

Sheep milk: physical-chemical characteristics and microbiological quality

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SUMMARY. Sheep milk is the third most consumed milk in Brazil. It is much appreciated for its nutritional status and is important for children that have problems with cow milk. Little information is known about the chemical, physical and microbiological composition of sheep milk from South Brazil. Thus, the aim of this study was to describe chemical and microbiological characteristics of sheep milk produced on two rural properties located in southern Brazil (Paran  and Rio Grande do Sul). The chemical composition of sheep milk was 17.32 g/100 g total solids, 5.86 g/100 g total protein, 4.46 g/100 g casein, 1.08 g/100 g whey protein, 7.28 g/100 g fat, 0.93 g/100 g ash, and 3.41 g/100 g lactose. High somatic cell count (1.7×10^6 cells/mL), total mesophilic bacteria (16.0×10^6 CFU/mL) and psychrotrophs (5.8×10^6 CFU/mL) were observed. Growth of *Staphylococcus aureus*, enterobacteria and coliforms occurred in 100% of the samples, and 45% of the samples showed growth of *Escherichia coli*. The sheep milk physical-chemical and microbiology parameters are similar to those presented in the literature for other countries but somatic cell count presented high levels.

Key-words: Ovine milk, composition, mastitis, hygiene.

RESUMO. Leite ovino: caracter sticas f sico-qu micas e qualidade microbiol gica. O leite de ovelha   o terceiro mais consumido no Brasil, e   muito apreciado devido ao seu aspecto nutricional. Pouca informa  o a respeito da composi  o qu mica e microbiol gica do leite de ovelha no Brasil   conhecida. O objetivo deste trabalho foi caracterizar o leite ovino produzido em duas propriedades rurais localizadas no Sul do Brasil (Paran  e Rio Grande do Sul). A composi  o qu mica do leite de ovelha foi 17,32 g/100 g de s lidos totais; 5,86 g/100 g de prote na total; 4,46 g/100 g de case na; 1,08 g/100 g de soro prote ico; 7,28 g/100 g de gordura; 0,93 g/100 g de cinzas e 3,41 g/100 g de lactose. Altas contagens de c lulas som ticas ($1,7 \times 10^6$ c lulas/mL), bact rias mes filas ($16,0 \times 10^6$ UFC/mL) e psicrotr ficos ($5,8 \times 10^6$ UFC/mL) foram observadas. Multiplica  o de *Staphylococcus aureus*, enterobact rias e coliformes ocorreu em 100% das amostras, 45% de amostras apresentou multiplica  o de *Escherichia coli*. Os par metros f sico-qu micos e microbiol gicos do leite ovino deste estudo s o semelhantes a literatura para outros pa ses, por m, a contagem de c lulas som ticas foi elevada.

Palavras-chave: Leite de ovelha, composi  o, mastite, higiene.

INTRODUCTION

In South Brazil, ovine livestock was dedicated to wool production until the 1990s. However, this traditional economical chain has been in a very deep economical crisis, due to the increasing popularity of synthetic fibers and there is no hope for recovery in the near future (1). An economic alternative for sheep farmers would be focusing their activities in other ovine products, such as meat and milk.

Sheep milk production by the 20 main producing countries was estimated at 8.3 billion tons in 2012, while bovine milk production was estimated at 458 billion tons in the same period (2). The website of the Food and Agricultural Organization does not present

data of sheep milk production in Brazil. According to Moraes (3), in Brazil, sheep raising for milk production is observed in the States of Rio Grande do Sul, Santa Catarina, Paran , S o Paulo, Minas Gerais, Rio de Janeiro, Distrito Federal and Bahia. National production of sheep milk is around 200,000 L/year. Few studies have been carried out to evaluate sheep milk composition in Brazil (4, 5).

There are differences in physical-chemical characteristics between cow and sheep milk. Sheep milk has higher specific gravity, viscosity, refractive index, titratable acidity, and lower freezing point (6), and contains higher total solids and greater nutrient contents than cow milk. The fat globule size is smaller in sheep milk (65% globules less than 3 μ m). This is advantageous for digesti-

bility and a more efficient lipid metabolism compared with cow milk fat (7).

Generally, sheep milk production is concentrated on cheese manufacture, usually conducted at farm level or in small local dairies. Composition characteristics favor sheep milk for cheese production. Over the years, Brazilian legislation had stipulated technical regulations about the identity and quality of milk and dairy products, however, up to date no specific legislation has been established for the identity and quality of sheep milk in Brazil. Recently, Normative Instruction 62 stipulated updated values of SCC and microbiological counts for milk, as well as physical-chemical parameters, applicable only to cow milk (8).

To enlarge data about sheep milk commercialized in Brazil, the objective of the present study was to describe the chemical and microbiological characteristics of sheep milk produced on two rural properties located in the States of Paraná and Rio Grande do Sul, in South Brazil.

MATERIAL AND METHODS

Experimental animals

Between April 2012 and March 2013, 22 samples from Lacaune sheep milk were obtained from two rural properties located in South Brazil, from Paraná (PR) and Rio Grande do Sul (RS) States. Monthly samples were collected.

The property located in PR (latitude 24°51'21"S, longitude 53°27'18"W and 780 m altitude) produces cheese and is inspected by the Municipal Inspection System (Serviço de Inspeção Municipal – SIM). The property located in RS (latitude 29°10'15"S, longitude 51°31'0de 8"W and 690 m altitude) produces cheese and yogurt and is inspected by the Federal Inspection System (Sistema de Inspeção Federal – SIF).

Physical chemical analysis

For the milk proximate composition, milk total solids (MTS) were determined by the direct forced air oven drying method (9). Protein analysis, total nitrogen (TN), non-casein nitrogen (NCN) and non-protein nitrogen (NPN) were determined by the Kjeldahl method (9). Total fat content was determined by Roese-Gottlieb methods and ash by muffle furnace (9). The milk lactose content was determined by difference. All measurements were performed in duplicate.

Density was determined by a Quevenne lactometer,

pH by electronic pH meter (TEC-3MP, Tecnal), acidity (as acid lactic %) by the Dornic method, and freezing point by electronic cryoscope (PZL-7000, PZL Indústria Eletrônica Ltda) (9).

Microbiological analysis

Milk samples were analyzed quantitatively for mesophilic bacteria, psychrotrophic bacteria, *Staphylococcus aureus*, enterobacteria, total coliforms, and *Escherichia coli*.

The mesophilic (35 °C/48 h) and psychrotrophic (21 °C/25h) bacterial counts were performed according to APHA (10). The 3M Petrifilm™ plate techniques were used to count the microbial populations as recommended by the manufacturer. STX Petrifilm™ plates were used to count *S. aureus* (36°C/24h), EB Petrifilm™ to enterobacterias (35 °C/24h), and EC Petrifilm™ to total coliforms (35 °C/24h) and *E. coli* (35 °C/48h).

Somatic Cell Count

Milk samples were collected in flasks containing bronopol (2-bromo-2-nitropane-1,3-diol). SCC was performed by flow cytometry (Somacount 300, Bentley Instruments).

Statistical analysis

The physical-chemical and microbiological data from the PR and RS properties were analyzed by the Mann-Whitney non parametric test. Differences were considered to be significant at $p < 0.05$. All data were analyzed using Statistics for Windows 7.0.

RESULTS

Table 1 presents the chemical composition and some physical characteristics of sheep milk produced in two rural properties located in southern Brazil obtained during one whole season. Milk samples from two different dairy sheep properties showed similar chemical composition and physical properties. Differences were only observed in mean total solids (PR= 16.47, RS= 18.71 g/100 g), whey protein (PR= 1.26, RS= 0.90 g/100 g), acidity (PR= 0.22. RS= 0.26 g lactic acid/100 g), and freezing point (PR= -0.571 °H, RS= -0.583 °H) ($p < 0.05$).

Freezing point in samples from PR (-0.572°H) and RS (-0.583°H) were statistically different ($p < 0.05$). The milk SCC forms the basis of abnormal milk control programs worldwide for goats, cows and sheep

(11). Mean SCC in samples from PR were not different from those from RS (Table 2).

The average mesophilic count in samples from PR

was not different from RS (Table 2). Total average mesophilic count was 16.0×10^6 . There was no difference on psychrotrophic count (Table 2) between the farms.

Table 1. Chemical composition and some physical characteristics of sheep milk from rural properties located at Paraná (PR) and Rio Grande do Sul (RS) States from April 2012 to March 2013.

Parameter (g/100 g)	Paraná	Rio Grande do Sul	Total
Total solids	$16.47b \pm 1.92$ 13.01 – 19.21	$18.17a \pm 1.28$ 16.56 – 19.81	17.32 ± 1.82
Total protein	5.71 ± 0.90 3.37 – 6.58	5.96 ± 0.67 4.73 – 6.92	5.83 ± 0.79
Casein	4.14 ± 0.88 2.30 – 5.07	4.78 ± 0.64 3.85 – 5.59	4.46 ± 0.82
Whey protein	$1.26a \pm 0.29$ 0.81 – 1.87	$0.90b \pm 0.13$ 0.67 – 1.06	1.08 ± 0.28
Fat	6.84 ± 1.11 5.00 – 8.50	7.73 ± 0.93 6.50 – 9.00	7.28 ± 1.10
Ash	0.94 ± 0.05 0.84 – 1.02	0.92 ± 0.04 0.84 – 0.97	0.93 ± 0.04
Lactose	3.24 ± 1.52 1.19 – 6.70	3.56 ± 0.54 2.93 – 4.86	3.41 ± 1.10
Density (mg/mL)	1.033 ± 0.003 1.027 – 1.037	1.035 ± 0.001 1.033 – 1.038	1.034 ± 0.004
pH	6.82 ± 0.38 6.32 – 7.42	6.69 ± 0.44 6.14 – 7.94	6.76 ± 0.41
Acidity (g lactic acid /100 g)	$0.22b \pm 0.05$ 0.11 – 0.27	$0.26a \pm 0.03$ 0.22 – 0.33	0.24 ± 0.05
Freezing point (°H)	$-0.571b \pm 0.087$ -0.556 – -0.583	$-0.583a \pm 0.125$ -0.572 – -0.619	0.577 ± 0.012

Results are presented as Mean \pm Standard Deviation. Range.

Values within the same row that have different superscript letter are significantly different by the Mann-Whitney Test ($p < 0.05$).

Table 2. Somatic Cell Count (SCC) and bacterial counts in sheep milk from rural properties located at Paraná (PR) and Rio Grande do Sul (RS) States from April 2012 to March 2013.

Parameter	Paraná	Rio Grande do Sul	Total
SCC ($\times 10^6$) (Cell/mL)	1.7 ± 1.33	1.6 ± 0.53	1.7 ± 1.01
Mesophilic ($\times 10^6$) (CFU/mL)	29.4 ± 59.30	1.3 ± 1.28	16.0 ± 44.32
Psychrotrophic ($\times 10^6$) (CFU/mL)	11.0 ± 34.03	0.2 ± 0.20	5.8 ± 24.69
<i>S. aureus</i> ($\times 10^5$) (CFU/mL)	$4.0a \pm 1.27$	$0.4b \pm 0.36$	2.2 ± 8.92
Enterobacteria ($\times 10^5$) (CFU/mL)	3.1 ± 5.83	2.2 ± 5.41	2.6 ± 5.50
Total coliforms ($\times 10^5$) (CFU/mL)	4.3 ± 7.22	2.0 ± 4.51	3.1 ± 5.92
<i>E. coli</i> (CFU/mL)	1.8 ± 5.39	0.8 ± 2.13	1.3 ± 4.03

Results are presented as Mean \pm Standard Deviation.

Values within the same row that have different superscript letter are significantly different by the Mann-Whitney Test ($p < 0.05$).

Regarding the indicator microorganisms evaluated, the presence of *S. aureus*, enterobacterias and coliforms was observed in 100 % of sheep milk analysed (Table 2). There was a difference in the *S. aureus* count in milk from PR (4.0×10^5 CFU/mL) and RS (3.8×10^4 CFU/mL) ($p < 0.05$). No difference was observed ($p > 0.05$) in the mean enterobacteria, total coliforms and *E. coli* counts.

DISCUSSION

In the present study, similar fat composition of Epirus mountain sheep milk from Egypt (7.85 g/100 g) was observed by Simos et al. (12). In contrast, they observed a higher mean protein content of 6.56 g/100 g and lactose content of 4.77 g/100 g. In Brazil, Brito et al. (4) and Ticiani et al. (5) described lower levels of fat (6.86 and 5.79 g/100 g) and total protein (4.93 and 4.46 g/100 g), and higher levels of lac-

tose (4.65 and 4.76 g/100 g, respectively) in milk from Lacaune ewes from Santa Catarina and 7/8 Lacaune ewes from Rio Grande do Sul, respectively. According to Morand-Fehr et al. (13), the macro- and micro-nutrient composition of sheep milk depends on the main production factors constituting the farming system: genotype and sanitary characteristics of animals, agro-climatic conditions, social and economic environment and farming methods such as feeding and milking. These factors contributed directly or indirectly on synthesis of milk constituents by secretory cells of mammal gland.

Fat is the component that more easily can present variation in milk composition. Diet is one of the factors that can promote this change (14), and presented variation over the study due to food availability on the properties. Pasture feeding, with high relation fiber/energy increase fat in milk, while fat is reduced with inclusion of concentrate in the diet (15). According to Azzara and Dimmick (16), the increase on SCC, observed in the present study, causes decrease on fat synthesis due to damage on secretory epithelium and lipolytic or proteolytic activity of leucocitary enzymes.

The difference observed in lactose levels among the different publications and the present study can be explained by the sanitary characteristic. It is well known that lactose concentration is reduced during clinical and subclinical mastitis in sheep (17). In February and March, cases of mastitis were detected in the herd in PR. In the same months, lower levels of lactose were also observed (1.41 and 1.19 g/100 g, respectively). Lactose also decreases during the lactation period (13).

The density found in the present study (Table 1) was lower than that observed by Brito et al. (4) in Brazil (1.036 mg/mL), and by Simos et al. (12) in Greece (1.037 mg/mL). The average density of sheep milk is 1.036 mg/mL, it increases until the middle, and decreases until the end of the lactation period, reaching a density of 1.034 mg/mL (14). The pH of Lacaune sheep milk usually ranges from 6.60 to 6.68 (14). Sixty percent of the samples were within this range. Sheep milk acidity was slightly higher in the present study compared to the literature. The increase in titratable acidity indicates accumulation of lactic acid from lactose fermentation by lactic acid bacteria. However, in this study, all samples presented stability in the boil proof (data not shown), evidencing the good stability of ca-

seins (18). A series of different milk components are responsible for the acidity level of the product, including acids, salts and proteins (mainly the caseins) (18). For that reason, changes in the concentrations of these components in the milk also affect the titratable acidity level, however, without affecting the alcohol stability of the milk protein.

Freezing point found in the present study was similar to those found by Assenat (14) in France (-0.570 to -0.575°C), and lower than those found by Mayer and Fiechter (19) in Austria (-0.544°C). Some samples showed high freezing point probably because water was added, which can be verified by the lower density values and a reduction in the levels of milk solids (20).

Mean SCC levels (1.6×10^6 cell/mL) were higher than those found by Leitner et al. (17) in uninfected sheep (4.2×10^5 cell/mL) and lower than those found in infected sheep (1.7×10^6 cell/mL). According to Raynal-Ljutovac et al. (21), breed, parity, lactation stage, type of birth, diurnal, monthly, and seasonal variations contribute significantly to changes of SCC in milk of dairy sheep.

Until this moment, Brazil and other countries have not presented legislation about SCC for sheep milk. Brazilian legislation for bovine milk indicates a maximum of 4.8×10^5 cell/mL (8), and it is from this parameter that supervision is based to evaluate mastitis on sheep herd. Thus, taking this value into account, three samples presented levels within the bovine milk legislation. However, small ruminants, such as sheep and goats, present SCC base levels higher than those observed in healthy cows. In cows, merocrine milk secretion is observed, while milk secretion in goats and sheep is largely apocrine in nature, and cytoplasmic particles are normal constituents of their milk (22).

Total average mesophilic count was above the maximum permitted level for raw cow milk (1×10^4 CFU/mL) (8). Milk microbial contamination is usually from external sources, as a result of the environment and milking conditions (14). According the European Council Directive 92/46/EEC, the mesophilic count limit for raw sheep milk is 1×10^6 CFU/mL (23), value that was not complied by both properties.

A higher proportion of psychrotrophic microorganisms was expected in sheep milk from RS, due to the storage time (48 h) under refrigeration, which would result in selection of these microorganisms (24). Nonetheless, this effect was not observed.

The counts of indicator microorganisms, at the levels observed, can implicate consumer's health. Furthermore, these microorganisms cause undesirable changes in cheeses produced with this milk, such as early gas formation (24).

Mechanical milking, good manufacturing practices (GMP) and hygiene are very important practices that contribute to microorganism reduction (25, 26). Both farms evaluated use mechanical milking. However, GMP has not been properly conducted, and new technical orientations and staff training must be offered.

CONCLUSION

The sheep milk physical-chemical and microbiology parameters are similar to those presented in the literature, except for the values of lactose, density and acidity. The somatic cell count presented high levels, and suggests the presence of infected animals in the herd. It is required that Brazilian government establishes a specific legislation to the ovine milk sector, that can give well defined parameters and a better quality of sheep milk can be achieved.

ACKNOWLEDGMENTS

To Coordination for the Improvement of Higher Education Personnel – CAPES, for the fellowship of Post-Doctoral National Program granted to Joice Si-fuentes dos Santos.

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Recibido: 11-02-2015

Aceptado: 18-05-2015