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Variations During Lactation in Total and Differential Leukocyte Counts, N-acetyl-ß-D-glucosaminidase, Antitrypsin and Serum Albumin in Foremilk and Residual Milk from Non-infected Quarters in the Bovine

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Östensson, K.: Variations during lactation in total and differential leukocyte counts, N-acetyl-β-D-glucosaminidase, antitrypsin and serum albumin in foremilk and residual milk from non-infected quarters in the bovine. Acta vet. scand. 1993, 34, 83-93. – Quarter samples of foremilk and residual milk were taken approximately every second week from 2 days post partum (pp) throughout lactation month 9, from 5 dairy cows in their second lactation period. Bacteriologically positive milk samples were excluded. The aim was to study the variation in total and differential leukocyte counts, N-acetyl-ß-D-glucosaminidase (NAGase), antitrypsin (ATR) and serum albumin (BSA) in milk during the lactation period and different stages of oestrous cycle. Also the between milkings variation was studied from lactation month 4 to 9. At 2 days pp, each fraction of milk contained significantly higher numbers of leukocytes and had a higher activity of NAGase and ATR than later in the lactation period. In foremilk the highest content of BSA was also recorded at 2 days pp. From lactation month 2 to 9, stage of lactation had, in general, a slight effect on the variation in the variables measured. The total leukocyte count in residual milk tended to increase as lactation proceeded. The proportion of monocyte-macrophages in foremilk was significantly decreased during the last 4 months. NAGase and BSA in both fractions and ATR in residual milk increased significantly towards the end of the lactation period. From lactation month 4 to 9 the highest recorded ranges of variation between milkings, within quarter and stage of lactation, in the total leukocyte count, proportions of neutrophils, lymphocytes, monocyte-macrophages, NAGase, ATR and BSA in foremilk were 215 x 10^3/ml, 42 %, 34 %, 54 %, 6.68 units, 0.36 units and 0.14 mg/ml respectively. The corresponding figures in residual milk were higher except for the variation in BSA which was slightly lower in residual milk than in foremilk. In residual milk there was a positive correlation between the proportion of neutrophils and the total leukocyte count, when calculated on data from all cows and the entire experimental period. During the oestrous periods, the proportion of neutrophils in residual milk was higher than during the dioestrous periods. Foremilk and residual milk differed in the total as well as the differential leukocyte counts in all the various stages of lactation, whereas the contents of NAGase, ATR and BSA were equal in both fractions. The exception was 2 days pp when the proportions of lymphocytes were equal in both fractions and BSA significantly higher in foremilk than in residual milk.

cow; stage of lactation; oestrus; dioestrus; fraction; differential counts; NAGase; BSA.

Introduction

The most common method of diagnosing subclinical mastitis is total somatic cell count (SCC) in milk (MSCC). However, little is still known about the differential cell count (DCC), particularly in milk from non-infected quarters. Knowing how to interpret the DCC and estimate what is "normal" is valuable not only for diagnostic but also for research purposes.

A majority of the cells in milk are leukocytes. Neutrophils are the predominant cell population in milk from udder quarters with clinical mastitis *(Blackburn et al.* 1955, *Saad & Östensson* 1990). In milk with low and moderately increased total cell counts (< 500 x 10^3 cells/ml) from non-infected quarters, monocyte-macrophages constitute the major population and the proportion of neutrophils has been reported to be in the range of 11 - 51 % *(Blackburn et al.* 1955, *Östensson et al.* 1988).

The DCC appears to depend on the fraction of milk analyzed *(Paape & Tucker* 1966, *Östensson et al.* 1988). Residual milk seems to be the fraction in which the DCC best reflects the condition of the udder tissues as the cell content in this fraction is least influenced by dilution and storage within the udder. This means that also small changes in the release of different leukocytes from the tissues to the milk could be detectable in the DCC in residual milk. Thus, in the present study, both foremilk and residual milk were analyzed.

Results from most of the previous studies on DCC in milk from non-infected udders are based on occasional samplings, so information about the variation during lactation is still small. The DCC seems to be influenced by the SCC also in milk from non-infected quarters with a fairly low SCC *(Blackburn et al.* 1955, *Östensson et al.* 1988). Consequently, all factors influencing the SCC could also influence the DCC. We know that this is true when infections are involved. However, factors that only slightly affects the MSCC could also be more obviously reflected in the DCC, being a more sensitive measure of alterations in the condition of udder tissues than the MSCC. The stage of lactation has a slight effect on the MSCC, which increases towards the end of the lactation period *(Miller et al.* 1983, *Brolund* 1985, *Emanuelsson et al.* 1988).

Experimentally induced high plasma oestrogen values in cows may cause an increase in the MSCC and the proportion of neutrophils in milk *(Guidry et al.* 1975, *Saad & Åström* 1988, *Saad et al.* 1990). The oestrous cycle has, however, not been shown to influence the above cytologic characteristics of milk *(Anderson et al.* 1983, *Berning et al.* 1987a).

The purpose of the present work was to study the variation in SCC and DCC in foremilk and residual milk from non-infected quarters during lactation, and between different stages of the oestrous cycle. Oestradiol-17ß and progesterone in blood plasma during the oestrous periods were determined. The variation between milkings, for individual quarters, was also studied from lactation month 4 to 9. N-acetyl-ß-D-glucosaminidase (NAGase), antitrypsin (ATR), and serum albumin (BSA) in milk are often used as indicators of the inflammatory condition of the mammary tissues and correlate to the MSCC *(Kitchen et al.* 1976, *Honkanen-Buzalski et al.* 1981a, *Sandholm et al.* 1984). For this reason NAGase, ATR and BSA in milk were also included in this study. As infections are known to influence the DCC strongly, thereby hiding minor influences on the DCC from other factors, this study was carried out on milk from non-infected quarters.

Material and methods

Animals

Five Swedish Red and White dairy cows in their second lactation were used. During their first lactation there had been no record of udder disease, as measured by monthly record-

ed MSCC in udder milk samples (< 150 x 10^3 cells/ml) or any disease that required veterinary treatment. The milk production per cow during their first lactation was comparable to the average milk production for Red and White dairy cows in Sweden, approximately 7300 kg of milk with a 4 % fat content (Anon. 1992). During the present study the cows were housed in an isolated barn and handled in the same way. They were fed hay and commercial concentrate on basis of their body weight and milk production. From 2 days post partum (pp) they were milked daily at 07^{30} am and 03^{30} pm using a Duovac milking machine (Alfa Laval, Tumba, Sweden) and according to the same routine.

Experimental design

Milk samples were taken on day 2 after parturition, during 3 consecutive oestrous periods (on day 1 in the oestrous cycle), starting at the first oestrus pp, and during the 2 intervening dioestrous periods (on day 11 in the oestrous cycle). The oestrous periods were detected on the basis of visible signs. Insemination of the cows started during their third oestrous period, after which milk samples were taken approximately every second week for 6 months. In the cows that failed to conceive after the first insemination the oestrous periods were in the following avoided as sampling occasions. The quarter milk was checked daily at the morning milking for visible signs of mastitis and CMT (California Mastitis Test) scores. Scandinavian scoring system (*Klastrup & Schmidt Madsen* 1974) was used in the CMT, which means that 1 is equivalent to less than approximately 150 x 10^3 cells/ml. Milk samples were taken for bacteriologic examination at every sampling and between the samplings if the CMT score at one morning milking was 4 or higher or if the CMT score was 2 to 3 for three successive days or if clinical signs of mastitis were observed. When infection in the udder had been diagnosed, additional samples for bacteriologic examination were taken once a week until the milk was bacteriologically negative. In case of clinical mastitis the animal was adequately treated. Samples from the quarters that were bacteriologically positive, with or without signs of clinical mastitis, were excluded from the data before the statistical calculations. Also samples that were taken from the infected quarters after the infection had ceased were decided to be excluded, until the total leukocyte concentration in foremilk had returned to values comparable to the values before the infection.

Sample collection

Quarter milk samples were collected at the morning milking. The foremilk samples (100 ml) were taken after milk had been checked for visible signs of mastitis and CMT scores. After the udder had been emptied by machine, followed by hand milking, 10 IU of oxytocin (Partoxin vet, 10 IU/ml, Pherrovet, Malmö, Sweden) were injected into the jugular vein. One to 2 min later the residual milk samples (100 ml) were collected. The milk samples for analyses of NAGase, ATR and BSA were stored at –20°C until analyzed.

At oestrus, blood samples were collected from the jugular vein in heparinized tubes and immediately centrifuged. Plasma was removed and stored at -20°C until analyzed for concentrations of oestradiol-17ß and progesterone according to standard procedures as used at the Department of Clinical Chemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Cell counts

Total and differential leukocyte counts of the milk were made by use of a flow cytometer (Cytofluorograph 50H, Ortho Diagnostic Instruments, Westwood, Mass., USA) after staining with supravital acridine orange as described *(Hageltorn & Saad* 1986). Leukocytes were differen-

tiated into neutrophils, lymphocytes and monocyte - macrophages. The leukocyte counts were made whithin 5 h after the milk collection. No other somatic cells but leukocytes are included in SCC.

Analysis of NAGase

Each milk sample was analyzed for NAGase after freezing and thawing samples twice. The NAGase activity in 10 µl of milk was determined, using the fluorometric substrate 4-methyl-umbelliferyl-N-acetyl-ß-D-glucosamine in a commercial kit (Milk NAGase test, Labsystems, Helsinki, Finland), designed for microtitration plate fluorometry *(Kitchen et al.* 1980, *Mattila & Sandholm* 1985,). A NAGase value of 100 represents a release of 5 picomol of product (4-methyl-umbelliferone) per min at 25°C, as catalyzed by 1µl of milk.

Analysis of ATR

The ATR activity was analyzed by measuring the trypsin-inhibiting capacity of the milk samples by a colorimetric procedure, using a commercial kit (Milk Antitrypsin kit, Labsystems, Helsinki, Finland) (Sandholm et al. 1984). A standard (dairy milk pool), a 100% control and a 0 control are included among the samples and the content of ATR is indicated in relation to the standard. For example, result 1 means that the content of ATR is the same in the sample as in the standard which is considered normal.

Analysis of BSA

The BSA content in the milk samples was determined by use of the radial immunodiffusion principle of *Mancini et al.* (1965).

Statistics

To compare statistically the average values during the various stages of the oestrous cycle and lactation for all cows, data were analyzed by least-squares analysis of variance using the GLM procedure of SAS (SAS Institute Inc. 1987). First a model including the effects of cow (n = 5), quarter position (n = 4), fraction (foremilk and residual milk), stage (2 days pp, oestrus, dioestrus, 4th and 5th, 6th and 7th, and, 8th and 9th month of lactation), and the combination of fraction and stage was used to check the effect of quarter position. The final analysis of variance from which the presented results are obtained (Table 1 and 2) was, however, performed according to a model including the effects of cow, fraction, stage and the combination of fraction and stage, i.e. the former model excluding the effect of quarter position. The mean of all quarters per cow and fraction at each sampling was calculated, and creates 1 observation in the final analysis of variance. This mean was calculated because the quarter samples per cow and sampling can not statistically be considered as independent observations. The correlation coefficient between the proportion of neutrophils and SCC was calculated within fraction, on data from all cows and the entire experimental period, using the Pearson product-moment correlation test (SAS Institute Inc. 1987). Also in the correlation test the above mean of all quarters per cow, fraction and sampling creates 1 observation. Logarithmic transformation ($\log_{10}$) was performed for the values of SCC, NAGase, ATR and BSA before the statistical analyses. Unless otherwise stated, level of statistical significance is $p < 0.05$.

Results

The data are obtained from a total of 18 samplings per cow and 360 quarter samples per fraction.

According to the statistical analysis of variance there was no overall effect of quarter position.

All the cows had normal parturitions and were clinically healthy, except for one episode of clinical mastitis in 2 of the cows (cow C and D

Table 1. Total leukocyte concentration in thousands/ml (SCC), proportion (%) of neutrophils (N), lymphocytes (L), monocyte-macrophages (M), content of N-Acetyl-ß-D-glucosaminidase (NAG, units), antitrypsin (ATR, units) and bovine serum albumin (BSA, mg/ml) in foremilk and residual milk from non-infected quarters during oestrus and dioestrus. Data are expressed as LS mean (the mean for samples from all the quarters per cow and fraction on each sampling occasion constitutes 1 observation) and median ( ). The median is given on a quarter basis.

	Within 3 months of lactation			
	Oestrus		Dioestrus	
	(n= 15)	(n= 60)	(n= 10)	(n= 40)
Foremilk				
SCC	101	(97)	114	(105)
N	21	(18)	17	(13)
L	5	(2)	4	(3)
M	74	(77)	79	(79)
NAG	7.24	(6.68)	7.01	(6.53)
ATR	0.86	(0.86)	0.86	(0.84)
BSA	0.10	(0.09)	0.10	(0.09)
Residual milk				
SCC	257	(225)	210	(185)
N	43[a]	(42)	32[b]	(32)
L	19	(17)	23	(19)
M	39	(35)	45	(43)
NAG	7.23	(6.62)	6.20	(6.19)
ATR	0.87	(0.86)	0.86	(0.84)
BSA	0.08	(0.08)	0.08	(0.06)

[a,b] LS means within each row, that have no superscript in common are significantly different ($p<0.05$).

in the 5th and 8th month of lactation, respectively). The milk was found to be bacteriologically positive in a total of 12 quarter samples per fraction which were distributed among cows as follows (cow, lactation month, number of quarters/sampling): A,8,1/1; C,5,1/1 and 2/1; D,8,1/1, 4/1 and 1/1; E,6,2/1. Corynebacterium bovis was isolated from cow A and E and Coagulase negative staphylococci from cow C and D. The SCC in the foremilk of the remaining 348 quarter samples were as follows: $\leq 100 \times 10^3$/ml in 158 samples, 101×10^3 - 200×10^3/ml in 173 samples, 201×10^3 - 300×10^3/ml in 15 samples and $> 300 \times 10^3$/ml in 2 samples (2 days pp). The average daily milk yield is presented in Table 2.
The first oestrus was detected 5 to 7 weeks pp. The avarage oestradiol-17ß and progesterone content in blood serum during oestrus was 53 pmol/l (range 28 to 91 pmol/l) and 0.4 nmol/l (-range 0 to 1.1 nmol/l), respectively.

Comparing foremilk and residual milk

In 90 % of the quarter samples of foremilk and residual milk the values were below the following: SCC, 170×10^3/ml and 500×10^3/ml, respectively; proportion of neutrophils, 34 % and 55 %; proportion of lymphocytes, 15 % and 29 %; NAGase, 13.7 and 19.5 units; ATR, 1.02 and 1.07 units; and BSA 0.17 and 0.16 mg/ml. In 90 % of the quarter samples of foremilk and residual milk the proportion of monocyte-macrophages was more than 51 % and 27 %, respectively.

Table 2. Total leukocyte concentration in thousands/ml (SCC), proportion (%) of neutrophils (N), lymphocytes (L), monocyte-macrophages (M), content of N-Acetyl-ß-D-glucosaminidase (NAG, units), antitrypsin (ATR, units) and bovine serum albumin (BSA, mg/ml) in foremilk and residual milk from non-infected quarters during lactation. Data are expressed as LS mean (the mean for samples from all quarters per cow and fraction on each sampling occasion constitutes 1 observation) and median ( ). The median is given on a quarter basis. The daily milk yield is expressed as mean, in l.

	Month of lactation									
	2 days pp		2 and 3 (dioestrus)		4 and 5		6 and 7		8 and 9	
	(n= 5)	(n=20)	(n= 10)	(n= 40)	(n= 20)	(n= 77)	(n= 20)	(n= 78)	(n= 19)	(n= 73)
Foremilk										
SCC	187^{b}	(176)	114^{a}	(105)	116^{a}	(100)	99^{a}	(85)	119^{a}	(108)
N	23	(21)	17	(13)	16	(14)	21	(20)	19	(17)
L	7	(5)	4	(3)	4	(3)	8	(6)	8	(6)
M	70^{b}	(74)	79^{a}	(79)	81^{a}	(82)	71^{b}	(70)	73^{b}	(74)
NAG	43.9^{b}	(38.0)	7.01^{a}	(6.48)	6.35^{a}	(5.76)	7.27^{a}	(6.12)	9.53^{c}	(9.48)
ATR	17.1^{b}	(13.6)	0.86^{a}	(0.84)	0.84^{a}	(0.83)	0.87^{a}	(0.87)	0.86^{a}	(0.86)
BSA	0.30^{b}	(0.29)	0.09^{a}	(0.09)	0.11^{ac}	(0.11)	0.12^{c}	(0.11)	0.12^{c}	(0.11)
Residual milk										
SCC	365^{b}	(264)	210^{a}	(185)	243^{a}	(195)	248^{a}	(195)	268^{a}	(204)
N	39^{ab}	(36)	32^{b}	(32)	40^{a}	(40)	38^{ab}	(38)	34^{ab}	(32)
L	11^{d}	(10)	23^{c}	(19)	16^{abd}	(14)	19^{bc}	(16)	14^{ad}	(14)
M	51	(50)	45	(43)	44	(43)	43	(40)	51	(50)
NAG	36.7^{b}	(32.8)	6.20^{a}	(6.23)	6.48^{a}	(6.06)	7.88^{a}	(6.11)	10.9^{c}	(8.87)
ATR	15.3^{b}	(11.3)	0.86^{a}	(0.84)	0.85^{a}	(0.83)	0.91^{ac}	(0.90)	0.91^{c}	(0.93)
BSA	0.11^{ac}	(0.10)	0.07^{b}	(0.06)	0.10^{a}	(0.10)	0.11^{ac}	(0.11)	0.12^{c}	(0.11)
Daily milk yield			26.3		24.0		20.7		16.5	

^{a,b,c,d} LS means within each row, that have no superscript in common are significantly different ($p<0.05$).

When the 2 fractions were compared (Tables 1 and 2) residual milk had, in all stages, significantly higher SCC, as well as higher proportion of neutrophils and lower proportion of monocyte-macrophages, than foremilk. In all stages, except for 2 days pp, the proportion of lymphocytes was higher in residual milk than in foremilk. There was no statistically significant difference between the 2 fractions regarding NAGase, ATR and BSA, except for 2 days pp when the BSA content in foremilk was higher than in residual milk.

In residual milk, the SCC correlated positively to the proportion of neutrophils ($r= 0.25$, $n= 89$, $p< 0.05$), calculated on data from all the cows and the entire experimental period. When the data were divided into 2 groups: samplings with a mean SCC in foremilk for all quarters of $\leq 100 \times 10^3$/ml (group 1) and $> 100 \times 10^3$/ml (group 2), the SCC and the proportion of neutrophils in residual milk, correlated significantly in both groups (group 1: $r= 0.39$, $n= 43$, $p= 0.01$; group 2: $r= 0.32$, $n= 46$, $p< 0.05$). In foremilk, significant correlation between the SCC and proportion of neutrophils were not found.

Variation between milkings, within quarter

The variation between milkings, for individual quarters, was calculated within cow and stage of lactation, from lactation month 4 to 9. The highest recorded ranges of variation were as follows: SCC, 215 x 10^3/ml in foremilk and 580 x 10^3/ml in residual milk; the proportion of neutrophils, 42 % and 48%; the proportion of lymphocytes, 34 % and 48 %; the proportion of monocyte-macrophages, 54 % and 59 %; NAGase, 6.68 units and 14.4 units; ATR, 0.36 units and 0.56 units; and BSA, 0.14 mg/ml and 0.13 mg/ml.

Variation between stages of lactation and oestrous cycle

The highest SCC, NAGase and ATR values in both fractions were measured 2 days pp (Table 2). In foremilk, BSA was also higher at this time than during the rest of the lactation.

From lactation month 2 to 9 the variations in the variables measured, were as follows (Table 2): the proportion of monocyte-macrophages in foremilk was significantly lower during lactation month 6 to 9, than earlier in lactation; the proportion of lymphocytes in residual milk was significantly lower during lactation month 4, 5, 8 and 9 than during lactation month 2 and 3; the highest NAGase and BSA in each fraction of milk and ATR in residual milk were recorded during the last 4 months of lactation.

During oestrus there was a significantly greater proportion of neutrophils in residual milk than during dioestrus ($p < 0.01$; Table 1).

Discussion

The MSCC is to some extent individual for the cow. The DCC seems to depend on the MSCC, so it is logical to assume that the DCC is also individual to a certain extent. Thus, to study the variation in DCC between milkings, comparisons should primarily be made within cow. This variation must be referred to as physiological, even if external causes, such as non-specific irritation due to improper mechanical milking and milking routines, are also probable. In this study the variation between milkings was, in general, fairly high in both fractions and in all variables measured. These findings support the results of *Duitschaever & Ashton* (1972) who reported large sample-to-sample variation in SCC and number of neutrophils throughout lactation. This variation between milkings indicates different conditions in the udder tissues at different milkings, also in non-infected udder quarters with low MSCC. The somewhat higher variations in residual milk, compared with foremilk, may suggest that cell counts in residual milk is a more sensitive measure of the inflammatory condition in the udder tissues.

Residual milk had a significantly higher SCC and proportion of neutrophils and, except for 2 days pp, also a higher proportion of lymphocytes than foremilk, as reported *(Östensson et al.* 1988). These differences between the fractions may be attributable to that cells, because of their close contact with the epithelium, are retained in the alveoli and ducts until residual milk is collected. It is notable that at 2 days pp, the proportions of lymphocytes were approximately the same size and low in both milk fractions. Why this relation between the fractions is different at this time, compared with later in lactation, can not be explained by the present study.

Leukocytes are, generally, accepted to be a major source of NAGase. NAGase in residual milk has been reported to be significantly higher than in foremilk *(Berning et al.* 1987b). Such a difference was not observed in the study reported here, probably because the difference in SCC between the fractions was less than in the study referred to. The positive correlation between the SCC and the proportion of neutrophils in residual milk, indicates that the SCC in this fraction gives a more reliable estimate of the inflammatory condition in the quarter, as indi-

cated by the proportion of neutrophils, than does the SCC in foremilk.

Stage of lactation had a slight effect on SCC and DCC in both fractions except for 2 days pp (Table 2). At this time, each milk fraction had significantly higher SCC and contained more NAGase and ATR than later in the lactation period. In foremilk the highest BSA content was also recorded at 2 days pp. These results may be explained by the increased capillary permeability in the udder around parturition, resulting in increased leakage of serum components into the milk. The high ATR activity in colostrum is known to, partly, be attributable to a trypsin inhibitor with a low molecular weight. The origin of the colostral inhibitor is not clear but results of *Honkanen - Buzalski & Sandholm* (1981a) indicate that also this inhibitor is blood derived.

The oestrous period is thought to influence the udder as indicated by higher incidence of clinical mastitis (see *Åström* 1972). Therefore the samples taken during the oestrous periods should not be compared with samples from the rest of the lactation period which are considered to be affected primarily by the stage of lactation. The samples collected at dioestrus should, however, be representative of lactation months 2 and 3, when studying the effect of stage of lactation. It is generally accepted that the MSCC increases towards the end of the lactation, owing to higher prevalence of mastitis, normal involution of the udder and decreased milk production which causes less dilution of the milk leukocytes *(Blackburn* 1966, *Miller et al.* 1983, *Brolund* 1985, *Emanuelsson et al.* 1988). However, *Duitschaever & Ashton* (1972) reported unchanged SCC throughout lactation, which agrees with results of the present study, where the SCC was unaltered in foremilk and only tended to increase in residual milk from lactation month 2 to 9. The daily milk yield per cow was never below 10 l and the milk was bacteriologically negative, which may explain why the effect of stage of lactation on the MSCC was so small. The alterations in proportions of the different leukocyte types were slight in both milk fractions throughout lactation. Our findings support those of Blackburn (1966) and Duitschaever and Ashton (1972) who reported unchanged proportion of neutrophils during the course of lactation. NAGase, ATR and BSA have been reported to be high early in lactation, lower in mid-lactation and increasing again towards the end of lactation *(Honkanen - Buzalski et al.* 1981b, *Mattila & Sandholm* 1985, *Miller & Paape* 1987, *Emanuelsson et al.* 1988). The results from both fractions in this study were in general agreement with these reports, with the highest values recorded at 2 days pp and during the end of the period studied. However, in foremilk ATR remained unaltered from lactation month 2 to 9.

In the study reported here, the proportion of neutrophils in quarter foremilk was below 51 % at all samplings (not presented in results). On one occasional sampling, this must probably be considered to be "normal" for milk in the present SCC range. As an average value from several samplings, the upper limit for the neutrophil proportion in milk from "healthy" quarters is probably lower. In this study 90 % of the quarter samples of foremilk and residual milk contained less than 34 % and 55 % neutrophils, respectively. *Blackburn et al.* (1955) report an avarage neutrophil proportion of 33 to 51 % in milk from non-infected quarters. *Duitschaever & Ashton* (1972) reported higher proportions of neutrophils (65 to 90%) in milk from non-infected quarters whereas *Lee et al.* (1980) found considerably smaller proportions of neutrophils ($\leq$ 11 %) identified by electron microscopy. Earlier studies by *Östensson et al.* (1988) on fraction-collected milk from non-infected quarters with a MSCC range comparable to the range in the present study, showed avarage

neutrophil proportions of 11 to 20 % in foremilk and 20 to 36 % in residual milk. As seen in Tables 1 and 2, the average neutrophil proportions correspond well to the latter study referred to. In colostrum, at 2 days pp, the proportion of neutrophils was comparable to results of *Jensen & Eberhart* (1981), although they reported higher SCC. *McDonald & Anderson* (1981), on the other hand, found a SCC similar to that reported here but considerably smaller proportion of neutrophils.

As clinical mastitis may appear more likely during oestrus than during dioestrus (see *Åström* 1972), many studies have been carried out to investigate the influence of oestrogens on the udder tissues (*Åström* 1972, *Anderson et al.* 1983, *Berning et al.* 1987a, *Saad & Åström* 1988, *Saad et al* 1990). Administration of oestrogens has been found to cause decreased milk production and an increase in the MSCC, the proportion of neutrophils, NAGase, ATR and BSA in milk. These effects of oestrogens have, however, not been observed during oestrus, except for the increase in BSA (*Anderson et al.* 1983, *Berning et al.* 1987a). In the study reported here, the proportion of neutrophils in residual milk was significantly higher during the oestrous periods than during the dioestrous periods (Table 1). The same tendency was seen in all cows. Avarage plasma oestradiol-17ß and progesterone during oestrus were comparable to those reported in normal cyclic cows (*Lemon et al.* 1975, *De Silva et al.* 1981). There was no significant difference in MSCC between the stages of the oestrous cycle. The difference in the proportion of neutrophils between oestrus and dioestrus that was evident in residual milk was not observed in foremilk, probably as a consequence of cell damage in this milk fraction, because of storage within the udder.

According to previous studies (*Östensson* 1988, *Saad & Östensson* 1990), the proportion of neutrophils appears to be a more sensitive measure of the inflammatory condition in the udder tissues than the SCC, and leukocyte counts in the residual milk seems to be a more sensitive measure of the condition in the udder quarters than leukocyte counts in the foremilk. Results from the present study confirm the previous indications.

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Sammanfattning

Variationer under laktationen, avseende innehållet av olika leukocyter, N-acetyl-β-D-glucosaminidas, antitrypsin och serum-albumin i förmjölk och residualmjölk från icke-infekterade juverfjärdedelar hos ko.

Från 5 mjölkkor togs prov av förmjölk och residualmjölk ungefär varannan vecka från 2 dagar post-partum (pp) till och med laktationsmånad 9. Alla bakteriologiskt positiva prover uteslöts. Syftet med arbetet var att studera variationen i det totala antalet leukocyter (SCC), proportionen av neutrofiler, lymfocyter och monocyt-makrofager samt innehållet av N-acetyl-ß-D-glucosaminidas (NAGase), antitrypsin (ATR) och serum albumin (BSA) i de 2 mjölk-fraktionerna under laktationsperioden. Variationen studerades speciellt med hänsyn till laktationsstadium och stadium i sexualcykeln (östrus, diöstrus) men också variationen mellan mjölkningar inom samma stadium undersöktes från och med laktationsmånad 4. Två dagar pp hade respektive mjölkfraktion signifikant högre SCC och högre aktivitet av NAGase och ATR än senare under laktationen. Det största innehållet av BSA i förmjölken uppmättes också 2 dagar pp. Från och med laktationsmånad 2 hade laktationsstadiet, generellt, ganska liten inverkan på de studerade parametrarna i båda mjölkfraktionerna. SCC i residualmjölk tenderade att öka från laktationsmånad 2 till 9. Proportionen monocyt-makrofager i förmjölk sjönk signifikant under de sista 4 månaderna. NAGase och BSA i båda mjölkfraktionerna och ATR i residualmjölk var högst mot slutet av laktationsperioden. Den största variationen mellan mjölkningar, för en enskild juverfjärdedel, angiven som skillnaden mellan det största och det minsta registrerade värdet inom respektive stadium och ko var för förmjölk följande: SCC, 215 x 10^3/ml; proportionen av neutrofiler, 42 %; proportionen av lymfocyter, 34 %; proportionen av monocyter-makrofager, 54 %; NAGase, 6.68 enheter; ATR, 0.36 enheter och BSA, 0.14 mg/ml. Motsvarande värden i residualmjölk var något högre utom avseende variationen i BSA som var något lägre i residualmjölken än i förmjölken. I residualmjölken förelåg en positiv korrelation mellan proportionen av neutrofiler och SCC, som inte observerades i förmjölken. Korrelationen beräknades på data från alla kor och hela laktationsperioden. Under östrus var proportionen av neutrofiler i residualmjölken större än under diöstrus. Denna skillnad observerades inte i förmjölken. Det var inte heller någon statistiskt säkerställd skillnad i SCC mellan östrus och diöstrus i någon av mjölkfraktionerna. Från laktationmånad 2 till 9 skiljde sig förmjölken och residualmjölken åt avseende både SCC och proportionen av respektive leukocyt-typ i alla stadier av laktationen och sexualcykeln medan innehållet av NAGase, ATR och BSA var lika i båda fraktionerna. 2 dagar pp var emellertid proportionen lymfocyter lika i båda fraktionerna och innehållet av BSA var signifikant högre i förmjölk än i residualmjölk.

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