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BULK TANK SOMATIC CELL COUNT IN MILK SAMPLES FROM STATE OF PARANÁ

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ABSTRACT: This research studied somatic cell count in bulk tank milk samples (BTSCC) from the state of Paraná, Brazil, at the Milk Quality Laboratory of the Dairy Herd Analysis Service of the Holstein Association of Paraná, the result of technical and scientific cooperation between UFPR and McGill University of Canada. A total of 1,950,034 bulk tank milk samples from ten regions of the state of Paraná were analyzed between January 2005 and April 2012. Fixed effects were the month and year of analysis, region and age of the sample. Means and standard deviations of BTSCC were 553,519 ± 545,532 cells/ml, respectively. All fixed effects were statistically significant (P<0.01). Highest values for BTSCC are observed in the month of February (554,000 cells/ml ± 1.45) and lowest values in September (450,000 cells/ml ± 1.47). Similarly, the highest values were found in the year 2010 (567,000 cells/ml ± 1.16), the lowest BTSCC was found in 2012 (444,000 cells/ml ± 1.57). The region effect was also significant with the highest values found in the South Center/ Guarapuava (668,000 cells/ml ± 0.87) and the lowest in the Southwest/ Francisco Beltrão (359,000 cells/ml ± 2.00). Both variables showed a reduction of their values with increasing age of the sample, from 518,000 ± 1.08 to 472,000 cells/ml ± 2.14 between the first and the seventh day, for the BTSCC. Coefficient of variation for BTSCC was 96.10%. The R^2 was 0.39 for BTSCC.
Key Words: age of the sample; mastitis; month and year of analysis; milk quality; region

CONTAGEM DE CÉLULAS SOMÁTICAS EM AMOSTRAS DE LEITE DE TANQUES NO ESTADO DO PARANÁ

RESUMO: A presente pesquisa avaliou o comportamento da contagem de células somáticas de leite de tanques (CCST) provenientes do Estado do Paraná, analisadas pelo Laboratório de Análise da Qualidade do Leite do Programa de Análise de Rebanhos Leiteiros do Paraná da Associação Paranaense de Criadores de Bovinos da Raça Holandesa, fruto do convênio entre UFPR e McGill University do Canadá. Analisou-se um arquivo contendo 1.950.034 amostras de leite de tanques, obtidas no período de janeiro de 2005 a abril de 2012, em dez regiões do Estado. Foram estudados os efeitos de meio, entre eles, mês e ano de análise, região e idade da amostra. As médias estimadas e desvios-padrão para CCST foram 553.519 ± 545.532 células/ ml respectivamente. Todos os efeitos incluídos no modelo foram significativos (P<0,01). Ocorreram importantes variações da CCST entre os meses de análise, destacando-se respectivamente as maiores médias e seus erros-padrão para o mês de fevereiro (554.000 células/ ml ± 1,45) e as menores para o mês setembro (450.000 células/ ml ± 1,47). Da mesma forma, o ano de análise apresentou variações significativas, em que as maiores médias ocorreram no ano de 2010 (567.000 células/ ml ± 1,16) e as menores em 2012 (444.000 células/ ml ± 1,57). Para as regiões, as maiores médias e seus erros-padrão foram de 668.000 células/ ml ± 0,87 (Centro Sul – Guarapuava) e as menores foram de 359.000 células/ ml ± 2,00 (Sudoeste - Francisco Beltrão). O efeito de idade da amostra, em dias, caracterizou redução da CCST de 518.000 ± 1,08 para 472.000 células/ ml ± 2,14 quando as amostras foram analisadas entre o primeiro e o sétimo dia de idade, para CCST. O coeficiente de variação para CCST foi de 96,10%. O R^2 para a CCST foi de 0,39%.
Palavras-chave: idade da amostra; mastite; intervalo de análise; qualidade do leite; região

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INTRODUCTION

The somatic cell count (SCC) is an established evaluation parameter used by programs that monitor udder health, milk quality improvement and payment for quality in traditional producer countries. (Schukken *et al*., 1993, Godkin, 2000, Fonseca, 2001).

The SCC has been widely used in developed countries since electronic devices made this practice available to producers (Fonseca and Santos, 2000; Santos, 2001 and Godking, 2000) In Brazil, it was introduced in 1991 by the Milk Quality Laboratory of the Dairy Herd Program Analysis of Paraná, from the Holstein Association of Paraná (APCBRH), Curitiba-PR. It was the result of technical and scientific cooperation between Universidade Federal do Paraná (UFPR) and McGill University in Canada.

The SCC has been used by industries as payment criteria for milk quality, making it a tool for management and quality monitoring, being directly related to programs to reduce production losses and to opportunities for milk higher pay (Harmon and Reneau, 1993, Rysanek and Babak, 2005).

High bulk tank somatic cell count (BTSCC) affects the composition of milk, the products shelf life, causing significant losses to dairy industry (Monardes, 1998).

According to Harmon & Reneau (1993), there is a significant association between BTSCC with the limit of 1,500,000 cells/ml. The percentage of infected udder quarters could reach up to 48% and the losses of milk yield up to 29 %.

According to Dürr *et al*. (2011), databases of herds' production performance are essential tools to the rational management of animals, to genetic evaluation of dairy cattle, to traceability programs of animals and milk products and to the dairy chain strategic planning.

The Milk Quality Laboratory of the Dairy Herd Program Analysis of Paraná of APCBRH provide services to monitor milk quality in compliance with National Program for Improvement of Quality Milk (PNMQL) through the Normative proceeding IN 51 (Brazil, 2002) and 62 (Brazil, 2011) of the Ministry of Agriculture, Livestock and Supply (MAPA). This work analyzed the BTSCC samples from dairy industries under its services.

This research objective was to evaluate how environment effects (month and year of analysis, and the region and age of the sample) could influence the BTSCC.

MATERIAL AND METHODS

There were used 1,950,034 bulk tank milk samples from production sites located in 10 regions, and respective main city, in the state of Paraná (PR). They were analyzed from January 2005 to April 2012.

The bulk tank milk samples were collected monthly by personnel trained by the dairy industry, before collecting the bulk tank milk is homogenized to analyze maintained in a sterilized jar that uses the default Bronopol, according to the procedures recommended by the manuals of Field Operations (Horst, 2008), and Sample Collection (Horst, 2010) of the Milk Quality Laboratory. The samples were placed in standard vials (70 ml), using the preservative bronopol (2-bromo-2-nitro propane-1,3-diol) and sent to Milk Quality Laboratory localized in Associação Paranaense de Criadores de Bovinos da Raçã Holandesa, Curitiba – PR – Brazil.

The number of days elapsed between the farm milk collection and the analysis in the laboratory, Curitiba-PR, was defined as the age of the sample. The milk samples were analyzed for

BTSCC, using equipment Somacount 500 ®, by flow cytometry.

The BTSCC data was prepared from the original Milk Quality Laboratory database to achieve better accuracy: BTSCC values equal to zero or negative as well as greater than 4,525,000 cells/ml were not considered. The same was valid for age of the sample lower than 1 and greater than 7 days.

All the database preparation, data pre-sorting and statistical analysis for BTSCC values were performed in the Department of Animal Science of the SCA of the UFPR in Curitiba-PR.

All data were analyzed through computer software SAS® version 9.3. Studied traits were analyzed through the following mathematical model:

$Y_{ijkl} = \mu + M_i + A_j + R_k + l_l + e_{ijkl}$

Where:

Y_{ijkl} = BTSCC values, collected in month ***i***, year ***j***, region ***k***, with age of the sample ***l*** and associated random error of each observation $e_{ijkl.}$

μ = general average;

M_i = effects of month of analyzes ***i***, being ***i*** = 1 (Jan.), 2 (Feb.), ... 12 (Dec.);

A_j = effects of year of analyzes ***j***, being ***j*** = 2005, 2006, ... 2012*;

R_k = effects of the region ***k***, being ***k*** = 1, 2, .., 10;

l_l = effects of the age of the sample in days ***l***, being ***l*** = 1, 2, ... 7;

e_{ijkl} = associated random error of each observation $Y_{ijkl.}$

*In 2012, there were milk samples collected only from the first four months of the year.

The evaluated dependent variable was BTSCC, as a result of the reading by flow cytometry equipment, Somacount 500 ® (Bentley Instruments, 1995b), in thousand cells/ml. Comparisons between means were performed using the Tukey test at 1% probability.

RESULTS AND DISCUSSION

The estimated mean, its standard deviation and the variation coefficient of BTSCC from the 1,950,034 analyzed samples from 2005 to 2012, were respectively 553,519 ± 545,532 cells/ml and 96.10%.

The large variability (CV 96.10%) in the BTSCC values is characterized by the standard deviation similar to the mean. Machado et al. (2000) and Paula et al. (2004) also found the same.

The average BTSCC found in this study is higher than those reported by Franks (2001) and Godkin (1999), considering averages of BTSCC in different countries. Franks (2001) observed the lowest average in Switzerland (112,000 cells/ml) and the highest in Israel (382,000 cells/ml), and Godkin (1999), in the Province of Ontario, Canada, observed an average of 250,000 cells/ml.

In Brazil, similar results were obtained by Machado *et al*. (2000), working with a smaller number of data (4785 samples). They found mean and standard deviation of 505,000 ± 593,000 cells/ml, and Paula *et al*. (2004) analyzed 257,540 bulk tank somatic cell count in the states of Santa Catarina, Paraná and São Paulo, from 1999 to 2001, and found an average of 486,812 ± 401,547 cells/ml with a variation coefficient of 62.35%.

According to Harmon & Reneau (1993), the limit of 500,000 cells/ml found in this study would be associated to 16% of infected udder quarters in herds and to 6% loss in milk production.

The SCC of milk from a cow indicates quantitatively the mammary gland degree of infection. Thus, SCC of milk from healthy animals is usually lower than 300,000 cells/ml (Philpot, 1998b; Machado, Sarríes & Pereira, 2000; NMC, 2001; Fonseca & Santos, 2000).

These results are considered high when compared with those countries with well-developed dairy industry, reflecting carelessness by producers regarding the health of the mammary gland. That seems to be the result of the lack of incentives by milk industries to establish programs to pay for milk quality based on SCC.

In Brazil, since the publication of the MAPA IN 51 (Brazil, 2002), the SCC criteria has been used to promote the improvement of milk quality. According to Fonseca (2001), a survey conducted in 93 dairy plants in the 1998 offseason, only 7% of the producers used the SCC test.

It is expected that, after the publication of IN 62 (Brazil, 2011), establishing to 2015 new legal limit of 400,000 cells/ml, industries and producers should give greater attention to SCC and that would decrease over the years. According Monardes (1998), the establishment of legal limits on economic blocks and countries like European Union (400,000 cells/ml), Canada (500,000 cells/ml) and New Zealand (400,000 cells/ml) respectively in 1992, 1994 and 1995 were decisive in enabling public policies for food security.

The results of variance analysis of BTSCC in Paraná are shown in Table 1. All the variables in the mathematical model were significant (P <0.01).

The month of analysis significantly influenced the BTSCC (P <0.01), as can be seen in the summary of the variance analysis (Table 1).

In Table 2, it can be identified higher estimates of adjusted means for BTSCC in summer, which corresponds to the period from January to March (525,000 to 532,000 cells/ml). Moreover, lower estimates were found in late winter and early spring from August to October (477,000 to 463,000 cells/ml). Those results resemble findings from other authors (Harmon & Reneau, 1993; Harmon, 1998b; Ott *et al*. 1999; Pritchard *et al*. 2001 and Paula *et al*. 2004).

According to Harmon & Reneau (1993), Harmon (1998b) and Paula *et al*. (2004), the stress of high temperatures and humidity, increasing the susceptibility to infection and the number of pathogens to which cows are exposed, make the summer the period with the highest incidence of clinical mastitis, mainly of environmental origin.

Table 2 shows that the months of March and April, June and July, June and November are similar to each other (P <0.01), why in the state of Paraná temperatures in these months are milder and therefore they have no differences significant.

In the summary of the variance analysis (Table 1), it is shown the year of the analysis significantly influenced the variable BTSCC (P <0.01).

Higher estimates of adjusted means (Table 3) were observed in 2010 (567,000 cells/ml) and the lowest in 2012 (444,000 cells/ml). Also, shows that in 2006 and 2007 they were similar (Table 3, P <0,01).

Several authors have also studied the effect of the years in BTSCC analysis (Schukken *et al*. 1990; Schukken *et al*. 1992a; Schukken *et al*. 1993; Sargeant *et al*. 1998; Godkin, 1999, and Paula *et al* ., 2004), showing a reduction in BTSCC over the years, due to the implementation of legal limits, mammary gland health monitoring programs and payment for quality.

Table 1 – Variance analysis summary of somatic cell count (BTSCC) in bulk tank samples in Paraná State.

SOURCE OF VARIATION	df	MEANS SQUARES
		BTSCC (x1,000 cells/ml)
Month of analysis	11	1,372,097.84**
Year of analysis	7	3,448,788.21**
Region	9	25,269,272.94**
Age of sample (days)	6	264,597.45**
Residual	1,950,000	282,964.74**
R^2	0.39	
CV (%)	96.10	

**(P<0.01)
R^2: Total variation accounted by the effects included in the model.
CV (%): Coefficient of variation

Table 2 - Number of observations (N), number of observations accumulated (%) estimates of adjusted means and standard errors by the least squares method of BTSCC, and months of analysis

MONTH OF ANALYSIS	N	%	BTSCC (x1,000 cells/ml)		
			Mean[1]	±	SE
January	165.665	8,50	525[a]	±	1,43
February	161.031	16,75	554[o]	±	1,45
March	183.159	26,15	532[c]	±	1,36
April	178.901	35,32	531[co]	±	1,37
May	170.074	44,04	516[e]	±	1,40
June	153.014	51,89	491[f]	±	1,48
July	155.888	59,88	489[fg]	±	1,47
August	157.926	67,98	477[n]	±	1,45
September	150.967	75,72	450[i]	±	1,47
October	159.212	83,89	463[j]	±	1,45
November	156159	91,90	491[fk]	±	1,46
December	158.038	100,00	500[l]	±	1,46
Total	1.950.034				

[1] Tukey Test. Means followed by at least the same letter don't differ statistically from each other (P<0.01)

Table 3 - Number of observations (N), number of observations accumulated (%) estimates of adjusted means and standard errors by the least squares method of BTSCC, according to the year of analysis

YEAR OF ANALYSIS	N	%	BTSCC (x1,000 cells/ml)		
			Mean[1]	±	SE
2005	125.257	6,42	459[a]	±	1,63
2006	186.438	15,98	495[o]	±	1,33
2007	262.332	29,44	493[oc]	±	1,16
2008	326.385	46,17	501[o]	±	1,07
2009	337.243	63,47	549[e]	±	1,08
2010	276.463	77,65	567[f]	±	1,16
2011	287.339	92,38	506[g]	±	1,12
2012[2]	148.577	100,00	444[n]	±	1,57
Total	1.950.034				

[1] Tukey Test. Means followed by at least the same letter don't differ statistically from each other (P<0.01)
[2] 2012 is represented only by milk samples collected on the first four months of the year.

In Table 4, it is shown that the region affected significantly (P <0.01) the characteristic studied (BTSCC).

The adjusted means and standard errors for the ten regions and their main cities are described in Table 4. The lowest BTSCC estimates were 359,000 cells/ml for the Southwest region, Francisco Beltrão - PR and highest 668,000 cells/ml for the Center South, Guarapuava - PR.

In table 4, the higher BTSCC means are represented by the regions of Center South, Guarapuava - PR; Metropolitan Curitiba - PR and Southeast, Irati - PR, with 668, 620 and 573 (x 1000 cells/ml) respectively. These regions, according to the Köppen classification (IAPAR, 1999), show temperate climate type Cfb, with average temperature in the coldest month between 15°C and 18°C, cool summers with average temperature in the warmest month below 22°C, without dry season and annual relative humidity ranging from 70 to 85%.

The BTSCC averages on Northwest regions, Umuarama - PR and Center East, Ponta Grossa - PR were similar (Table 4, P<0,01).

Ott *et al.* (1999) and Norman *et al.* (2001) obtained similar results in the United States. They found significant differences between regions in the BTSCC study, more likely caused by differences in weather: dry climate regions had lower BTSCC and hot and humid climate higher.

Emanuelson & Funke (1991); Allore *et al*. (1997); Wells & Ott (1998); Ott *et al*. (1999) and Norman *et al*. (2001) found differences between regions, ascribed by the level of technology, production and herd size.

In Brazil, Noro (2004) and Paula *et al*. (2004) found similar results to this study, highlighting the differences found in herd size, production system, level of technology adopted, implementation of mammary gland health control programs and milk quality payment, stimulated by the dairy industries of those regions.

In the variance analysis summary (Table 1), it is shown that the age of the sample significantly influenced the BTSCC (P <0.01). These results contrast with those obtained by Monardes *et al*. (1996), who found no significant effect of the sample age on the SCC.

Table 4 - Number of observations (N), number of observations accumulated (%) estimates of adjusted means and standard errors by the least squares method of BTSCC, by region

REGION MUNICIPALITY	N	%	BTSCC (x1,000 cells/ml) Mean[1]	±	SE
1. Northwest Umuarama	61.991	3,18	442[a]	±	2,18
1. Central West Campo Mourão	414.991	24,46	405[b]	±	0,85
2. Central North Londrina	145.630	31,93	520[c]	±	1,46
3. Pioneer North Cornélio Procópio	35.372	33,74	533[d]	±	2,85
4. Central East Ponta Grossa	138.721	40,86	435[ae]	±	1,50
5. West Cascavel	135.252	47,79	461[f]	±	1,50
6. Southwest Francisco Beltrão	73.903	51,58	359[g]	±	2,00
7. Center South Guarapuava	502.468	77,35	668[h]	±	0,87
8. Southeast Irati	51.142	79,97	573[i]	±	2,40
10. Curitiba	390.564	100,00	620[j]	±	0,94
Total	1.950.034				

[1]Tukey Test. Averages followed by at least the same letter don't differ statistically of each other (P<0.01)

Table 5 presents the adjusted means for BTSCC that show similar values for two and three days, as four, five and six days are similar to each other (P<0.01).

However, as a tendency, when the age of the sample increased from 1 day to 7 days the BTSCC decreased from 518 to 472 (x 1000 cells/ml).

Paula *et al*. (2004), found that from the first to the seventh day of age the samples had a reduction in the BTSCC values, contrasting with Kennedy *et al*. (1982), cited by Ostrensky (1999), who observed that the SCC values in the first three days remained virtually unchanged, and with Ostrensky (1999), who found an increase in the SCC values.

Table 5 – Number of observations (N), number of observations accumulated (%), estimates of adjusted means and standard errors, the least squares method of BTSCC, according to the age of the sample

AGE OF SAMPLE (in days)	N	%	BTSCC (x1,000 cells/ml) Mean[1]	±	SE
1	282.619	14,49	518[a]	±	1,08
2	685.983	49,67	513[b]	±	0,75
3	358.766	68,07	511[bc]	±	0,99
4	258.364	81,32	500[d]	±	1,13
5	206.047	91,88	499[de]	±	1,29
6	88.496	96,42	498[ef]	±	1,87
7	69.759	100,00	472[g]	±	2,14
Total	1.950.034				

[1]Tukey Test. Averages followed by at least the same letter don't differ statistically of each other (P<0.01)

Kennedy *et al*. (1982), cited by Ostrensky (1999), as in this study, also reported that the SCC was reduced as the age of the sample increased, showing a reduction of 28% (Ostrensky, 1999).

Table 5 shows that 68.07% of the samples were analyzed by the third day, while 96.42% until the sixth day. Monardes *et al*. (1996) reported that in Canada, 93% of the samples were analyzed by the third day and 99% by the seventh day after collection.

CONCLUSION

The averages for BTSCC in the State of Paraná are high compared to countries with well-developed dairy industry, reflecting a high level of mastitis in the studied herds associated with a significant amount of infected udder quarters that would lead to significant milk losses. Based on the results found in this study, it is suggested a demand for producers to reassess the management of mastitis control programs that would focus on the health of the mammary gland. The dairy industries must also reevaluate its payment programs for milk quality, correlating BTSCC with the reduction on production losses and improving opportunities for larger returns on milk production. The new legal limit to be implemented, as an instrument of public policy in favor of food security, should

signal that Brazil can compete in the dairy products international market. It would show that the milk quality, internally, also follows standards established for over 30 years by traditional producing countries.

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INFORMATIVE NOTES

The research project was approved (protocol 024/2011) by the Ethics Committee on Animal Use (CEUA), Sector of Agricultural Sciences (CAS) of UFPR.

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