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Review Article

Somatic Cells in Relation to Udder Health and Milk Quality-A Review

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Abstract | The production of high-quality milk is a prerequisite for sustaining a profitable dairy industry. The number of somatic cells (macrophages, polymorphonuclear neutrophils, lymphocytes and epithelial cells) per mL of milk, usually called somatic cell count (SCC) is routinely used to identify subclinical mastitis and define quality standards. Elevated SCCs are associated with changes in milk composition, resulting in poor quality of milk and milk products. SCC in milk is influenced by many factors, such as animal species, milk production level, lactation stage and also the individual and environmental factors besides management practices. A threshold of <200,000 cells/mL is considered to be of the most practical value for determining the health of mammary quarter. This review aims to highlight the importance of somatic cells for improving udder health and quality of milk as well as milk products.

Keywords | Mastitis, Somatic cell count, Milk quality, Immunity, Management

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INTRODUCTION

Mastitis, an inflammatory reaction of the mammary gland, is regarded as the most common infectious disease of dairy cows resulting in considerable economic losses for both dairy farmers and milk processors (Halasa et al., 2007; Geary et al., 2012). It is characterized by physical, chemical and bacteriological changes in the milk and pathological transformations in the glandular tissue of the udder. Almost all cases of bovine mastitis are associated with bacteria; however yeasts, fungi or algae may also be involved (Hogan et al., 1999). Mastitis is initiated after an infective dose of a pathogenic organism enters the udder through the teat canal. This is followed by bacterial growth, production of toxins and then progression to either subclinical or clinical states or resolution of the infection as a result of the cow's 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). Main changes in the udder include; leakage of ions, proteins and enzymes into the milk due to an increased vascular permeability, decreased synthesis of caseins and lactose and invasion of phagocytising cells into the milk compartment (Østerås, 2000). Therefore the chief alterations in milk composition are increased levels of sodium, chloride, and serum proteins and reduced calcium, lactose and casein (Kitchen, 1981). However, the degree of these changes depends on the nature of the infectious agent and the extent of inflammatory response. Despite intensive research and implementation of various mastitis control strategies over the last few decades, bovine mastitis has not yet disappeared and is insurmountable in the dairy profitability. The gold standard to measure the degree of udder inflammation is the cytological examination i.e. milk somatic cell count (SCC) (Hamann, 2002). Somatic cells are indicators of both resistance and susceptibility of cows to mastitis and can be used to monitor the level or occurrence of subclinical mastitis in herds or individual cows. Yet many producers fail to completely understand the implications of SCC for udder

Table 1: Characteristics and composition of somatic cells in healthy milk of different species

	Macrophages	PMNs	Lymphocytes	Epithelial cells	References
Morphological characteristic	Big nucleus	Plurilobary nucleus	Dense and round nucleus	Round nucleus	ISO13366-1/ IDF148-1
Biological functions	Phagocytosis -Intracellular killing of bacteria -Presentation of antigen -Secretion of chemo-attractants	-Phagocytosis -Intracellular killing of bacteria -Secretion of antibacterial factors -Detection of chemo-attractants	-Specific immune system -Production of immune regulatory cytokines -Lysis of demaged host cells	-Secretion of chemoattractants	Burvenich et al., 2003; Paape et al., 2003
Percentage of each cell type in milk of different species					
Bovine	35-79	3-6	16-18	2-15	ISO13366-1/ IDF148-1
	26	16	23	36	Benic et al., 2012
Sheep	46-84	2-28	11-20	1-2	Gonzalez-Rodriguez et al., 1995
	57	31	8	2	Morgante et al., 1996
Goat	15-41	45-74	9-20	6	Dulin et al., 1983
	11	79	10	-	Boulaaba et al., 2011

health. Therefore, this article highlights the importance of SSC in relation to udder health, in order to improve milk quality on dairy farms.

Somatic Cells

Somatic cells (SCs) are present as a part of the innate immune system of the udder. They include 75 to 85% leucocytes (macrophages, polymorphonuclear neutrophils (PMNs), lymphocytes) and 15 to 25% epithelial cells (Barrett, 2002). In a healthy udder, the somatic cell count (SCC) is nearly constant, the exception being the first weeks postpartum. A relatively constant number of somatic cells are being secreted into the milk over the lactation (Miller et al., 2004). However, when the udder is infected, the resident somatic cells signal to a resting population of white blood cells in the bloodstream, and a massive influx of polymorphonuclear neutrophil cells takes places into the milk (Shuster et al., 1995). These cells kill bacteria, and when the infection is eliminated the cell count of milk returns to normal. The measurement of the number of somatic cells in milk is taken as the gold standard for ruling out the severity of mastitis. 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). An elevation of above 2, 00,000 cells/mL is an indication of mastitis (Harmon, 2001). Bulk tank SCC (BTSCC) values are routinely used to define the national and international regulatory standards that govern hygienic milk production. The national standards for BTSCC vary from <400,000 cells/mL (EU, Australia, New Zealand and Canada) to <1,000,000 cells/mL (Brazil) (USDA, 2013). However, minimum international export requirements for milk quality are becoming more important than national regulations. The legal maximum BTSCC for bovine milk in most US states, Germany, Canada remains at 1×10^5, 5×10^5 , and 7.5×10^5 cells.mL−1, respectively (FDA, 2011; Olechnowicz and Jaskowski, 2012).

Defensive Role of Somatic Cells

SCs are known to be one of the major defense components of the mammary gland against intramammary infections (Sharma et al., 2011). The four main cell types composing SCC, are briefly presented in Table 1.

Macrophages are generally the predominant cell type in healthy cow milk. They can fight against bacterial invasion quickly by engulfing action. In the case of infection, macrophages release chemical messengers or chemoattractants that are detected by PMNs and direct PMNs in turn towards the infection site. Both macrophages and PMNs are capable of ingesting microbial cells by phagocytosis and play an essential role in the innate immune system. Moreover, macrophages participate in the specific immunity as do lymphocytes (Burvenich et al., 2003).

Polymorphonuclear neutrophils can be recruited and increase milk SCC when the infection continues. At the site of infection, PMNs phagocytise microorganisms and kill them by using a combination of oxidative and non-oxidative mechanisms (Pham, 2006). Lymphocytes have a determinant role in the specific immune system. They are the only cells capable of recognizing the antigens through specific membrane receptors for invading pathogens (Sordillo et al., 1997).

Table 2: Enzyme activity of somatic cells in milk and milk products

Somatic cells	Enzymes	Activities	Reference
Macrophage	Cathepsin-B	Protease	Guha and Padh, 2008
	Cathepsin-D		Diment et al., 1988; Guha and Padh, 2008
	Cathepsin-H		Guha and Padh, 2008
	Cathepsin-L		Guha and Padh, 2008
	Cathepsin-G		Campbell et al., 1989; Considine et al., 2002
	Cathepsin-S		Guha and Padh, 2008
	Elastase	----	Campbell et al., 1989; Prin-Mathieu et al., 2002.
	Lipoprotrin lipase		Azzara and Dimick, 1985a
	Collagenase		Prin-Mathieu et al., 2002.
	Myeloperoxidase		Considine et al., 2002
PMNs	Cathepsin-B		Magboul et al., 2001
	Cathepsin-C		Travis and Fritz, 1991
	Cathepsin-D		Baggionlini et al., 1978
	Cathepsin-L		Travis and Fritz, 1991
	Cathepsin-G		Baggionlini et al., 1978; Considine et al., 2002
	Cathepsin-S		Considine et al., 2002
	Elastase	----	Baggionlini et al., 1978; Travis and Fritz, 1991; Prin-Mathieu et al., 2002.
	Lipoprotrin lipase		Azzara and Dimick, 1985b
	Collagenase		Prin-Mathieu et al., 2002.
	Myeloperoxidase		Mukherjee et al., 2004.
Lymphocytes	Elastase	----	Prin-Mathieu et al., 2002.
Epithelial cells	Cathepsin-B	Protease	Guha and Padh, 2008
	Cathepsin-D		Seol et al., 2006; Guha and Padh, 2008
	Cathepsin-L		Lah et al., 1996
Unknown cell	Cathepsin-K		Moatsou, 2010
	Catalase		Kitchen, 1976

Table 3: Changes in milk constituents with elevated SCC

Milk constituent	SCC ($\times 10^3$ cells/ml)				Reason for change
	<100	<250	500-1000	>1,000	
Decrease (in g/100 ml)					
Lactose	4.90	4.74	4.60	4.21	Reduced synthesis
Casein	2.81	2.79	2.65	2.25	
Fat	3.74	3.69	3.51	3.13	
Increase (g/100 ml)					
Whey proteins (Total)	0.81	0.82	1.10	1.31	Leakage from blood
Serum albumins	0.02	0.15	0.23	0.35	
Immunoglobulins	0.12	0.14	0.26	0.51	
Chloride	0.091	0.096	0.121	0.147	
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	

Source: Schallibaum, Melchior. National Mastitis Council, Inc. 40th Annual Meeting Proceedings. 2001.

Mammary epithelial cells are the milk secreting cells. They are shed from the mammary epithelium during lactation (Boutinaud and Jammes, 2002). The epithelial cells act as the first defense line of the mammary glands, and they may

participate in the immunity of neonates in various species (Boutinaud and Jammes, 2002).

Besides the immune defense role in the udder, somatic cells are believed to continue their protective function in milk. Additionally, some components being identified to be from SCs help to enhance the host defense. For example, PMNs have bactericidal and respiratory burst activities and they can eliminate the invading bacteria by releasing granular enzymes and reactive oxygen species (ROS) (Paape et al., 2002). Some antibacterial proteins such as macrophage scavenger receptor types I and II, lymphocyte cytosolic protein 1, PMN peptidoglycan recognition protein and cathelicidins, identified in bovine milk also arise from SCs. They can continue to exert their protective properties when they are in skim milk, whey, or milk fat globule membranes (Hettinga et al., 2011).

The enzymes released from SCs (Table 2) play an important protective role in milk. The function of the lysozyme (SC endogenous enzyme) is well recognized for the ability to destroy bacteria (Paape et al., 2003). Certain proteinases secreted by PMNs, such as cathepsin G, elastase, and proteinase 3, furnish antimicrobial activities during phagocytosis of the invading microbes. They could also cleave the bacterial virulence factors and contribute to the extracellular killing of microorganisms by cleaving their bacterial virulence factors as shown in mice (Pham, 2006). Catalase, one of the main antioxidant enzymes in milk, is an endogenous enzyme from PMNs and is suspected of being responsible for changed redox potential of milk that limits the survival capability of microorganisms (Hamed et al., 2008).

Estimation of Somatic Cell Counts

Though SCC is subjected to variation, it is still used as an indicator of milk quality in several species, especially in ruminants and humans (Hunt et al., 2013). For measuring SCC, the direct microscopic method is commonly used. However, because of the complexity and laboriousness of this method, indirect methods have become widespread.

Fluorescence flow cytometry is an indirect method for counting somatic cells after staining their nuclei with special reagents. The automated system based on this method consists of an analyzer, PC, specialized software and sometimes a device for automatic supply of the cuvettes with the samples. Another indirect method based on the measurement of electrical conductivity of milk is the conductometric method. Since mastitis milk/ milk with high concentration of somatic cells possesses high electric conductivity because of the increased concentration of chlorine ions. However, the instrument that operates on this method can serve as a mere indicator of the variations in the number of somatic cells in milk, rather than precise somatic cell counter. Furthermore, viscosimetric method based analyzers measure the outflow time of the tested sample through a special capillary with known diameter and display the results in accordance with the calibration chart. The time of milk mixture outflow in this method increases accordingly with the increase of somatic cell concentrations. There is however very little information on the specific application of these methods in ewe milk (Berglund et al., 2004) on account of the higher content of total solids than cow milk.

High SSC *Vs* Quality of Milk and Milk Products

Unlike milk production loss, there is a direct relationship between SCC and quality of milk (Table 3) and dairy products. The negative correlation between milk yield and SCC is well documented by many authors (Juozaitiene et al., 2006; Jia-zhong et al., 2010). Elevated SCCs have been associated with changes in milk composition because of reduced synthetic activity in the mammary tissue (Lindmark et al., 2006). Cinar et al. (2015) reported SSC to have a negative correlation with lactose (%) and positive correlation with total solids (%), milk fat (%) and protein (%). The decrease in the lactose in high somatic cell counted milk, causes delayed acidification and impairs the hygienic safety of the end product (Sharma, 2007). The negative consequences of the presence of high SCCs are however more strongly related to shorter shelf life and undesirable organoleptic characteristics of the final dairy products, due to enzymatic activities of somatic cells (Töpel, 2004). Elevated SCC decreases fat and protein content in cheese; increases cheese moisture level and reduces moisture-adjusted cheese yields (O'Brien et al., 2004). The increase in cheese moisture may be due to weak coagulation on account of altered milk protein composition, mineral disproportion and an increased milk pH (Auldist, 2000). Somatic cell count may also have negative consequences on cheese flavor as it affects lipolysis in cheese (Chen et al., 2010). Furthermore, though SCC in milk did not increase the extent of proteolysis and viscosity of yoghurt. However, free fatty acids increase with elevation in SCC and reduce the shelf life of yoghurt (Fernandes et al., 2007). Based on these results the authors suggested that raw milk used to produce yoghurt should not contain more than 400 000 somatic cells/mL.

Factors Affecting Somatic Cell Count

Apart from intramammary infection, there are a lot of factors that influence the milk somatic cell count both at individual and herd level. The ability to correctly interpret somatic cell counts depends on the understanding of the factors which may affect the number of somatic cells.

Udder Infection

Infection status of the udder is the most important factor affecting the somatic cell count in milk (Dohoo and Meek, 1982; Vissio et al., 2018). The degree and nature of

the cellular response are proportional to the severity of the infection. The average bulk tank milk SCC increases with an increase in the number of infected quarters (Meek et al., 1980).

Stage of Lactation

SCC increases aslactation progresses regardless of whether the cow is infected or not (Dohoo and Meek, 1982; Mijić et al., 2012). The percentage of lymphocytes decreases while that of neutrophils increases in early and late lactation. Usually, higher SCC's (>10^6 cells/mL) are encountered at the time of parturition which then decreases to 10^5 cells/mL in 7 to 10 days post-partum (Jensen and Eberhart, 1981).

Milking Frequency

Some investigations showed that SCC varies with milking frequency. A shift from two times a day to three times a day milking decreases bulk milk SCC and the proportion of high somatic cell counted cows (Hogeveen et al., 2001), while very short milking intervals (4 h and less) increase SCC (Hamann, 2001). Long milking intervals with automatic milking systems (AMS) increase bulk milk SCC (Pettersson et al., 2002).

Age/ Breed

Somatic cell count usually increases with age of the dairy cow (Beckley and Johnson, 1966), primarily due to an increased prevalence of infection in older cows. Variations in SCCs have also been noted between different breeds of dairy animals. The high-producing exotic cattle breeds usually have a higher presence of SCs/mL in milk in contrast to indigenous breeds (Krol et al., 2013).

Parity

Parity has been reported to influence the SCC (Bombade et al., 2017). As parity advances, cows have a greater probability of developing high SSCs in milk (Skrzypek et al., 2004). The increased SCC with parity may be attributed to the increased prevalence of intramammary infections and greater cellular response to certain pathogens. However, there is little change in SCC of uninfected quarters as the number of lactations increases (Sheldrake et al., 1983).

Seasonal/ Diurnal Variation

Seasonal variation in SCC for dairy herds has been consistently reported (Summer et al. 2007; Cicconi-Hogan et al., 2013). Generally, somatic cell counts are highest during summer and lowest during winter (Khate and Yadav, 2010; Bombade et al., 2017). The probable reason of high SSC during summer could be the increased growth and number of environmental bacteria in the bedding material of housed stock due to favorable temperature and humidity (Harmon, 1994). Diurnal variationsin SSC have also been proposed (Alhussien and Dang, 2017). In general, SCC is lowest prior to milking, increases rapidly up on stripping, and may persist for up to 4 hours after milking and then gradualy declines. These differences in high and low SCC for individual quarters vary from 4 to 70-fold (White and Rattray, 1965). Studies have also shown that two consecutive milkings from the same cow could fluctuate in SCC by 30%.Out of all the milk somatic cells, neutrophils exhibit maximum diurnal variation in their numbers (Alhussien and Dang, 2017).

Reduction And Management of High Somatic Cell Counts

The primary reason for dairy producers to reduce SCC is to decrease milk losses due to mastitis. Milk processors want decreased SCCs because it reflects increased cheese yield and keeping quality of the milk. Though mastitis is a bacterial disease occurring in individual animals but for its abatement mastitis control programmes need to be implemented at the herd level. Numerous studies have indicated that effective implementation of best management practices result in reduced bulk tank somatic cell counts (Olde Riekerink et al., 2010; Dufour et al., 2011). However, control of mastitis requires a multidisciplinary approach that is focused on prevention of new infections and appropriate intervention for infected cattle. Since bacterial invasion mostly occurs during the dry period, particularly during advanced gestation. Therefore to control SCC and reduce the occurrence of mastitis, prevention strategies should be followed during the dry period. Neave et al. (1969) developed the 5-point plan that is the basis for control of intramammary infections caused by contagious pathogens. However, on many modern dairy farms, the BTSCC is low but environmental pathogens continue to cause excessive cases of clinical mastitis (Oliveira and Ruegg, 2014). To address the increased incidence of mastitis caused by environmental pathogens, the national mastitis council expanded the 5-point plan to 10-points that focus on comprehensive mastitis control (NMC, 2013). Based on these plans, 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 emphasize on 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 Considerations

Supplementation of antioxidant vitamins and minerals de-

creases milk SCC. This is because of the fact that mastitis is associated with release of free radicals, increased total oxidant capacity and decreased total antioxidants capacity in milk (Atakisi et al., 2010). Vitamin A is an important factor in improving immune function and attenuating oxidative stress (Jin et al., 2014). In addition, beta-carotene (precursor of Vit A) appears to function as an antioxidant and plays an important role in protecting udder tissue from the harmful effect of free radicals by reducing superoxide formation within phagocytes. Jukola et al. (1996) reported low concentrations of Vitamin A (<0.8µg/mL) and beta-carotene (<2µg/mL), linked with severity of bovine mastitis.

Vitamin E acts as a lipid-soluble cellular antioxidant, free radical scavenger, and protects against lipid peroxidation (Yang et al., 2011). Supplementation of vitamin E (at 500 IU/animal/d) and selenium (at 6 mg/animal/d) alone or in combination for two months during early lactation reduced the SCC from 29.39×105 to 8.28×105 cells/mL of milk (Sharma and Maiti, 2005). A meta-analysis by Moyo et al. (2005) to estimate the magnitude and significance of the effect of vitamin E status on udder health demonstrated that vitamin E supplementation was on average associated with a 14% reduction in the risk of occurrence of clinical mastitis. Moeini et al. (2009) reported that milk SCC of heifers significantly decreased (193,000/mL vs. 179,000/mL) upon Se and α-tocopheryl acetate supplementation.

Zinc is essential for maintaining the integrity of keratin lining the streak canal. Low Zn status leads to poor quality milk with high SCC (Gaafar et al., 2010). Popovic (2004) reported zinc methionine to significantly reduce SCCs in cows from 45 days pre-calving until 100 days post-calving. Moreover, Scaletti et al. (2003) reported that copper supplementation reduced the SSC and severity of signs during experimental E. coli mastitis; however, the duration of mastitis was unaffected.

CONCLUSION

Understanding the relationship between somatic cells and the production of high-quality milk is fundamental for the profitability of the dairy business. Optimum outputs can be achieved by reducing somatic cell counts at the herd level. Routine screening tests, improved sanitation, dry period therapy and improvement in management, as well as feeding, are needed to reduce somatic cell counts and prevent the occurrence of udder infections.

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CONFLICT OF INTEREST

The authors declare that they have no competing interest.

AUTHORS CONTRIBUTION

All authors contributed equally in collecting the relevant information and finalising the manuscript.

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