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SOMATIC CELL SCORE IN BULK TANK MILK IN THE STATE OF PARANÁ

Newton Pohl Ribas[1], Paulo Rossi Junior[1], Humberto Gonzalo Monardes[2], José Augusto Horst[3], Daiane Regonato[1], Maycon Cesar Almeida[1]

[1] UFPR
[2] McGill University, Canada
[3] APCBRH
Correspondência: Newton Pohl Ribas: newtonribas.ufpr@gmail.com

ABSTRACT: Since 1983, the logarithmic transformation of the somatic cell count of the bulk tank milk, the somatic cell score (BTSCS), has been used as an indicator of milk quality in the herd. This research studied BTSCS in bulk tank milk samples from milk processors of the state of Paraná, Brazil, analyzed 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. The effect of BTSCS in bulk tank samples from dairy farms in the State of Paraná, Brazil was studied in a total of 1,950,034 tank milk samples from ten regions of Paraná tested for somatic cell count from January 2005 to April 2012. Environmental effects such as the region and age of the sample as well as the year and month of the analysis were considered. Variance components for the trait were estimated and the distribution of samples in the different classes of BTSCS was also studied. BTSCS estimated mean was 4.83 with a standard deviation of 1.52. All the effects were significant (P <0.01). BTSCS varied in the different years and months, with February and 2010 showing the highest averages, 4.87 and 4.85, respectively. There were significant variations between regions, the highest BTSCS found was 5.28 in South Center/ Guarapuava region. BTSCS values decreased with age of the sample, bringing them down from 4.75 to 4.66 in five days. It was observed that 65.95% of all samples had a score five or less. This study confirmed that the logarithmic transformation of BTSCC in BTSCS presents statistical properties superior to those of BTSCC, including more accurate hypothesis, smaller standard errors and coefficients of variation and larger coefficients of determination.
Key Words: sample age; mastitis; month and year of analysis; milk quality; region

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

RESUMO: Trata-se da conversão da CCS em dez categorias, utilizado desde 1983, auxiliando a avaliação da qualidade do leite no rebanho. A presente pesquisa avaliou o comportamento do ECS em amostras de leite de tanques provenientes de indústrias de laticínios 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, McGill University e APCBRH. Foram analisadas 1.950.034 amostras de leite de tanques, obtidas no período de janeiro de 2005 a abril de 2012, em dez regiões do Estado. Os efeitos de meio, entre eles, mês e ano de análise, região e idade da amostra foram estudados. Estimaram-se os componentes de variância para a característica e analisou-se a distribuição das amostras nas classes de ECS. A média estimada e desvio-padrão do ECS foi de 4,83 ± 1,52. Todos os efeitos incluídos no modelo foram significativos (P<0,01). Ocorreram variações do ECS entre os meses de análise, destacando-se maior média para o mês de fevereiro (4,87). Da mesma forma, o ano de análise apresentou variações significativas, em que a maior média ocorreu no ano de 2010 (4,85 ± 0,0031). Ocorreram significativas variações também entre regiões, em que a maior média e seu erro-padrão foi de 5,28 ± 0,0023 (Centro Sul – Guarapuava). O efeito de idade da amostra apresentou redução de 4,75 para 4,66 do primeiro ao quinto dia. Das amostras analisadas, 65,95% apresentaram escore cinco ou mais. Este estudo confirmou que a transformação logarítmica dos BTSCC em BTSCS apresenta propriedades estatísticas superiores aos de BTSCC, incluindo a hipótese mais precisa, os erros padrões menores e maiores coeficientes de variação e de determinação.
Palavras-chave: idade da amostra; mastite; mês e ano de análise; qualidade do leite; região

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INTRODUCTION

The logarithmic transformation of bulk tank somatic cell count (BTSCC) into a somatic cell score (BTSCS) is been used to monitor milk quality in dairy herds since BTSCS presents statistical properties superior to those of BTSCC. In the United States and Canada, the milk quality control programs aim at reaching 80% of the herds with a maximum linear score of three in order to get better payment for the milk (Ribas, 1999).

The advantages of using the logarithmic transformations of BTSCC are:

1) hypothesis tests are more accurate in BTSCS than in BTSCC. BTSCS analysis of variance F values are approximately twice those of the corresponding BTSCC values. The standard errors and confidence intervals are relatively lower in BTSCS than in BTSCC (Shook & Ruegg, 1999);

2) BTSCS features a normal frequency distribution, desirable in statistical analysis, and the distribution of BTSCC is clearly skewed with the average substantially greater than the median (Hunt & Anderton, 1993; Shook & Schutz, 1994, Shook & Ruegg, 1999);

3) BTSCS average is close to the median with approximately 50% of the values positioned on either side of the mean, simplifying the interpretation. BTSCC average is considerably higher than its median, and this difference is highly variable, because is strongly influenced by a small percentage of extreme values (Hunt & Anderton, 1993; Shook & Schutz, 1994). According to Shook (1982), Shook & Schutz (1994) and National Mastitis Council (1999), dairy cattle management programs in many countries have created a linear score by distributing the log transformations and their original values in ten categories going from zero to nine (Table 1).

Table 1 – Linear score and bulk tank somatic cell count (BTSCC).

LINEAR SCORE	Average point	BTSCC (x 1,000 cells/ mL) Variation		
0	12.5	0	to	17
1	25	18	to	34
2	50	35	to	70
3	100	71	to	140
4	200	141	to	282
5	400	283	to	565
6	800	566	to	1,130
7	1,600	1,131	to	2,262
8	3,200	2,263	to	4,525
9	6,400	above	of	4,525

Source: Shook (1982)

The BTSCS is used in quality control programs of dairy herds (Shook & Ruegg, 1999) all over the world. It produces a range of values of approximately 10 points, with each BTSCS point up doubling the somatic cell count (SCC) and each point down splitting the SCC in two (Hunt & Anderton, 1993). Ostrensky (1999) compared BTSCS and BTSCC and some environmental factors influencing BTSCC in Paraná. He found a coefficient of determination (R^2) of 0.4282 and a coefficient of variation (CV) of 121.07% for BTSCC and of 0.6444 and 25.25% for BTSCS, with BTSCS showing smaller standard errors than BTSCC.

Paula (2002) compared BTSCC and BTSCS and found 0.5009 for R^2 and 62.35% for CV in BTSCC, while BTSCS were 0.5350 and 18.62% respectively, with lower standard errors for BTSCS than for BTSCS. The same author observed that 65.95% of the bulk tank milk samples fitted in the score five or less indicating a probably higher incidence of mastitis in the herds studied (Paula, 2002).

BTSCS used in this study were collected under Normative Instructions number 51 (Brazil, 2002) and 62 (Brazil, 2011) of Brazil's Ministry of Agriculture. The objectives of this study were to study the effects environmental factors such as month and year of test and region and age of the sample at analysis on the SCS of bulk tank milks in the state of Parana. The study of the distribution of samples in the different

BTSCS classes was also an objective of this study.

MATERIAL AND METHODS

BTSCS was calculated in 1,950,034 samples of refrigerated pooled milk tanks from farms in 10 regions of the state of Paraná (Northwest – Umuarama, Central West - Campo Mourão, Central North – Londrina, Pioneer North - Cornélio Procópio, Central East - Ponta Grossa, West – Cascavel, Southwest - Francisco Beltrão, Central South – Guarapuava, Southeast – Irati and Curitiba) between January 2005 and April 2012, analyzed 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 in Canada.

To obtain better accuracy, Ostrensky (1999) and Paula (2002) proposed the following restrictions to the database: discard BTSCC values less than or equal to zero and above 4,525,000 cells/ml and keep samples between one and seven days old.

BTSCS is a logarithmic transformation of the BTSCC developed by Shook (1982), obtained by the equation: BTSCS = log2 (BTSCC/100.000) + 3.

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

Yijkl = μ + Mi + Aj + Rk + Il + eijkl

Where:

Yijkl = BTSCS of samples from milk collected in the month ***i*** of the year ***j*** in the region ***k***, age ***l*** and with the random error associated to each observation being **e***ijkl*, wherein:

μ = overall mean;

Mi = effect of the month of analysis ***i***, where i = 1 (Jan), 2 (Feb), ..., 12 (Dec);

J = effect of the year of analysis ***j***, where j = 2005, 2006, ..., 2012 *;

Rk = effect of the region where the sampling took place ***k***, where k = 1, 2, ..., 10;

Il = effect of the age of the sample at analysis, in days, with ***l*** = 1, 2, ..., 7;

eijkl = random error associated to each observation Yijkl.

* In 2012, samples were collected only in the first four months of the year.

Tukey Test (1% probability) was used to compare means. BTSCC dependent variable was obtained using flow cytometry technology (Somacount 500®, Bentley Instruments, 1995b), in thousand cells/ml and BTSCS was obtained by logarithmic transformation of BTSCC (Ali & Shook, 1980, Shook, 1982), using the equation BTSCS = log2 (BTSCC/100) + 3. BTSCS values corresponding to the BTSCC range zero to 12,000 cells/ml were forced to be equal to zero in order to avoid negative numbers.

RESULTS AND DISCUSSION

Table 2 shows the BTSCC and BTSCS means with their respective standard deviations and coefficients of variation and the age of the samples obtained from 2005 to 2012.

Table 2 – Number of observations (N), means, standard deviations (SD) and coefficients of variation (CV) of Bulk Tank Somatic Cell Counts (BTSCC) and Scores (BTSCS) and age of samples in days.

CHARACTERISTICS	N	AVERAGE	±	SD	CV(%)
BTSCC*	1,950,034	553	±	545	96.10
BTSCS	1,950,034	4.83	±	1.52	30.06
Age of sample (days)	1,950,034	2.98	±	1.57	-

*(x 1,000 cells/ mL)

BTSCC averages were higher than those found by Franks (2001) and Godkin (1999) 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 Ontario, Canada, found an average of 250,000 cells/ml. Working with less data in Brazil, Machado and Pereira Sarries (2000), found the BTSCC mean to be

505,000 with a SD of 593,000 cells/ml. Their estimates, however, were similar to those observed in this study.

BTSCS values found by Ostrensky (1999) and Paula (2002) in Paraná were similar to the ones found here (4.461 ± 1.789 and 4.839 ± 1.235, respectively). The conclusion was that in spite of all efforts from the dairy organizations in the State of Paraná, its milk quality given by either BTSCC or BTSCS standards remained unchanged in the last twelve years. Values from this study are higher than those found in countries with developed dairy farming and may reflect poor hygiene and health management and little incentive from the industries in paying milk according to its quality, using BTSCC as a base of payment.

Accordingly, the upper limit average of 500,000 cells/ml found by Harmon & Reneau (1993), was associated with 16% of infected quarters in herds and with 6% less milk production. Table 3 shows the distribution of BTSCC samples in BTSCS classes in this study.

Table 3 - Classes of bulk tank somatic cell scores (BTSCS), corresponding range of bulk tank somatic cell count (BTSCC), number of observations (N) in each class and cumulative percentage of observations (%).

BTSCS	BTSCC VARIATION[1]	N	%
0	0 a 17	30,439	1.56
1	18 a 34	42,581	3.74
2	35 a 70	63,934	7.02
3	71 a 140	167,315	15.60
4	141 a 282	384,531	35.32
5	283 a 565	597,231	65.95
6	566 a 1,130	454,298	89.25
7	1,131 a 2,262	170,923	98.01
8	2,263 a 4,525	38,782	100.00
TOTAL		1.950.034	

[1](x 1,000 cells / mL)

BTSCS was five or lower in 65,95% of samples, a result below the goal set by the milk quality control services in the United States and Canada that is 80% of the herds having a maximum score of three (Ribas, 1999). In comparison, only 15.60% of the samples in Parana showed a score of 3 or less (Table 3). The high number of samples with BTSCS of above five shows how important is to implement milk payment programs according to quality and policies to regulate the legal limits for milk quality in Brazil (IN 62, Brazil, 2011) aiming at bringing down the limit of 600,000 cells/ml of 2012 to 400,000 cells/ml by 2015.

Table 4 – Analysis of variance of the bulk tank somatic cell score (BTSCS) in the State of Paraná.

SOURCE OF VARIATION	df	MEANS SQUARES
		BTSCS
Month of analysis	11	1,193.58**
Year of analysis	7	1,425.27**
Region	9	37,404.30**
Age of sample (days)	6	350.56**
Residual	1,950,000	2.113**
R^2		0.46
CV (%)		30.06

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

The following R^2 and CV for BTSCS were observed: 0.46 and 30.06%, respectively. The standard errors were lower for the BTSCS, across the effects studied. These results are similar to those found by Shook & Ruegg (1999), Ostrensky (1999) and Paula (2002), who observed higher R^2, smaller CV and smaller standard errors with the use of logarithmic transformations for the BTSCC, thus consolidating the statistical point of view for using logarithmic transformations of BTSCC.

The effect of the month of the analysis on BTSCS played a significant role (P <0.01), as can be seen in Table 4. Table 5 shows that the largest estimates of adjusted means for BTSCS were in late summer and early fall, from February to April, and the lower estimates were found in late winter and early spring, during the months of August, September and October. Other authors, like Harmon & Reneau (1993), Harmon (1998b); Ott, Wells & Smith (1999) and Pritchard, Anderson & Myers (2001), found similar results to this study.

According to Harmon & Reneau (1993), Harmon (1998b), Paula (2002) and Magalhães *et al.* (2006), summer is the period with the highest incidence of clinical mastitis, mainly due to factors

such as the heat stress and humidity that increase susceptibility to infection and the proliferation of pathogens to which cows are exposed.

Table 5 – Leasts squares means, number of observations (N) and cumulative percentage of observations (%), by month of analysis.

MONTH OF ANALYSIS	N	%	BTSCS		
			Mean[1]	±	SE
January	165,665	8.50	4.78[a]	±	0,0039
February	161,031	16,75	4.87[b]	±	0,0039
March	183,159	26.15	4.80[ac]	±	0,0037
April	178,901	35.32	4.81[cc]	±	0,0037
May	170,074	44.04	4.77[ae]	±	0,0038
June	153,014	51.89	4.69[f]	±	0,0040
July	155,888	59.88	4.68[fg]	±	0,0040
August	157,926	67.98	4.63[h]	±	0,0039
September	150,967	75.72	4.57[i]	±	0,0040
October	159,212	83.89	4.60[j]	±	0,0039
November	15,159	91.90	4.67[gk]	±	0,0040
December	158,038	100.00	4.70[fkl]	±	0,0039
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)

The effect of year of analysis significantly influenced BTSCS (P <0.01), as it can be seen in Table 6. This table shows that the highest estimates of adjusted means were in 2010 (4.85) and the lowest in 2012 (4.65). In 2012 we used only information from the first four months of the year. The rising trend of BTSCS adjusted averages from 2005 (4.60) to 2010 (4.85) was evident. The following year, 2011, showed a clear decrease, 4.70, and 2012 seems promising because the four months collected in 2012 are supposed to be the highest in a normal year, as shown in Table 5.

Table 6 – Leasts squares means, number of observations (N) and cumulative percentage of according to year of analysis.

YEAR OF ANALYSIS	N	%	BTSCS		
			Mean[1]	±	SE
2005	125,257	6.42	4.60[a]	±	0.0044
2006	186,438	15.98	4.72[b]	±	0.0036
2007	262,332	22.44	4.68[c]	±	0.0031
2008	326,385	46.17	4.71[e]	±	0.0029
2009	337,243	63.47	4.81[e]	±	0.0029
2010	276,463	77.65	4.85[f]	±	0.0031
2011	287,339	92.38	4.70[fg]	±	0.0030
2012[2]	148,577	100.00	4.65[h]	±	0.0043
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.

Between 1998 and 2003, Noro (2004), described an upward trend of SCS from 3.08 to 3.58 in Rio Grande do Sul. Likewise Paula (2002), saw an increase from 4.74 to 4.76 between 1999 and 2001 in Paraná. Results from the present study suggest that farmers need to re-evaluate their mastitis control programs focusing on the health of the mammary gland. The industries, on their turn, should rethink the way they pay for the milk using a payment-by-quality policy based on BTSCC or BTSCS, correlating these values with a reduction in production losses and providing opportunities for the farmers to get more money for milk of better quality . Brazil's Ministry of Agriculture wants to bring down somatic cell count maximum legal level to 400,000 cells/ mL of milk by 2015 (Brazil, 2011), consolidating the expectations of the National Program for the Improvement of Milk Quality.

BTSCS was significantly affected by the region (P <0.01) of sampling, as seen in Table 7). It was observed that the lowest BTSCS estimate was 4.20 in Francisco Beltrão (Southwest Paraná) and the highest 5.28 in Guarapuava (Center Paraná) with all the BTSCS adjusted averages differing significantly(P <0.01) (Table 7). Other high BTSCS values were found in Curitiba (5.18) and Irati (5.02). All these regions of high BTSCS values show the Cfb temperate climate type, according to the Köppen classification (IAPAR, 1999), with winter average temperatures between 15°C and 18°C. Summers are cool with average temperature in the warmest month below 22 °C, not a well defined dry season and the annual relative humidity between 70 and 85%.

Paula (2002) and Noro (2004) found similar results to this study in different regions of Brazil. Herd size, production systems, level of technology adopted, health control programs and payment of milk according to quality would be the factors behind the BTSCS differences found.

Table 7 – Leasts squares means, number of observations (N) and cumulative percentages of observations (%) according to region.

REGION MUNICIPALITY	N	%	BTSCS Mean[1]	±	SE
1. Northwest Umuarama	6,991	3.18	4.48[a]	±	0.0059
2. Central West Campo Mourão	414,991	24.46	4.23[g]	±	0.0023
3. Central North Londrina	145,630	31.93	4.76[c]	±	0.0039
4. Pioneer North Cornélio Procópio	35,372	33.74	4.80[d]	±	0.0078
5. Central East Ponta Grossa	138,721	40.86	4.53[e]	±	0.0041
6. West Cascavel	135,252	47.79	4.66[f]	±	0.0041
7. Southwest Francisco Beltrão	73,903	51.58	4.20[g]	±	0.0054
8. Central South Guarapuava	502,468	77.35	5.28[b]	±	0.0023
9. Southeast Irati	51.142	79,97	5,02[i]	±	0,0065
10. Curitiba	390,564	100.00	5.18[j]	±	0.0025
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).

The age of sample also played a role (P <0.01) influencing BTSCS (Table 8).

Table 8 presents BTSCS averages adjusted by the age of sample. One day old BTSCS (4.75) was lower than the five days old BTSCS (4.66). However, BTSCS values of two, four and six days old were all similar (P <0.01).

Table 8 – Leasts squares means, number of observations (N) and cumulative percentages of observations (%) according to the age of sample.

AGE OF SAMPLE (in days)	N	%	BTSCS Mean[1]	±	SE
1	282,619	14.49	4.75[a]	±	0.0029
2	685,983	49.67	4,71[b]	±	0.0020
3	358,766	68.07	4.67[c]	±	0.0027
4	258,364	81.32	4.70[d]	±	0.0030
5	206,047	91.88	4.66[e]	±	0.0035
6	88,496	96.42	4.70[bcd]	±	0.0051
7	69,759	100.00	4.80[a]	±	0.0058
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).

Comparing one and eight days old samples, another study showed a decrease in BTSCS from 28% to 10.7%, a fall justified by the cell lyses seen in aging samples (Kennedy *et al.*, 1982; Ostrensky, 1999). In the present study, 68.07% of the samples were three days old or less and 96.42% were analysed by the sixth day after the sampling, because difficulties in logistics and transportation, while in Canada 93% of all samples were analysed before the third day and 99% before the seventh day (Monardes *et al.*, 1996).

CONCLUSION

BTSCS averages are high in Paraná when compared to those found in countries with developed dairy industries. Dairy organizations have tried their best to ameliorate this picture by using BTSCS to monitor milk quality. Nevertheless milk quality in Parana has remained unchanged for the last twelve years.

This study confirmed that the logarithmic transformation of BTSCC in BTSCS presents statistical properties superior to those of BTSCC, including more accurate hypothesis, smaller standard errors and coefficients of variation and larger coefficients of determination. The industries involved should also reevaluate their payment policies rewarding better the milk with higher quality standards given by the BTSCS values.

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INFORMATIVE NOTES

The study was approved by the Ethics Committee on Animal Use of the Federal University of Paraná (protocol 024/2011).

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