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Relationship between somatic cell count, polymorphonuclear leucocyte count and quality parameters in bovine bulk tank milk

Erik Wickström[1], Karin Persson-Waller[2,3], Helena Lindmark-Månsson[4], Karin Östensson[2] and Åse Sternesjö[1]*

[1] Department of Food Science, Swedish University of Agricultural Sciences, PO Box 7051, SE-750 07 Uppsala, Sweden
[2] Department of Clinical Sciences, Swedish University of Agricultural Sciences, PO Box 7054, SE-750 07 Uppsala, Sweden
[3] Department of Animal Health and Antimicrobial Strategies, National Veterinary Institute, SE-751 89 Uppsala, Sweden
[4] Swedish Dairy Association, Ideon Science Park, SE-223 70 Lund, Sweden

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The somatic cell count (SCC) in bovine bulk tank milk is presently used as an indicator of raw milk quality, reflecting the udder health status of the herd. During mastitis, SCC increases, mostly owing to an influx of polymorphonuclear leucocytes (PMN) from blood into milk, with a concomitant change in milk composition. Bulk tank milk samples were categorized according to their SCC, as well as polymorphonuclear leucocyte count (PMNC), to study relationships between SCC, PMNC and various raw milk quality traits, i.e. contents of total protein, whey protein, casein, fat and lactose, casein number, proteolysis and rheological properties. The proportion of PMN, obtained by direct microscopy, was significantly higher in samples with high SCC compared with low SCC samples. SCC and PMNC were strongly correlated, yielding a correlation coefficient of 0·85. High SCC samples had lower lactose and casein contents, lower casein number and more proteolysis than low SCC samples. Samples with high PMNC had a lower casein number than low PMNC samples. Samples with high and low SCC or PMNC did not differ in respect to rheological properties. Our results do not indicate that PMNC is a better biomarker than SCC for raw bulk tank milk quality, as previously proposed.

Keywords: Neutrophils, milk composition, bulk tank milk proteolysis.

Bulk tank milk somatic cell count (SCC) is a general indicator of the udder health of the herd and it is used as an indirect measure of milk quality (Schukken et al. 2003). An elevated SCC, especially in quarter or cow composite milk, is correlated with changes in milk composition, e.g. less casein and more serum-derived whey proteins, as well as increased proteolytic and lipolytic activities (Auldist & Hubble, 1998). SCC may, however, vary greatly depending on factors such as number of lactations, stage of lactation, season and milking frequency, irrespective of the udder health status of the animal (Harmon, 1994; Pyörälä, 2003). In bulk tank milk, where the total volume of milk will dilute effects from affected quarters, SCC appears to be less sensitive and specific as a biomarker for milk quality, e.g. suitability for cheese production (Leitner et al. 2006).

Somatic cells in milk consist of macrophages, lymphocytes and polymorphonuclear leucocytes (PMN; also denoted as neutrophils). As a response to invading pathogens, PMN migrate across the blood-milk barrier and act as the first line of cellular defence by phagocytosis (Paape et al. 2003). Therefore, PMN is the cell type that contributes mostly to the increase in SCC in mastitis. In milk from healthy udder quarters, <25% of the somatic cells consist of PMN, but in milk from a quarter with mastitis this proportion can in severe cases exceed 90% (Saad & Östensson, 1990; Kehrli & Shuster, 1994; Kelly et al. 2000).

In addition to increased cell traffic across the blood-milk barrier, a large number of different serum components pass into the milk during inflammation. The major indigenous proteinase of milk, plasmin and its zymogen plasminogen, both leak into the milk (Bastian & Brown, 1996). In the milk, plasminogen is activated to plasmin, catalysing hydrolysis of β-casein. PMN contain urokinase and tissue-type plasmin activators, both of which act to increase the proteolytic activity (Ballou et al. 1995; Politis, 1996; Haddadi et al. 2006). In this way, PMN may indirectly contribute to increased proteolysis. PMN also contain a broad range of intracellular proteinases, including

*For correspondence; e-mail: ase.sternesjo@lmv.slu.se

elastase, cathepsin (B, D, and G) and matrix metalloproteinases, which can hydrolyse caseins (Hurley et al. 2000; Le Roux, 2003; Somers et al. 2003; Mehrzad et al. 2005; Haddadi et al. 2006; Kelly et al. 2006) and may affect the quality and yield of dairy products, e.g. shorten the shelf life time of liquid milk (Barbano et al. 2006) and reduce the cheese curd yield (Leitner et al. 2008).

Because of the described negative effects on milk components associated with PMN, several authors have suggested the PMN count (PMNC) as a more specific biomarker than SCC for milk quality (Kelly et al. 2000; Pillai et al. 2001; Vangroenweghe et al. 2002; Le Roux, 2003; O'Brien et al. 2003). There are, however, to our knowledge no studies conducted on the importance of PMNC for the quality of bulk tank milk. The objective of this study was therefore to investigate relationships between SCC, PMNC and milk quality parameters in bulk tank milk samples.

Material and Methods

Bulk tank milk samples

Bulk tank milk samples (n=319) were collected from Swedish dairy farms in different geographical areas. On one occasion at each farm, a milk sample was collected by the tanker driver just before the tank was emptied. The sample consisted of commingled milk from four different milkings. One set of samples (n=228) was used only for determination of SCC, the proportion of PMN (PMN%) and PMNC. These samples were prepared for microscopic cell differentiation on one occasion at the national central laboratory for milk grading (Eurofins Steins Laboratorium AB, Jönköping, Sweden). The other set of samples (n=91) was collected in cooperation with Milko dairy cooperative, and was, in addition to the analysis of SCC, PMN% and PMNC, analysed for contents of total protein, whey protein, casein, fat and lactose, casein number, proteolysis and rheological properties. The samples were collected at the dairy plant and kept at 4 °C during transportation to the University laboratory during the same day. All analyses were performed on fresh milk, except for the evaluation of proteolysis, where sample aliquots were stored at −70 °C until analysis. Sample aliquots for evaluation of rheological properties were treated with bronopol for preservation (2-bromo-2-nitropropane-1,3-diol; VWR International AB, Stockholm, Sweden).

Bulk tank milk somatic cell count and polymorphonuclear leucocyte count

Bulk tank milk SCC was determined using direct electronic fluorescence-based cell counting (Fossomatic 5000, Foss, Hillerød, Denmark). Slides for direct microscopy counting of PMN were prepared and counted (IDF, 2006) using a modified Newman staining solution. The staining solution was prepared by dissolving 6·0 g methylene blue (SIGMA Chemical Co, St. Louis MO, USA) in 520 ml 95% ethanol (Altia Oyj, Rajamäki, Finland). Xylene (440 ml; Merck KGaA, Darmstadt, Germany) and acetic acid (40 ml; Merck KGaA) were added to the solution, which was subsequently filtered. For each slide, 100 cells were counted from a random area and classified either as PMN or other types of somatic cells. PMN were characterized by their densely stained multilobed nucleus, and cytoplasm containing small dense granules. Each milk smear was counted twice and the average number of PMN was used to express PMN%. PMNC was calculated by multiplying PMN% by SCC.

Determination of contents of total protein, whey protein, casein, fat and lactose and casein number

Total protein, fat and lactose contents were measured by mid-infrared spectroscopy (Fourier Transform Instrument, FT 120, Foss). Casein content was determined using an indirect method, whereby the whey fraction of the protein was determined by mid-infrared spectroscopy after rennet coagulation of the caseins. In short, 60 µl calcium chloride (48%) was added to the milk (40 ml) and the sample was incubated at 40 °C in a water bath. Upon reaching this temperature, 200 µl rennet (180±10 International Milk Clotting Units; IMCU) was added and the sample was mixed and incubated at 40 °C for 15–20 min to allow the milk to coagulate. The resulting curd was cut into small pieces and passed through a filter (42 µm) to separate caseins from whey proteins. Casein content was finally calculated by subtracting whey protein from total protein. Casein number was obtained by dividing casein content by total protein and multiplying by 100.

Proteolysis

Proteolysis was estimated by measuring free amino terminals according to a fluorescamine method (Wiking et al. 2002). In brief, intact milk proteins were precipitated with trichloracetic acid and subsequently removed by centrifugation. The proteolytic activity in the sample was evident as peptides in the supernatant. Fluorescamine was added, and after reaction with the amino terminals, it fluoresced. The resulting fluorescence was measured in a LS-50B Luminescence Spectrophotometer (Perkin Elmer Corporation, Norwalk CT, USA) at excitation 390 nm and emission 480 nm. Results were compared with a standard curve constructed by analysis of leucine diluted in HCl, and expressed as leucine-equivalents.

Rheology

Analyses of the rheological properties of the milk were, with minor modifications, performed as described by Hallén et al. (2007). Curd firmness was assessed with a Bohlin VOR Rheometer (Malvern Instruments Nordic AB,

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Uppsala, Sweden). In short, milk samples (12 ml) with 0·02% bronopol solution (20% w/v bronopol) were incubated for 60 min at 30 °C before addition of 20 µl chymosin solution. Chymax Plus, with a strength of 200 IMCU/g (Christian Hansen A/S, Hørsholm, Denmark) was used instead of pure chymosin. Subsequently, the sample was transferred to a cup fitted with a 2 gcm torsion bar, and the temperature was kept constant at 30 °C. Each measurement lasted for 30 min and gel strength was determined 25 min after chymosin addition. Clotting time was recorded as the time from chymosin addition until the strength of the gel reached 5 Pa.

Statistical analyses

To study the distribution of PMNC between udder health classes based on SCC, all samples (n=319) were categorized into the following classes: SCC ⩽100, 101–200, 201–300, 301–400 and >400 × 1000 cells/ml. Differences in PMN% and PMNC between these categories were analysed with Student's unpaired t test using SAS Version 9.1 (SAS Institute Inc., Cary NC, USA). To assess relationships between SCC, and PMN% and PMNC, Pearson correlation and linear regression was used. To investigate relationships between SCC, PMNC and milk quality parameters, samples (n=91) were categorized as below the 25th percentile (low), 25th to 75th percentile (medium), or above the 75th percentile (high) for SCC and PMNC, respectively. Student's unpaired t test was used to assess differences in milk quality parameters between cell categories within SCC and PMNC. In all analyses, SCC, PMNC and proteolysis were log-transformed to obtain normal distribution. A level of P<0·05 was accepted as statistically significant.

Results

Somatic cell count in relation to proportion and number of polymorphonuclear leucocytes

Milk samples with high SCC also contained a high proportion of PMN (Fig. 1). Cells in samples allotted into the different SCC categories, i.e. ⩽100, 101–200, 201–300, 301–400, and >400 × 1000 cells/ml, consisted of (mean ± SD) 16·1 ± 6·7, 21·2 ± 8·2, 24·0 ± 9·1, 24·5 ± 9·5 and 34·2 ± 10·5% PMN, respectively. All SCC groups, except the groups containing 201–300 and 301–400 × 1000 cells/ml, differed in PMN%.

Likewise, PMNC increased with increasing SCC (Fig. 1). On average, milk samples of the different SCC categories contained (mean ± SD) 12·3 ± 6·7, 30·8 ± 13·9, 56·8 ± 22·7, 84·9 ± 34·2 and 226·5 ± 122·3 × 1000 PMN/ml, respectively. All SCC groups differed significantly with respect to PMNC.

Both Pearson correlation (r=0·849) and linear regression analyses (P<0·001) indicated a strong relationship between SCC and PMNC (Fig. 2). When comparing SCC and PMN%, the correlation coefficient was 0·393 (P< 0·001; data not shown).

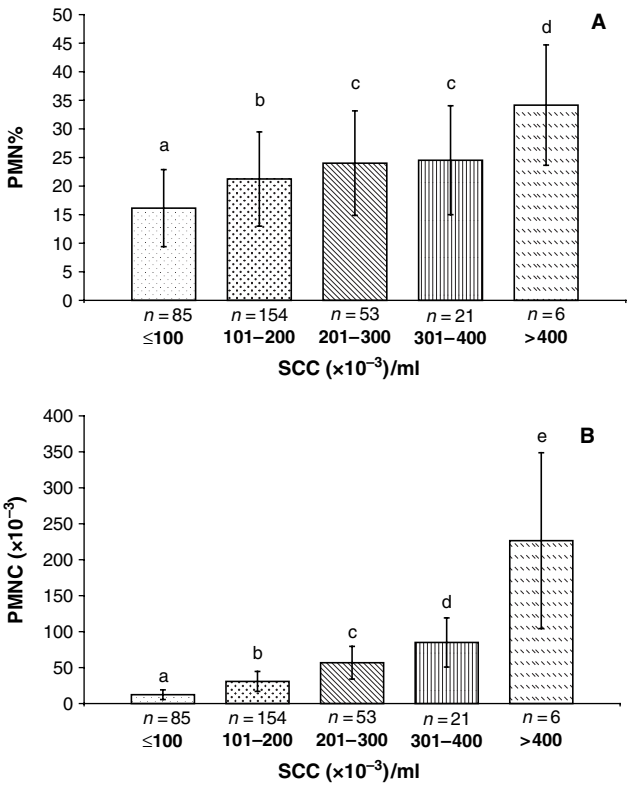

Fig. 1. Proportion of polymorphonuclear leucocytes (PMN%; A) and PMN count (PMNC; B) in bulk tank milk samples (n=319) categorized in somatic cell count (SCC) classes. Bars illustrate means ± SD. Categories with different letters differ significantly (P<0·05).

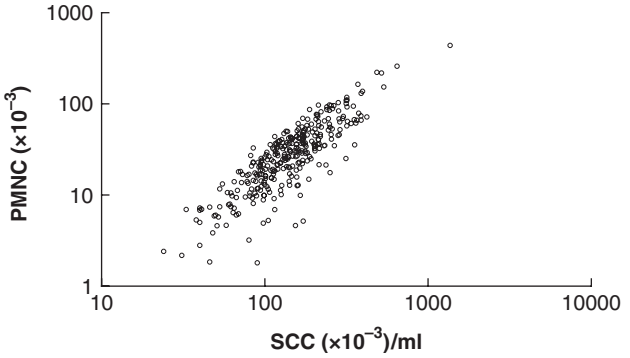

Fig. 2. Relationship between somatic cell count (SCC) and polymorphonuclear leucocyte count (PMNC) in 319 bulk tank milk samples (r=0·849; P<0·001).

Somatic cell count and polymorphonuclear leucocyte count in relation to milk quality parameters

Low and high SCC samples differed in contents of lactose and casein, casein number and proteolysis (Table 1).

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Table 1. Differences in quality parameters between bulk tank milk samples (n=91) with low, medium and high somatic cell count (SCC). Means ± SD within a row with different superscripts are significantly different (P<0·05)

	Low SCC†	Medium SCC‡	High SCC§
n	23	45	23
SCC ($\times 10^{-3}$/ml)	74·70 ± 22·40^{a}	155·60 ± 31·25^{b}	392·22 ± 237·10^{c}
PMNC¶ ($\times 10^{-3}$/ml)	16·66 ± 8 270^{a}	45·33 ± 17 850^{b}	133·35 ± 84·80^{c}
PMN, %	21·6 ± 6·8^{a}	28·5 ± 6·6^{b}	33·6 ± 7·4^{c}
Fat, g/100 ml	4·68 ± 0·47^{a}	4·39 ± 0·44^{b}	4·47 ± 0·28^{ab}
Lactose, g/100 ml	4·65 ± 0·13^{a}	4·61 ± 0·10^{ab}	4·57 ± 0·08^{b}
Total protein, g/100 ml	3·60 ± 0·22	3·53 ± 0·17	3·50 ± 0·15
Casein content, g/100 ml	2·63 ± 0·16^{a}	2·58 ± 0·13^{ab}	2·54 ± 0·11^{b}
Whey protein, g/100 ml	0·96 ± 0·07	0·96 ± 0·04	0·95 ± 0·05
Casein number	73·17 ± 0·69^{a}	73·06 ± 0·56^{b}	72·51 ± 0·70^{b}
Proteolysis, mM	1·03 ± 0·06^{a}	1·07 ± 0·06^{ab}	1·08 ± 0·07^{b}
Clotting time, s	125·8 ± 44·9	118·6 ± 40·9	116·7 ± 44·0
Gel strength, Pa	348·3 ± 123·0	353·2 ± 97·9	348·2 ± 149·2

† <25th percentile of the sample values [SCC range (33–104) × 1000/ml]
‡ 25th–75th percentile of the sample values [SCC range (105–221) × 1000/ml]
§ >75th percentile of the sample values [SCC range (222–1397) × 1000/ml]
¶ Polymorphonuclear leucocyte count, PMNC.

Table 2. Differences in quality parameters between bulk tank milk samples with low, medium and high polymorhonuclear leucocyte count (PMNC). Means ± SD within a row with different superscripts are significantly different (P<0·05)

	Low PMNC†	Medium PMNC‡	High PMNC§
n	23	45	23
SCC¶ ($\times 10^{-3}$/ml)	80·61 ± 29·96^{a}	157·89 ± 47·68^{b}	381·83 ± 243·30^{c}
PMNC ($\times 10^{-3}$/ml)	15·45 ± 6·73^{a}	43·66 ± 13·07^{b}	137·82 ± 81·16^{c}
PMN, %	19·11 ± 4·66^{a}	28·10 ± 5·11^{b}	36·72 ± 5·36^{c}
Fat, g/100 ml	4·60 ± 0·42	4·39 ± 0·48	4·55 ± 0·28
Lactose, g/100 ml	4·63 ± 0·13	4·62 ± 0·11	4·58 ± 0·07
Total protein, g/100 ml	3·57 ± 0·22	3·52 ± 0·19	3·53 ± 0·12
Casein content, g/100 ml	2·61 ± 0·16	2·57 ± 0·14	2·56 ± 0·08
Whey protein, g/100 ml	0·97 ± 0·06	0·95 ± 0·05	0·95 ± 0·05
Casein number	73·22 ± 0·70^{a}	72·98 ± 0·67^{a}	72·61 ± 0·52^{b}
Proteolysis, mM	1·05 ± 0·07	1·07 ± 0·06	1·07 ± 0·07
Clotting time, s	123·61 ± 43·16	116·93 ± 40·93	122·22 ± 45·71
Gel strength, Pa	349·39 ± 123·69	354·18 ± 95·04	345·17 ± 152·17

† <25th percentile of the sample values [PMNC range (5–25) × 1000/ml]
‡ 25th–75th percentile of the sample values [PMNC range (26–80) × 1000/ml]
§ >75th percentile of the sample values [PMNC range (81–437) × 1000/ml]
¶ Somatic cell count, SCC.

Lactose and casein contents and casein number were lower in the high SCC group compared with the low SCC group, while the opposite was the case for proteolysis. The fat content was lower in the medium SCC group compared with the low SCC group, but there was no difference in fat content between low and high SCC samples. Differences in contents of total protein and whey protein, clotting time and gel strength between SCC groups were not observed.

Table 2 presents the milk quality parameters of samples with low, medium and high PMNC. The only significant difference between the group categories was in casein number, where samples with a high PMNC had a lower casein number than samples with low PMNC.

Discussion

During mastitis, the increase in milk SCC is primarily due to the large recruitment of PMN. Therefore, in milk from a quarter with high SCC, the proportion and number of PMN will be higher than in milk from a healthy quarter. For bulk tank milk, consisting of commingled milk from many individuals and several milkings, the variation in PMN% is less pronounced. In the present study, the bulk tank milk samples on average contained 20·8% PMN, and the average proportion of PMN among samples with >400 × 1000 cells/ml (n=6) was 34%. One of the samples contained as much as 1.4 million cells/ml with 39% PMN, indicating that the prevalence of subclinical mastitis in the

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herd was high. Compared with values previously reported, the proportions of PMN observed in our study may seem rather low. Notably, only a limited number of studies have been performed with bulk tank milk, since most studies are with milk from individual quarters or cow composite milk. Using direct microscopy, Gargouri et al. (2008) investigated 80 bulk tank samples with exceptionally high SCC [range (600–1700) × 1000 cells/ml] and found that PMN on average constituted 32% of the cells, which is in agreement with our results. O'Brien et al. (2003) found that more than 30% of samples with a SCC range of (200–400) × 1000 cells/ml contained over 70% PMN. These results should, however, be taken with some caution since the method used is an in-house ELISA by O'Sullivan et al. (1992)

Since microscopic counting of stained cells is laborious, and the results very much depend on the person counting the cells, flow cytometry is frequently used in cell differentiation. In the present study, initial attempts to use a flow cytometry method described by Dosogne et al. (2003) for cell differentiation of low-SCC milk were discontinued. Considering that the bulk tank milk samples used consisted of commingled milk from four different milkings, the age and condition of the PMN were regarded as limiting factors. Most flow cytometry methods for cell differentiation in milk involve several steps of centrifugation. During centrifugation, there is a risk that the macrophages will associate with the cream layer, and thus be partly removed. This will create a higher proportion of PMN in studies based on cell suspensions as compared with whole milk. Microscopy based on smears of cell suspensions may be criticized for the same reason and therefore, smears of whole milk samples were used in this study. However, the variation in results between methods based on microscopy and flow cytometry, respectively, as well as differences between these two techniques, is substantial. As an example, various authors have reported proportions of PMN in milk from healthy udders of 3–95% (Schröder & Hamann, 2005).

Owing to the dilution effect in bulk tank milk, the correlation between SCC and PMNC was expected to be of lower magnitude compared with correlations reported for quarter milk (Kelly et al. 2000). However, in the present study, a strong correlation between SCC and PMNC was found (r=0·85). O'Brien et al. (2003) studied milk at udder quarter, cow composite and bulk tank level, and found correlation coefficients between SCC and PMNC of 0·92, 0·87 and 0·73, respectively. Gargouri et al. (2008) reported a correlation coefficient of 0·88 between SCC and PMNC, using bulk tank milk samples with very high SCC. Kelly et al. (2000) conducted a study including bulk tank milk samples in a SCC range more similar to ours, and found a correlation coefficient of 0·69 between SCC and PMNC

Whereas relationships between SCC and milk quality parameters have been frequently studied in bulk tank milk, studies on the relationship between PMNC and milk quality are rare. Partly owing to its content of intracellular proteinases, several authors have suggested that PMN could be important for milk quality, and might also serve as a marker of milk quality (Kelly et al. 2000; Pillai et al. 2001; Vangroenweghe et al. 2002; Le Roux, 2003; O'Brien et al. 2003). In contrast to such expectations, the findings of the present study do not indicate that PMNC in bulk tank milk is related to milk quality to a large extent. Casein number was the only milk quality parameter that differed between PMNC groups, with decreasing casein number associated with increasing number of PMN, despite no significant differences in contents of total protein, whey protein or casein. When samples were grouped according to PMN%, no differences were found between low, medium and high PMN% samples (data not shown).

In contrast to PMNC, SCC was related to several milk quality parameters in the present study. High SCC milk had lower lactose content, which also has been found by other authors. This finding is due to leakage in the blood-milk barrier as well as reduced biosynthetic ability of the secretory cells (Shuster et al. 1991; Berning & Shook, 1992). High SCC milk also contained less casein and had a lower casein number and higher activity of proteolysis. Similar results have been reported for quarter and cow composite milk (Somers et al. 2003; Larsen et al. 2004). The effect on casein can be explained both by reduced synthesis of milk-specific components and an increase in proteolytic enzymes in milk, originating in serum and/or somatic cells. The milk concentration of plasmin increases with increasing SCC and plays an important role in proteolysis of casein (Kelly et al. 2006). Although the method used to estimate the extent of protein degradation in this work is rather insensitive (Harayani et al. 2003; Le et al. 2006; Åkerstedt et al. 2008) statistically significant differences between bulk tank milk with high and low SCC were observed. It may be argued that this difference is of minor practical importance. Considering the limited sensitivity of the method, these differences may be underestimated and with a more sensitive method, e.g. HPLC, observed differences might have been more pronounced. Even though milk with high SCC contained less casein and had a lower casein number, SCC had no effect on rennet coagulation properties of bulk tank milk. Politis & Ng-Kwai-Hang (1988) showed a decline in cheese yield for high SCC milk after adjustment for casein, indicating that casein is only one of several variables affecting cheese yield. Leitner et al. (2006) reported negative effects on rheological properties when using high SCC milk, with longer clotting time and lower curd yield. Our results are, however, in agreement with a recent study by Leitner et al. (2008), where SCC was not a factor determining clotting time and curd yield. There may be several possible reasons for a weak association between SCC and clotting time and gel strength in bulk tank milk. The decrease in casein associated with elevated SCC could be too small to have an effect on rheological properties, and the coagulation process is probably too complex to be explained by variations

in individual milk components. Leitner et al. (2006) found differences in gel strength depending on species of bacteria present in the sample. They hypothesized that bacteria-specific effects on the inflammatory response could explain changes in milk composition and therefore predict milk quality for cheese production.

The fact that SCC was more correlated with milk quality parameters than PMNC may be due to a significant proteolytic effect of other types of somatic cells, such as the macrophages. The number of macrophages will also increase during mastitis (Azzara & Dimick, 1985; Sandholm et al. 1995) and their proteinases could thus contribute significantly to the breakdown of milk proteins. Cathepsin D, a lysosomal aspartic proteinase found in lysosomes of different tissues, is sometimes regarded as a macrophage proteinase (Kelly et al. 2006) and is strongly correlated with SCC. Macrophages also contain the plasminogen activator urokinase, which induces conversion of plasminogen to plasmin, and thus contributes to proteolysis (Caroprese et al. 2007). Studies on relationships between macrophages and milk quality are therefore warranted.

The current study is, to our knowledge, the first relating PMNC in bulk tank milk to milk quality parameters. The study showed a strong correlation between SCC and PMNC in bulk tank milk, but the correlations observed between total SCC and milk quality parameters were not found when the effect on milk quality of one individual cell type, PMN, was evaluated. Therefore, our results do not support the idea that PMNC would be a better indicator of bulk tank milk quality than SCC. The results might, however, be different if the quality of the resulting dairy products, e.g. cheese, UHT milk and powder milk was studied, since effects associated with PMN might be more pronounced over time.

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