Composition and colour indices of sheep’s bulk-tank milk are influenced by production practices

Lorena Jiménez Sobrino, Justa María Poveda Colado, Ana Isabel Garzón Sigler, Andrés Luis Martínez Marín, Nieves Núñez Sánchez, Jesús Romero Asensio, María Dolores Pérez-Guzmán Palomares and Ramón Arias Sánchez

Center Regional de Selección y Reproducción Animal (CERSYRA), Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal (IRIAF), Valdepeñas, Ciudad Real, Spain; Departamento de Producción Animal, Facultad de Veterinaria, University of Córdoba, Córdoba, Spain; Laboratorio Interprofesional Lácteo de Castilla-La Mancha (LILCAM), Talavera de la Reina (Toledo), Spain

ABSTRACT
Over the past few years, there has been a great development of the sheep dairy sector in Spain and a wide variability in the production outcomes of farms, although most of the farms have similar environmental and economic constraints. Milk quality evaluation is important not only for cheese production, but also for other dairy products. Therefore, the aim was to evaluate the effect of some factors on bulk-tank milk characteristics of Manchega sheep, with special attention to casein and urea contents and colour indices. Factors of variation such as season, annual milk yield of ewe (AMYE), the enrolment (or not) in a breeding programme (BPM) and the feeding strategy of farms have been considered. The study included 77 farms distributed along La Mancha region, in Spain. Data about production practices and feeding strategy were also collected from these farms. A total of 308 analytical determinations of milk characteristics including fat, protein, total solids, casein, lactose, protein/total solids, casein/fat, pH, urea content, colour indices and somatic cell count were analysed by automated methods. The results of the present work indicate that season was the source of variation with the greatest effect on chemical characteristics of bulk-tank milk, except for casein/fat ratio for which the difference was smaller. The feeding strategy effects on characteristics of milk have also been confirmed. Moreover, it has been demonstrated that the feeding strategies are applied more coherently according to their productive characteristics in farms with small and medium size than in farms with the largest herd size.

Introduction
It is estimated that more than three million metric tons of sheep’s milk are produced each year in the European Union. Spain represents 17% of the European sheep milk production, with a total of 2,647,670 dairy sheep, producing a total of 580,900 tons of milk, mainly used for cheese production (INLAC 2017). Manchega sheep is the largest autochthonous dairy breed of Spain. It comes from Castilla-La Mancha and it is characterised by high production level and easiness to adapt to different climates and farming systems. Milk from Manchega sheep is almost completely used to produce cheese under the protected designation of origin (PDO) Manchego Cheese, contributing to the protection of the Manchego breed and the preservation of the entire value chain of Manchego Cheese (Arias et al. 2012). A notorious recession of dairy sheep activity has been observed, leading to changes in type and intensity of land utilisation and thus, environmental and landscape degradation. These changes are forcing farmers to adapt to a more competitive milk production that reveal new types of organisation, which should be attended by technical programmes for controlling sheep production systems (Rivas et al. 2015).

In order to assure high quality of milk, quality assurance programmes take into consideration several chemical and sanitary parameters of milk, such as protein and fat content or somatic cells counts (SCC), the latter as a hygienic indicator (Gonzalo et al. 2006). Automated methods of milk determination have been...
gradually become available and the same instrument can be used for determination of milk fat and protein, as well as new parameters such as casein and urea contents. On one hand, casein is the key component in making up the curd matrix in cheese-making that entraps the fat and some authors consider looking at casein/fat to forecast the cheese yield. This ratio is critical in controlling the final fat in the dry matter of the finished cheese (Wendorff 2017). On the other hand, the interest in using milk urea as a biological indicator of the efficiency of dietary protein utilisation, including reduced nitrogen excretion into the environment and improved fertility has been studied in cattle (Pytlewski et al. 2010). However, studies on urea concentrations in sheep’s milk are scarce and less detailed than those for cow’s milk. Besides milk composition, colour indices may provide interesting information in food science, because they are directly responsible for product appeal and consumer acceptability. In regard with sheep’s milk colour, Assenat (1991) indicated that it possess a white nacreous aspect by visual evaluation. The instrumental evaluation, using the recommended industrial system of CIELAB by spectrophotometry method, offers the same perception as that of the human eye with the advantage of being free of external subjective and variable influences; by contrast, it provides objective results, though the three-dimensional Lab colour space (Ramirez-Navas 2010). Studies about colour assessment have focussed on characterisation of cheeses (Pavia et al. 1999; Maegenis et al. 2014), but the lack of knowledge about colour characterisation using CIELAB system of sheep bulk-tank milk and its main sources of variation requires further studies, especially due to its possible repercussion on the colour of cheeses.

The aim of this work was to study the effect of the season and different farm characteristics (annual milk yield of ewe, herd size, enrolment or not in a breeding programme and the feeding strategy) on several milk quality parameters such as the chemical characteristics, somatic cell count and milk colour, in order to identify possible sources of variation.

Materials and methods

Animals and sampling

Between January and December 2012, a total of 308 bulk-tank milk samples were collected from 77 dairy sheep herds belonging to a total population of 798 Manchega sheep farms and reared in the Castilla-La Mancha region of Spain. The bulk-tank milk samples were collected with seasonal periodicity: winter was considered from December to February, spring from March to May, summer from June to August and autumn from September to November. The farms were selected through stratified random sampling according to the herd size (≤600; from 600 to 1200 and ≥1200 sheep). The lactation length was 130 days in average, with herds undergoing reproductive intensification to achieve three lambing every second year and 30 days of lamb’s breast-feeding.

All samples were collected during the morning milking. Previous to milk sampling, milk was mechanically mixed for 10 min and homogenised bulk-tank milk sample was taken and added with 133 ml of azidiol (BOE 2011) in a sterile container (200 ml). All the samples were placed directly into cool boxes and processed within 48 h in the laboratory.

On-farm data collection

As a starting point, a survey about on-farm management practices was filled by the researcher using a predesigned questionnaire. Information about herd size, AMYE, the enrolment (or not) in a BPM and the feeding strategy was considered and is summarised in Table 1. The information about AMYE was calculated only from the bulk-tank milk produced by each farm thorough 2012, divided by the number of sheep that had each farm. Two groups of farms were recorded based on the 50th percentile of the AMYE values distribution: low milk production (LAMYE ≤133 litres) and high milk production (HAMYE >133 litres).

Table 1. Characteristics of Manchega milk sheep farms.

| Item                        | Small (≤600 sheep) | Medium (600–1200 sheep) | Large (≥1200 sheep) |
|-----------------------------|--------------------|--------------------------|---------------------|
| Farms, n                      | 32                 | 25                       | 20                  |
| AMYE* (≤133 litres)         | 19                 | 10                       | 6                   |
| AMYE* (>133 litres)         | 13                 | 15                       | 14                  |
| Breeding programme enrolled| 5                  | 11                       | 11                  |
| Not enrolled                 | 27                 | 14                       | 9                   |
| Group-feeding                |                    |                          |                     |
| Applied                      | 4                  | 9                        | 17                  |
| Not applied                  | 28                 | 16                       | 3                   |
| Indoor feeding               |                    |                          |                     |
| CTMRb                        | 10                 | 12                       | 6                   |
| FMTMRc                       | 0                  | 0                        | 9                   |
| FPCMd                        | 22                 | 13                       | 5                   |
| Feeding by-product applied   | 11                 | 15                       | 10                  |
| Not applied                  | 21                 | 10                       | 10                  |
| Type of forage               |                    |                          |                     |
| Hay                          | 32                 | 25                       | 12                  |
| Silage                       | 0                  | 0                        | 8                   |

*Annual milk yield of ewe.

**Commercial total mixed ration.

*Farm-made total mixed ration.

*Forage plus concentrate mix.
The classification of farms depending of BPM was based on whether the farms apply or donot apply the Manchega breeding selection in the farm. The breeding objective for Manchega sheep breed is an increase in milk yield, measured in kg of milk produced over a lactation period of 130 days (MAPAMA 2017). For feeding characteristics, use of group-feeding (GF) (applied or not applied), the type of indoor feeding (TIF) (commercial total mixed ration (CTMR), farm-made total mixed ration (FMTMR) and forage plus concentrate mix (FPCM), feeding by-products (BP) (applied or not applied) and the type of forage (TOF) (hay or silage) were considered. The classification of sheep in different GF depending on physiological status, stage of lactation, milk yield and body condition score (applied-not applied) was studied. Moreover, the classification of TIF in different types of feed (CTMR or FMTMR as mixture of ingredients, and FPCM, as discrete ingredients), was studied. Besides, the categorisation of BP based on wet brewers grains, which is included in the diets in different share according to days in milk of the sheep, was applied or not applied. Finally, the classification of TOF was based on different methods of alfalfa storage (silage-hay).

**Chemical characteristics and somatic cell count**

The following milk composition parameters were obtained: Fat g/100g of milk, total protein (Tp, g/100g of milk), total solids (Ts, g/100g of milk), total casein (Tc, g/100g of milk), lactose (La, g/100g of milk) and urea (U, mg/L of milk). Somatic cell count of bulk-tank milk (BTSCC, cells/mL) and pH were also evaluated. The milk samples were processed at the Interprofessional Milk Laboratory of Castilla-La Mancha (LILCAM) using automated facilities. Milk samples were analysed with a mid-infrared spectrometer MilkoScan 6000 FT (Foss Electric, Hillerød, Denmark) while somatic cell count was analysed with Fossomatic FC (Foss Electric, Hillerød, Denmark), in both cases specific calibrations for sheep milk was used. The pH analysis was performed with a Crison Basic 20 pH metre with an electrode for liquids (Crison Instruments, Barcelona, Spain).

**Colour indices analysis**

Colour was measured with a Konica Minolta CM-2300d spectrophotometer (Konica Minolta Business Technologies, Inc., Osaka, Japan), following the method described by Buffa et al. (2001). The values for lightness (L*; 100 = white, 0 = black), redness (a*; +60 = red, −60 = green), and yellowness (b*; +60 = yellow, −60 = blue) were recorded. Four measurements were performed on each milk sample and mean values were calculated.

**Statistical analysis**

Statistical analyses were undertaken using SAS version 9.3 (SAS Institute 2011). A total number of 308 observations belonging to 77 farms were included in the analysis. Univariate analysis was performed to determine measures of central tendency, dispersion and distribution characteristics. Means, standard deviation (SD), 25, 50 and 75th percentile and 95% CI (confidence interval) were calculated for the Fat, Tp, Ts, Tc, La, Tp/Ts, Tc/Fat, U, pH, somatic cell and colour indices (L*, a* and b*). Somatic cell count was normalised by log10 transformation and named logBTSCC. Spearman correlation coefficients between indicators of milk quality (chemical composition, colour indices and logBTSCC) were determined using the CORR procedure.

Prior to multivariate analysis, we selected different variables that provided enough information to explain the differences in the level of composition parameters, colour indices and logBTSCC. The farm and herd size were not included in the model to avoid redundant information. The effects of season of the year, AMYE, the enrolment (or not) in a BPM, GF, TIF, feeding BP and TOF on chemical characteristics, colour indices and logBTSCC were studied using PROC GLM according to the following linear model:

\[ y_{ijklmno} = \mu + SEA_i + AMYE_j + BPM_k + GF_l + TIF_m + BP_n + TOF_o + e_{ijklmno} \]

where \( y_{ijklmno} \) were the chemical characteristics mentioned, colour indices (L*, a* and b*) and logBTSCC; \( \mu \) was the general mean; \( SEA_i \) was the fixed effect of season of the year (winter, spring, summer and autumn); \( AMYE_j \) was the fixed effect for annual milk yield of ewe (low milk production (LAMYE) ≤ 133 litres and high milk production (HAMYE) > 133 litres: according to 50th percentile for the variable); \( BPM_k \) was the fixed effect of the enrolment in BPM (enrolled or not enrolled); \( GF_l \) was the fixed effect of group-feeding (applied or not applied); \( TIF_m \) was the fixed effect type of indoor feeding (CTMR:commercial total mixed ration, FMTMR:farm-made total mixed ration and FPCM:forage plus concentrate mix); \( BP_n \) was the fixed effect of feeding by-product (applied or not applied); \( TOF_o \) was the fixed effect type of forage (hay or silage) and \( e_{ijklmno} \) was the residual effect. Finally, the associations between chemical composition, colour
indices and BTSCC of bulk-tank milk samples with season and production practices were analysed using a multivariate correspondence analysis, to find a dimensional graphical representation of the rows and columns of a contingency table. Given that only categorical variables can be included in this analysis, continuous variables (chemical composition and colour indices) were first categorised based on the 50th percentile of the distribution and BTSCC was categorised by the recommended threshold proposed by Ariznabarreta (1999), considering that a herd was assumed to have good sanitary conditions when BTSCC was <500,000 cells/mL.

Results

Manchega bulk-tank milk composition

A description of the distribution of the chemical composition, logBTSCC and colour indices values is shown in Table 2. Also, the correlations between the chemical properties, logBTSCC and colour indices are presented in Table 3. The highest positive correlations were among Fat, Ts, Tp and Tc (ranged between 0.93 and 0.64); however, La was intermediary and inversely related with the above-mentioned components (ranged between −0.61 to −0.55). U content was moderately positive correlated with the nitrogen parameters of milk: Tp and Tc. In addition, significant and negative correlation was found between U and La (r = −0.19) and between urea and logBTSCC (r = −0.13). L* index of milk showed a negative and significant correlation with Ts, Tp and Tc in a range from −0.18 to −0.24, obtaining greater correlations with pH (r = −0.38) and U content (r = −0.29). No correlation between L* index and Fat was obtained (r = −0.07). Correlations between a* and b* indices with chemical parameters showed a similar trend, being moderate and positive with Fat, Ts, Tp and Tc and negative with La.

Table 2. Descriptive statistics of milk characteristics (n = 308).

| Percentile | Mean  | SD  | CV  |
|------------|-------|-----|-----|
| Chemical   |       |     |     |
| Fat, g/100g| 7.87  | 0.83| 10.51|
| Total protein (Tp), g/100g | 6.06  | 0.52| 8.54 |
| Total solids (Ts), g/100g | 19.40 | 1.17| 6.05 |
| Total casein (Tc), g/100g | 4.83  | 0.41| 8.59 |
| Lactose (La), g/100g | 4.61  | 0.24| 5.29 |
| Tp/Ts | 0.31  | 0.44| 14.44 |
| Tc/Fat | 0.62  | 0.05| 8.14 |
| Urea (U), mg/L | 490   | 120 | 24.53 |
| pH | 6.61  | 0.15| 2.22 |
| Log bulk-tank somatic cell count (logBTSCC), cells/mL | 6.02  | 0.23| 3.85 |
| Colour indices | | | |
| Lightness | 81.55 | 0.69| 0.85 |
| Redness | −1.48 | 0.22| −14.44 |
| Yellowness | 8.47  | 0.78| 9.18 |

SD: standard deviation; CV: coefficient of variation.

Relationship between sheep milk characteristics with season, annual milk yield of ewe, breeding programme and feeding strategy of dairy farms

Least square means of the chemical characteristics and logBTSCC of bulk-tank milk samples collected according to season, production practices and feeding strategy are shown in Table 4. Season conditioned chemical characteristics and colour indices of bulk-tank milk are recorded. In this way, the lowest values (p < 0.05) of Fat, Tp, Ts, Tc, Tp/Ts, U and pH were observed in spring and summer; in opposite the La values were higher in spring and summer than in autumn and winter. The effect of season was significant on Tc/Fat ratio, however, the differences were small. Regarding to the influence of AMYE, lower values (p < .05) of Tp and Tc, Tp/Ts ratio and pH were observed in farms with sheep showing a higher production level. As for the enrolment in a BPM, farms enrolled in a BPM showed lower values of La (p < .05) and lower values of logBTSCC (p < .05) with respect to not enrolled farms. With regard to feeding factors, results showed lower values (p < .05) of Tp, Ts and Tc on farms applying GF, compared to farms where GF were not applied. Also, lower values (p < .05) of U and logBTSCC were obtained on farms feeding BP in comparison to farms where BP were not used. Finally, significant lower values (p < .05) of Tp/Ts and Tc/Fat ratios were obtained on farms where silage was applied; in opposite, high values of La were observed on farms where hay was applied.
Least square means for colour index parameters of bulk-tank milk samples collected according to season, production practices and feeding strategy are shown in Table 5. The lowest values for L*/C₃ (p < .05) were obtained in winter and autumn, compared to spring and summer. In relation to a*/C₃ and b*/C₃, lower values were obtained in winter for both, indicating a tendency towards green and blue. Furthermore, lower values for a*/C₃, indicating a tendency to green colour, were obtained on farms enrolled in a BPM and applying silage, compared to those not enrolled in a BPM and where hay was applied.

**Overview of the associations between milk characteristics, season and production practices of dairy farms**

Figure 1 showed the results of multiple correlations between chemical characteristics and colour indices of bulk-tank milk and season. This overview showed, on one hand, the strong association between the low levels of Fat, Tp and Tc (Fat₁, Tp₁ and Tc₁), high level of La (La₂), with the colour indices A₁ and B₁; on the other hand, the association between the high levels of Fat, Tp and Tc (Fat₂, Tp₂ and Tc₂), low level of La (La₁), with the colour indices A₂ and B₂. Also, the figure showed, on one hand, the association between small and middle herd size (HS₁ and HS₂), with lower AMYE (LAMYE), no application of GF (NGF), the use of CTMR and FPCM and the application of hay as forage (HA); on the other hand, the association between large herd size (HS₃) with the addition of FMTMR and silage as forage (SI), with the application of GF (GF) and the higher AMYE (HAMYE). Two joined dimensions explained 45.02% of total variation, with SI and FMTMR being the more discriminatory variables.

**Discussion**

**Manchega bulk-tank milk composition**

Values observed for Fat, Tp and Ts were in agreement with previous reports for Manchega breed bulk-tank milk data by Arias (2009). The results for these parameters are within the range reported by the Spanish regulation (BOE 2011), which establishes a standard of identity and quality for Manchego cheese, providing limits for Fat, Tp and Ts. Nevertheless, the values were higher than previous reports for high-yielding milk sheep breeds, as Assaf and Lacaune breeds reared in Spain (MAPAMA 2017) and Sarda and Massese autochthonous breeds located in other Mediterranean areas (Pirisi et al. 2000; Martini et al. 2008). In this way, it was verified that the composition of sheep milk depends on the breed, genetics, farming methods and environment, similar to what was observed by Morand-Fehr et al. (2007).
The content of Tc obtained in the present study was higher than previously reported by Park et al. (2007), which mentioned an average composition of Tc of 4.20 g/100 g in sheep milk. The value is higher that the range presented by Sevi et al. (2004) (from 3.60 to 4.60 g/100 g) in bulk-tank milk samples from Comisana sheep. Nevertheless, it must be taken into account that concentration of Tc in ovine milk varies between breeds, because of the occurrence of genetic polymorphisms for all milk proteins (Selvaggi and Tufarelli 2012). Tc content has not been included in the quality control system of bulk-tank milk in a routine way, although it plays a crucial role in cheese structure and stability; furthermore, reduction in Tc content in milk reduces cheese yield (Pellegrini et al. 1997). There are works that make reference to look at casein relationships with other constituents to forecast the potential cheese quality and cheese yield; hence, the Tc/Fat ratio is critical in controlling the final fat in the dry matter of the finished cheese. The mean and the variations throughout the year for Tc/Fat ratio were within the range showed in different sheep breeds by Wendorff (2017), which reaffirms the usefulness of the bulk-tank milk from these farms for the cheese-making industry, which are mainly interested in standardised sheep milk throughout the year. The U content in sheep bulk-tank milk is used to monitor dietary protein intake and is indicative of dietary nitrogen adequacy in dairy sheep. In the present study, the mean value for U content in bulk-tank milk was within a range of 340 to 550 mg/L reported by Sallato et al. (2013), who considers that results within this range are indicators of well-balanced diets in dairy sheep. Nevertheless, the high variability for U content in this study may be due to the use of non-homogenous diets between the farms. About 25% of the milk samples showed a mean value greater than 567 mg/L, which is more than the U upper limit for the pasteurisation process, which is 500 mg/L. This result can be associated with the feed composition, indicating the necessity of using a complete balanced diet to avoid high levels of urea in milk in these farms.

### Table 4. Least square means (LSM ± SD) of sources of variation studied for milk characteristics.

| Chemical Characteristics | Fat ± | Total protein ± | Total solids ± | Total casein ± | Lactose ± | Tp/Ts ± | Tc/Fat ± | Urea ± | pH ± | logBTSCC ± |
|--------------------------|-------|----------------|---------------|---------------|----------|--------|---------|--------|------|-----------|
| **Season**               |       |                |               |               |          |        |         |        |      |           |
| Winter                   | 7.99 ± 0.12 | 6.20 ± 0.06 | 19.79 ± 0.15 | 4.79 ± 0.05 | 4.70 ± 0.03 | 31.33 ± 0.21 | 0.60 ± 0.01 | 564 ± 16.55 | 6.71 ± 0.07 | 9.6 ± 0.03 |
| Spring                   | 7.47 ± 0.12 | 6.66 ± 0.06 | 18.79 ± 0.15 | 4.62 ± 0.06 | 4.84 ± 0.03 | 30.14 ± 0.20 | 0.62 ± 0.01 | 493 ± 17.99 | 6.5 ± 0.02 | 9.8 ± 0.03 |
| Summer                   | 7.56 ± 0.12 | 5.72 ± 0.06 | 18.75 ± 0.15 | 4.56 ± 0.05 | 4.72 ± 0.03 | 30.51 ± 0.21 | 0.60 ± 0.01 | 478 ± 16.55 | 6.58 ± 0.02 | 9.8 ± 0.03 |
| Autumn                   | 8.11 ± 0.11 | 6.21 ± 0.06 | 19.91 ± 0.15 | 5.00 ± 0.05 | 4.51 ± 0.03 | 31.15 ± 0.20 | 0.62 ± 0.01 | 560 ± 16.35 | 6.69 ± 0.02 | 9.9 ± 0.03 |
| **Breeding programme**   |        |                |               |               |          |        |         |        |      |           |
| Applied                  | 7.66 ± 0.10 | 5.93 ± 0.06 | 19.21 ± 0.14 | 4.71 ± 0.05 | 4.75 ± 0.03 | 30.82 ± 0.18 | 0.62 ± 0.01 | 526 ± 15.04 | 6.65 ± 0.02 | 9.7 ± 0.02 |
| Not applied              | 7.91 ± 0.10 | 5.97 ± 0.05 | 19.41 ± 0.12 | 4.77 ± 0.04 | 4.64 ± 0.02 | 30.74 ± 0.17 | 0.61 ± 0.01 | 495 ± 13.73 | 6.64 ± 0.02 | 9.7 ± 0.03 |
| **Group-feeding**        |        |                |               |               |          |        |         |        |      |           |
| Enrolled                 | 7.69 ± 0.09 | 5.88 ± 0.05 | 19.15 ± 0.12 | 4.68 ± 0.04 | 4.72 ± 0.03 | 30.69 ± 0.17 | 0.62 ± 0.01 | 515 ± 13.62 | 6.65 ± 0.02 | 9.8 ± 0.03 |
| Not enrolled             | 7.87 ± 0.11 | 6.02 ± 0.05 | 19.47 ± 0.15 | 4.81 ± 0.05 | 4.67 ± 0.03 | 30.87 ± 0.20 | 0.61 ± 0.01 | 506 ± 16.40 | 6.61 ± 0.02 | 9.9 ± 0.03 |
| **Feeding by-product**   |        |                |               |               |          |        |         |        |      |           |
| Applied                  | 7.75 ± 0.10 | 5.93 ± 0.05 | 19.26 ± 0.13 | 4.72 ± 0.05 | 4.70 ± 0.02 | 30.74 ± 0.17 | 0.61 ± 0.01 | 531 ± 13.85 | 6.64 ± 0.02 | 9.8 ± 0.03 |
| Not applied              | 7.82 ± 0.10 | 5.97 ± 0.05 | 19.35 ± 0.13 | 4.77 ± 0.05 | 4.69 ± 0.03 | 30.83 ± 0.18 | 0.61 ± 0.01 | 490 ± 14.43 | 6.63 ± 0.02 | 9.9 ± 0.03 |
| **Type of forage**       |        |                |               |               |          |        |         |        |      |           |
| Hay                      | 7.71 ± 0.10 | 6.06 ± 0.05 | 19.22 ± 0.12 | 4.83 ± 0.04 | 4.59 ± 0.02 | 31.57 ± 0.16 | 0.63 ± 0.01 | 497 ± 13.02 | 6.61 ± 0.02 | 9.8 ± 0.03 |
| Silage                   | 7.85 ± 0.20 | 5.83 ± 0.11 | 19.40 ± 0.26 | 4.66 ± 0.09 | 4.80 ± 0.05 | 30.00 ± 0.35 | 0.59 ± 0.01 | 537 ± 27.89 | 6.66 ± 0.03 | 9.9 ± 0.05 |

### Table 5. Least square means (LSM ± SD) of sources of variation studied for milk characteristics.

| Colour Indices | Lightness | Redness | Yellowness |
|----------------|-----------|---------|------------|
| **Season**     |           |         |            |
| Winter         | 81.17 ± 0.08 | -1.66 ± 0.03 | 8.11 ± 0.11 |
| Spring         | 81.76 ± 0.08 | -1.55 ± 0.03 | 8.35 ± 0.11 |
| Summer         | 82.20 ± 0.08 | -1.52 ± 0.03 | 8.46 ± 0.11 |
| Autumn         | 81.09 ± 0.08 | -1.47 ± 0.04 | 8.30 ± 0.11 |
| **Breeding programme** |           |         |            |
| Enrolled       | 81.56 ± 0.07 | -1.54 ± 0.03 | 8.37 ± 0.10 |
| Not enrolled   | 81.55 ± 0.06 | -1.57 ± 0.02 | 8.25 ± 0.09 |
| **Group-feeding** |           |         |            |
| Enrolled       | 81.58 ± 0.07 | -1.59 ± 0.03 | 8.24 ± 0.10 |
| Not enrolled   | 81.54 ± 0.06 | -1.51 ± 0.02 | 8.37 ± 0.09 |
| **Feeding by-product** |           |         |            |
| Enrolled       | 81.54 ± 0.06 | -1.57 ± 0.02 | 8.25 ± 0.09 |
| Not enrolled   | 81.57 ± 0.08 | -1.54 ± 0.03 | 8.37 ± 0.10 |
| **Type of forage** |           |         |            |
| Hay            | 81.58 ± 0.06 | -1.48 ± 0.02 | 8.53 ± 0.09 |
| Silage         | 81.53 ± 0.13 | -1.63 ± 0.05 | 8.29 ± 0.18 |

*Annual milk yield of ewe.

Distinctive superindices: a, b, c, d: p < 0.05.
considered as high by Sallato et al. (2013) and which might trigger health risks in sheep and problems in milk production. The results indicate the convenience to include the evaluation of U content in milk quality assurance programmes.

Data referring to Manchega bulk-tank milk L*a*b* colour assessment is inexistent. In the present work, samples showed a high L* value, indicating a tendency to white colour. Moreover, all the samples showed low a* and b* values, indicating lesser tendency to the green and yellow colour, respectively. The present result for a* was higher (−1.48) than the range between −4.25 and −3.79 obtained in cow milk by McDermott et al. (2016), indicating a tendency towards red colour for sheep milk compared to cow milk. Although these results occurred probably because of the presence of pigments, as cited by Mestdagh et al. (2011), no information was available in the present study on the milk’s pigments content.

McDermott et al. (2016) reported a positive correlation between L* parameter and Fat, Tp and Tc, contrary to the results observed in this work. These results occurred probably because of the differences between cow and sheep species, not only in milk’s physical structure, but also because of the dispersion of casein micelles and fat globules in milk, which is responsible for the diffusion of incident light and is related to lightness (Raty and Peiponen 1999). The positive correlation noted in the present study between a* parameter and the main components of sheep milk (Fat, Tp and Tc) was different compared to the correlations between these parameters in cow milk according to McDermott et al. (2016). This fact could be explained by the content of milk’s pigments, which is species-dependent (Prache et al. 2005; Kuhnen et al. 2013). Such findings suggest that all the knowledge about the characterisation of cow’s milk colour should be adapted to sheep milk.

**Relationship between sheep milk characteristics with season, annual milk yield of ewe, breeding programme and feeding strategy of dairy farms**

Previous studies have confirmed the influence of season and production systems in dairy sheep, which relates to compositional changes of bulk-tank milk (Pulina et al. 2006; Martini et al. 2008; Bonanno et al. 2013). Under the new competitive dairy sheep production chain, milk quality is essential for cheese production and for that reason, the evaluation of the sources of variation on milk composition and colour indices are crucial in fully understanding the changes in Manchega sheep bulk-tank milk.

The results showed that season was the main source of variation which affected the chemical characteristics of milk, in accordance with different studies in sheep milk (Martini et al. 2008; Sitzia et al. 2015). The milk produced in spring and summer was lower in Fat, Tp, Ts and Tc, and higher in La, concurring with the higher milk-yield lactational stage of this breed noted by Arias et al. (2012). The explanation for these results is the well-known antagonism between milk yield and milk composition (Pulina et al. 2006), that has been confirmed in our study with the results obtained for annual milk yield of ewe. Similarly, the lower U content obtained in spring and summer could be influenced for the same antagonism; nevertheless, similar values of U content have been found in other seasons and breeds (Prpić et al. 2005), suggesting the influence of additional productive practices that could affect the variation of U content. Moreover, from the perspective of cheese technology, the stability of Tc/Fat ratio throughout the year gives the cheesemakers the opportunity to have a standardised milk with respect to fat and Tc concentrations, which is essential in

![Figure 1. Multiple correspondence analysis of studied variables according to season (squares where SP: Spring; SUM: Summer; AUT: Autumn and WI: Winter) and general characteristics and bulk-tank somatic cell count (BTSCC) (dots where Fat1: low fat; Fat2: high fat; Tp1: low total protein; Tp2: high total protein; Tc1: low total casein; Tc2: high total casein; La1: low lactose; La2: high lactose; U1: low urea; U2: high urea; L1: low lightness (black); L2: high lightness (white); A1: low redness (green); A2: high redness (red); B1: low yellowness (blue); B2: high yellowness (yellow); LSCC: low bulk-tank somatic cell count and HSCC: high bulk-tank somatic cell count).](image-url)
making up the curd in cheese-making process (Wendorff 2017). Regarding to the variation of colour index parameters throughout the year, it is important to note that the decrease of some components of milk determines that the colour of the summer and spring milk and is seen to show a tendency towards white colour, which is according to the correlations obtained in the present study. The significant tendency to green and blue colour of the milk in winter could be explained by the higher levels of some milk pigments, such as riboflavin, as was mentioned in cow milk by Poulsen et al. (2015), who explained that feeding silage in winter may affect riboflavin synthesis. Moreover, the graphical display from Figure 1 played a complementary role to understand the relationships between chemical characteristics and colour indices of bulk-tank milk with season. The associations between the characteristics of sheep milk, on one hand, with spring and summer (black oval) and on the other hand, with winter and autumn (yellow oval) were obtained. This could be possible due to the highest percentage of Manchega sheep whose lamb are pregnant in late winter-early spring and in mid lactation during the subsequent summer and thus play a key role in increasing the proportion of these animals in the herd (ESROM 2016).

From the present study, it is relevant the effect that the enrolment in a BPM has on quality of bulk-tank milk. According to Spanish regulation, Manchega sheep farms included in a breeding programme have greater milk yield because the main breeding objective in the selection of Manchega breed is the increase of the milk yield per lactation (MAPAMA 2017). Thus, the average for annual milk yield of ewe in farms enrolled in a breeding programme reaches the value of 201 litres of milk, higher than the value of 143 litres of milk in farms not enrolled in a breeding programme (AGRAMA 2017). This could explain the lower levels of the main constituents of milk obtained in our research, especially the Fat content, for farms enrolled in a BPM. Nevertheless, the ratio Tc/Fat is similar for both, enrolled and not enrolled farms. It would be interesting to control the value of this ratio to avoid results outside the range proposed by Wendorff (2017), which

Figure 2. Multiple correspondence analysis of studied variables according to feeding management, herd size, annual milk yield of ewe and bulk-tank somatic cell count (BTSCC) (squares where NGF: not groupfeeding; GF: group-feeding; CTMR: commercial total mixed ration; FMTMR: farm-made total mixed ration; FPCM: forage plus concentrate mix; HA: hay; SI: silage; HS1: small herd size; HS2: medium herd size; HS3: large herd size; LAMYE: low annual milk yield of ewe; HAMYE: high annual milk yield of ewe; LSCC: low bulk-tank somatic cell count and HSCC: high bulk-tank somatic cell count) and chemical characteristics (dots where Fat1: low fat; Fat2: high fat; Tp1: low total protein; Tp2: high total protein; Tc1: low total casein; Tc2: high total casein; La1: low lactose; La2: high lactose; U1: low urea; U2: high urea).
could determine the milk quality for cheese-making in the future. Moreover, several studies have contributed to explain the negative effect of high somatic cell counts on milk. On one hand, a higher milk somatic cell count is associated with a higher bacterial count, with the consequent impact that this count could have in terms of animal productive capacity and public health (Gonzalo et al. 2006; Arias et al. 2016). On the other hand, the detrimental effect of high somatic cell count on milk composition has additional effects on the cheese-making process, with several studies reporting slower milk coagulation, weak curd consistency and lower cheese yields after processing high somatic cell count milk (Pirisi et al. 2000; Caballero et al. 2015). In our study, the lower values of logBTSCC from farms enrolled in a BPM may indicate better mammary health, but also may denote higher hygienic, sanitary and technological quality of the milk from the enrolled versus not enrolled farms.

As previously reported by Bocquier and Caja (2001), feeding management is one of the main factors of variation of sheep milk composition, which is also being confirmed in the present study through some feeding strategies. First of all, the application of GF requires to use balanced feeding according to the characteristics of each group, being a practice more common in farms with larger size of herd and greater milk yield when compared to smaller and less productive farms (Rivas et al. 2015). In our study, the lower values of logBTSCC from farms enrolled in a BPM may indicate better mammary health, but also may denote higher hygienic, sanitary and technological quality of the milk from the enrolled versus not enrolled farms.

In our study, GF was a significant source of variation on the chemical characteristics of milk. This fact was mainly observed on protein nitrogenous constituents of bulk-tank milk (Tp, Ts and Tc), with lower values for these components in farms where GF were applied. This is probably due to the well-known dilution effect, because according to the results from the survey (Table 1), the allocation of sheep into GF is more common practice in larger farms. Although, Fat content was not affected by the application (or not) of GF, which suggests the influence of other factors that could determine the lower values obtained for protein nitrogenous constituents. This could be elucidated by the results obtained for U content in this study, with greater values in farms where GF were applied, that could demonstrate an unbalanced feeding according the characteristics of each group in terms of the dietary protein or energy supplied, being in agreement with results from Lagriffoul et al. (1999). On the basis of the obtained results, further studies in Manchega sheep will be required about how to adjust the rations based on sheep’s characteristics. Secondly, the effect of BP on different milk components was observed in the present study. Our results showed higher U content on bulk-tank milk from farms where BP was applied and despite not being extremes, in one hand, could compromise health status of animal with pathologies linked with nitrogenous excess (mastitis, laminitis, enterotoxaemia, alkalosis, etc.) (Sallato et al. 2013) and on the other hand, demonstrating protein unbalanced rations. Many authors evidenced this last statement (Cannas et al. 1998; Lagriffoul et al. 1999), explaining the necessity of synchronisation between rumen degradable protein and fermentable energy to reduce the nitrogen excesses and thus rumen ammonia and blood and milk urea concentrations. The application of BP was linked with higher logBTSCC, contrary with the results showed by Firkins et al. (2002), not obtaining a change of somatic cell count in cows. Additional studies should be conducted to clarify this relationship in the future.

Thirdly, the TOF used in the studied farms (HA or SI) affected the value of some milk components. The farms using silage registered higher significant values for La, demonstrating an increase of milk production. Similar results were observed by Badran et al. (2014), who studied the effects of feeding SI on Assaf sheep milk quality and quantity. Moreover, the use of SI produced a significant decrease on Tp/Ts and Tc/Fat values, which could be due to the drop of Tp and Tc, but also to the increase of Fat in milk. The decrease of protein nitrogenous constituents of bulk-tank milk could be due to the increase of non-protein nitrogen in forage storages as SI compared to HA (Martin et al. 2004). This explanation was corroborated in our study due to the higher U content in farms using SI versus farms using HA. Besides this explanation, other reasons such as the greater ruminal degradation of dietary crude protein in forage harvested as SI than as HA could be considered. In relation to this fact, Vagnoni and Broderick (1997) in dairy cows, reported that an excessive ruminal degradation of SI nitrogen could exceed the availability of energy for microbial protein synthesis, with the subsequent waste of nitrogen not used. Moreover, the TOF used in farms had a significant effect on a*. Compared with HA, milk from farms added SI showed lower level of a*, which could be related with higher riboflavin content of this milk. Some studies about the contents of riboflavin in cow’s milk could support this statement (Lee et al. 1998; Shingfield et al. 2005). More studies about the evaluation of pigments pattern of milk from sheep production systems could be done in the future not only due to their impact on milk, but also in dairy products (Prache et al. 2005).

Finally, the strong association (Figure 2) between the small and medium size of herd and their
mentioned feeding strategies, with chemical characteristics of bulk-tank milk (yellow oval) should be highlighted on one hand, and on the other hand the weak association between large size of herd and its mentioned feeding strategies, with chemical characteristics of bulk-tank milk (black oval) should also be observed. These results illustrated that the feeding strategies from the small and medium herd sizes are applied more coherently according to their productive characteristics when compared to the largest herds. These findings are according with Rivas et al. (2015), who highlighted the difficulty of feeding management on Manchega sheep farms.

Conclusions

The quality of sheep’s bulk-tank milk was influenced by season, annual milk yield of ewe, the enrolment in a breeding programme and the feeding strategies of farms. Season was the source of variation with the greatest effect on chemical characteristics of milk compared to the remaining factors, except for Tc/Fat ratio for which the differences were smaller. The relative stability of Tc/Fat gives the cheesemaker the opportunity to have a more standardised milk, which is essential in making up the curd in cheese-making process. Moreover, between all the studied farm characteristics, it is worth mentioning the influence of the feeding strategies of farms on the quality of milk. In this way, the application of group-feeding decreased the major components of milk compared to the farms where group-feeding was not applied. Besides, the addition of hay increased Tp/Ts and Tc/Fat ratios, together with a tendency to red colour of milk, compared to the addition of silage. The report also demonstrates that the feeding strategies from the small and medium size of herd which are applied more coherently according to their productive level, when compared to the largest herds. This study serves as a base for future studies about the role of feeding strategies in the current sheep production systems. whose knowledge constitutes an advantage to monitor the quality of milk in dairy sheep farms.

Acknowledgements

The authors thank PDO Manchego Cheese and Breeder’s Association of Manchega sheep breed (AGRAMA, Spain) for valuable collaboration in this work.

Disclosure statement

None of the authors have any conflicts of interest that could inappropriately influence their work.

Funding

This study was supported by project RTA2011-00057-C02-01 of the National Institute of Research and Agrarian and Food Technology (INIA), Government of Spain.

ORCID

Lorena Jiménez Sobrino http://orcid.org/0000-0001-8570-643X
Justa María Poveda Colado http://orcid.org/0000-0002-2843-2249
Ana Isabel Garzón Sigler http://orcid.org/0000-0002-0543-5164
Andrés Luis Martínez Marín http://orcid.org/0000-0003-0052-9724
Nieves Núñez Sánchez http://orcid.org/0000-0003-3909-8597
Ramón Arias Sánchez http://orcid.org/0000-0001-8608-8274

References

AGRAMA. 2017. National Manchega breeders association; [accessed 2017 Jun 9]. http://www.agrama.org
Arias R. 2009. Somatic cell count and quality of sheep milk in Castilla-La Mancha [Doctoral Thesis]. Castilla-La Mancha, Spain: Universito de Castilla-La Mancha.
Arias R, Oliete B, Ramón M, Arias C, Gallego R, Montoro V, Gonzalo C, Pérez-Guzmán MD. 2012. Long-term study of environmental effects on test-day somatic cell count and milk yield in Manchega sheep. Small Rum Res. 106:92–97.
Arias R, Gallego R, Altares S, Garzón A, Romero J, Jiménez L, Oliete B, Arias C, Caballero J, Martínez A, et al. 2016. Quality of milk in Manchego sheep flocks. A review. Arch. Zootec. 65:469–473.
Ariznabarreta A. 1999. Somatic cell count as indirect method of subclinical mastitis in Churra sheep breed [Doctoral Thesis]. Castilla y León, Spain: Universito de León.
Assenat. 1991. Sheep’s milk. In: F.M. Luquet editor. Milk and dairy products. Ed. Acribia S.A., Zaragoza (Spain), Vol. 1, 277–311.
Badran I, Alqaisi R, Salman M, Fatafta M. 2014. The effects of feeding silage on Assaf sheep milk quality and quantity. In: Tielkes E, editor. Bridging the gap between increasing knowledge and decreasing resources. Tropentag 2014, Book of abstracts. Hamburg: International Research on Food Security, Natural Resource Management and Rural Development, p. 170.
Bocquier F, Caja G. 2001. Effects of nutrition on ewe’s milk quality. INRA Prod Anim. 14:129–140.
Bonanno A, Tornambé G, Bellina V, De Pasquale C, Mazza F, Maniaci G, Di Grigoli A. 2013. Effect of farming system and cheesemaking technology on the physicochemical characteristics, fatty acid profile, and sensory properties of Caciocavallo Palermitano cheese. J Dairy Sci. 96:710–724.
BOE. 2011. Royal decree 752/2011, of 27 May, that established basic control rules for agents to control raw milk.
from sheep and goat sector. Official State Gazette (Spain) N° 137, 9/06/11. http://www.boe.es/ (accessed 2017 Feb 10).

Buffa MN, Trujillo AJ, Pavia M, Guamis B. 2001. Changes in textural, microstructural, and colour characteristics during ripening of cheeses made from raw, pasteurized or high-pressure treated goats’ milk. Int Dairy J. 11:927–934.

Caballero J, Garzón A, Oliete B, Arias R, Jiménez L, Núñez N, Martínez A. 2015. Relationship of somatic cell count and composition and coagulation properties of ewe’s milk. Miljekarstvo. 65:138–143.

Cannas A, Pes A, Mancuso R, Vodret B, Nudda A. 1998. Effect of dietary energy and protein concentration on the concentration of milk urea nitrogen in dairy ewes. J Dairy Sci. 81:499–508.

ESROM. 2016. Report about the selection scheme for Manchega sheep (ESROM); [accessed 2017 Feb 10]. p. 146. http://www.agrama.org/.

Firkins J, Harvatine DJ, Sylvester JT, Eastridge ML. 2002. Lactation performance by dairy cows fed wet brewers grains or whole cottonseed to replace forage. J Dairy Sci. 85:2662–2668.

Gonzalo C, Carriedo JA, Beneitez E, Juárez MT, De La Fuente LF, San Primitivo F. 2006. Short communication: bulk tank total bacterial count in dairy sheep: factors of variation and relationship with somatic cell counts. J Dairy Sci. 89:549–552.

INLAC. 2017. Interprofessional Dairy Organisation; [accessed 2017 Feb 10]. http://www.inlac.es/.

Kuhnen S, Moacry JR, Trevisan R, Pinheiro LC, Maraschin M. 2013. Carotenoid content in cow milk from organic and conventional farms in Southern Brazil. J Food Agrc Environ. 11:221–224.

Lagriggoul G, Guitard JP, Arranz JM, Autran P, Drux B, Penteado M, Silvio do Egito A, Daguer H. 2014. Rheological, physicochemical and authenticity assessment of Minas Frescal cheese. Food Control. 45:22–28.

MAPAMA. 2017. Ministry of Agriculture and Fisheries, Food and Environment (Spain); [accessed 2017 Jun 9]. http://www.mapama.gob.es

Martin B, Fedele V, Ferlay A, Grolier P, Rock E, Gruffat D, Chilliard Y. 2004. Effects of grass-based diets on the content of micronutrients and fatty acids in bovine and caprine dairy products. Grassl Sci Eur. 9:876–886.

Martini M, Mele M, Sclozzi C, Salari F. 2008. Cheese making aptitude and the chemical and nutritional characteristics of milk from Massese ewes. Ital J Anim Sci. 7:419–437.

McDermott A, Visentin G, McParland S, Berry DP, Fenelon MA, De Marchi M. 2016. Effectiveness of mid-infrared spectroscopy to predict the color of bovine milk and the relationship between milk color and traditional milk quality traits. J Dairy Sci. 99:1–7.

Mestdagh F, Kerkhaert B, Cucu T, De Meulenaer B. 2011. Interaction between whey proteins and lipids during light-induced oxidation. Food Chem. 126:1190–1197.

Morand-Fehr P, Fedele V, Decandia M, Le Frileux Y. 2007. Influence of farming and feeding systems on composition and quality of goat and sheep milk. Small Rum Res. 68:20–34.

Park YW, Juárez M, Ramos M, Haenlein GFW. 2007. Physicochemical characteristics of goat and sheep milk. Small Rumin Res. 68:88–113.

Pavia M, Guamis B, Trujillo AJ, Capellas M, Ferragut V. 1999. Changes in microstructural, textural and color characteristics during ripening of Manchego-type cheese salted by brine vacuum impregnation. Int Dairy J. 9:91–98.

Papić Z, Konjačić M, Vnučec I, Ramljak J, Ivanković A. 2005. Non-nutritional factors of milk urea concentration. Stocarstvo. 59:173–187.

Pellegrini O, Remue F, Rivemale M, Barillet F. 1997. Renneting properties of milk from individual ewes: influence of genetic and non-genetic variables, and relationship with physicochemical characteristics. J Dairy Res. 64:355–366.

Pirisi A, Piredda G, Corona M, Pes M, Pintus S, Ledda A. 2000. The effect of somatic cell count on the composition of sheep milk and the characteristics of the dairy product. OVIS (Aula Veterinaria) 66:21–27.

Poulsen NA, Rybicka I, Poulsen HD, Larsen LB, Andersen KK, Larsen MK. 2015. Seasonal variation in content of riboflavin and major minerals in bulk milk from three Danish dairies. Int Dairy J. 42:6–11.

Prache S, Cornu A, Berdagué JL, Priolo A. 2005. Traceability of animal feeding diet in the meat and milk of small ruminants. Small Rum Res. 59:157–168.

Pulina G, Nudda A, Battacone G, Cannas A. 2006. Effects of nutrition on the contents of fat, protein, somatic cells counts, aromatic compounds, and undesirable substances in sheep milk. Anim Feed Sci Tech. 131:255–291.

Ptylewski J, Antkowiak I, Skrzypek R. 2010. Relationships between somatic cells counts and urea level in the milk of polish Holstein-Friesian cows of Black-and-White and red-White varieties. Acta Sci. Polonorum Zootech. 9:39–50.

Ramirez-Navas JS. 2010. Characterization of milk and cheeses by spectro colorimetry. Latin American Dairy Tech. 61:52–58.

Raty J, Peiponen K. 1999. Reflectance study of milk and its color in the UV-Visible Range. Applied Spectroscopy. 53:1123–1127.

Rivas J, Perea P, Angón E, Barba C, Morantes M, Dios-Palomares R, García A. 2015. Diversity in the dry land mixed system and viability of dairy sheep farming. Ital J Anim Sci. 14: 179–186.

Sallato O, Fidelle F, Arranz JM. 2013. The rate of urea in sheep milk: an indicator of well-balanced ration; [accessed 2017 Feb 10]. CDEO Centre Départemental de l’Élevage Ovin. http://www.gis-id64.fr/

SAS Institute Inc. 2011. SAS Online Version 9.3. Cary (NC): SAS Institute Inc.
Selvaggi M, Tufarelli V. 2012. Caseins of goat and sheep milk: analytical and technological aspects. In: Anthony M. Ventimiglia, JM. Birkenhager editors. Casein: production, uses and health effects. New York (NY): Nova Publishers, Inc.; p. 1–26.

Sevi A, Albenzio M, Marino R, Santillo A, Muscio A. 2004. Effects of lambing season and stage of lactation on ewe milk quality. Small Rum Res. 51:251–259.

Shingfield KJ, Salo V, Pahkala E, Toivonen V, Jaakkola S, Piironen V, Huhtanen P. 2005. Effect of forage conservation method, concentrate level and propylene glycol on the fatty acid composition and vitamin content of cow’s milk. J Dairy Res. 72:349–361.

Wendorff WL. 2017. Sheep milk: processing of sheep milk. In: YW Park, GFW Haenlein editors. Handbook of milk of non-bovine mammals. 1ed. Oxford (UK): Blackwell Publishing; p. 297.

Sitzia M, Bonanno A, Todaro M, Cannas A, Atzori AS, Francesconi AHD, Trabalza-Marinucci M. 2015. Feeding and management techniques to favour summer sheep milk and cheese production in the Mediterranean environment. Small Run Res. 126:43–58.

Vagnoni DB, Broderick GA. 1997. Effects of supplementation of energy or ruminally undegraded protein to lactating cows fed alfalfa hay or silage. J Dairy Sci. 80:1703–1712.