Artigo DOI: 10.14295/2238-6416.v69i5.368

QUALITY OF RAW REFRIGERATED MILK BASED ON SCC AND TBC INDICATORS IN THE SOUTHWEST OF MINAS GERAIS STATE, BRAZIL

Qualidade do leite cru refrigerado obtido através dos indicadores CCS e CBT no sudoeste mineiro

Odila Rigolin-Sá[1], Norival França[1], Keyla Cristiane Pereira Esper[1], Douglas de Pádua Andrade[1]*

ABSTRACT

Subclinical mastitis is a disease of dairy herds that causes serious financial losses to the producer resulting in reduced production and, in some cases, discarding of milk. Due to this problem, the aim of this study was to evaluate the presence of mastitis in cattle state of raw refrigerated milk producers in a total of 11 dairy farms in the southeast of Minas Gerais state, Brazil, in 2012, and verify suitability to Brazilian Standards IN-62. Duplicated samples were collected every month from April to November 2012 in 11 dairy farms in Southwest of Minas Gerais, bringing a total of 188 milk samples. These samples were collected in cooling tank and stored in sterilized bottles preserved in bronopol for SCC testing and azidiol for coliform microbiological and TBC testing. Among all the analysed variables, SCC results from all the dairy farms differed most from the control group ($p \leq 0.001$). Consequently, according to legislation, all the dairy farms can be classified as contamination risk areas for subclinical mastitis considering that they all exceeded the limit values established by law.
Keywords: mastitis; milk quality; microbiological analysis.

RESUMO

A mastite subclínica é uma enfermidade do rebanho leiteiro que causa grandes prejuízos econômicos ao produtor, provocando uma queda na produção e

1 Fundação de Ensino Superior de Passos (FESP/UEMG), Rua Sabará, 164, Centro, 37900-004, Passos, MG, Brasil. E-mail: odilarigolin@yahoo.com.br
* Autor para correspondência.

Recebido / Received: 14/08/2013
Aprovado / Approved: 10/09/2014

até mesmo o descarte do leite produzido. Devido a esta problemática objetivou-se neste estudo avaliar a presença de mastite em bovinos produtores de leite cru refrigerado, produzido em 11 propriedades leiteiras do Sudoeste Mineiro no período 2012 e verificar a adequação à IN-62. Foram realizadas duplicatas de amostras mensais no período de abril a novembro de 2012 em 11 propriedades leiteiras do sudoeste mineiro, totalizando 188 amostras de leite. As amostragens de leite foram realizadas nos tanques de resfriamento, onde as amostras foram coletadas em frascos esterilizados com conservante bronopol para analise de CCS e com conservante azidiol para análise microbiológica de coliformes e CBT. Entre todas as variáveis analisadas, os resultados CCS de todas as propriedades foram os que mais diferiram com o controle ($p \leq 0{,}001$). Desta forma, de acordo com a legislação todas as propriedades podem ser classificadas como áreas de risco de contaminação por mastite subclínica, todas as propriedades excederam os valores limites estabelecidas pela legislação.

Palavras-chave: mastite; qualidade do leite; análise microbiológica.

INTRODUCTION

Milk production is considered one of the main agricultural activities in Brazil. Its social and economical importance is reflected in the position it occupies in Brazilian agribusiness and it is one of the key sectors for national income generation and tax collection.

In view of its composition, milk is considered one of the most complete nutritional food products and essential for the human diet. For this same reason, it is an excellent substrate for the development of a significant diversity of microorganisms, including pathogens. The availability of milk nutrients and its almost neutral pH makes this medium extremely favourable for microbial growth. The action of bacteria or their enzymes on its components causes several alterations to milk and its by-products. These changes include unpleasant flavours and aromas, reduced validity period, interference of technological processes and diminished yield, especially for cheese (HICKS et al., 1982; CHAMPAGNE et al., 1994). Leite Júnior et al. (2003); Timm et al. (2003) emphasize constant concerns and discussions among technicians and health authorities about the consequences and factors that affect milk quality, especially in relation to the risk of transmitting microorganisms that can cause outbreaks of food-related diseases.

In order to promote milk and by-product quality improvements, add value to Brazilian dairy products and safeguard the health of consumers, in 1996, the Federal Government, through the Ministry of Agriculture, Livestock and Supply – Ministério da Agricultura, Pecuária e Abastecimento, created a strategic public policy for agribusiness in Brazil called the National Milk Quality Improvement Program regulated in 2002, through the Inspection Department of Animal Products (Departamento de Inspeção de Produtos de Origem Animal – DIPOA), published Standard Instruction nr. 51 to establish milk quality identification parameters and criteria for collection, cooling and bulk transportation, with maximum and minimum limits of variables for product quality evaluations. Subsequently, to better adapt some parameters to the reality of brazilian producers, Normative Instruction 62 (BRASIL, 2011) became effective, altering IN-51 (BRASIL, 2002) and amending the fixed limit for variables, Somatic Cell Count – SCC and Total Bacterial Count – TBC. These parameters, together with coliform count, are important tools to determine the incidence level of udder mastitis in a given herd.

Currently the most common mastitis prevention and control indicator is CSS (SOUZA et al., 2005), which, in addition to microbiological parameters, can more effectively and precisely determine the level of contamination caused by mastitis. Cunha et al. (2008) list the factors that can influence SCC variation, such as order of delivery, lactation period and seasonality. However, the occurrence of intra-mammary mastitis is the key factor responsible for SCC variation (HARMON, 1994).

According to Radostits (2000), subclinical mastitis is the inflammation of a mammary gland that results in pathological alterations in the glandular tissue and causes a series of physical, chemical and biological alterations to milk, directly affecting its quality and composition. Of all the different pathologies that affect dairy cattle, this disease causes the most serious financial losses to producers, resulting in reduced production or, in some cases, discarding of milk.

Within the context of this worldwide problem, the aim of this study was to evaluate the presence of subclinical mastitis in cows producers of refrigerated raw milk using the Somatic Cells Count, TBC and coliforms to evaluate the sanitary-hygienic quality during milking in 11 dairy farms in the southweast of Minas Gerais and verify suitability to IN-62/2011.

MATERIAL AND METHODS

Duplicated samples were collected every monthly from April to November 2012 in 11 dairy farms in the southwest of Minas Gerais, totalling 188 milk samples. This region produces approximately 176 million litres of milk per year and this agricultural sector is one of the most socially and economically important sectors in the region. The selection of the properties is: milking type and acceptance of the participants by the milk products. The milking types are: 1) Closed system with holding tank; 2) Closed system without holding tank; 3) Open system with holding tank (Table 1).

Milk samples were collected in the refrigerators at each farm, where they were stored in sterile jars and preserved in bronopol for SCC and azidiol of microbiological testing. The samples were then conserved in isothermal recipients with recyclable ice with approximate refrigeration temperature of 4 ºC and sent for testing at the Food Product and Environmental Analysis Laboratory (FPEAL) – LAAPA in the Passos Foundation of Higher Education (FESP/UEMG).

The multiple tube technique was adopted for total and thermotolerant coliforms. For total coliforms, the Brilliant Green Bile Broth 2% bacteriological culture medium was used with incubation at 36 ºC for 24 – 48 hours. The contaminated samples were subsequently transferred to tubes with *E. coli* broth to determine the presence of thermotolerant coliforms in a 24-hour period with incubation at 44.5 ºC. The Most Probable Number (MPN/mL) was used in all coliform samples, as described in the Compendium of Methods for the Microbiological Examination of Foods (VANDERZANT; SPLITTSTOESSER, 1992).

Total Bacterial Count was measured using the method described in the Compedium of Methods for the Microbiological Examination of Foods (Swanson et al. 2001), which consists of quantifying a group of bacteria found in milk by inoculating the samples on plates and incubating at 36 ºC for 48 hours. The culture medium used in the plates was Plate Count Agar that contains nutrients that allow bacterial multiplication. Results were obtained by counting the colonies expressed in a Colony Forming Unit – CFU.

Somatic cell count of milk samples was based on the 300 system of Bentley, by flow cytometry. Results of milk sample tests were compared using IN-62 standards (BRASIL, 2011).

Data were analysed using two-way analysis of variance (ANOVA) associated with the Tukey multiple comparison test to determine whether the parameters studied in each farm differed from the control. The control group was based on limit values for each parameter according to IN-62. Original data of average values for each variable were transformed into base-10 logarithms. All statistical data were analysed using the SigmaPlot 11.0. programme.

RESULTS AND DISCUSSION

Table 02 shows results obtained from microbiological testing and CSS of raw refrigerated milk conducted at the 11 studied dairy farms, and limits of each variable established by IN-62 (Control) (BRASIL, 2011).

Currently, there is a widespread debate about the importance and standards of SCC and TCB to guarantee hygiene and quality of milk (GODKIN, 2000), and on how to ensure that producers produce better quality milk and subsequently add value to their end product. However, whilst attempting to adapt legal reference values to the reality of

Table 1 – Technical characteristics of each studied dairy farm

Farm	Production (L^{-1}/day)	Municipality	Milking Type	Dipping	Milking Hygiene
A	11,000	São João Batista do Glória	Closed Circuit (Holding tank)	Before and After	Yes
B	2,300	Passos	Closed Circuit (Holding tank)	Before and After	Yes
C	500	Passos	Closed Circuit (Same level)	Before	Yes
D	450	Passos	Open Circuit (Same level)	Before	No
E	1,700	Passos	Closed Circuit (Same level)	Before	Yes
F	1,250	Passos	Closed Circuit (Same level)	Calf at foot	Yes
G	2,500	São João Batista do Glória	Closed Circuit (Holding tank)	Before and After	Yes
H	2,200	Passos	Closed Circuit (Holding tank)	Before	Yes
I	650	Passos	Open Circuit (Same level)	Before	No
J	750	Passos	Open Circuit (Same level)	Before and After	Yes
K	1,300	Passos	Open Circuit (Holding tank)	Before	Yes

Brazilian producers, MAPA altered the limit values of SCC from 6.00 x 10^5 to 4.00 x 10^5, and of TBC from 5.00 x 10^5 to 10.00 x 10^5, thus increasing strictness of compliance with two of the key parameters of milk quality indicators. The total and thermotolerant values of this present study were compared to those of the Compedium of Methods for the Microbiological Examination of Foods (SWANSON et al., 2001).

Among the analysed variables, SCC results of all the farms differed most from the control ($p < 0.001$). Consequently, according to IN-62, all daily farms can be classified as risk areas for subclinical mastitis contamination and as producers of lower quality milk. In spite of reducing concentrations of TBC and SCC according to IN-62, all the farms exceeded the limit value established by law. Therefore, the analysed milk indicates lack of

Table 2 – Average, standard deviation, minimum e maximum of Total Bacterial Count, Total Coliforms, Thermotolerant Coliforms and Somatic Cell Count of raw refrigerated milk in dairy farms from April/2012 to November/2012

Farm	TBC (UFC/mL)	Total Coliform (NMP/mL)	Thermotolerant Coliform (NMP/mL)	SCC SCC (cs/mL x 1000)
Control	1.00 x 10^5	1.00	1.00	4.00 x 10^5
A	1.26 x 10^2 ± 1.13 x 10^2 0.05 x 10^2 - 4.00 x 10^2	148.70 ± 36.10 2.10 - 120.00	0.20 ± 0.16 0.01 - 0.40	765.60 ± 402.88* 241.00 - 1331.00
B	7.98 x 10^5 ± 1.20 x 10^6 0.30 x 10^1 - 3.80 x 10^6	22.32 ± 18.94 2.30 - 46.00	10.58 ± 14.17 0.01 - 46.00	1805.80 ± 713.44* 800.00 - 2835.00
C	1.26 x 10^5 ± 1.57x10^5 2.00 x 10^1 - 5.20 x 10^5	88.18 ± 34.91 0.90 - 110.00	47.18 ± 50.26 0.01 - 110	843.00 ± 330.40* 405.00 - 1276.00
D	1.44 x 10^6 ± 1.90 x 10^6 0.22 x 10^1 - 6.20 x 10^6	72.66 ± 44.81* 9.30 - 110.00	37.92 ± 32.06 0.30 - 110.00	2266.80 ±1208.88* 605.00 - 5289.00
E	1.10 x 10^5 ± 1.31 x 10^5 5.00 x 10^1 - 3.00 x 10^5	66.92 ± 51.70 2.30 - 110.00	23.32 ± 34.67 0.01 - 110.00	610.40 ± 112.24* 486.00 - 891.00
F	2.23 x 10^3 ± 3.5 x 10^4 3.00 x 10^1 - 1.1 x 10^5	88.42 ± 34.53* 2.10 - 110.00	32.32 ± 32.54 0.30 - 100.00	590.2 ± 410.72* 108.00 - 1617.00
G	6.40 x 10^4 ± 7.67 x 10^4 0.60 x 10^1 - 1.70 x 10^5	45.50 ± 26.20 1.50 - 110.00	29.98 ± 32.01 0.01 - 110.00	1812.40 ± 1319.44* 680.00 - 5111.00
H	2.45 x 10^6 ± 3.81 x 10^6 3.30 x 10^2 - 1.20 x 10^7	75.20 ± 41.76 0.01 - 110.00	6240.00 ± 38.08 0.01 - 110.00	3420.40 ± 1534.72* 606.00 - 5636.00
I	1.86 x 10^5 ± 2.23 x 10^5 0.46 x 10^1 - 5.40 x 10^5	51.12 ± 47.10 2.30 - 110.00	47.78 ± 49.78 0.30 - 110.00	743.60 ± 248.16* 281.00 - 1364.00
J	1.02 x 10^7 ± 1.51 x 10^7 0.20 x 10^1 - 4.80 x 10^7	23.46 ± 34.62 0.40 - 110.00	23.00 ± 34.80 0.01 - 110.00	500.80 ± 251.76* 203.00 - 1032.00
K	0.38 x 10^1 ± 4.05 x 10^1 0.00 - 9.80 x 10^1	1.32 ± 1.58 0.00 - 4.30	0.06 ± 0.10 0.01 - 0.30	670.60 ± 182.72* 489.00 - 1000.00

* Results that significantly differ (p = 0,01) from control.

mastitis control in the studied farms, as SCC is used to designate all cells found in the milk, including haematopoietic cells (leucocytes) and squamous epithelial cells, that increase significantly when there is swelling in the mammary gland (NATZKE, 1981).

In addition to increased cell count, mastitis causes alterations in the three main components of milk: fat, protein and lactose. Enzymes and minerals are also affected. The extent of increased SCC and changes in milk composition are directly related to the surface of mammary tissue affected by the inflammatory reaction (SCHAELLIBAUM, 2000). Furthermore, fat and lactose tend to drop as SCC increases.

In the study by Paiva et al. (2012) the percentage to SCC, in milk sample in 2008 increased in 60% according to the IN-62. The authors concluded that the seasonal variations influenced both the refrigerated raw milk quality and the TBC increase.

Seasonality is considered a primordial factor of SCC variations in cattle. In a study conducted by Bueno et al. (2005) that compared the SCC of milk during seasons showed a correlation between environment temperature and SCC variation, being that the highest averages of SCC occurred in the dry season (September and October). Magalhães et al. (2006), observed a greater tendency of increased SCC in the summer (January to March), correlating this variable with increased temperature, meaning that SCC increases as the temperature increases. However, Peller et al. (2000) emphasized that higher SCC associated to risk factors of subclinical mastitis is proportionally related to a greater number of calvings and start and end of lactation.

Another indicating factor that can cause CSS variation is related to the different milking systems used for dairy cattle. Milking can be manual or mechanical by means of an open or closed system. The milking location may or may not have a holding tank.

Comparing SCC results of the studied farms, the only differences were between Farm H and Farm A ($p = 0.04$), E ($p = 0.36$), F ($p = 0.01$) and J (0.01), which confirms that milking systems did not directly influence the high incidence of mastitis in the farms as these differences were detected in farms that used the same milking system (Table 01).

Results also showed that farms (A and K) with closed circuit freestall milking systems with tanks caused a reduction in somatic cell count, while other farms where production was mostly in the pastures presented increased SCC. The freestall cattle confinement system can influence contamination because after milking the cows are guiding to feeding stalls and remain standing until closing of the sphincter, thus reducing the possibility of environmental mastitis contamination.

According to Carvalho et al. (2013), the SCC variation is more closely related to a lack of equipment and tool maintenance or their inadequate usage, than to the actual system.

In a study carried out by Borges et al. (2013) in the mesoregions of Minas Gerais Central and west parts, it was believed that the values of the Somatic Cell Counting (SCC) were in the legally established limit of 600,000 cell/mL, except in the production range above of 1,000L/day, according to the Normative Instruction n. 62 (IN-62/2011). The total bacterial counting was the most critical factor. The average values of bacterial counting surpassed the current prescribed limit, and the cattle with daily production of over 500 L/day were the ones which presented a higher number of results which are not according to the legal standards.

Given the high concentration of somatic cells, there is also a tendency for increased TBC, although TBC parameters, as in the case of coliform testing, and considering the non-standardization of limits in the laws of dairy products, are more closely associated to better hygiene-sanitary quality of milk than to the incidence of subclinical mastitis.

TBC results showed that eight dairy farms presented differences in relation to the control group, indicating that the incidence of mastitis is related to TBC. According to Eneroth et al. (2000), contamination of dairy products by TBC bacteria may be the result of inadequate quality of the water supply and hygiene procedure deficiencies, and not only mastitis. Therefore, hygiene procedures conducted in the productive chain of milk are critical for the obtainment of a high quality raw material.

Cousin (1982); Hogan et al. (1988) state that TBC is influenced by humidity and temperature of the environment, which directly reflects on hygiene of the animal, environment, equipment, milking procedures and cooling. Considering the potential for multiplication, milk bacteria can cause alterations, such as the degradation of fat, protein and carbohydrates, and render the product unfit for consumption and industrial processing.

Paiva et al. (2012) verified a significant increase in the milk average gotten by the producer/month and in the amount of milk received by a milk processing industry in Minas Gerais and an improvement in the TBC parameters established by the IN 51 for 2008 and 2011, when the industry implemented a pay program according to the quality.

The TBC increase poses a risk for the consumers' health because of the disease traffic and, for the industry; it brings damages regarding sensorial problems in the milk derivatives (Mendonça et al., 2001).

Results related to contamination of milk by the total coliform group and thermotolerants all the dairy farms got different with the controlling. It is therefore increasingly important to produce good quality milk. Hygiene of the animal and hands of the milker are essential to achieve this objective (SANTOS; FONSECA, 2007).

In these contexts, the quality of raw milk is affected by several conditions, especially zootechnical factors, handling, feeding and genetic potential of cattle and factors related to the collection and storage of freshly milked milk (CARVALHO et al., 2002). Temperature and time of milk storage are also important, as these factors are directly related to the multiplication of microorganisms found in milk (GUERREIRO et al., 2005). Consequently, sanitary and hygiene control of herds and milking is fundamental to ensure the ideal composition of milk and reduce the risk of pathogenic agent transmission.

Good practices in milk production applicable to the areas of animal health, milking hygiene, nutrition, animal well-being and environmental conditions were established with follow-up and guidelines to producers by means of monthly reports. Results were also discussed to identify critical contamination points, support improvements of milk production and ensure that raw materials were collected from healthy animals and maintained under acceptable conditions. It was also emphasized that these activities should be constant, progressive and personalized to guarantee better adaptation to the reality of each farm and current legislation.

CONCLUSIONS

The sanitary hygienic quality of milk are not suitable to requiring IN 62/2011. The microbiological quality of milk indicates the necessity to improve the production system of milking, equipment sanitizing and the sanitary hygienic handling to obtain milk.

The implementation of a system of payment for milk quality producer is necessary to improve the quality indices of the raw material, which does not occur at the study site.

ACKNOWLEDGMENTS

The authors would like to thank Fundação de Amparo a Pesquisa de Minas Gerais

(FAPEMIG) and Fundação de Ensino Superior de Passos / Universidade do Estado de Minas Gerais (FESP / UEMG) for financing the project and helping in the logistical support in which the work can be performed successfully. We also thank all trainees for help in the field and in laboratory.

REFERENCES

BORGES, L. R. et al. Milk quality according to the daily range in farm production in the Mesoregion Central Mineira and Oeste of Minas Gerais regions, Brazil. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 65, n. 4, p. 1239-1243, 2013.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Departamento de Inspeção de Produtos de Origem Animal. Instrução Normativa nº 51, de 18 de setembro de 2002. Aprova os Regulamentos Técnicos de Produção, Identidade e Qualidade do Leite tipo A, do Leite tipo B, do Leite tipo C, do Leite Pasteurizado e do Leite Cru Refrigerado e o Regulamento Técnico da Coleta de Leite Cru Refrigerado e seu Transporte a Granel, em conformidade com os Anexos a esta Instrução Normativa. **Diário Oficial da República Federativa do Brasil**, Brasília, 20 set. 2002. Seção 1, p. 8-13.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 62, de 29 de dezembro de 2011. Aprova o Regulamento Técnico de Produção, Identidade e Qualidade do Leite tipo A, o Regulamento Técnico de Identidade e Qualidade de Leite Cru Refrigerado, o Regulamento Técnico de Identidade e Qualidade de Leite Pasteurizado e o Regulamento Técnico da Coleta de Leite Cru Refrigerado e seu Transporte a Granel, em conformidade com os Anexos desta Instrução Normativa. **Diário Oficial da República Federativa do Brasil**, Brasília, 30 dez. 2011. Seção 1, p. 6.

BUENO, V. F. F. et al. Contagem celular somática: relação com a composição centesimal do leite e período do ano no Estado de Goiás. **Ciência Rural**, v. 35, n. 4, p. 848-854, 2005.

CARVALHO, L. A. et al. **Embrapa Gado de Leite. Sistema de Produção.** 2002. Disponível em: <www.cnpgl.embrapa.br> Acesso em: 05 jul. 2013.

CARVALHO, T. S. et al. Qualidade do leite cru refrigerado obtido através de ordenha manual e mecânica. **Revista do Instituto de Laticínios Cândido Tostes**, v. 68, n. 390, p. 05-11, 2013.

CHAMPAGNE, C. P. et al. Psychrotrophs in dairy products: their effects and their control. **Critical Reviews in Food Science and Nutrition**, v. 34, n. 1, p. 1-30, 1994.

COUSIN, M. A. Presence and activity psychrotrophic microorganisms in milk and dairy products. **Journal of Food Protection**, v. 45, n. 2, p. 172, 1982.

CUNHA, R. P. L. et al. Mastite subclínica e relação da contagem de células somáticas com número e lactações, produção e composição química do leite em vacas da raça Holandesa. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 60, n. 1, p. 19-24, 2008.

ENEROTH, A. et al. Contamination of milk with Gram-negative spoilage bacteria during filling of retail containers. **International Journal of Food Microbiology**, v. 57, n. 1-2, p. 99-106, 2000.

GODKIN, A. Qualidade do leite ao redor do mundo: O papel da CCS. In: SIMPÓSIO INTERNACIONAL SOBRE QUALIDADE DO LEITE, 2., 2000, Curitiba. **Anais...** Curitiba: CIETEP/FIEP, 2000. p. 9-16.

GUERREIRO, P. K. Qualidade microbiológica de leite em função de técnicas profiláticas no manejo de produção. **Ciência e Agrotecnologia**, v. 29, n. 1, p. 216-222, 2005.

HARMON, R. J. Physiology of mastitis and factors affecting somatic cell counts. **Journal of Dairy Science.** v. 77, n. 7, p. 2103-2113, 1994.

HICKS, C. L. et al. Psychrotroph bacteria reduce cheese yield. **Journal of Food Protection**, v. 45, n. 4, p. 331-334, 1982.

HOGAN, et al. Bacterial and somatic cell counts in bulk tank milk from nine well managed herds. **Journal of Food Protection**, v. 51, n. 12, p. 930-934, 1988.

LEITE JÚNIOR, A. F. S. et al. Qualidade microbiológica do leite tipo C pasteurizado, comercializado em João Pessoa, Paraíba. **Revista Higiene Alimentar**, v. 14, n. 74, p. 45-49, 2003.

MAGALHÃES, H. R. et al. Influência de fatores de ambiente sobre a contagem de células somáticas e sua relação com perdas na produção de leite de vacas da raça Holandesa. **Revista Brasileira de Zootecnia**, v. 35, n. 2, p. 415-421, 2006.

MENDONÇA, A. H. et al. Qualidade físico-química de leite cru resfriado: comparação de diferentes procedimentos e locais de coleta. In: CONGRESSO NACIONAL DE LATICÍNIOS, 18., 2001, Juiz de Fora. **Anais...** Juiz de Fora: Templo. 2001.p. 276-282.

NATZKE, R. P. Elements of mastitis control. **Journal of Dairy Science.** v. 64, n. 6, p. 1431-1442. 1981.

PAIVA, C. A. V. et al. Annual evolution of raw Milk quality processed in a dairy industry of Minas Gerais state, Brazil. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 64, n. 2, p. 471-478, 2012.

PEELER, E. J. et al. Risk factors associated with clinical mastitis in low somatic cell count British dairy herds. **Journal of Dairy Science**, v. 83, n. 11, p. 2464-2472, 2000.

RADOSTITS, O. M. et al. **Clínica veterinária.** Rio de Janeiro: Guanabara Koogan, 2000. 1119p.

SANTOS, M. V.; FONSECA, L. F. L. (Eds). **Estratégias para controle de mastite e melhoria da qualidade do leite.** São Paulo: Manole. 2007. 314p.

SCHAELLIBAUM, M. Efeitos de altas contagens de células somáticas sobre a produção e qualidade de queijos. In: SIMPÓSIO INTERNACIONAL SOBRE QUALIDADE DO LEITE, 2., 2000, Curitiba. **Anais...** Curitiba: CIETEP/FIEP, 2000. p. 21-26.

SOUZA, G. N. et al. Fatores de risco para alta contagem de células somáticas do leite do tanque em rebanhos leiteiros da Zona da Mata de Minas Gerais, Brasil. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 57, supl. 2, p. 251-260, 2005.

SWANSON, K. M. J, PETRAN, R. L. & HANLIN, J. H. Culture methods for enumeration of microorganisms. In: DOWNES, F. P. & ITO, K. (eds), **Compendium of Methods for the Microbiological Examination of Foods**, 4th ed. Washington: American Public Health Association (APHA), 2001. Chapter 6, p. 53-67.

TIMM, C. D. et al. Avaliação da qualidade microbiológica do Leite Pasteurizado integral, produzido em micro-usinas da região sul do Rio Grande do Sul. **Revista Higiene Alimentar**, v. 17, n. 106, p. 88-93, 2003.

VANDERZANT, C.; SPLITTSTOESSER, D. F. **Compendium for the microbiological examination of foods.** Washington, DC: American Public Health Association, 1992. 1219p.