Fractionized milk composition in dairy cows with subclinical mastitis

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ABSTRACT: Mastitis is the inflammatory reaction of the udder to invading pathogens. One of the most apparent reactions is the increased influx of immunoreactive cells from blood into milk inducing a dramatic increase of milk somatic cell counts (SCC). We have investigated (i) the relationship between log SCC/ml in infected quarters being >6 (n = 8, group I) or varying between 5.4 and 6 (n = 8, group II) and concentration of dry matter (DM), fat, protein, lactose, insulin-like growth factor (IGF)-1, insulin, prolactin, tumor necrosis factor (TNF)-α, sodium, potassium, chloride, electrical conductivity and osmolarity as compared with the contralateral (healthy) quarter (log SCC/ml <5.2); and (ii) composition of fractionized milk [cisternal milk, quartiles of alveolar milk and residual milk (after i.v. injection of 10 u.i. oxytocin)] during machine milking of infected and healthy quarters. SCC were higher ($P < 0.05$) in infected than in healthy quarters. Concentrations of fat, sodium, chloride, and IGF-1 were higher ($P < 0.05$), while that of lactose was lower ($P < 0.05$) in infected than in healthy quarters (group I). Concentrations of fat and chloride in both groups, of DM (in group II), and electrical conductivity and sodium (in group I) increased from the cisternal to alveolar (100%) fractions in infected quarters, while fat and DM concentrations similarly increased in healthy quarters. In conclusion, several but not all milk traits changed in a different manner during the course of milking in infected and non-infected quarters.

Keywords: cows; machine milking; mastitis; somatic cell counts

Subclinical infections, although lacking clinical symptoms, are characterized by reduced milk secretion and altered milk composition (Kitchen, 1981; Fox et al., 1985; Harmon, 1994). Within few hours after the invasion of pathogenic microorganisms in the udder, somatic cell counts (SCC) in milk increase in response to activated inflammatory mediators (Craven and Williams, 1985; Paape and Capuco, 1997; Smits et al., 1998; Wittmann et al., 2002; Schmitz et al., 2004). The enhanced paracellular diapedesis of leukocytes through the epithelium causes reduced tight junction integrity (Stelwagen et al., 1999) and hence exchange of constituents between blood and milk. Lactose, which is synthesized exclusively by mammary epithelial cells, partially leaks into blood circulation through the damaged blood-milk barrier (Mielke et al., 1985). Furthermore, microbial toxins and enzymes from damaged cells cause injury of secretory cells (Kitchen, 1981). Therefore, the ability of the mammary epithelium to synthesize and secrete the major specific milk constituents is reduced (Schultz, 1977; Fox et al., 1985; Eberhart et al., 1987), while the secretion of other proteins like lactoferrin is simultaneously up-regulated (Schmitz et al., 2004). The concentration of caseins is reduced in infected quarters due to reduced secretion and due to destruction by blood-borne proteases like plasmin (Politis et al., 1992). Simultaneously, the amounts of blood borne components, such as serum albumin and sodium (Na^+) and chloride (Cl^-) ions increase in milk of infected quarters (Kitchen, 1981; Harmon, 1994; Zank and Schlatterer, 1998). Increased Na^+ and Cl^- ion concentrations cause an elevation of electrical conductivity (Wheelock et al., 1966). The goal of this study was to investigate changes in milk composition during the course of milking in subclinically infec-

ted quarters of variable severity in order to possibly provide indicative parameters for early detection of mammary infection in addition or alternatively to somatic cell counts (SCC).

MATERIAL AND METHODS

Animals and husbandry

Sixteen primiparous (n = 2) and multiparious (n = 14) Red Holstein × Simmental (n = 8), Brown Swiss (n = 3) and Holstein (n = 5) cows from the herd of the Swiss Federal Research Station Agroscope Liebefeld Posieux (ALP), Switzerland, were used. Quarter foremilk samples from all udder quarters were obtained to identify infected quarters using the California Mastitis Test (CMT).

The animals were divided into two groups of eight cows with different SCC levels, based on quarter foremilk SCC and independent of the pattern of the pathogenic microorganisms. In group I quarter log SCC/ml foremilk was >6.0, while group II contained cows with log SCC/ml from 5.4 to 6.0. The contralateral quarters with log SCC/ml <5.2 in the foremilk were used as controls.

Sampling procedures

Machine milkings were carried out with a quarter milking claw (Surge RX, Westfalia Landtechnik GmbH, Oelde, Germany) and milk fractions were collected from the infected and the contralateral quarters. To obtain the cisternal fraction separately before the occurrence of alveolar milk ejection, milking was performed without prestimulation. The cisternal sample was the first milk obtained after the start of milking. During further milking, 100-ml milk samples were collected for every 0.5 kg of sequentially removed milk from both quarters until milk flow ceased. Based on the actual quarter milk yield, the obtained fractions were selected and mixed to obtain milk fractions corresponding to 25%, 50%, 75% and 100% of removed milk. An additional fraction was collected during removal of residual milk after i.v. injection of 10 i.u. oxytocin. The collected six fractions were used for measurements of major constituents and insulin-like growth factor-1 (IGF-1), electrolytes and electrical conductivity. In addition, the whole milk of the respective quarters was collected including residual milk to obtain a proportional sample of the total quarter yield. Parameters analyzed are shown in Table 1.

Table 1. Quarter milk composition (including residual milk) in infected and non-infected (contralateral) quarters

Parameter		Group I (log SCC/ml >6.0)		Group II (log SCC/ml = 5.6–6.0)	
		infected quarter	contralateral quarter	infected quarter	contralateral quarter
Somatic cells	log/ml	6.40 ± 5.71*	5.26 ± 4.81	5.77 ± 4.63*	5.26 ± 4.68
Dry matter	g/l	128 ± 5	124 ± 4	126 ± 8	135 ± 9
Fat	g/l	54.6 ± 4.6*	44.1 ± 1.9	42.7 ± 4.6	49.5 ± 3.2
Protein	g/l	35.3 ± 1.7	34.4 ± 1.2	36.7 ± 1.3	36.4 ± 1.2
Lactose	g/l	43.8 ± 1.0*	48.1 ± 0.6	46.0 ± 0.8	47.6 ± 1.1
IGF-1	µg/l	12 ± 2*	7 ± 1	14 ± 7	12 ± 5
Insulin	µg/l	0.67 ± 0.11	0.41 ± 0.07	0.63 ± 0.11	0.49 ± 0.07
T3	nmol/l	0.30 ± 0.04	0.26 ± 0.04	0.23 ± 0.04	0.22 ± 0.04
PRL	µg/l	27 ± 4	21 ± 3	20 ± 6	18 ± 4
TNF-α	µg/l	0.33 ± 0.02	0.29 ± 0.05	0.27 ± 0.02	0.23 ± 0.03
Na+	mmol/l	30 ± 3*	22 ± 1	29 ± 3	24 ± 3
K+	mmol/l	39 ± 2	42 ± 1	36 ± 2	39 ± 2
Cl–	mmol/l	38 ± 2*	31 ± 2	37 ± 2	34 ± 2
El. conductivity	mS/cm	6.06 ± 0.14	5.64 ± 0.11	5.84 ± 0.18	5.45 ± 0.23
Osmolarity	mosm/l	288 ± 10	295 ± 9	291 ± 25	295 ± 30

*means of infected and contralateral quarters are significantly different ($P < 0.05$) within groups I or II

Laboratory methods

Samples of foremilk were obtained aseptically and were analyzed bacteriologically using Quick-Mastitis test (Labo Basi S.A., 6853 Ligornetto, Switzerland). Samples were centrifuged for 15 min at 1 000 × g, then the sediment was streaked on the agar and incubated for 24 h at 37°C. Colonies were identified as staphylococci, streptococci, *Escherichia coli* (*E. coli*) or unidentifiable bacteria based on the guidelines of the manufacturer. Cows were retained in this study only if the bacteriological result from contralateral quarters was negative.

Concentrations of fat, total protein, lactose and SCC in milk were measured in the laboratory of Swiss Simmental Breeding Association, Zollikofen, Switzerland. All other traits were determined in our own laboratory.

For dry matter (DM) determination, 1 g of whole milk was dried for 3 h at 105°C, followed by measuring of the remaining weight. Concentrations of fat, protein and lactose were determined by infra-red spectroscopy (Milko-Scan 605; Foss Electric, 3400 Hillerod, Denmark). SCC were determined by the fluoro-opto-electronic method (Fossomatic; Foss Electric, 3400 Hillerod, Denmark).

Concentrations in milk serum of insulin, IGF-1, prolactin (PRL), 3.5.3'-triiodothyronine (T_3) and tumor necrosis factor-α (TNFα) were analysed as described by Ontsouka et al. (2003) and Kenison et al. (1990). To obtain serum, samples were defatted by double centrifugation at 4° (for 15 min at 1 000 × g). Then, the infranatant was centrifuged again (for 30 min at 20 000 × g). The resulting infranatant was used for hormone determinations by radioimmunoassay (RIA). Concentrations of IGF-1 were determined in samples of whole milk by RIA as described by Hadorn et al. (1997).

Milk Na, K and chloride (Cl) ions were measured in milk serum by means of ion selective electrodes (Cobas Mira *Plus*, F. Hoffmann-La Roche, 4070 Basle, Switzerland), as described by Ontsouka et al. (2003).

The osmolarity was determined in whole milk using indirect measurement of osmotic pressure by the Multi-Osmette Model 2430 Automatic Osmometer (Precision Sampling Inc., San Rafael California, USA) based on measurement of freezing point depression, which is proportional to the concentration of the solute (Blagden's law). Electrical conductivity was measured at 25°C using the LDM electrode from WTW (Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany).

Statistical analyses

Values of different traits measured in milk are presented as means ± SEM. For statistical evaluations the SAS program package release 6.11 (SAS, 1995) was used. Differences of milk traits between infected quarters and their respective contralateral quarter were tested for significance ($P < 0.05$) by paired *t*-test by using the UNIVARIATE procedure. In addition, the Pearson correlation coefficients between parameters were calculated.

Changes of the content of milk traits during the course of milking in cows of both groups were tested for significance ($P < 0.05$) based on repeated measures analysis of variance employing the MIXED Procedure of SAS. The individual animal was used as the repeated subject.

RESULTS

Bacteriological analyses

In 16 foremilk samples from quarters with elevated SCC, the presence of *Streptococcus* spp., *Staphylococcus* spp., *Proteus* spp., and *E. coli*, was diagnosed 10, 3, 2 and 1 times, respectively. In group I mastitis in 50% was associated with *Streptococus* spp., in 38% by *Staphylococus* spp., and in 12% by *E. coli*. In group II, mastitis was in 75% associated with *Streptococus* spp. and in 25% with *Proteus* spp.

Milk composition in proportional quarter samples

In total quarter milk samples, SCC were significantly higher ($P < 0.05$) in the infected than in the contralateral quarter in both groups. Mean SCC in total quarter samples were higher in all total quarter samples than in the foremilk which was the criterium of grouping. In group I, concentrations of fat, Na, Cl and of IGF-1 (Table 1) were higher ($P < 0.05$) in infected than in contralateral healthy quarters, whereas those of lactose were lower ($P < 0.05$). The same parameters were numerically but not significantly different between infected and contralateral quarters in group II. Concentrations of DM, protein, K, T_3,

Table 2. Composition of milk fractions of infected and non-infected (contralateral) quarters of group I and group II

Parameter	Quarter status	Milk Fractions					
		Cisternal	25%	50%	75%	100%	Residual
Dry matter (g/l)	Infected Group I	130 ± 22	125 ± 7	125 ± 5	138 ± 7	135 ± 5	147 ± 5
	Contralateral Group I	103 ± 9^{B}	115 ± 8^{AB}	134 ± 8^{AB}	133 ± 7^{AB}	140 ± 10^{AB}	154 ± 9^{A}
	Infected Group II	105 ± 4^{C}	116 ± 3^{BC}	128 ± 3^{ABC}	135 ± 5^{ABC}	145 ± 5^{AB}	160 ± 6^{A}
	Contralateral Group II	108 ± 10^{B}	130 ± 15^{B}	126 ± 7^{B}	138 ± 6^{B}	141 ± 7^{B}	176 ±7^{A}
Fat (g/l)	Infected Group I	23.2 ± 5.3^{C}	42.1 ± 8.8^{BC}	55.2 ± 7.9^{BC}	69.2 ± 10.8^{AB}	79.2 ± 9.1^{AB}	93.5 ± 9.0^{A}
	Contralateral Group I	26.1 ± 5.2^{C}	23.0 ± 3.6^{C}	50.4 ± 7.0^{BC}	63.7 ± 6.1^{AB}	71.1 ± 5.5^{AB}	92.7 ± 9.1^{A}
	Infected Group II	24.6 ± 3.8^{D}	27.3 ± 4.0^{CD}	36.0 ± 4.0^{CD}	53.5 ± 4.3^{BC}	70.8 ± 6.6^{AB}	88.1 ± 6.0^{A}
	Contralateral Group II	24.8 ± 4.5^{D}	25.5 ± 3.8^{D}	38.0 ± 3.0^{CD}	53.8 ± 5.9^{BC}	74.5 ± 10^{B}	103 ± 9.1^{A}
Lactose (g/l)	Infected Group I	44.7 ± 1.7	46.9 ± 1.3	45.1 ± 1.3	43.2 ± 1.3	42.0 ± 0.8	41.7 ± 0.8
	Contralateral Group I	46.6 ± 1.1	49.7 ± 0.7	48.5 ± 1.2	49.0 ± 0.9	47.3 ± 0.6	45.3 ± 1.3
	Infected Group II	44.1 ± 1.5	48.3 ± 1.0	47.1 ± 0.7	46.2 ± 0.9	44.4 ± 0.8	44.6 ± 1.2
	Contralateral Group II	46.0 ± 2.0	49.2 ± 1.3	49.1 ± 1.2	48.1 ± 1.13	47.1 ± 1.1	46.4 ± 1.2
Protein (g/l)	Infected Group I	35.8 ± 1.9	37.9 ± 1.8	36.2 ± 1.4	36.6 ± 1.8	35.6 ± 1.7	36.8 ± 2.6
	Contralateral Group I	34.4 ± 1.7	36.5 ± 1.6	34.7 ± 1.3	35.2 ± 1.1	33.8 ± 1.2	31.8 ± 1.5
	Infected Group II	35.0 ± 1.5	37.8 ± 1.4	38.0 ± 1.12	36.5 ± 1.2	35.8 ± 1.2	35.2 ± 1.1
	Contralateral Group II	35.0 ± 1.7	37.7 ± 1.3	37.2 ± 1.3	36.1 ± 1.3	35.0 ± 1.2	33.7 ± 1.2
IGF-1 (μg/l)	Infected Group I	10.2 ± 2.3	9.1 ± 2.5	9.2 ± 2.1	11.2 ± 2.9	12.8 ± 2.9	13.3 ± 2.8
	Contralateral Group I	8.9 ± 1.4	9.3 ± 2.1	7.7 ± 1.9	7.9 ± 1.5	9.9 ± 2.5	7.5 ± 2.0
	Infected Group II	13.6 ± 4.6	15.4 ± 5.7	14.4 ± 5.2	12.9 ± 5.7	13.8 ± 5.7	13.9 ± 5.2
	Contralateral Group II	12.2 ± 5.1	12.9 ± 6.2	11.8 ± 5.1	12.2 ± 5.9	10.9 ± 5.2	10.3 ± 4.8
SCC (log/ml)	Infected Group I	6.15 ± 5.35^{C}	6.20 ± 5.75^{BC}	6.28 ± 5.65^{BC}*	6.41 ± 5.61^{ABC}*	6.50 ± 5.72^{AB}*	6.60 ± 5.77^{A}*
	Contralateral Group I	5.06 ± 4.57	5.14 ± 4.04	5.02 ± 4.72	5.15 ± 4.89	5.26 ± 4.84	5.57 ± 5.25
	Infected Group II	5.89 ± 5.28^{B}	5.64 ± 4.72^{B}	5.69 ± 4.85^{B}	5.93 ± 5.47^{B}	6.08 ± 5.41^{AB}*	6.28 ± 5.56^{A}*
	Contralateral Group II	5.12 ± 4.23	5.09 ± 4.62	5.06 ± 4.63	5.15 ± 4.62	5.33 ± 4.76	5.54 ± 4.85

^{A,B,C}means without common superscript differ ($P < 0.05$) during the course of milking within line

*means of infected and contralateral quarters within group and milk fraction are significantly different ($P < 0.05$)

Table 3. Electrical conductivity and electrolyte concentrations in milk fractions of infected and non-infected (contralateral) quarters

Parameter	Quarter status	Milk Fractions					
		Cisternal	25%	50%	75%	100%	Residual
El. conductivity (mS/cm)	Infected Group I	$6.62 \pm 0.20^{A*}$	5.80 ± 0.22^{B}	$6.02 \pm 0.21^{B*}$	6.23 ± 0.20^{AB}	$6.28 \pm 0.17^{AB*}$	$6.15 \pm 0.21^{AB*}$
	Contralateral Group I	6.00 ± 0.21	5.40 ± 0.18	5.36 ± 0.07	5.58 ± 0.22	5.54 ± 0.15	5.33 ± 0.17
	Infected Group II	6.37 ± 0.27	5.81 ± 0.15	5.89 ± 0.14	5.82 ± 0.17	5.99 ± 0.27	5.73 ± 0.25
	Contralateral Group II	6.07 ± 0.34	5.49 ± 0.25	5.65 ± 0.27	5.46 ± 0.22	5.24 ± 0.21	5.21 ±.25
Na^+ (mmol/l)	Infected Group I	$30 \pm 2^{B*}$	23 ± 3^{C}	29 ± 5^{BC}	$33 \pm 4^{AB*}$	$38 \pm 3^{A*}$	$38 \pm 2^{A*}$
	Contralateral Group I	19 ± 2	20 ± 3	20 ± 2	19 ± 1	25 ± 2	26 ± 1
	Infected Group II	26 ± 3	27 ± 4	27 ± 3	31 ± 4	31 ± 4	34 ± 3
	Contralateral Group II	22 ± 2	23 ± 3	23 ± 3	25 ± 3	27 ± 3	27 ± 3
K^+ (mmol/l)	Infected Group I	36 ± 2	41 ± 2	39 ± 2	38 ± 1	37 ± 2	39 ± 2
	Contralateral Group I	36 ± 3	40 ± 2	42 ± 2	42 ± 1	40 ± 1	42 ± 2
	Infected Group II	32 ± 2	35 ± 2	36 ± 2	37 ± 2	34 ± 2	35 ± 2
	Contralateral Group II	35 ± 2	3 ± 2	39 ± 2	37 ± 2	38 ± 2	37 ± 2
Cl^- (mmol/l)	Infected Group I	$34 \pm 3^{B*}$	31 ± 3^{B}	37 ± 3^{AB}	$39 \pm 3^{AB*}$	$43 \pm 2^{A*}$	$44 \pm 2^{A*}$
	Contralateral Group I	27 ± 2	28 ± 4	30 ± 2	27 ± 2	33 ± 2	34 ± 1
	Infected Group II	32 ± 2^{B}	33 ± 3^{B}	35 ± 2^{AB}	38 ± 2^{A}	37 ± 2^{A}	41 ± 3^{A}
	Contralateral Group II	30 ± 3	30 ± 4	33 ± 3	34 ± 3	36 ± 3	36 ± 3

^{A,B,C}means without common superscript differ ($P < 0.05$) during the course of milking within line

*means of infected and contralateral quarters within group and milk fraction are significantly different ($P < 0.05$).

PRL, insulin, and TNF-α, osmolarity and electrical conductivity (Table 1) were not significantly different between infected and contralateral quarters in both groups, although electrical conductivity was numerically higher in infected than in the contralateral quarters. Na concentration was positively correlated with Cl concentrations ($r = 0.94$, $P < 0.001$), but negatively with K concentrations in the infected quarters ($r = –0.72$, $P < 0.05$). There was a positive correlation of TNF-α concentrations with IGF-1 concentrations ($r = 0.82$, $P < 0.05$) in the infected quarters.

Composition of milk fractions during the course of milking

Concentrations of DM increased throughout milking in all quarters except for infected quarters in group I, where the values were already high in the cisternal fraction, however, not significantly higher than in the corresponding contralateral quarters. In both infected and contralateral quarters the fat concentration (Table 2) increased during milking ($P < 0.05$) from cisternal to alveolar (100%) fractions. Concentrations of lactose, protein, IGF-1, and K (Tables 2, 3) did not significantly change during milking in any of the investigated quarters or groups. Concentration of Na and electrical conductivity (Table 3) were high in the cisternal fraction of the infected quarter in group I, reached their lowest values in the 25% alveolar fraction and increased thereafter towards the end of milking. No significant changes of Na concentrations and electrical conductivity were observed in the other quarters. Concentrations of Cl increased in the infected quarters of both groups throughout milking and increased numerically, but not significantly in the contralateral quarters. SCC increased throughout milking in all quarters and were much higher in the 100% alveolar fraction and in residual milk than in the cisternal milk. Only in the infected quarters of group I the SCC were higher in the cisternal than in the 25% alveolar fraction.

DISCUSSION

Milk composition in infected quarters in groups I and II

Streptococcus spp. were the most frequently diagnosed microorganisms in quarters with elevated SCC, followed by *Staphylococcus* spp., *E. coli* and *Proteus* spp. These bacteria were most likely the reason for the subclinical mastitis. Their spectrum corresponds with the general situation in Swiss dairy herds (Hartmann, 1990; Busato et al., 2000). Although SCC were much lower in healthy quarters, i.e. in quarters with negative CMT and bacteriological result and low foremilk SCC, total quarter milk SCC were unexpectedly high. This elevation was caused by the final alveolar fractions and the residual milk, which were shown to have much higher SCC than foremilk (Table 2) and which contributed to the total quarter milk.

The high milk fat content in infected quarters as compared with contralateral quarters in group I did not agree with findings of others (Schultz, 1977; Kitchen, 1981; Eberhart et al., 1987), which reported milk fat depression during mastitis. Causes of the rise in our study are not obvious. On the other hand, a slight fat depression was noted in infected quarters in group II. The elevated fat content was possibly the consequence of reduced lactose synthesis and therefore reduced milk volume while there was no or only slight depression of fat synthesis in this subclinical stage of the infected quarters in this study.

The low lactose concentrations in infected quarter in the group I were in accordance with others investigators (Schultz, 1977; Fox et al., 1985; Mielke et al., 1985; Harmon, 1994). Because lactose is the main osmotically active component in milk of healthy quarters, lactose concentration can only be depressed if the reduced lactose synthesis is compensated by the increased influx of blood-borne electrolytes. Therefore, the low lactose concentrations is dependent on the severity of damage to the tight junctions (Stelwagen et al., 1999). Possibly, lactose concentrations in group II with relatively low SCC were not affected because the intact mammary epithelium prevented extensive electrolyte influx. This is in accordance with the fact that concentrations of Na and Cl were more elevated in infected quarters in group I than in group II and with earlier studies (Harmon, 1994).

Concentrations of DM, protein, insulin, T_3, PRL, TNF-α, K, and also electrical conductivity and osmolarity were not significantly different between infected and healthy quarters in both groups. In contrast, Na, Cl and IGF-1 were elevated in the infected quarters of group I. Therefore, the blood-borne electrolytes Na and Cl seem to pass into milk already at a low level of disturbance. They are more

powerful parameters for mastitis detection than electrical conductivity, which is indirectly dependent on electrolyte concentration (Wheelock et al., 1966), but obviously also influenced by other milk constituents like fat (Fernando et al., 1981; Woolford et al., 1998). This is especially true towards the end of milking, when high electrolyte concentrations do not cause increased electrical conductivity at a simultaneously high fat content in these milk fractions. Electrical conductivity was highest in the cisternal fraction in all groups, likely because concentrations of Na and Cl were relatively high and because the fat content was low in this milk fraction. Towards the end of milking, when electrolyte concentrations reached their highest values, the electrical conductivity increased only slightly and not significantly. This was obviously due to the dramatically increased fat concentration towards the end of milking. Thus, in defatted milk samples, the electrical conductivity clearly increased towards the end of milking (Wittkowski et al., 1979).

Osmolarity was not significantly different between infected and contralateral quarters in both groups, possibly because the decline of lactose concentrations in infected quarters was counterbalanced by the rise of other osmotic active substances, such as Na and Cl. This finding could be expected because the water in milk, determining its volume, passes into milk via a difference of osmotic pressure.

The IGF-1 can leak from blood plasma into milk as a consequence of epithelial damage or is synthesized by immune cells in milk like macrophages and lymphocytes by immuno-activation was also elevated in infected quarters of group I as shown previously during acute mastitis (Bruckmaier et al., 1993; Liebe and Schams, 1998). Despite this rise, IGF-1 seems not to be a suitable parameter for the detection of mastitis in dairy practice because its concentration is low and its detection is only possible via immunological tests. A significant release of TNF-α may only occur in cases of acute and severe inflammation, such as caused by endotoxin administration, which was not the case in our study (Blum et al., 2000).

Fat concentration increased significantly from the cisternal to the alveolar (100%) fractions in the infected and contralateral (healthy) quarters in groups I and II. Due to the polarity of the membranes of the fat droplets, they are more than other milk components subjected to adhesive forces. This may be an important reason that fractions with the highest fat concentration are ejected and removed at the end of milking. Similarly as fat, the SCC increased continuously and significantly from cisternal to alveolar fractions in infected quarters in group I, while in the infected quarters of group II the rise of SCC was only significant in residual milk. The rise of SCC during milking may indicate, that cells are primarily present in the alveolar fraction, hence rise towards the end of milking. Similarly as fat droplets, milk cells have a polar membrane. It may be speculated that the somatic cells are subjected to the same adhesive forces as fat droplets or are even partially attached to the milk fat fraction.

In conclusion, alterations in milk composition depend on the severity of mammary infection. The amounts of some milk components synthesized in the mammary gland or being transported into milk did not vary during the course of milking of infected as compared with noninfected quarters. These findings need to be considered in the interpretation of mastitis data, which are mostly based only on the measurement of SCC. For the detection of mastitis by means of physico-chemical parameters the direct measurement of milk electrolytes seems to be more powerful than electrical conductivity.

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