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# Study of the differential microbiological quality of sheep milk relative to the standard plate counts

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**Abstract.** Sheep milk production is a valuable sector for the European Mediterranean countries, and in the case of Castilla-La Mancha region (Spain), the production of sheep milk is linked with a high quality product. The hygienic quality of sheep milk is related to different groups of microorganisms, although farmers only have information about standard plate counts (SPC). The aim of this study was to evaluate the different bacterial counts (thermodurics, psychrotrophics, coliforms, *Staphylococcus* coagulase positive (SCP) and coagulase negative (SCN)) in relation with (SPC). A total of 283 bulk milk samples belonging to 26 herds distributed in Castilla-La Mancha region were collected during 2011. The mean values (log cfu/ml) of SPC, thermodurics, psychrotrophics, coliforms, *Staphylococcus* coagulase positive and coagulase negative were 5.49, 3.29, 4.89, 2.92, 2.68 and 4.10 respectively. In general, samples are distributed around low and intermediate intervals of microorganisms counts. It has been also confirmed a tight relationship between SPC and other microorganisms groups when counts are low. This relationship is more diffuse when high counts are considered. This fact could indicate the presence of other factors which only affect certain microorganisms groups. This work evidences the necessity of going through in the study of the global quality of ewes' milk destined to cheese-making.

**Keywords.** Differential microbiological quality – Plate Standard Count – Sheep milk.

***Etude de la qualité microbiologique différentielle du lait de brebis par rapport à la flore aérobie mésophile***

***Résumé.*** *La production du lait de brebis est un important secteur dans les pays méditerranés de l'Europe. Spécifiquement dans le cas de la région de Castilla-La Mancha (Espagne), la production du lait de brebis est liée à l'élaboration de fromage de haute qualité. La qualité hygiénique du lait de brebis est reliée avec différents groupes de microorganismes, bien que les fermiers aient seulement information sur la flore mésophile aérobie totale (SPC). L'objective de cette étude est évaluer la relation entre les dénombrements de différentes groupes de microorganismes (thérmoduriques, psychotrophes, coliformes,* Staphylococcus *coagulasse positive -SCP et coagulasse négative-SCN), et la SPC. On a récupéré 283 échantillons du lait des tanks de 26 fermes de la région de Castilla-La Mancha pendant 2011. La valeur moyenne (log ufc/ml) de SPC, thérmoduriques, psychotrophes, coliformes, SCP et SCN sont 3,29, 4,89, 2,92, 2,68 and 4,10. En général, les échantillons se sont distribués dans les intervalles basse et intermédiaire dans les dénombrements bactériennes. On a aussi confirmé l'existence d'une étroite relation entre SPC et les autres groupes de microorganismes quand les dénombrements sont bas. Par contre, cette relation est plus diffuse quand les dénombrements sont hauts. Ce résultat pourrait indiquer la présence d'autres facteurs qui affectent seulement certes groupes de microorganismes. Ce travail met en évidence la nécessité d'approfondir dans l'étude de la qualité du lait de brebis destiné à l'élaboration de fromage.*

***Mots-clés.*** *Qualité microbiologique différentialle – Flore mésophile aérobie totale – Lait de brebis.*

## I – Introduction

Sheep milk production in Mediterranean Europe is a valuable sector, from the environmental and economic point of view, and from the maintenance of the rural society. Moreover, these countries are linked to the production of traditional dairy products with difereciated quality assesments.

Sheep milk quality shows a great importance in obtaining dairy products with high quality, and thus the hygienic and sanitary aspects of milk are essential. Nowadays, the quality control systems only consider not-specific-indicators such as the Standard Plate Count (Pirisi *et al*., 2007). Moreover, there are many factors that may have some effect on this indicator (Sevi *et al*., 2000, 2004; Gonzalo *et al.*, 2006), and that could modify the kind of microorganisms of milk in each farm. In cow milk, some studies have been performed about the differential diagnostic of microorganisms in bulk tank milk and about the criteria to a correct explanation of them (Jayarao *et al.*, 2004; Elmoslemany *et al.*, 2009). In these studies some mircroorganisms such as psychrotrophics, resistant to pasteurization – thermodurics, coliforms, *Staphylococcus*, etc. are of special interest. However, in sheep milk, the milk production system, the herd management characteristics and the hygienic-sanitary conditions of farms determine the differential microbiological diagnostic (Muehlherr *et al.*, 2003). Moreover, the studies that analyse these aspects are scarce.

Castilla-La Mancha region, with around 140 x $10^6$ litres (MAGRAMA, 2011), is the second sheep milk productive region in Spain. So that, our research group has started some research works with the final objective of studying the global quality of sheep milk used in cheese-making. The first results about the differential microbiological quality of bulk tank milk are shown in this work.

## II – Material and methods

Between November 2010 and October 2011, a total of 283 bulk-tank milk samples were collected from 26 sheep farms, distributed in the region of Castilla-La Mancha (Spain). The farms were selected by the size, the production system and the milking system, with farm agents that represent the variability of farm management conditions of the region. Farms ranged in size from 200 to 2500 ewes, had a semi-extensive production system and milking parlour.

Milk sampling was performed before homogenization, transported to the Dairy Laboratory of CERSYRA under refrigerated conditions (below 5ºC) and analysed within 24 h for the differential counts of micro-organisms.

### 1. Microbiological analysis

Samples (1 ml) were homogenized in 9 ml of sterile 0.1% peptone-water solution (w/v). Appropriated serial decimal dilutions were made and inoculated on several specific media. A fixed quantity of 0.1 ml of the corresponding dilution was plated for different microbiological analyses by surface plating.

The total bacterial count or standard plate count (SPC), thermodurics, and psychrotrophics were plated in the plate count agar (PCA) (Panreac, Barcelona, Spain). SPC and thermodurics (after milk pasteurization, 62.8ºC for 30 min) were incubated in aerobic conditions at 30ºC for 72 h (ISO 4833:2003). Psychrotrophics were incubated at 6.5º for 10 days (ISO 6730/IDF 101:2005). The determination of coliforms was achieved with CromoIDTM Coli (bioMérieux, Madrid, Spain) and plates were incubated 37ºC for 24 h. Finally, for *Staphylococcus* coagulase positive (SCP) and coagulase negative (SCN), Agar Baird Parker RPF (bioMérieux, Madrid, Spain) was used and plates were incubated at 37ºC for 24 h. Results for all counts were reported as the number of CFU per ml.

### 2. Statistical analysis

Results of microbiological counts were transformed into respective decimal logarithms to fit a normal distribution of values. All data were analyzed using the SAS statistical package.

The mean values of the different microbiological groups have been calculated. Moreover, the analysis of the samples distribution considering some bibliographic thresholds has been perfor-

med. The SPC thresholds have been proposed by the European Regulation 853/2004; psychrotrophics thresholds have been indicated by Gonzalo *et al*. (2013); coliforms thresholds are used in Roquefort region (France) (Pirisi *et al*., 2007); SCP thresholds have been established following the indications made by Marco *et al*. (2012). Thermodurics and SCN, due to the lack of bibliographic references, have been studied taking into account the frequency distribution obtained in this work.

Moreover, a correspondence analysis has been performed in order to determine the association between the different groups of microorganisms.

## III – Results and discussion

The obtained results indicate that the milk from the studied farms has a high microbiological quality. SPC, an indicative of the hygienic quality, shows a mean value lower than 500,000 cfu/ml, which is the maximum threshold established by the Regulation 853/2004 for milk for cheese-making without a thermal treatment. The differential microbiological analysis of milk gives a clear idea about milk quality and the process to obtain it (Table 1). There are different groups of microorganisms that inform about the hygienic conditions of the productive system of farms: thermodurics (those microorganisms that survive the pasteurization conditions), psychrotrophics (those microorganisms that develop at refrigeration conditions), and coliforms. On the other hand, staphylococci indicate the incidence of intramammary infections which can be clinic (SCP) or subclinic (SCN). The obtained results of mesophiles, psychrotrophics and coliforms are similar to those obtained by Sevi *et al*. (2000, 2004) when studied Comisana sheep milk, or the psychrotrophic counts obtained by Nuñez *et al*. (1984).

**Table 1. Means and standard error for microbiological counts (log cfu ml$^{-1}$) in raw ewe's milk**

| Microorganisms | Means ± SE |
|---|---|
| SPC | 5.49 ± 0.062 |
| Thermodurics | 3.29 ± 0.060 |
| Psychrotrophics | 4.89 ± 1.290 |
| Total coliform | 2.92 ± 0.057 |
| *Staphylococcus* coagulase positive | 2.68 ± 0.062 |
| *Staphylococcus* coagulase negative | 4.10 ± 0.042 |

In order to know the situation of flocks studied, it has been performed a distribution study of the groups of microorganisms, taking into account some bibliographic references (Fig. 1). SPC distribution in the studied flocks indicates that almost two thirds of the samples (62.26%) show lower values than 500,000 cfu/ml, which is the maximum threshold fixed by the Regulation 853/2004 to use milk for raw milk cheese-making. From them, 35.53% showed lower counts than 100,000 cfu/ml, threshold that would indicate excellent milk hygienic quality. It is important to stand out the small percentage of samples (9.16%) with counts between 500,000 and 1,500,000, maximum threshold to thermal treated milk for cheese-making. These results show that, in general, milk samples used in this study show an adequate hygienic quality (Fig. 1A). It has been observed that SPC increase as a result of inadequate hygiene practices in the farms and milking parlours (Gonzalo *et al*., 2006).

**Fig. 1. Frequency distribution of bulk SPC (A), thermodurics (B), psychrotrophics (C), coliforms (D), SCP(E) and SCN(F) counts for sheep bulk tank milk.**

When taking into account the thermoduric microorganisms, it is observed that 1000 ufc/ml threshold distributes samples approximately in two halves. In Fig. 1B it is observed that the highest percentage of samples (74%) are between 100 and 1000 ufc/ml. Due to the low frequencies in the highest counts (log-thermodurics >4.5), it could be affirmed that in general milk is obtained in an adequate way, since the increase of these microorganisms has been related to the deficient hygiene of milk parlour (Oliete *et al*., 2011).

The distribution of psychrotrophics, based on the studies of Gonzalo *et al*. (2013), indicates that most of the samples (60.72%) show lower values than 200,000 ufc/ml (Fig. 1C). This result would indicate that in general the refrigeration process does not condition the development of this group of microorganisms in sheep milk, but it is related to days in refrigeration. Moreover, it is also related to the hygienic conditions of farms (Oliete *et al*., 2011). Only 13.89% of samples show higher psychrotrophic counts than 2,500,000 cfu/ml, indicating long storage times, that would affect the cheese-making process due to their high enzymatic content (Nuñez *et al*., 1984).

Coliform count has been considered in the control quality systems for years in some dairy regions (Pirisi *et al*., 2007) due to its relationship with the hygienic conditions of farms (Jayarao *et al*., 2004). Moreover, from the technological point of view, coliforms are correlated to cheese yield (Jiménez *et al*., 2011). In Fig. 1D it is shown the distribution of coliform counts. It is observed that 72.66% of the samples show lower values than 2500 ufc/ml, indicating good hygienic quality in the studied farms. However, there are still an important percentage of milk samples (27.34%) with high coliform counts that would indicate the necessity of persevering in the application of efficient cleaning and disinfecting programs in farms.

The distribution of SCP counts (Fig. 1E) shows that 69.11% of the samples are under 1000 ufc/ml, threshold of the presence of SCP thermostable toxin cited by certain researchers (Marco *et al*., 2012). In our case, the most part of the samples (50.79%) shows counts between 100 and 1000 ufc/ml. This result would indicate the satisfactory quality of milk from the point of view of these microorganisms related to mammary health.

In the same way, it has been studied the group of SCN, the most prevalent microorganisms in subclinical mastitis in dairy sheep herds (Gonzalo *et al*., 2002). Moreover, this group of microorganisms is correlated to curd quality (Jiménez *et al*., 2011). In Fig. 1F, it can be appreciated that most of SCN counts are located between log SCN = 3.5 – 4.5, and around half of the samples show values higher than 10,000ufc/ml.

Apart from knowing the distribution of samples in relation to the different microbiological groups independently, it is necessary to know the relationships between these groups. It has been studied the association between the groups related to the hygienic conditions of farms (SPC, thermodurics, psycrotrophics and coliforms) considering the thresholds previously commented. In Fig. 2, the two first dimensions of the correspondence analysis are plotted.

**Fig. 2. Multiple correspondence analysis of SCP, thermodurics, psychrotrophics and coliforms counts of sheep bulk tank milk.**

Dimension 1, which explains 32.95% of the variation, clearly groups low counts of SPC (≤ 100,000 ufc/ml), thermodurics (≤ 1000), psycrotrophics (≤ 200,000) and coliforms (≤ 500) (in green). This clear association would indicate that in general, low counts of SPC implicate low counts of the other groups. When high counts are obtained –SPC (> 1,500,000), thermodurics (> 1000), psycrotrophics (> 2,500,000), coliforms (> 2500)– the group obtained is much more disperse (in green). This fact would indicate the presence of some variation factors that would only affect certain groups of microorganisms. So the hygienic management conditions would affect with different intensity the groups of microorganisms. In this group it is also included the intermediate psychrotrophic counts

(200,000-2,500,000 cfu/ml) that would indicate the scarce capacity of this first dimension to explain the difference between intermediate and high psychrotrophic counts. However, this difference can be clearly justified by Dimension 2. Furthermore, Dimension 2 (18.86% of the variation) clearly groups intermediate SPC and psychrotrophic counts (in orange), that would indicate higher relationship between these two groups of microorganisms than with other microorganisms.

## IV – Conclusions

In view of the obtained results, it can be concluded that the microbial counts related to the hygienic and sanitary quality of milk, are adequate. In general, most part of the samples show low and intermediate levels of microbial counts. Moreover, it has been verified the tight relationship between SPC and the other groups of microorganisms related to the hygienic conditions of farms, when their counts are low. This relationship is somehow more diffuse when high microbial counts are obtained, that would indicate the presence of other factors that only affect certain groups of microorganisms. This work demonstrates the necessity of going in depth on the effect of hygienic-sanitary management conditions on the different groups of milk microorganisms and on the relationship with the global quality of milk used for cheese-making.

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## References

**Elmoslemany A.M., Keefe G.P., Dohoo I.R. and Jayarao B.M., 2009.** Risk factors for bacteriological quality of bulk tank milk in Prince Edward Island dairy herds. Part 1: Overall risk factors. In: *Journal of Dairy Science,* 92, pp. 2634-2643.

**Gonzalo C., Ariznabarreta A., Carriedo J.A. and San Primitivo F., 2002.** Mammary pathogens and their relationship with somatic cell count and milk yield losses in dairy ewes. In: *Journal of Dairy Science*, 85, pp. 1460-1467.

**Gonzalo C., Carriedo J.A., Benitez E., Juárez M.T., De La Fuente L.F. and San Primitivo F., 2006.** Bulk Tank Total Bacterial Count in Dairy Sheep: Factors of Variation and Relationship with Somatic Cell Count. In: *Journal of Dairy Science,* 89, pp. 549-552.

**Gonzalo C., 2013.** Programas de Calidad, Seguridad y Certificación de la Leche del Consorcio de Promoción del Ovino. 131 pp.

**ISO 4833:2003.** Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of microorganisms – Colony-count technique at 30 degrees C.

**ISO 6730/IDF 101:2005.** Milk-Enumeration of colony-forming units of psychrotrophic micro-organisms.

**Jayarao B.M., Pillai S.R., Sawant A.A., Wolfgang D.R. and Hegde N.V., 2004.** Guidelines for Monitoring Bulk Tank Milk Somatic Cell and Bacterial Counts. In: *Journal of Dairy Science*, 87, pp. 3561-3573.

**Jiménez L., Oliete B., Garzón A., Romero J., Arias C., Calatayud J.R., Pérez-Guzmán M.D. and Arias R., 2011.** Relación de los parámetros tecnológicos de leche de tanque de oveja Manchega con su composición y carga microbiana. Resultados Preliminares. In: *XXXVI Congreso de la Sociedad Española de Ovinotecnia y Caprinotecnia (SEOC)*. San Sebastián. Fecha: 5-7 de octubre de 2011. Libro de Actas, pp. 176-180.

**MAGRAMA, 2011.** Producción anual y destinos de la leche (todas las clases de leche) en las explotaciones agrarias. http://www.magrama.gob.es/es/estadistica/temas/estadisticas-agrarias/ganaderia/estadisticas-industrias-lacteas/estadistica-lactea-anual/#para3. Consulted 28th October 2013.

**Marco J.C., Jacquin C., Esnal A., Jiménez R., Sánchez M. and Gonzalo C., 2012.** Los Programas de Mejora de la Calidad de la Leche: su impacto en la rentabilidad de las explotaciones de ovino lechero. In: *Revista Tierras Caprino* nº 2 (año 2012). Monografía Sanidad en el Ganado Caprino, pp. 8-19.

**Muehlherr J.E., Zweifel C., Corti S., Blanco J.E. and Stephan R., 2003.** Microbiological quality of raw goat's and ewe's bulk tank milk in Switzerland. In: *Journal of Dairy Science*, 86, pp. 3849-3856.

**Nuñez J.A., Chavarri F.J. and Nuñez M., 1984.** Psychrotrophic bacterial flora of raw ewes' milk, with particular reference to Gram negative rods. In: *Journal of Applied Bacteriology*, 57, pp. 23-29.

**Oliete B., Arias R., Garzón A., Jiménez L., Romero J., Arias C., Calatayud J.R. and Pérez-Guzmán M.D., 2011.** Variación del recuento de diferentes grupos de microorganismos en la leche de tanque de oveja Manchega en relación con las condiciones higiénicas de las ganaderías. Resultados preliminares. In: *XXXVI Congreso de la Sociedad Española de Ovinotecnia y Caprinotecnia (SEOC)*. San Sebastián. Fecha: 5-7 de octubre de 2011. Libro de Actas, pp. 181-184.

**Pirisi A., Lauret A. and Dubeuf J.P., 2007.** Basic and incentive payments for goat and sheep milk in relation to quality. In: *Small Ruminant Research*, 68, pp. 167-178.

**Reglamento (CE) nº 853/2004 del Parlamento Europeo y del Consejo de 29 de abril de 2004**, modificado por el **Reglamento (CE) Nº 1662/2006**, por el que se establecen normas específicas de higiene de los alimentos de origen animal. (DO L 139 de 30.4.2004, p. 55/205).

**Sevi A., Taibi L., Albenzio M., Muscio A. and Annicchiarico G., 2000.** Effect of parity of milk yield, composition, somatic cell count, renneting parameters and bacteria counts of Comisana ewes. In: *Small Ruminant Research*, 37, pp. 99-107.

**Sevi A., Albenzio M., Marino R., Santillo A. and Muscio A., 2004.** Effects of lambing season and stage of lactation on ewe milk quality. In: *Small Ruminant Research*, 51, pp. 251-259.