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Physical properties of mammary secretions in relation to chemical changes during transition from colostrum to milk

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We examined the physical and chemical changes in milk during early lactation, and how these changes were affected by leaving one quarter unmilked in either the first or second milking, with the purpose of discriminating between colostrum and normal milk. Milk samples were collected from each quarter of 17 cows during the first 5 d after calving and then after about 7 d and 14 d. Samples were analysed for somatic cell count (SCC), fat, protein, casein, lactose, IgG_1, colour, plasmin, pH and coagulation properties. Large variations occurred in both chemical and physical properties throughout the study period. Within six milkings, the concentration of casein decreased by 60%, IgG_1 by 94%, and lactose increased by 34%. At milking number 6, rennet coagulation time was lowest and curd firmness was highest. The pH increased from 6·4 to 6·7 over the period of the experiment, and the colour changed from yellow (reddish) to white. Coagulation properties and the pH fell within the range of normal milk after five milkings. Measurement of colour and density appeared to be a potential method for detection of milk unsuitable for the dairy factory. Effects of omitting one quarter in one milking differed between milk components, but seemed to be of little importance to the physical properties.

Keywords: Colour, pH, coagulation properties, fat, protein, casein, lactose, IgG_1, plasmin, SCC.

During the first week after calving, the composition of bovine mammary secretion changes markedly and, in domestic ruminants, the main difference between colostrum and milk is the high concentration of immunoglobulins. It is important for the cheesemaker to be aware that the protein composition of milk available for processing is subject to variation. This is particularly so in relation to the ratio of the various proteins (Dalgleish, 1992; Feagan, 1979), and major whey proteins other than Igs are also found in higher concentrations in colostrum than in mature milk (Levieux & Ollier, 1999).

Physical properties of colostrum are also different from milk. For example the colour of colostrum is reddish-yellow (Edelsten, 1988), the density is higher than that of milk (Haggag et al. 1991) and the pH is lower (Edelsten, 1988). Physical properties of the milk proteins, e.g., curd firmness and heat stability, influence the quality of the manufactured product.

Data on the physical properties of raw milk are important since they can influence the design and operation of dairy processing equipment or can be used to determine the concentration of specific components in milk, or to assess the extent of biochemical changes in the milk during processing (Fox & McSweeney, 1998). The lowest-cost production of uniform high-quality products is best achieved if the processor obtains milk of consistent or at least predictable protein composition (Feagan, 1979). For particular milk proteins, the composition sought by the manufacturer may best be manipulated at farm level (e.g., bovine serum albumin, BSA). Further modification of the milk may best be done at the factory, e.g., pH adjustment to produce a firmer curd for cheesemaking and a milk with better heat stability. Adjustments on farm take longer and are more difficult to achieve than the day to day adjustments that can be made to the milk after it reaches the factory. Factory adjustment, however, is not always achievable and can add to the cost of processing (Feagan, 1979).

Zawistowski & Mackinnon (1993) found that 89% of 400 tested milk samples from one state in Canada were

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contaminated with colostrum as the content of IgG was >0·1%. Information on the technological consequences for the milk industry of adding early milk is scarce and inconsistent. There is a need for more precise studies of the technological implications of IgG and other whey proteins in milk in order to be able to harmonize legislation concerning the time for withholding post-parturition milk (Levieux & Ollier, 1999) or, for instance, in order to develop computerized separation of milk in the automatic milking systems (AMS).

Owing to these complications, it is necessary to know how the composition of milk changes during transition from colostrum to milk. This will make it possible to classify and separate the milk and use it in the most profitable way. The objectives of this investigation were to study the physical and chemical changes in milk during the transition period, and to determine how the chemical changes affect physical properties. Effects of omitting the first milking *post partum* of one quarter and the second milking of another quarter were also measured in order to study the transition effects within cow.

Materials and Methods

Experimental animals

The experiment used 17 cows from the Danish Cattle Research Centre (DK-8830 Tjele, Denmark) (9 Danish Holsteins, 4 Danish Jerseys, and 4 Danish Red Breed) in early lactation and different lactation numbers (14 cows in second lactation and 1 cow in each of lactation numbers 1, 3 and 4). The Danish Cattle Research Centre houses 120 cows and three automatic milking systems (AMS). The cows were fed a total mixed ration (TMR) containing wheat- and grass silage and spring barley. Average 305-d milk yield was 7550 kg. The 17 cows used in the experiment were selected by choosing cows that calved within a certain period of time. After calving, the calf stayed with the cow for 1 d but the udder was covered to prevent the calf from sucking.

Milk samples and recordings

For the first 5 d *post partum*, the cows were led by hand to the AMS for milking twice daily at 06.00 and 16.30. At the first two milkings, only three of the four quarters were milked, leaving out the left rear at the first milking and the left front in the second milking. The right quarters served as normal controls. This strategy allowed us to study the effect of local or systemic control of milk composition within the cow and check the consequences of an attachment failure of the AMS. For simplicity, milking number refers to milking number of the cow, meaning that milking no. 1 is missing for left rear and milking no. 2 is missing for left front. Milk samples were obtained from all milked quarters for the first 10 milkings. From day 5 *post partum*, the cows were allowed to enter the AMS freely. The withhold period of colostrum is normally accounted <5 d but in order to catch a possible tailing effect, the cows were sampled at the two following Thursday evenings representing about 1 week and 2 weeks of milking. On those days, the cows were milked after having been kept away from the AMS for 5 h, and milk samples from individual quarters were obtained. Milk yield for each quarter was read from the Free Flow Meters of the AMS (DeLaval VMS, SE-14721 Tumba, Sweden). Those samples that were not analysed within 24 h were either frozen at −20 °C or preserved, as appropriate (Broad Spectrum Microtabs, D&F Control Systems, Inc., CA-94583, USA).

The foremilk of four quarters of four cows contained clots in all 11 samples and was scored as visually abnormal (clinical mastitis). If a quarter had one observation with >10 000 000 cells/ml or >500 000 cells/ml in milking number 9, then the quarter was classified as ‘infected’ during the entire period. This resulted in 17 out of 68 quarters being classified as infected. The colour of the four quarters was compared at each individual milking and samples with a reddish tinge were noted as being blood contaminated. In all, 36 samples from 12 quarters of six cows were so classified.

Analyses

Somatic cell counts (SCC) were determined at Steins Laboratory, DK-7500 Holstebro, Denmark using a Fossomatic (Foss Electric, DK-3400 Hillerød, Denmark). Fat, protein, casein, lactose and density were measured by use of a MilkoScan FT120 (Foss Electric, DK-3400 Hillerød, Denmark) after having heated the samples to 38 °C. Immunoglobulin IgG_1 was analysed by radial immunodiffusion (RID) by a commercial test kit (VMRD, Pullman, WA-99163, USA). Samples were diluted 0 to 20 times with PBS pH 7·4 to fall into measurable levels. Two people read the diameters of the resulting rings. An enlarger with incorporated scale was used to ensure accuracy. If a reading from the two persons differed by more than 0·1 mm, it was repeated. Using standard solutions and standard scales, the concentrations were calculated from the diameters. Colour was measured by a Chroma meter (CR-300, Minolta Co., Osaka 541-8556, Japan) where 2 ml of sample was transferred to a black capsule and colour was measured immediately. Colour was expressed on three scales within the visible spectrum: ‘L’, a light/dark scale that runs from 0 (black) to 100 (white); ‘a’, a red/green scale (− is green, and + is red); and ‘b’, a blue/yellow scale (− is blue, and + is yellow). The pH was measured by use of a pH meter (PHM 220, LAB pH meter, Radiometer Copenhagen, DK-2700 Brønshøj, Denmark) on samples from 12 cows. Coagulation properties were examined by means of a Formagraph (Foss Electric, DK-3400 Hillerød, Denmark). Fresh sample (10 ml) was heated to 32 °C over 30 min; 0·2 ml of rennet (Standard 190, Chymosin (rennin) content: 63%, bovine pepsin content: 37%, Danish Home Production, DK-5853 Ørbæk, Denmark) was added and

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the sample was transferred to the Formagraph. The course of the coagulation over 60 min was printed on graph paper and coagulation time (R=time from rennet addition to separation of the forks), time for aggregation (K_{20}=time when the amplitude of the forks reached 20 mm) and curd firmness (A_{30} and A_{50}=amplitude of the forks after 30 and 50 min, respectively) were measured from the graph. Samples from 11 cows were tested.

The reaction of the samples to pasteurization was quantified visually. Fresh samples were placed in a water bath (63 °C) for 30 min (low temperature, long time (LTLT) pasteurization), and then allowed to cool for 60 min before visual inspection. A scale from 1 to 4 was used where 1 was normal (no reaction) and 4 was coagulation (butter-like). Samples from 12 cows were examined.

Activity of plasmin (EC 3.4.21.7) was determined by a modification of the method described by Politis et al. (1989) and Korycka-Dahl et al. (1983) using the chromogenic substrate S-2403 (pyroGlu-Phe-Lys-pNA·HCl) (Haemochrom Diagnostica AB, DK-2000 Frederiksberg, Denmark). Preparation of samples included centrifugation (1040 ***g*** at 4 °C for 10 min twice), removal of fat, and dilution (5 times) with PBS-EDTA (50 mM-NaH_2PO_4, 100 mM-NaCl, 10 mM-EDTA, pH 7·4). Enzyme activities were determined in microtitre plates. For determination of plasmin activities, wells contained 100 µl PBS-EDTA, 50 µl diluted milk sample and 50 µl 5·6 mM-substrate solution. Substrate cleavage was determined by immediate reading of the absorbance at 405 nm each minute at 37 °C for 45 min after addition of the substrate and mixing of the reaction mixture. Rate of pNA formation was calculated from the linear portion of the absorbance *v.* time curve. Enzyme activity was expressed as the increase in ΔA_{405} min^{-1} ml^{-1}. Samples from milkings 1, 2, 3, 4, 6 and 10 from all 17 cows were analysed.

Statistical analyses

Data were analysed using a MIXED procedure in the program package SAS© (SAS, 1999). The following model was used in the analysis of variance:

$$Y=\mu+\alpha_i+\beta_j+\alpha\beta_{ij}+\gamma_k+\kappa_l+\lambda_m+\varepsilon_{ijlkm} \quad (1)$$

where:

Y=dependent variable
μ=mean
α=effect of quarter (i=Right Front, Right Rear, Left Front, Left Rear)
β=effect of milking number (j=1, 2,…, 12)
γ=effect of breed (k=Red Danish, Danish Holsteins, Danish Jersey)
κ=effect of visible clots in the milk (l=0, 1)
λ=effect of blood in the milk (m=0, 1)
$\alpha\beta$=effect of interaction between quarter and milking number
ε=residual. ε_{ijlkm} are independent and $N(0, \sigma^2)$.

Cow was included as a random effect to test differences between breeds, and interaction between cow and quarter was included as a random effect to test differences between milking numbers. Milking number was treated as a repeated measurement of the subject quarter within cow to account for repeated measurements. The Satterthwaite adjustment was used to adjust the degrees of freedom for the missing values (systematic for milkings 1 and 2, and randomly for the remaining missing observations).

To account for non-normal distribution, IgG_1 and SCC were transformed using the $\log_{10}$ function and plasmin was transformed using the square root. The colour 'b' was added to the value 10 before $\log_{10}$ transformation to avoid negative values, which resulted in a normal distribution of data. If the effect of a factor was not significant, the factor was removed from the model for the component in question. Differences in physical properties between milking numbers were calculated for samples where appearance was normal and blood was not present. A variable 'Norm' was created from the colour data. Each of the colour variables L, a and b was divided into three groups and 'Norm' was produced according to the following rules:

L-norm	1	L darker than L-norm=2
	2	L is within the average$\pm 2\sigma$ for that breed for milking 6–12
	3	L lighter than L-norm=2
a-norm	1	a more red than a-norm=2
	2	a is within the average$\pm 2\sigma$ for that breed for milkings 3–12
	3	a more green than a-norm=2
b-norm	1	b more yellow than b-norm=2
	2	b is within the average$\pm 2\sigma$ for that breed for milkings 11–12
	3	b more blue than b-norm=2
Norm	1	Samples where the three norms above are all 1
	2	Samples where one or more of the norms above are 2 or 3

Results

Overall changes in milk yield, composition and colour are given in Table 1 and changes in physical properties are presented in Table 4. Average milk yield for the milked quarters decreased from milking 1 to milking 2, and increased thereafter until milking 10. The decrease from milking 1 to milking 2 would have been larger if all quarters had been included in all milkings. Leaving out the left rear quarter in milking number 1 led to a compensatory increase in milk yield in milking number 2 for that quarter, increasing the overall average milk yield for milking number 2. The decrease in milk yield seen in Table 1 in milkings 11 and 12 was probably due to the shorter interval (minimum 5 h) between automatic milkings at this point compared with the interval between the

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Table 1. LS means (n=17) and significance of changes in milk including milk yield per quarter, cell count per ml (log SCC), immunoglobulin IgG_1, casein, and colour (lightness, L; redness, a; and yellowness, b). Quarters with blood or clots in the milk are excluded from the LS means. An indication is given of the milking number after which no further statistical change was noted

Milking no.	Yield, kg	Log SCC	Log IgG_1	Casein, %	L	a	b
1	2·16^{bc}	5·93^{ab}	3·77^{a}	9·24^{a}	34·1^{a}	−1·97^{a}	12·16^{a}
2	1·68^{a}	5·98^{a}	3·54^{b}	7·24^{b}	35·3^{b}	−2·45^{bc}	10·15^{b}
3	2·12^{b}	5·76^{bc}	3·13^{c}	4·72^{c}	36·3^{c}	−2·75^{d}	6·91^{c}
4	2·28^{b}	5·76^{bc}	2·82^{d}	3·93^{d}	37·1^{d}	−2·65^{d}	7·11^{c}
5	2·57^{cd}	5·65^{cd}	2·59^{e}	3·63^{e}	37·5^{e}	−2·62^{cd}	6·11^{d}
6	2·56^{cd}	5·56^{d}	2·41^{f}	3·50^{ef}	38·0^{f}	−2·48^{bc}	6·28^{d}
7	2·72de	5·40^{e}	2·33^{g}	3·41^{efg}	38·1^{f}	−2·50^{c}	4·99^{e}
8	2·78de	5·37^{e}	2·16^{h}	3·35^{fg}	38·2^{gh}	−2·44^{bc}	4·90^{e}
9	2·80de	5·30^{ef}	2·12^{i}	3·26^{g}	38·3^{h}	−2·36^{bc}	4·23^{f}
10	2·95^{d}	5·21^{fg}	2·08^{j}	3·21^{gh}	38·3^{h}	−2·44^{bc}	3·64^{f}
11†	2·67de	5·12^{g}	1·94^{k}	3·02^{h}	38·1^{fg}	−2·45^{bc}	2·75^{g}
12†	2·72de	4·93^{h}	1·85^{l}	2·73^{i}	38·0^{f}	−2·34^{b}	2·44^{g}
N	722	681	720	718	714	714	714
SD	0·61	0·14	0·01	0·36	2·9	0·11	2·80
Quarter	***	NS	***	***	*	NS	NS
Milking no.	***	***	***	***	***	***	***
Q × M	***	NS	**	***	NS	NS	NS
Breed	NS	NS	NS	NS	NS	*	NS
Visual	NS	***	NS	NS	NS	NS	NS
Blood	*	***	NS	NS	***	***	*
No change after milking number:	5	12	12	12	6	2	11

† Note that milking numbers 11 and 12 are the evening milkings on the two Thursdays following milking 10
Values within a column without a common superscript letter indicate significant differences between milkings (P<0·05)

Table 2. LS means (n=17) of casein yield during the first four milkings after calving (g)

Quarter	1st milking	2nd milking	3rd milking	4th milking
RF	169^{b}	58^{d}	73^{d}	75^{d}
LF	190^{ab}	—	114^{cd}	71^{d}
RR	256^{ab}	86^{cd}	88^{cd}	96^{cd}
LR	—	273^{a}	115^{c}	102^{cd}

Values without a common superscript letter indicate significant differences between milkings and quarters: P<0·01 (SEM=14·5)

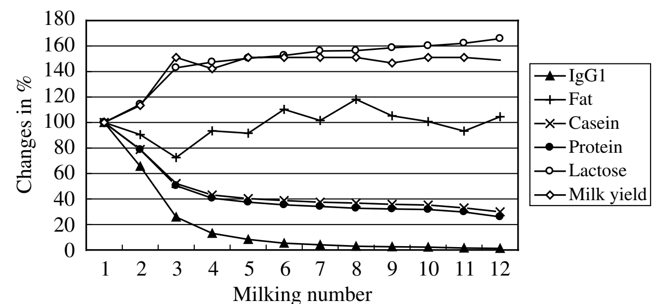

Fig. 1. Relative changes (%) in concentration of IgG_1, fat, casein, total protein, lactose and milk volume during the first milkings after calving. Concentration in milking number 1 is 100%. (n=51 for milking numbers 1 and 2, n=68 for milking numbers 3–12.)

first ten milkings. Milk yield of quarters containing blood was 0·45 kg less than the others (P<0·05). SCC decreased from about 1 000 000 cells/ml for milkings 1 and 2 to <100 000 at milking 12 of quarters not having abnormal milk. Quarters with clots in the milk were about 7-fold higher and quarters containing blood about 2-fold higher than quarters with visually normal milk (P<0·001).

Lactose concentration increased from 2·6% to 4·4% throughout the period and was 0·34 percentage units higher (P<0·001) in samples without visual abnormalities (results not shown). Protein decreased from 13·90 to 3·55% (results not shown) and casein decreased from 9·24 to 2·73% during the period (P<0·001) and effects of quarter and interaction between quarter and milking number were also significant (P<0·001). Immunoglobulin IgG_1 declined (P<0·001) during the period, and there was also a significant effect of quarter (P<0·001). Relative changes of milk yield and the major components of milk and colostrum are shown in Fig. 1.

Omission of a milking had no significant effect on the percentages of fat and lactose or on SCC. The left quarters, for which one milking was omitted, had higher concentrations of protein and casein than the right quarters (results not shown). The rear quarters had a higher casein yield (percentage × milk yield) than the front quarters. Additionally, from Table 2 it can be seen that leaving out the left rear in the first milking resulted in neither significant accumulation nor loss of casein. The yield that would have

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Table 3. LS means (n=17) for yield (content × milk yield) of IgG_1 (g). For clarity, not all milking numbers are included in the table

Quarter	1st milking	2nd milking	3rd milking	4th milking
RF	$94·9^{a}$	$28·2^{cde}$	$19·9^{efg}$	$11·9^{g}$
LF	$105·4^{a}$	—	$37·9^{bcd}$	$12·6^{g}$
RR	$157·5^{a}$	$41·5^{bc}$	$23·8^{def}$	$16·0^{fg}$
LR	—	$168·6^{a}$	$44·0^{b}$	$21·0^{defg}$

Values without a common superscript letter indicate significant differences between milkings and quarters: $P<0·01$ (SEM = 8·3)

been expected if the left rear had been milked in milking 1 was simply delayed to milking 2, but in milkings 3 and 4 the difference between the two rear quarters was no longer significant. Omitting the left front in the second milking seemed to cause little accumulation since the yield in milking 3 was higher (NS) for the left front than it was for the right front in milking 2. However, in milking 3 there was no significant difference between the front quarters and in milking 4 there was no significant difference between the four quarters.

For protein and casein, the concentrations of IgG_1 were higher in the two left quarters (data not shown). Yields of IgG_1 in milkings 1–4 are presented in Table 3. Yield of IgG_1 decreased markedly during the first milkings but omission of a milking of a quarter acted mainly as mentioned for casein. Differences between quarters were neutralized in milking 4, so leaving a quarter unmilked during one of the first milkings after calving did not significantly affect total IgG_1 yield.

Colour of the samples changed with time from calving ($P<0·001$); it became lighter (lightness, L, increased over the first six milkings), less red (redness, a, decreased and stabilized after the second milking) and less yellow (yellowness, b, decreased during the entire period) (Table 1). Effect of quarter was significant for L ($P<0·05$). This was not an effect of omission of a milking, rather it seemed that the front quarters in general had higher L-values than the rear quarters. Effect of breed was significant ($P<0·05$) in that Jersey cows had less reddish milk than Danish Holsteins and the Red Danish Breed. Not surprisingly blood in the milk also affected the colour significantly; it caused the sample to be darker ($P<0·001$), less yellow ($P<0·05$), and of course more red ($P<0·001$). Although both number of milking and presence of blood in the milk were highly significant, the F-value of the statistical analysis for blood was 44 times that of milking number for the colour redness, indicating that any effect of colostrum would easily be hidden by the presence of blood. Two examples of colour changes are given in Fig. 2.

Density decreased ($P<0·001$) to a stable level during the first six milkings (Table 4). Effect of quarter was significant ($P<0·01$), and this was caused by higher density in the right quarters where one milking was omitted. The pH increased during the entire period ($P<0·001$), and effect of quarter was significant ($P<0·05$). However, quarters with clots in the milk had numerically a slightly higher pH (0·03, NS) than other quarters and after omission of these, the effect of quarter was reduced ($P<0·10$) and there was no effect of leaving a quarter unmilked. Activity of plasmin decreased during the period ($P<0·01$), and at milking 10 it was only about 50% of the level of milking number 1. There was no effect of omitting one quarter in one milking.

Coagulation properties were all strongly affected by milking number ($P<0·001$). Milk did not clot during heating (pasteurization) after three milkings, and the coagulating properties (rennet coagulation time, RCT; time for aggregation, K_{20}; curd firmness, A_{30} and A_{50}) seemed to have an optimum around milking numbers 6 and 7 (Table 4 and Fig. 3). Changes in RCT were explained primarily by changes in pH and casein content ($P<0·001$), where the increase in pH accounted for an increase of 0·22 log units and the decrease in casein for a decrease of 0·46 log units from milking 1 to 12. A 10-fold increase in SCC (one log unit) was related to an increase in RCT of 0·06 log units. Similar trends were found for K_{20}. A_{30} and A_{50} decreased significantly with the increase in pH and increased with the decrease in casein ($P<0·001$).

In Table 5, the variable 'Norm' is used to show the relationship between the colour measurements and the composition of milk. 'Norm' was created so that 'Norm' = 1 covers samples with the typical colour of colostrum within breed and 'Norm' = 2 covers the remainder of the samples. Combining the two best measurements of colour (L and b) made it possible to separate the milk even further into two classes: typical colostrum (L = 1 and b = 1) and normal milk (Norm = 2) with no overlap in IgG content and hardly any in protein (Table 5). However, a very large group of samples had intermediate results. Fat percentage and density were not separated by this method.

Discussion

Very large variations occurred in physical properties of the milk and these changes were related to chemical composition. For milk samples with 'Norm' = 1, protein concentration ranged from 3·5 to 13·5%, but for 'Norm' = 2 the concentration was only 3·4 to 4·7% (Table 5), so the colour measurements were not without relation to the composition of the samples. Similar results were found for IgG_1. These results imply that colour measurements could distinguish between colostrum and milk. However, the method needs refinement, which can be seen from the samples with 'Norm' = 1 but only 3% protein (false positive).

The white colour of milk results from dispersion of reflected light by the fat globules and the colloidal particles of casein and calcium phosphate (Fox & McSweeney, 1998; Edelsten, 1988). Homogenization of milk results in a whiter product owing to increased scattering of light by smaller, homogenized fat globules (Fox & McSweeney,

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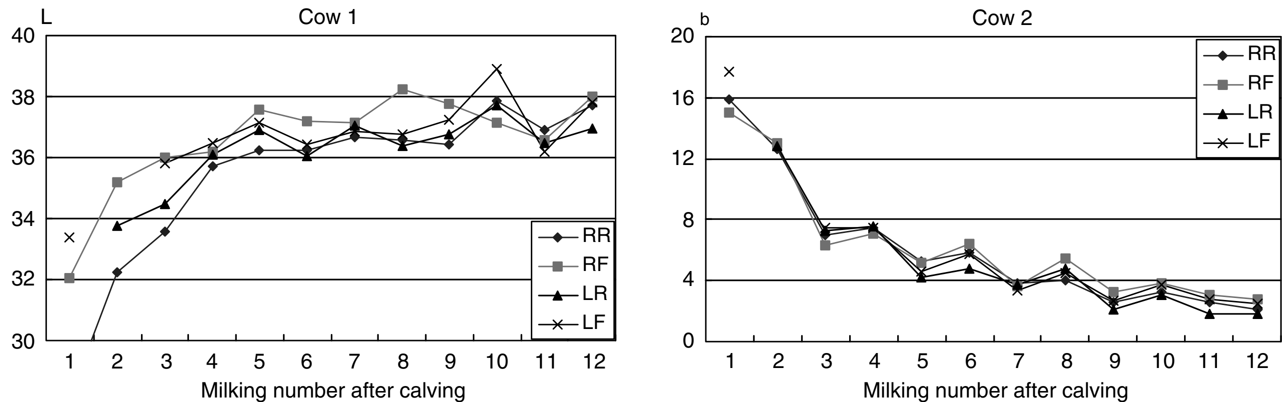

Fig. 2. Two examples of the colour 'L' (light/dark) and 'b' (yellow/blue) of two individual cows. The quarter RR of cow 1 was darker than the others for milkings 2 and 3 owing to blood in the milk. All quarters of cow 2 became bluer (less yellow) as lactation progressed, irrespective of the omission of a milking.

1998), so the increase in L-value and thus the lighter colour of samples over time may be due to a more homogeneous product containing smaller particles. Milk ranges in colour from bluish-white to golden-yellow, depending on breed, ration, and the amount of fat and solids. The yellow colour is due to carotene (Edelsten, 1988), and since carotene is not synthesized by the animal but is obtained from plant sources, feed has a major effect on the colour of milk and milk products (Fox & McSweeney, 1998). Since animals in our experiment were fed the same ration throughout the experiment, feed is unlikely to explain the changes we saw. Colostrum has a slightly reddish-yellow colour (Edelsten, 1988) and as little as 0·1 ml blood/l can be detected visually and that 0·4 ml/l turned milk very pink (Linzell & Peaker, 1975). Ouweltjes & Hogeveen (2001) obtained repeatable results when measuring the colour of normal composition milk, which means that standard values, which are the basis for detecting abnormalities, are feasible. Espada & Vijverberg (2002) examined the possibility of using colour measurements for detection of abnormal (both colostrum and mastitic) milk. They found colostrum to have a less blue colour than the herd average, and conclude that colour is a useful tool to identify abnormal milk. From Table 1 and Fig. 2 it can be seen that 'b' decreased considerably meaning that the colour became bluer during the period, agreeing with the findings of Espada & Vijverberg (2002).

Density of milk declined during the first five to six milkings (Table 4), and it was also during these milkings that the most important changes in milk composition occurred (Fig. 1). Haggag et al. (1991) found that specific gravity of buffalo colostrum decreases from 1·040 to 1·032 within 48 h *post partum*, which is similar to our results. Milk from the left rear quarter that was omitted in the first milking, had a higher density than milk from the other quarters, which fits nicely with the higher concentrations of immunoglobulins and casein found in this quarter. This indicates that omission of the first milking after calving results in higher milk density because of increased concentrations of immunoglobulins and casein.

The pH increased from 6·4 to 6·7 during the first 2 weeks *post partum*. The pH of bovine milk is, between 6·5 and 6·7, with 6·6 as the normal value when measured at temperatures of around 25 °C (Edelsten, 1988), so from Table 4 it can be seen that our samples reached the normal pH around milking number 5. Values >6·7 usually denote mastitis and those <6·5 indicate the presence of colostrum or bacterial deterioration (Edelsten, 1988). Haggag et al. (1991) thus found that the pH of mastitic milk from buffalo cows rose to over 7, and that of colostrum was 6·43±0·02 compared with 6·57±0·04 for normal milk. Politis & Ng-Kwai-Hang (1988c) found that RCT, rate of curd firming, and curd firmness at cutting increase by 3·52, 3·41 min and decrease by 9·45 mm, respectively, for every unit increase in milk pH. So, pH is an important factor that might be used for the detection both of colostrum and mastitic milk. However, as pH was not affected by omission of one milking, but the concentration of IgG_1 was, pH measurements might not be sensitive enough to detect colostrum in milk.

Pasteurization of the samples showed that the milk from the first milkings after calving behaved differently from more mature milk. Salt balance and acidity are two of the most important factors in the heat stability of milk, and the low heat stability of colostral milk is ascribed to a higher level of ionic calcium (Parry, 1974). According to Fox & McSweeney (1998), heat coagulation time increases with increasing pH from 6·4 to about 6·7. Thus, the coagulation seen in our samples from milkings 1 and 2 might be due to the low pH in these samples or to the low heat stability of the immunoglobulins (Parry, 1974).

About 50% of milk in the EU is used for cheese production (Fox & McSweeney, 1998), and coagulation is the most important stage in cheesemaking. The amount of whey retained in the curd affects the texture and acidity of the cheese and may influence flavour by affecting the

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Table 4. LS means (n=17) and significance of physical changes in density, pH, pasteurization (% of quarters with a score different from 1), plasmin activity, rennet coagulation time (Log_{10}RCT, min), time for aggregation ($Log_{10}K_{20}$, min), and curd firmness (A_{30}, mm). Quarters with blood or clots in the milk are excluded from the LS means. An indication is given of the milking number after which no further statistical change was noted

Milking no.	Density	pH	Past	Plasmin	RCT	K_{20}	A_{30}
1	1·048[a]	6·37[a]	80[a]	293[a]	1·232[ab]	1·113[a]	43·1[bc]
2	1·042[b]	6·42[b]	43[b]	297[a]	1·296[a]	1·040[b]	44·7[b]
3	1·038[c]	6·42[b]	5[c]	276[ab]	1·152[c]	0·837[d]	51·1[a]
4	1·034[d]	6·45[b]	0[d]	234[bc]	1·116[c]	0·826[d]	51·3[a]
5	1·033[de]	6·49[c]	0[d]		1·093[cd]	0·724[d]	51·0[a]
6	1·032[f]	6·50[c]	0[d]	204[c]	1·048[d]	0·689[d]	51·5[a]
7	1·033[ef]	6·54[d]	0[d]		1·083[d]	0·701[d]	51·1[a]
8	1·031[f]	6·56[de]	0[d]		1·091[cd]	0·732[cd]	48·8[ab]
9	1·032[f]	6·59[e]	0[d]		1·132[c]	0·778[bc]	45·2[bc]
10	1·032[f]	6·63[f]	0[d]	147[d]	1·143[c]	0·770[c]	45·8[bc]
11†	1·032[f]	6·68[g]	0[d]		1·151[bc]	1·886[b]	42·5[c]
12†	1·030[g]	6·73[h]	0[d]		1·189[b]	1·956[a]	34·7[d]
N	718	457	482	408	425	425	425
SD	0·000	0·01	—	225	0·015	0·033	89·5
Quarter	**	*	NS	NS	NS	NS	NS
Milking no.	***	***	***	***	***	***	***
Q × M	**	NS	NS	NS	NS	NS	NS
Breed	NS	NS	NS	NS	NS	NS	**
Visual	NS	NS	NS	NS	NS	NS	*
Blood	NS	NS	NS	NS	**	NS	NS
No change after milking number:	6	5	4	10	3	3 (12)	1 (12)

† Note that milking numbers 11 and 12 are the evening milkings on the two Thursdays following milking 10
Values within a column without a common superscript letter indicate significantly differences between milkings ($P<0·05$)

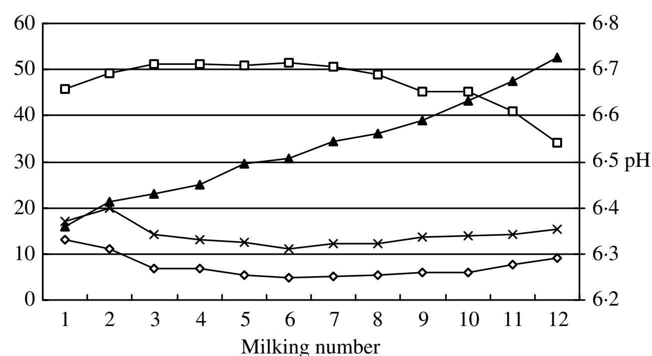

Fig. 3. Development in coagulation time (RCT, min, ×), agglutination time (K_{20}, min, ◇), curd firmness (A_{30}, mm, □) and pH (▲) during transition from colostrum to milk. Values are simple means of all samples. (n=33 for milking numbers 1 and 2; n=44 for milking numbers 3–12.)

growth of bacteria or other ripening agents (Beeby et al. 1971). In our rennet coagulation measurements, early milk showed unique behaviour. RCT decreased during the first six milkings and then increased at a slower rate. Curd firmness, on the other hand, increased during the first milkings and then decreased (Table 4 and Fig. 3). So, the fastest coagulation and the firmest curd were achieved around milking 6 and at a pH of about 6·5, which was also found by Klimes et al. (1986).

Firmness of the coagulum (curd tension) is reduced by decreasing casein, and it is little affected by lactose (Beeby et al. 1971). RCT increases when the pH increases, and it decreases when the concentration of protein increases (Fox & McSweeney, 1998). From this, the reason for the decrease in RCT from the first to the sixth milking is not clear, but the subsequent increase might be explained by the increase in pH and the decrease in protein. The decrease in curd firmness from milking 6 might be explained by the same factors.

Cell count also affects coagulation properties. Okigbo et al. (1985) found that coagulation properties of normal (<200 000 cells/ml) and abnormal milk drawn from individual quarters are significantly different, with abnormal milk having longer RCT (18–23 min) and forming weaker curd (A_{30}=14–35 mm) than normal milk (RCT=12–17 min, A_{30}=28–50 mm). Assuming that RCT of 12–17 min indicates normal milk and RCT >17 min indicates abnormal milk, then our samples were normal after milking number 2. Average A_{30} for our samples was >35 mm in all milkings, so from these criteria only samples from milkings 1 and 2 would be classified as abnormal milk.

Politis & Ng-Kwai-Hang (1988c) also found that elevated SCC is associated with a significant increase in RCT. An increase from 100 000 to 500 000 cells/ml resulted in an increase of 2·1% in RCT and 2·2% in K_{20}. A further increase to 1 000 000 cells/ml resulted in an increase of

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Table 5. Relationships between colour measurements (L, a and b; lightness, redness and yellowness, respectively) and concentration of IgG_1, protein and fat plus milk density (95% confidence intervals)

	'Norm'	*n*	IgG_1 (mg/ml)	Protein (%)	Fat (%)	Density
L	1	343	68–6341	3·79–15·13	1·99–8·52	1·029–1·050
	2	297	67–682	3·37–5·53	3·22–9·62	1·027–1·036
	3	115	62–360	3·34–5·01	3·26–9·68	1·025–1·036
a	1	252	65–6250	3·31–15·13	3·72–9·93	1·026–1·049
	2	285	64–1177	3·50–6·03	3·06–8·88	1·028–1·036
	3	218	107–5402	4·31–12·78	1·83–6·05	1·031–1·048
b	1	586	87–5402	3·86–14·00	2·47–9·67	1·027–1·047
	2	128	59–605	3·28–5·55	2·30–7·36	1·028–1·037
	3	42	54–130	3·18–4·36	1·83–4·82	1·029–1·037
'Norm'	1	698	67–5196	3·54–13·47	2·38–9·49	1·027–1·046
	2	57	62–205	3·38–4·66	2·66–5·81	1·029–1·037
L=1 and b=1		271	207–6535	4·41–15·37	1·99–8·85	1·029–1·050

Table 6. Earliest milking number that falls within specified limits

Limits	Protein (%)	Casein (%)	IgG_1 (mg/dl)	SCC $\times 10^{-3}$	pH	A_{30} (mm)	Colour, Norm
Min.	3·5	3·0	0	0	6·5	25	2
Max.	4·2	3·5	100	400	6·7	66	2
Milking no.	>10	6	>10	>11	5	1	11
5–95% of samples	7–12	4–11	8–12	3–12	3–12	2–12	6–12

20·7 and 13·4% in RCT and K_{20}, respectively. Politis & Ng-Kwai-Hang (1988b) concluded that there is a direct link between SCC, milk protein composition, cheese composition, and losses of milk components in the whey. The lower fat and casein contents in milks with high SCC, the two most important components in terms of cheese yield, are expected to result in lower yields (Politis & Ng-Kwai-Hang, 1988a). This implies that because of its high cell count (Table 1), colostrum should not be sent to the dairy.

Proteinases are technologically important for milk and dairy products (Fox, 1989), and proteinase activity in colostrum is about twice that of mature milk. Plasmin is the major proteinase normally present in milk (Manjunath & Bath, 1990). We found that the activity of plasmin was about twice as high in milkings 1 and 2 as in milking 10 (Table 4). Pyörälä & Kaartinen (1988) also found a numerical decrease (NS) in plasmin activity during the first week after calving. A decrease after calving would be expected because quarters with subclinical mastitis show elevated SCC and higher activity of plasmin than healthy quarters (Urech et al. 1999). Thus, colostrum with high SCC would also be likely to show raised levels of plasmin activity.

Bastian et al. (1991) found no effect of plasmin activity on clotting time and curd firmness. However, Mara et al. (1998) added purified plasmin to skim milk and then measured the clotting properties. Increasing plasmin addition, with concomitantly increased levels of casein hydrolysis, resulted in increased curd firming time and reduced curd firmness. With low levels of plasmin addition, however, the magnitude of these effects was small.

In general, physical properties were affected by chemical composition of the samples and all compounds and properties were affected by milking number. Still, even though the major constituents, fat, protein, casein and immunoglobulins, were affected by quarter, these effects were not reflected in the coagulation properties. Only density was affected by quarter in that the left quarters, which were omitted from the first milking, had a higher density ($P<0·01$).

Changes in visual appearance (clots or flakes) were related to lactose content and SCC, but none of the physical properties reacted to this. Blood in the sample had a more pronounced effect as it affected the colour and the coagulation properties. Thus, it appears that colour may be a good indicator of abnormal coagulation patterns.

Table 6 shows when the milk could be sent to the dairy factory when certain minimum and maximum limits for composition and properties are applied. Depending on the criteria established, the milk could be delivered more than a week after calving or it could be delivered immediately after calving. Typical characteristics for colostrum are the high content of immunoglobulins and the high cell count, and these are still high after milking 10. On the other hand, pH and curd firmness reach acceptable levels very soon after calving. Thus, if the objective is to deliver milk of a specific composition, then the milk should be withheld for 5–7 d, but if the objective is to deliver milk that can coagulate with rennet, then 2 d would suffice.

Okigbo et al. (1985) suggest that because of the large differences between milk from healthy and infected quarters, it would seem necessary to withhold milk from all

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four quarters. A better approach might be to withhold milk that shows signs of abnormality. There could, however, be ethical arguments against this practice.

Another important factor is natural variation between cows (Table 6). At a given time from calving, milk from different cows will vary in composition and properties. For example, the concentration of IgG_1 was <0·1% in milking number 8 for one cow but was still >0·1% in milking number 12 for another cow (Table 6). Lightness (L) was >38 in milking number 4 for some cows but was still <38 in milking number 12 for others. Similarly, pH was 6·5 in milking number 1 for some cows but for others the pH reached 6·5 in milking number 7. So even though an absolute time limit for withholding milk after parturition would be a simpler rule, it cannot be a very precise rule when based on criteria of milk composition.

Colostrum is normally withheld from newly calved cows for about 3 or 4 d corresponding to six to eight milkings with twice-daily milking. Alternatively, farmers look at the colour and viscosity for the sorting. It is technically possible to measure the colour of the milk and set specific limits for no further change within breed and herd (the Norm-values in Table 5) as to when the fluid should be regarded as milk rather than colostrum. However, colour is not directly related to the main components of colostrum – the immunoglobulins – rather to the casein content, but the correlations between IgG, protein, and casein are high enough (>0·9) for the method to be usable in practice. Statistical models including 'L' and 'b' could explain about 77–79% (R^2) of the variation in protein, casein, and IgG_1 whereas density explained about 49–68%. However, the combination of colour and density explained 84% of the variation in IgG_1 and 93–94% of the variation in protein and casein. Thus, combining information about the milk colour and the density, it might be possible to develop a system that can not only detect milk showing an abnormal coagulation pattern (via the colour measurements) but which could also detect milk with increased levels of casein and IgG_1 (via the density), i.e., milk contaminated with colostrum.

In conclusion, these results showed that great variations occur in both composition and physical properties of the mammary secretion during transition from colostrum to milk. Despite significant differences between quarters for some compositional constituents, most of the physical properties were not affected by quarter or omission of a milking. However, blood in the sample affected colour, RCT and A_{30}, and it also affected IgG_1 and SCC. Since the colour measurements were effective in detecting blood, and blood affected both chemical and physical properties, colour measurements seem to be a potential method for detecting milk that is unsuitable for dairy processing. However, to do this, standard values for normal milk must first be developed. Colour measurements were not very effective in detecting high concentrations of casein and IgG_1 as a result of omission of a milking. Density appeared to be a better indicator of such changes. The combination of colour measurements and density explained the changes in protein and casein content well and slightly better than the changes in IgG_1.

Because of the variations seen between animals and even between quarters, it might be better to separate milk based on the composition of the milk instead of time from calving. It is easier, however, to lay down a certain number of days for which milk should be withheld. From our results, this period should not be shorter than 3 d if technical properties are the criteria and not shorter than 5 d if milk composition is the criterion as it is not until this time that the SCC and IgG_1 come close to levels expected of normal milk.

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