



Evolution and factors influencing somatic cell count in raw milk from farms in Viçosa, state of Minas Gerais

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ABSTRACT. The somatic cell count (SCC) of refrigerated raw milk reflects the occurrence of mastitis in the herd and the management of rural property. Therefore, this study aimed to evaluate the SCC of the refrigerated raw milk and its determinant factors in dairy properties of Viçosa, State of Minas Gerais. Monthly, milk samples were taken from 44 cooling tanks during 2012. A structured questionnaire was applied to evaluate the production and management characteristics of the herds. There was a significant variation ($p < 0.05$) in the mean SCC of the milk, which was lower from June to August. Higher mean SCC and percentages of samples above the limit set by the legislation were observed in months of higher rainfall and ambient temperature ($p < 0.05$). Mechanical milking, lower milk yield, productivity, inadequate milking procedures, equipment hygiene, and water quality were all factors that influenced ($p < 0.05$) the SCC of raw refrigerated milk. Milker training is required for production of low SCC milk.

Keywords: environment, SCC, mastitis, milking, seasonality.

Evolução e fatores que influenciam a contagem de células somáticas em leite cru de fazendas em Viçosa, Minas Gerais

RESUMO. A contagem de células somáticas (CCS) do leite cru refrigerado reflete a ocorrência de mastite no rebanho e manejo da propriedade rural. Portanto, este trabalho objetivou avaliar a CCS do leite cru refrigerado e seus fatores determinantes em propriedades leiteiras de Viçosa, estado de Minas Gerais. Mensalmente, foram coletadas amostras de leite de 44 tanques de refrigeração durante o ano de 2012. Um questionário estruturado foi aplicado para avaliar as características de produção e manejo dos rebanhos. Houve variações significativas ($p < 0,05$) na CCS média do leite, sendo menores nos meses de junho a agosto. Maiores CCS médias e porcentagens de amostras acima do limite estabelecido pela legislação foram observadas em meses de maiores precipitações de chuva e temperatura ambiente ($p < 0,05$). A ordenha mecânica, menor produção de leite, produtividade, procedimentos inadequados de ordenha, higiene dos equipamentos e qualidade da água são fatores que influenciaram ($p < 0,05$) a CCS do leite cru refrigerado. A capacitação dos ordenhadores é necessária para produção de leite com baixa CCS.

Palavras-chave: ambiente, CCS, mastite, ordenha, sazonalidade.

Introduction

The production of quality milk results from several factors related to the management in the rural property, which determines the health of the mammary gland of the cows. Bovine mastitis, inflammation of the mammary gland due mainly to pathogenic microorganisms, causes a series of alterations in the composition, volume and physical-chemical characteristics of the milk (Bueno et al., 2005; Wickström, Persson-Waller, Lindmark-Månsson, Östensson, & Sternesjö, 2009; Vargas et al. 2014).

Losses in milk production during the infectious process can reach up to 26% of the total production,

according to the degree of intensity of the inflammatory process and the stage of lactation at which the infection occurs. Mastitis causes economic damage and risks to human health due to the transmission of infectious agents (Martins et al., 2007).

One of the consequences of mastitis is the increase of somatic cells in milk. These are the epithelial cells of desquamation of the mammary gland and defense cells of the organism, such as neutrophils, macrophages and lymphocytes. These cells impair the processing of dairy products, altering taste and industrial yield (Paiva, Cerqueira, Souza, & Lana, 2012).

The somatic cell count (SCC) is indicative of the occurrence of intra mammary inflammation in the

animal, if it reaches values above 200,000 cells mL⁻¹ milk (Beaudeau, Fourichon, Seegers, & Bareille, 2002; Dias, 2007). In milk of the cooling tank, the SCC may reflect the inadequate management in the dairy properties, such as the lack of hygiene during milking and in the environment of the animals. Other factors related to the occurrence of mastitis and consequent increase in SCC in dairy herds are: rearing system, diet balance, stress sources, stage and order of lactation (Guimarães & Langoni, 2005).

Due to the seasonality of the Brazilian territory, the seasons of the year are well defined in the period of drought and rain, which influences the occurrence of mastitis (Bueno et al., 2005; Martins et al., 2006; Noro, González, Campos, & Dürr, 2006; Roma, Montoya, Martins, Cassoli, & Machado, 2009). The incidence of the disease is higher in times of higher rainfall and temperature, due to the accumulation of organic matter in the environment and favoring the proliferation of infectious microorganisms. This results in higher SCC in the milk of expansion tanks (Bueno, Mesquita, Oliveira, Nicolau, & Neves, 2008), being able to extrapolate the current maximum parameter of 500,000 cells mL⁻¹ set by the Ministry of Agriculture, Livestock and Supply (MAPA).

The small seasonal variation provides better planning for the processing of dairy products by the industry at certain times of the year (Takahashi, Cassoli, Zampar, & Machado, 2012). Moreover, SCC is a well-considered criterion for the payment of milk, as it affects yield and quality of products (Coelho et al., 2014). Therefore, the objective of this study was to evaluate the evolution of the CCS of the refrigerated raw milk and the factors related to the production and management characteristics of dairy herds in Viçosa, State of Minas Gerais.

Material and methods

Samples of refrigerated raw milk from 44 refrigeration tanks in rural properties of Viçosa, State of Minas Gerais, were collected monthly during the year 2012. The collection was performed with a ladle disinfected with 70% alcohol after stirring the milk for five minutes. Samples were packaged in Pleion bottles containing Bronopol® preservative, to be sent under refrigeration to the Laboratory of Quality of Milk of Embrapa (Brazilian Company of Agricultural Research).

CCS was determined by flow cytometry using the Bentley Combi System 2300® (Bentley Instruments Incorporated, Chaska, USA) according to the methodology established by the International Dairy Federation (IDF). The results were expressed in cells mL⁻¹.

A questionnaire was applied to the employees and owners of the herds in January (summer) and July (winter) of 2012. It was considered the main aspects of management and characteristics of dairy properties reported in the literature that may interfere with the occurrence of mastitis and, consequently, in the SCC of the produced milk (Prestes, Filappi, & Cecim, 2002; Guimarães & Langoni, 2005; Souza, Brito, Moreira, Brito, & Bastos, 2005; Svensson, Nyman, Waller, & Emanuelson, 2006; Oliveira, Galvão, Paixão, & Munhoz, 2010; Coentrão, Souza, Brito, Brito, & Lilenbaum, 2008; Zafalon, Langoni, Benvenuto, Castelani, & Broccolo, 2008; McDougall, Parker, Heuer, & Compton, 2009).

The production characteristics evaluated were the type of milking (manual or mechanical), milking with calf at foot (yes or no), lactating animals (below and above 20 animals), milk production (below or above 100 L day⁻¹), milk yield (below or above 5 L day⁻¹) and lactation phase (beginning and end).

The management aspects analyzed were: adequate facilities (yes or no), adequate milking procedures (yes or no), milker hygiene (yes or no), equipment hygiene (yes or no), adequate feeding of cows (yes or no), sanity of the herd (yes or no), clinical mastitis treatment (yes or no), treatment of dry cow (yes or no), hygiene of cows' environment (yes or no), hygiene of calves' environment (yes or no), hygiene of heifers' environment (yes or no) and water quality (yes or no).

Samples of water were taken in sterile plastic bags in the milking parlor, flaming the faucets with 98% alcohol. The analyses were performed for pH, color, turbidity, hardness, chlorides, total and thermo tolerant coliforms, following methodology established by MAPA. In order to consider adequate the water samples, the results were compared with the parameters established by the Ministry of Health.

To check the influence of seasonality on milk SCC, average data of rainfall (mm) and monthly temperatures (°C) throughout 2012 in Viçosa were obtained from the National Institute of Meteorology (INMET). The results were subjected to linear regression at the 5% level of significance.

SCC data were subjected to logarithmic transformation and subjected to the Kruskal Wallis non-parametric test, at the 5% level of significance, for comparison of means over the months.

To verify the risk factors, SCC was categorized into properties with more and less than 600,000 cells mL⁻¹, a parameter established by MAPA at the time of sample collection. The results were subjected to statistical analysis of frequency, using the Fisher's

test at the level of significance of 5%, obtaining the Odds Ratio. All statistical analyses were run using Stata 12.0 software (StataCorp LP, Texas, USA).

Results and discussion

Most of the animals in the evaluated properties consisted of crossbred animals (Holstein x Gyr), kept year-round on pasture and supplemented with forage during drought periods. Herds had on average 15 lactating animals characterized as little specialized, producing on average 80 liters per day and predominantly milked manually. Pastures were formed by tropical forages and many pens were unpaved, with no area reserved exclusively for milking.

During collection of milk samples from the tanks, lower rainfall volumes tended to occur in the middle of the year, mainly from June to August (Figure 1). Higher volumes tended to occur at the beginning and end of the year, however, little rainfall was observed in February and April. The average temperatures presented the same behavior, with lower temperatures in the middle of the year.

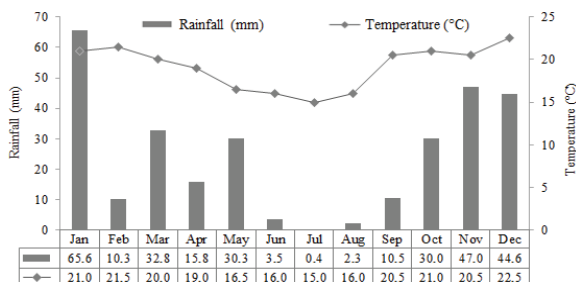


Figure 1. Average monthly rainfall and temperature in Viçosa, State of Minas Gerais, in 2012.

Source: INMET.

According to Lima et al. (2004), pronounced variations in temperature and rainfall in Brazil occur in winter and summer periods, culminating in seasonal variations in forage growth rates. From May to October, it is observed a drier climate with pastures of lower nutritional quality, which results in lower content of milk solids. Rainfall usually occurs from November to April, when the grasses present superior nutritional quality and higher solid contents.

There was a significant variation ($p < 0.05$) in the mean SCC of the refrigerated raw milk, which was lower from June to August (Figure 2), that is, in months of lower temperatures and rainfall. The coefficients of variation indicate that the data varied differently in each month, being higher in September and lower in August.

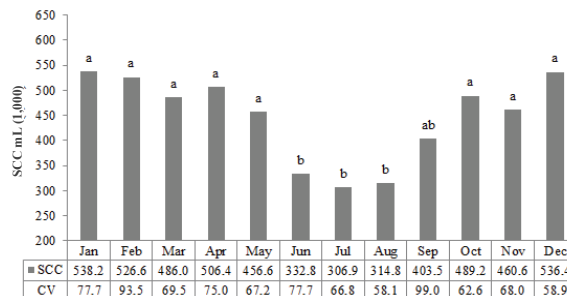


Figure 2. Mean monthly variation in SCC of refrigerated raw milk from rural properties in Viçosa, State of Minas Gerais, in 2012.

Mean values with distinct letters are significantly different by the Kruskal Wallis test ($p < 0.05$).

The regression analysis indicated that rainfall volumes and average temperatures influenced ($p < 0.05$) indirectly the mean SCC throughout the year. Higher SCC values were observed in months of higher rainfall ($r = 0.805$) and ambient temperature ($r = 0.511$).

During spring, summer and fall, months are characterized by high rainfall volumes, which increase the exposure of herds to pathogens that cause mastitis (Henrichs, Macedo, & Karam, 2014). Unhygienic conditions were observed in the pastures, with accumulation of mud and feces. The animals lay down and are contaminated with excessive organic matter, which is carried to the pens where the animals are milked and to the milking equipment.

The reduced immunity of animals due to heat stress increases the risk of being affected by mastitis and, consequently, increases the SCC of the milk of animals on pasture (Teixeira, Freitas, & Barra, 2003; Roma et al., 2009). In the present study, the stress caused by heat in months with high temperature may have influenced the higher SCC, since the animals were raised in an extensive system.

As to the percentage of milk samples that did not comply with national legislation, there were variations between 6.8 and 25.0% samples above the limit set by the legislation at the time of sample collection, i.e., above 6×10^5 cells mL^{-1} (Figure 3).

Along the year, the volume of rainfall and average temperatures also influenced ($p < 0.05$) indirectly the percentage of samples with SCC above the limit established by the legislation. Higher percentages of samples above the limit were found in periods of higher rainfall ($r = 0.629$) and ambient temperature ($r = 0.740$).

In properties with mechanical milking, daily milk production of less than 80 L day^{-1} and yield lower than $5 \text{ L cow}^{-1} \text{ day}^{-1}$, the milk SCC was higher ($p < 0.05$) than 6×10^5 cells mL^{-1} in the summer

(Table 1). The chances of SCC being high in milk from properties with mechanical milking, low production and yield is 8.7, 7.3 and 5.6 times higher, respectively, than in properties with manual milking, high production and yield.

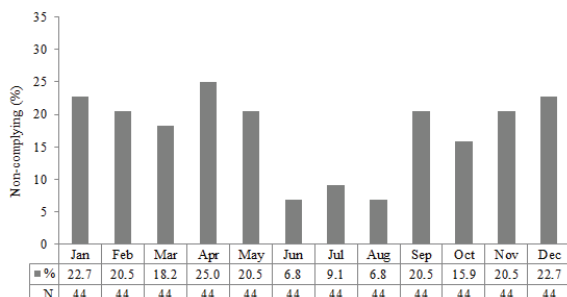


Figure 3. Percentage of milk samples from rural properties in Viçosa, State of Minas Gerais, which did not comply with the Brazilian legislation regarding SCC in 2012.

In Viçosa (Minas Gerais state), Nero, Viçosa and Pereira (2009) observed that small farmers had poor knowledge of milk management, due to lack of information from technical assistance. The farmers were unaware of the measures to produce quality milk and keep herd healthy. Poor knowledge of small farmers regarding milk quality and lack of technical assistance may have influenced the high SCC observed in the present study.

Nevertheless, Braga, Brietzke, Araújo, Garcia and Peixoto (2006) observed that the increase in the level of milk production in Marechal Cândido Rondon, State of Paraná, was related to the increase in the percentage of farmers supplying milk with SCC above 1×10^6 cells mL^{-1} , a limit established at the time. Braga et al. (2006) associated low milk SCC in properties of low milk yield with the ease of milking and cow environment. Because they have fewer animals, farmers spend more time on daily tasks.

Table 1. Production characteristics determining SCC of refrigerated raw milk from rural properties of Viçosa, State of Minas Gerais, during the summer and winter of 2012.

Variable	Category	Summer		P	Odds Ratio	Winter		P	Odds Ratio
		< 6×10^3 cells mL^{-1}	> 6×10^3 cells mL^{-1}			< 6×10^3 cells mL^{-1}	> 6×10^3 cells mL^{-1}		
Type of milking	mechanical	5	6	0.008*	8.7	9	4	0.005*	29.8
	manual	29	4			31	0		
Milking with calf presence	with calf	16	5	1.000	1.1	20	2	1.000	1.0
	without calf	18	5			20	2		
Lactating animals	< 15 cows	17	8	0.072	4.0	24	4	0.279	6.1
	> 15 cows	17	2			16	0		
Milk production	< 80 L day^{-1}	12	8	0.027*	7.3	15	4	0.029*	14.8
	> 80 L day^{-1}	22	2			25	0		
Milk yield	< 5 L $\text{cow}^{-1} \text{day}^{-1}$	10	7	0.030*	5.6	21	3	0.614	2.7
	> 5 L $\text{cow}^{-1} \text{day}^{-1}$	24	3			19	1		
Lactation phase	< 3 months	23	4	0.149	3.1	24	1	0.300	4.5
	> 3 months	11	6			16	3		

*Significant association between SCC and the variable by Fisher's test at 5% significance.

In the winter, productivity had no association ($p > 0.05$) with milk SCC in the present study. The chances of milk SCC being high in properties with mechanical milking and low production were 29.8 and 14.8 times higher than in properties with manual milking and high production. This indicates that in properties with high SCC in milk, the management of mechanical milking and low production are determinant factors, given the increased chances of SCC being high in the winter.

Incorrect use of the mechanical milking machine, lack of preventive maintenance and lack of knowledge of the farmers regarding these practices may have increased the risk of transmission of infectious diseases among animals, impairing the udder health and milk SCC. The incidence of mastitis due to the influence of milking machines is because this equipment can carry pathogens from one cow to another or between breast quarters of the same cow, which increases the SCC of the milk stored in the tank (Coentrão et al., 2008).

The SCCs higher ($p < 0.05$) than 6×10^5 cells mL^{-1} in properties with inadequate milking procedures such as poor drying of teats with paper towel and use of pre- and post-dipping, in addition to inefficient equipment hygiene and poor water quality (Table 2). In summer, the chance of SCC of milk being higher in properties with inadequate milking procedures, equipment hygiene and poor water quality is 15.7, 7.0 and 41.3 times higher, respectively, than in properties with adequate practices.

In the Zona da Mata, State of Minas Gerais, Coentrão et al. (2008) reported that the risk factors for milk CCS related to milking equipment were fissures or cracks in the rubber parts of the equipment, inadequate condition of the teat cups and poor cleaning of the equipment.

Table 2. Management practices of dairy herds determining SCC of refrigerated raw milk from rural properties of Viçosa, State of Minas Gerais, during the summer and winter of 2012.

Variable	Category	Summer		P	Odds Ratio	Winter		P	Odds Ratio
		< 6 x 10 ⁵ cells mL ⁻¹	> 6 x 10 ⁵ cells mL ⁻¹			< 6 x 10 ⁵ cells mL ⁻¹	> 6 x 10 ⁵ cells mL ⁻¹		
Adequate facilities	Yes	26	8	1.000	1.2	30	4	0.559	3.1
	No	8	2			10	0		
Milking procedures	Yes	21	1	0.004*	15.7	33	1	0.032*	14.1
	No	12	9			7	3		
Hygiene of the milker	Yes	16	3	0.474	2.1	25	4	0.282	5.5
	No	18	7			15	0		
Equipment hygiene	Yes	28	4	0.015*	7.0	29	2	0.569	2.6
	No	6	6			11	2		
Equipment hygiene	Yes	22	6	0.451	1.8	28	3	1.000	1.3
	No	8	4			12	1		
Sanity of the herd	Yes	25	6	0.449	1.8	25	2	0.634	1.7
	No	9	4			15	2		
Clinical mastitis treatment	Yes	27	6	0.237	2.6	31	3	1.000	1.1
	No	7	4			9	1		
Treatment of dry cow	Yes	15	7	0.281	2.9	18	3	0.334	3.7
	No	19	3			22	1		
Hygiene of cows' environment	Yes	14	2	0.283	2.8	17	0	0.147	6.7
	No	20	8			23	4		
Hygiene of calves' environment	Yes	24	6	0.701	1.6	27	2	0.596	2.1
	No	10	4			13	2		
Hygiene of heifers' environment	Yes	15	3	0.489	1.8	16	1	1.000	2.0
	No	19	7			24	3		
Water quality	Yes	31	2	0.001*	41.3	35	1	0.033*	15.0
	No	3	8			5	3		

*Significant association between SCC and the variable by Fisher's test at 5% significance.

Inadequate condition and lack of cleanliness of the milking machine can carry microorganisms that cause mastitis into the udder, which increases the SCC of the milk. In farms where milkers were not trained in maintaining the equipment, the animals were 2.51 times more likely to have SCC above 200,000 cells mL⁻¹.

In winter, the SCC of the tank was not associated ($p > 0.05$) with equipment hygiene. The low rainfall and lower environmental temperatures favor the cleaning of milking equipment due to the low risk of contamination by organic matter in the environment. The hygienic detergents work efficiently, since the risk of contamination by organic matter is low (Lima et al., 2004; Bueno et al., 2005; Cavalcanti, Cavalcanti, Souza, & Araújo, 2010).

Milk production requires sufficient drinking water to meet animal needs, cleaning and sanitizing the teats, equipment and facilities (Brito, Brito, & Verneque, 2000). In agreement with Guerreiro, Machado, Braga, Gasparino and Franzener (2005), environmental sources of milk contamination include water used for cleaning equipment and other tasks. It is important to use potable water for these purposes, with low contamination by coliforms and other microorganisms, such as *Pseudomonas* spp. and *Bacillus* spp.

In winter, the chances of SCC being high in milk from tanks of properties with inadequate water quality decreased to 15.0 times more than properties

with adequate water quality. The water quality has a greater influence on the occurrence of mastitis and increase in the SCC of the milk of the tank in the rainy season, because the risk of water contamination is higher and the udders of the animals are washed more frequently. The chance of SCC being higher in properties with inadequate milking procedures did not show a significant variation.

Conclusion

SCC of refrigerated raw milk from dairy herds from Viçosa, State of Minas Gerais, varies throughout the year. The higher rainfall and temperatures caused by seasonality provide higher SCC in the milk of the tank.

Mechanical milking, lower milk production, yield, inadequate milking procedures, equipment hygiene, and water quality are factors that influence the SCC of refrigerated raw milk. Training of milkers is mandatory for the production of milk with SCC below the limits set by the legislation.

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