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Monitoring of the Somatic Cells Count for Improving Milk and Dairy Products Quality

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Running title: **Monitoring of Somatic Cells in Milk**

Abstract
This review discusses current knowledge of the impact of mastitis on milk composition and processing properties. Dairy product quality defects resulting from mastitis are due to enzymatic breakdown of milk protein and fat. Somatic cell count (SCC) values are routinely used to detect the inflammation that results from intramammary infection of dairy cattle. Subclinical mastitis may cause more losses in a herd than clinical mastitis, since the animal does not exhibit typical symptoms of the disease. A useful tool for subclinical mastitis diagnostic is SCC. The increase of SCC in milk is associated with development of several undesirable sensorial defects in dairy products as salty flavors due to a change in milk mineral balance, rancid and bitter off-flavors due to increased lipase and protease activity, respectively. Proactive management of mastitis infections by using of SCC as a diagnostic tool could be extremely effective for improving milk and dairy products quality.

Practical applications
The production of high quality milk is a requirement to sustain a profitable dairy industry. Losses due to mastitis include decreased milk production, increased treatment costs, discarded milk, premature culling, death and loss of milk quality premiums. Somatic cell count values are routinely used to identify subclinical mastitis and played role as quality parameter of raw milk. This review presents the current knowledge about the usage of SCC as a diagnostic tool for subclinical mastitis. Such knowledge will help for development and implementation of more complete udder health programs and monitoring systems in order to improve milk quality on dairy farms.

Key words: somatic cell count, mastitis, milk, dairy products, quality

Introduction

Mastitis is a complex disease described as an inflammatory reaction of the mammary gland of all mammals. Almost all mastitis occurring in dairy cows is caused by bacteria, although some cases are caused by yeasts, fungi or algae (Hogan et al. 1999). Mastitis is initiated after an infective dose of a pathogenic organism enters the udder through the teat canal, followed by bacterial growth during an incubation period, production of toxins that are harmful to the mammary gland and then progression to either subclinical or clinical states or resolution of the infection as a result of the cows immune response (Ovideo-Boyso et al. 2007). Subclinical mastitis is 15 to 40 times more prevalent than clinical mastitis and causes high economic losses in most dairy herds (Schultz et al., 1978). As a result of the inflammation process the mammary tissue is damaged. This causes increased vascular permeability and leakage of blood constituents, serum proteins, enzymes, and salts into the milk; decreased synthesis of caseins and lactose; and decreased fat quality (Østerås, 2000; Harmon, 1994). (A 10; A 20). Therefore the main alterations in milk composition as a result of mastitis are related with reduced calcium, lactose and casein levels, and increases in sodium, chloride, and levels of serum proteins (Kitchen, 1981).

Mastitis is causing great economic losses due to reduction in milk yield, lowering its nutritive value. The most important negative effects of mastitis on the dairy industry include lower industrial yield and reduced shelf life of dairy products, due to undesirable sensory attributes caused mainly by proteolytic and lipolytic enzymes (Kitchen, 1981). The milk from an affected animal usually contains microorganisms which are potentially pathogenic for humans (Barbano, 1989).

Somatic cells in milk

When a cow gets infected, the resident somatic cells signal to a resting population of white blood cells in the blood stream, and a massive influx of mostly polymorphonuclear cells into the milk takes places (Shuster et. al., 1995). These cells kill bacteria, and when the infection is eliminated then usually within a few weeks cell count of milk returns to normal. An example of such a response is presented in Figure 1, where data are presented of an experimental *E. coli* infection.

Therefore, the somatic cells are mostly cells of the immune system (Sordieedinlo et al., 1997). They are part of the natural defense mechanism and include lymphocytes, macrophages, polymorphonuclear cells and some epithelial cells (Table 1) (Pillai et al., 2001) Somatic cells are the result from the inflammatory response to an intramammary infection.

Somatic cell count, or a parameter derived from this count, is often used to distinguish between infected and uninfected quarters. There is a general agreement between infection status and the inflammatory response to this infection as measured by an increased SCC. As with any diagnostic test, errors will occur when solely depending on a single test. To minimize the amount of error, diagnostic test parameters such as sensitivity and specificity are calculated at various cut-off values in the SCC continuum (Schepers et al., 1997). So to be able to distinguish between infected and uninfected quarters it was repeatedly shown that a cut-off of approximately 200 000 to 250 000 cells was optimal to reduce diagnostic error (Doho and Lesli, 1991; Leavens et al., 1997; Leslei et al., 1997, Schepers et al., 1997). At this cut-off value, diagnostic sensitivity was shown to be approximately 75%, while specificity was approximately 90% (Schepers et al., 1997).

Normally, in milk from a healthy mammary gland, the SCC is lower than 10^5 cells/ml, while bacterial infection can cause it to increase to above 10^6 cells/ml (Bytygi et al., 2010). Enumeration of the somatic cell count (SCC) of milk has long been used as a tool for measuring milk quality (Dohoo and Leslie 1991). Bulk tank SCC (BTSCC) values are routinely used to define the national and international regulatory standards that govern hygienic milk production. In Europe, the EEC directive 92/46 in April 1992 stated that milk with a somatic cell count (SCC) over 400 000 cells per mL may not be used for fluid milk and starting in 1998 not even for human consumption. In North America limits at 750 000 (USA) and 500 000 cells (Canada) are in place (Sargeant et al., 1998).

Factors affecting somatic cells count

There are plenty of factors that influence milk somatic cell count at individual and herd level apart from intramammary infection. The ability to correctly interpret somatic cell counts depends on an understanding of the factors which may affect the number of somatic cells.

The most important factor affecting the somatic cell count of the milk is the infection status of the quarter (Dohoo and Meek, 1982). The degree and nature of the cellular response are likely to be proportional to the severity of the infection. The

average number of milk SCC increases in a bulk tank sample with an increase in the number of quarters infected (Meek et al., 1980).

SCC increases with progressing lactation (late lactation) regardless of whether the cow is infected or not (Dohoo and Meek, 1982). SCC elevation has been linked with an animal's innate immune response in preparation for calving and to enhance the mammary gland defense mechanism at this critical calving time (Reichmuth, 1975). During early and late lactation the percentage of neutrophils tends to increase while the percentage of lymphocytes decreases (McDonald and Anderson, 1981). At parturition SCC are usually higher than one million per ml and decreases to 100,000 cells/ml in the 7 to 10 days post-partum (Jensen and Eberhart, 1981).

Some investigations showed that SCC increases with increasing age (Beckley and Johnson, 1966). This increase is primarily due to an increased prevalence of infection in older cows (Reichmuth, 1975). SCC variation has been noted between breeds of dairy animals. The high-producing cattle breeds such as Brown Swiss and Black and white Holstein have higher presence of SCC/ml in milk.

Somatic cell counts are generally lowest during the winter and highest during the summer season (Khate and Yadav, 2010). During summer, the growth and number of environmental bacteria is increased due to favorable temperature and humidity (Harmon, 1994). Free radicals are generally produced during stress due to milking techniques, environmental and infectious organisms (teat injury).

Monthly monitoring indicated that BTSCCs peak during the summer months (July through September) when higher temperatures and humidity increase stress on cows and provide conditions more favorable for bacterial growth.

Methods for determination of SCC

Direct or reference (arbitration) method of somatic cells count in milk is a microscopic method which involves the counting of the stained somatic cells using a microscope. It is a very time- and labour-consuming method which also requires highly qualified personnel. It is because of the complexity and laboriousness of the microscopic method indirect methods of somatic cells count have become widely spread.

Indirect method of SCC could be summarized in the fallowing three groups: Viscosimetric method, Conductance-measuring method and Optofluoroelectronic method/ fluorescence flow cytometry method.

There are automated systems for automatic counting of Somatic Cells (where Somatic Cells' nucleus stained with special reagents are counted) which are based on the achievements of optofluoroelectronic method/ fluorescence flow cytometry method. They include an analyzer, PC, specialized software and sometimes a devise for automatic supply of the cuvettes with the samples.

The more affordable option is DCC Somatic Cells Counter by DeLaval, which is based on the same principle of the automatic direct count of the Somatic Cells number in a milk sample. DCC Somatic Cells Counter allows measurement of the precise number of somatic cells in each quarter of the cow's udder, as well as in a milk tank.

Another indirect method of determining of the somatic cells number is conductance-measuring/ conductometric method which is based on the measurement of electrical conductivity of milk. It is well known that inflammation in the udder causes changes not only of the qualitative composition of milk but also its physical and chemical properties change, in particular electric conductivity. Mastitis milk/ milk with high concentration of somatic cells is characterized by increased content of chlorine ions, which leads to the increase of its specific electric conductivity. However the instruments that use this method can rather serve as indicators of the variations in the number of somatic cells in milk, rather than precise somatic cells counters.

The devices of this type are useful for veterinarians for fast detection of both clinical and sub-clinical mastitis of animals as well as for fast sorting of healthy and sick animals in the herd.

The devises of indirect action which are based on viscosimetric method (viscosimetric somatic cells analyzers) measure the viscosity of milk. Tested milk sample is mixed up with a special chemical preparation (Mastoprim) which destroys somatic cells' membranes (leukocytes), resulting in DNA molecules come in a solution, increasing its viscosity.

The higher somatic cells number in a tested milk sample the more viscous (thick and stringy) is the mixture. Viscosimetric somatic cell analyzer measures/ determines the time of outflow of the tested sample through a special capillary with a known diameter and displays the results in accordance with the calibration chart that reflects the compliance of the outflow time with the somatic cells number. The time of milk mixture

outflow increases accordingly with the increase of somatic cells concentration.

Impact of SCC on milk and dairy products quality

Many studies have been carried out to determine the effect of SCC on the yield and quality of milk and dairy products, especially cheeses. Subclinical mastitis alters the composition of the milk in addition to suppressing milk yield (Bramley, 1992; Harmon, 1994). Unlike milk production loss, there is a direct relationship between SCC and milk quality (Table 6).

According to Harmon (1994), mastitis or elevated SCC is associated with a decrease in lactose, casein and fat in milk because of reduced synthetic activity in the mammary tissue. The largest negative consequences of the presence of SCC are related to shorter shelf life and less sensory content or un-desirable organoleptic characteristics of the final product, due to enzymatic activities of somatic cells (Töpel, 2004). The higher levels of free fatty acids in high cell count milk may produce a rancid flavor. The high presence of SCC in milk affects the activity of yogurt fermentation (Tamime and Robinson, 1999), and can even stop this process. Fernandes et al. (2007) studied the effect of SCC in raw milk on the chemical and physical properties of yogurt. The authors found that SCC in milk did not increase the extent of proteolysis of the resulting yoghurt. Over the 30 d of storage there was no change in viscosity in yoghurt manufactured from milk with low and intermediate SCC. In contrast, viscosity of yoghurt with high SCC increased with storage time. The investigations showed that increased SCC in milk led to an increase in FFAs in the resulting yoghurt during storage for 30 d that may result in a decrease in its shelf life. Based on these results the authors suggested that raw milk used to produce yoghurt should not contain more than 400,000 cells.mL-1.

Cheese production from milk with high somatic cells count has been reported to be lower than from low cell-count milk (Everson, 1980). Lipolysis in cheese was clearly dependent on somatic cell counts, which may have important consequences for cheese flavor (Chen et al., 2010). The proteolytic effects of somatic cells on main caseins in cheese are different if cheeses are made with raw or pasteurized milk. The effects of somatic cells on cheese ripening also depend on the pretreatment of milk.

Approaches for reduction of somatic cells in milk

A number of control programs aimed at all udder health issues have been designed. Bacterial invasion occurs mostly during the dry period, particularly during late gestation. To reduce the occurrence of mastitis and control SCC, prevention strategies should be followed during the dry period. The standard mastitis control program decreases the prevalence of intramammary infections with contagious pathogens (Hillerton et al., 1995), but it has been rated relatively low in success in prevention of clinical mastitis from environmental pathogens (Lam et al., 1997; Barkema et al., 1999). Control of mastitis requires a multidisciplinary approach that is focused on prevention of new infections and appropriate interventions. Implementation of successful mastitis control can be summarized in three practical recommendations:

1. Each farm should routinely work with their advisors to develop an annual udder health plan that includes clear goals for milk quality.
2. The annual udder health plan should emphasise prevention of new infections.
3. Farmers must identify and manage chronically infected cows. Cows that maintain more than 2 months of individual SCC >200,000 cells/mL and cows that experience repeated (>2 episodes) of clinical mastitis can be considered to be chronically infected.

Nutritional supplementation with vitamins and minerals enhances the immunity of the animal and therefore decreases SCC numbers.

The currently used primary parameters to analyse the herd situation in the mastitis control program are:

- bulk milk somatic cell count;
- percentage of cows with SCC >250,000 cells/ml per test-day;
- percentage of cows with new infections;
- culling rate because of mastitis.

Conclusion

The level of mastitis infection in a dairy herd can have a significant impact on herd profitability. It is inevitable that more complete udder health programs and monitoring systems have been developed and implemented. Somatic cell count in cow milk is commonly used as an effective index of udder health in dairy cows. There is a general agreement between infection status and the inflammatory response to this infection as measured by an increased SCC. Implementation of complete udder health programs should be

accompanied by research efforts to further fine-tune these complete udder health control and monitoring programs.

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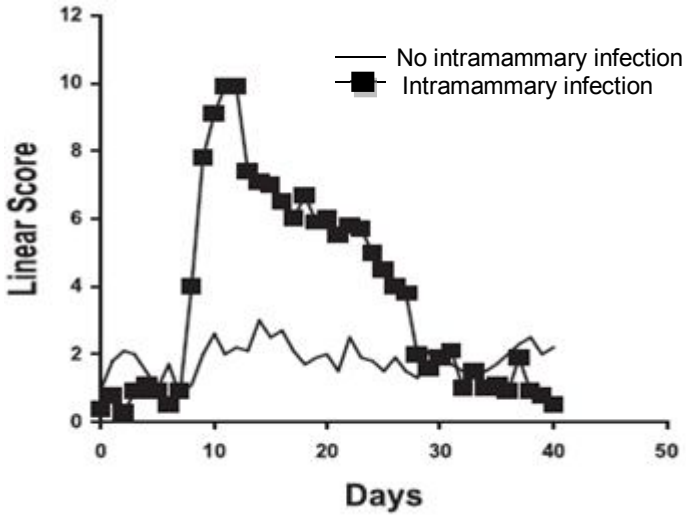
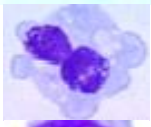
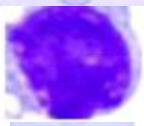


Figure 1. Somatic cell count pattern during a successful immune response to an incoming E. coli bacterial infection (addopted by Schukken et al., 2003).

Table 1. Cell types found in normal bovine milk.

Cell type	*% cells (range)*	*Image of somatic cells*
Neutrophil (PMNs)	0-11	
Macrophage	66-88	
Lymphocytes	10-27	
Epithelial (ductal)	0-7	

[1]From Lee et.al., 1980 ; [2]ISO 13366-1/IDF148-1

Table 3. Changes in milk constituents with elevated SCC.

Milk consistent	*SCC (x 10^3 cell/ml)*				*Reason for change*
	< 100	**<250**	**500-1.000**	**>1.000**	
Decrease					
Lactose	4.9	4.74	4.6	4.21	
Casein	2.81	2.79	2.65	2.25	Reduced
Fat	3.74	3.69	3.51	3.13	synthesis
Increase					
Whey protein (Total)	0.81	0.82	1.10	1.31	
Serum albumins	0.02	0.15	0.23	0.35	Leakage
Immunoglobulins	0.12	0.14	0.26	0.51	from
Chloride	0.091	0.096	0.121	0.147	blood
Sodium	0.057	0.062	0.091	0.105	
Potassium	0.173	0.180	0.135	0.157	
pH	6.6	6.6	6.8	6.9	