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EVALUATION OF METHODS OF ANALYSIS TO DETERMINE THE SOMATIC CELL COUNT IN RAW MILK, KEPT IN THE COOLING TANK

Avaliação de métodos rápidos para análises da contagem de células somáticas no leite cru de tanques de resfriamento

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ABSTRACT

We analyzed the quality of raw milk from eight dairy property in Rio Grande do Norte, Brazil, stored in a cooling tank, in order to evaluate methods for determining somatic cell counts (SCC). The Somaticell® kit and a portable Direct Cell Counter (DCC) were compared with each other and with the MilkoScan™ FT+ (FOSS Denmark), which uses Fourier Transform Infrared Spectroscopy (FTIS). Direct cell counter data were processed for somatic cell scores (log-transformed somatic cell count) and analyzed with the SAS®, Statistical Analysis System. Comparison of means and correlation of somatic cell scores were conducted using Pearson's correlation coefficient and the Tukey Test at 1%. No significant difference was observed for comparison of means. The correlation between somatic cell scores was significant, that is, 0.907 and 0.876 between the MilkoScan™ FT + and the Somaticell® kit and Direct Cell Count (DCC) respectively, and 0.943 between the Somaticell® kit and Direct Cell Count (DCC). The methods can be recommended for monitoring the quality of raw milk kept in a cooling tank in the production unit.

Keywords: somatic cell score; mastitis; milk quality.

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RESUMO

A qualidade do leite cru mantido em tanque de resfriamento de oito propriedades do Rio Grande do Norte foi analisada, com o objetivo de avaliar métodos para determinação da contagem de células somáticas (CCS). Foram usados o kit Somaticell® e o equipamento portátil Direct Cell Counter (DCC), sendo comparados entre si e com o MilkoScan™ FT+ (FOSS) que usa a metodologia do Infravermelho com Transformada de Fourier (IVTF). Os dados de CCS foram transformados para escore de células somáticas ECS log (CCS) e analisados pelo pacote estatístico SAS®. Foram feitas a comparação das médias e a correlação dos escores de células somáticas por meio do coeficiente de correlação de Pearson, sendo aplicado o Teste de Tukey a 1%. Não foi observada diferença significativa para a comparação das médias. A correlação entre os escores de células somáticas foi significativa, sendo de 0,907 e 0,876 entre o MilkoScan™ FT + e o kit Somaticell® e o Direct Cell Count (DCC) respectivamente, e de 0,943 entre o kit Somaticell® e o Direct Cell Count (DCC). Os métodos avaliados podem ser recomendados para o monitoramento da qualidade do leite cru refrigerado mantido em tanque de resfriamento em nível de unidade produtiva.

Palavras-chave: escore de células somáticas; mastite; qualidade do leite.

INTRODUCTION

Brazil created the "Programa Nacional de Melhoria da Qualidade do Leite (PNQL)'' – (Milk Quality Improvement Program) aiming to implement measures focused on improving the quality of dairy and dairy products. In 2011, Ministry of Agriculture, Livestock and Provision published the Normative Instruction Nº 62 approving the technical regulations of production, identity and quality of milk – types A, B and C, pasteurized milk and refrigerated raw milk as well as technical regulations for the collection of refrigerated raw milk and its transportation (BRASIL, 2011).

The somatic cell count (SCC) of milk from Brazilian herds started to be monitored by the requirement NI 62/2011, with maximum values – by geographic region and deadlines to be achieved. That is due to the fact that the SCC is accepted worldwide as a leading indicator of milk quality which encourages the assessment of presence or absence of subclinical mastitis in the herd, providing data for the estimation of production losses at a production unit level (FONSECA; SANTOS, 2000). The determination of SCC in the milk from the refrigerated tank is connected mainly to the idea of – product quality in terms of consumption and processing, and the milk analysis is part of the mastitis control in terms of prevalence of the subclinical disease, evolution of acute and chronic infections and loss of milk production (RODRIGUES, 2008).

Despite the benefits of SCC in monitoring milk quality, technologies for quantification of somatic cells are still restricted to the laboratory, hindering its adoption by the producer. In this context, both direct and indirect methods of estimation of SCC have been undergoing changes.

As an example of indirect methods, we have: the California Mastistis Test (CMT), Wisconsin Mastitis Test (WMT) and Somaticell®. The latter is an adaptation of WMT, sold as fast kit is a qualitative test for essence but presents conversion to SCC/mL, facilitating its adoption by the producer in the properties.

At the other extreme are the goals, methods for official control of the Brazilian Network for Quality Milk (RBQL). These procedures employ electronic equipment that use different techniques for determining the somatic cell count in raw milk. Among the technologies used by electronic equipment, we have: flow cytometry equipment used by Somacount (Bentley Instruments Inc.) and Fossomatic (FOSS Denmark), the technique of infrared spectrometry Fourier transform used in the equipment MilkoScan TM FT + (FOSS Denmark). The optical lens is also available to the producer through a device portrait. Direct Cell Counter (DCC), which is a battery-powered capable of determining the somatic cell count in a sample of raw milk about, forty five seconds (DE LAVAL, 2013). The DCC with its portability and ease of use is likely to be used in the production unit (RUEGG et al. 2005).

The current situation of the Brazilian dairy cattle guides towards the necessity of having fast and reliable information on the number of SCC in the milk at a production unit level, hence the importance of assessing the number of SCC in the cooling tank, which favors quick decision making to correct possible occurrences. By sending samples to one of the operating units of the "Rede Brasileira de Laboratórios de Controle da Qualidade do Leite'' (Brazilian Network of Milk Quality Control Laboratories), despite abiding the legislation, it has as a main drawback, the time elapsed between the collection of milk and access to the results, that, depending on the geographic region, might take a long time, plus the shipping fees and analysis (ARAÚJO et al., 2012).

The aim of this study was to evaluate methods of analysis for determining the somatic cell count (SCC) in loco, in raw cattle milk, held in the production units'cooling tanks, located in the state of Rio Grande do Norte, Brazil.

MATERIAL AND METHODS

From January 2010 to January 2011, raw milk samples were collected monthly and kept in cooling tanks, from eight property specialized in the production of cattle milk, located in Mesoregions: Agreste Potiguar in the state of Rio Grande do Norte, Brazil. In each property, eight samples were collected in vials with a capacity of 40 mL, four of them without preservative and in the other four, Bronopol® preservative tablets were added. The vials were packed in insulated box containing ice, and four samples with no preservative were analyzed by Somaticell® and Direct Cell Counter® (DCC), and four other samples containing the preservative were sent to the Milk Clinic Laboratory (ESALQ / USP) for electronic analysis of somatic cell count by Fourier Transform Infrared Spectroscopy (FTIR) using the equipment MilkoScan™ FT + (FOSS, Denmark). The collection procedure was performed according to recommendations of the Instructions Manual for collecting and sending samples of milk for analysis, CASSOLI; MACHADO, 2006.

The Somaticell® analysis was repeated to each sample, with the tube in an upright position, the reagent was added to the 2 ml mark, and then with the aid of the pipette, the milk was placed until it reached the 4 ml mark. The tube was held vertically and the solution was stirred with the aid of a straw, making up 30 moves up and down, during a period of 20 to 24 seconds. After the homogenization, the tube was closed with a proper lid up to its lock and turned, leaving the lid down for 30 seconds. Afterwards, the tube was turned over again, for 5 seconds and then preceded the reading of the remaining liquid on the graduated scale on the tube itself.

The samples for analysis in the DCC® were refrigerated, and analyzed in the morning after the day of collection. The sample was homogenized and placed in a disposable cup and with the aid of a disposable

cassette, aspired 0.6 ml of milk, then the cassette containing the sample was placed in the reading apparatus which emits a beam of light passing through the cassette and enables the machine to read the sample and to count individual cells. The reading time per sample was approximately 45 seconds, with the result being presented in SCC / microl on the device's display.

The results of the analyses performed by the laboratory during January 2010 to January 2011 were used as a reference for the comparison with the results obtained by Somaticell® and the Direct Cell Counter. The vials containing milk samples were packed in insulated boxes with recyclable ice and shipped the next morning, on the day of collection to the Milk Clinic Laboratory (ESALQ-USP). The SCC results were determined by Fourier Transform Infrared Spectroscopy (FTIR) using the equipment MilkoScan™ FT + (FOSSTM).

The data collected in this research were organized and evaluated using descriptive statistics, analysis of variance (ANOVA) and correlation analysis. For this purpose, the homogeneity of variances and normality of data were tested. These tests demonstrated the need to perform mathematical transformation of the variable SCC to make it possible to achieve normality and homogeneity of variances (SHOOK; RUEGG, 1999). We used the logarithm (LOG) of (x +1) on the base 10, where "x" corresponds to the measured value of the variable SCC. The data from transformed SCC were called somatic cell score (SCS) and showed normal distribution and homogeneity of variance. The results were analyzed using the SAS statistical package®, Statical Analysis System (SAS Institute, 1998). In ANOVA, in order to compare the range, the Turkey's test was performed at 1% of significance and we used the Pearson's correlation coefficient for correlation analysis.

RESULTS AND DISCUSSION

It can be observed in Table 1 that the maximum values – recorded in the three methods are in disagreement with the Normative Instruction No. 62 (IN-62) (BRASIL, 2011). However, when the range obtained from SCC is analyzed, it can be noted that it complies with the inherent period in the study, except for the deadline established for the implementation of IN-51. We can see that the Somatic Cell Count range is consistent with data found in several regions of the country. Machado et al. (2000) in 4785 samples from tanks in the state of São Paulo and northern Minas Gerais state found an average of 505,000 cells/mL and standard deviation of 593 000 cells/mL. Silva et al. (2009) in tanks from southwestern Goiás describe SCC values – for dry and wet seasons of 524,000 and 529,000 cells / mL respectively. Souza et al. (2006) studied 91,618 samples from southeastern Espírito Santo, Minas Gerais and Rio de Janeiro from July 1st, 2005 to June30th, 2006, and they found 510,000 cells / mL and standard deviation of 522 000 cells / mL. Although the SCC range complies with the ranges found in other regions of the country.

By analyzing the SCC range per property (Figure 1), it appears that, for the standards required by Normative Instruction nº 51 (NI-51) (BRASIL, 2002), – the number five property has not reached the threshold in any of the three methods used. On the other hand, by considering the maximum values – of SCC required at the end of deployment of NI-51, we noticed that only the property number three would be producing milk within the required standards of SCC.

The comparison of log-transformed results of SCC from Somaticell® with MilkoScan™ FT + showed a correlation of $r = 0.91$, $p<0.0001$ (Table 2). The comparison of log-transformed results (SCC) showed a significant correlation with $r = 0.88$, $p<0.0001$ (Table 2). The comparison of the SCC range between Somaticell® and Direct Cell showed

a positive correlation with r = 0.94 (p<0.0001) (Table 2). Rodrigues (2008) described a significant correlation (p <0.001) of 0.92 when he compared the SCC results from Somaticell® with the results of Fossomatic. Araújo et al. (2012) described a Tests Somaticell® and DeLaval Cell Count® present satisfactory results as an alternative methodology for determination of somatic cell count in raw milk cooling tanks.

Findings from Ruegg et al. (2005) studied 800 samples of milk with DCC and with automated equipment. The evaluation results by DCC were compared to those of direct microscopy in order to determine the SCC in goat milk presenting positive correlation (BERRY; BROUGHAN, 2007) and also with the correlations for sheep milk (GONZALO et al., 2006).

Table 2 – Number of samples, Pearson's correlation coefficients (r) to the MilkoScan in relation to Somaticell® and DCC and Somaticell® in relation to CHD

Variance	N	r	Value of p
Milkoscan x DCC	67	0,88	< 0,0001
Milkoscan x Somaticell®	82	0,91	< 0,0001
DCC x Somaticell®	52	0,94	< 0,0001

Table 1 – Medium, minimum and maximum values, and standard deviation of somatic cell count (SCC*1000)

Variance	N	Range	Standard	Value	
			Deviation	Minimum	Maximum
MilkoScan™ FT+	97	525,40	321,19	100,00	1963,75
DCC	67	580,28	316,60	112,50	1759,00
Somaticell®	82	597,39	257,96	136,00	1478,00

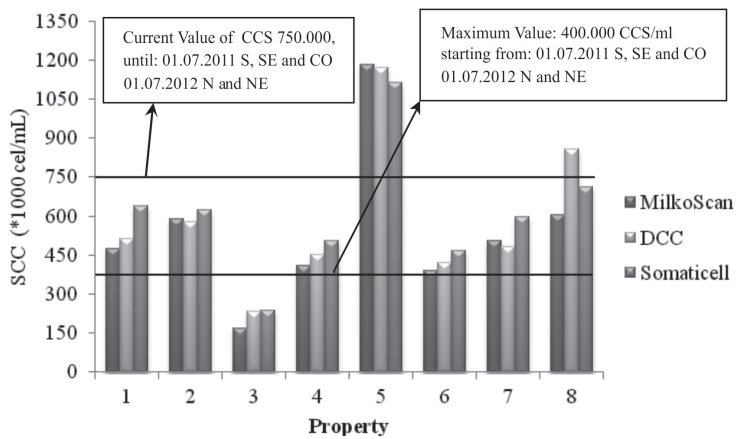

Figure 1 – Comparison of the results of SCC range, obtained by MilkoScan™ FT +, DCC and Somaticell® with the requirements of Normative Instruction nº 51

CONCLUSION

The Somaticell® Kit and Direct Cell Counter (DCC) were suitable for in loco determination of somatic cells count in raw milk cattle, held in cooling tanks.

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