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Relationships between chemical and physical parameters of bulk milk from Valle del Belice sheep

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ABSTRACT

In this study, we aimed to evaluate the relationships between the constituents of bulk milk of Valle del Belice sheep. During two years of investigation, 693 milk samples were collected and analysed to assess the major physicochemical parameters. Comparative analysis of partial and simple correlations revealed a strong correlation between milk constituents without the effect of third-party parameters.

HIGHLIGHTS

- Milk composition of Valle del Belice ewes showed an interesting aptitude for cheese-making
- The comparative analysis of partial and simple correlations has allowed us to better understand the relationships between the parameters of the chemical-physical composition of sheep's milk
- The study highlighted the lack of relationship between the total bacterial count of milk and the chemical-physical parameters of sheep's milk

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Introduction

Goat and sheep dairy farming is a vital part of the national economy of many countries, especially those in the Mediterranean region. In Italy, dairy sheep and goat farming systems vary from extensive (marked seasonal milk production, dual-purpose breeds, low feed supplementation, transhumance, hand milking, absence of farm facilities, farm-made cheese) to intensive systems (seasonal or continuous milk production, improved local breeds or crosses, exploitation of forage crops, high feed supplementation, machine milking and housing facilities, and industrial cheeses) in accordance with the economic relevance of the production chain and the specific environment and breed (Todaro et al. 2015). In farming systems based on grazing pastures, milk production frequently depends on climatic conditions and meteorological events that influence the quantity and quality of the pastures (Pulina et al. 2006). Accordingly, in Sicily, the production of sheep milk varies greatly throughout the year; it is essentially linked to the seasonal availability of grazed forage, on which periods of mating activity and

lambing depend (Todaro et al. 2014). In this inland area, dairy sheep production is an important resource for the local economy, and the Valle del Belice ewe is the main local breed reared on the island, with approximately 150,000 sheep heads. The Valle del Belice breed originated in 1980 from hybridisation among the native Pinzirita and Comisana breeds and Sarda rams. Selective interbreeding has given rise to a new sheep biotype that combines the characteristics of the three original breeds, resulting in very productive and rustic animals suitable for dairy farming (Portolano 1987). The breed is subject to limited breeding programs to improve milk production traits, and it shows excellent adaptability to local environments, sometimes under harsh conditions (Mastrangelo et al. 2017).

Information on the composition and physicochemical characteristics of sheep milk is essential for the successful development of the dairy sheep industry, as well as for marketing products. Several studies have been conducted on the milk quality of Valle del Belice sheep (Cappio Borlino et al. 1997; Giaccone et al. 2000; Todaro et al. 2014; Tolone et al. 2019), but none have reported the relationships between the

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main chemical and physical components of bulk milk used for producing Sicilian cheeses.

Several parameters contribute to milk quality (Bencini and Pulina 1997), and the correlations between them make their interpretation and optimisation difficult. The multiple comparison between simple and partial correlations allows the understanding of certain phenomena that will remain hidden due to the effect of third variables.

The aim of this study was to address the relationship patterns among parameters of bulk milk produced by Valle del Belice ewe.

Materials and methods

Milk samples were collected as part of routine animal milk collection in breeding farms, and is a non-experimental, veterinary practice. No animal discomfort was caused for sample collection for the purpose of this study. The Directive 2010/63/EU of the European parliament and the council and the Italian D.lgs 26/2014 do not apply to non-experimental practices. An ethical review by the Animal welfare body was therefore not required.

The composition and quality of bulk sheep milk were monitored for two consecutive years in 17 farms, located in different areas of central-western Sicily, which rear Valle del Belice ewes. In the two years of the survey, 811 bulk milk samples were collected every two weeks and, 693 samples were used for statistical analysis after eliminating the samples that presented missing data.

Physico-chemical analysis

After using a HI 9025 pH-meter (Hanna Instruments, Ann Arbor, MI, USA) to determine pH, the samples were transported at 4 °C, without the addition of preservatives, to the milk laboratory at the Istituto Zooprofilattico Sperimentale della Sicilia 'A. Mirri'. Within 6 h of collection, samples were analysed for

determination of fat, protein, lactose, and somatic cell count (SCC) *via* the infra-red method (Combi-Foss 6000, Foss Electric, Hillerød, Denmark); total bacterial count (TBC) by BactoScan instrument (Foss Electric, Hillerød, Denmark). The SCC and TBC were logarithmically transformed to normalise their distributions.

Total nitrogen (TN), non-casein nitrogen (NCN), and non-protein nitrogen (NPN) were determined using FIL-IDF standard procedures (1964,1993) and the Kjeldahl method. From these nitrogen fractions, the casein $((TN - (NCN \times 0.994)) \times 6.38)$ and whey protein $((NCN - NPN) \times 6.38)$ contents were calculated.

Freezing point values were determined using an Astor Cryoscope 4000 SE, titratable acidity by the Soxhlet–Henkel method ($^{\circ}\text{SH } 100 \text{ mL}^{-1}$), and urea level by the enzymatic method using the difference in pH (CL-10 Plus, Eurochem, Roma, Italy).

Statistical analysis

Two different approaches were used for the statistical analysis of milk components: Pearson simple (rSP) and partial correlation (rPP), statistical analysis was carried out utilising the procedures CORR and FACTOR of SAS software (ver. 9.1, SAS Institute Inc., Cary, NC).

Results and discussion

Physico-chemical parameters of bulk milk

Simple statistics of the physico-chemical parameters of Valle del Belice ewe milk are reported in Table 1. Fat and protein percentages detected are in accordance with those reported for this breed (Cappio-Borlino et al. 1997) but, as is well known, they vary according to the season of production (Todaro et al. 2014). The average percentage of casein was 4.59%, which represented 82% of the total proteins, indicating that casein varies from 76–83% of the total proteins (Park et al. 2007). Milk urea level had an average of 37 mg/dL, which is close to the value (35 mg/dL) considered acceptable for dairy ewes (Cannas et al. 1998). However, the high

Table 1. Physico-chemical parameters of Valle del Belice Ewe's milk.

Parameters	Mean value	Standard deviation	Minimum value	Maximum value
Fat (%)	6.95	0.94	3.79	10.14
Protein (%)	5.59	0.47	4.00	7.79
Casein (%)	4.59	0.41	2.97	5.84
Whey protein (%)	1.36	0.20	0.91	2.86
Urea (mg/dl)	37.21	11.47	6.55	70.63
Lactose (%)	4.60	0.20	3.51	5.89
pH	6.59	0.21	4.76	7.76
Titratable acidity (TA, $^{\circ}\text{SH}/100 \text{ mL}$)	9.81	2.56	5.98	35.60
Somatic Cell Count (SCC, Log ₁₀)	6.16	0.47	4.00	7.04
Total bacterial count (TBC, Log ₁₀ CFU)	5.55	0.35	4.49	6.68
Freezing point (MFP, $^{\circ}\text{C}$)	-0.561	0.01	-0.624	-0.502

standard deviation indicates a high variability of this parameter, which is explained by the fact that the milk urea curve usually follows the availability and quality of the pastures, with high values during spring months (Molle et al. 2008; Todaro et al. 2014).

The percentage of lactose in the milk of Valle del Belice sheep was 4.6%; the low variability can be attributed to the fact that it is the main osmotically active component of milk, and its content remains substantially unchanged during lactation in healthy animals. Similar values have been reported for other Italian (Martini et al. 2008; Manca et al. 2016) and foreign (Garzon et al. 2021; Tatar et al. 2021) sheep breeds.

Milk pH and titratable acidity (TA) resulted within the range of those of fresh ovine milk (Park et al. 2007), indicating the degree of acid production in milk.

The Somatic Cell Count (SCC) had an average of 6.16 logarithm value that corresponded to 1.4 million SCC per millilitre of milk; this mean value was lower than those reported in previous studies on individual samples of the same breed (Riggio et al. 2010; Tolone et al. 2013). SCC changes are caused by environmental and genetic factors, but such increase is predominantly due to udder infections (Raynal-Ljutovac et al. 2007; Leitner et al. 2008), which worsen milk quality and considerably damage curd and cheese yields. A study conducted by Sutura et al. (2018) confirmed that high levels of SCC in Valle del Belice milk are associated with milk yield losses and variations in fat and protein percentages. The estimated loss in milk yield according to the SCC level was approximately 16%.

The mean total bacterial count in milk was 5.55 logarithmic points, which corresponded to 355,000 CFU/mL. This value is below the standard for raw sheep milk production (500,000 CFU/mL) and reflects the hygienic conditions of milk production in the investigated flocks.

The milk freezing point (MFP) has an average of -0.561°C according to a survey of bulk sheep milk

produced in Sicily (Scatassa et al. 2017). MFP is an important quality indicator and is closely dependent on water-soluble compounds; however, it depends on many factors, including milk components (mainly lactose and chlorides, as well as Ca, K, Mg, and phosphates), sheep breed, milk yield, year and month of production, animal feed, and health status (Hanus et al. 2015). Furthermore, the MFP at the beginning and end of lactation had values close to zero; therefore, MFP, as an indicator of the adulteration of milk with water, should not be used in this physiological phase of lactation (Scatassa et al. 2017).

Correlation analysis

Correlation analysis is a statistical method used to discover if there is a relationship between two variables and how strong that relationship may be, whereas partial correlation analysis allows estimating the association between two quantitative variables after eliminating the influence of other variables (Vargha et al. 2013).

Table 2 reports the Pearson correlations (below the diagonal) and partial correlation coefficients (above the diagonal). A preliminary step with proc Factor of SAS software is the assessment of the suitability of the dataset for this statistical approach. The evaluation was performed by comparing simple (rSP) and partial (rPP) Pearson correlations between the observed variables. According to Manca et al. (2016), a marked decrease in rPP compared with rSP supports the factor hypothesis of an underlying latent structure that regulates the correlations of the multivariate system. The Kaiser index measure the sampling adequacy (MSA; Cerny and Kaiser 1977). The value of MSA in this study was 0.65, intermediate between the values of 0.57 reported by Manca et al. (2016) and 0.77 reported by Garzon et al. (2021) in similar dataset.

From the analysis of the simple correlations between the percentage of fat and those of protein

Table 2. Pearson correlations below the diagonal and partial correlation coefficients above the diagonal.

	Fat	Protein	Urea	Casein	Whey protein	Lactose	SCC	pH	TA	TBC	MFP
Fat	1	0.25***	-0.22***	0.15***	-0.01	-0.14***	-0.01	0.01	0.02	0.04	-0.22***
Protein	0.60***	1	0.08*	0.71***	0.35***	-0.29***	-0.04	0.02	0.06	0.07	-0.03
Urea	-0.14***	0.08*	1	0.00	0.27***	0.27***	-0.11**	0.16***	0.03	0.09*	-0.26***
Casein	0.54***	0.81***	0.08*	1	-0.14***	0.17***	-0.05	0.10**	-0.00	-0.10**	-0.02
Whey protein	0.23***	0.48***	0.27***	0.31***	1	-0.11**	0.12**	0.02	-0.12***	-0.00	-0.07
Lactose	-0.33***	-0.37***	0.31***	-0.19***	-0.21***	1	-0.17***	0.01	0.01	-0.07	-0.20***
SCC	0.01	-0.03	-0.16***	-0.08*	0.08*	-0.22***	1	0.09*	0.05	-0.04	0.02
pH	0.01	0.14***	0.11***	0.17***	0.19***	-0.08*	0.08*	1	-0.60***	-0.04	0.26***
TA	0.05	-0.03	-0.05	-0.06***	-0.16***	0.05	-0.04	-0.66***	1	0.11**	-0.06
TBC	0.05	0.04	0.05	-0.04	0.02	-0.07	-0.03	-0.14***	0.18***	1	0.04
MFP	-0.21***	-0.17***	-0.29***	-0.16***	-0.12***	-0.20***	0.13***	0.31***	-0.25***	-0.04	1

SCC: somatic cells count (Log10); TA: titratable acidity; TBC: total bacterial count (Log10); MFP: milk freezing point.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

and casein, a strong and positive correlation emerges, in agreement with what is reported in the literature on sheep's milk (Manca et al. 2016; Scatassa et al. 2017; Konečná et al. 2019; Kawęcka et al. 2020; Tatar et al. 2021), where the correlations between fat and protein vary from 0.41 (Konečná et al. 2019) to 0.85 (Kawecka et al. 2020) whereas those between fat and casein vary between 0.48 (Manca et al. 2016) and 0.82 (Kawecka et al. 2020). Likewise, a negative correlation was found between fat and lactose, even though values ranging between -0.15 (Scatassa et al. 2017) and -0.59 (Tatar et al. 2021) have been reported in the literature. These correlation coefficients, while remaining significant, decreased when the rPP values were considered.

Milk SCC was negatively correlated with the lactose percentage, considering both rSP (0.22 ; $p < 0.001$) and rPP (0.17 ; $p < 0.001$). Similar correlations have been previously reported (Kawecka et al. 2020; Scatassa et al. 2017). It is well known that SCC depend of many environmental factors (Albenzio et al. 2004), however the high SCC value of bulk milk suggests the presence of mastitis in the herd. Bianchi et al. (2004) reported lactose concentration of 4.80 and 4.51 g/100 mL, respectively in healthy and infected udders of Sarda sheep breed. In addition, other researchers (Vivar-Quintana et al. 2006; Paschino et al. 2019) observed a decrease in lactose content and an increase in SCC in sheep milk. These authors explained the decrease in lactose as a change in blood flow to the udders due to epithelial cell damage and inflammation, which can reduce glucose available for lactose synthesis. In addition, Albenzio et al. (2004) indicated that in milk with high SCC, the decrease in lactose is caused by the replacement of that carbohydrate with other osmotically active components. The possible presence of mastitis in milk with high SCC values was confirmed by the simple (0.08 ; $p < 0.05$) and partial (0.12 ; $p < 0.01$) correlation coefficients between SCC and whey protein. Milk SCC was negatively correlated with urea (0.16 ; $p < 0.001$), which is consistent with previous evidence reported in dairy ewes (Scatassa et al. 2017; Nudda et al. 2020). Milk pH was positively correlated with all major milk constituents, but rPP analysis showed that only correlations with urea (0.16 ; $p < 0.001$) and casein (0.10 ; $p < 0.01$) remained significantly positive. The pH of milk is positively correlated with urea because urea is a strongly alkaline substance that increases milk pH. Moreover, a strong negative correlation was observed between pH and titratable acidity. Concerning TA, the only significant and weak correlation was observed with whey proteins rSP

(-0.16 ; $p < 0.001$) and rPP (-0.12 ; $p < 0.001$). This correlation could be explained by hypoacidic milk samples with low TA, probably from herds with a high frequency of mastitis, where the milk had a high whey protein content.

The total bacterial count of the milk is attributable to the environmental pollution of the milk after milking. The correlation analyses (simple and partial) did not yield unique results, with the exception of titratable acidity, highlighting a positive correlation between rSP (0.18 ; $p < 0.001$) and rPP (0.11 ; $p < 0.01$). This finding was consistent with the results obtained by Scatassa et al. (2017). Milk bacteria, particularly lactic acid bacteria, use lactose to produce lactic acid, increasing the titratable acidity, which expresses the quantity of lactic acid.

Regarding the rSP between MFP and all the main milk constituents, these presented significant negative coefficients, according to other studies (Scatassa et al. 2017; Hanus et al. 2015); in particular, Hanus et al. (2015) justified these findings assuming that MFP is less dependent on milk conductivity (on the concentration of ions and osmotic pressure) and is more influenced by the major ewes' milk components. When the rPP coefficients were considered, only fat, urea, and lactose had significant and negative correlation coefficients, explaining their interactions with MFP. In fact, the presence of fat in milk creates a barrier that prevents the formation of ice crystals, leading to a lower freezing point; milk urea is a non-electrolyte solute that does not participate in hydrogen bonding with water molecules, thus reducing the freezing point of the liquid, whereas lactose interferes with hydrogen bonding between water molecules, leading to a reduction in the freezing point of the liquid.

Conversely, MFP was positively correlated with pH, rSP (0.31 ; $p < 0.001$), and rPP (0.26 ; $p < 0.001$) according to Pavić et al. (2002), who reported higher values ($r = 0.65$; $p < 0.01$). This is because the hydrogen ion (H^+) concentration in an acidic solution affects hydrogen bonding between water molecules, which in turn affects the freezing point of the liquid.

Conclusion

In this study, the relationships between all the main components of bulk milk produced by the Valle del Belice sheep were reported. The comparative analysis of partial and simple correlations made it possible to better understand the relationships between the parameters of the chemical-physical composition of

milk. The study highlighted the lack of relationship between the total bacterial count of milk and the chemical-physical parameters of sheep's milk.

Disclosure statement

No potential conflict of interest was reported by the authors. The authors alone are responsible for the content and writing of this article.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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