COMPOSITION AND MICROBIOLOGICAL QUALITY OF REFRIGERATED RAW MILK IN TWO SEASONS OF THE YEAR IN SANTA HELENA, WESTERN PARANÁ

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ABSTRACT: This study aimed at quantifying the occurrence of proteolytic psychotropic microorganisms and total coliforms in refrigerated raw milk, in addition to assessing the quality through its physical-chemical composition. The samples were collected in 10 dairy farms in the municipality of Santa Helena – Western Paraná. Two collections were made, one during the spring and the other during the summer. Samples of refrigerated raw milk were carried out directly from the cooling tank, aseptically, packed in sterile bottles and transported under isothermal conditions (± 4 ºC) to the laboratory where the physical-chemical composition and microbial populations were determined. The levels of fat had great amplitude between the seasons, being higher in the summer, while there was no variation for the contents of protein, lactose, minerals, and non-fat solids. The total coliform count did not differ between seasons. The values obtained for proteolytic psychotropic counts were higher in the summer. A significant positive correlation was found between the total coliform counts, the proteolytic psychotropic counts ($r=0.73$), the levels of protein ($r=0.45$), non-fat solids ($r=0.45$), and minerals ($r=0.46$). Also, the proteolytic psychotropic counts showed a positive correlation with the cryoscopic index. The milk components met the requirements of NI76. The quality of refrigerated raw milk in the municipality of Santa Helena, Western Paraná was not satisfactory for total coliforms, due to its high incidence, indicating the need for good practices in milking management. Proteolytic psychotropic bacteria presented low proliferation, thus not affecting milk quality.
KEYWORDS: Contamination. Proteolytic psychotropics. Total coliforms.

COMPOSIÇÃO E QUALIDADE MICROBIOLÓGICA DO LEITE CRU REFRIGERADO EM DUAS ESTAÇÕES DO ANO EM SANTA HELENA, OESTE DO PARANÁ

RESUMO: O presente estudo teve como objetivo quantificar a ocorrência de microrganismos psicrotróficos proteolíticos e de coliformes totais em leite cru refrigerado além de avaliar a qualidade por meio da sua composição físico-química. As amostras foram coletadas em 10 propriedades leiteiras no município de Santa Helena – Oeste do Paraná. Foram realizadas duas coletas, uma durante a primavera e outra no verão. As amostragens do leite cru refrigerado foram realizadas diretamente no tanque de resfriamento, de forma asséptica, acondicionado em frascos esterilizados e transportado sob condições isotérmicas (± 4ºC) ao laboratório onde foram determinadas a composição físico-química e as populações microbianas. Os teores de gordura tiveram grande amplitude entre as estações do ano, sendo superior no verão, enquanto que não houve variação para os teores de proteína, lactose, minerais e sólidos desengordurados. A contagem de coliformes totais não diferiu entre as estações. Os valores obtidos para contagens de psicrotróficos proteolíticos foram superiores no verão. Foi constatada correlação significativa positiva entre as contagens de coliformes totais com as contagens de psicrotróficos proteolíticos ($r=0,73$) e os teores de proteína ($r=0,45$), sólidos desengordurados ($r=0,45$) e minerais ($r=0,46$). Além disto, as contagens de psicrotróficos proteolíticos apresentaram correlação positiva com o índice crioscópico. Os componentes do leite atenderam as exigências da IN76. A qualidade do leite cru refrigerado no município de Santa Helena, Oeste do Paraná não foi satisfatória em relação aos coliformes totais, devido sua alta incidência, indicando a necessidade de boas práticas no manejo de ordenha. As bactérias psicrotróficas proteolíticas tiveram baixa proliferação, não afetando a qualidade do leite.
PALAVRAS-CHAVE: Contaminação. Psicrotróficos proteolíticos. Coliformes totais.

COMPOSICIÓN Y CALIDAD MICROBIOLÓGICA DE LA LECHE CRUDA REFRIGERADA EN DOS ESTACIONES DEL AÑO EN SANTA HELENA, OESTE DE PARANÁ

RESUMEN: El presente estudio tuvo como objetivo cuantificar la ocurrencia de microorganismos psicrotróficos proteolíticos y de coliformes totales en leche cruda refrigerada, además de evaluar la calidad a través de su composición fisicoquímica. Las muestras fueron recolectadas en 10 granjas lecheras del municipio de Santa Helena – Oeste de Paraná. Se realizaron dos

colecciones, una en primavera y otra en verano. Las muestras de leche cruda enfriada se realizaron directamente en el tanque de enfriamiento, de forma aséptica, se empacaron en botellas estériles y se transportaron en condiciones isotérmicas (± 4ºC) al laboratorio donde se determinó la composición fisicoquímica y las poblaciones microbianas. Los niveles de grasa tuvieron gran amplitud entre las estaciones, siendo más altos en verano, mientras que no hubo variación para los contenidos de proteínas, lactosa, minerales y sólidos desgrasados. El recuento total de coliformes no difirió entre temporadas. Los valores obtenidos para los recuentos de psicotrópicos proteolíticos fueron mayores en verano. Se encontró una correlación positiva significativa entre los recuentos de coliformes totales y los recuentos de psicrótroficos proteolíticos (r=0.73) y los niveles de proteínas (r=0.45), sólidos desgrasados (r=0.45) y minerales (r=0.46). Además, los recuentos de psicrótroficos proteolíticos mostraron una correlación positiva con el índice crioscópico. Los componentes de la leche cumplieron con los requisitos de IN76. La calidad de la leche cruda refrigerada en el municipio de Santa Helena, Oeste de Paraná no fue satisfactoria en relación a los coliformes totales, debido a su alta incidencia, lo que indica la necesidad de buenas prácticas en el manejo del ordeño. Las bacterias psicrotróficas proteolíticas tuvieron baja proliferación, no afectando la calidad de la leche.

PALABRAS CLAVE: Contaminación. Psicotróficos proteolíticos. Coliformes totales.

Introduction

Milk is considered a complete food because it contains proteins, fats, carbohydrate minerals, vitamins, and water, but the quality of milk can be affected due to physical and chemical factors (KUMAR *et al.*, 2018). According to Gottardi *et al.* (2008), the hygienic-sanitary conditions in which the milking and storage of the milk occurs are determinant in its quality. Bacterial contamination can come from udder dirt, the hands of the milker, milking equipment, and poorly sanitized drums and buckets (JAMAS *et al.*, 2018). The groups of microorganisms most commonly used as indicators of hygiene in milk are mesophilic, psychrotrophic, and coliform aerobes (PERIN *et al.*, 2019).

The National Program for the Improvement of Milk Quality, following the requirements of Normative Instruction nº76 - NI76, determines the physical-chemical and microbiological parameters for raw milk, among other aspects (BRASIL, 2018). The control of the growth of microorganisms can be considered as a key point in the quality and safety control of dairy products (PERIN *et al.*, 2019).

Inadequate storage temperatures can lead to an increase in total coliforms in refrigerated raw milk (Maciel *et al.*, 2008). The cooling of milk prevents the development of mesophilic bacteria (PINTO; MARTINS; VANETTI, 2006), but allows the development of psychrotrophic bacteria, such as Pseudomonas, characterized by having intense metabolic activity in the temperature range between 4 and 7˚C (JAY; LOESSNER; GOLDEN, 2005), in addition to Bacillus, which predominate in the temperatures of cooling margins from 8˚C to 10˚C (PERIN *et al.*, 2019).

Most psychrotrophic are destroyed by heat treatments (RIBEIRO JÚNIOR *et al.*, 2018), however, some of these bacteria can produce heat-resistant proteolytic and lipolytic enzymes, maintaining their enzymatic activity even after heat treatment (CAPODIFOGLIO *et al.*, 2016). Proteolytic enzymes promote a reduction in the yield of dairy products, in addition to organoleptic changes in milk, such as bitter taste, due to the presence of peptides originating from the breakdown of casein (PERIN *et al.*, 2019).

In this context, the present study aimed to quantify the occurrence of proteolytic psychrotrophic microorganisms and coliforms and to evaluate the quality of milk through the physical-chemical composition.

Material and Methods

Samples of refrigerated raw milk were collected, from 10 rural dairy farms, located in the municipality of Santa Helena - Western Paraná, with two collections, one during the spring and the other in the summer, totaling 20 collections. Dairy farms were characterized as small and medium-sized. The average number of animals milked in each herd was 20 animals, composed of primiparous and multiparous cows, in different stages of lactation. In all explorations the milking performed was of the mechanical type.

Samples of refrigerated raw milk were carried out directly in the cooling tank, aseptically, collecting 200 mL of refrigerated raw milk after homogenization, with the aid of a stainless steel ladle, which was stored in sterile bottles and transported under isothermal conditions (± 4ºC) to the Microbiology laboratory of Unioeste, Marechal Cândido Rondon, Paraná, where the physico-chemical composition and the populations of total proteolytic and coliform psychrotrophic microorganisms were determined.

For the physical-chemical analysis of milk, the automatic Milkoscope Expert analyzer (Scope Electric, Razgrad, Bulgaria) was used. The contents of fat, protein, lactose, solids non-fat, minerals, and the density of the samples were determined.

The microbial population was determined using culture techniques according to Silva *et al.* (1997), where test tubes containing 9 mL of sterile peptone water (0.1%) were used for decimal dilutions of milk samples, up to the 10^{-3} dilution, and from these, inoculum sowing was performed.

For the determination of proteolytic psychrotrophic, Nutrient Agar was added plus 10% skimmed-milk powder reconstituted to 10%, freshly prepared, melted, and cooled to 45ºC (APHA, 2014). The plates were incubated inverted at 7ºC for 10 days (MARSHALL; GOFF; HARTEL, 2003). Proteolytic psychrotrophic microorganisms appear in the form of a transparent halo around the colonies, resulting from the conversion of casein into soluble nitrogen compounds.

Total coliforms were quantified using the sowing method, using the selective culture medium Violet Red Bile Agar, maintained at 35ºC for 24 hours. After the incubation periods, the plates were counted with the aid of the Quebec type colony counter.

The data regarding the counting of microorganisms were transformed into a log base, and all data were submitted

to statistics with the aid of the SAS program (Version 9.3, SAS Institute, Cary, NC, EUA). Initially, a descriptive analysis of the data was performed and later they were subjected to analysis of variance, and the seasons (referring to collections) were compared by the *Tukey* test, adopting a significance level of 5%. The data of the physical-chemical composition were also subjected to *Pearson's* correlation about total proteolytic and psychrotrophic coliforms of milk.

Results and Discussion

The fat levels ranged between 2.36 and 8.37%, with a mean of 5.22% (Table 1). This range may be due to different breeds of animals present in each herd, as the fat content is generally higher in *Bos indicus* than in *Bos taurus* (KUMAR *et al.*, 2018). Despite the wide range of variation, the average is by Normative Instruction nº 76 (BRASIL, 2018), which must be at least 3.0%. For protein, lactose, and minerals, the range of variation was smaller, and all the parameters studied meet the minimum limit allowed by NI 76.

For the variables protein, lactose and minerals, these did not have oscillations between their levels in the studied properties (Table 1) and the average values are by NI 76, which stipulates minimum values of 2.9% and 4.3% for protein and lactose, respectively. Solids non-fat obtained an average of 8.09%, is below the minimum established (8.4%) by the NI 76 (BRASIL, 2018).

Among the properties evaluated, the disparity between coliform counts was found, ranging from 1.0 to 4.54 log CFU mL^{-1} (Table 1). This result is probably due to the particularities of management of each property, as according to Gottardi *et al.* (2008), even though there is the same technical guidance in all properties, there are differences regarding aspects of handling animals in milking such as hygiene, obtaining and storing milk, reflecting on the hygienic quality of the milk obtained. Another factor that contributes to this contamination by total coliforms may be the contact of milk with contaminated surfaces or via intramammary

secretion of cows with mastitis (JAMAS *et al.*, 2018).

There is no legislation determining the quantities of total coliforms in raw milk, only for enterobacteria (NI76). However, Chambers (2002) reported that counts above 100 CFU mL^{-1}, that is, greater than 2 log CFU mL^{-1} of bacteria in the coliform group, indicate flaws in hygiene during and between milking. In the present study, the average values of total coliforms were higher than these, showing the need for hygiene in milking.

The count of proteolytic psychrotrophic also showed great oscillation between the samples collected, ranging from 2.60 to 6.25 log CFU mL^{-1}. There are not parameters in the current legislation on the values allowed for proteolytic psychrotropics. However, they are considered indicators of milk quality, as their presence is due to flaws in the hygienic-sanitary handling of the equipment used (REIS *et al.*, 2014). These microorganisms can cause problems such as changes in taste and odor, loss of consistency, or gelatinous appearance in dairy products (ÂNGELO *et al.*, 2014), reducing their shelf life and the products' credibility with consumers.

The main sources of psychrotrophic contamination are poorly sanitized milking equipment and water (SILVA *et al.*, 2018). In addition to deficiencies in hygiene procedures aimed at milking management (ARCURI *et al.*, 2008), mastitis in milked animals (PINTO; MARTINS; VANETTI, 2006), the time elapsed from milking to cooling and the cooling temperature (ARCURI *et al.*, 2008).

Under adequate conditions of milking and preservation, this group generally represents 10% of the total bacterial count (TBC) of raw milk, but when the milk is obtained in poor hygiene conditions, it can represent about 75% of the total raw milk microbiota (PERIN *et al.*, 2019). According to NI 76, the maximum allowed for TBC as 300.000 CFU mL^{-1} (BRASIL, 2018), however, 10% of this TBC corresponds to 4.48 log CFU mL^{-1}. Therefore, the mean value obtained for proteolytic psychrotrophic (4.34 log CFU mL^{-1}) was below this value, demonstrating that these microorganisms would not affect the quality of the milk.

Table 1 - Physical-chemical composition and coliform and psychrotrophic counts in milk from dairy herds in the municipality of Santa Helena - Paraná

	n	Mean	SD	Minimum	Maximum
Fat (%)	19	5.22	2.00	2.36	8.37
Protein (%)	19	2.96	0.11	2.78	3.29
Lactose (%)	19	4.44	0.16	4.16	4.93
Solids non-fat (%)	19	8.09	0.30	7.59	9.98
Minerals (%)	19	0.67	0.02	0.63	0.74
Density (%)	19	26.96	1.92	23.01	29.98
Cryoscopic index (°C)	19	-0.522	0.023	-0.594	-0.493
Total coliforms (log CFU mL^{-1})	20	2.88	0.99	1.00	4.54
Proteolytic psychrotrophic (log CFU mL^{-1})	19	4.34	1.05	2.60	6.25

n = number of observations; SD = Standard Deviation.

When comparing the seasons, the fat content was higher in the summer than in the spring (Table 2). This difference is possibly related to the composition of the diet offered to the animals, which in the summer period was mostly composed of tropical forages, which contributes to

a greater production of rumen acetate with a consequent increase in the levels of fat in the milk (VAN SOEST, 1994).

Table 2 - Physical-chemical composition and coliform and psychrotrophic counts in milk from dairy herds in the municipality of Santa Helena - Paraná at different seasons

	Summer	Spring	SEM	P-Value
Fat (%)	6.78	3.39	1.115	<0.01
Protein (%)	2.98	2.95	0.112	0.57
Lactose (%)	4.46	4.41	0.169	0.56
Solids non-fat (%)	8.13	8.03	0.306	0.51
Minerals (%)	0.67	0.66	0.024	0.29
Density (%)	26.38	27.62	1.859	0.16
Cryoscopic index (°C)	-0.534	-0.508	0.019	0.01
Total coliforms (log CFU mL^{-1})	3.16	2.62	0.978	0.23
Proteolytic psychrotrophic (log CFU mL^{-1})	4.83	3.81	0.941	0.03

SEM = Standard error of the mean.

Milani *et al*. (2016) when evaluating the quality of milk in different production systems and seasons, they observed means values of fat in the spring of 3.33%, 3.60% and 3.53% and the summer means of 3.64%, 3.71% and 3.70% for specialized, semi-specialized and extensive production systems. These values were similar to those obtained in the present study during the spring, with mean of 3.52%, however, they were lower than those obtained in the summer with mean of 6.46%.

The protein percentages did not vary (P=0.57) between seasons, similar to what was observed in the study by Botaro *et al*. (2008). The levels of lactose (P=0.56) and solids non-fat (P=0.51) also did not differ between seasons, being in disagreement with that reported by Lacerda, Mota and Sena (2010), who observed a tendency towards a reduction in the concentration of lactose and total solids in milk during periods of high temperatures, such as in the summer. There was no difference (P=0.29) between seasons for mineral content and density (P=0.16) of refrigerated raw milk.

The cryoscopic index was lower in summer than in spring (Table 2). This variable indicates the depression of the freezing point of milk about that of water, and can be used as an indicator of adulteration in milk through the addition of water (ALVES; DANTAS; GUSMÃO, 2020). Milk without adulteration is considered when the cryoscopic index is between -0.512ºC and -0.536ºC (BRASIL, 2018). For this, it is known that the freezing temperature of the milk is lower than that of water, however, the addition of water causes the cryoscopic index to approach zero, diluting the milk components, such as lactose and minerals (ALVES; DANTAS; GUSMÃO, 2020).

When comparing the seasons, there were no differences (P>0.05) for the coliform population in milk (Table 2). The lack of significance is also due to the hygienic-sanitary management adopted in the properties. A higher population of proteolytic psychrotropics was observed in samples collected in the summer (P=0.03). The summer period corresponds to the period with the highest rainfall incidence (D'ORAZIO BORTOLUZZI *et al*., 2019). According to Gomes *et al.* (2018), the rainy season may result in higher counts of proteolytic psychrotropics, proving in his research by observing a higher count of proteolytic psychrotropics in this period about the drought period (2.0 x 10^8 to 5.0 x 10^8 CFU mL^{-1}).

A significant positive correlation was found between total coliforms and proteolytic psychrotropics, protein, solids non-fat, and minerals (Table 3).

Table 3 - Pearson's correlation coefficients for physical-chemical composition and total proteolytic and psychrotrophic coliforms of milk from dairy herds in the municipality of Santa Helena – Paraná

	Total coliforms	Proteolytic psychrotrophic
Proteolytic psychrotrophic	0.73 P < 0.01	
Fat	-0.01 P = 0.98	0.43 P = 0.14
Protein	0.45 P = 0.05	0.35 P = 0.15
Lactose	0.45 P = 0.06	0.35 P = 0.15
Solids non-fat	0.45 P = 0.05	0.35 P =0.14
Minerals	0.46 P = 0.05	0.37 P = 0.12
Density	0.34 P = 0.16	-0.01 P = 0.99
Cryoscopic index	-0.39 P = 0.10	-0.56 P = 0.01

The significant positive correlation between total coliforms and proteolytic psychrotropics may be due to the presence of bacteria belonging to the genera *Citrobacter* and *Klebsiella*, which although they belong to the group of total coliforms, are also considered psychrotrophic microorganisms, and, although these have not been identified in this study, can have contributed to this correlation (MARTIN *et al*., 2011). The correlations observed with the solids non-fat content were expected since they consist of protein, lactose, and minerals (GOMES *et al*., 2018).

The proteolytic psychrotropics showed a positive correlation with the cryoscopic index (Table 3). This can be explained by the fact that the increase in water activity favored the proliferation of this microbial group, which by their nature proliferate at low temperatures and high values of water activity, such as refrigerated raw milk (JAY; LOESSNER; GOLDEN, 2005).

Conclusion

The milk components met the requirements of Normative Instruction nº76/2018. It was found that the fat content and the cryoscopic index differed between the seasons. The quality of refrigerated raw milk in municipality of Santa Helena, western of Paraná, was not satisfactory about total coliforms, due to its high incidence, indicating the need for good practices in milking management. Proteolytic psychrotrophic bacteria had low proliferation, not affecting milk quality.

References

ALVES, M. P.; DANTAS, T. N. P.; GUSMÃO, T. A. S. Avaliação da qualidade de leite produzido no município de Caturité. **Revista Brasileira de Gestão Ambiental**, Pombal, v. 14, n. 1, p. 17-27, jan./mar. 2020.

ÂNGELO, F. F. *et al*. Bactérias psicrotróficas em leite cru refrigerado. **Revista Científica Eletrônica de Medicina Veterinária**, São Paulo, n. 22, p.1-14, jan. 2014.

APHA. **Standard methods for the examination of water and wastewater**. American Public Health Association. v. 20, 2014.

ARCURI, E. F. *et al*. Counting, isolation and characterization of psychrotrophic bacteria from refrigerated raw milk. **Ciência Rural**, Santa Maria, v. 38, n.8, p. 2250-2255, nov. 2008.

BOTARO, B. G. *et al*. Effect of betalactoglobulin polymorphism and seasonality on bovine milk composition. **Journal of Dairy Research**, London, v. 75, n. 2, p. 176-181, jan. 2008.

BRASIL. Instrução Normativa n. 76 de 26 de novembro de 2018. Dispõe sobre Regulamento Técnico de Identidade e Qualidade de leite cru refrigerado. **Diário Oficial da República Federativa do Brasil**, Brasília, Seção 1, n.230,

p.9, 30 nov. 2018.

CAPODIFOGLIO, E. *et al*. Lipolytic and proteolytic activity of *Pseudomonas* spp. isolated during milking and storage of refrigerated raw milk. **Journal of Dairy Science**, London, v. 99, n. 7, p. 5214-5223, jul. 2016.

CHAMBERS, J. V. **The microbiology of raw milk**. *In:* ROBINSON, R. K. Dairy microbiology handbook: the microbiology of milk and milk products. 3ª ed. New York: John Wiley and Sons, 2002.

D'ORAZIO BORTOLUZZI, D. *et al*. Precipitação mensal provável no Noroeste do Paraná. **Revista Brasileira de Agricultura Irrigada**, Fortaleza, v. 13, n. 2, mai. 2019.

GOMES, A. C. *et al*. Diagnóstico de propriedades em regime de economia familiar e da qualidade do leite no município de Gouveia-MG. **Revista Agropecuária Técnica**, Paraíba, v. 39, n. 1, p. 96-106, 2018.

GOTTARDI, C. P. T. *et al*. Goat's milk quality by coliform's and staphylococci counting. **Ciência Rural**, Santa Maria, v. 38, n. 3, p. 743-748, mai./jun. 2008.

JAMAS, L. T. *et al*. Quality parameters of bovine milk from family farms. **Pesquisa Veterinária Brasileira**, Rio de Janeiro, v. 38, n. 4, p. 573-578, abr. 2018.

JAY, J. M.; LOESSNER, M. J.; GOLDEN, D. A. **Modern food microbiology**. Aspen Gaithersburg. 7ª ed. 2005.

KUMAR, D. *et al*. Compositional quality of milk and its contaminants on physical and chemical concern: a review. **International Journal of Current Microbiology and Applied Sciences**, India, v. 7, n. 5, p. 1125-1132, mai. 2018.

LACERDA, L. M.; MOTA, R. A.; SENA, M. J. Somatic cell counts, composition and total bacterial counts of milk from dairy farms in the cities of Miranda of Norte, Itapecurú-Mirim and Santa Rita, Maranhão. **Arquivos do Instituto Biológico**, São Paulo, v. 77, n. 2, p. 209-215, abr./jun. 2010.

MACIEL, J. F. *et al*. Microbiological quality of raw milk marketed in Itapetinga- BA. **Revista Brasileira de Saúde e Produção Animal**, Bahia, v. 9, n. 3, p. 443-448, 2008.

MARSHALL, R. T.; GOFF, H. D.; HARTEL, R. W. **Standard methods for the examination of dairy products**. 6ª ed. New York, USA: Plenum Publisher. 2003.

MARTIN, N. H. *et al*. When cheese gets the blues: *Pseudomonas fluorescens* as the causative agent of cheese spoilage. **Journal of Dairy Science**, London, v. 94, n. 6, p. 3176-3183, jun. 2011.

MILANI, M. P. *et al*. Milk quality in different production systems, year and weather seasons. **Revista Brasileira de Ciência Veterinária**, Niterói, v. 23, n. 3-4, p. 206-211, out. 2016.

PERIN, L. M. *et al*. **The microbiology of raw milk.** *In:* Raw milk, Academi Press, 2019. p. 45-64.

PINTO, C. L. O.; MARTINS, M. L.; VANETTI, M. C. D. Microbial quality of raw refrigerated milk and isolation of psychrotrophic proteolytic bacteria. **Food Science and Technology**, Campinas, v.26, n. 3, p. 645-651, jul./set. 2006.

RIBEIRO JÚNIOR, J. C. *et al.* The main spoilage-related psychrotrophic bacteria in refrigerated raw milk. **Journal Dairy Science**, London, v. 101, p. 75-83, jan. 2018.

REIS, D. L. *et al*. Quality and microbiological safety of fermented bovine dairy produced in Federal District, Brazil. **Semina: Ciências Agrárias**, Londrina, v. 35, n. 6, p. 3161-3172, nov./dez. 2014.

SILVA, C. G. *et al*. Influence of water treatment and milking practices on milk quality. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, Belo Horizonte, v. 70, n. 2, p. 615-622, mar./abr. 2018.

SILVA, N.; JUNQUEIRA, V.C.A.; SILVEIRA. N.F.A. **Manual de métodos de análise microbiológica de alimentos**. São Paulo: Livraria Varela, 1997.

VAN SOEST, P. J. **Nutritional ecology of the ruminant**. Ithaca: Constock Publishing Associates, 1994.