



Natural Variations of Milk Somatic Cell Count in Dairy Cows

Naturliga variationer i celltal i komjök

by

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Photo: Marta Woloszyn, 2006-07-20

**Institutionen för husdjurens
utfodring och vård**

**Swedish University of Agricultural Sciences
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**Examensarbete 243
MSc Thesis**

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ABSTRACT

The main objectives of this thesis were to find out which factors affect the day-to-day variations of somatic cell count (SCC) in bovine milk and how the results of on-line measurements of SCC can be applied in automatic milking systems. The effect of milking interval and milk fraction during sampling on SCC was investigated.

Mastitis is regarded as one of the most costly diseases in the world in dairy production. Most of the mastitis cases are without visible symptoms (subclinical) and pass unnoticed. Cows having subclinical mastitis contribute the most to the total production loss for the farmer. With the new technology of on-line measuring of milk SCC comes the possibility to measure the SCC at every milking at a very low cost, getting an good overview of the variations in SCC. But it is of great importance to be able to interpret these consecutive SCC values and to know how and why the variations of milk SCC fluctuate.

After investigating what factors that have an impact on an uninfected gland two experiments were performed, the first regarding the effect of milking interval on SCC and the second, the importance of the selected milk fraction. Both factors are assumed having an effect on the SCC.

In the first experiment data from 83 different cows during a four-month period were examined for the influence of milking interval on the SCC. The cows were housed in a barn with the automatic milking system VMS, provided by DeLaval. The VMS was equipped with an on-line cell counter, OCC. The data was analysed using SAS REG procedure. Even though a trend between lower SCC and longer milking interval could be seen on all milking data, the correlation was not significant on total milkings level. However, 82% of the single cows had a negative correlation between SCC and milking interval, of which 34% were significant ($P < 0.05$). Although the results of this study showed that milking interval affects an uninfected udder, there was no possibility of creating a compensating algorithm applicable on all cows.

The second experiment was performed in Switzerland during 40 days. A special milking machine with four separate milk containers was used enabling the measuring of quarter SCC. In total, 3420 milk samples from 19 cows were tested for SCC. All analyses were made by the Institution of Veterinary Physiology, University of Bern, using the Wilcoxon Signed Rank Test in SigmaStat. The result of this study showed that foremilk SCC did not very well represent the SCC of total milk of single quarters or whole udder milk. Foremilk SCC was significantly higher ($P < 0.001$). The study also showed that single quarter milk was not representative when estimating whole udder milk SCC. The mean of measured SCC of whole udder milk was significantly higher ($P < 0.001$) than the volume corrected SCC calculated from total quarter milk.

SAMMANFATTNING

Huvudsyftena med detta examensarbete var att ta reda på vilka faktorer som påverkar dag-till-dagvariationen av somatiska celler i komjölk och hur resultaten av on-line registrering av celltalet kan appliceras i automatiska mjölkningssystem. Effekten på celltalet av mjölkningsintervall och olika mjölkfraktioner under provtagning undersöktes.

Mastit anses vara en av de mest kostsamma sjukdomarna i världen inom mjölkindustrin. De flesta fall av mastit sker utan några synliga symptom, så kallad subklinisk mastit, och passerar obemärkt. Kor med subklinisk mastit är de som orsakar den största delen av lantbrukarens totala produktionsförlust. Med den nya on-lineregistreringsteknologin kommer möjligheten att mäta celltalet under varje mjölkning till ett mycket lågt pris. Detta ger en mycket god överblick på celltalsvariationerna för varje enskild ko. Det är av stor vikt att kunna tolka dessa på varandra följande värden och veta hur och varför celltalet varierar.

Efter att ha undersökt vilka faktorer som påverkar celltalet i ett friskt juver utfördes två försök; det första med avseende på hur mjölkningsintervallet inverkar på celltalet och det andra betydelsen av vald mjölkfraktion. Båda dessa faktorer antas ha en inverkan på celltalet.

I det första experimentet undersöktes data från 83 kor under en fyramånadersperiod med avseende på hur mjölkningsintervallet inverkar på celltalet. Korna inhystes i ett stall med det automatiska mjölkningssystemet VMS från DeLaval. VMS:en var utrustad med en on-linecellräknare, OCC. All data analyserades med "SAS REG procedure". Trots att det fanns en trend mellan lägre celltal och längre mjölkningsintervall då all celltalsdata för alla kor analyserades, var korrelationen inte signifikant. Däremot hade 82% av de enskilda korna en negativ korrelation mellan celltal och mjölkningsintervall, varav 34% var signifikant ($P < 0.05$). Trots att resultatet av detta försök visade att mjölkningsintervallet hade en inverkan på celltalet, var det inte möjligt att skapa en kompenserande algoritm som gick att tillämpa för alla kor.

Det andra experimentet utfördes i Schweiz under 40 dagar då en särskild mjölkningmaskin som tillät fjärdedelsmjölkning användes. Totalt mättes celltalet på 3420 mjölkprover från totalt 19 kor. All statistisk analys utfördes av institutionen för veterinärfysiologi, Berns universitet, med ett "Wilcoxon Signed Rank Test" i SigmaStat. Resultatet av detta försök visade att celltalet i förmjölken inte representerade celltalet i helmjölken från de enskilda fjärdedelarna och inte heller celltalet i heljuvermjölken. Celltalet i förmjölken var signifikant högre ($P < 0,001$). Försöket visade också att helmjölken från de enskilda fjärdedelarna inte var representativ vad gäller estimering av celltalet i heljuvermjölk. Medeltalet av det uppmätta celltalet i heljuvermjölken var signifikant högre ($P < 0,001$) än det volymkorrigerade celltalet uträknat från den totala fjärdedelsmjölken.

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INTRODUCTION

Mastitis is regarded as one of the most costly diseases in the world in dairy production. It causes great economical losses for the farmer, health problems and reduced welfare for the cow and problems with the raw milk quality for the dairy industry. It is not unusual to find that up to 40 per cent of the cows in a herd have mastitis. Most of the mastitis cases are without visible symptoms (subclinical) and pass unnoticed. Generally, producers put an emphasis on clinical mastitis, when there are visible symptoms, and underestimate the significance of subclinical mastitis, not realizing that for every clinical case in the herd there are 15 to 40 subclinical cases contributing to an elevated milk somatic cell count (SCC). Cows with subclinical mastitis contribute the most to the total production loss for the farmer.

Detection of subclinical mastitis is very problematic, as the milk looks normal and the udder isn't red or swollen. The cow looks healthy and there is no sign of fever, depression or dramatic drop in milk yield in the infected quarter. Yet, the subclinical mastitis cause a large overall reduction in milk yield and the undetected cows contribute to spreading infection in the herd when the mastitis is caused by bacteria.

Inflammations in the udder are followed by an increase of milk somatic cells. It is a sign that the cow's immune system is activated. Milk SCC is the best indicator of the extent to which the gland is involved in fighting an infection, and thus the best way to detect subclinical mastitis. SCC is the most widely accepted criterion for indicating the udder health status of a dairy herd.

Many countries have a milk recording program for monitoring SCC in the herds. In Sweden, this test is done on individual cow level once every month by the official milk recording. The dairies analyse the milk regarding bulk tank SCC once a week. The data from the dairy can tell the farmer a great deal about the overall udder health status of his herd, but it cannot pinpoint which cows that have mastitis. In the month between two measurements in the milk recording program a lot can happen regarding inflammation and SCC on cow level. A whole infection may pass unnoticed. The information received is very much dependent on the test day. Further, an elevation in SCC at the once monthly recording may be of other reasons than inflammation, which makes it difficult to draw accurate conclusions. A more frequent sampling would give more accurate information if the elevated SCC is due to inflammation.

With the new technology of on-line measuring of milk SCC comes the possibility to measure the SCC at every milking at a very low cost. It is of great importance to be able to interpret these consecutive SCC values and to know how and why the variations of milk SCC fluctuate. When is the elevation in SCC caused by natural variations and when is there an ongoing or new infection? At what point should the farmer react? The main focus in this thesis is how the results of on-line measurements of SCC can be applied. The implementation for the on-line cell counting will mainly be in automatic milking systems.

In this thesis I have chosen to concentrate only on SCC as indicator of mastitis. Other methods for detection of mastitis exist but are in this context not of interest since it is the on-line measuring of SCC that is in focus.

OBJECTIVE

The main objectives of this thesis were to find out which factors affect the day-to-day variations of somatic cell count in bovine milk, at what point the variation no longer should be treated as natural and how the results of on-line measurements of SCC can be applied in automatic milking systems. The effect of milking interval and milk fraction during sampling on SCC was investigated.

LITERATURE REVIEW

1. Biological background

Somatic cell count

General

Milk contains cells that derive from the blood – *somatic cells*. The somatic cells are primarily white blood cells, i.e. *leukocytes* (Östensson, 1993). They are present as part of the immune system of the udder and protective mechanisms of the mammary gland. The leukocytes include *macrophages*, *lymphocytes* and *polymorphonuclear neutrophils*, so-called *PMN* (Harmon, 2001). Epithelial cells, the cells that produce milk, can also be found in milk but to a much lesser extent, ranging from 0 to 7% of the total somatic cell population (Lee *et al.*, 1980).

In a healthy udder the SCC is nearly constant between days at udder composite milk level, the exception being the first weeks postpartum. A relatively constant number of cells are being secreted into the milk over the lactation (Emanuelson & Persson, 1984; Miller *et al.*, 1993). When the udder is infected, the cow's immune system is activated and the number of somatic cells increases. The measurement of the number of somatic cells in milk is known as SCC and is taken as a control of the udder health status and is also a measure of the severity of the mastitis.

Levels of SCC

At the whole udder level and udder quarter level the normal SCC in uninfected quarters is generally below 200'000 cells/ml milk but may be below 100'000 in first lactation animals (Harmon & Reneau, 1993). It has been suggested that cows with SCC of less than 200'000 cells/ml are not likely to be infected with major mastitis pathogens, but cows with SCC above 300'000 cells/ml milk are probably infected (Brolund, 1985; Smith, 1996). According to a study performed by Eberhart and co-workers (1979) 50% of uninfected cows had SCC under 100'000 cells/ml and 80%

were under 200'000 cells/ml. The authors state that an elevation above a level of 200'000 cells/ml is an indication of inflammation of the udder, which is caused by bacteria infection or is recovering from an infection. A study of 44 uninfected cows in their first to third lactation showed that the geometric mean SCC was 49'400 cells/ml (Laevens *et al.*, 1997). A 16-month survey done at the University of Kentucky showed that 4213 bacteriologically negative quarters had a geometric mean SCC of 29'000 cells/ml. Thus, an elevation above the 200'000 cell/ml level is generally considered abnormal and an indication of inflammation in the udder (Harmon, 2001).

A threshold of 200'000 cells/ml milk is the most commonly used when discussing the definition at what milk SCC level that indicates mastitis (Dohoo, 2001). The CMT (Californian Mastitis Test) is a commonly used diagnostic tool for mastitis. According to the Scandinavian scoring system, a score of 3 or higher (SCC exceeding 300'000 cells/ml) indicates udder disturbances. The American milk recording association DHIA (Dairy Herd Improvement Association) has set the same threshold value to DHI score no 5, equivalent to 283'000 cells/ml.

It is also of importance when discussing SCC to distinguish whether the measurement is made on whole udder or udder quarter level. The udder consists of four separate milk-producing glands independent of each other and often only one quarter is infected. Timms & Schultz (1984) showed in one study that 70-80% of the infected cows were only infected in one quarter in that particular study. Elevated SCC in one inflamed quarter may be masked by the dilution effect from the other, healthy quarters, especially since milk yield decreases in udder quarters with high SCC. In a study performed by Berglund *et al.* (2004) it was observed that in composite milk samples with less than 100'000 cells/ml, 12% of the milk samples contained one or more udder quarter with CMT 3 and above.

The inflammation process

The number of infections is in direct proportion to the amount and the species of the microorganisms surrounding the cow. How many cows that have an ongoing infection is determined by two main factors: how often the udder is exposed to mastitis causing substances and what possibility the cow has to fight the infection (Reneau, 1986).

It is important to distinguish between the terms infection and inflammation when discussing mastitis. Infection is the bacterial invasion whereas inflammation is the animal's immune reaction to either microorganisms or trauma. Both infection and inflammation may occur independent of each other.

As mentioned, bacterial infection is the most common inflammation cause and is caused when the bacteria invade the mammary gland through the teat canal. Inflammation may also be the result of local trauma and tissue damage or in rare cases due to hormonal imbalances. During infection the SCC can rise to several million cells/ml milk.

As bacteria grow in the mammary gland they release toxins and other metabolites, which stimulate defensive responses from the host. The macrophages initiate the inflammatory process by releasing a range of active substances stimulating similar responses among other leukocytes. These signals stimulate the migration of PMN from the blood into the mammary gland. The influx of PMN is the major cause of the increase in SCC. In a healthy udder the macrophage is the predominant cell type whereas in mastitic milk over 90% of the somatic cells are influxed PMN cells (Sandholm, 1995). On arrival in the mammary gland, the leukocytes ingest and kill the invading bacteria. This process results in the production and release of other pharmacologically active compounds causing further inflammation. (Bramley, 1992)

The inflammation process goes on even after the bacteria have been eliminated and until healing of the gland occurs. Schultz (1977) reported that it might take from a few days to several weeks (or longer) for the SCC to decrease after the elimination of pathogens from the mammary gland. This aspect should be kept in mind whenever meeting the problem of high SCC in milk with negative bacteriological culture results. Another explanation of the negative bacteriological culture results is that the number of bacteria might be too low to be detected or that the bacteria hide (Sandholm, 1995).

In the early stages of an infection, i.e. the acute phase, the SCC peak is reached within hours or days depending on the infecting pathogen. The SCC closely parallels the number of bacteria in milk. The peak is then followed by a small decline in SCC as the leucocytes kill bacteria. The degree of the decline in SCC varies considerably depending on the environmental factors, the pathogen involved and the individual cow (Harmon & Reneau, 1993).

The defence mechanism may successfully eliminate the infection or the infection may persist, partly controlled by the host defence, for a longer period of time and often in the state of subclinical mastitis. In some cases, when the bacteria thrive well in the udder, the mastitis can develop into a chronic state. The changes in enzyme activity as a consequence of the inflammation make the milk a good growth medium for the bacteria (Sandholm & Ali-Vehmas, 1995). A vicious circle is initiated, in which the inflammatory response actually enhances bacterial growth and this predisposes the udder to recurrent infections. In chronic infections, both the SCC and number of bacteria tend to fluctuate up and down, and lead to repeated cases of clinical mastitis (Sears *et al.*, 1990).

Most infections occur during the lactation period, but a relatively large proportion of the cows are infected during the dry period and at calving. Infections that occur during the dry period may remain latent and easily become acute during the first weeks of lactation. On average 20% of all quarters are infected during the dry period and approximately 10% of the quarters are infected at calving (Sandholm & Pyörälä, 1995b).

Subclinical and clinical mastitis

Definitions

An inflammation in the mammary gland, **mastitis**, is the cow's defence against, and the reparation of damage that has affected the tissue in the mammary gland. The most common cause is when microorganisms, usually bacteria, infect the gland (Crist *et al.*, 1997; Harmon, 1994; Sheldrake *et al.*, 1983).

Clinical mastitis occurs when the inflammation is substantial and there are visible signs of the disease. Mild signs are clots or flakes in milk and a slight swelling of the infected quarter. In severe cases of clinical mastitis the milk secretion is abnormal and the udder is warm and swollen. The cow often has a loss in appetite, may have fever and rapid pulse. In rare cases the mastitis can result in death (Crist *et al.*, 1997).

Most cases of mastitis are **subclinical**. There are usually no visible signs of the disease and the inflamed mammary gland is therefore difficult to detect. However, the SCC of the milk will be elevated and through monitoring the SCC inflammation can be revealed (Crist *et al.*, 1997).

The consequence of subclinical infections

Generally, producers put an emphasis on clinical mastitis and underestimate the significance of the subclinical state. Subclinical mastitis causes the greatest financial lost to dairy farmers through lowered milk production and maintains infection foci in the herd (Sandholm, 1995). For every clinical case of mastitis, there are 15 to 40 subclinical cases (Bailey, 1996; Crist *et al.*, 1997).

2. Consequences due to mastitis

High somatic cell count in milk is highly associated with mastitis, which is the most common and economically important production disease among dairy cows in the developing countries. Milk SCC has proven to be a good indicator of udder health and is the most frequently used indicator of subclinical mastitis (Östensson, 1993). An increase in SCC above 100'000 cells/ml milk has been associated with a progressive decrease in milk yield and an adverse impact on dairy product quality (Jones *et al.*, 1984; Jones, 1986).

Milk yield

Decrease in milk production

The most significant consequence of mastitis from the economical point of view is the reduction in milk yield; it contributes to the loss of income with 70 per cent of the total losses caused by mastitis (Taponen & Myllys, 1995; Bailey, 1996). The reduction is caused by degeneration of the milk producing tissue during inflammation. According to the British Department of Agriculture it is assumed that mild cases of mastitis result in 5% reduction in lactation yield, while severe cases are associated with a yield loss of 15% (Kossaibati *et al.*, 1998). Table 1 shows the effect

of elevated SCC on the milk yield. As the milk somatic cell count exceeds 100'000 cells/ml, milk quantity begins to decrease.

Table 1. The effect of SCC in bulk milk on the milk yield (Korhonen & Kaartinen, 1995).

SCC in bulk milk (cells/ml)	Reduction in yield (kg/cow/year)	Reduction in yield (%)
< 250'000	-	-
250'000-500'000	200	4
500'000-750'000	350	7
750'000-1000'000	750	15
> 1000'000	900	18

It has been indicated that when one or more udder quarters are infected, the uninfected quarters to some extent compensate for the milk loss. Despite the compensatory effect there is still a large reduction in total milk yield. (Hamann & Reichmuth, 1990)

Reduced production after recovery from mastitis

Inflammation has pathological consequences, among them, tissue damage and alteration of secretory function. Mastitis infections during early lactation may damage milk secretory cells and thus have extended negative effects on cumulative milk yield. Reduced activity of milk secretory cells during early lactation continues to depress milk yield throughout the remaining lactation (Miller *et al.*, 2004). Regardless of the time of occurrence during the lactation, mastitis has a long-lasting effect on milk yield. Rajala-Schultz *et al.* (1999) reported that cows with mastitis did not reach their premastitis milk yields during the remainder of the lactation after onset of the disease.

In addition to the carry-over effect of elevated SCC within lactation, residual effects of an elevated SCC during a previous lactation may also exist for milk yield for a subsequent lactation. Fetrow and colleagues (1991) found that the effect of increased SCC during second lactation on third-lactation milk yield was significant. In a literature review (Fetrow *et al.*, 2000) different studies show that the carry-over effect of mastitis impact on the subsequent lactation ranged from 9 up to 50%.

Changes in milk composition

Physiological changes

Inflammatory reactions change the composition of milk both in terms of quantity and quality. As the degree of inflammation increases the chemical composition of milk approaches more and more that of blood. The permeability of the blood vessels increases resulting in the passage of ions, proteins and inflammatory cells from the blood circulation into the mammary gland. The milk producing epithelial cells break down and enzymes are released. The degradation of epithelial cells makes the milk production less efficient and the quantity of milk decreases (Korhonen & Kaartinen, 1995).

When the udder tissue is inflamed milk synthesis diminishes resulting in a dry matter fall with 5-15%. There are contradictory results in the literature dealing with the change of fat content in mastitic milk. Most investigations have shown a fat decrease with less than 10%. Although the change due to the inflammation in fat content in mastitic milk based on composite milk is relatively minor, there are differences between milk fractions (Sarikaya *et al.*, 2006). This study showed an increase in fat in the cisternal milk and in the first 400 g of the alveolar milk with increasing SCC. Also, the fat composition changes considerably as SCC increases (Korhonen & Kaartinen, 1995; Walstra *et al.*, 2006a). The total amount of fatty acids remains unchanged but the amount of short-chained fatty acids (C4-C12) increases whereas long chained fatty acids (C16-C18) decrease (Massart-Leen *et al.*, 1994; Walstra *et al.*, 2006a). Milk with a high SCC is more susceptible to spontaneous lipolysis and this causes breakdown of triglycerides, oxidation of fatty acids and rancid off-flavours. The quantity of free fatty acids increases, whereby the quality of milk is decreased.

In milk from a healthy udder approximately 80% of milk protein is casein and the remaining 20% is whey protein. The total quantity of protein does not decrease clearly until the SCC is approx. 1'000'000 cells/ml milk. The ratio between the different proteins, however, changes at a much lower SCC. With increased SCC the proteolytic (protein degrading) activity of milk increases and that results in a decreased proportion of casein. Whey proteins, on the other hand, increase with increased SCC (Korhonen & Kaartinen, 1995). Casein is a very important milk component in the cheese industry, as low casein content in milk means reduced cheese yield and deteriorated quality properties resulting in bitter taste and reduced shelf life in milk products (Korhonen, 1995; Walstra *et al.*, 2006b).

A highly negative correlation exists between SCC and lactose content (Berglund *et al.*, 2007). The amount of lactose in mastitic milk is reduced by approximately 10%. Lactose is the most important osmotic component in milk and a decreased lactose level leads to a disturbed balance between milk and blood. To maintain the osmotic balance, large amounts of sodium and chloride ions diffuse from blood to milk. When sodium and chloride become more concentrated in milk, the amount of calcium, phosphorus, magnesium and potassium decrease considerably. The change in salt balance weakens the curdling ability of milk and also reduces heat tolerance and the organoleptic properties (Korhonen & Kaartinen, 1995).

See Figure 1 for changes in milk production and different milk components with increasing SCC.

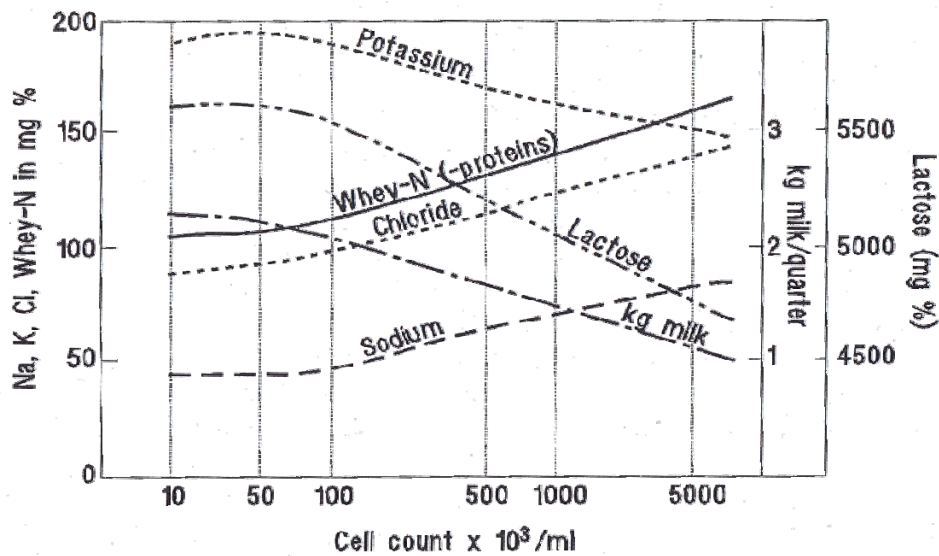


Figure 1. Changes in milk production and different milk components with increasing SCC (Korhonen & Kaartinen, 1995).

Factors affecting the payment from the dairy

In many countries dairies have a premium and deduction system according to the quality of the milk. In this part I have concentrated on the components that influence the quality parameters of the payment system of Swedish dairies. The EU and the Swedish Board of Agriculture regulate the quality parameters. Besides the content of fat and protein the quality factors are:

- SCC
- Bacteria count
- Spores
- Freezing point (included in payment as from 2006-04-10 for Arla Foods)
- Residues from antibiotics and other medicine
- Visibly changed milk

(Swedish Dairy Association, 2006-09-22).

In Sweden there is a joint agreement between the seven largest dairies, which together stand for over 99% of the Swedish milk production (Swedish Dairy Association, 2006-09-22). The authorities set the quality parameters as well as their limits, but regarding the payment model, the dairies can decide on their own.

The principle for the milk payment is that every producer shall get paid as much as possible for their milk. From the milk price, deductions or premiums are made depending on the quality of the milk. How the farmer is affected depends on which dairy association he or she belongs to. The milk price is also affected by season, geographic location and varying government subsidies.

All dairies in Sweden analyse the milk regarding the bulk tank SCC once a week. Somatic cell measures are significant because high quality milk with low SCC is a better raw material for the processing industry (Korhonen & Kaartinen, 1995). The premium and deduction system for SCC of Arla Foods, the major dairy in Sweden and Denmark, is shown in Table 2.

Table 2. The premium and deduction system (Arla Foods, 2006-04-20). Milk price in Sweden is currently SEK 2.70 (from April 1st 2006) for farmers within Arla Foods.

Limits in somatic cell count (x 1000 cells/ml)	Premiums / deductions (% of milk price)
<201	+2%
201-300	+1%
301-400	0
401-500	-4%
>500 ¹⁾	-10%

¹⁾ If shipped milk exceeds somatic cell limit three times in a row a deduction of 20% will follow. Fourth time and beyond results in a 30% deduction. The deduction is made in per cent of the raw material value of milk (approx. SEK 2.2). After three months of geometric mean > 400'000 cells a first warning is given. The second warning is given two months after the first if the SCC level doesn't decrease. One month after the second warning, the farmer is suspended. –Arla Foods (2006-04-20)

In European countries members of the EU, Australia and New Zealand the upper limit of bulk tank milk is 400'000 cells/ml. Canada has set the limit to 500'000 cells/ml and the US to 750'000 cells/ml. (Fetrow *et al.*, 2000)

Fat and protein content highly affect the payment of the milk but are not included in the quality factors established by the authorities. The monthly means in protein- and fat content serve as the basis for the payment of the milk (for the raw material value). As mentioned, mastitis has a negative effect on nutritional milk components making fat and protein content decrease at high SCC levels. This results in a reduction in milk payment. In Sweden there is still no distinction of the *quality* of fat or protein in milk, but the discussion of analysing for free fatty acids is highly topical. In Holland for example, an analysis of free fatty acid content is compulsory (Swedish Dairy Association, 2006-09-22).

To sum up, mastitis affects many quality parameters as it has an effect on cell count, bacteria count, losses in protein and fat content, smell and taste, and perhaps even antibiotics. Having a good health status and low mastitis incidence in the herd gives the producer a higher payment for the milk.

Decreased reproduction ability

In a study including around 1000 Holstein dairy cows on two commercial dairy farms in central California, Santos and colleagues (2004) found that cows that had a case of clinical mastitis prior to their first breeding, had an extended interval from calving to first insemination. Cows that had mastitis prior to pregnancy diagnosis had decreased conception rates when compared to the controls. In addition, there was an increase in abortions in cows with clinical mastitis compared with healthy cows.

These results are in accordance with Schrick *et al.* (2001) who saw that services per conception were 2.1 for cows with subclinical mastitis and 3.0 for cows with clinical mastitis, compared with only 1.6 for uninfected cows. The study showed that subclinical mastitis reduced reproductive performance of lactating cows similar to clinical mastitis. Subclinical mastitis followed by clinical mastitis resulted in the most severe loss in reproductive performance. The mechanism(s) by which subclinical or clinical mastitis may influence reproductive performance is still unknown. The authors discussed that changes in hormone balance due to mastitis may affect the follicular and oocyte development and therefore decrease the reproductive ability.

Diseases related to mastitis

Mastitis can sometimes be related to other diseases. Ill cows have a weakened immune system, which can predispose to mastitis. And also, cows with mastitis can have a predisposition to other diseases. By preventing mastitis the consequential risk of the cow contracting other diseases is significantly reduced.

Metabolic stress and other metabolic related diseases

During early lactation the cow often comes in a negative energy balance because of the large increase in milk production. The cow cannot consume enough feed to satisfy the metabolic demands causing metabolic stress. A recent study (Holtenius *et al.*, 2004) showed a difference in the metabolism and immune status between herds with high or low yearly mastitis treatment. Blood from 271 cows from 20 high yielding dairy herds was examined. Ten of the selected herds represented low mastitis treatment incidence and ten herds had high mastitis treatment incidence. Herds with high mastitis treatment incidence had a significantly lower eosinophil count, significantly lower concentration of the amino acids tryptophan, glutamine and arginine in plasma and a significantly higher concentration of nonesterified fatty acids. This indicates an increased metabolic stress in cows from herds with higher mastitis treatment incidents.

Ketosis or acetoanaemia is a common metabolic disease occurring during the first two months after calving in high-producing cows. The peak frequency of ketosis occurs during the fourth week and coincides with the peak of milk production. The primary type of ketosis impairs the cow's immune system and makes her predisposed to other diseases like mastitis. Results from a Dutch study (Kremer *et al.*, 1993) showed that ketonemic cows had more severe mastitis than non-ketonemic animals. Secondary ketosis often occurs in a cow that has some other disease that depresses her appetite, for instance an ongoing mastitis infection.

Cows with mastitis can suffer from a depression in appetite. At parturition this will result in a reduction of the total available amount of calcium from the diet. If the cow cannot meet the increasing need for calcium near calving, she will get hypocalcaemia, also called paresis or milk fever (Eddy, 2004). If the cow isn't treated immediately, death will occur within 10 to 24 hours.

Between 80 and 90% of the cases of left displaced abomasum (LDA) occur during the first month after calving when the cow is most susceptible to new diseases. Low feed consumption around parturition is a risk factor for LDA, which can make cows with a depressed appetite caused by mastitis more prone to the disease. Mastitis, metabolic stress, ketosis and milk fever are all factors that predispose a cow to abomasum displacement (Geishauser, 1995).

Culling and additional costs

In Sweden mastitis is one of the three major reasons why a cow is removed from the herd. The culling reasons “high cell count” and “mastitis” together stand for almost one fourth of all the cullings (Swedish Dairy Association, 2006-04-20). Mastitis leads to an increased involuntary culling and high replacement costs.

When a cow is being treated for mastitis costs like additional time for the herdsman, veterinary fees, costs of drugs and treatments and discarded milk due to antibiotics have to be added to the economical loss. The distribution of different costs is shown in Figure 2.

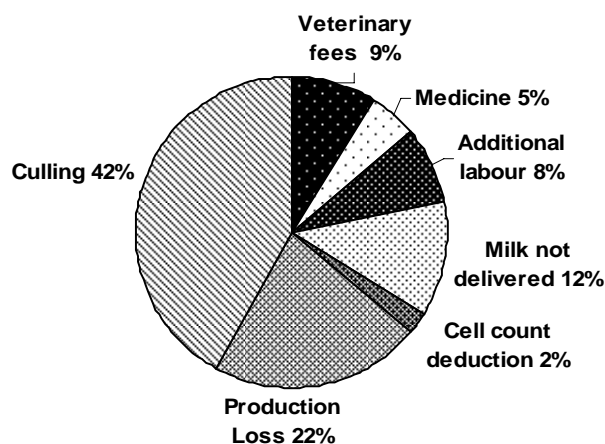


Figure 2. Distribution of costs associated with mastitis in an average Swedish dairy herd (SHS, 1996).

3. Natural factors affecting the variation of SCC

These factors are defined as natural in this thesis because a) they are not related to inflammation and b) because we cannot affect them from milking to milking (in an automatic milking system). According to Sjaunja (1986) the relative day-to-day variation in SCC for an individual cow is 9-10% (uninfected cow). This variation was not due to reasons related to inflammation. The study was made on cows milked twice daily with uneven milking intervals. How the day-to-day variation is affected when the cows are milked in an automatic milking system has not yet been investigated.

Variations related to milking

When evaluating the SCC it is very important to take the time of sampling into consideration. The SCC varies significantly with the fraction of milk collected during udder evacuation and with milking intervals.

Milk fractions during udder evacuation

The first fraction, i.e. the first squirts, include milk left behind from previous milking (residual milk) having high cell count, thus making the first fraction high in somatic cells. The SCC then decreases in the foremilk and further in the composite milk. The last streams of milk, strippings milk, have a high SCC (Östensson, 1993). The SCC curve during a milking is similar to the SCC curve throughout the whole lactation (see Figure 2) (Paape & Tucker, 1966). When the udder is healthy the differences in SCC between fractions are minor but this changes as the SCC increases. In an experiment performed by Sarikaya and Bruckmaier (2006) a significant change was observed in SCC during milking in quarters with a total SCC above 100'000 cells/ml. The strict foremilk sample, which only represented for 0.3% of the total milk volume, had a fivefold increase in SCC (cells/ml) compared with the total milk SCC. Further, the experiment showed that there are differences also within the fractions. The foremilk sample was categorized into 6 fractions consisting of one handstripped milk jet each. There was a significant decrease in SCC during milking of the foremilk fractions, meaning that any interpretation of the milk SCC must consider the fraction from which the milk sample was removed.

Milking intervals and milking frequency

The diurnal variation is largely caused by variation in milking intervals (Reneau, 1986). As mentioned, the SCC is at its highest in strippings during milking and immediately after milking. The SCC then remains high for 3-4 hours, followed by a steady decline until the next milking. The lowest cell count is obtained just before milking due to the dilution effect; as the milk yield increases the SCC decreases (Saloniemi, 1995a). After 10-12 h the milk secretion rate slowly starts to decline and after 25-30 h it has stopped completely. How the secretion rate declines and when it stops is individual for each cow, depending on the cisternal size (Hamann & Dodd, 1992).

Stage of lactation

In long term *uninfected* cows the number of cells in milk follows an approximate inverse lactation curve (Brolund, 1985; Schepers *et al.*, 1997), as seen in Figure 3.

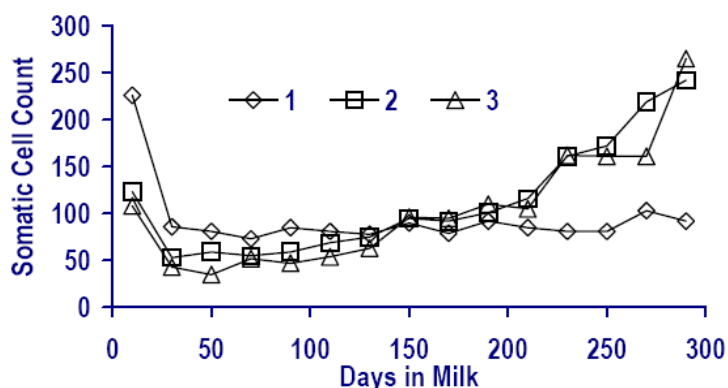


Figure 3. The upper 95% confidence limit of SCC in uninfected cows. The numbers in the legends indicate the three parity groups: first parity, second parity and third and higher parities. (Adapted from Schepers *et al.*, 1997).

The number of somatic cells is high immediately after calving but drops rapidly during the first week of lactation. The high cell count the first days of lactation is due to the high immunoglobulin content in the colostrum. If the cow has an infected udder the SCC decreases more slowly (Saloniemi, 1995a).

It has generally been observed that SCC increases with advancing stage of lactation as drying-off approaches. However, the change in milk SCC from *uninfected* quarters displays little change (Eberhart *et al.*, 1979; Saloniemi, 1995a; Sheldrake *et al.*, 1983). For cows with subclinical mastitis the SCC increases significantly towards end of lactation.

Recognition of inflammation caused by bacteria becomes more difficult towards drying-off because the SCC naturally increases. According to Saloniemi (1995a) the cell count of a healthy udder increases slightly after the daily milk production has fallen below 4 kg and the raise in SCC can be explained by increased concentration of somatic cells; an inverted dilution effect.

Age

SCC and mastitis incidence tends to increase as a cow gets older. However, age per se does not affect the SCC if the cow is healthy. The somatic cell reaction against infectious pathogens increases with age, which makes the cow more prone to new infections. Older cows with previous infections (may have been subclinical and passed unnoticed) have a higher SCC in general and tend to have an elevated cell count even in a healthy udder. They also tend to have infections that are longer and cause more extensive tissue damage and the SCC decreases relatively slowly after infection (Saloniemi, 1995a). Mastitis is increasing throughout number of lactations (Table 3).

Table 3. Mastitis incidence in different parities in Swedish dairy herds in 2005. The number of mastitis incidences per 100 cows in parities 1 through >5, and below the percentage of total disease incidents (Swedish Dairy Association, 2006-04-20).

Breed / Parity	1st	2nd	3rd	4th	5th	>5th
Swedish Red	9,10 (47%)	13,21 (52%)	18,60 (47%)	21,21 (43%)	24,10 (42%)	24,51 (43%)
Swedish Friesian	13,08 (53%)	18,92 (57%)	22,80 (49%)	26,51 (45%)	27,81 (43%)	28,34 (42%)

Season

The highest SCC generally occurs during the summer with the lowest counts occurring during the winter (Emanuelson & Persson, 1984; Kennedy *et al.*, 1982). This coincides with an increased incidence of clinical mastitis during the summer months. The seasonal effect is not physiological but a result of increased bacterial contamination during weather that provides better conditions for bacterial growth and increased frequency of teat damage during pasture (Reneau, 1986).

Breed and genetics

There are significant differences in SCC between different breeds, where Swedish Friesian have higher SCC than Swedish Red (Table 4) (Brolund, 1985; Saloniemi, 1995a; Swedish Dairy Association, 2006). However, these differences are considered as minor compared with the impact of bacteriological status. Also, the differences between breeds will not be of importance when evaluating the variation in SCC within an individual cow.

Table 4. Mean SCC (cells/ml milk) in two different Swedish breeds in parities 1 through 4 and total for all parities (Swedish Dairy Association, 2006-04-20).

Breed / Parity	1st	2nd	3rd	4th	Total
Swedish Red	59'000	88'000	114'000	135'000	86'000
Swedish Friesian	74'000	106'000	135'000	158'000	103'000

Since the heritability for mastitis and somatic cell count is very low ($h^2 = 0.02$), the breeding progress is slow (Emanuelson *et al.*, 1988; Norberg *et al.*, 2006, Svensk Avel, 2006). Nevertheless, since breeding for increased production leads to decreased resistance against mastitis (Emanuelson *et al.*, 1988), it is important to incorporate mastitis resistance in the breeding goals. Many countries have monthly measures of the SCC from individual cows for breeding purposes. In Sweden, health traits have been a part of the breeding goals for centuries and big effort has been made on those traits that lead to healthier cows (Svensk Avel, 2006).

Individual variation

Cow variances within a herd reflect the relative importance of genetic effects and permanent environmental effects characteristic for the individual cow. These variances are consistent across test-day observations within lactation. According to a study by Kennedy and co-workers (1982) the average cow variance for SCC was

30.8% of the total variance (ranging from 23.8 to 34.7%). A study by Emanuelson and Persson (1984) shows that the most important factor affecting uninfected cows is variation of the individual cow, which accounted for 38-46% of the total variation. Lindström *et al.* (1981) came to similar conclusions with the variation of the individual cow accounting for 35% of the total variation of SCC. This in accordance with Schepers *et al.* (1997) who also found that the effect of 'cow nested within herd' showed a large portion of the variation of SCC in those cows with negative bacteriological culture results.

Udder conformation

The teat canal is the gateway through which the microorganisms have to invade in order to establish an infection in the mammary gland. Jørstad and co-workers (1989) found that teat canal diameter, teat injury and increased sphincter patency have a strong positive association with high SCC. The study indicated further a highly significant association between leakage of milk and high SCC. In a study by Persson Waller *et al.* (2003) a relationship was found between increased milk leakage and automatic milking systems, which is becoming a more and more common milking system today.

If the udder is of poor shape, i.e. the teat-floor distance is short; there is a higher risk of teat ramp and environmental contamination. Studies have indicated that cows with dish-shaped or well-attached rounded udders have less mastitis than cows with a pendulous shaped udder. A short distance from the udder to the ground is associated with a predisposition to mastitis (Saloniemi, 1995b).

4. Other important factors affecting the SCC

Increase in SCC due to bacteriological infection

The single most important factor affecting the SCC in milk is the infection status of the mammary gland. This holds true at the quarter, cow, or bulk tank level (Crist *et al.*, 1997; Harmon, 1994; Sheldrake *et al.*, 1983).

Different types of pathogens

The most common organisms that infect the udder can be divided into two groups; major and minor pathogens.

The major pathogens cause a strong inflammation response that result in the greatest SCC increase and compositional change of milk. They include: *Staphylococcus aureus*, *Streptococcus* spp. and coliforms (Harmon & Reneau, 1993). The minor pathogens include *Corynebacterium bovis* and coagulase-negative staphylococci (CNS) and usually only cause a moderate increase in SCC.

The gram-positive bacteria of *Staphylococcus* and *Streptococcus* are the most common casual agent of mastitis (Table 5). 95% of subclinical cases of mastitis and more than 60% of the clinical cases in the Nordic countries are caused by gram-

positive cocci. Of these, the most common pathogen is *Staphylococcus aureus*. This bacterium is the most problematic and significant of the bovine mastitis pathogens (Pyörälä, 1995).

The importance of coagulase-negative staphylococci (CNS) as a pathogen has increased during recent years. The bacteria of the CNS group cause nearly as many cases of both subclinical and clinical mastitis as *Staph. aureus* (Pitkälä, 2006). Another common pathogen, which causes almost one forth of the subclinical and clinical mastitis in the Nordic countries, is *Str. dysgalactiae*. *Str. agalactiae* however, which continues to be a problem in several countries in Europe and in the U.S., is only isolated from less than 1% of the samples in Scandinavia (Pyörälä, 1995).

The most common method of spread is from infected udders to other udders at milking time (Harmon & Reneau, 1993). Approximately one fifth of all cases of clinical mastitis in the Nordic countries are caused by coliforms of environmental origin, of which 85% are *E. coli*. In the rest *Klebsiella* and other enterobacteria are isolated (Sandholm & Pyörälä, 1995a). The source of environmental pathogens is the surroundings of the cow, e.g. the bedding, manure and soil (Harmon, 1994).

Table 5. The most frequent bacteria causing mastitis in the Nordic countries (Pyörälä, 1995). “-” means that no frequency data has been found.

Type of bacteria	Frequency subclinical mastitis	Frequency clinical mastitis
<i>Staph. aureus</i>	30-40%	20-30%
CNS	30%	20%
<i>Str. dysgalactiae</i>	20-25%	-
<i>Str. agalactiae</i>	-	1%
Coliforms	-	20%

Herd management and milking routines

Management factors

Mastitis is a disease significantly related to herd management (Reneau, 1985). Control of mastitis by procedures that reduce the rate of new infections has been very effective. Consistent use of teat dip, dry cow therapy, antibiomatic treatment of clinical mastitis, good milking technique, well-maintained machines and specific milking order has a great impact on lowering the herd SCC (Barkema *et al.*, 1998, Hutton *et al.*, 1990)

Management factors like type of housing, bedding and stall maintenance, and manure handling also have a significant influence on the infection rate (Reneau, 1986). A study performed by Barkema *et al.* (1999) found a relationship between SCC and management style. The farmers that had a “clean and accurate” style had lower bulk milk SCC than the “quick and dirty”. The first group of farmers worked precisely rather than fast; the latter group worked quickly rather than precisely. As a result, the farms with herds that had a low bulk milk SCC had better hygienic conditions than

those farms with herds that had a high bulk milk SCC. Farmers who kept better records and were more familiar with each cow in their herds had a lower bulk milk SCC. It was also important to have consistent routines as cows are creatures of habit and easily react on changes.

Nutrition

Feed and water deprivation has a great impact on milk production and results in reduced milk yield and elevated SCC. The increase in SCC can partly be interpreted as a dilution phenomenon (Reneau, 1986). The deprivation is often caused by a disease, for instance metabolic stress as mentioned earlier, that not only makes the cow eat less, but in addition weakens her immune system and makes her more prone to new infections. It is very important that the hygienic quality of feed and water is good to restrict infections caused by environmental bacteria.

Stress

Generally, cows harbouring subclinical mammary infections respond to stress with significant increases in milk SCC. Uninfected cattle, however, do not appear to respond in any significant proportion (Wegner *et al.*, 1976; Sears *et al.*, 1990). The exception is cows under heat-stress. When a cow is exposed to high temperatures, often combined with high humidity, she suffers from heat-stress. During heat-stress cows show reduced feed intake, decreased activity, increase respiratory rate, and increase both peripheral blood flow and sweating. These responses have a significant negative effect on milk production of the lactating cow (West, 2003). As mentioned earlier, a reduced feed intake has a big impact on milk yield. The same applies for water deprivation, which is not uncommon during high temperature conditions. During hot weather blood flow shifts to peripheral tissues for cooling purposes and may alter nutrient metabolism and contribute to lower milk yield (McGuire *et al.*, 1989). The total decline in milk yield results in a higher SCC.

Automatic milking systems and SCC

The difference in automatic milking systems (AMS) compared to conventional tied-up or parlour milking is that the cows on a voluntary basis decide when they are going to be milked. Within present time limits this results in a higher variation in the milking intervals and in most cases more than two milkings per day. The increased milking frequency gives rise to a higher milk yield and the higher daily milk yield may decrease the SCC due to the dilution effect (Kelly, 1998). According to Hillerton (1991) more frequently milked quarters had a lower SCC and fewer positive bacteriological tests compared to twice daily milking. The likely cause is that with increased milking frequency bacteria is removed of from the udder before they manage to cause inflammation.

The system also allows milking at quarter level basis, which enables gentler teat treatment with individual automatic detachment of teat cups and less risk of cross contamination between quarters. Both of these factors decrease the risk of infection and may contribute to a lower SCC level in the herd.

In the automatic milking system there is no visual inspection of the milk during pre-milking, and the milk has to be controlled by the use of different sensors on-line. The detection capability of the milk robot is highly demanded.

5. Summary of variation factors

As the literature review has shown, there are many factors affecting the milk SCC. Many of the factors are natural, others are external and unwanted. The single most important factor affecting the SCC in milk is infection status. If the mammary gland is infected, other factors like age and stress will also have a negative effect on the SCC. If the gland is uninfected, however, few other factors will have an impact on milk SCC. The factors that do have an impact on an uninfected gland are:

- Normal diurnal variation – the milking interval
- Milk fraction collected throughout milking
- Colostrum milk first weeks after calving
- When the milk production is low close to drying-off
- Deprivation of water and feed
- Day-to-day variation (10%)

Apart from first weeks after calving, the factor that affects the SCC the most if the udder is uninfected is milking interval. Due to the dilution effect, the SCC decreases from approximately four hours after previous milking until next milking. In practical terms this means that the farmer may observe if an increase in SCC is due to a shorter milking interval. In automatic milking systems there are no fixed intervals and the interpretation of the SCC may be more difficult; with fixed intervals it is much easier to compare the cell counts. The first part of this thesis investigates the effect of the milking interval and if it is possible to compensate for it in an automatic milking system.

Depending on which milk fraction during milking is analysed, the cell count varies. Most analytical methods measure the SCC in the foremilk. In the automatic milking system VMS™ (DeLaval, Sweden) the analysis is done on the total milk. The second part of this thesis examines if an estimation of total milk SCC can be done based on single quarter foremilk and single quarter main milk.

In Sweden the cell count on individual cows is measured only at the national milk recording performed once a month. The farmer gets an overview of his cows' health status but a big disadvantage is the large time gap between measurements. Another method of testing for mastitis is by using the Californian mastitis test (CMT). This test is easy to perform but usually the farmer needs an indication of an udder problem before testing a cow. Moreover, the CMT can only be used to detect very high somatic cell counts. The test is good at detecting infections in quarters that have SCC > 500'000 cells/ml but infected quarters may be missed when the cell count is lower. The relationship between SCC values and CMT is not precise because of the high degree of variability in SCC values of each CMT score (Ruegg *et al.*, 2005). With the

advantage of measuring the SCC at every milking the farmer is given a whole new level of knowledge and control. The mastitis infection could be detected in a much earlier stage benefiting both the farmer's economy and the welfare of the cows.

MATERIAL AND METHODS

Two different experiments were performed in this study. In the first experiment the effect of milking interval on SCC was studied and in the second experiment mastitis detection and estimation of total milk SCC based on single quarter foremilk and single quarter main milk was evaluated.

1. The effect of milking interval on SCC

The experiment was carried out at Kungsängen Research Centre, Uppsala Sweden, from May 18th to August 26th 2006.

Animals

The cows used in the experiment were those housed in the stable with an automatic milking system during the three-month period. There were normally 54 to 56 cows housed at a time. During the whole study there were 83 different cows housed in the stable. The cows were of the breed Swedish Red and were in parities ranging from 1st to 7th. The calvings were evenly distributed over the year with a slightly increased calving rate during the winter months.

Feeding

The cows were on pasture during the whole experimental period. In the barn they were fed with silage in *ad lib.* and concentrate according to their individual milk yield.

Milking

Since the cows were housed in the stable with an automatic milking system, there were no fixed milking intervals except for the milking permission, which was given after 6 hours since last milking. The cows came and went as they wanted from pasture. The cows were milked with the automatic milking system VMSTM, provided by DeLaval (DeLaval Voluntary Milking System VMSTM). The VMSTM was equipped with an on-line cell counter, OCCTM (DeLaval On-line cell counter OCC), which recorded the SCC at every milking.

The OCCTM

The OCCTM is based on the same technique as the DeLaval Cell Counter (DCCTM) and has the same specifications. Studies have showed that the DCCTM correlates very well with the fluorescence based electronic cell counter (Fossomatic, Foss Electric, Hillerød, Denmark) (Hamann & Redetzky, 2004; Ruegg *et al.* 2005), which is the generally accepted standard cell counting method (Holtorp, 1989).

Repeatability of the DCC™ and OCC™ (DeLaval, 2004)

- 12% at 100'000 cells/ml
- 8% at 400'000 cells/ml
- 7% at 1'000'000 cells /ml

The DCC™ is a portable and battery operated optical cell counter that delivers fast measuring results. A special cassette is used to collect the milk for analysis. This cassette contains a small amount of reagents, which when mixed with the milk reacts with the nuclei of the somatic cells. After insertion of the cassette into the DCC™, the cassette is exposed to light, which gives rise to fluorescent signals. This is recorded in an image, and that image is used to determine the number of somatic cells in the milk.

The OCC™ uses the same measuring technique but here the cell counter is built-in in the VMS™. The collection of a representative milk sample is done during milking from each cow and no cassettes have to be used. The whole procedure runs automatically and the cell count for each cow is stored in the herd management database. The OCC™ will be on the market as of 2007 as optional equipment to the VMS™.

Experimental design and data recording

The study was done to investigate the influence of milking interval on SCC for each cow. During four months milk SCC and milking intervals were recorded. After every milking the on-line cell counter stored the specific data in a log file. From the herd management program ALPRO™ (DeLaval, Sweden) data such as lactation number, calving date and milk yield was extracted.

Data handling and statistical analysis

In total 8019 milkings from 83 cows were analysed. No discrimination was done regarding infection status. Some cows were not housed in the VMS™ stable during the whole test period and the OCC™ was sometimes out of function. For these reasons the cows included in the study have different amounts of milking data.

All SCC data from the OCC™ and information regarding the milkings from May 18th to August 26th was compiled to an excel file. The SCC was transformed into the natural logarithm to get a normal distribution. The data was analysed using SAS REG procedure (SAS version 9.1) regarding how the length in milking interval affects the cell count.

B: Mastitis detection and estimation of total milk SCC based on single quarter foremilk and single quarter main milk.

The experiment was carried out at ALP Research Centre, Posieux Switzerland, from June 21st to July 31st 2006. It was one part of a series of experiments investigating different aspects of the SCC.

Animals

Data was collected during 40 days from 19 cows of mixed Swiss breeds. The five cows in each group were in 1st to 5th parity, except in the first group where the cow from 1st parity was removed because of stress.

Feeding and management

The cows were housed in a conventional stable and fed twice daily, just before milking. They were fed manually with approx. 12 kg of hay produced on the farm (85% DM), 6 kg of maize silage and approx. 4 kg of concentrates, given on the feeding table, per day. After the evening milking the cows were released on pasture. Between morning and evening milking they were kept in the barn because of the high outdoor temperature. Water was available ad libitum.

Milking

The cows were milked twice daily; at 05.30 and 15.30. A special milking machine with four separate milk containers was used to enable the measuring of quarter SCC. See picture 1. One cow was milked at a time. The udder was stimulated for approx. 30 seconds and a foremilk sample was taken from each quarter. The cow was then milked with the milking equipment allowing separation of quarter milk. Quarter milk yields were recorded by weighing and a milk sample was taken from each quarter container. Finally, the quarter milk was mixed and a composite udder milk sample was taken. Totally, nine samples were taken from each cow at every milking. Between cows the milking equipment was cleaned.



Picture 1. Milking equipment used for milking of separate quarters (Photo: Marta Woloszyn).

Experimental design and data recording

The cows were divided into four groups with five cows in each group. These five cows were from parity 1 through 5, except in the first group where the cow from parity 1 was removed because of stress. All cows in each group were approximately in the same stage of lactation, starting at quite early in lactation for the first group to late in lactation for the fourth group. Each group was tested for foremilk SCC on each quarter after udder stimulation, total quarter SCC and total udder milk SCC for subsequent 10 days; a total of nine milk samples per milking from each cow. This routine was repeated for all four groups and resulted in 3420 milk samples from 19 different cows from 40 days in total.

Data handling and statistical analysis

All foremilk samples, quarter milk samples and total milk samples were tested for SCC using the DeLaval Cell Counter (DCC™). In total, 3420 milk samples were tested for SCC. In addition to the total milk SCC measurement the SCC was calculated by the quarter milk SCC corrected for quarter milk yield.

All analyses were made by the Institution of Veterinary Physiology, University of Bern, Switzerland. A paired t-test was done using the Wilcoxon Signed Rank Test in SigmaStat version 3.5. All results are after Bruckmaier and Wellnitz (2006).

RESULTS

A: The effect of milking interval on SCC

Total cow level

Even though a trend between lower SCC and longer milking interval could be indicated from all milking data, as seen in Figure 4, the correlation was not significant on total milkings level.

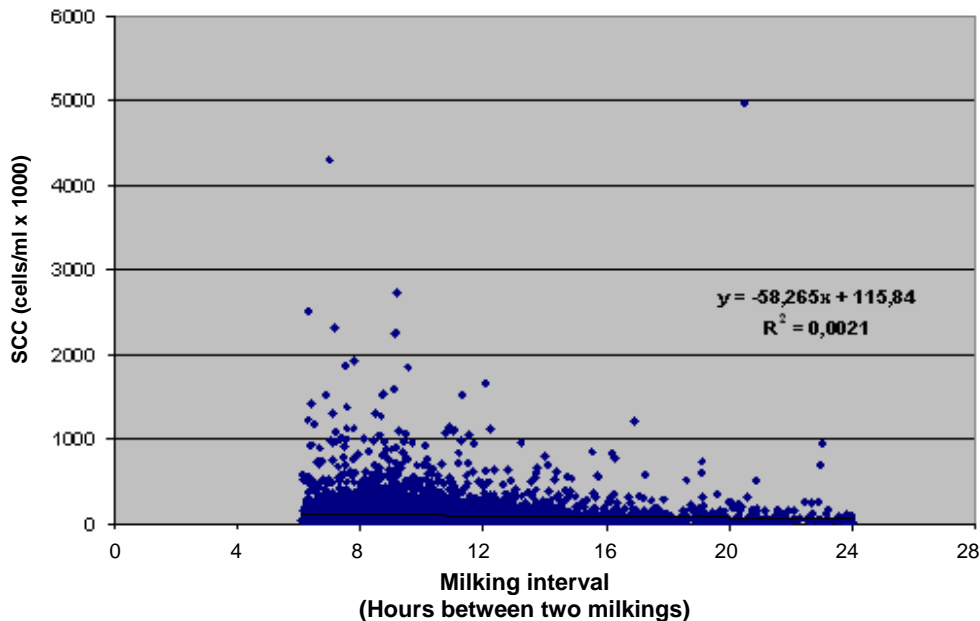


Figure 4. Milk SCC (cells/ml x 1000) from the OCC™ compared to the milking interval in hours between two milkings, all milkings (n = 8019). All data starts at a 6 h interval, which is the minimum milking interval in the AMS.

After an examination of the exceptionally high cell counts, especially after long milking intervals, the following milkings were excluded from the data: (Figure 5 shows the data after the exclusion.)

1. **Cow 1121** – Had chronic mastitis with highly irregular SCC during the whole period she was in stable. All data was removed.
2. **Cow 1182** – One high SCC after a long milking interval. The succeeding data showed a further increase in SCC due to a new infection. Only the first milking with high SCC and long interval was removed.
3. **Cow 1231** – Had severe mastitis: 4'965'000 cells/ml after a 20.5 hour milking interval. Only one milking was recorded and the cow was then removed from the AMS-stable. The data was removed.
4. **Cow 1241** – Two milkings after a long milking interval at two separate occasions were removed, both indicating an initial mastitis infection. The succeeding data at both occasions showed a further increase in SCC due to a new mastitis infection.

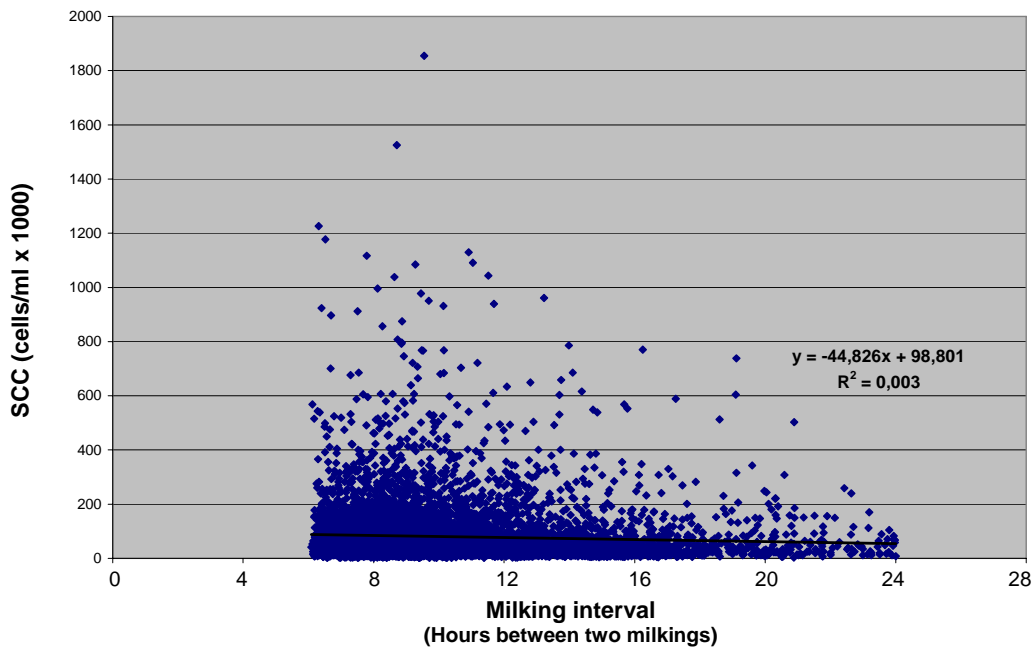


Figure 5. Milk SCC (cells/ml x 1000) from the OCC™ compared to the milking interval in hours between two milkings after the exclusion of four cows, n = 7821. All data starts at a 6 h interval; the minimum milking interval in the AMS.

These data were excluded because the infection status had such a clear influence on the SCC. We could not determine the effect of milking interval in the beginning of a new infection or when there were only a few scattered milking data. To further illustrate the effect of milking interval on the SCC, all milkings (after exclusion) were divided into three groups according to milking interval; 6-12 hours, 12-18 hours and 18-24 hours.

The distribution of milkings in each milking interval group is seen in Figure 6. As expected the largest number of milkings was in interval group 6-12 hours and only a very small number of milkings in the interval group 18-24 hours. During the period when the cows were out on pasture no manual collection of cows from pasture to barn was made.

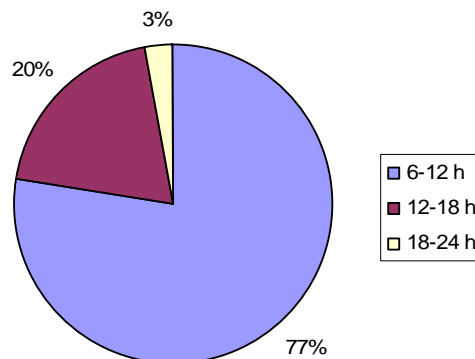


Figure 6. Distribution of milkings in the three milking interval groups 6-12 h, 12-18 h and 18-24 h, n = 7821.

Since the number of milkings was different between three groups, the data was converted into the relative percentage of SCC distribution. Figure 7 shows the relative distribution of SCC in the three milking interval groups.

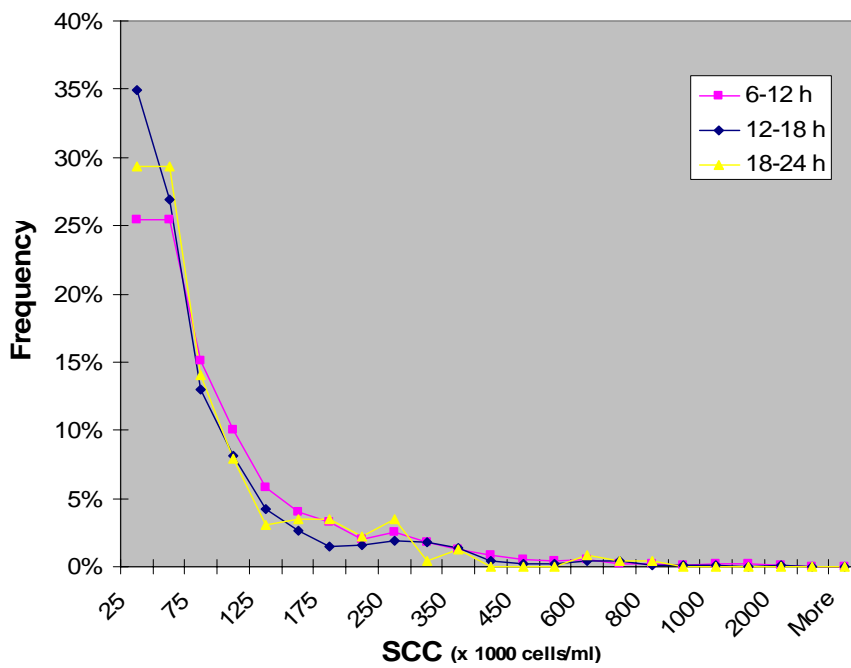


Figure 7. The relative distribution of milk SCC (cells/ml x 1000) in the three milking interval groups 6-12 h, 12-18 h and 18-24 h, n = 7821.

After an 18 hour milking interval the milk production has most likely stopped completely, and the SCC is no longer affected by the milking interval in the sense that there is no more dilution effect. In Figure 8 the group with the longest milking interval; 18-24 h, has been removed.

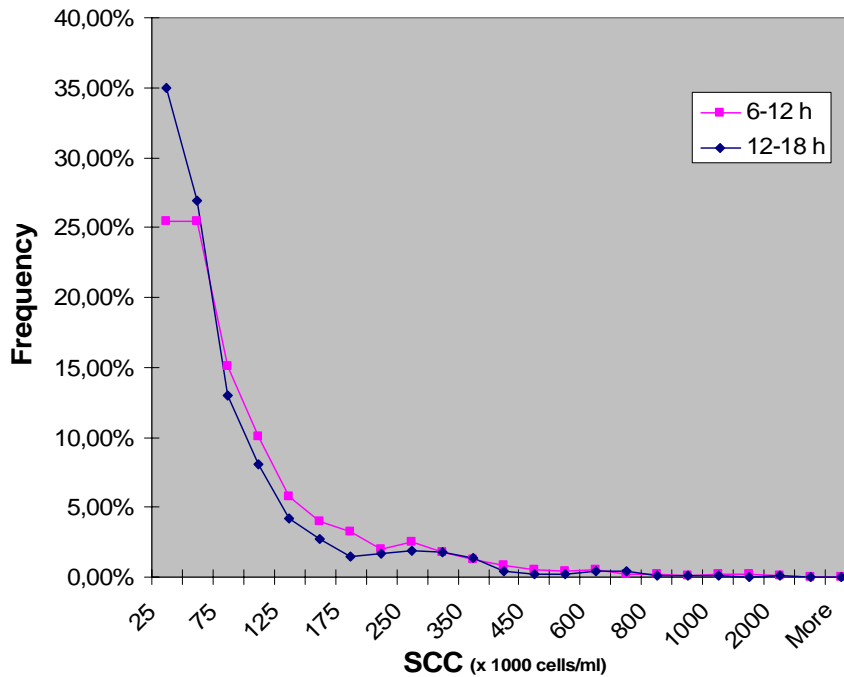


Figure 8. The relative distribution of milk SCC (cells/ml x 1000) in the interval groups 6-12 h and 12-18 h.

At low SCC (< 75'000 cells/ml) there was a slight indication that long milking intervals tend to have lower cell counts. The longer milking intervals (12-18 h) had a higher frequency of low SCC than in interval 6-12 h. As the SCC increased, so did the relative frequency for the shorter milking interval.

Individual cow level

There was a negative correlation between milking interval and SCC for 82% of the cows (68 of 83). Regarding the cows having a positive correlation (18%), the correlation could to a great extent be explained by:

- a) The cow had mastitis over a part of the test period
- b) One or a few very low, strongly influential values were affecting the inclination of the curve. The most likely explanation was an error in cell counting in the OCC™ giving exceptionally low SCC values.

An example of a cow with a positive correlation having both mastitis and a low influential value is shown in Figure 9.

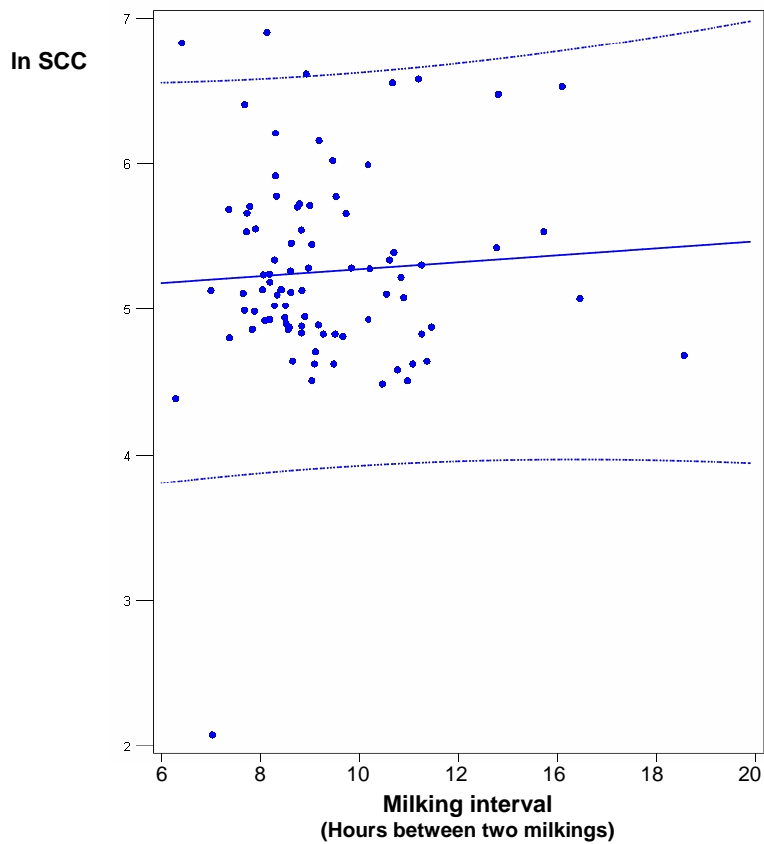


Figure 9. An example of the positive correlation between milking interval and SCC (ln SCC).

28 of the 83 cows (34%) had a significant correlation between SCC (ln SCC) and milking interval. Table 6 shows those cows divided in significance levels.

Table 6. Cows with significant correlation between SCC and milking interval. Total no of cows = 28.

Number of cows	Significance level
28	P < 0.05
16 (of 28)	P < 0.01
9 (of 28)	P < 0.001

Regression analysis on milkings from each cow showed that the variation due to milking interval was very large and the correlation for each cow was very low. The regression lines were too different between cows to be able to create a compensation algorithm applicable to all cows.

B: Mastitis detection and estimation of total milk SCC based on single quarter foremilk and single quarter main milk.

This part of the results has previously been presented in DeLaval research report (Bruckmaier and Wellnitz, 2006).

Estimation of total milk SCC based on single quarter main milk

The mean of measured SCC of whole udder milk was higher ($P < 0.001$) than the SCC of total milk of single quarters where the cell count was corrected for the quarter milk yield. The same phenomenon was observed in cell count mean when comparing morning and evening milkings. (See Figures 10 and 11. Notice that the scaling starts at 100 cells/ μ l).

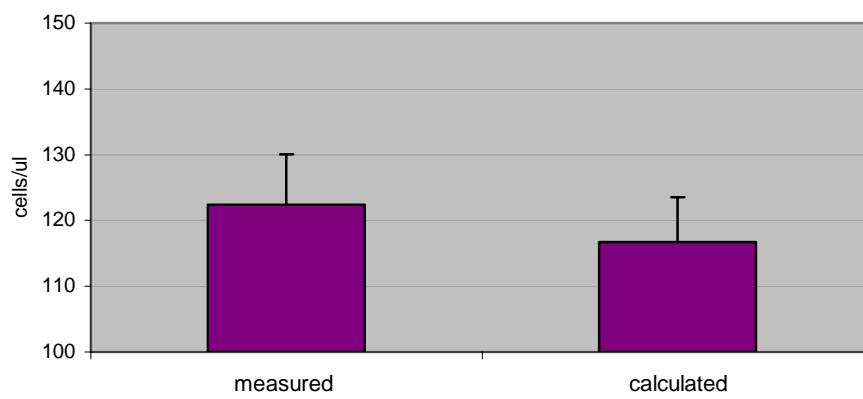


Figure 10. Measured and calculated total quarter SCC (n = 379) (Bruckmaier & Wellnitz, 2006).

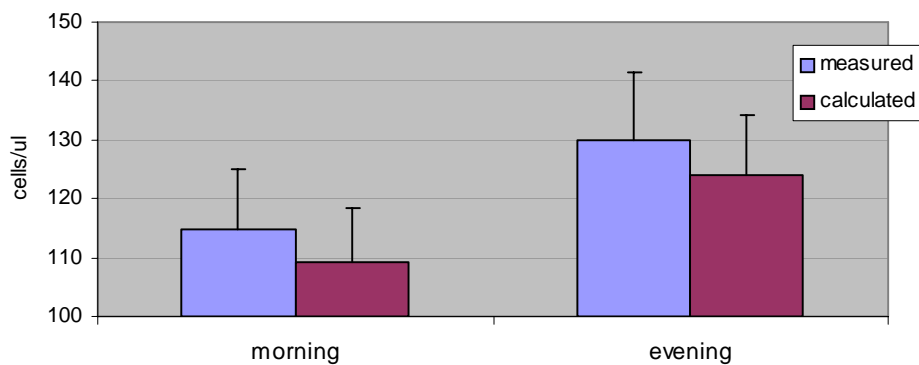


Figure 11. Measured and calculated total quarter SCC based on morning and evening milkings (Bruckmaier & Wellnitz, 2006).

Estimation of total milk SCC based on single quarter foremilk

Foremilk SCC was significantly higher ($P < 0.001$) than total quarter milk SCC, as seen in Figure 12. Therefore, foremilk samples are not representative of the total milk SCC of single quarters and SCC of the whole udder.

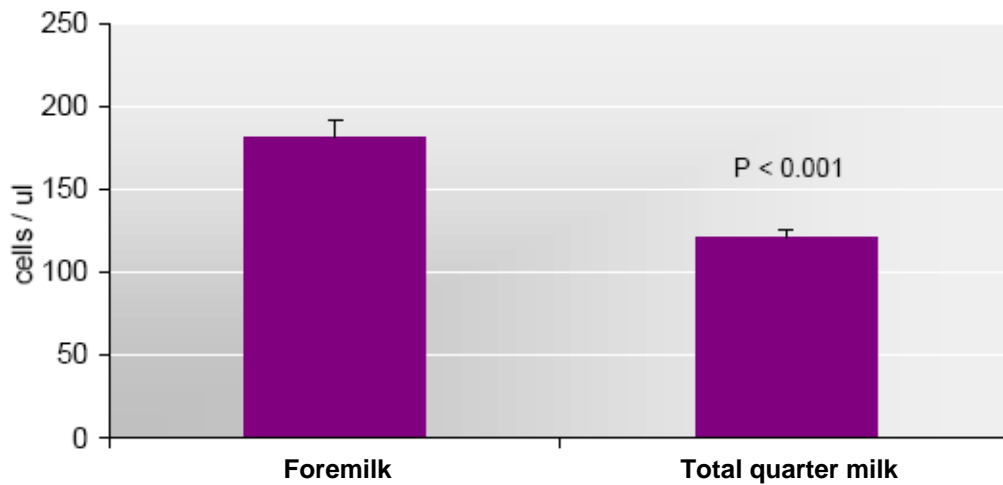


Figure 12. SCC in foremilk and total milk of single quarters (Bruckmaier & Wellnitz, 2006).

The correlation between foremilk SCC and total quarter SCC for all measurements was 0.70, but at SCC levels above 1000 cells/ μ l the correlation was much lower ($R^2=0.46$). (See figures 13a-c).

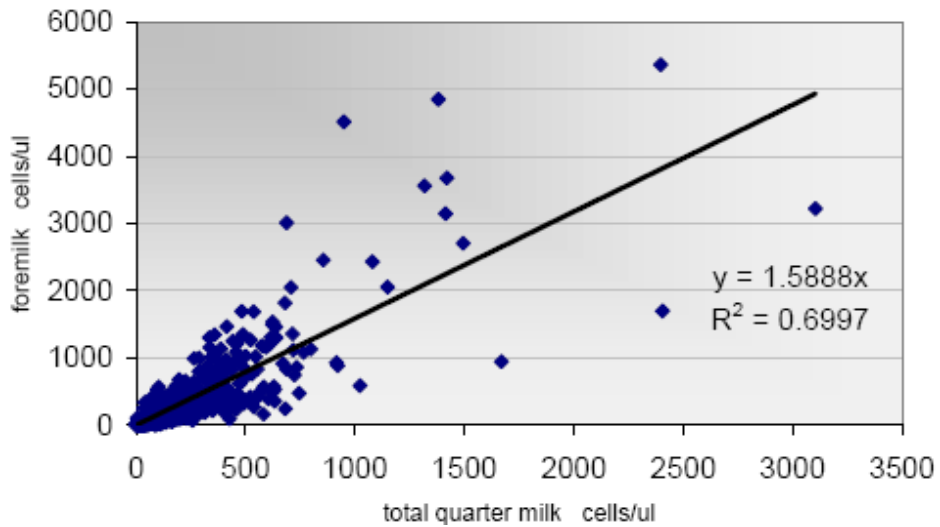


Figure 13a. The correlation between foremilk SCC and total quarter milk SCC for all measurements (Bruckmaier & Wellnitz, 2006).

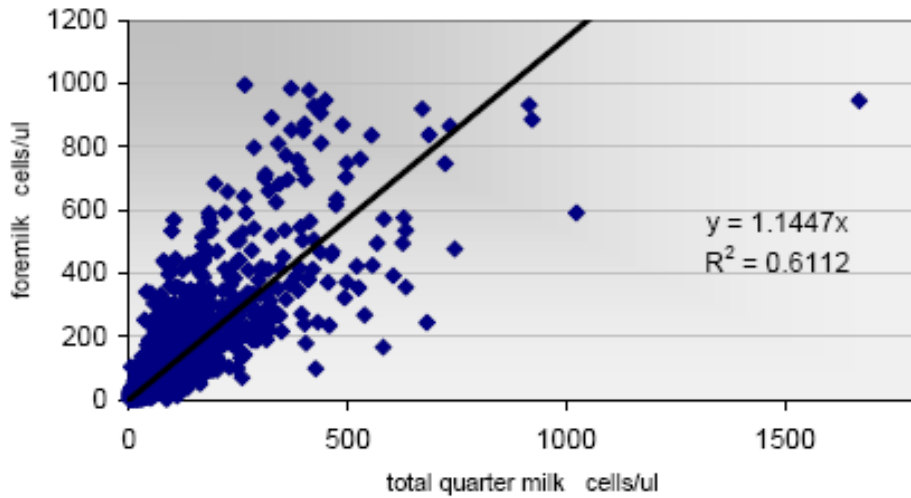


Figure 13b. The correlation between foremilk SCC and total quarter milk SCC for samples < 1000 cells/μl milk in foremilk (Bruckmaier & Wellnitz, 2006).

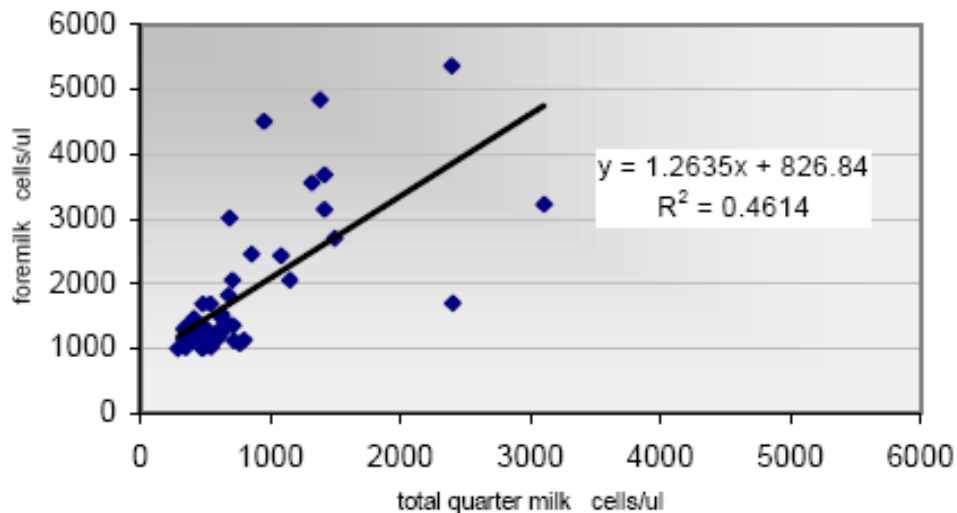


Figure 13c. The correlation between foremilk SCC and total quarter milk SCC for samples > 1000 cells/μl milk in foremilk (Bruckmaier & Wellnitz, 2006).

At SCC levels below 1000 cells/μl foremilk SCC was defined as $SCC_{\text{foremilk}} = 1.14 + SCC_{\text{total}}$ and the SCC of foremilk and total quarter milk were regarded as fairly similar. However, the correlation at levels below 1000 cells/μl was only 0.61 which implies that the estimation of total quarter milk SCC from foremilk samples will be difficult. At SCC levels above 1000 cells/μl the estimation becomes even more problematical due to even lower correlation.

Masking effect of one quarter on total milk

In some cases the differences between foremilk SCC and total quarter milk SCC within the same cow varied tremendously. The foremilk SCC from one quarter (or more) did not correspond to the SCC in single quarter main milk from the same quarter(s) or with the total quarter milk. Figure 14 shows variations in the foremilk measurements of cows having an average of below 80'000 cells/ml (80 cells/μl) in

the total udder milk. The figure illustrates that variation between foremilk and total quarter milk was not necessarily related to high SCC levels.

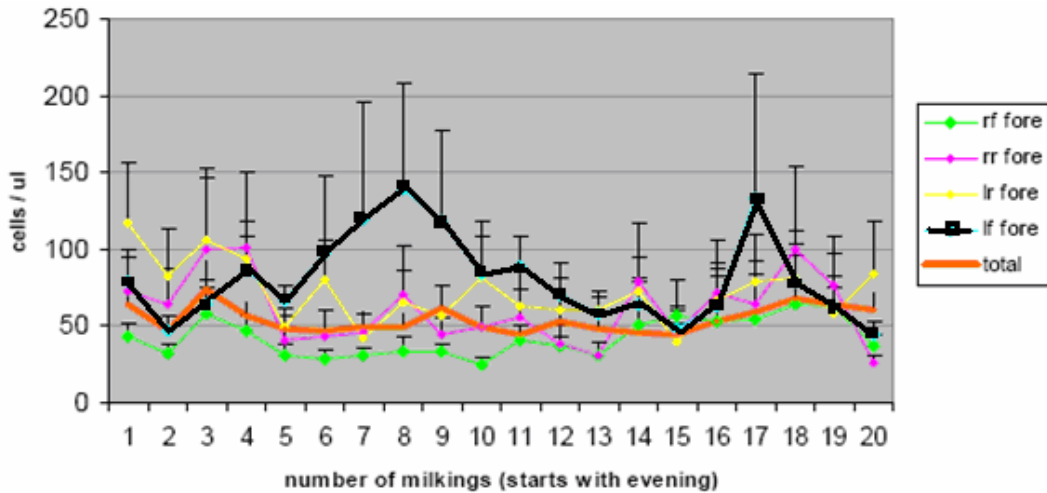


Figure 14. Variations in foremilk of cows with total udder milk $\leq 80'000$ cells in average (n=10). Rf fore is foremilk from right front teat, rr fore is from right rear, lr fore is from left rear and lf fore is from left front. Total means the measured SCC from the total udder milk (Bruckmaier & Wellnitz, 2006).

Furthermore, the SCC of single quarters did not always represent the SCC in total quarter milk. Figure 15 shows an example of how an infection in the left front teat is masked in both the SCC calculated from the separate quarters and the measured total udder SCC.

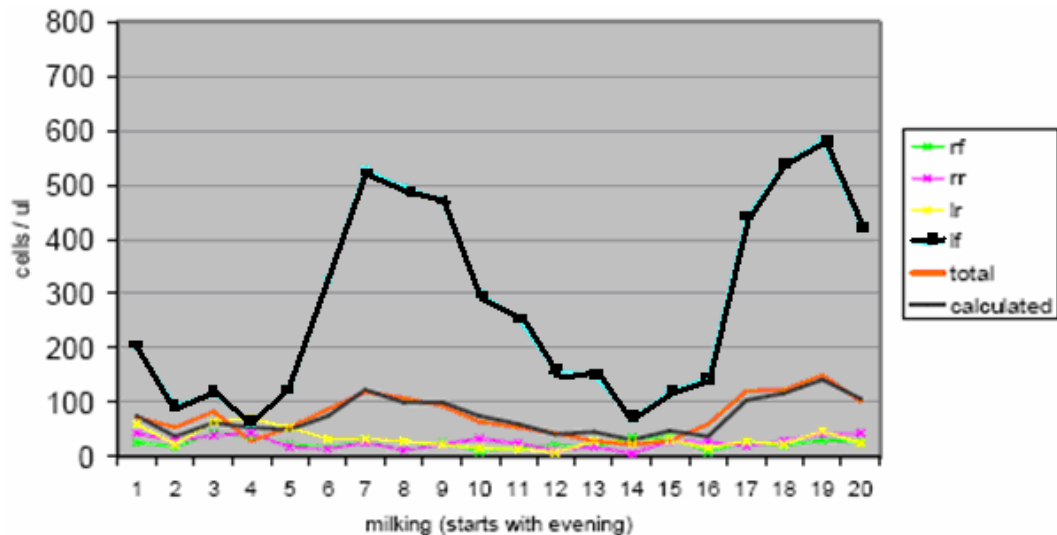


Figure 15. Variations in quarter milk SCC in one cow during the 10-day test period. Rf is total quarter milk from right front teat, rr is from right rear, lr is from left rear and lf is from left front. Total means the measured SCC from the total udder sample and calculated is the total milk SCC in the quarter milk (Bruckmaier & Wellnitz, 2006).

DISCUSSION

A: The effect of milking interval on SCC

The results from this study showed that the SCC in an uninfected udder is affected by the milking interval. 82% of the cows had a negative correlation between SCC and milking interval, of which 34% were significant ($P < 0.05$). The remaining 18% of the cows either had an elevation in SCC caused by mastitis or the inclination of the curve was affected by exceptionally low and incorrect values given by the OCC™. However, this study was not able to find a solution to create a SCC compensating algorithm for milking interval applicable to all cows. The variation in SCC due to milking interval was too different between cows.

The explanation of the relatively large amount of milkings in milking interval group 18-24 h is that the cows were on pasture. Normally, the cows that have not been milked after a certain time interval (decided by the farmer) are manually driven into the AMS. When the cows are out on pasture no manual collection of cows is being done at the research station. Furthermore, it is highly probable that the pasture makes the cows less eager to go and be milked, and that the intervals will automatically shorten once the cows are housed inside.

Earlier studies comparing different milking intervals have shown similar results on the influence of milking interval on SCC. A study performed by Smith and co-workers (2002) showed that somatic cell scores (according to the DHI scoring system) and weighted somatic cell counts were lower for herds milking three times daily than herds milking two times. Herds milking three times daily had a higher percentage of somatic cell scores in the low range (0 to 3) and a lower percentage in the high range (7 to 9). A recent study (Clark *et al.*, 2006) compared the effect of milking frequency (once vs. twice-daily milking) and breed (Holstein-Friesians vs. Jerseys) on different milk parameters, SCC among others. Their results showed that milking once daily increased somatic cell count throughout the year in both breeds. According to Saloniemi (1995a) the lowest cell count is obtained just before milking due to the dilution effect, and as long as the cow is still producing milk, a longer milking interval should give a lower cell count. All previous studies of the effect of milking interval on SCC have been performed on fixed milking intervals and not in automatic milking systems.

To answer the question at what point the variation of SCC no longer should be treated as natural, i.e. due to age, breed, stage of lactation etc., is not an uncomplicated matter. The SCC is affected by many factors and to say what variation is unnatural is very dependent of the individual cow herself. It's very difficult to make a general statement and we must rather make our decisions on herd and cow level.

As already described in the literature review, an uninfected udder is not affected by as many factors as an infected, and SCC should in principle at all times be low. But there are a few things to consider: The milking interval affects the SCC significantly

(Reneau, 1986) but as the results of the first study shows, it doesn't explain the total variation in SCC. Older cows with previous infections (may have been subclinical and passed unnoticed) have a higher leukocyte number in general and tend to have an elevated cell count even in a healthy udder. It is fairly unusual that a cow has passed totally without any infections throughout her lactations (Swedish Dairy Association, 2006-04-20), and this is important to consider when deciding if the variation is natural or not. Having many prior infections can either be just an elevation in leucocytes or an actual infection. If the variation is large, the latter is the most likely, as cows with many mastitis incidents are more prone to new infections. Studies have shown that the most important factor affecting SCC in uninfected cows is variation of the individual cow (Emanuelson & Persson, 1984; Schepers *et al.*, 1997), and the relative day-to-day variation of 10% (Sjaunja, 1986) is also an explanation of the variation in SCC.

To have this study made on cows at Kungsängen Research Centre had both its advantages and disadvantages. A huge advantage is that there was good surveillance and monitoring of the cows, the possibility to move infected cows and regular SCC and bacteriological tests. No bacteriological tests are presented in the results. The cows were overall very healthy. This made it easier to determine how the milking interval affects the SCC when there is no risk that an infection is affecting the SCC. The disadvantage was that because of the herd's unusually low SCC level compared with the average Swedish farm, the data was more difficult to interpret due to the small differences. The cows' overall low cell counts could explain the steep drop in the relative distribution of SCC in Figure 7 and this may also explain why the difference between the two interval groups wasn't so large as the SCC increased. Kungsängen Research Centre did not represent the general situation on Swedish farms. Another limitation of this study was the bacteriological status; to know the bacteriological status of all cows at all times, i.e. preferably daily testing, would have been optimal.

B: Mastitis detection and estimation of total milk SCC based on single quarter foremilk and single quarter main milk.

For a long time a 10 ml milk sample taken after the first squirts has been considered compatible with a 10 ml sample from the total quarter milk regarding the SCC level (Vahlberg, 1975). Later on experiments have shown that foremilk samples have higher SCC than strip milk samples (Östensson, 1993). Therefore, it was questionable how sensitive foremilk samples were to provide representative data of total cell counts and to detect early changes in SCC. The result of this study showed that foremilk SCC did not very well represent the SCC of total milk of single quarters or whole udder milk of the cow. Foremilk SCC was significantly higher ($P < 0.001$) than SCC of total quarter milk and whole udder milk. Testing on foremilk samples does not allow a prediction of exact levels of SCC in quarters on the basis of foremilk SCC. However, they can be useful for early detection of increased SCC, although exact cell numbers can not be predicted.

A recent study (Sarikaya & Bruckmaier, 2006) indicated the importance of measured milk fraction for estimating total milk SCC. The decline in SCC between foremilk and cisternal milk was 50%, and between cisternal and first 400 g of alveolar milk another 80% in milk with a SCC of 100'000 cells/ml in total milk. However, changes in milk with low or very low SCC were marginal during milking. Further, the study showed that single cows can have a dramatic variation in foremilk SCC depending also on the foremilk fraction. A significant decline in the foremilk fractions was observed from the first hand-stripped milk jet to the sixth at concentrations of >350'000 cells/ml in total quarter milk. Although one of these hand-stripped foremilk fractions presented only 0.1 to 0.2% of the total milk, the SCC was two to three times greater than the total quarter milk SCC. This study also concluded that the foremilk SCC is not very well related to total quarter milk and whole udder milk.

When estimating total milk SCC it is of great importance to consider which fraction that is used as measuring sample. As already discussed, milk SCC can show a great variations during milking. When the foremilk SCC is relatively low, the variation in SCC in the remaining milk is marginal, indicating that there is no ongoing infection (Wellnitz *et al.*, 2007). However, at higher SCC levels, the variation in SCC in different fractions during milking shows larger differences, both in small fractions as in the strict foremilk and between cisternal and alveolar milk. The higher the SCC is in total milk, the greater differences in SCC between the foremilk and the alveolar milk (Wellnitz *et al.*, 2007). When the quarter foremilk SCC levels increase, so does the SCC in the total quarter milk. However, this increase in total milk SCC is disproportional and the total milk SCC can still be so low that the quarter is considered as healthy. At high SCC levels in the total milk, the differences within the foremilk can be so great, that making a reliable prediction for the total milk SCC from one sample is not possible. With increasing SCC, foremilk samples can be used to predict elevated SCC at a whole quarter level, while the quality of estimation of the exact SCC value is decreasing. The threshold for mastitis at quarter level must always be based on what sample that is measured and one must consider the variations even within small milk fractions.

The result from the performed study also showed that single quarter milk was not representative when estimating whole udder milk SCC. The mean of measured SCC of whole udder milk was higher ($P < 0.001$) than the SCC calculated from total quarter milk.

The sampling equipment of the VMS™ takes representative composite milk samples so that the SCC can be compared directly with the SCC from the monthly milk recording. The aim in the VMS™ is to somehow have the measuring of the SCC done before the cow has finished milking and to be able to automatically divert the milk if the SCC is too high. The problem in this case is that it will be impossible to take a sample from the whole udder milk, and a suggestion has been to take it from the foremilk. Results from the study shows that a sampling method using the foremilk would not give a representative sample.

Another idea is for the VMS™ to take a sample at quarter level, one test per milking, meaning that one quarter is tested every fourth time. Assuming a cow has a milking frequency at 2-3 times a day this would mean that an individual quarter would be tested every two days at the most. The performed study showed that single quarter SCC did not represent the whole udder milk SCC and is thus not a representative method. However, if the sampling is carried out consistently maybe that is not of great importance. If the comparison is made within the individual cow at all times we will have the advantages of eliminating the masking effect we get in composite milk samples and keeping the costs at the same level. Another suggestion is to take two separate samples at every milking: either from both front quarters or both rear quarters. In this way a good intercomparison is obtained since individual quarters show a parallelism in SCC (Berglund *et al.*, 2007; Cullen, 1968). Sampling all four quarters at one milking would be even better, but is too expensive due to the costs of the staining reagent used during cell counting. This method, with sampling at quarter level, will not be suitable when wanting to estimate bulk tank SCC.

Normally a level of 200'000 cells/ml is set as the general threshold (Dohoo, 2001). A SCC above that level needs to be reviewed on the basis of the situation in the herd and the individual animal. Different farmers have different tactics whether to tolerate high SCC and at what level. If the bulk tank SCC is at reasonable level, moderate and high cell counts are more tolerated.

The carry over effect from sample to sample in the OCC™ could cause false positives if a cow with low SCC is milked after a cow with high SCC. However, there is a program in the OCC™ designed to compensate for this.

Conclusions

According to the results of this study there is no possibility of creating a general compensatory algorithm when evaluating the effect of the milking interval on SCC. We know that the milking interval affects the SCC but the impact is very different between individual cows. However, this study was only performed on one farm with cows with very low cell counts, and perhaps further studies using more farms have to be done to obtain more reliable results.

The OCC™ makes it possible to detect mastitis infection at an early stage, when it is easy and cost effective to treat it and before it spreads within the herd infecting other cows. Since the OCC™ measures at every milking it gives an excellent overview of each individual cow and her SCC development. Regarding the milk sampling method the optimal would be to keep the present and to develop another method sampling every quarter (on total quarter milk). The farmer could then decide which method to apply at what times. To collect the milk samples before the milk is pumped to the tank would solve the diverting problem, if the milk was pumped to an intermediate container while the OCC™ was measuring SCC. After SCC results are obtained the

milk is either pumped on to the tank or to a separate milk container. Exactly how the sampling device should be constructed needs to be further investigated.

Adding more production and health parameters into the herd management program will give the most optimal view of the cow's status. For instance activity measurements and feed consumption as possible factors affecting the SCC. Tracking the individual quarters regarding milk production is also very important, as a decrease in yield in one quarter can be a sign of an early infection, possibly missed by the OCC™ because of the masking effect. However, it is very important to make the herd management program very user friendly and that all the different parameters are shown clearly and are in easy-to-grasp tables and figures, so that the farmer can get as much information out of them as possible.

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