# Studies of the Microbiological and Physico-Chemical Composition of Goat´s Milk from North-Western Spain

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## Abstract
Twenty-seven samples of raw goat's milk were collected from four farms in the North-West of Spain with the aim of analysing counts of various mesophilic bacteria and of determining the physical and chemical composition during different seasons of the year. Overall mean values for mesophilic aerobic micro-organisms (Standard Plate Counts, SPCs) were above 5.6 log cfu/ml, very similar to the counts on M17 agar (presumptive *Lactococcus*, 5.6 log cfu/ml) and higher than the counts on MSE agar (presumptive *Leuconostoc*, 4.6 log cfu/ml) and on MRS agar (presumptive *Lactobacillus*, 4.4 log cfu/ml). Counts were generally higher in winter than in spring and summer, although no statistically significant differences were found. The microbiological quality of raw goat's milk produced in the farms investigated in the North-West of Spain is only partially in accordance with European Union standards. Hence, hygiene practices should be improved so as to decrease the standard plate counts on some of the farms checked. The physical and chemical composition tests also showed higher values for fat, protein and dry extract during the winter season. The average fat content (5.5 g/100 ml) and dry extract (14.7 g/100 ml) were higher than the values noted in other studies, making this of interest for cheese making.

*Keywords: goat's milk, microbiological counts, lactic acid bacteria, physical and chemical composition*

## Introduction
Spain is one of the world leaders in goat's milk production (1) and the second biggest producer of goat's milk and cheese in the entire European Union. It accounts for 25% of the E.U.'s cheese production (2), and in the last five years there has been an upward tendency in Spain's goat population (3). Castile and Leon is an Autonomous Region located in the North-West of Spain. It is one of the largest single regions in the whole European Union, with an area of nearly 100,000 square kilometres and a population of goats reaching 153,590 head, the sixth highest in Spain out of nineteen regions.

On the world stage, goat's milk is often consumed as a liquid without being transformed into some other dairy product. This is why its composition and chemical characteristics are of considerable importance both nutritionally and medically, in the prevention of food allergies (4). However, it is most often used for cheese making (5). It is white in colour and has a characteristic pleasant taste and smell, differing from bovine or human milk in having more digestible protein and fat, greater alkalinity and buffering capacity, having particular uses in medicine and human nutrition (4, 6). Goat's milk contains substantially more protein (3.5%) and ash (0.8%), but less lactose (4.1%), than human milk, its average fat content being 3.8% (6).

It is an important option as a substitute for cow's milk that may be consumed by the elderly and children, thanks to its high digestibility, and by those allergic to bovine milk (7). As occurs with the milk of other species, its composition is directly influenced by a range of factors, such as breed, age, stage of lactation, amount of milk produced, state of general and udder health, physiology, geographical location, genetic factors, and diet (8).

In respect of productive breeds, the Alpine and the Murciano-Granadina, the latter being a native Spanish breed, are widely distributed in Spain. The first breed accounts for 73% of the total number of goats in the Castile and Leon Region (3). Milk production by these breeds is similar (600 to 700 litres per lactation), with a fat content varying from 3.7% for the Alpine to 5.6% for the Murciano-Granadina, according to data from the Spanish Ministry of Agriculture and Food (3).

Studies of the microbiology of goat's milk in Spain are few in number (9–12) and some of these are more related to the behaviour of various different micro-organisms during the ripening of cheese (13, 14). However, goat's milk is an ideal medium for multiple micro-organisms. Lactic acid bacteria (LAB) make up a significant part of the microbiota, gaining access to milk from a variety of sources (5). They are considered the chief organisms responsible for the transformation of milk into cheese and other fermented products, and also for the inhibition and inactivation of other micro-organisms, including pathogens.

The aim of the work presented here was to analyse counts of the mesophilic microbiota (mainly lactic acid bacteria: LAB), together with the physical and chemical composition of goat's milk produced in the North-West of Spain at different seasons of the year, thus contributing to the limited information hitherto published about this type of milk, which is of increasing interest to the dairy industry and specially to cheese producers.

**Table 1: Raw goat's milk samples collected**

| Farm (herd size) | Samples (n = 27) | Period | Breed |
|---|---|---|---|
| **A** (> 800) | 6 | 2, winter; 2, spring; 2, summer | Murciano-Granadina |
| **B** (> 500) | 6 | 2, winter; 2, spring; 2, summer | Murciano-Granadina |
| **C** (< 500) | 4 | 2, winter; 2, spring | Alpine |
| **D** (> 800) | 11 | 3, winter; 3, spring; 5, summer. | Alpine |

## Materials and Methods

**Sample Collection:**

Twenty-seven samples of raw goat's milk were taken from bulk tanks refrigerated to below 8°C on 4 farms (A to D) in the Province of Leon in the Castile and Leon Region of Spain between February and October 2015 (Table 1). On all the farms, production was intensive, with animals kept indoors, mechanically milked, and fed with a mixture of forage, mainly alfalfa, corn, and oats, and also with fodder grains (on farm D, silage was used as well). All the farms were subject to the European, Spanish, and regional sanitary legislation, with no recent reports of clinical or sub-clinical mastitis in the individual flocks. The samples were transported to the laboratory in an insulated box at refrigeration temperatures for their later analysis within twenty-four hours.

**Microbiological Analysis:**

Standard plate counts (SPCs) of mesophilic aerobic organisms were determined on plate count agar (PCA, Oxoid, Basingstoke, U.K.) at 30^C for 72 hours in accordance with ISO 4833-2 (15). LAB were counted using the following media: De Man, Rogosa and Sharpe (MRS) Agar (Oxoid) pH 5.2 incubated in anaerobic jars at 37°C for 72 hours for presumptive *Lactobacillus* (16, 17), on Mayeux, Sandine and Elliker (MSE) agar (Oxoid) at 30°C for 4 days for presumptive *Leuconostoc* (18), and on M17 Agar (Pronadisa, Madrid, Spain) at 30°C for 24 hours for presumptive *Lactococcus* (19).

**Compositional Analysis:**

Analysis of total solids, fat, protein, lactose content, and somatic cell counts (SCCs) was undertaken at the Regional Animal Health Laboratory of Castile and Leon at Villaquilambre in the Province of Leon, Spain, using a MilkoScan FT 6000 device (Foss Iberia, Barcelona, Spain). The pH was measured directly in the samples with a Testo 205 pH-meter (Testo Instruments, Lenzkirch, Germany).

**Statistical Analysis:**

Statistical analysis was performed using the SPSS program for Windows (I.B.M., New York, U.S.A.). The one-way ANOVA test was used to analyse the varying counts between different seasons of the year and breeds to test the data for normality and variance homogeneity. When the differences were significant, the individual means were compared with Tukey's test. Significant difference was set at a level of $p < 0.05$.

## Results and Discussion

Table 2 and Figure 1 give the outcomes of the microbiological counts. A comparison of the results obtained by other authors is presented in Table 3, showing large differences between studies (a range of counts ≥ 3 log cfu/ml for all the culture media).

The average SPC, at 5.66 log cfu/ml, was below the limits established by European criteria for raw milk of species other than cows, which are 6.18 log cfu/ml for milk that is intended for heat treatment and 5.69 log cfu/ml for milk to be used in the manufacture of raw milk products (20). These European limits are higher than those in the U.S.A., at 5.00 log cfu/ml for grade "A" goat's milk (21), or in Canada, at 4.7 log cfu/ml (22). They must be seen as extremely high, indicative of very deficient hygiene practices, and leading to a drastic reduction in the quality and shelf life of milk (23).

Analysis of the results over different seasons showed 6.43 ± 1.55 log cfu/ml in winter, 5.69 ± 1.30 log cfu/ml in spring and 4.85 ± 0.54 log

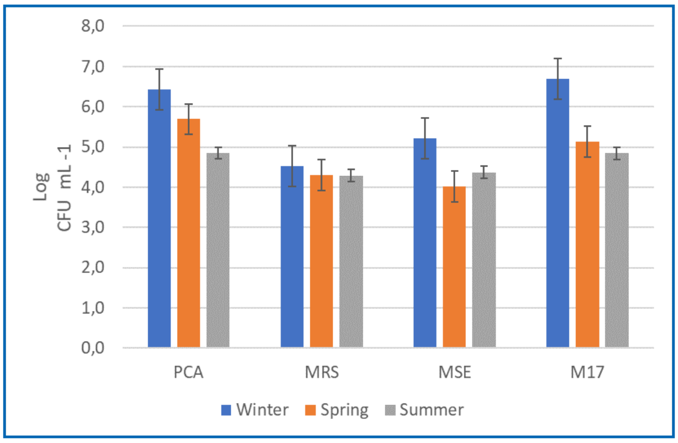


**Figure 1: Microbiological counts on different media (mean ± standard deviation, log cfu/ml) for twenty-seven samples of goat's milk from three seasons of the year (nine samples per season). No significant differences were found among samples taken during different seasons ($p < 0.05$). PCA, SPC; MRS, presumptive *Lactobacilli* spp.; MSE, presumptive *Leuconostoc* spp.; M17, presumptive *Lactococcus* spp.**

**Table 2: Microbiological counts (mean ± standard deviation, log cfu/ml) and somatic cell counts (SCC × 103 cells /ml) from the farms investigated**

| Farm | Breed | SPC | MRS | MSE | M17 | SCC × 103 |
|---|---|---|---|---|---|---|
| A (n = 6) | MG | 4.48 ± 0.44 | 3.79 ± 0.49 | 3.80 ± 0.58 | 5.41 ± 1.93 | 1487.50 ± 226.73 |
| B (n = 6) | MG | 4.73 ± 0.84 | 3.51 ± 0.29 | 3.31 ± 0.13 | 4.22 ± 0.38 | 1568.17 ± 201.38 |
| C (n = 4) | ALP | 5.79 ± 0.67 | 4.09 ± 0.28 | 3.89 ± 0.07 | 4.50 ± 1.59 | 2476.25 ± 120.43 |
| D (n= 11) | ALP | 6.75 ± 1.40 | 5.27 ± 0.48 | 5.91 ± 1.23 | 6.77 ± 1.49 | 1676.82 ± 199.75 |
| **Mean (n = 27)** | | **5.66 ± 1.43** | **4.38 ± 0.87** | **4.56 ± 1.41** | **5.57 ± 1.82** | **1730.61 ± 369.35** |

MG, Murciano-Granadina Breed; ALP, Alpine Breed; SPC, Standard Plate Count; MRS, presumptive *Lactobacilli* spp.; MSE, presumptive *Leuconostoc* spp.; M17, presumptive *Lactococcus* spp. n, number of samples.
No significant differences among breeds were found between samples taken in different seasons ($p < 0.05$).


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cfu/m in summer (Figure 1). On these figures, only winter samples would be above those limits. Nevertheless, no significant differences were found from one seasons to another, although the high standard deviations of certain average values may in some way have affected results (Figure 1). In France (24), no significant differences were found between three different sampling periods, the range being 5.40 to 5.90 log cfu/ml. However, in Canada a wider range was found, running from 3.79 to 5.81 log cfu/ml (25). For farms A and B, the SPCs, at 4.48 and 4.73 log cfu/ml, respectively, would be below the legal limits set in the European Union, which are 6.18 log cfu/ml for milk that is intended for heat treatment and 5.69 log cfu/ml for milk destined for the manufacture of raw milk products. In contrast, on farm C, the count of 5.79 cfu/ml would be acceptable for heat-treated products, but too high for milk intended for the manufacture of raw milk products. Finally, for farm D, the SPC would be above both these limits (20), as can be seen from Table 2. Farm D also yielded the highest counts on MSE and MRS agars, while the highest figures on M17 were found in the case of farm A (Table 2). On all the farms, the conditions were similar: intensive indoor production, mechanical milking, refrigerated tanks, and milk kept under refrigeration until analysis (carried out within 24 hours). Except on farm D, no cleaning and disinfection of udders was normally carried out, as farmers stated that they did not find it necessary.

Other studies performed on raw goat's milk in Spain found a wide range of counts, running from 4.75 to 7.66 log cfu/ml (10, 11, 12, 13, 14, 32), as may be seen from Table 3. In France (21), reported results are very similar to those noted here. Lower counts were found in Italy, the Netherlands (28), Switzerland (39), Canada (25) and the U.S.A. (21), as indicated by Table 3. As previously mentioned, variation in SPCs from season to season can be striking, which makes comparisons between countries difficult. Nevertheless, various hygiene measures could be implemented on several of the farms investigated in order to reduce SPCs. Delgado-Pertiñez et al. (26) found that the establishment

**Table 3: Comparison of microbiological counts (mean, log cfu/ml) in goat's milk in this study and other reports**

| Country | SPC | MRS | MSE | M17 | References |
|---|---|---|---|---|---|
| Spain | 5.66 | 4.38 | 4.56 | 5.57 | This study |
| Spain | 6.72 | 4.84 | 5.37 | 6.72 | 14 |
| Spain | 5.54 | 3.59 | 5.10 | 5.39 | 10 |
| Spain | 7.66 | 6.13 | 6.91 | 7.57 | 13 |
| Spain | Nd | 4.11 | 3.78 | 5.39 | 11 |
| Spain | 4.80 | Nd | Nd | Nd | 12 |
| Spain | 4.75 | 2.72 | Nd | Nd | 32 |
| Italy | 4.70 | 3.48 | Nd | 3.53 | 33 |
| France | 5.50 | 4.50 | 4.70 | 5.40 | 21 |
| The Netherlands | 4.30 to 4.40 | Nd | Nd | Nd | 28 |
| Switzerland | 4.70 | Nd | Nd | Nd | 39 |
| Canada | < 5 | Nd | Nd | Nd | 25 |
| U.S.A. | < 5 | Nd | Nd | Nd | 21 |

SPC, Standard Plate Count; MRS, presumptive *Lactobacillus* spp.; MSE, presumtve *Leuconostoc* spp.; M17, presumptive *Lactococcus* spp. Nd, not determined.

of appropriate hygiene conditions and sanitary management on farms improved bacteriological quality and SCCs. These measures included applying teat-seals after milking and an increased level of cleanliness of the milking area, milking equipment, animals, and the farm in general. The SCC average, at 1,730.61 × $10^3$ cells per ml, and counts in each of the three seasons, as shown in Table 2, were within the threshold values indicated for goat's milk in the absence of mastitis, according to Paape et al. (27), who quote a figure of between 270 × $10^3$ and 2,000 × $10^3$ cells per ml, higher than in bovine or sheep milk. On farm C, counts close to 2,500 × $10^3$ cells per ml were found. These data point to good sanitary conditions of the herds on three out of the four farms investigated. There were no significant differences among the seasons, although the highest levels were found in winter (1,792 × $10^3$ cells per ml). By farms, no correlation was found between SPC and SCC counts, although farm C had the lowest SPC numbers and the highest SCC counts, as seen in Table 2.

These results concur with the situation in the Castile and Leon Region as a whole, where most samples (64.7%) had more than 1,700 × $10^3$ cells per ml (23). However, the levels found in other studies carried out in countries such as Canada, where 8% of the samples had fewer than 750 × $10^3$ cells per ml (25), or the Netherlands, where the mean was around 1,000 × $10^3$ cells per ml (28), were much lower. In the European Union as a whole (20), there are no criteria for SCC in goat's milk, although in cow's milk the limit is fewer than 400 × $10^3$ cells/ml. In the USA, criteria are set by the Grade "A" Pasteurized Milk Ordinance, and SCCs in raw goat's milk are not permitted to exceed 1,500 × $10^3$ cells per ml (29).

Of the LAB found, samples were dominated by presumptive *Lactococcus*, with counts on M17 agar of 5.57 versus 5.66 log cfu/ml on PCA (Table 2). Similar or higher counts have been noted elsewhere in Spain (Table 3). In France, Delavenne et al. (24) also found this group dominant, with similar counts, whilst studies in Italy yielded lower counts (Table 3). The genus *Lactococcus* appears to be a major element in the natural microbiota of goat's milk and can play a role of some relevance in the production of craft or artisanal raw milk cheeses (30).

Presumptive *Leuconostoc*, counted on MSE agar, showed an average of 4.56 log cfu/ml, these being the second most frequent group of lactic acid bacteria found, as was also the case in studies in France (Table 3). High counts of *Leuconostoc* are regularly found in goat's milk (Table 3), and this seems to be related to the mode of nutrition (31).

Finally, the counts for presumptive *Lactobacillus* on MRS agar, at 4.38 log cfu/ml, were lower than the other LAB groups investigated, and very similar to, or lower than, those found elsewhere in Spain (Table 3). In Italy, counts were lower, whilst in France they were similar to those reported here (Table 3).

No significant differences were found between samples taken at different seasons on any of the culture media used, even though counts were regularly higher in winter than in the spring and summer seasons (Figure 1). This was also the case when the results for the different breeds were compared (Table 2). LAB as a whole constitute an essential part of the natural microbiota of goat's milk, in view of the fact that the ratio between lactic acid bacteria and mesophilic counts found in this study was almost 1. There is a probable overlap between the counts for the different LAB groups, since the media used are not particularly selective in differentiating between them (32–34). A high standard deviation was found in the means by farms, particularly in respect of the M17, PCA and MSE counts (Table 2). This may principally be due to the differences in levels found over the three seasons investigated.

The chemical composition of the goat's milk analysed in this study is

**Table 4: Chemical composition (g/100 ml) and pH of the goat's milk samples investigated (mean ± standard deviation)**

| Farm | Breed | Fat | Protein | Lactose | Dry Extract | pH |
|---|---|---|---|---|---|---|
| **A** (n = 6) | MG | 5.98 ± 0.47 | 4.01 ± 0.45 | 4.66 ± 0.05 | 15.41 ± 1.01 | 6.76 ± 0.09 |
| **B** (n = 6) | MG | 5.35 ± 0.45 | 3.65 ± 0.15 | 4.72 ± 0.04 | 14.39 ± 0.67 | 6.89 ± 0.10 |
| **C** (n = 4) | ALP | * | 3.74 ± 0.42 | 4.50 ± 0.02 | * | 6.79 ± 0.04 |
| **D** (n = 11) | ALP | 5.25 ± 0.26 | 3.66 ± 0.09 | 4.72 ± 0.04 | 14.32 ± 0.33 | 6.61 ± 0.15 |
| **Mean** (n = 27) | | **5.52 ± 0.54** | **3.78 ± 0.36** | **4.67 ± 0.09** | **14.71 ± 0.91** | **6.73 ± 0.16** |

MG, Murciano-Granadina; ALP, Alpine; n, number of samples.
No significant differences among breeds were found between samples taken in different seasons ($p < 0.05$). *No sampling (farm closed).

shown in Table 4 and Figure 2. A comparison of the results obtained by different authors is presented in Table 5. The differences in the fat and protein contents between studies are similar (around 2 g/ml), but higher in the case of dry extract (2.84 g/100 ml). The range of values for lactose was smaller (0.75) and the pH varied from 6.51 to 6.73. There were significant differences between samples taken in different seasons for all chemical components, except lactose, which remained stable (Table 4). The averages for fat, protein and dry extract were higher in the winter (Figure 2). Changes in the composition of goat's milk occur over the seasons, because towards the end of lactation, the fat, protein, solids and mineral contents increase, while the lactose content decreases (4).

The average fat content noted, 5.52% (Table 4) was much higher than the average concentration of 3.80% reported for this species (6) in other studies carried out elsewhere in Spain and in France, Greece and Portugal, as may be seen from Table 5 (1). This content was similar in the two breeds investigated (Table 4). According to data from the Spanish Ministry of Agriculture, the average fat content of 3.7% in Alpine goat's milk is lower than the figure of 5.25% found here, as can be seen in Table 4 (3). This may be an indication of a need to review the official data. Fat content is influenced by many factors, including the time of the year, stage of lactation, breed and type of feeding. In the present study, feeding was similar on all the farms during the three seasons of the year considered.

Protein was slightly higher in the winter, at 4.05 g/100 ml (Figure 2), a result differing from those observed by other authors (4), who found no significant differences among the seasons. The average of the three periods (3.75 g/100 ml) was similar to the data observed elsewhere in Spain (35) and higher than the levels found in France and Portugal (36). In Greece, the data were very variable (36, 37), as is shown by Table 5.

The protein content, like the fat content, is influenced by breed, season, genetics and diet (36, 38).

Lactose, at 4.67% (see Table 4) exceeded the desired minimum of 4.10% (19) and the average for goat's milk (6). It was higher than the figures recorded by other authors for other European countries (Table 5). Contents were similar in all three seasons in the present study. Lactose is one of the most stable nutrients in the chemical composition of milk, and is directly related to the regulation of osmotic pressure; higher amounts of lactose point to higher milk production.

With regard to the results relating to the various chemical components, dry extract at 14.71% (see Table 4) was well above the desired minimum required for cheese making of 12.97 g/100 ml and was higher than reported elsewhere, as may be seen from Table 5. Finally, the average pH value of 6.73 found fell within the normal range of values, 6.50 to 6.80, for goat's milk, this indicating the milk's freshness. There were statistically significant differences from season to season, with the lowest value of 6.63 being found in the winter (Figure 2). This may be linked to a bacterial group characterized by its acidifying capacity, and related to the highest values of presumptive *Lactococci* (counts on M17; Figure 1) during this season. The time between sampling and processing of the samples did not vary by season. No deterioration in udder health was observed. The time between milking and analysis was under 24 hours for all the farms and refrigeration temperatures were always correctly maintained. No significant differences in chemical composition were found between the breeds (Table 4).

The results obtained in this study allow the conclusion that the microbiological quality of raw goat's milk samples taken from large farms in the North-West of Spain is only partially in accordance with European Union standards. This suggests that it could be advisable to improve the hygienic conditions on some of the farms investigated. In addition,

**Table 5: Comparison of the physical and chemical composition of goat's milk noted in this study and in published reports**

| Country | Fat (g/100 ml) | Protein (g/100 ml) | Lactose (g/100 ml) | Dry extract | pH | References |
|---|---|---|---|---|---|---|
| **Spain** | 5.47 | 3.75 | 4.67 | 14.64 | 6.73 | This study |
| **Spain** | 3.79 | 3.83 | ----- | 13.21 | 6.72 | (34) |
| **Greece** | ----- | ----- | ----- | ----- | 6.72 | (32) |
| **Spain** | 5.26 | ----- | ----- | 14.08 | 6.63 | (11) |
| **Portugal** | 4.27 | 3.49 | 4.21 | 12.80 | 6.59 | (30) |
| **France** | 3.44 | 3.44 | 4.56 | 11.80 | 6.51 | (30) |
| **Greece** | 4.80 | 5.41 | 3.92 | 14.40 | 6.63 | (30) |
| **Greece** | 4.12 | 3.44 | 4.26 | 12.57 | 6.65 | (35) |

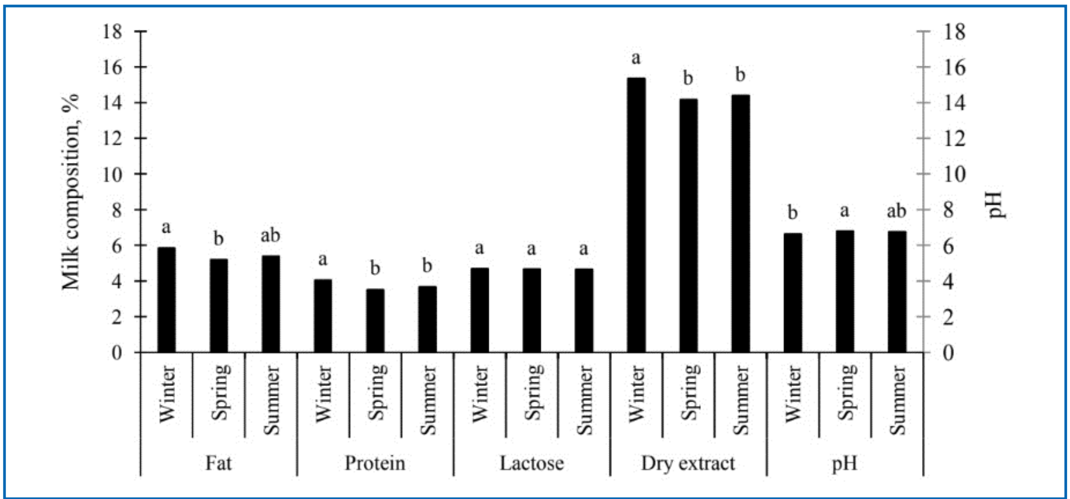


**Figure 2: Chemical composition (g/100 ml) and pH (mean ± standard deviation) of twenty-seven samples of goat's milk from three seasons of the year (nine samples per season).**

**Values for the same parameter with different letters are significantly different ($p < 0.05$)**

the samples examined proved to have the composition and physical and chemical characteristics that are to be desired in this foodstuff, both for consumption in liquid form and for the production of cheese and other dairy products with a noteworthy high content of fat and of dry extract, characteristics that would make this milk of interest to the cheese industry.

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Disclosure of Conflicts of Interest

All persons involved in this study declare no conflict of interests.

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