

Physiological Aspects of Milk Somatic Cell Count in Dairy Cattle

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Abstract

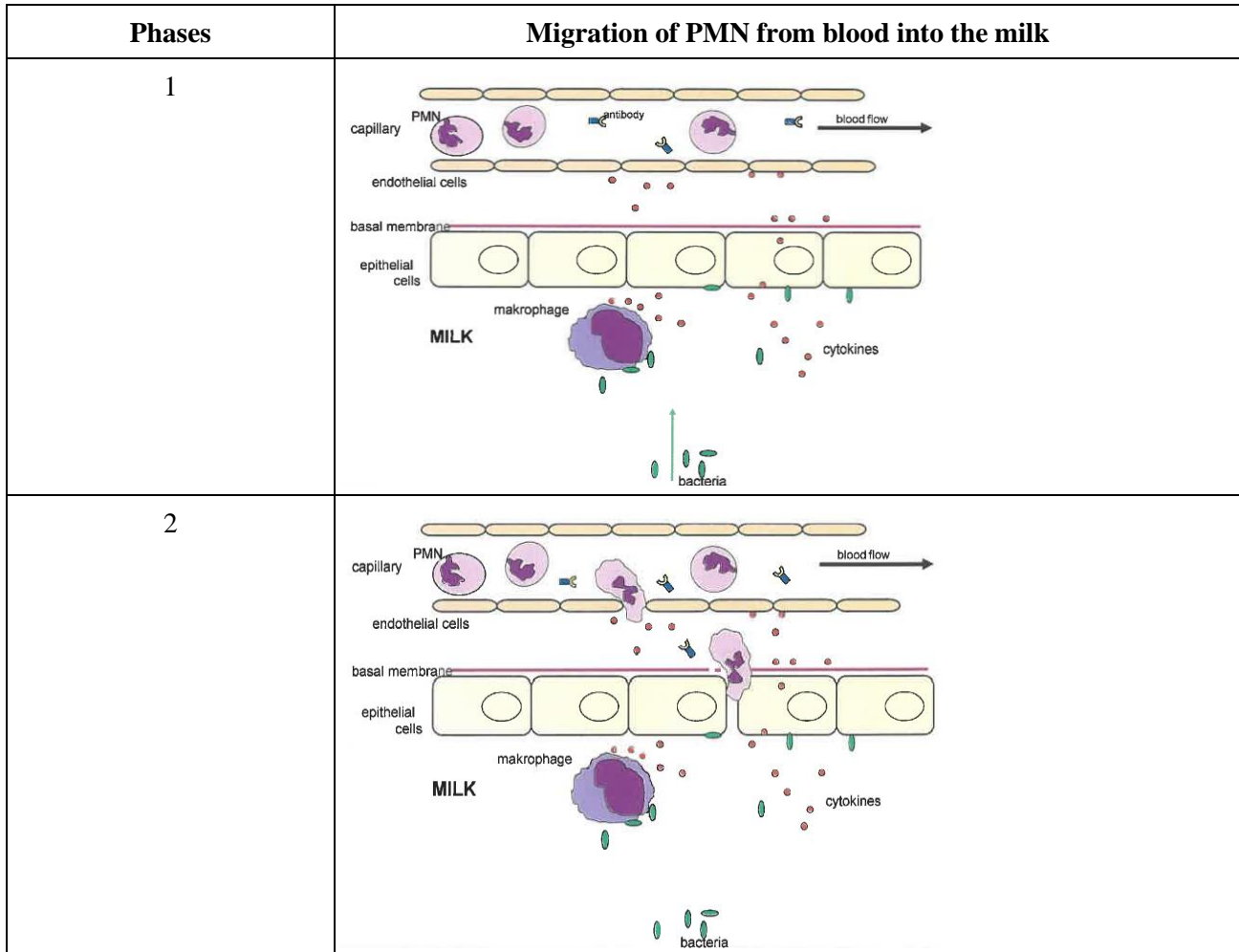
An assessment criterion for the raw milk quality and the udder health of dairy cattle is the milk somatic cell count (SCC). Somatic cells are part of the udders' immune system and the protective mechanisms of the mammary gland. They are always present in milk, but increase when an infectious agent enters the udder or the udder is injured. The present study sheds light on some physiological aspects of milk SCC in dairy cattle. Somatic cell counts of up to 100,000 cells/ml milk at the individual animal level are referred to as the normal physiological range. However, the number and the differential cell pattern of these cells in healthy dairy cattle is determined by physiological factors such as milking fraction, lactation stage, parity and breed of the dairy cattle. The following proportions of SCC are found in the milk produced in a healthy udder: macrophages (58%), polymorphonuclear neutrophils (PMN) (12 %), lymphocytes (28%) and epithelial cells (2%) of the total SCC. If the udder is contaminated, the SCC in the milk increases sharply and the PMN content of the milk also increases significantly (up to 90%). It is noteworthy that not every increase in SCC in milk indicates an infection of the udder. There are around 15% milk samples that contain a higher number of cells and at the same time no pathogens are present in the udder. Furthermore, the SCC level and the milk quality depend on the milking technology and routine. Thus, the milking machine is seen as an important factor in milk performance and quality. Due to the observed effects, the use of a quarter individual milking system "MultiLactor" has a positive significant impact on udder health and therefore the SCC remains in the range of the physiological level. In addition, it is necessary to adapt the housing system to the requirements of the dairy cattle to stabilize a low SCC and healthy udders. It is also noted that increasing SCC in milk is a decrease in raw milk quality, which affects milk processing. Therefore, the researcher suggests that the upper limit for SCC for cheese milk should be 100,000 per ml.

In conclusion, prevention is the key to long-term low milk SCC. This can be achieved through the use of the right milking technology, appropriate husbandry, good feeding and implementation of hygiene measures.

Keywords: Cow, Milk, Milking Machine, MultiLactor, Milk processing, Physiology, Somatic Cell Count

Introduction

Somatic milk cells are the body's own cells that enter the milk of dairy cattle from the blood and mammary gland tissue. The SCC is quantified as cells per milliliter milk. All milk from dairy cattle contains somatic cells (SC). Many countries in the world use SCC as an indicator of raw milk quality, to monitor mastitis prevalence in dairy cattle and as a general indicator of the hygienic conditions of milk production on farms (Hamann, 2010; Burgess 2010; Hunt *et al.*, 2013; Alhussien and Dank 2018; Deshapriya *et al.*, 2019). However, SCC is often used to distinguish between infected and uninfected udder quarters (Schukken *et al.*, 2003). The majority of somatic cells are white blood cells and a small number of epithelial cells and non-cellular units (Paape *et al.*, 2002; Sarikaya, 2006; Burgess, 2010; Sharma *et al.*, 2011; Zajac *et al.*, 2018). The secretory parenchyma of the mammary gland is partially permeable to the blood cells. This permeability increases under abnormal conditions of milk secretion and leads to an increase in somatic cells, predominately white blood cells, which help fight infection by attacking invaders and consuming infected or dead cells (Figure 1).



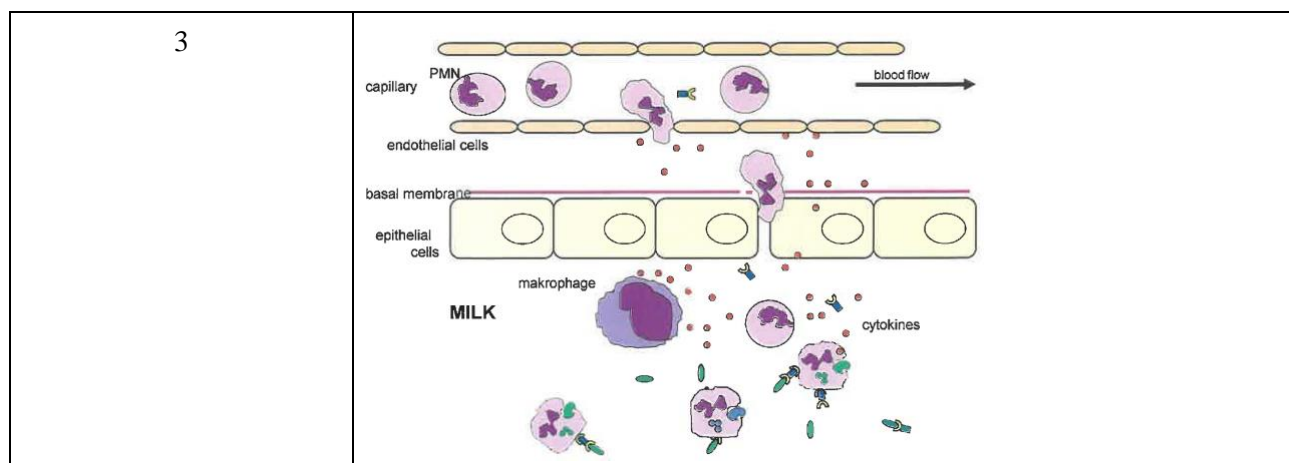


Figure 1: Phases of migration of PMN through epithelial cells from the blood into the milk; by Bruckmaier (2013) with some changes.

The white blood cells in the milk are composed of polymorphonuclear neutrophils (PMN), macrophages and lymphocytes (Fig. 2).

Microscopical pictures of the somatic cells				
SC Diameter	5-10 μm	8-30 μm	4-9 μm	15-30 μm
Name of the SC	lymphocyte	macrophage	PMN*	epithelial cell

Figure 2: Somatic cells in the milk using the microscope. PMN*: polymorphonuclear neutrophils granulocytes (Mielke 1994; Sarikaya, 2006; Zajac *et al.*, 2018).

However, the distribution of these cells depends on udder health. In the healthy udder, the proportions of SC are as follows: macrophages with 58%, PMN cells with 12 %, lymphocytes with 28% and epithelial cells with 2% of the total SCC (Leitner *et al.*, 2000; Winter, 2010; Alhussien and Dank, 2018). In the event of contamination of the udder, the SCC in the milk increases very sharply and the relative proportions of the cell types present in the milk change significantly. In particular, the PMN content increased (up to 90%) in order to protect the udder from invading microbes (Burvenich *et al.*, 1994; Paape *et al.*, 2002; Alhussien *et al.*, 2016; Alhussien and Dank, 2018) (Table 1). This means that PMNs migrate from the blood to the udder in response to a variety of inflammatory mediators to phagocytize and kill bacterial pathogens (Persson *et al.*, 1993). Milk SCC of an individual dairy cow indicates its udder health status, but tank milk SCC can indicate the general state of the udder health in a herd of dairy cattle (Hamann, 2010). The increase of the SCC in milk affects lipolysis and the physiochemical composition of milk (Ma *et al.*, 2000; Gargouri, 2008).

Table 1: Cell distribution in whole quarter milk of different SCC levels (Sarikaya *et al.*, 2004)

Parameters	SCC < 100 x 10 ³	SCC 100-400 x 10 ³	SCC > 400 x 10 ³
SCC (log ₁₀ cells/ml)	4.57±0.10	5.39±0.06	6.15±0.07
Lymphocytes (%)	20.9±1.2	11.4±1.4	3.3±0.3
Macrophages (%)	45.6±1.9	25.1±3.5	9.5±1.9
PMN (%)	33.5±1.7	63.5±3.8	87.2±1.8

In the following sections, the physiological aspects of SCC in milk in dairy cows are presented and discussed.

I. Physiological Level of Milk Somatic Cell Counts in the Dairy Cattle

According to the current state of knowledge, SCCs of up to 100×10^3 cells/ml milk at the individual animal level are referred to as the normal physiological range (Hillerton, 1999; Leitner *et al.*, 2000; Skrzypek, 2002; Krömker 2015) and research from North America and Europe has showed that uninfected quarters had a mean SCC of approximately 70×10^3 cells (Dohoo and Leslie, 1991; Schepers *et al.*, 1997; Djabri *et al.*, 2002). This SCC varies between 10×10^3 and 100×10^3 cells/ml in healthy udders. However, the number and the differential cell pattern of these cells in healthy dairy cattle is determined by physiological factors such as milking fraction, stage of lactation, parity and breed of the dairy cattle (Table 2). The SCC may change during the milking process (Ontsouka *et al.*, 2003; Bruckmaier *et al.*, 2004) and a high SCC can be observed at the beginning and at the end of the milking process (Sarıkaya and Bruckmaier 2006) as well as at the beginning and end of the lactation period (Sheldrake *et al.*, 1983; Zecconi and Smith, 2000; Singh and Dang, 2002; Wingren, 2018; Kaskous 2020). Moreover, with increasing lactation number, the SCC in the milk increases (Smith, 1995; Schukken *et al.*, 2003; Saravanan *et al.*, 2015; Goncalves *et al.*, 2018; Wingren, 2018; Kaskous, 2020).

Table 2: SCC in milk by breed in Bavaria, Germany (milk recording- report 2019)

Breed	Number of cows	Number of samples	SCC x 10^3
Simmental Cattle	919011	6757036	211
Brown Swiss	133205	1049440	237
Yellow Cattle	1904	13625	272
Holstein Cattle	103006	766451	258
Red Holstein	25902	196713	252
Jersey Cattle	870	6569	288

The table clearly showed the low SCC content in the Simmental breed compared to other breeds. However, the SCC in this table applies for the entire year 2019 and for all dairy cows in Bavaria under different environmental conditions such as feeding, husbandry, milking technology and hygienic measures.

II. Increase in Somatic Cell Counts above Physiological Level

A rapid increase in the SCC above physiological values reflects the activation of the mammary immune response to an intramammary infection or an injury to the udder (Schukken *et al.*, 2003; Hamann, 2010; Burgess 2010; Nolan 2017). This increase in SCC not only fights infection, but also repairs tissue damage (Alhussein and Dang, 2018). Therefore, the risk of mastitis in herds with an SCC of several hundred thousand per ml is significantly higher than in herds with a tank milk SCC of less than 100×10^3 per ml (Hamann, 2010). But not every increase in the SCC in milk indicated an infection of the udder. There are around 15% of milk samples that contain a higher number of cells while at the same time no pathogens are present in the udder (Sharma *et al.*, 2011) (Fig. 3). In this case, environmental factors have influenced the increase in SCC in milk, such as: feeding, husbandry, temperature, humidity, etc.

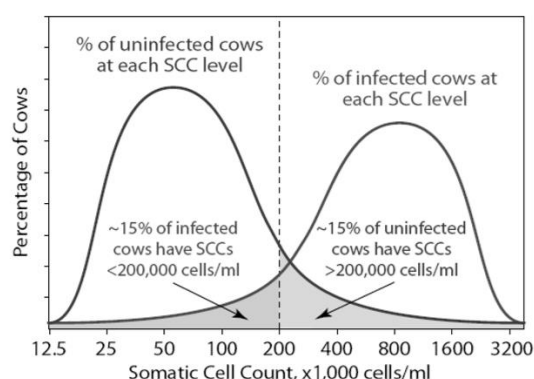


Figure 3: Relationship between SCC and udder infection status of cow (Sharma *et al.*, 2011)

In order to be able to differentiate between infected and non-infected quarters under practical conditions, it has been shown repeatedly that a cut-off of approximately 200×10^3 to 300×10^3 cells was optimal in order to reduce

diagnostic errors. Therefore, milk with an SCC greater than 400×10^3 is classified by the European Union as not suitable for human consumption. The limit of 400×10^3 is not viewed as the physiological cell concentration in milk that distinguishes healthy from unhealthy udders, but as an operating threshold of practical value under field conditions, not the ultimate goal for udder health and the production of top-quality milk.

Generally, a lower SCC indicates better animal health, as somatic cells originate only from inside the animal's udder. Some studies have reported that low SCC is regarded as a sign of poor immune response, but in general this does not have to be true; it can happen that there is only a minor current infection. Therefore, immune response is best measured by how quickly the immune system reacts to the disease challenge, not how high the SCC is before infection occurs.

Regarding the problems mentioned after the increase in SCC in milk, legal standards for the number of somatic cells are set in many countries, but these can vary widely between 100×10^3 and 750×10^3 cells/ml (Schwarz *et al.*, 2011; Olechnowicz and Jaskowski, 2012). Nowadays the lower level is a common standard in some countries because it is accepted that SCCs above 100×10^3 /ml give a strong indication that there is mastitis (subclinical) in the cattle. When changing the environment of the dairy cattle or irritation and inflammatory conditions of the udder quarter, the animal's immune system is activated and the SCC increases. An SCC of 20-50 million/ml milk and more can be reached (Urech *et al.*, 1999; Sarikaya *et al.*, 2004). These are primarily non-specific defense reactions based on an intense influx of neutrophilic granulocytes into the infected udder and its milk. However, the mammary gland has various cellular, anatomical, and humoral defense mechanisms which help in preventing the invasion and establishment of pathogens in the gland (Rainard and Riollet, 2006; Alhussein and Dang, 2018). It is noteworthy that higher activities of antioxidant enzymes have been observed in milk with a high PMN content. This may indicate that PMN caused changes in the antioxidant status of the mammary glands (Hamed *et al.*, 2008). Thus, milk SCC, particularly PMN, could generate a situation of oxidative stress in the mammary gland. Pathogens usually gain entry to the quarter through the teat canal, which generally remain closed until certain times, like before, during and after the milking process.

High milk SCC also changes its chemical composition. By changing the permeability of the blood vessels or cell walls, the anion-cation-level (Na^+ , K^+ , Cl^-) changes and as a result, the conductivity and pH value are increased. In addition, the lactose content of the milk decreases, but the variations are relatively low, since lactose is involved in the osmotic system of udder.

On the other hand, many reports have shown that with increasing milk SCC, a decrease in daily milk yield has been observed and the loss in milk yield can be up to 35% and more (Valde *et al.*, 1997; Dürr *et al.*, 2008; Colla *et al.*, 2011; Hand *et al.*, 2012; Cinar *et al.*, 2015; Goncalves *et al.*, 2018) and the milk composition and quality were affected (Cinar *et al.*, 2015). However, milk loss was high during the early stage of lactation and decreased in middle of lactation, and highest milk losses were observed during the late stage of lactation (Goncalves *et al.*, 2018). Potter *et al.* (2018) found that a cow with relatively high SCC (250×10^3 cells/ml) compared with a cow with a relatively low SCC (50×10^3) produces, on average, 1.6 kg/d less milk. Similarly, Silva *et al.* (2018) found that the increase in the SCC had a negative impact on milk production and composition in Holstein cows in the semi-arid zone in Brazil. The results of a study clearly showed that with an increase in the SCC from 100×10^3 to 800×10^3 cells/ml in the milk and more, the milk yield per lactation of the black and white cow decreased to 658 kg (14.4%) (Juozaitiene *et al.*, 2006). Furthermore, high milk-producing cows are under stress of milk production, and their immunity becomes low, leading to a higher SCC in their milk (Mukherjee and Dank, 2011).

III. Dynamic Influence of the Milking Machines on the Physiological SCC Level

For 50 years it has been known that the design and function of milking machines can be directly related to the degree of mastitis in dairy cattle. In addition, the SCC level and milk quality depend on the milking technology and milking routine (Dang and Anand 2007; Petrovska and Jonkus 2014; Kaskous, 2018; 2020). Studies have shown that a high cell count in milk is due to poor milking technology (Fehlings 2006). This means that the milking equipment and routine must be adapted to the physiological mechanism of the animals in order to achieve optimal milk yield and to keep the SCC in the milk at a physiological level. Kaskous (2020) found that despite the high milk yield of the dairy cows, the SCC in the milk was low during the study period because the correct "MultiLactor" milking machine was used on the farm. The author explained that during the milking process no stress was caused because the "MultiLactor" has a special stimulation system, which is necessary for the complete emptying of the udder. This

plays a crucial role in maintaining the SCC in milk at a physiological level. Therefore, the SCC remained below 100×10^3 cells/ml during the study period (one year). Stress during milking is one of the significant factors that negatively affect the SCC in milk, as can be found in automatic milking systems compared to conventional milking parlors (Gygas *et al.*, 2008; Castro *et al.*, 2015). During the milking course, timing of oxytocin release and milk ejection before milk removal begins is very important for the subsequent milking performance (Bruckmaier *et al.*, 2001) and any interruption in the milk ejection process can disturb milk removal. The result is inflammation of the udder (Petrovska and Jonkus, 2014) which could result in an increase in the SCC in the removed milk (Bruckmaier 2001).

Thus, the milking machine is considered as an important factor in milk yield and quality including SCC level during the course of milk removal in dairy cows (Kaskous and Fadelmoula, 2015; Kaskous, 2020). After a study of 7000 Bavarian farms, Fehlings (2006) reported that observed problems with udder health and a high SCC above the physiological level of milk are often due to deficiencies in milking technology. Kaskous (2018) found that a quarter individual milking system “MultiLactor” had a significant impact on udder health by continuously reducing the number of somatic cells in the milk during the study period in different breeds from different dairy farms (Fig. 4).

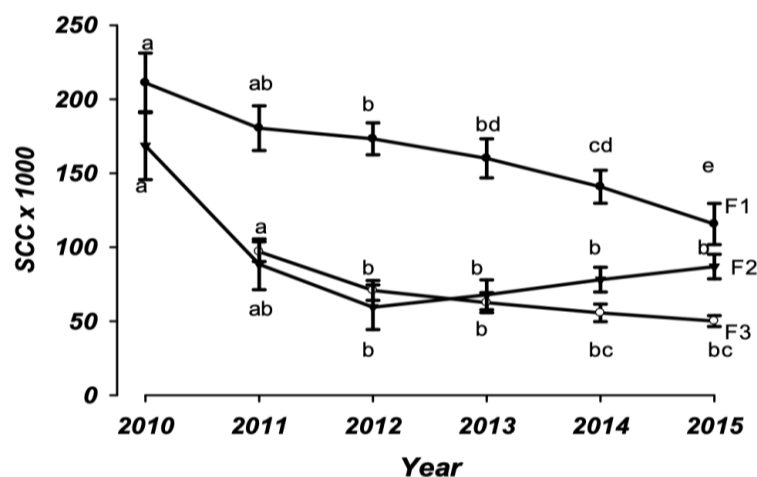


Figure 4: Development of SCC after using “MultiLactor” milking system in different breeds from different dairy farms. F1: (Holstein breed); F2: (Holstein/Red breed); F3: (Brown Swiss breed) (Kaskous, 2018).

It is equally important that the milking routine and hygiene must be optimal so that the SCC remains below the physiological level. It is possible to have a properly installed and well-functioning milking machine available on the farm and at the same time the cows will be poorly milked. Kline *et al.* (2018) indicated that hygiene practices and the milking process can influence SCC. Using a new and disposable cotton towel or paper to wipe the udders before milking can result in a lower SCC and bacteria than using the same wet towels. Furthermore, it has been shown that milking practices strongly affect the bulk tank SCC (Zucali *et al.*, 2011). An earlier study has shown that milking practices that were associated with a low bulk tank SCC were the use of post-milking teat disinfection and not drying after wet cleaning of the udder before milking (Barkema *et al.*, 1998).

IV. Dynamic Influence of Husbandry, Including Feeding, on the Physiological SCC Level

It is necessary to adapt the housing system to the requirements of the dairy cattle to stabilize a low SCC and healthy udders. Valde *et al.* (1997) found that herds with tie stalls had a higher clinical rate of mastitis, a significantly higher proportion of herds with ketosis and a lower mean fertility status index. Moreover, studies have shown that cows in organic herds have higher SCC compared to cows in conventional herds (Hovi and Roderick, 2000; Ahlman, 2010). Diet also often plays a critical role. Hence, nutrition is involved in maintaining the immunity of the dairy cow’s udder. In particular, stable metabolic situations without ketoses, rumen acidosis and mineral metabolism disorders are essential prerequisites for a strong and healthy udder with a low SCC. A non-adequate feeding routine affects the udder health and cell count negatively. Hickman (2019) reported that one of the most important foundations for healthy, high-performance dairy cows is the basic feed. A ration that is insufficiently enriched with raw fibers for the dairy cattle leads to disturbances of the rumen fermentation and as a result the cell number of the milk increases significantly. It should also be noted that a lack of energy and excess of crude protein in the fed diet greatly affects

the SCC of the milk. It is known that an excess of protein in the diet burdens the liver and if there is insufficient energy supply, the effect is exacerbated, because the detoxification of ammonia to urea in the liver is very energy-consuming. As a result, the development of udder inflammation is promoted and the cell count of the milk increases drastically. Osten-Sacken (2000) reported that urea level in milk is positively correlated with SCC. However, in Polish Holstein-Friesian cattle variety a decrease in urea level was shown with an increase in the SCC in milk (Pytlewski *et al.*, 2010).

It is important to meet the energy needs after calving by adequate feed intake to avoid subclinical or clinical ketosis (Zhang and Ametaj, 2020). Studies showed that the negative energy balance in the animal increases the SCC of the milk and weakens the resistance to mastitis pathogens (Suriyasathaporn *et al.*, 2000). According to Klucinski *et al.*, (1988), ketone bodies and ammonia in milk inhibit the udder's barrier against invading microorganisms. Therefore, increased milk ketone body concentrations are usually associated with increased cell counts (Suriyasathaporn *et al.*, 2000; Zhang and Ametaj, 2020). As a result, ketosis is associated with an increased risk of clinical mastitis (Correa *et al.*, 1993; Oltenacu and Ekesbo, 1994). While it may be controversial whether or not ketone bodies reduce the capacity of leukocytes for phagocytosis *in vivo*, several studies have clearly indicated that the killing capacity of leukocytes is impaired by ketone bodies *in vitro* and *in vivo* (Suriyasathaporn *et al.*, 2000). Thus, the impairment of the udder defense mechanism in negative energy balance cows seems related to hyperketonemia. The results of Glindemann (2006) showed that changes in the parameters of energy metabolism during the pre-partum period are related to udder health in dairy cattle and the composition of the different cell structure. Dry management phase is very important to reduce the SCC of the milk and to avoid mastitis infections (Zarwel, 2019), because both the new infection rate and the chance of healing are the highest in this phase.

The supply of vitamins and minerals also plays a major role in milk quality, especially in udder health and stabilization of the milk SCC at the physiological level. The effects of organic sources of zinc (Zn), copper (Cu) and selenium (Se) on SCC in dairy cows were studied. The results showed that the number of new and total cases of subclinical mastitis was lower for the group of cows fed with organic sources of Zn, Cu and Se compared to animals receiving inorganic sources (Cortinhas *et al.*, 2010). Good management is therefore the best defense against high SCC in milk. Supplementing the dairy cows' feed with trace minerals can reduce SCC and help the dairy cows to produce good quality milk. Also, if the mammary gland is not supplied with the necessary minerals (such as selenium) and vitamins (such as vitamin E, beta-carotene), it can lead to an increased SCC of the milk.

Mycotoxins (metabolic products of mold) represent a major problem in connection with high SCC and udder diseases. Potential sources for these are silage, concentrated feed, straw and hay. In practice it is not infrequently observed that the supply of mold components through feeding has a significant negative impact on udder health and can significantly increase the number of cells. In addition, it was found that poor feed quality, contaminated with mold, high Mycotoxins and higher nitrate content has an influence on the SCC of the milk.

Housing conditions also have a major influence on udder health and thus on milk quality (Fahr, 2002). Poor air conditions in the barn, high temperatures, insufficient lying comfort, especially unclean lying areas, insufficient water absorption and water quality, overcrowding or contact with sick animals mean more or less stress and possible consequences for the udder health of the animals. However, the following measures are important for the dairy farms: the resting areas should be dry, clean and comfortable, the walks wide enough, and the barn very light and well ventilated. The calving area should be dry, clean and after each calving must be cleaned, strewn and regularly disinfected. In fact, every mistake in husbandry and feeding, every health and metabolic disorder can weaken the animal's body defenses and the immune system, and thus increase the SCC in the milk.

V. Dynamic Influence of the SCC Level on Milk Quality and Processing

A major consequence of an increasing SCC in milk is a decrease in raw milk quality, which affects milk processing (Politis and Ng-Kwai-Hang, 1988; Urech *et al.*, 1999; Sarikaya *et al.*, 2004; Pytlewski *et al.*, 2010; Talukder and Ahmed, 2017). This can be manifested in one or more of the following ways:

- Enzymic activity of the infecting bacteria causing degradation of the milk fat and protein (Le Roux *et al.*, 1995; Klei *et al.*, 1998; Burgess, 2010; Skeie 2010; Reis *et al.*, 2013; Bezman *et al.*, 2015).
- SCC containing lipolytic and proteolytic enzymes lead to degradation of major nutrients fats and proteins, respectively (Talukder and Ahmed, 2017).

- The somatic cells promote a plasmin and other enzymes (proteolytic enzymes) which hydrolyze β - and α 2-casein into peptides with a simultaneous clear increase in γ -casein (Barbano *et al.*, 1991; Auldism *et al.*, 1996; Urech *et al.*, 1999; Santos *et al.*, 2003; Leitner *et al.*, 2006; Talukder and Ahmed, 2017). However, plasmin is present in milk from both healthy udders and udders with elevated SCC, but in the case of udder infections, the other enzymes increase more significantly (Verdi and Barbano, 1988). It is also noted that casein hydrolysis in the udder starts between milking intervals (Schaar 1985; Urech *et al.*, 1999) and even in early stages as a physiological regulator of net casein secretion (Wilde *et al.*, 1991; Urech *et al.*, 1999).
- Milk from quarters with subclinical mastitis showed elevated SCC and had increased activity of n-Acetyl- β -D-glucosaminidase (NAGase) (Urech *et al.*, 1999)
- Presence of pathogens (infecting bacteria) (Deshapriya *et al.*, 2019).
- Reduces cheese yield (Politis and Ng-Kwai-Hang, 1988; Auldism *et al.*, 1996; Santos *et al.*, 2003; Talukder and Ahmed, 2017).
- Flavour defects (bitterness, rancidity) (Ogola *et al.*, 2007; Ma *et al.*, 2000).

Furthermore, milk with a higher SCC (>500,000 per ml) reduces cheese yield (Auldism *et al.*, 1996), and such milk is associated with higher proteolytic activity (Talukder and Ahmed, 2017). This means a lower concentration of fat and casein and a higher concentration of whey proteins, especially serum albumin and immunoglobulin (Skeie, 2010). Based on results showing a marked decrease in cheese yield at an SCC of over 100×10^3 per ml milk it was suggested that the upper limit for SCC for cheese milk should be 100×10^3 per ml (Barbano *et al.*, 1991). Cheese made from milk with high SCC exhibited a decreased firmness and elasticity and an increased stickiness and off-flavour (Garndison and Ford, 1986). However, the effect of a high milk SCC was more detrimental to cheese quality in late lactation than in early lactation (Auldism *et al.*, 1996). If the milk has a high SCC, it leads to cheesemaking deterioration due to a longer coagulation time and a weak coagulum agent. This means that the cheese has an increased moisture content and a lower dry matter yield (Rogers and Mitchell 1994; Auldism *et al.*, 1996). Mazal *et al.* (2007) have produced cheese from high milk SCC (> 600×10^3 cells/ml) and low milk SCC (< 200 cells/ml) and they found significantly higher total protein and non-protein nitrogen, lower true protein and casein concentrations, and higher proteolysis during maturation in high SCC milk than in low SCC milk. Talukder and Ahmed (2017) have reported that maintaining low SCC will allow more good quality raw milk and provide a better product to milk processors whether used as fluid milk or converted to milk-based products. Investigations by Fahr and Lengerken (1997) have shown that milk from cows with a significantly increased cell count (> 400×10^3 ml) had a longer coagulation time of more than 20%.

In summary, it is important to avoid milk with high SCC during milk processing, or to reduce the SCC very efficiently on the cow farm.

Conclusion

The content of somatic cells in the milk is the most important inflammatory parameter of the udder. Cell numbers from 10×10^3 to a maximum of 100×10^3 cells/ml of milk are defined as a normal physiological range. In order to maintain this SCC level in the milk, we must take into account the following measures: The SCC must be regularly determined in the milk and documented so that we can recognize new infections.

Conflict of Interests

There is no conflict of interest.

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