Seasonal variation of Serrana goat milk contents in mountain grazing system for cheese manufacture

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SUMMARY
The aim of this study was to evaluate the seasonal variation of milk content and somatic cells count (SCC) during the year on Serrana dairy goats reared in mountain. For this study, 1195 milk samples were taken from the bulk tank of 42 Serrana goat dairy herds, SCC, milk components and total bacteria count were determined along one year. The mean±SD percentage of milk protein, fat, lactose and solids-not-fat were 3.63±0.24, 5.22±0.70, 4.72±0.25 and 8.92±0.44, respectively. The mean fat:protein ratio, Log 10 SCC and Log 10 of total bacteria count per mL were 1.45±0.21, 3.39±0.19 and 2.55±0.53, respectively. A significant effect of month in all these parameters was found (P<0.001), and polynomial correlations were observed between months and protein (r=0.61; R\textsuperscript{2}=0.38; RSE=0.19), fat (r=0.63; R\textsuperscript{2}=0.40; RSE=0.53), lactose (r=0.68; R\textsuperscript{2}=0.46; RSE=0.14), solids-not-fat (r=0.71; R\textsuperscript{2}=0.51; RSE=0.27) or Log 10 SCC (r=0.39; R\textsuperscript{2}=0.15; RSE=0.18). These results confirm that the kidding season and level of milk production can be associated with concentration of solids components and particularly fat content in milk. This seasonal variation of milk composition and SCC are relevant information to establish SCC thresholds and to detect intramammary infections and to evaluate milk quality as a benchmark. Finally, this information about seasonal changes in bulk milk composition of Serrana goats can benefit cheese makers and help them for a better understanding of the effects on cheese making and final product composition.

Keywords: Intramammary infections; milk composition; milk quality; somatic cells count

RESUME
La variation saisonnière de la composition du lait de chèvre de la race Serrana dans le système de pâturage de montagne pour la fabrication de fromage

Le but de cette étude a été d'évaluer la variation saisonnière annuelle des composants du lait et du comptage de cellules somatiques (CCS) sur les chèvres laitières de la race Serrana élevées en montagne. Pour cette étude, 1195 échantillons de lait ont été prélevés dans le tank à lait de 42 troupeaux. Les composants du lait, CCS et nombre total de bactéries ont été déterminés sur l’année 2015/2016. Le pourcentage moyen (±SD) de protéines de lait, matières grasses, lactose et solides non gras était de 3.63±0.24, 5.22±0.70, 4.72±0.25 et 8.92±0.44, respectivement. Le rapport moyen graisse / protéine, Log 10 CCS et Log 10 du nombre total de bactéries / mL était de 1.45±0.21, 3.39±0.19 et 2.55±0.53. Un effet significatif du mois sur tous ces paramètres (P<0.001) et des corrélations polynomiales entre les mois et les protéines r = 0.61; R\textsuperscript{2}=0.38 ; RSE = 0.19), la graisse (r = 0.63 ; R\textsuperscript{2}=0.40 ; RSE = 0.53), lactose (r = 0.68 ; R\textsuperscript{2}=0.46 ; RSE = 0.14), solides non gras (r = 0.71 ; R\textsuperscript{2}=0.51 ; RSE = 0.27) ou Log 10 CCS (r = 0.39 ; R\textsuperscript{2}=0.15 ; RSE = 0.18) ont été observés. Ces résultats confirment que la saison de mise bas et le niveau de production de lait peuvent être associés à la concentration de composants solides et en particulier à la teneur en matières grasses du lait. Cette variation saisonnière de la composition du lait et du CCS est une information pertinente pour établir les seuils de CCS, pour détecter les infections intramammary et pour évaluer la qualité du lait en tant que benchmark. Finalement, cette information sur les changements saisonniers dans la composition du lait des chèvres Serrana peut profiter aux fromagers et les aider à mieux comprendre les effets sur la fabrication du fromage et la composition du produit final.

Mots-clés : Infections intramammaries; composition du lait; qualité du lait; comptage de cellules somatiques

Introduction
Making saleable goat cheese may pose a problem with respect to composition because goat milk is variable in composition [13]. Milk goat composition and quality are affected by a wide array of factors: udder health, breed, season and year, stage of lactation, diet, and system of production such as reported in different studies with French, Spanish, Greek and Portuguese dairy goats [14, 25, 32]. Mastitis is an inflammation of the mammary gland and represents an enormous animal health problem, it reduces milk quantity, alters milk composition, lowers the hygienic value of milk, impair the processing milk properties, causing high economical losses in dairy farms [21, 48]. Hence, mastitis is always a hot topic for goat farmers and the somatic cells count (SCC) is the criteria commonly available to evaluate the existence of mastitis; this is an indirect and inexpensive detection method used in ruminants [27]. However, it is unclear if goat SCC can be used in a comparable way due to the great variations in SCC and the significant higher cellular counts without meaning the incidence of intramammary infections (IMI), characteristic from this species [17, 46]. Although it is unanimous that IMI have a higher influence on the increase of SCC, the relation between IMI and SCC values in not so linear as in dairy cows as both physiological and pathological factors may influence SCC in goat milk [17, 46, 48].

By other hand, there are seasonal SCC variations because the binomial photoperiod-temperature influences on milk production and, indirectly, the SCC [31]. Understanding seasonal SCC variations, knowing associated factors...
and taking them into account when using the SCC for a
diagnostic purpose in goats, can provide a tool on treatment
and controlling IMI and also manage to establish legal limits
for SCC in goat milk. Therefore, research in this topic is
especially important to understand SCC behavior in this
species according to their specific production system and
herd management.

Portugal has five goat indigenous breeds officially
recognized: Serrana, Bravia, Serpentina, Algarvia and
Charnequieira; the last three breeds are on the brink of
extinction, with fewer than 4000 females each [49]. The
Serrana Goat breed is reared in the northeastern mountain
regions of Portugal under grazing system, referred to as the
most representative one in Portugal, with a population around
20,000 females, representing nearly 50% of the national
herd of indigenous goats [49]. According to the farmer’s
association (Associação Nacional de Caprinicultores da
Raça Serrana) there were 17,411 animals registered in the
genalogic herd-book, corresponding to 224 farms [47]. It is
generally bred to produce milk for cheese-making and the
farms on which this goat breed is raised are small, with small
herds handled according to extensive systems, which are
highly dependent on the season.

Season is considered a variation factor for milk
composition and SCC due to the sum of several factors
(grazing fodder offer, temperature and milk yield curve
influenced by kidding season) [18, 35].

In a recent study, it has been observed a geometric mean
of 2196*10³ cells per ml of milk from bulk tank in Serrana
goats. Moreover, a multiple correlation (R=0.43; p<0.001)
was observed between log10 SCC and protein, fat and
lactose milk contents, and total bacterial count [23]. This low
correlation evidenced that non-infectious factors can play a
significant role in this breed and system production has been
reported. So, the aim of the present study was to describe
the monthly variation of bulk tank SCC and milk contents
of Serrana goats, expecting a season influence (as a sum of
factors).

Materials and methods

The Serrana goat herds were located at northeast of
Portugal, in mountain region, at latitude 41ºN and L 7º W.
This region is known as Trás-os-Montes and it is included
in the temperate Mediterranean climate region (Csb)
according to the Köppen climate classification [20]; its
average temperatures and precipitation can be consulted in
YR [51]. These dairy herds are characterized by their small
dimension, normally up to 100 adult animals. The mean
parity is estimated at 4.2 for females kidding in July, which
decrease to 2.9 until February of next year [45]. Goats daily
graze in mountain areas and lactating females are individually
supplemented with concentrated feed. Due to the annual
biomass variation of lands some forages are cultivated to feed
goats. In general, rye grass hay is available in farm, mainly
in January and February; by July goats access to cultivated
sorghum pastures; and, goats are also feed with oat hay and
oat grain in fall.

Only Serrana goat Transmontano ecotype reared under
pastoral system contribute with milk to Protected designation
of Origin - “DOP” cheese production [36, 37, 38]. Herds with
more than 50 adult goats were considered for the present
study. The 150-days normalized milk production in this
ecotype is 96.1 L (95% CI: 95.9 to 96.4 L; n=144,921) with
lowest milk production for goats kidding in summer season
[45]. The standard procedures in these herds were the hand-
milking and refrigeration of milk in bulk tank and it transfer,
twice in week, to cheese factory.

Animals and samples

A total of 1195 milk samples were obtained between
October 2015 and September 2016 from the bulk tank of
42 Serrana goat dairy herds, which are more than 15% of
the total breed herds. All milk samples were taken from
milk received for “Protected Designation of Origin Serrana
cheese” manufacture [36, 37, 38] and the flock effect was
not considered for the present study. Repeated samples
were taken during this period from the same bulk tank in the
same farms. From each herd bulk tank, after milk homogenization,
approximately 45 ml of milk were weekly or monthly collected
(Table 1) to a sterile container with up to 4 drops (160 ul) of
sodium azide (azidiol) as preservative [1]. After collection,
all samples were immediately refrigerated and delivered to the
laboratory (ALIP - Associação Interprofissional do Leite
e Lactícinos) until the 24 hours after their collection. At
laboratory, these refrigerated samples were analyzed until 24
hours after their reception.

Milk contents determination

Bulk milk SCC (SCC per mL) was determined with Fossomatic™ FC (Foss, Hillerød, Denmark) [42]. To
determine milk composition, fat (%), protein (%) and
lactose (%) were measured with Milkoscan 6000 (Foss
Electric, Hillerød, Denmark) [41] Bulk milk total bacterial
count (TBC per mL) was measured with BactoScan™ FC (Foss Instruments) [4] The equipment was calibrated with
cow milk, which is the standard calibration method [18]. The
freezing point was also evaluated in all milk samples in order
to detect milk adulteration with water [5]. All results came
negative.

Statistical analysis

The weighted arithmetic means of each milk component
were calculated considering the samples number contribution
per month of each dairy herd. Both SCC (Log SCC) and TBC
(Log TBC) were transformed in a base-10 log scale.

Anova and Tukey HDS test were used to estimate the
effect of month in milk goat contents and the fat/protein
ratio. Independently, the monthly pattern of milk goat contents was estimated by polynomial degree 3 correlations. Pearson correlations were also used to correlate lactose and protein or Log 10 SCC.

The JMP11 package [43] was used for all evaluations. Descriptive results are showed as mean ± SD.

Results

The mean percentage of milk protein, fat, lactose and solids-not-fat were 3.63±0.24, 5.22±0.70, 4.72±0.25 and 8.92±0.44, respectively. The mean fat/protein ratio, Log 10 SCC and Log 10 TBC were 1.45±0.21, 3.39±0.19 and 2.55±0.53, respectively. A significant effect of month in all these parameters was found (Table II).

Protein concentration increased to peak values of 4.05% in October and decreased to 3.48% in June and July (P<0.001), remaining under the mean level of 3.58% between April and August. Fat concentration rose 6.75% in October, with the highest value (P<0.001), and declined to 4.92% in April, with similar values until August and remaining under the mean value. Fat/protein ratio increased to peak values of 1.68% in October and decreased to 1.37% in April (P<0.001), remaining under the mean level of 1.53% between May and September. Lactose concentration increased to peak values of 4.91% in December and decreased to 4.42% in August (P<0.001), remaining under the mean level of 4.69% between June and October. Total solids-not-fat concentration increased to peak values of 9.47% in December and decreased to 8.47% in July and August, remaining under the mean level of 8.87% between May and September. A slightly increase

![Table II: Serrana milk goat contents at month level (mean±SD).](image)

** Table I: Frequency of milk samples collected and number of herds which contributed in each month.**

**Table II: Serrana milk goat contents at month level (mean±SD).**
on Log 10 SCC can be seen on summer and autumn (peak value of 3.51 in October) during the breeding season and it decreased in winter and spring months (lower values of 3.25 in February; P<0.001).

In some months, TBC and SCC showed a correspondence; this was the pattern for both minimums and maximums, in February and October respectively. However, this pattern was not consistent in other months.

Significant (P<0.001) polynomial correlations were observed between months and protein (r=0.61; R²=0.38; RSE=0.19), fat (r=0.63; R²=0.40; RSE=0.53), lactose (r=0.68; R²=0.46; RSE=0.14), solids-not-fat (r=0.71; R²=0.51; RSE=0.27) or Log 10 SCC (r=0.39; R²=0.15; RSE=0.18). The regression curves are reported in Figure 1.

![Figure 1: Estimation (polynomial correlations) of monthly pattern for Serrana milk goat contents](image)

The Pearson correlation between percentages of protein and lactose in milk was r=0.17 (R²=0.03; RSE=0.24; n=1195; P<0.001). Besides, a low negative correlation between Log 10 SCC and lactose was observed (r=−0.41; R²=0.17 RSE=0.19; n=1195; P<0.001).

**Discussion**

In this study, differences due to season were highly significant for the milk composition. These results suggest that the kidding season and, hence, the level of milk production is associated with the concentration of total solids and specially the fat content in milk [9]. This last component is recognized as the most important one in terms of cost, nutrition and physical and sensory characteristics of the product [3]; its lowest fat level coincides with the highest production period, around May in Serrana goats [45]. A similar seasonal pattern was found for fat in commingled goat milk from the northeast of the United States of America [13]. On the other side, the highest content of protein was found in the winter months; these results are different from the results of a study conducted in Spain with Murciano-Granadina goats, bred more intensively, where this peak was observed in spring and the lower values were found in winter [9].

On the other hand, factors like parity, stage of lactation, n° of lactations, milk yield, estrus, breed and age and the pathogen involved (minor or major pathogenic bacteria), contribute to greater ranges of SCC in dairy goat milk, making it difficult to establish a reliable SCC thresholds value [6, 17, 19]. Milk yield is lower on primiparous dairy goats, while the highest production is for parity 3 or 4, with a negative correlation with SCC, a highest average of SCC (sudden SCC elevations several times during lactation and high variations from day to day) in first lactation animals decreasing until the age of six, depending also on the stage of lactation [16, 53]. The seasonal variation of parity as well the 150-days normalized milk production level was also reported in Serrana Goats [45] and can explain, at least partially, the results of the present study. Moreover, we also recently observed that the occurrence of some reproductive alterations (abortions and stillbirths) also affected negatively the 150-day normalized milk production of goats [44].

The results of the present study indicate that there is a seasonal pattern for SCC. This seasonal variation on SCC may be related to lactation stage, as SCC tends to increase during and towards the end of lactation period. Physiologically, dairy goats have SCC with an upward trend corresponding to the progression of the productive period [34], revealing an inverse relationship with milk production [40], the SCC being higher at the end of lactation. This final increase in SCC during the lactation could be explained due to a dilution effect, since it is expressed in cells / ml of milk [17, 19, 44]. Hence, it is unclear if this negative correlation between SCC and milk production reflects the effect of an IMI, or if it is the result of that dilution effect. Manlongat et al. [22] described an increase on somatic cell chemotactic activity in late lactation non mastitic goats, possibly due to mammary gland involution, increasing SCC in this period. It is not clarified how important this mechanism is in explaining the high SCC in late lactation compared with what can be attributed to a concentration effect caused by a decreased milk yield [17, 19]. Several authors have consistently reported a negative correlation between SCC and milk yield in this species suggesting that high SCC can be associated with lower milk yield and vice versa [2, 26, 52, 53]. Within the seasonal variation of SCC and related with the fact that in most farms there is a seasonality in kidding, a higher variation on SCC during autumn/breeding season was described, showing higher counts during the first days of estrus [48]. On the other hand, some authors analyzed the influence of the binomial photoperiod-temperature on milk yield and effect on SCC, reporting that the spring season with the increased photoperiod, mild temperatures and more food available tend to increase milk production and therefore the SCC is reduced [8]. García-Hernández et al. [11] associated effects of long day photoperiod on increased per cent milk fat and decreased SCC (<1,705 x 10⁴ SC mL⁻¹).

The current study also shows an increase of the SCC in the summer; which can be expected because the SCC tends to increase as temperatures decrease the production [8, 30, 31];
In this region, the warmest months are July and August, with an average daily (24 h) temperature per month of 21.3 and 21.0°C, respectively [51]. In contrast, in the autumn months the situation tends to be opposite. What is more, according to Zeng and Escobar [52], the decrease on the amount of milk produced during late lactation leads to higher levels of SCC and of pathogens in milk. These individual factors associated with season have not been studied in this work because the aim was to study the global season effect; however it is already known that the season effect sums several factors (grazing diet and fodder offer, temperature and milk yield influenced by kidding season) [18, 35].

Thus, the cellular concentration of goat milk is so high that, according to Corrales et al. [7], at the end of lactation it is impossible to distinguish between uninfected and healthy glands through SCC. Therefore, the variations during this period are so important and without necessarily the incidence of IMI that some authors have proposed not using SCC for the mastitis diagnosis [6, 7, 44]. Other authors showed significant variations in weekly and monthly SCC in goats [17, 33].

Furthermore, it has been found some correspondence for TBC and SCC in some cases [12]; however, in general and as it has been showed in this study, this relationship has not been persistent [10, 28, 50]. Apart from that it is widely accepted that the increase of SCC is related to the pathogenicity of the etiological agents of the Intra-mammary infection [17]; however, in this study only TBC has been considered and bacteria have not been identified.

On the other hand, mastitis is associated with a decrease in the level of milk lactose in ruminant species, except for goat milk for which previous studies are conflicting [21]. Nevertheless, the negative correlation between SCC and lactose concentration in goat milk has been showed by other authors [15, 53], the loss of functional mammary tissues due to mastitis, causes the increase of SCC and the decrease in the lactose concentration; although Pasquini et al. [29] did not observe this effect on milk from individual goats. There is a similar conflict between total content of protein and mastitis impact for goat milk [21]. In this way, no association was found between SCC and protein content in this study for Serrana goats; however, some authors had previously associated high SCC with high protein content [24, 29, 50]. Similarly, SCC are not correlated to fat content in the current study, like other authors already showed [29, 50].

The evidence of high SCC and important direct losses in milk yield and technology, and the food safety linked to subclinical mastitis show the need of mastitis-control programs in herds in order to a final economic return to producers [39]. Besides, the implementation of programs for improving health and hygiene quality in bulk tank milk would be more efficient if both TBC and SCC were considered [12].

In conclusion, these results confirm that kidding season and consequently the level of milk production and stage of lactation can be associated with concentration of solids components and particularly fat content in milk. There is a seasonal influence on milk composition and SCC and it should be considered when establishing SCC thresholds to diagnose IMI and to evaluate milk quality. These thresholds should be adapted according to the production system and the goat breed. What is more, further studies should be conducted to evaluate the feasibility of SCC for the diagnosis of certain IMI. The information about seasonal changes in bulk milk composition of Serrana goats can benefit cheese makers and help them for a better understanding of the effects on cheese making and final product composition. This knowledge can be used to design programs to reduce variability, as breeding and feeding schemes or strategies for milk standardization. Further research about the interactions between the factors affected by seasonality would be interesting.

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Conflict of interest

The authors declare that they have no conflict of interest.

References


51. - YR: Weather statistics for Província de Trás-os-Montes e Alto Douro, Bragança (Portugal). 2018. Available at: https://www.yr.no/place/Portugal/Bragan%C3%A7a/Prov%C3%ADncia_de_Tr%C3%A1s-os-Montes_e_Alto_Douro/statistics.html.