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Effect of different milking intervals on the composition of cisternal and alveolar milk in dairy cows

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Effects of six different milking intervals on the distribution of milk between cistern and alveoli were studied in a randomized, incomplete Latin Square experiment with four lactating Holstein cows. Cisternal and alveolar milk was measured 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 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, 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 did 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 passed 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.

Keywords: Cisternal milk, alveolar milk, milking interval, milk composition.

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 used to assess machine milking ability of dairy ruminants (Labussière, 1988; Le Du et al. 1994; Peris et al. 1996). Species differ greatly in the proportion of total milk that can be stored within the cistern. After a normal milking interval (8–16 h) dairy cows store <30% of the total milk yield in the cistern (Bruckmaier et al. 1994; Pfeilsticker et al. 1996; Ayadi et al. 2003a) whereas, in contrast, the corresponding value for dairy goats is 57–88% (Peaker & Blatchford, 1988; Knight et al. 1994a). In sheep, large differences occur in cisternal milk, with values ranging from >50% for dairy breeds (McKusick et al. 2002; Rovai et al. 2002) to <30% for meat breeds (Caja et al. 1999), reflecting effects of selection on udder morphology.

Partitioning of milk between alveolar and cisternal compartments in the udder also differs between species according to milking interval (Peaker & Blatchford, 1988; Knight et al. 1994b; McKusick et al. 2002), and the concentration of milk components is markedly affected by the site of milk storage in the gland. Distribution and accumulation of cisternal and alveolar milk fractions changes with milking interval in dairy cows (Knight et al. 1994b; Stelwagen et al. 1996; Ayadi et al. 2003a). Davis et al. (1998) and Stelwagen (2001) report that, together with cisternal size, the ability of alveoli to drain (actively or passively) to the cistern is important in explaining the change of milk yield and milk composition that occurs when milking interval is extended to 24 h. However, only Davis et al. (1998) report the changes observed

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simultaneously in yield and composition of each milk compartment of the cow udder for long milking intervals (24 and 40 h).

Concentration of milk fat is lower in the cistern than in the alveoli of dairy cows (Labussière, 1985; Davis et al. 1998; Waldmann et al. 1999) and dairy ewes (Labussière, 1969; McKusick et al. 2002). Differences between alveolar and cisternal contents of milk protein and lactose, however, are minimal (Labussière, 1985; Davis et al. 1998). Milk protein content varies less than milk fat in response to changes in milking interval (Villiers & Smith, 1976) and milking frequency (DePeters et al. 1985; Holmes et al. 1992). An increase of milking frequency from 1 to 2 times a day (Carruthers et al. 1993; Stelwagen et al. 1994) or from 2 to 3 times a day (Szuchs et al. 1986; Erdman & Varner, 1995) decreased fat and protein contents in the milk of dairy cows. These results do not agree with those of 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 >3 times a day, milk composition in dairy cows varied only slightly (Van der Iest & Hillerton, 1989; Ipema & Benders, 1992). Moreover, Labussière (1985) report 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 when devising appropriate management systems and milking routines, especially when using automatic milking (Weiss et al. 2002). Moreover, knowing how milk composition varies with milking interval can be useful for establishing the sampling schedule for automatic milk recording with variable milking intervals (Friggens & Rasmussen, 2001; Peeters & Galesloot, 2002).

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

Materials and Methods

Animals and their management

Four Holstein cows were used for a 5-week period. Cows (215±25 d in milk and 20±3 l/d milk yield) were kept in tie-stalls and given a total mixed ration *ad libitum* (5·02 MJ Net Energy for Lactation/kg and 161 g crude protein/kg dry matter) 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. 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 <0·5 l/min), and teat dipping. Udder health was checked weekly using a Californian Mastitis Test (CMT) reagent (Pitman-Moore España S.A., Porriño, Pontevedra, Spain) in the foremilk collected during udder preparation for a regular morning milking.

Experimental procedures

The design was 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. To prevent the milk ejection reflex and to enable recording of cisternal and alveolar milk fractions separately (Knight et al. 1994b; Bruckmaier et al. 1997), 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 m Ø × 10 cm length; VP 23 N Aesculap, Hanover, Germany) and volumes recorded. Alveolar milk was obtained after an i.m. injection of oxytocin at a supraphysiological dose ($20{\cdot}0\times10^{-3}$ i.u./kg BW; Veterin Lobulor, Lab. Andreu, Barcelona, Spain) to remove residual Atosiban from the oxytocin receptors and to induce milk ejection. Alveolar milk was machine milked 10 min later, using a quartered claw. Milk fractions per quarter were weighed after collection to an accuracy of 10 g, the weights were recorded and representative samples from each fraction and quarter were collected for analysis. Milk fat and protein contents were determined using an NIRS analyser (Infra Alyser 450 D, Bran+Luebbe, Norderstedt, Germany), as described by Albanell et al. (1999).

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 between 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 used the REPEATED statement in PROC MIXED using milking interval as a regression variable.

Table 1. Milk yield and milk composition of dairy cows at different milking intervals

Values are means ± SEM for $n=4$

Milking interval, h	Yield, l	Fat, %	Protein, %	Estimated ECM†, 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±0·13d	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%)

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

[a,b,c,d] Values in the same column without a common superscript letter are significantly different ($P<0·05$)

Table 2. Milk yield and milk composition in the front and rear udder quarters at different milking intervals in dairy cows

Values are means ± SEM for $n=4$

Fraction	Milking interval, h	Milk, l		Fat, %		Protein, %	
		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,c,d] Values in the same column without a common superscript letter are significantly different ($P<0·05$)

Results

Using CMT, no subclinical mastitis was detected in any udder quarter during the experimental period and udders were considered healthy. Changes in total milk yield and milk composition according to milking interval are summarized in Table 1. Linear regression analysis indicated a significant relationship between daily yield of 3·5% energy-corrected milk (ECM) and milking frequency, such that:

$$3{\cdot}5\%\ \text{ECM (l/d)} = 1{\cdot}604 \times \text{milkings per day} + 17{\cdot}25,$$
$$r^2 = 0{\cdot}91,\ P<0{\cdot}01,\ \text{RSD} = 0{\cdot}36.$$

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 with the 12-h milking interval. No differences were observed in milk yield or milk composition for the left and right udder quarters but 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 per quarter was 0·10–1·89 l (15–42% of the total milk in the udder) for the 4–24-h milking interval (Table 2), being 30% on average. Cisternal milk volume increased slowly until 12 h after milking (16%) but more rapidly thereafter, reaching a plateau after 20 h (42%). Rear quarters stored, on average, 34% more cisternal milk (25–44%) 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 2) according to milking interval, but its pattern of accumulation differed markedly from that of 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 (16–34%) 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 indicated a marked change ($P<0·01$) in the milk storage pattern in the udder between 12 h (17 : 83) and 20 h (43 : 57).

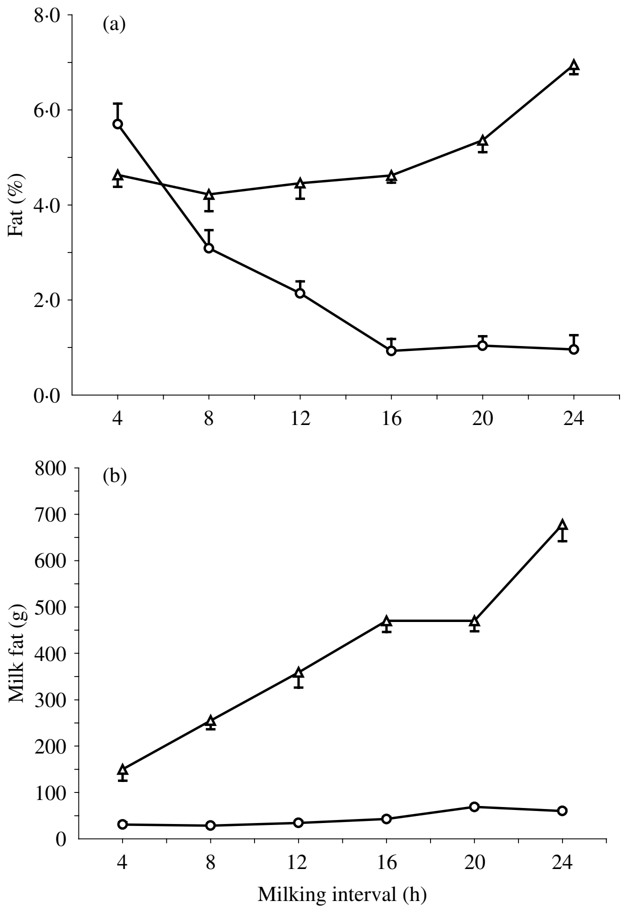

Fig. 1. Changes in (a) fat content and (b) fat yield for cisternal (○) and alveolar (△) milk fractions for different milking intervals in four dairy cows.

Values are means ± SEM

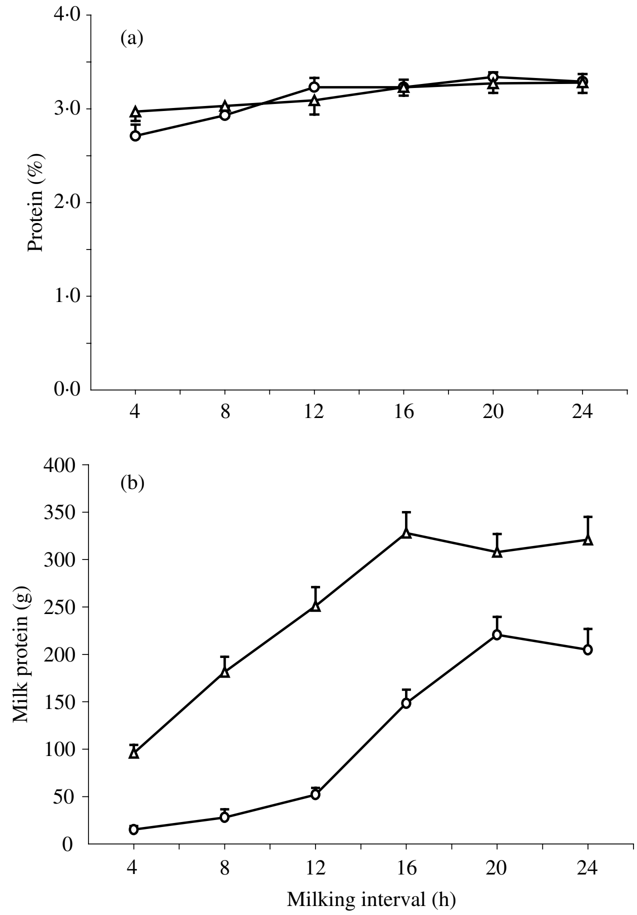

Fig. 2. Changes in (a) protein content and (b) protein yield in cisternal (○) and alveolar (△) milk fractions for different milking intervals in four dairy cows.

Values are means ± SEM

Fat content in total milk was high and changed quadratically with milking interval (R^2=0·77; $P<0·05$) in a concave shape (Table 1). It was lowest for the 16 and 20-h milking intervals, but there were no differences ($P>0·05$) in milk fat content between the extreme milking intervals (4 and 24 h). Milk composition of cisternal and alveolar fractions varied inversely at different milking intervals (Fig. 1a). Milk fat percentage decreased in cisternal milk ($P<0·001$) and increased in alveolar milk ($P<0·001$). There were no differences between left and right quarters (P=0·64), but the percentage of fat in cisternal milk tended to be higher ($P<0·07$) in front quarters than in rear quarters, at the 4-h and 8-h milking intervals (Table 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 ($P<0·05$) thereafter (Fig. 1a). The final value of fat percentage in mean alveolar milk (6·95 %) was higher than the initial (5·62 %; $P<0·05$) and final (0·96 %; $P<0·001$) values of mean cisternal milk, as can be calculated from Table 2. Because of the fractional changes in volume and composition, daily fat yield tended to increase for cisternal milk (P=0·10), and increased significantly for alveolar milk ($P<0·001$) at longer milking intervals. Fat yield was greater ($P<0·001$) in both milk fractions (Fig. 1b) in the rear quarters than in the front quarters (results not shown).

Changes in cisternal and alveolar milk protein with changes of milking interval are shown in Fig. 2a. Milk protein content varied less with milking interval than did milk fat content. As milking interval increased, protein content increased in the cisternal milk fraction ($P<0·001$) and tended to increase in the alveolar milk fraction (P=0·08). Initial and final milk protein values did not differ between cisternal and alveolar fractions or between front and rear quarters (Table 2). Protein yield increased ($P<0·05$) with milking interval in both fractions as shown in Fig. 2b and was greater in the rear quarters than in the front quarters ($P<0·01$) for both milk fractions (Table 2).

Discussion

Changes of cisternal and alveolar milk with change of milking interval agreed closely with earlier observations (Knight et al. 1994b; Davis et al. 1998) and with current models of milk accumulation (Stelwagen, 2001) as indicated by Ayadi et al. (2003a). Nevertheless the methodology used in our experiment only allowed us to see the direct and short-term effects of milking frequency in late lactation. Under these conditions, there was a significant linear effect of the number of milkings per day on the daily milk yield, which agrees with the hypothesis of a fixed effect of the number of milkings as proposed by Erdman & Varner (1995). 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 twice to thrice a day. Moreover, the estimated reduction in milk yield when only one milking per day was done (10%) was 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. Ayadi et al. (2003b) report only a slight reduction in milk yield (1·1 l/d; 3·7%) without any important change in any milk component or milk persistency in dairy cows when one milking is omitted weekly.

Milk fat is the most variable component in ruminant milk. Fat content in cisternal milk was very high at the first 4-h milking interval because of drainage of the residual milk stored in the upper part of the mammary gland caused by its displacement by freshly secreted milk. Thereafter, fat content in cisternal milk decreased as milking interval increased because of dilution in the cisternal compartment (Labussière, 1969, 1985; Stelwagen, 2001). In contrast, fat content in the alveolar milk was constant during the first 16 h of milking interval and increased when tight junction leakage would be expected to start (17–18 h; Stelwagen et al. 1997). Similar results are 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 4–24-h milking intervals. Moreover, according to Stelwagen et al. (1997), the diameter of milk fat globules is greater than the intercellular joints. The changes observed in the present study agree with the milk accumulation model of Stelwagen (2001). Moreover, 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 suggests that the transfer of milk fat from the alveoli to the cistern stopped, leading to 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 was only obtainable if milk ejection occurred during milking. McKusick et al. (2002) report that alveoli can store up to 70% of total fat yield in dairy ewes in keeping with their greater cisternal milk percentage.

The decrease in total milk fat with milking interval was consistent with the changes 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 decreased total milk fat with increasing milking interval (4–12 h) in early lactation but no effect in mid and late lactation, as in our results with longer milking intervals (4–24 h). Total milk fat decreases markedly with increasing milking interval (4–24 h) in dairy sheep (McKusick et al. 2002). Nevertheless, results are not directly comparable because of differences in experimental procedures. Thus, McKusick et al. (2002) and Weiss et al. (2002) removed the residual milk 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 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 owing to its homogeneous distribution in the colloidal phase of milk. Protein content in total milk was constant after 12 h. No corresponding results are available in dairy cows for comparison, but McKusick et al. (2002) reported the same pattern of change in dairy sheep according to milking interval. Moreover, milk protein content does not vary (Holmes et al. 1992) or increases slightly (Klei et al. 1997) when milking frequency is reduced 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 newly secreted milk; for milking intervals of 16, 20 and 24 h, protein content in cisternal milk was constant. This may be a result of decreased milk protein synthesis and accumulation of the feedback inhibitors of lactation within the alveoli at longer milking intervals (Wilde et al. 1995). Protein content in alveolar milk showed a similar trend but no significant effects were detected for any milking intervals, as reported by Labussière (1985). The tendency of protein content to increase for extended milking intervals in our experiment may be explained by 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 accord with the changes in milk yield and in milk fractions for the different milking intervals and agree with other results (Stelwagen et al. 1997; Auldist & Prosser, 1998).

Extrapolating to the equivalent milking frequency from 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 >3 milkings/d, because of the more complete removal of residual milk.

In conclusion, varying the milking interval in dairy cows induced major changes in milk concentrations 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 milking interval is extended owing to the change of the contribution of alveolar and cisternal fractions. A larger cisternal compartment in the udder could give rise to lower milk yield losses and to smaller milk composition changes when moving to lower milking frequencies.

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