Fractionized Milk Composition During Removal of Colostrum and Mature Milk

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ABSTRACT

Experiments were designed to study compositional differences in colostrum and mature milk and during the course of milk removal. Fractionized milk samples during the course of machine milking were analyzed in single (right rear) quarters in the cisternal fraction, after 25, 50, 75, and 100% of spontaneously removed milk, in residual milk, and in composite samples from all quarters on d 2 (colostrum) and in wk 4 (mature milk) of lactation. Somatic cell counts; concentrations of dry matter, total protein, insulin-like growth factor-I, insulin, prolactin, tumor necrosis factor- α , Na, and Cl; γ -glutamyltransferase activity; and electrical conductivity were higher, whereas lactose concentration was lower on d 2 than in wk 4. Concentrations of fat, potassium chloride, and osmolarity did not differ between lactational periods. During the course of milking, concentrations of dry matter, fat, lactose, and potassium, and osmolarity increased, whereas somatic cell counts, protein, insulin like-growth factor-I, insulin, prolactin, and sodium concentrations, electrical conductivity and γ -glutamyltransferase activity decreased on d 2, and protein, sodium, and electrical conductivity decreased in wk 4. In conclusion, various milk constituents differed considerably between lactational periods (colostrum and mature milk). Milk isotonicity was only in part associated with lactose concentration. Electrical conductivity was associated with Na, K, and fat concentrations and was highest in the cisternal fraction. Changes in milk constituents during milking need to be considered if milk samples are taken for analytical purposes and to evaluate the health status of the udder. (**Key words:** Dairy cow, milk composition, colostrum, mature milk)

Abbreviation key: γ GT = γ -glutamyltransferase; PRL = prolactin; TNF- α = tumor-necrosis factor- α .

INTRODUCTION

Colostrum and milk contain fat, proteins, lactose, and minerals, which are of nutritional importance. In addition, they contain vitamins, immunoglobulins, hormones, growth factors, cytokines, enzymes, and other bioactive peptides (Koldovsky, 1980; Campana and Baumrucker, 1995; Swaisgood, 1995; Blum and Hammon, 2000), metabolites derived from alveolar epithelial cells (Peaker and Linzell, 1975), and immunocompetent cells (Lee et al., 1980). Breed, age, nutrition, and health status of the cow are well known to influence milk composition. Colostrum differs greatly in composition from mature milk and meets the nutritional requirements of the newborn (Blum and Hammon, 2000; Blum and Baumrucker, 2002).

Colostrum and milk components are secreted by different mechanisms (Patton and Jensen, 1975). Secretion is regulated by both local and systemic factors. Local factors include intramammary pressure (Bruckmaier and Blum, 1998) and an autocrine feedback inhibitor of lactation (Wilde and Peaker, 1990). Milk is secreted between milkings and accumulates in alveolar and cisternal compartments (Bruckmaier et al., 1994a; Davis et al., 1998; Knight et al., 1994).

During milking the cisternal fraction is first removed. Removal of the alveolar fraction requires milk ejection by oxytocin, which commences in dairy cows at about 1 min after tactile udder stimulation has started, resulting in transfer of milk to the cistern for removal (Bruckmaier and Blum, 1996). Milk ejection is a continuous process throughout milking (Bruckmaier et al., 1994b). Milk ejection is delayed if only small amounts of milk are stored in the udder, i.e., towards the end of lactation and if intervals between milkings are short (Bruckmaier et al., 1994b; Bruckmaier and Hilger, 2001). Because milk ejection is a continuous process throughout milking, it can be hypothesized that there are also continuous changes in milk composition during the course of milking.

The objective of this study was to measure the concentration of nutritional and nonnutritional milk components in colostrum (on d 2 of lactation) and in mature

Received March 6, 2002.

Accepted October 21, 2002.

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milk (at wk 4 of lactation) in fractionized milk samples. Analyses were performed in one quarter only, because the duration of milk flow of individual quarters is variable (Wellnitz et al., 1999), i.e., an exact analysis of compositional changes of milk constituents on a whole udder basis is not possible. Such data are of importance if milk constituents are analyzed to monitor animal health and to determine which components may be ingested by suckling calves.

MATERIALS AND METHODS

Animals and Husbandry

Sixteen Red Holstein $\times$ Simmental cows were used. All animals belonged to the dairy herd of the Swiss Federal Research Station of Animal Production, Posieux, Switzerland and were in their second to fourth lactation.

Cows were fed a standard diet (consisting of grass silage, hay, and concentrates) used at the research station for lactating dairy cows and according to recommendations (Jans and Kessler, 1999).

Cows were tested weekly for SCC and milk composition. All animals were free of clinical udder health problems, i.e., SCC of mature milk were below 100,000 cells/ml in all quarters at the end of the previous lactation and after wk 1 in the current lactation until the end of the experiment. A prophylactic antibiotic treatment was routinely performed at drying off. Cows were milked twice daily at 0600 and 1600 and were kept in a tie-stall barn.

Experimental Procedures

Experimental milkings were carried out during routine milking time using a quarter milking claw (Surge RX, Westfalia Separator GmbH, Oelde, Germany). Milking was performed at a vacuum level of 45 kPa and a pulsation rate of 60 cycles/min at a ratio of 70:30. Milk samples were collected from the right rear quarter during one evening milking.

In eight cows, milking was performed and quarter milk samples were taken during the colostrum period on d 2 of lactation (third milking). In another eight cows, samples were taken during one afternoon milking in wk 4 of lactation. To obtain a sample of the cisternal fraction before milk ejection, milking was performed without prestimulation (Bruckmaier and Blum, 1996). The cisternal sample was the first 100 ml of 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 the right rear quarter 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. An additional sample was collected from the total milk obtained from all quarters.

Laboratory Procedures

For DM determination, 1 g of whole milk or colostrum was placed for 3 h at 105°C for water evaporation followed by measuring the remaining weight.

Concentrations of fat, protein, and lactose were determined by infrared spectroscopy (Milko-Scan 605; Foss Electric, 3400 Hillerød, Denmark). The SCC were determined by the fluoro-opto-electronic method (Fosso-matic; Foss Electric, 3400 Hillerød, Denmark).

For the determination of γ -glutamyltransferase (γ -GT) activity, milk samples were defatted by centrifugation at 4°C (for 15 min at 3000 $\times g$) after casein coagulation by addition of chymosin. The γ -GT activity was measured with a kit (# 0751510) from F. Hoffmann-La Roche (Basle, Switzerland) using an automatic analyzer (Cobas Mira Plus, F. Hoffmann-La Roche).

Concentrations of IgG were measured in milk serum after immunodiffusion in agarose by precipitation with a rabbit anti-bovine IgG serum as described (Vacher and Blum, 1993). To obtain milk whey, samples were defatted by centrifugation at 4°C (for 15 min at 3000 $\times g$). For IgG measurement, the colostrum was diluted 1:10.

Concentrations of insulin and prolactin (PRL) were determined in whey of colostrum and mature milk. To obtain milk whey, samples were defatted by centrifugation at 4°C (for 15 min at 3000 $\times g$). Then, the infranatant was centrifuged again (for 30 min at 20,000 $\times g$). The infranatant was used for hormone determinations by radioimmunoassay as described by Hadorn et al. (1997). Concentrations of IGF-I were determined in samples of whole milk and in colostrum by radioimmunoassay as described by Hadorn et al. (1997). Immuno-reactive tumor necrosis factor- α (TNF- α) in whey of milk and colostrum (3 $\times$ centrifugation) was measured by specific double antibody radioimmunoassay as described by Blum et al. (2000).

The elements Na, K, and Cl were measured in milk whey by means of ion selective electrodes (Cobas Mira Plus, F. Hoffmann-La Roche).

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). The sample osmolarity was computed by measurement of freezing point depression, which was proportional to the

Table 1. Contents of composite milk from all quarters on d 2 and in wk 4 of lactation.

		Day 2 (colostrum)	Week 4 (mature milk)
Parity		2.8 ± 0.9	2.9 ± 0.8
Daily milk yield	kg	16.8 ± 1.7*	33.9 ± 0.9
Dry matter	g/kg	159 ± 16*	117 ± 12
Fat	g/L	70.5 ± 8.5	57.6 ± 6.7
Protein total	g/L	52.0 ± 3.2*	32.5 ± 1.0
Lactose	g/L	43.9 ± 0.9*	49.9 ± 0.5
Na	mmol/L	27 ± 1*	20 ± 1
K	mmol/L	44 ± 1	41 ± 1
Cl	mmol/L	38 ± 2*	29 ± 1
Elec. conductivity	mS/cm	5.6 ± 0.2*	4.9 ± 0.2
Osmolarity	mOsm/L	295 ± 2	274 ± 9
Somatic cells	×10 ⁶ /L	1479 ± 585*	41 ± 15
IGF-I	μg/L	103 ± 21*	4 ± 1
Insulin	μg/L	4.55 ± 1.04*	0.37 ± 0.02
Prolactin	μg/L	120 ± 16*	15.4 ± 1.0
TNF-α	μg/L	5.0 ± 0.6*	1.8 ± 0.2
γ-GT	μkat/L	137 ± 9*	24 ± 8
IgG	g/L	28.3 ± 5.7*	1.5 ± 0.1

*Means are significantly different ($P < 0.05$) between d 2 and wk 4.

concentration of the solute (Blagden's law). Sample results were then computed by linear interpolation using the average standard readings.

Electrical conductivity was measured at 25°C using the LDM electrode from WTW (Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany).

Statistical Analyses

Values of milk traits are expressed as means ± SEM. Differences between stages of lactation and changes during the course of milking at each stage of lactation were tested for significance ($P < 0.05$) by analysis of variance using the MIXED models procedure of SAS. Because SCC could not be assumed to be normally distributed the SCC values were converted to log value for statistical calculations. The MIXED models included period of lactation, the animal, and the milk fraction (if applicable) as class variables. For all calculations on composite milk samples, the animal was the repeated subject during the course of milking. Differences were localized by Bonferroni's t-test. In addition, Pearson's correlation coefficients among various parameters were calculated.

RESULTS

Characteristics of Colostrum and Mature Milk in Milk from all Quarters

Major constituents. Dry matter, protein concentration (Table 1), and SCC were higher ($P < 0.05$) on d 2 (colostrum), and lactose concentration was lower ($P < 0.05$) compared with wk 4 (mature milk). Concentration of fat was numerically but not significantly higher on

d 2 than in wk 4. On d 2, protein was correlated with IGF-I ($r = 0.76$, $P < 0.05$), insulin ($r = 0.71$, $P < 0.05$), γ-GT ($r = 0.75$, $P < 0.05$), IgG ($r = 0.88$, $P < 0.05$), and nonsignificantly with PRL ($r = 0.68$, $P = 0.06$).

Milk electrolytes, osmolarity and electrical conductivity. Concentrations of Na and Cl (Table 1) were significantly ($P < 0.05$) higher in colostrum than in mature milk. Osmolarity was numerically, but not significantly higher on d 2 than in wk 4. Electrical conductivity (Table 1) was higher ($P < 0.05$) on d 2 than in wk 4. On d 2, Na was highly correlated with Cl ($r = 0.9$, $P < 0.05$), electrical conductivity was closely and negatively correlated with lactose ($r = -0.92$, $P < 0.05$), and osmolarity was closely correlated with γ-GT ($r = 0.94$, $P < 0.05$) and nonsignificantly with total protein ($r = 0.80$, $P = 0.06$).

Hormones, enzymes, and immunoglobulin G. The concentrations of IGF-I, insulin, PRL, TNF-α, and IgG and γ-GT activity were higher ($P < 0.05$) in colostrum (d 2) than in mature milk (Table 1). The IgG concentration was closely correlated with Na and Cl on d 2 ($r = 0.92$, $P < 0.05$).

Milk Characteristics During the Course of Milking

Major constituents. As shown in Table 2, concentrations of DM and fat increased ($P < 0.05$) from cisternal to 100% alveolar and residual fractions during milking on d 2 and in wk 4. The concentration of lactose (Table 2) was lowest in the cisternal fraction and increased ($P < 0.05$) in alveolar fractions. The concentration of protein (Table 2) on d 2 decreased ($P < 0.05$) transiently from the cisternal to the 25% alveolar fraction and decreased further towards the end of milking. In wk 4, protein

Table 2. Fractionized milk composition and osmolarity and electrical conductivity on d 2 and wk 4 of lactation.

Traits		Stages of lactation	Milk fractions					
			Cisternal	25%	50%	75%	100%	Residual
Dry matter	g/L	Day 2	126 ± 14 ^C	131 ± 8 ^C	144 ± 12 ^C	171 ± 19 ^B	210 ± 23 ^A	218 ± 22 ^A
		Week 4	110 ± 10 ^C	121 ± 8 ^C	126 ± 4 ^C	136 ± 2 ^C	166 ± 8 ^B	217 ± 11 ^A
Fat	g/L	Day 2	25.9 ± 7.9 ^E	44.5 ± 10.8 ^D	69.9 ± 7.7 ^C	93.2 ± 11.6 ^B	124.5 ± 12.9 ^A	129.2 ± 17.5 ^A
		Week 4	22.4 ± 4.1 ^E	33.6 ± 4.9 ^{DE}	45.3 ± 4.1 ^{CD}	55.3 ± 3.8 ^C	84.3 ± 4.6 ^B	130.7 ± 9.4 ^A
Protein	g/L	Day 2	53.0 ± 3.6 ^A	49.3 ± 2.0 ^B	51.6 ± 3.2 ^A	51.8 ± 3.1 ^A	49.6 ± 2.9 ^B	46.1 ± 1.8 ^B
		Week 4	33.3 ± 1.3 ^{AB}	34.1 ± 1.0 ^A	33.5 ± 1.0 ^{AB}	33.1 ± 1.0 ^{AB}	31.8 ± 0.9 ^B	29.9 ± 0.7 ^C
Lactose	g/L	Day 2	37.2 ± 1.03 ^B	44.8 ± 1.0 ^A	43.8 ± 0.9 ^A	43.3 ± 0.9 ^A	43.2 ± 1.0 ^A	43.0 ± 0.9 ^A
		Week 4	46.8 ± 1.1 ^B	51.8 ± 0.6 ^A	51.3 ± 0.6 ^A	50.3 ± 0.6 ^A	48.3 ± 0.5 ^B	47.5 ± 0.5 ^B
SCC	log/L	Day 2	8.77 ± 8.58 ^{BC}	8.44 ± 7.99 ^C	8.81 ± 8.47 ^{ABC}	8.94 ± 8.68 ^{AB}	8.99 ± 8.69 ^{AB}	9.06 ± 8.74 ^A
		Week 4	7.77 ± 7.49	7.14 ± 6.0	7.14 ± 6.6	7.28 ± 6.70	7.68 ± 7.08	7.81 ± 6.48
Na	mol/L	Day 2	31 ± 3 ^A	26 ± 2 ^C	27 ± 2 ^{BC}	30 ± 3 ^{AB}	32 ± 4 ^A	31 ± 3 ^A
		Week 4	23 ± 2 ^B	17 ± 1 ^C	19 ± 2 ^C	19 ± 1 ^C	25 ± 2 ^A	24 ± 1 ^A
K	mmol/L	Day 2	38 ± 3 ^D	45 ± 2 ^A	45 ± 1 ^A	44 ± 2 ^A	44 ± 2 ^A	45 ± 2 ^A
		Week 4	33 ± 3 ^D	36 ± 3 ^{CD}	34 ± 3 ^{CD}	38 ± 3 ^{BC}	38 ± 2 ^B	44 ± 2 ^A
Cl	mmol/L	Day 2	42 ± 3	38 ± 2	39 ± 2	42 ± 3	43 ± 3	42 ± 3
		Week 4	26 ± 4	24 ± 2	21 ± 3	27 ± 2	27 ± 4	32 ± 1
Osmolarity	mOsm/L	Day 2	263 ± 7 ^B	288 ± 4 ^{AB}	294 ± 3 ^{AB}	293 ± 4 ^{AB}	295 ± 4 ^A	296 ± 4 ^A
		Week 4	254 ± 41 ^B	313 ± 18 ^{AB}	271 ± 41 ^{AB}	292 ± 1 ^B	299 ± 66 ^A	295 ± 5 ^B
Conductivity	mS/cm	Day 2	6.05 ± 0.14 ^A	5.70 ± 0.17 ^A	5.93 ± 0.22 ^A	5.61 ± 0.15 ^{AB}	5.43 ± 0.21 ^{BC}	5.25 ± 0.20 ^C
		Week 4	5.63 ± 0.26 ^A	5.25 ± 0.23 ^A	4.86 ± 0.16 ^B	4.87 ± 0.11 ^B	4.75 ± 0.13 ^B	4.36 ± 0.11 ^C

^{A,B}Means of milk fractions with different letters are different ($P < 0.05$).

concentration decreased ($P < 0.05$) from the 25% to the 100% alveolar fraction and decreased further in residual fractions. SCC decreased numerically but not significantly from high values in the cisternal fraction to lowest values in the first alveolar fraction and increased further during the course of milking ($P < 0.05$) with highest values in the residual fraction; these changes were not significant in wk 4.

Milk electrolytes, osmolarity, and electrical conductivity. Concentrations of Na (Table 2) transiently decreased ($P < 0.05$) from the cisternal fraction to 50 or 75% alveolar fractions, then increased towards the end of milking on d 2 and in wk 4. The concentration of K and osmolarity (Table 2) increased ($P < 0.05$) from cisternal to alveolar fractions, while electrical conductivity decreased ($P < 0.05$) from cisternal to 100% alveolar fractions and still in residual fractions. Cl did not change significantly during milking. Osmolarity (Table 2) had its lowest values in the cisternal milk and increased in the alveolar fractions during milking.

Hormones, enzymes, and immunoglobulin G in colostrum. On d 2, concentrations of IGF-I decreased ($P < 0.05$) from cisternal to alveolar fractions. Concentrations of insulin and PRL, and γ -GT activities transiently decreased on d 2 ($P < 0.05$) in the 25% alveolar fraction, and increased thereafter (Table 3). Concentrations of IgG did not significantly change during milking.

DISCUSSION

Milk Components in Composite Milk on d 2 and in wk 4 of Lactation

Of the various characteristics measured, concentrations of fat, K, and osmolarity did not differ significantly between d 2 and wk 4 of lactation, and thus, behaved slightly different from what is observed during milking on practical dairy farms, likely because residual milk was included.

The DM content was higher in colostrum than in mature milk due to greater amounts of milk solids in colostrum. The high total protein concentration in colostrum was largely due to high amounts of IgG (Guidry et al., 1980b; Butler, 1983; Larson, 1992; Vacher and Blum, 1993). Concentrations of other protein fractions (not measured in this experiment) such as lactoglobulin, lactoferrin, and transferrin are known to be also higher in colostrum than in mature milk (Sanchez et al., 1988; Ye-Xiuyun and Yoshida, 1995). The decreasing total milk protein concentration in mature milk was likely in part due to dilution resulting from increased milk production.

Lactose production causes water influx in milk through osmotic effects, and values were lower in colostrum than in mature milk. However, concentrations of Na and Cl, which are, osmotically active molecules in milk, were elevated in colostrum compared with mature

Table 3. Hormones, γ -GT, and IgG in colostrum (d 2 of lactation).

Traits		Milk fractions					
		Cisternal	25%	50%	75%	100%	Residual
IGF-I	$\mu\text{g/L}$	129 ± 30^A	93.2 ± 20.3^C	93.6 ± 16.6^C	106 ± 23^B	103 ± 20^{BC}	97 ± 19^{BC}
Insulin	$\mu\text{g/L}$	6.27 ± 1.97^A	3.62 ± 0.75^C	4.18 ± 0.04^{BC}	4.85 ± 1.12^{ABC}	6.19 ± 2.1^A	5.60 ± 1.57^{AB}
PRL	$\mu\text{g/L}$	168 ± 45^A	113 ± 15^C	118 ± 13^{BC}	138 ± 24^{AB}	148 ± 32^{AB}	134 ± 20^{BC}
γ -GT	mkat/L	157 ± 17^A	127 ± 9^C	138 ± 8^{BC}	152 ± 11^{AB}	162 ± 14^A	109 ± 12^C
IgG	g/L	38.3 ± 8.6	20.6 ± 3.1	24.3 ± 3.9	34.5 ± 7.7	36.2 ± 11.3	38.3 ± 10.9

^{A,B}Means of milk fractions with different capital letters are significantly different ($P < 0.05$).

milk. Thus, the electrolyte transfer from blood into milk through leaky tight junctions (Nguyen and Neville, 1998) is expected to increase the milk volume during the colostrum period despite relatively low lactose secretion.

The SCC were much higher in colostrum than in mature milk. This is in accordance with previous results (Emanuelson and Persson, 1984; Hallberg et al., 1995; Andrew, 2001). Mastitis pathogens are not infrequently found in colostrum (Andrew, 2001). However, in our study there were no indications for a clinical mastitis in the dry or periparturient period. Furthermore, colostrum appearance was always normal (milky, thickened) according to the criteria described by Hallberg et al. (1995). The SCC increases during infection in mature milk as well as in colostrum (Hallberg et al., 1995). However, the SCC in colostrum measured in the multiparous cows (1479×10^3 cells/mL) of our study were markedly below mean values (2458×10^3 cells/mL) found by Andrew (2001), and below the lowest values measured in the fall season (2580×10^3 cells/mL) in the study of Hallberg et al. (1995) in primiparous cows. In addition, the California Mastitis Test was always negative and SCC measured at 7 to 14 d after parturition (i.e., in mature milk) were always $<100 \times 10^3$ cells/mL (data not shown). Somatic cell count would still have been elevated at this time if there would have been mastitis during the dry or periparturient period. Therefore, udders on d 2 of lactation were considered healthy; i.e., high SCC on d 2 of lactation were of physiological nature and were most likely due to penetration of cells through leaky tight junctions between the mammary epithelial cells (Nguyen and Neville, 1998). In wk 4 (mature milk) the SCC were low because we have tested only cows with healthy udders ($<100 \times 10^3$ cells/mL).

The IGF-I concentration dropped drastically from colostrum to mature milk in accordance with Ronge and Blum (1988), Vega et al. (1991), and Campana and Baumrucker (1995). The high amounts of PRL and insulin on d 2 of lactation were probably the consequence of a marked influx from blood into milk through leaky tight junctions (Nguyen and Neville, 1998) or by transcellular routes (Ollivier-Bousquet, 1993). In addition,

the TNF- α concentration was higher in colostrum than in mature milk. TNF- α also has chemotactic activity and is involved in the influx of somatic cells from blood into milk. Gene expression of TNF- α by milk somatic cells was increased in quarters with increased immunological activity (Wittmann et al., 2002). Thus, TNF- α may be produced by somatic cells, in part, to regulate further influx of cells into milk.

The γ -GT is known to be localized on the outer surface of the alveolar cell membrane to promote absorption of some amino acids across alveolar cell membranes (Baumrucker and Pocius, 1978). The activity of γ -GT was much higher on d 2 than in wk 4, which agrees with previous findings (Hadorn et al., 1997).

The IgG concentration was higher on d 2 than in wk 4, demonstrating enhanced passage from blood into milk through leaky tight junctions and epithelial secretory cells (Butler, 1983; Ollivier-Bousquet, 1993). After the colostrum period, the IgG is transported from blood into milk only by transcellular selective receptor-mediated transcytosis (Hammer and Mossman, 1976; Guidry et al., 1980; Butler, 1983), thus explaining reduced concentrations in wk 4.

Concentrations of Na and Cl were closely correlated with those of IgG on d 2. This implies that Na and Cl may pass into milk simultaneously with IgG, although transport mechanisms are expectedly much different from IgG. Their appearance in milk at these stages is primarily regulated by a passage through epithelial cells (Peaker and Linzell, 1975).

Electrical conductivity was higher in colostrum than in mature milk because it is related to greater amounts of Na, Cl, and K in colostrum and in mature milk (Wheelock et al., 1966).

Changes in Milk Composition During the Course of Milking

Amounts of DM increased during milking both on d 2 and in wk 4 of lactation. This was due to increased concentration of some milk components. Thus, fat concentration increased continuously from the cisternal to the alveolar (100%) fraction and increased further in

the residual fraction during milking. Milk fat has a lower specific gravity than water and before milk removal, fat may ascend within the alveolar lumina, thus causing an increasing fat content during removal of the alveolar fraction. In addition, fat droplets during milking may move less rapidly than the aqueous phase, and are influenced by capillary and adhesive forces. Therefore, fractions with the highest fat concentration are removed at the end of milking. Thus, incomplete milk ejection and removal causes a considerable reduction in milk fat. Calves, if consuming only a portion of the stored milk, may ingest only small amounts of fat.

Milk protein concentration in colostrum was higher in the cisternal fraction than in subsequent fractions and reached lowest values in the residual fraction. The high concentration in cisternal colostrum may in part be due to high IgG levels in this fraction, which represents a considerable percentage of the total protein. In mature milk, the protein content did not change significantly until the 75% fraction, but also decreased at the end of milking, with lowest values in the residual fraction. In mature milk, this phenomenon was shown previously (Wittkowski et al., 1979); the reasons, however, are unclear.

The pattern of SCC during the course of milking, i.e., high values in cisternal milk, low values in the first alveolar fraction and a steady rise until the end of milking was in accordance with previous investigations (Wittkowski et al., 1979). Usually, quarter cisternal milk samples are analyzed for udder health monitoring. Milk ejection can occur within 40 s after the start of a tactile teat contact (Bruckmaier and Hilger, 2001). Thus, quarter milk sampling must be performed within 40 s to avoid a decline of SCC due to a dilution caused by alveolar milk ejection. Concentrations of PRL, insulin, and γ -GT activities (measured only on d 2 of lactation because concentrations or activities were too low in wk 4) changed significantly during milking with high values in the cisternal milk, lowest values in the 25% fraction. This finding suggests similar mechanisms of distribution in the mammary gland compartments and milk fractions. Thus, IgG, γ -GT, and IGF-I are transported into the alveolar space by para- and/or transcellular mechanisms, and PRL is secreted primarily transcellularly into milk, similar to casein (Ollivier-Bousquet, 1993).

Concentrations of Na and K varied significantly during milking and among animals, while the concentration of Cl did not differ during milking, suggesting that these substances are transported differently into and distributed among udder compartments. Although the concentrations of Na and Cl as osmotically active substances were relatively high in the cisternal fraction, osmolarity was always low at the start of milking and

increased thereafter on d 2 and in wk 4, indicating that increasing lactose concentration in the alveolar milk exerts the main osmotic activity.

Electrical conductivity was highest in the cisternal fraction at both periods of lactation, and associated with relatively high concentrations of Na and Cl. Despite an overall trend of increasing electrolyte concentrations toward the end of milking, of electrical conductivity decreased. This was most likely due to the simultaneous increase in milk fat content, which has been identified as a factor decreasing electrical conductivity (Fernando et al. 1981; Hamann et al., 1995). Measurement of electrical conductivity to show changes electrolyte concentrations due to mastitis-related damage of tight junctions (Nguyen and Neville, 1998) may show highest sensitivity in the cisternal fraction where electrolyte concentrations are high and milk fat concentration is low.

In conclusion, changes in concentrations of milk components in different milk fractions illustrate that some constituents such as fat are not equally distributed within udder compartments after their secretion. These changes have to be considered if milk samples are taken for analysis of their constituents. Increased osmolarity, despite a significant decrease in lactose concentration toward the end of milking, illustrates that electrolytes are involved in the regulation of milk isotonicity.

ACKNOWLEDGMENTS

This study was supported in part by the Swiss Federation for Artificial Insemination, Zollikofen, Switzerland and by Central Federation of Swiss Milk Producers, Berne, Switzerland. We thank the Swiss Simmental Association, Zollikofen, for performing milk analyses. The technical assistance of C. Morel and Y. Zbinden, Div. of Animal Nutrition and Physiology, Faculty of Veterinary Medicine, Univ. of Berne, is greatly appreciated.

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