Importance of the Sampled Milk Fraction for the Prediction of Total Quarter Somatic Cell Count

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ABSTRACT

This study investigated the changes in somatic cell counts (SCC) in different fractions of milk, with special emphasis on the foremilk and cisternal milk fractions. Therefore, in Experiment 1, quarter milk samples were defined as strict foremilk (F), cisternal milk (C), first 400 g of alveolar milk (A1), and the remaining alveolar milk (A2). Experiment 2 included 6 foremilk fractions (F1 to F6), consisting of one hand-stripped milk jet each, and the remaining cisternal milk plus the entire alveolar milk (RM). In Experiment 1, changes during milking indicated the importance of the sampled milk fraction for measuring SCC because the decrease in the first 3 fractions (F, C, and A1) was enormous in milk with high total quarter SCC. The decline in SCC from F to C was 50% and was 80% from C to A1. Total quarter SCC presented a value of approximately 20% of SCC in F or 35% of SCC in C. Changes in milk with low or very low SCC were marginal during milking. Fractions F and C showed significant differences in SCC among different total SCC concentrations. These differences disappeared with the alveolar fractions A1 and A2. In Experiment 2, a more detailed investigation of foremilk fractions supported the findings of Experiment 1. A significant decline in the foremilk fractions even of F1 to F6 was observed in high-SCC milk at concentrations $>350 \times 10^3$ cells/mL. Although one of these foremilk fractions presented only 0.1 to 0.2% of the total milk, the SCC was 2- to 3-fold greater than the total quarter milk SCC. Because the trait of interest (SCC) was measured directly by using the DeLaval cell counter (DCC), the quality of measurement was tested. Statistically interesting factors (repeatability, recovery rate, and potential matrix effects of milk) proved that the DCC is a useful tool for identifying the SCC of milk samples, and thus of grading udder health status. Generally, the DCC provides reliable results, but one must consider that SCC even in strict foremilk can differ dramatically from SCC

in the total cisternal fraction, and thus also from SCC in the alveolar fraction.

Key words: foremilk, milk fraction, somatic cell count

INTRODUCTION

The SCC in milk is an indicator of the activity of the cellular immune defense of the udder (Sordillo et al., 1997; O'Brien et al., 1999; Leitner et al., 2000). These somatic cells, mainly leukocytes, are part of the natural defense mechanism, and SCC is often used to distinguish between infected and uninfected quarters. Milk from uninfected quarters generally contains a physiological basal cell count of $<100 \times 10^3$ somatic cells/mL (Hillerton, 1999). A striking elevation of SCC greater than this concentration is abnormal, and high individual cow SCC are known to be positively correlated with mastitis (Kehrli and Schuster, 1994; Kelly et al., 2000). A rapid increase in SCC reflects activation of the mammary immune response in the early acute phases of infection.

Mostly, foremilk or composite quarter milk samples are used for SCC measurement, and results have shown the high impact of SCC on the interpretation of udder health status (Woolford et al., 1998; Schukken et al., 2003). In addition, herds and cows with very low SCC have been examined, and there is evidence that risk of severe mastitis is increased in those with low SCC in comparison with those with greater SCC before infection (Sol et al., 2000; Suriyasathaporn et al., 2000; Sarikaya et al., 2006).

It is generally accepted that cells are important in the defense of the udder and the SCC is used to monitor udder health status. Because SCC differs in foremilk and composite milk, we hypothesized that SCC would also differ between fractions of the foremilk. A new cell-counting technology allows sample sizes of $<100 \mu\text{L}$. Therefore, it is important to know the influence of a specific milk fraction on the SCC results. In this context, the study aimed to investigate the importance of the sampled milk fraction to predict total quarter milk SCC and udder health status.

MATERIALS AND METHODS

Cows and Husbandry

Two experiments were carried out. In Experiment 1, 36 Brown Swiss cows in their first to fifth lactation were

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used. Ten animals were in early stages of lactation (12 to 98 d), 15 were in midlactation (112 to 206 d), and 11 in a late stage of lactation (224 to 421 d). Experiment 2 included 25 Brown Swiss cows in their first to fourth lactation. Ten cows were in early (10 to 87 d), 8 in mid- (120 to 201 d), and 7 in late lactation (233 to 395 d).

Cows were fed a diet consisting of 22 kg of maize silage, 12 kg of grass silage, 2 to 3 kg of hay, and 6 to 8 kg of concentrates. Water was available ad libitum. Average milk production on the day of investigation was 23 ± 2 kg per cow. Cows were kept in a loose-housing barn and were milked twice daily at 5 a.m. and 4 p.m.

Experimental Design

Experiment 1. This study included fractionized milking during routine milking times with special quarter milking equipment (Sarıkaya et al., 2005). This device allowed separation of single quarter milk samples into 4 fractions: strict foremilk (**F**), cisternal milk (**C**), first 400 g of alveolar milk (**A1**), and the remaining alveolar milk (**A2**). To obtain F and C free of alveolar milk, milking was performed without any udder preparation to avoid milk ejection and mixing of milk fractions (Bruckmaier and Blum, 1996). According to previous studies (Bruckmaier and Hilger, 2001), no milk ejection is expected during the first 50 s after initiating tactile teat stimulation. Therefore, the F and C samples were removed during this period. In this case, F represented the first 2 stripped jets of milk, and C was the remaining milk before milk ejection (i.e., within 50 s after initiating sampling). The A1 fraction consisted of the first 400 g of milk after milk ejection. This fraction contained mainly alveolar milk, but it is possible that a portion of cisternal milk also was included. The remaining alveolar milk was defined as A2.

Experiment 2. This study also included fractionized milking, but with a different setup of the fractions. Milking was performed without any udder preparation. The first 6 fractions (**F1** to **F6**) consisted of one hand-stripped milk jet each (i.e., each fraction represented the volume of the teat cistern capacity). By definition, F1 to F6 were all fractions of strictly foremilk. The remaining quarter milk was collected by normal machine milking and defined as remaining milk (**RM**). This fraction included the remaining cisternal and the entire alveolar milk.

In both experiments, each cow was sampled only once. Later, samples were collected from a single quarter of each cow. All samples were immediately stored at 4°C and transferred for further processing on the same day.

Measurement of SCC

As a basis for this study, the validity of measuring SCC by an automated cell counter [DeLaval cell counter

Table 1. Grouping and number of investigated quarter milk samples according to their total quarter SCC in Experiments 1 and 2

SCC group	Experiment 1, SCC/mL	No.	Experiment 2, SCC/mL	No.
1	$<20 \times 10^3$	5	$<50 \times 10^3$	8
2	$20 \text{ to } 50 \times 10^3$	14	$50 \text{ to } 100 \times 10^3$	6
3	$50 \text{ to } 100 \times 10^3$	9	$100 \text{ to } 350 \times 10^3$	6
4	$>100 \times 10^3$	8	$>350 \times 10^3$	5

(**DCC**); DeLaval, Tumba, Sweden] was tested. The validation considered the statistical traits of repeatability, recovery rate, and potential matrix effects in various milk samples.

Mathematical Calculations

For calculation of repeatability, each sample was measured twice. Hereby, the REML method of estimating variance components was performed. The recovery rate, which emphasizes the reliability of the measurement, was determined by adding different volumes of cell suspensions with a defined number of cells to untreated low-SCC milk. To achieve a suspension with a defined number of cells, milk was centrifuged for 30 min at $1,500 \times g$ at 4°C. The separated cell pellet was washed with PBS and resuspended in PBS. Afterward, the cell count of the suspension was determined by hemocytometric counting with a Neubauer chamber (Sarıkaya et al., 2004). Hereafter, the cell count per milliliter of suspension was calculated. Five different amounts of cell suspensions (i.e., with 5 different cell numbers) were then added to the untreated low-SCC milk. Each step was measured in addition to the original milk sample. The recovery rate showed the ratio of the difference between the measured SCC (before and after adding the cells) and the expected value. Individual sample recovery also was calculated.

Potential matrix effects were investigated by performing 6 serial dilutions of milk samples with PBS buffer. Dilution factors ranged from 1 to 6. In each series of dilutions, Pearson's coefficient of correlation was calculated. The correlation coefficient is a quantity that gives the quality of a least-squares fitting to the original data.

SCC

The SCC of all milk samples in Experiments 1 and 2 was measured with a DCC (DeLaval). The DCC was particularly suitable for the low amounts of milk available, because it requires a minimum sample size of only 60 μ L (Sarıkaya et al., 2006). Milk samples were categorized into 1 of 4 groups based on the total quarter SCC (Table 1).

Figure 1. Regression curves of the repeatability (A) and recovery rate (B) resulting from the validation of SCC as measured by the DeLaval cell counter (DeLaval, Tumba, Sweden).

Statistical Analyses

Data are presented as means $\pm$ standard errors of the means (SEM). Differences between itemized fractions and SCC groups were tested for significance by ANOVA using the MIXED models procedure of SAS (SAS Inst., Inc., Cary, NC). The MIXED model included the cow and milk fraction as class variables. Cow was the repeated term during the course of milking. The matrix structure of compound symmetry was used. Statistical significance between fractions was tested by the least significance difference test.

RESULTS

Mathematical Calculations (Statistical Parameters of Validation)

A repeatability of 0.99 was achieved based on 180 samples that were measured in duplicate. The recovery, calculated as the ratio between the difference of the measured SCC before and after adding cells and the expected value, was $99.3 \pm 0.8\%$ based on 30 measurements (Figure 1). Individual sample recovery ranged from 93 to 106%. Potential matrix effects, investigated by performing serial dilutions of milk with buffer, produced a Pearson's correlation coefficient of >0.99 in each series of dilutions.

Milk Cells

All investigated milk samples were assigned to 1 of 4 categories according to their total quarter milk SCC. Mean SCC in groups 1, 2, 3, and 4 were 15 ± 1 , 32 ± 2 , 68 ± 4 , and $198 \pm 24 \times 10^3/\text{mL}$, respectively, in Experiment 1, and 26 ± 4 , 79 ± 5 , 181 ± 33 , and $864 \pm 135 \times 10^3/\text{mL}$, respectively, in Experiment 2.

Experiment 1. No significant changes in SCC were detected during the course of milking in groups 1, 2, and

3. The SCC in group 4, however, decreased ($P < 0.05$) from F to C and further to A1, and increased slightly again toward A2. The decrease in SCC of A2 was significant only in the F fraction. The SCC of fractions F and C was lowest in group 1 and increased ($P < 0.05$) with the SCC group number. This significance was not detected in fractions A1 and A2 (Figure 2). The mean volume of each fraction and its respective proportion of total milk harvested are summarized in Table 2.

Experiment 2. No significant changes in SCC were detected during the course of milking in groups 1, 2, and 3. A decrease ($P < 0.05$) in SCC during milking was observed in group 4. Here, the greatest SCC concentrations were achieved in F1. A slight decrease in SCC took place in fractions F2 and F3. Fractions F4 and F5

Figure 2. Changes in SCC in the defined milk fractions of strict foremilk (F), cisternal milk (C), first 400 g of alveolar milk (A1), and the remaining alveolar milk (A2) obtained via fractionized milking in Experiment 1. Fractions were additionally categorized into 4 different SCC groups according to their total quarter SCC. ^{a,b}Means with different superscript letters within a milk fraction differ ($P < 0.05$) between groups. ^{A-C}Means with different superscript letters within a group differ ($P < 0.05$) between milk fractions.

Table 2. Classification of collected milk fractions (Experiment 1)

Abbreviation	Milk fraction	Volume, ¹ mL	Percentage of total milk ²
F	Strict foremilk ³	9.4 ± 0.8	0.3 ± 0.0
C	Cisternal milk ⁴	140.5 ± 12.5	4.3 ± 0.4
A1	First 400 g of alveolar milk	396 ± 8	13.6 ± 1.1
A2	Remaining alveolar milk	2,820 ± 184	81.8 ± 1.2

¹Fraction mean volume ± SEM.²Proportion of total milk harvested.³First 2 stripped jets of milk without any udder preparation.⁴Remaining milk before milk ejection.

contained fewer ($P < 0.05$) SCC than F1. This decrease ($P < 0.05$) continued in F6 and RM. The SCC of all 6 foremilk fractions (F1 to F6) of group 4 were increased ($P < 0.05$) compared with the SCC of their identical fractions in groups 1, 2, and 3. The RM showed a numerical increase from group 1 to 4 (Figure 3). The mean volume of each fraction and its representative proportion of total milk harvested are summarized in Table 3.

DISCUSSION

As the trait of interest, SCC was measured directly by use of the DCC. The quality of measurement was tested. Statistically interesting factors (repeatability, recovery rate, and potential matrix effects) proved that

the DCC is a useful tool for identifying the SCC of milk samples and thus for grading the health status of the udder.

Furthermore, our results demonstrated changes in milk SCC based on the milk fraction and total quarter SCC. In Experiment 1, collected milk fractions from a single quarter were defined as strict foremilk (F), cisternal milk (C), first 400 g of alveolar milk (A1), and remaining alveolar milk (A2). All investigated milk fractions of each quarter were assigned to groups (1 to 4) according to their total quarter SCC.

The SCC groups 1 to 3, representing the quarters with a total SCC of $<100 \times 10^3/\text{mL}$, showed minor changes during milking. A significant change in SCC during milking was observed in SCC group 4 belonging to quarters with a total SCC above $100 \times 10^3/\text{mL}$. Milk samples containing somatic cells above this concentration were assumed to emanate from inflamed quarters, subclinically or clinically. The F in SCC group 4 had the greatest concentrations and represented a fivefold increase in somatic cells per milliliter, compared with the total SCC, even though it represented only 0.3% of the total milk volume. A significant decrease in SCC was observed from F to C and further to A1. Fraction C, representing the next 4% of total milk, already showed half the SCC of F. In A1, the SCC was 10% of F. The main milk fraction composite as A2 had an SCC similar to A1. These changes during milking, indicating the importance of the sampled milk fraction for measuring SCC as the change in the first 3 fractions, were remarkable. Fractions F and C also showed significantly different SCC concentrations among the 4 SCC groups. This difference could not be observed in the later A1 and A2 fractions. Thus, the expressiveness of the SCC changes was according to which SCC fraction was used. Fraction F presented an alarming SCC that indicated a highly inflamed udder, even in a clinical way. Fraction C ranged in the subclinical inflammation area, whereas A1 showed a slight increase in SCC.

Taking into account the results of Experiment 1, we conducted Experiment 2. Here, the foremilk fraction was

Figure 3. Changes in SCC in the defined milk fractions F1 to F6, consisting of one hand-stripped milk jet each, and the remaining cisternal plus alveolar milk (RM) in Experiment 2. Fractions were additionally assigned to 4 different SCC groups according to their total quarter SCC. *Means within a milk fraction differ ($P < 0.05$) between groups. ^{A-C}Means within a group without common superscript letters differ ($P < 0.05$) between milk fractions.

Table 3 Classification of collected milk fractions (Experiment 2)

Abbreviation	Milk fraction	Volume, ¹ mL	Percentage of total milk ²
F1	First foremilk jet	2.7 ± 0.3	0.1 ± 0.0
F2	Second foremilk jet	2.3 ± 0.3	0.1 ± 0.0
F3	Third foremilk jet	3.1 ± 0.3	0.1 ± 0.0
F4	Fourth foremilk jet	3.3 ± 0.3	0.1 ± 0.0
F5	Fifth foremilk jet	3.4 ± 0.3	0.2 ± 0.0
F6	Sixth foremilk jet	3.7 ± 0.3	0.2 ± 0.0
RM	Remaining cisternal and alveolar milk	2,560 ± 236	99.2 ± 0.1

¹Fraction mean volume ± SEM.²Proportion of total milk harvested.

investigated in greater detail. Therefore, foremilk was categorized into 6 fractions consisting of one hand-stripped milk jet each. In this case, the SCC groups 1 to 3 represented the quarters with an SCC of $<350 \times 10^3/\text{mL}$, and they showed no significant changes during milking. A significant decrease in SCC was observed in group 4. Even in the 6 foremilk fractions F1 to F6, the decrease was significant. This was very interesting because one fraction presented only 0.1 to 0.2% of the total milk harvested. Fraction F6 represented only two-thirds and RM only one-third of the SCC of F1. Fractions F1 to F6 of group 4 also were increased significantly to their identical fractions in groups 1, 2, and 3. Surprisingly, this significance was not detected in RM. In this context, Bruckmaier et al. (2004) investigated the effect of milk ejection on the sensitivity of mastitis indicators such as physicochemical factors and somatic cells. They showed that the significances between high- and low-SCC quarters before milk ejection were striking.

In conclusion, SCC measurement by the DCC provided reliable and precise results; in particular, in those quarters having a high SCC, the sampled milk fraction had a crucial influence on the measured SCC value. One must consider that even SCC in strict foremilk can dramatically differ from that in the total cisternal fraction. The practical consequence of our findings was that any interpretation of the milk SCC must consider the fraction from which the milk sample was removed.

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