Artigo Técnico

EFFECTS OF STORAGE TEMPERATURE AND MILK SAMPLE AGE ON THE SOMATIC CELL COUNT OF GOAT MILK

Efeitos da temperatura de armazenamento e do tempo na contagem de células somáticas no leite de cabra

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SUMÁRIO

A temperatura e o tempo de armazenamento de amostras de leite de cabra têm sido identificados como possíveis fatores que influenciam na contagem de células somáticas (CCS). Com objetivo de estabelecer o efeito da temperatura e do tempo de armazenamento de amostras de leite de cabra, foram realizadas em 320 alíquotas de amostras de leite de 20 cabras a CCS utilizando o equipamento Somacount 300 (Bentley Instruments). Foi adicionado o conservante bronopol nas amostras de leite e estas mantidas a temperatura de As amostras de leite foram 5°, 10°, 20° e 30°C e analisadas com 1, 3, 5 e 7 dias após a coleta. A temperatura e tempo de armazenamento não influenciaram significativamente a CCS (P>0,05). Os resultados de CCS em amostras de leite armazenadas a 10°, 20° e 30°C foram sensivelmente menor em relação as amostras armazenadas a 5°C. Considerando uma variação menor de 9%, os resultados da CCS em condições experimentais podem ser aceitos para monitoramento da qualidade do leite e saúde da glândula mamária em rebanhos caprinos leiteiros.

Termos para indexação: leite de cabra, amostras de leite, contagem de células somáticas, temperatura e tempo de armazenamento

SUMMARY

In goat milk, storage temperature and milk age has been identified as one of possible factors affecting the somatic cell count (SCC). To establish the effect of the storage temperature and milk age used for SCC in goat milk, counts were performed on 320 aliquots of milk sample from 20 goats using Somacount 300 (Bentley Instruments). The milk samples were preserved with bronopol, kept at 5°, 10°, 20° and 30°C and analyzed 1, 3, 5 and 7 day post-collection. The temperature storage and milk age did not modify the SCC of the milk samples significantly (P>0,05). The results of SCC in milk samples incubated at 10°, 20° and 30°C were slightly lower than those incubated at 5°C. Considering SCC

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variation lower than 9%, the results of SCC under experimental conditions can be acceptable for monitoring both the udder health and milk quality in dairy goats.

Index Terms*:* goat milk; milk samples; somatic cell count; temperature and time storage

1 INTRODUCTION

The somatic cell counts (SCC) are widely used monitors of udder health and milk quality in dairy goat industry (HAENLEIN, 2002; PAAPE et al., 2007). In the United States (US) the legal limit of SCC established by Food and Drug Administration for dairy goats is 1,000,000 mL^{-1} (PAAPE et al., 2007). The values of SCC less than 400,000 cells/mL can be used to classify uninfected goat in early lactation (McDOUGALL et al., 2001).

In milk-testing laboratories, the most commonly used method of enumerating SCC is the fluoro-optical eletronic or counter method. This method is based on the formation of a fluorescent complex when ethidiubromide dye penetrates the cell and comes in contact with nuclear DNA (INTERNATIONAL DAIRY FEDERATION, 1995). Indeed, only cell-counting procedures that are specific for DNA should be used for SCC in goats milk, due to the presence of anuclear particles (DULIN et al., 1982). SCC of goat milk were from 25 to 27% lower when the eletronic equipment was calibrated with cow milk standard. (ZENG, 1986; ZENG et al.; 1999). The eletronic equipments calibrated with cow milk standard can be used to SCC in goat milk with until 2,549,000 cell mL^{-1} (ARCURI et al., 2004).

Accuracy of SCC in milk samples is very important to most dairy farmers and to the dairy industry. Factors affecting SCC in electronic equipments, such as the preservative used in milk samples, the analytical temperature, the storage temperature and milk age have been evaluated in goat milk (ARCURI et al., 2004; SÁNCHES et al., 2005). The studies of Arcuri et al. (2004) and Sánches et al. (2005) have established the effects of these factors and their interactions on the accuracy of SCC. Using a DNA-specific electronic counter (Somacount 300; Bentley Instruments Inc., Chaska, MN). The SCC of goat milk samples preserved with bronopol presented less variation than counts conducted on unpreserved samples (ARCURI et al., 2004). No effect was observed on SCC of goats milk samples preserved with bronopol kept at lower than 5°C and analyzed 1, 2, 3 and 4 day post-collection (ARCURI et al., 2004).

In dairy laboratories, the use of electronic instruments for SCC requires that the test conditions be optimized for determining several variables in the same milk sample. Information on the different methods of preservation, storage temperature, and interactions with storage time could help to optimize analyses. The effects of these factors must also be taken into account for quality control intra- and inter-laboratory comparisons (SÁNCHES et al., 2005).

Currently, the bronopol has been used as a preservative for milk samples by milk quality laboratories in Brazil. Knowing the variation of goat SCC according to temperature and time of storage may aid in the interpretation of results. Information about the variation of goat SCC in function of time and temperature of storage is scarce in Brazil. This information can be used to establish a collect milk samples protocols for goat milk. The present study was designed to determine the effects of the storage temperature and storage time on the SCC of goat milk samples preserved with bronopol.

2 MATERIAL AND METHODS

Saanen and Toggenburg goats from the same herd were randomly selected for the study. The animals were at different stages lactation, ranged from 1 to 5 parities, and showed no clinical sign of mastitis. Before routine milking, twenty 1800-mL composite milk samples were obtained from the goats selected. Sample were kept at 4°C and immediately transported to laboratorory (Milk Quality Laboratory - Embrapa Dairy Cattle - Brazilian Agriculture Research Corporation). Immediately after mixing, by inverting 20 times, each sample was divided into sixteen 40-mL aliquots, wich were then assigned to the sixteen experimental groups as shown in Table 1.

A total of 320 counts were obtained for the original 20 milk samples. All of the aliquots were put into in plastic vials 50-ml and preserved with bronopol (0.04 mg/mL; Broad Spectrum Microtabs II; D&F Control Systems, Inc., Dublin, CA).

The milk age tested were 1, 3, 5 and 7 day post-collection. All of the aliquots were prepared and stored within 6 h of collection. Before testing, milk aliquots were heated to 40°C for 10 minutes, as this has been defined as equipment manufactor (Somacount 300, Bentley Instuments Inc., Chaska, MN). The SCC was determined by citometer flow method by eletronic equipment (Somacount 300, Bentley Instuments Inc., Chaska, MN) according to international IDF standard 148A (INTERNATIONAL DAIRY FEDERATION).

Table 1 – Experimental groups to which aliquots taken from 20 goat milk samples were assigned (16 aliquots per sample preserved with bronopol).

Storage temperature (°C)	Sample age (day post-collection)
5	1, 3, 5 and 7
10	1, 3, 5 and 7
20	1, 3, 5 and 7
30	1, 3, 5 and 7

Before and during the experiments, interlaboratory quality control tests and calibration of the equipment to check for inter-sample variability was undertaken by reference laboratory VALACTA (Ste Anne de Bellevue, Canada) using cows milk standards available. During the experimental counts, the cell counter was adjusted to a slope (*b*) = 1.00 and intercept (*a*) = 0.

Statiscal analysis was carried out using the general linear model (GLM) (MORGAN et al., 2001). The SCC logarithm was used to normalize the distribution of SCC and used as the dependent variable. Means were compared using GLM procedures. In the model applied, the effect of the goat was random, and the remaining effects were fixed such that:

$$Y_{ijk} = \mu + G_i + S_j + T_k + ST_{jk} + e_{ijk}$$

Where Y_{ijk} = dependent variables for logSCC ; μ = mean; G_i = goat effect (20 levels); S_j = storage temperature effect (4 levels: 5°, 10°, 20° and 30°C); T_k = milk age effect (4 levels: 1, 3, 5 and 7 day post-collection); ST_{jk} = effect of interaction storage temperature x milk age; and e_{ijk} = random residual.

3 RESULTS AND DISCUSSION

The results obtained for the individual samples ranged from $6{,}90x10^4$ to $2{,}62x10^6$ cells/mL and the arithmetic mean and median were $9{,}71x10^5$ and $8{,}04x10^5$ cells/mL, respectively. The test storage temperature and milk age as well as

Table 2 – ANOVA of variations in the logSCC variables

Source of variation	df	*F*	P	ES
Corrected Model	16	0.342	0.992	0.018
Goat	1	3.234	0.073	0.011
Storage temperature	3	0.192	0.902	0.002
Day post-collection (time)	3	0.376	0.770	0.004
Temperature x Time	9	0.059	1.000	0.002
Error	303			

Table 3 – Least square means of logSCC, geometric mean (GM) and arithmetic mean (AM) of goat somatic cell count (SCC) by storage temperatures x day post-collection

Temperature (°C)	Day post-collection	LSM (logSCC)	SCC (x 10^3 mL^{-1})	GMSCC (x 10^3 mL^{-1})	AM
5	1	2.873[a]	747	1.010	
	3	2.875[a]	791	1.028	
	5	2.874[a]	794	1.035	
	7	2.865[a]	782	1.020	
10	1	2.898[a]	751	1.005	
	3	2.866[a]	734	986	
	5	2.871[a]	724	984	
	7	2.860[a]	688	940	
20	1	2.900[a]	748	1011	
	3	2.860[a]	744	999	
	5	2.852[a]	711	964	
	7	2.801[a]	662	911	
30	1	2.893[a]	745	978	
	3	2.838[a]	748	970	
	5	2.821[a]	713	847	
	7	2.784[a]	682	848	

LSM - last square means; SCC - somatic cell count; [a] Means with the same superscripts in te same column no differ significantly (P > 0.05).

the interaction storage temperature x milk age showed no significant effect on logSCC variation (Table 2). Despite of no significant effects of storage temperature and milk age were observed, the effect of storage temperature was twice higher than effect of milk age.

Table 3 shows the last square means of logSCC obtained for the 16 different test conditions. Consistent with SCC results described in previous study (SÁNCHES et al., 2005), the results of SCC obtained at 1 post-collection in milk samples with bronopol at refrigeration temperature (5°C) were considered as a reference value. The least square means of the logSCC were slightly greater (P>0,05) when the samples were analyzed 1 day post-collection and kept at 5°C in relation others.

The SCC geometric means of goat milk samples kept at 10°, 20° and 30°C decreasing 8,4%, 11,5% and 5,5%, respectively, when analyzed 7 day post-collection. The relative drop of 8,7% was observed in the geometric means of SCC obtained for the milk samples kept at 5°C and analyzed 1 day post-collection compared with the milk samples kept at 30°C and analyzed 7 day post-collection. During storage at 5°C, the SCC did not change from 1 to 7 day post-collection, in agreement with previous results obtained in goat milk samples kept refrigerated for 3 and 4 days (ZENG et al., 1999; ARCURI et al., 2004). In other study, Sánches et al. (2005) observed relative decrease ranging from 4% to 7%, in the geometric means of the SCC in goat milk samples preserved with bronopol and kept at refrigeration temperature for different day post-collection. However, in milk samples stored at 10° to 30°C the SCC remaining constant throughout the study period. The low variation of SCC within different experimental groups suggesting the suitability of several storage temperature and milk age was due to preservative presence (bronopol). A decreasing lower than 10% has been observed with little significance in the practical interpretation of the SCC. This variation could have some economic effects as the milk bonus payment using SCC and remaining below the legal limit established by official agencies. This variation could also provide wrong interpretation about the milk quality and udder health of goat herd.

4 CONCLUSION

The storage temperature and milk age did no modify the SCC in samples preserved wity bronopol. In milk samples kept at 5°C from 1 to 7 day post-collection did not vary but those kept from 10° to 30°C for 7 day post-collection changed only slightly, nearly 12% of arithmetic mean in day 1. This results suggesting that temperature storage more appropriated was 5°C with SCC variation lower than 9%. The results of SCC under experimental conditions can be acceptable for monitoring both the udder health and milk quality in dairy goats. Finally, despite the scarce significance of variations attributable to storage temperature and milk age, the effects of these factors should nevertheless be taken into account for intra- and inter-laboratory quality control.

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