage, d	56 ± 13	151 ± 18	311 ± 21
Milk partitioning[2]			
Alveolar, L	$2.45^a \pm 0.18$	$2.06^a \pm 0.15$	$0.66^b \pm 0.11$
Cisternal, L	$1.22^a \pm 0.16$	$0.62^b \pm 0.09$	$0.49^b \pm 0.06$
Total, L	$3.67^a \pm 0.24$	$2.68^b \pm 0.20$	$1.15^c \pm 0.12$
Cistern area[3], cm^2	$12.10^a \pm 1.30$	$8.00^b \pm 1.70$	$4.20^c \pm 0.52$
Cisternal:Total, %	$33.20^b \pm 6.60$	$23.10^c \pm 4.50$	$42.60^a \pm 5.00$

[a,b,...d] Different letters in the same column indicate significant differences at $P < 0.05$.

[1] Values are means ± SEM.

[2] Measured in front quarters 12 h after milking.

[3] Average of both sides measured at 12 h after milking by ultrasonography according to Ayadi et al. (2003a).

The correlation between cisternal area and volume of cisternal milk was significant ($P < 0.01$) and positive at early ($r = 0.82$), mid ($r = 0.74$) and late lactation ($r = 0.80$), as well as when pooled data were used ($r = 0.80$). The high correlation between cisternal milk and cistern area at different stages of lactation in our study confirms that the methodology used by Ayadi et al. (2003a) was adequate to obtain repeatable images of the udder cistern.

Moreover, the positive correlation between the scanning area and the volume of cisternal milk agrees with those previously reported by Bruckmaier et al. (1994) and Ayadi et al. (2003a) in dairy cows.

Linear regression analysis indicates a significant relationship between daily milk yield (L/d) and cisternal size (cm^2) and the regression equation was:

$$\text{Milk yield} = 6.827 + 1.331 \times \text{cistern area}$$

$$r = 0.70,\ P < 0.01,\ \text{RSD} = 1.66.$$

The positive correlation between daily milk yield and cisternal size measured by ultrasonography observed in our study is similar to the correlation between udder volume and milk yield previously reported in dairy cows (Knight and Dewhurst, 1994). These results indicate that animals with large cisterns should be more efficient producers of milk.

As lactation advanced, alveolar and cisternal milk volumes, and cistern area measured by ultrasonography decreased significantly ($P < 0.01$), as shown in Table 6.1. Moreover, alveolar milk volume did not change between early and mid lactation, but decreased by 68% ($P < 0.01$) between mid and late lactation. Both cisternal milk and cistern area decreased but the decline in the cistern area was more evident (Table 6.1). Thus, although cisternal milk volume decreased by 49% ($P < 0.01$) between early and mid lactation and remained unchanged thereafter, cistern area only decreased by 34% ($P < 0.01$) between early and mid lactation, and continued decreasing by 48% ($P < 0.01$) between mid and late lactation. This decrease is in accordance with the results of Dewhurst and Knight (1993), Pfeilsticker et al. (1996) and Bruckmaier and Blum (1998) who observed that cisternal milk decreased throughout lactation in dairy cows.

Cisternal milk fraction represented 33% of total milk stored in the udder at early lactation, while this percentage decreased at mid lactation (23 %) and increased at late lactation (43 %), as shown in Table 6.1. These results do not agree with those reported by Wilde et al. (1996) who observed an increment in the proportion of cisternal milk as lactation advanced in dairy cows. Dewhurst and Knight (1993) observed an increase of cisternal milk fraction as lactation advanced in dairy cows. Moreover, Rovai et al. (2002) reported in dairy ewes, that cisternal milk volume and area of cisterns (measured by ultrasonography using 5 MHz sectoral probe at 8 h milking interval) decreased throughout lactation. This decrease in cisternal milk stored in the udder can be explained by the decline in secretory tissue throughout lactation by apoptosis (Dewhurst and Knight, 1993; Wilde et al., 1997).

6.3.2. Experiment 2

The udder cistern filled with milk was clearly evident as a dark area (anechogenic) and the glandular parenchyma as a grey-white area (echogenic) in the scans, as previously described by Cartee et al. (1986), Ruberte et al. (1994b) and Ayadi and Caja (2000). Ultrasonographic images obtained in our work (Figure 6.1) were similar to those previously reported in cows (Bruckmaier and Blum, 1992; Bruckmaier et al., 1994a; Ayadi et al., 2003a).

Cistern area before OT injection ranged from 2.8 to 27.7 cm^2, differed between cows and varied ($P < 0.01$) according to milking interval (8h, 7.6 ± 1.5 cm^2; and 16 h, 11.1 ± 2.4 cm^2).

Mean cistern area increased dramatically (93%) at 3 min after OT injection, reaching its maximum value (17.8 vs 9.2 cm^2) as shown in Figure 6.2. These results agree with those of Bruckmaier and Blum (1992) and Ayadi and Caja (2000) where cistern area increased by 41% and 120%, respectively after OT injection. This variation in the increase of cistern area between authors may be due to the use of different types of probe (linear vs. sectoral), but not to differences in udder morphology (i.e cistern size). The increase in cistern area after OT injection was due to milk transfer from alveoli to cistern when myoepithelial cells contracted by effect of OT.

At 3 min after OT injection, average cistern area increased dramatically (0 min, 8.7 cm^2; and, 3 min, 16.8 cm^2; $P < 0.001$), and decreased slowly thereafter (14.6, 13.5 and 12.8 cm^2 at 15, 30 and 60 min, respectively). Change from 0 to 3 min was greater ($P < 0.05$) for 8h (136%) than for 16 h (60%) milking intervals, but their maximum value did not differ (Figure 6.2). These results show that the udder reached its maximum distension 3 min after OT injection in dairy cows, regardless of the quantity of milk stored in the udder. As a consequence the maximum elasticity of the cistern can be calculated by difference between the areas before and after the OT injection at a fixed milking interval.

After OT injection, cistern area significantly ($P < 0.01$) decreased for both milking intervals: 8h (13.5, 22.5 and 28.6%) and 16 h (7.9, 13.2 and 25.7%), respectively for 15, 30 and 60 min. This variation in the decrease of cistern area between the 8 and 16 h milking intervals could be due to the fact that cistern milk volume after 16 h was greater than after 8 h. At 8 h after milking, cisternal milk only represents 10% (Bruckmaier et al., 1994a) and 14% (Ayadi et al., 2003a) of total milk yield and alveoli were not totally full of milk.

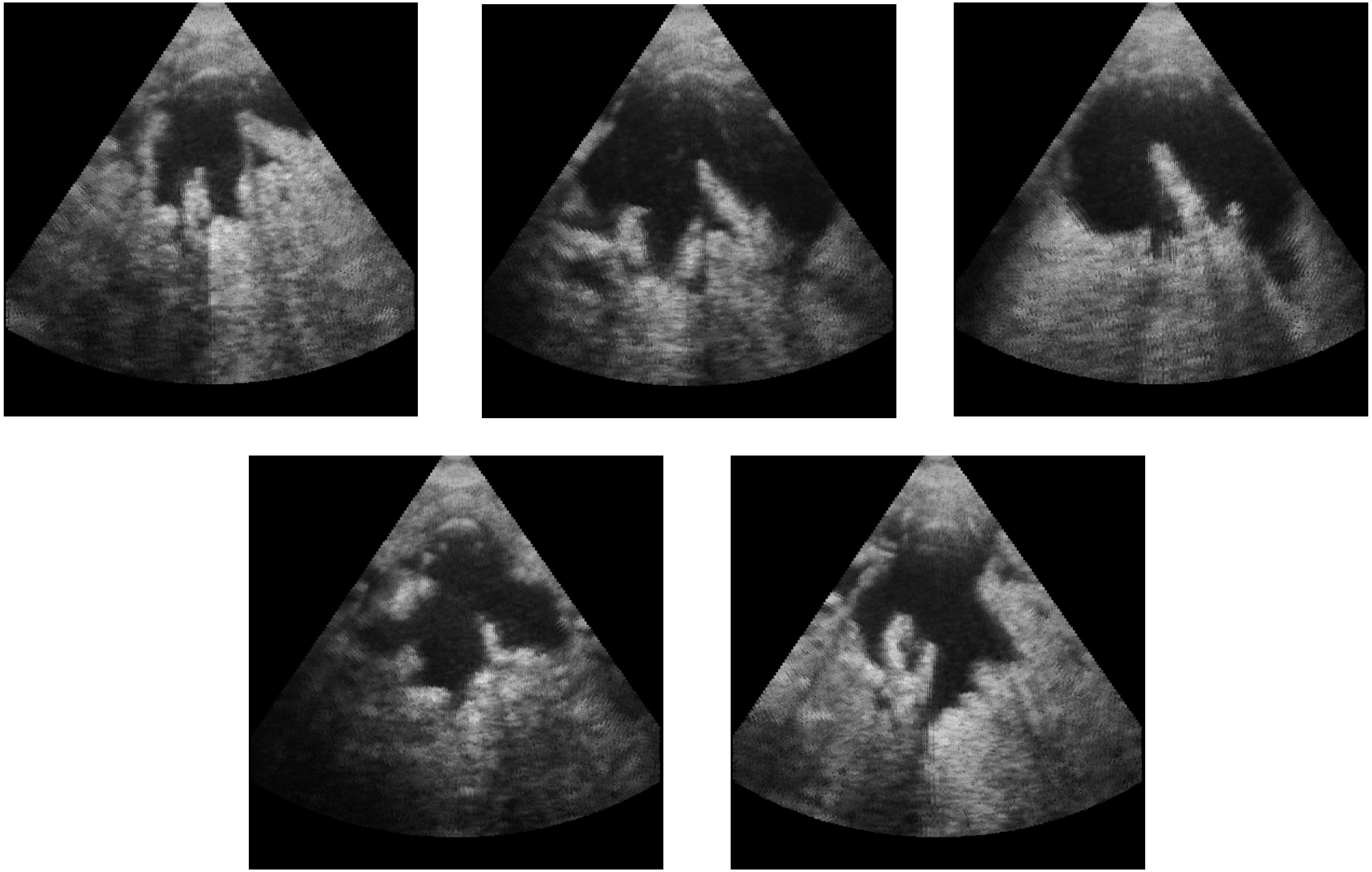

Figure 6.1. Ultrasonographic of the left front quarter cistern of dairy cow (#111) at 8h milking interval according to time after OT injection. Top line: From left to right (0, 3, and 15 min). Bottom line: From left to right (30 and 60 min).

On the other hand, cisternal milk as a percentage of total milk was 20% for 10-14h after milking (Bruckmaier et al., 1994a; Knight et al., 1994a) and 32% for 16 h (Ayadi et al., 2003a) and ducts were more full of milk and a lower percentage of alveolar milk would move to the cisterns when OT was injected at 16 h milking interval, according to the model proposed by Stelwagen (2001).

Decrease in cistern area was only significant after 15 min ($P < 0.05$). The reduction in cistern area, as a consequence of the decrease of cisternal milk volume in our results, did not agree with the results of Pfeilsticker et al. (1996) who reported non significant differences in the amount of cisternal milk, measured at 10 h milking interval, when teats were stimulated 15, 60 or 120 min before milking. Nevertheless, as Pfeilsticker et al. (1996) did not use an OT blocking agent, these last results could have been influenced by a new OT secretion during the drainage of cisternal milk.

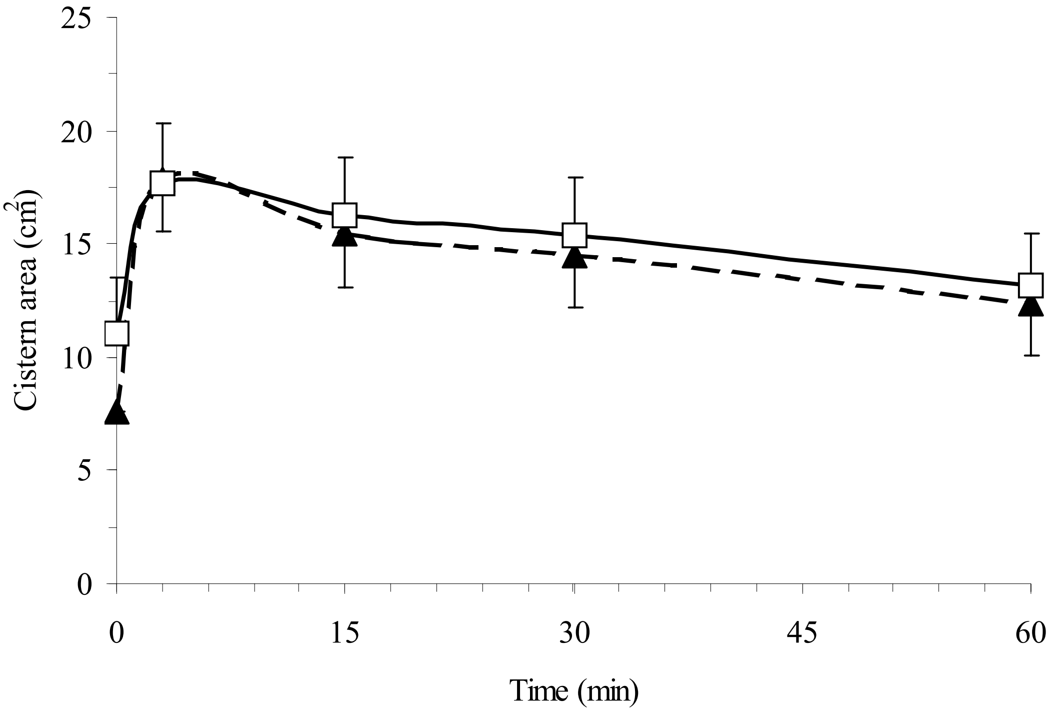

Figure 6.2. Change in cistern area measured by ultrasonography in the front quarters of dairy cows at 8 (---▲---) and 16 h (—□—) milking intervals according to time after oxytocin injection (Values are means for 7 cows).

The decrease in cistern area in our work was interpreted to be a consequence of the return of milk to the ductal and alveolar compartments of the cow udder following the completion of milk letdown and we call this 'cisternal recoil'.

This cisternal recoil effect may be explained by the suction of cisternal milk when myoepithelial cells that surround the alveoli and smooth muscular fibers of ducts are relaxed. Our results confirm the ‘cisternal recoil’ effect suggested by Knight (1994).

The decrease of cistern area after OT injection at 16 h milking interval may also be related to the increase of the permeability of tight junctions between the mammary cells observed at 16 to 17 h (Stelwagen et al., 1997). Thus, transfer of lactose from milk to blood plasma may reduce the osmotic pressure which partly induces the return of milk richer in lactose from cisternal to alveolar compartments to maintain a constant osmotic pressure within the alveoli.

A delay between the activation of the milk letdown reflex and milk evacuation from the udder can negatively affect milk yield. Labussière (1981) reported that milk yield decreased by 5, 16 and 30% when milking was delayed by 2, 6 and 13 min, respectively.

Several reports in dairy cows confirm this phenomena when milking was delayed after stimulation (Murray and Lightbody, 1962; Mayer et al., 1984). Nevertheless, Phillips (1984) did not observe milk losses when teat cup attachment was delayed by 3 or 12 min as compared with immediate attachment after udder stimulation, and Pfeilsticker et al. (1996) did not observe losses in milk when teats were stimulated 15, 60 or 120 min before milking.

In practice, to profit from the short and beneficial effect of endogenous OT (3 at 4 min) it is important to reduce the time between preparation of the udder and evacuation of milk by machine milking (Labussière, 1993). For the most common milking schedule, specially on conventional milking farms, groups of cows should stay in the waiting parlour before milking. Noise from the milking machine and the presence of the milkman can stimulate milk ejection and produce milk letdown to the cistern. As a consequence, milk could return to alveoli if time between milk ejection and milk evacuation is long.

Milk returned to the alveoli would be considered as residual milk if no new OT is secreted. Recently, the introduction of the automatic milking system in some intensive farms (Ipema and Benders, 1992; Lind et al., 2000; Veysset et al., 2001) may reduce time between milk ejection and milking or, on the contrary, increase dramatically time after stimulation if cluster attachment is failed or the cow rejected for milking until the next opportunity.

6.4. Conclusions

We conclude that, ultrasonography is a useful method to evaluate changes of cisternal milk throughout lactation in dairy cows and that udder cistern decreases as lactation stage advances. There is clear evidence of the return of milk to the ductal and alveolar compartments if cows are not milked immediately after milk letdown. We termed this effect ‘cisternal recoil’. Moreover, the udder reached its maximum distension 3 min after OT injection regardless of the quantity of milk stored in the udder in dairy cows, this is considered to be a means to evaluate the maximum cistern area and the cisternal elasticity. New research on the effects of the cisternal recoil on the loss of milk yield in dairy cows is required.

CAPÍTULO 7:

Conclusiones

CAPÍTULO 7: Conclusiones

Del conjunto de experiencias realizadas, se extraen una serie de conclusiones, generales y específicas, sobre el estudio de la estructura interna de la ubre mediante ecografía y los efectos de los intervalos entre ordeños sobre los cambios productivos y morfológicos de la ubre en vacas lecheras.

7.1. Conclusiones específicas

7.1.1. Supresión de un ordeño por semana en vacas lecheras

1. La supresión de un ordeño a la semana a mitad de la lactación produce una pequeña pérdida de producción de leche (3,7%), sin afectar su composición ni la persistencia de la lactación y el estado sanitario de la ubre de forma significativa.
2. Se observa una relación negativa entre la cantidad de leche perdida al suprimir un ordeño por semana y el área cisternal evaluada mediante ecografía, así como con el volumen de leche cisternal.
3. No se observa ninguna relación entre la pérdida al suprimir el ordeño del domingo por la tarde y el nivel de producción de leche o el volumen de la leche alveolar.
4. La supresión de un ordeño por semana produce una importante disminución en la producción de leche, el porcentaje de grasa y el RCS en el día de la supresión, y un incremento compensatorio de estos parámetros en los dos días siguientes. Sin embargo, el contenido en proteína y lactosa de la leche no varía de forma importante. Dichas variables vuelven a sus niveles medios a las 72 h del día de la supresión, sin afectar las medias semanales.

7.1.2. Efectos de distintos intervalos entre ordeños sobre la composición química de la leche cisternal y alveolar en vacas lecheras

1. La evolución de la cantidad de leche cisternal a diferentes intervalos entre ordeños sigue una evolución sigmoidal, con una saturación a las 20 h, mientras que la evolución de la leche alveolar es lineal hasta las 16 h, a las que se satura posteriormente. La relación media entre leche cisternal : alveolar en la ubre de vacas lecheras es de 30 : 70, con un cambio muy importante en el almacenamiento de la

leche en la ubre entre las 12 y las 20 h de intervalo entre ordeños, en las que aumenta fuertemente el porcentaje de leche cisternal.

2. La relación entre los cuartos anteriores : posteriores, tanto en leche cisternal como en alveolar, es de 40 : 60. El contenido en proteína de la leche es mayor en los cuartos posteriores en ambas fracciones. Sin embargo, el porcentaje de grasa tiende a ser mayor en los cuartos anteriores en ambas fracciones.
3. El contenido en grasa de la leche cisternal disminuye progresivamente hasta las 16 h para mantenerse constante posteriormente. Al contrario, el contenido en grasa de la leche alveolar se mantiene constante hasta las 16 h, aumentando rápidamente hasta las 24 h. En la leche total, el porcentaje de grasa evoluciona de forma cuadrática, disminuyendo con el aumento del intervalo entre ordeños.
4. El contenido en proteína de la leche cisternal aumenta y tiende a aumentar en la leche alveolar con el intervalo entre ordeños, sin diferencias entre ambas fracciones y entre los cuartos anteriores y posteriores. En la leche total, el porcentaje de proteína bruta se incrementa con el aumento del intervalo entre ordeños.
5. El aumento de la producción de grasa con el intervalo entre ordeños es mucho más marcado en el compartimento alveolar que en el cisternal. Sin embargo, dicho aumento en la producción de proteína es similar para ambas fracciones
6. No se observan casos de mamitis subclínicas como consecuencia directa de la aplicación de diferentes intervalos entre ordeños.

7.1.3. Evolución de la leche cisternal a lo largo de la lactación y con el tiempo transcurrido desde la estimulación del reflejo de eyección en la ubre de vacas lecheras.

1. Se observa una correlación alta y positiva (r = 0.80) entre el volumen de leche cisternal y el área de las cisternas evaluada mediante ecografía en diferentes estados de lactación.
2. A lo largo de la lactación, disminuye el volumen de leche cisternal y alveolar, así como el área cisternal evaluada mediante ecografía.
3. El área de las cisternas glandulares aumenta de forma importante (+93%) después de la inyección de oxitocina (3 min.), disminuyendo posteriormente hasta los 60 min.

cuando no se evacua la leche de la ubre, lo que evidencia la existencia del llamado ‘reflujo cisternal’.

4. El valor máximo del área cisternal 3 min después de la inyección de oxitocina es independiente del intervalo entre ordeños y de la cantidad de leche almacenada. La cisterna resulta así una estructura fibrosa poco elástica que limita el almacenamiento de leche.

7.2. Conclusiones generales

1. La pérdida de leche ocasionada por la supresión de un ordeño por semana se ve afectada por las características de las cisternas glandulares, y por tanto, la evaluación del tamaño cisternal a través ultrasonografía puede ser un método adecuado para seleccionar vacas que se adaptan mejor a la reducción de la frecuencia de ordeño.
2. La supresión de un ordeño por semana puede no ocasionar problemas de comercialización de la leche, a pesar de las variaciones en la composición y el RCS de la leche durante la semana. Es recomendable aplicar este sistema de ordeño sólo en vacas con un buen estado sanitario de la ubre.
3. El control lechero cuando se suprima un ordeño a la semana, a efectos de no alterar sus resultados, debería realizarse después de 5 ordeños a fin de evitar las modificaciones en la composición de leche por efecto de la supresión del ordeño.
4. A corto plazo, el aumento del intervalo entre ordeños produce una disminución en el contenido en grasa y un incremento en la proteína bruta de la leche total, como consecuencia del cambio en la contribución de las fracciones de leche cisternal y alveolar en los distintos intervalos entre ordeños.
5. Existe una cierta dificultad en el drenaje de los glóbulos grasos de la leche desde la zona alveolar hacia las cisternas. Sin embargo, el drenaje de las micelas de caseína entre los dos compartimentos es fácil y fluido entre ordeños.
6. Las vacas con cisternas grandes, además de minimizar la pérdida de leche, pueden también reducir la variación de la composición de leche, en el caso de la reducción de la frecuencia de ordeño.
7. La ecografía mamaria es una metodología adecuada para la evaluación del tamaño cisternal a lo largo de la lactación en vacas lecheras.

8. Hay una clara evidencia del retorno de la leche desde el compartimento cisternal a los conductos y alvéolos, si las vacas no son ordeñadas después del drenaje de leche. Este fenómeno se denomina “reflujo cisternal” o “*cisternal recoil*”.
9. La distensión máxima de la cisterna de la ubre, observada 3 min después de la inyección de oxitocina, puede ser considerada como una metodología para evaluar el tamaño máximo de las cisternas en vacas lecheras.
10. Se recomienda la disminución del tiempo de espera de las vacas antes de entrar a la sala de ordeño, a fin de aprovechar el efecto corto y positivo de la oxitocina endógena y evitar la retención de la leche.

CAPÍTULO 8:

Referencias bibliográficas

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