



Uncovering milk quality in local sheep breeds: From bioactive constituents to genetics

Johanna Ramirez-Diaz^{a,1}, Arianna Manunza^{a,1}, Giorgia Stocco^{b,*}, Alessio Negro^{c,d},
Claudio Cipolat-Gotet^b, Stefano Biffani^{a,b}

^a Institute of Agricultural Biology and Biotechnology, National Research Council, Milano, Italy

^b Department of Veterinary Science, University of Parma, Parma, Italy

^c Ufficio Studi, Associazione Nazionale della Pastorizia, Roma, Italy

^d Department of Agricultural and Environmental Sciences (DISAA), University of Milan, Milano, Italy

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ABSTRACT

The increasing global population has highlighted the necessity for nutritious, equitable, and sustainable food alternatives. Sheep milk cheese has emerged as a promising alternative to traditional dairy products, providing consumers with a unique and healthier nutritional composition, while also presenting an opportunity for rural economic development. The growing demand for this product could catalyze further innovation and investment in the coming years. To improve the quality of sheep milk and its technological properties, a deep understanding of the factors underlying milk composition is essential. This study focused on characterizing milk composition and mineral content of the Comisana and Massese sheep breeds to understand their genetic and non-genetic background. Results suggest that ovine milk quality can be enhanced in the short and medium term by appropriate technology and breeding strategies, eventually supporting the dairy industry.

1. Introduction

Sheep milk production has carved a distinct and important niche within the global milk supply. According to FAO (2022), the main contributors are Asia, Europe, and Africa, accounting for approximately 45.6 %, 30.6 %, and 23.5 % of global sheep milk production, respectively, with the Americas adding only a negligible portion. In European southern countries, sheep farming systems, especially those focusing on local breeds, are also pivotal for preserving genetic diversity and supporting rural economies (Di Trana et al., 2015). These systems often rely on traditional, extensive farming methods that are well-adapted to Mediterranean climates and landscapes. Moreover, they provide a nutrient-rich dairy source and support traditional and local dietary practices. Specifically, the milk from these autochthonous sheep breeds is mainly used for producing high-quality cheeses, often recognized under the Protected Designation of Origin (PDO) label, such as Roquefort, Pecorino Romano and Manchego.

The quality of these cheeses is intrinsically linked to the unique composition of milk from local sheep breeds, with mineral content

playing a key role. Minerals are essential for various physiological functions and significantly contribute to both milk processing and the nutritional profile of dairy products. For example, calcium (Ca) and phosphorus (P) influence the coagulation process during the initial stages of cheese production (Fox et al., 2017; Stocco et al., 2021), while sodium (Na) and potassium (K) influence the growth dynamics of microbial communities during cheese aging, potentially affecting flavour development and other quality traits (McMahon et al., 2014). Additionally, several recent studies have demonstrated that multi-elemental analysis combined with chemometric tools are effective in discriminating cheeses based on geographical origin, manufacturing practices, and milk source. For instance, significant differences in mineral and trace element profiles were observed in Northern Spanish PDO cheeses depending on the use of sheep milk, brine salting, and ripening conditions (Moreno-Rojas et al., 2012). Similar approaches have been applied to distinguish high-value Italian Pecorino cheeses (such as Pecorino Romano, Sardo, and Farindola) based on their elemental composition and origin with high accuracy (Di Donato et al., 2021). Even in non-European contexts, such as Brazil, mineral profiling and chemometric

* Corresponding author.

E-mail address: giorgia.stocco@unipr.it (G. Stocco).

¹ These authors share first authorship.

classification have successfully authenticated artisanal cheeses and classified them by region and type, underscoring the global relevance of these techniques (de Andrade et al., 2022). Enhancing the mineral profile of milk could thus offer notable health benefits and processing advantages. For dairy sheep farmers, this improvement could represent a valuable opportunity to increase the economic value of their milk production. Despite these potential benefits, there is a notable lack of research exploring the factors that contribute to variations in milk mineral content of sheep. While it is established that dietary mineral and vitamin supplementation, as well as farming practices, can temporarily improve the mineral profile and composition of sheep milk (Adinepour et al., 2022; Arshad et al., 2021), studies evaluating strategies for the permanent enhancement of sheep milk mineral content and overall composition are limited and yield variable findings (Casoli et al., 1989; Jones & Wilson, 2022; Marshall et al., 2024).

In contrast, research on dairy cattle have shown that physiological stages (Patel et al., 2018; Toffanin et al., 2015) and genetic factors influence milk mineral content (Denholm et al., 2019; Zaalberg et al., 2021). Additionally, several studies identified genomic regions associated with variations in mineral content of cattle milk (Sanchez et al., 2021; Singh et al., 2022). We hypothesize that the limited research on dairy sheep in this area is primarily due to the cost, resource-intensive nature, and time-consuming process of reference analysis for mineral content (Soyeurt et al., 2009), which requires large sample sizes and poses substantial challenges. Thus, the aim of this study was to investigate the genetic and non-genetic factors influencing milk yield, composition, and mineral content of two Italian sheep breeds and to provide news insights to support dairy industry in achieving permanent improvements in the quality of sheep milk.

2. Materials and methods

2.1. Sampling of milk and pedigree information of the ewes

The present study was carried out as a part of the Sheep4Cheese Project (M.D. No. 28921 of 18/07/2019; Sheep4Cheese project). The project objective was to investigate the impact of genomics on cheese-making properties of sheep milk not only focusing on increasing the sector competitiveness but also safeguarding biodiversity. To this end, two local breeds, Comisana and Massese, were studied. All sampled animals belonged to a private genetic nucleus farm (Asso.Na.Pa.) located in Asciano, Tuscany (Italy), at 200 m a.s.l., where both breeds are raised and conserved. Comisana is a local breed from central and southern Italy, while Massese originates from the Italian Apennines. Traditionally, these breeds have been reared in semi-extensive and extensive farming conditions, but they are also well-adapted to intensive production systems. In the present study, milk samples (about 0.5 L/ewe) from Comisana ($N = 429$) and Massese ($N = 311$) were collected by farm technicians using volumetric milk meters during morning milking sessions. The ewes were chosen to ensure a representative sample of both breeds, considering factors such as parity, days in milk (DIM) and the overall health status. Along with the milk samples, information related to milk yield (MY; kg/sampling), parity, and days in milk was recorded. These collections took place across eleven sampling sessions (~70 ewes/sampling) conducted between November 2021 and March 2023. Pedigree records for Comisana and Massese included up to fifty generations for individuals that have undergone phenotyping. The complete pedigree datasets included 742,851 animals for Comisana (15,033 sires and 172,481 dams) and 132,685 animals for Massese (3014 sires and 45,626 dams).

2.1.1. Milk quality and mineral profile analyses

Individual milk samples were analysed for fat, protein, lactose and casein percentages using a MilkoScan FT3 infrared analyser (Foss Electric A/S, Hillerød, Denmark) calibrated according to the reference methods (ISO 1211/IDF for fat (ISO-IDF, 2010a), ISO 8968-2/IDF 20–2

for casein (ISO-IDF, 2014), and ISO 26462/IDF 214 (ISO-IDF, 2010b) for lactose). The somatic cell count (SCC, cells/ml) was measured using a Fossomatic 7 DC somatic cell counter (Foss Electric A/S, Hillerød, Denmark) and transformed into the logarithmic score, $SCS = [\log_2(SCC \times 10^{-5}) + 3]$ (Ali & Shook, 1980). Milk pH was measured with a portable pHmeter (sensION Portable Meter fitted with a 5050 T pH electrode (Hach company, CO, USA).

For the mineral profile (mg/kg), Ca, P, Na, K, Mg, S, and Cl were assessed via Wavelength Dispersive X-ray fluorescence (WDXRF; S6 jaguar, Bruker Corporation, Billerica, Massachusetts, USA). This analytical technique has recently gained popularity for the analysis of milk and dairy products, alongside techniques such as Atomic Absorption Spectrophotometry (AAS) and Inductively Coupled Plasma (ICP). One of the key advantages of WDXRF is its ability to analyse samples without requiring decomposition. Moreover, it allows for the simultaneous quantification of minerals and trace elements (Pashkova et al., 2018). To ensure analytical validity, the WDXRF technique was calibrated by correlating the elemental concentrations obtained from a set of samples analysed by both WDXRF and inductively coupled plasma optical emission spectrometry (ICP-OES). Specifically, the technique exploits the photoelectric effect, where incident X-rays excite electrons within the sample atoms, leading to the emission of element-specific fluorescence radiation. Each chemical element emits unique fluorescence radiation, facilitating precise identification and quantification of constituent atoms within the sample. For liquid samples such as milk, no specific preparation is necessary. Approximately 3 mL of milk per sample is placed in a disposable cup and positioned within the instrument for analysis. Helium saturation in the reading chamber was employed to optimize measurement accuracy. Milk samples were scanned at 30 kV to detect P, Mg, S, Na, and Cl, and at 50 kV for Ca and K, with a total scanning time of 3 min. Calibrations were established using Bruker's Spectra.Elements Advance v.3 software.

2.2. Statistical analysis

2.2.1. Data quality and model selection

Prior to statistical analysis, data were checked for incomplete information regarding MY, milk gross composition (fat, protein, casein and lactose), pH, somatic cell score, and mineral content (Ca, P, Na, K, Mg, S, and Cl). Values with missing or erroneous data (e.g., biologically implausible values) were discarded. For each trait, a stepwise regression analysis was conducted to identify the subset of variables producing the best model performance. Briefly, the process involves iteratively adding and removing predictors to the model, calculating an F-statistic for each added predictor, and comparing it to a critical value from the F-distribution. Predictors are considered significant and retained if their F-statistic exceeds the critical value. This process continues until no additional variables can be included or excluded without significantly affecting model performance (Galvão & Araújo, 2009). Moreover, to mitigate and account for possible bias due to overfitting, additional model selection criteria such as Cp, Akaike information criterion (AIC), bayesian information criterion (BIC), and adjusted R^2 , were used to select the best model. Finally, the final model was selected based on both a combination of statistical significance and biological plausibility. The effects of DIM (class 1: 8–30d, class 2: 31–50d; class 3: 51–70d; class 4: 71–90d; class 5: 91–110d; class 6: 111–130d; and class 7: 131–148d), parity order (1,2;3,4; > 5), MY (class 1: 0.3–0.55 kg; class 2: 0.6–0.79 kg; class 3: 0.8–0.97 kg; class 4: 1–2.33 kg), number of samplings, milk gross composition, pH and SCS, were included in stepwise regression. In contrast, MY, milk gross composition, pH and SCS were excluded as predictors when treated as dependent variables. Milk protein also was removed from the casein model because of their high correlation. All the analyses were conducted using the *olsrr* (Hebbali, 2024) R package. The two breeds were analysed separately; however, to assess potential statistical differences between them, a bootstrap resampling test was conducted. Briefly, random samples with replacement were drawn for each

breed (with the same size as the original samples), and the difference between the two-sample means was calculated. This process was repeated 10,000 times to generate a distribution of bootstrap differences. A 95 % confidence interval for the mean differences was then calculated. If the confidence interval includes zero, this suggests that there is not sufficient statistical evidence to conclude a significant difference between the two groups.

2.2.2. Pearson correlation among daily milk yield, quality and mineral profile

Pearson correlations were calculated to quantify the relationships between the evaluated traits, including MY, milk gross composition, pH, somatic cell score, and mineral content, using the *Corrr* R package (Kuhn et al., 2022).

2.2.3. Genetic models

Variance components and genetic parameters were estimated for MY, milk gross composition, somatic cell score, pH and mineral content. This analysis provides insights into underlying genetic architecture of these traits within breed, which in turn shape breeding strategies to optimize dairy sheep performance. To reach this goal, a Bayesian approach with Markov-chain Monte Carlo methods (Sorensen & Gianola, 2002) was employed in a single-trait analysis for all traits. The general animal model was expressed as follows:

$$y = X\beta + Wc + Zu + e$$

where y is a vector of records for the single trait; β is the vector of systematic effects; c is a vector of random sampling effects (uncorrelated) and u is a vector of random direct additive genetic effects and e is a vector of random residual effects. Incidence matrices X , W and Z relate β , c and u to observations. We assumed y as normally distributed: $y | \beta, c, u \sim N(X\beta + Wc + Zu, I\sigma_e^2)$ (Sorensen & Gianola, 2002) and for the effects in W , Z and R , flat priors were assumed.

A Gibbs sampler implemented on Gibbsf90+ software (Misztal et al., 2022) was used to obtain the marginal posterior distribution (MPD) of genetic parameters, using a single chain with 500,000 cycles with a burn-in of 50,000 and then, one out of every 50 iterations was stored. Convergence was verified using graphical analysis from Heidelberger and Welch (1983) and Gweke (1992) methods, as well the *coda* R package (Plummer et al., 2006). For all the analysis, convergence was achieved. Posterior means, standard deviation and the lower and upper bounds of the 95 % highest posterior probability density regions (HPD) were calculated using PostGibbsf90+ program (Misztal et al., 2022). Heritability (h^2) for each trait was estimated as:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2}$$

where: h^2 is the heritability; σ_a^2 is the additive genetic variance; σ_e^2 is the residual variance.

3. Results and discussion

3.1. Descriptive statistics of milk yield, quality and mineral profile

Table 1 presents the descriptive statistics (mean $\pm$ standard deviation) for MY, quality, and mineral content for Comisana and Massese ewes, whereas results from the bootstrap resampling test are in Supplemental Table S1. The average MY per sampling was 0.82 ± 0.33 kg for Comisana ewes, and 0.85 ± 0.36 kg for Massese ewes. Comisana ewes had an average fat content of 6.67 ± 1.10 %, while Massese ewes had 6.80 ± 1.24 %. The average protein content was 5.36 ± 0.59 % for Comisana and 5.48 ± 0.61 % for Massese ($P < 0.05$). Comisana ewes showed an average casein content of 4.16 ± 0.49 %, slightly lower than Massese ewes at 4.26 ± 0.48 % ($P < 0.05$), resulting in a similar casein-

Table 1

Descriptive statistics (mean $\pm$ SD) of milk production, composition and minerals of Comisana and Massese ewes.

	Comisana		Massese	
	Mean	SD	Mean	SD
<i>Production. kg/sampling</i>				
Milk yield	0.82	0.33	0.85	0.36
<i>Composition</i>				
Fat, %	6.67	1.10	6.80	1.24
Protein, %	5.36	0.59	5.48	0.61
Casein, %	4.16	0.49	4.26	0.48
Lactose, %	4.85	0.27	4.76	0.33
pH	6.53	0.09	6.52	0.17
SCS ¹	4.33	2.39	4.81	2.66
<i>Minerals, mg/kg</i>				
Ca	2154	256	2198	264
P	1780	206	1701	216
Mg	227	39	222	44
S	553	68	565	79
K	1260	119	1159	123
Na	721	186	770	221
Cl	962	108	948	125

¹ SCS = $\log_2(\text{somatic cell count} \times 10^{-5}) + 3$.

to-protein ratio (0.78) for both breeds. The average lactose content was 4.85 ± 0.27 % for Comisana and 4.76 ± 0.33 % for Massese ($P < 0.05$). Comisana ewes had a lower SCS ($P < 0.05$) of 4.33 (corresponding to about 250,000 cells/mL of milk), while Massese ewes had 4.81 (corresponding to about 350,000 cells/mL of milk). The milk yield per lactation of the Massese breed highly varied, with historical estimates ranging from 107 to 300 kg, influenced by multiple pregnancies during the year and seasonal factors (Giulioti et al., 2023). Massese milk is particularly valued for cheese-making, notably used in Pecorino della Montagna Pistoiese, a local product of the Tuscany region (Martini et al., 2008), while the Comisana breed is further characterized by its high milk yield (Stocco et al., 2024), with studies indicating that optimized grazing schedules can enhance both yield and quality of milk (Valenti et al., 2017; Zaïdi et al., 2008). In this study, the Ca levels in Comisana and Massese milk were different ($P < 0.05$) and of 2154 ± 256 mg/kg and 2198 ± 264 mg/kg, respectively. The P content was significantly higher in Comisana milk (1780 ± 206 mg/kg) compared to that of Massese milk (1701 ± 216 mg/kg; $P < 0.05$). Moving to Mg, both breeds showed relatively close levels, with Comisana having 227 ± 39 mg/kg and Massese 222 ± 44 mg/kg.

Also, S content in milk displayed significant difference between the two breeds, with Comisana at 553 ± 68 mg/kg and Massese at 565 ± 79 mg/kg. The K was on average at 1260 ± 119 mg/kg in Comisana and 1159 ± 123 mg/kg in Massese milk ($P < 0.05$). The Na was 721 ± 186 mg/kg in Comisana milk, whereas in Massese it was slightly higher at 770 ± 221 mg/kg ($P < 0.05$). Lastly, Cl content in Comisana was similar to that of Massese, with 962 ± 108 mg/kg and 948 ± 125 mg/kg, respectively. Despite the considerable knowledge on MY and quality of milk from Massese and Comisana, there is a noticeable gap in the scientific literature on their milk mineral profile.

Combining multi-elemental analysis with chemometric techniques has proven to be an effective strategy for authenticating cheeses based on factors such as geographical origin, production methods, and milk source. This approach has demonstrated global relevance as, for example, in Brazil, mineral profiling alongside chemometrics was successfully used to authenticate artisanal cheeses and distinguish them by type and region (de Andrade et al., 2022). In Europe, similar successes have been noted: Northern Spanish PDO cheeses showed significant differences in mineral and trace element content, influenced by variables like sheep milk usage, brining, and aging conditions (Moreno-Rojas et al., 2012). Italian Pecorino varieties, including Romano, Sardo, and Farindola, have also been accurately classified by origin through their elemental composition (Di Donato et al., 2021), further

underscoring the worth of this analytical approach.

3.2. Non-genetic effects on milk yield and quality

Table 2 provides a detailed overview of the non-genetic effects selected through stepwise regression and included in the genetic animal model. These effects are economically important enhancing our ability to predict and improve milk minerals effectively. The parity order and DIM were two critical factors that influenced the variability of milk yield and composition in dairy ewes. Parity had a wider effect on Massese breed, impacting a broader range of milk components and MY in comparison to Comisana breed. This difference highlights the varying responses of the two breeds to reproductive cycles and suggests the need for tailored management strategies. In the scientific literature, there are contrasting findings on the effect of parity on milk composition in sheep. For instance, Sevi et al. (2000) found no statistically significant effect of parity on MY in a study involving 24 Comisana ewes. However, the authors observed higher milk quality in terms of protein, casein, fat content, and coagulation properties in ewes during their third parity. In contrast, Pazzola et al. (2014) indicated better coagulation properties in first-parity ewes of Sarda breed compared to those in later parities. These findings may be due to breed-specific traits, differences in management practices, and dynamic physiological changes during different lactation stages. Similarly to parity, DIM had a broader effect on the Massese ewes, affecting all traits, whereas in the Comisana breed, it primarily influenced fat, protein, and lactose. The unique reproductive characteristics and breeding practices of Massese sheep, such as multiple lambings in short intervals and irregular lactation lengths (Giulioti et al., 2023), might explain the broader effect of DIM on milk traits compared to the Comisana breed. These breed-specific factors affect mammary gland development and nutritional requirements, which can, in turn, amplify the effects of lactation stage on milk yield and composition. Again, the effect of MY had a more extensive influence on Massese breed, affecting all traits, compared to the Comisana breed, where it impacted fat, protein, casein, and SCS.

Regarding milk components, breed was an important factor in determining the levels of components in milk. In Comisana breed, fat affected both casein and lactose (Table 2), whose Pearson's correlations were positive (0.44) and negative (−0.29), respectively (Table 3). Like Comisana, fat and casein levels in Massese showed a positive correlation (0.56). Fat content in milk is often positively correlated with casein levels, due to their concurrent synthesis in the mammary gland (Weaver & Hernandez, 2016). This observation aligns with fundamental literature, which highlights that casein plays a critical role in the structure and stability of milk. Casein proteins aggregate into micelles, which help to stabilise fat globules in milk (Fox et al., 2017). Additionally, in Massese breed, casein also influenced lactose levels. Lactose synthesis plays a key role in milk secretion by regulating osmotic balance and the metabolic environment in the mammary gland (Van Vliet & Walstra, 2017), which may influence casein production and explain the observed correlation with fat (−0.37; Table 3). For Comisana breed, lactose content was crucial for fat (−0.29) and SCS (−0.42), while in Massese breed, lactose was significantly correlated with MY (0.32), casein (−0.37), and SCS (−0.34). These findings align with Van Vliet and Walstra (2017), who reported that lactose is inversely related to other milk components but positively related to milk yield, due to its role in osmotic regulation.

Milk pH did not have a notable effect on any traits in Comisana breed. On the contrary, in Massese breed, milk pH influence lactose content and SCS (Table 2). A possible explanation is that variations in milk pH can be indicative of changes in udder health (Bianchi et al., 2004; Pir, 2008), especially when SCS is high, as in the case of Massese. In this breed, higher milk pH was associated with increased SCS, whereas lactose content showed an inverse relationship, likely reflecting the effects of udder infections (Costa et al., 2020; Stocco et al., 2019). The SCS content was found to be important for MY and lactose content in the Comisana breed indicating that the lower content of somatic cells could affect only osmotic aspects related to milk production. In the Massese breed, SCS content was important for MY, fat, and protein content, reflecting the effect of the high content of SCS in milk.

Table 2
Non-genetic and genetic effects included in the Bayesian animal model (*) for milk yield (MY), composition and minerals for Comisana and Massese ewes.

	Milk yield and composition							Minerals, mg/kg						
	MY	Fat	Protein	Casein	Lactose	pH	SCS ¹	Ca	P	Mg	S	Na	K	Cl
Comisana														
Non-genetic effects														
Parity Order	*	*		*			*			*		*	*	*
DIM				*	*		*	*	*	*	*	*	*	*
MY		*	*	*			*		*			*	*	
Fat				*	*			*		*	*	*		
Casein		*						*	*		*	*		
Lactose		*					*			*	*	*	*	*
pH											*			
SCS ¹	*				*				*	*		*		*
Genetic effects														
Direct additive genetic effects	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Sampling	*	*	*	*	*	*	*	*	*	*		*	*	*
Residual	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Massese														
Non-genetic effects														
Parity Order	*	*	*	*	*		*	*	*	*	*		*	*
DIM	*	*	*	*	*	*	*						*	
MY		*	*	*	*		*	*	*	*	*	*	*	*
Fat	*			*	*		*	*		*	*	*		*
Casein		*			*		*	*	*	*	*	*		
Lactose	*		*	*		*	*	*		*	*		*	*
pH					*		*	*	*	*	*	*	*	*
SCS ¹	*	*	*			*			*			*	*	*
Genetic effects														
Direct additive genetic effects	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Sampling	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Residual	*	*	*	*	*	*	*	*	*	*	*	*	*	*

¹ SCS = log₂(somatic cell count × 10^{−5}) + 3.

Table 3
Pearson correlation matrix among milk yield (MY), composition and minerals in Comisana (above the diagonal) and Massese (below the diagonal) breeds.

	Comisana													
	Milk yield and Composition, %										Minerals, mg/kg			
Composition	MY	Fat	Protein	Casein	Lactose	pH	SCS ¹	Ca	P	Mg	S	K	Na	Cl
	1	-0.28***	-0.15***	-0.09	0.22***	0.00	-0.20	-0.11***	-0.03	-0.06	-0.13**	0.10	-0.10***	-0.11***
	Fat, %	1	0.45***	0.44***	-0.29***	-0.07	0.14	0.57***	0.23***	0.44***	0.47***	-0.16***	0.21***	0.05
	Protein, %	-0.27***	0.31***	1	0.96	-0.18	-0.05	0.17	0.43***	0.40***	0.42***	-0.15***	0.34***	0.06
	Casein, %	-0.36***	0.56***	0.61***	1	-0.16	-0.05	0.17	0.43***	0.39***	0.44***	-0.13***	0.34***	0.06
	Lactose, %	0.32***	-0.37***	-0.10	-0.37***	1	0.01	-0.42**	-0.14***	-0.09	-0.28***	-0.24***	0.25***	0.04
Massese	pH	0.16***	-0.08	-0.06	-0.15***	1	-0.09	0.01	-0.10	0.09	0.27***	0.02	0.06	-0.07
	SCS ¹	-0.14	0.29***	0.17***	0.22***	-0.34***	-0.00	1	0.15***	0.24	0.14	-0.18***	0.27	0.08***
	Ca	-0.13	0.37***	0.21***	0.31***	-0.04	0.07	0.15	1	0.36***	0.65***	-0.01	0.37***	0.13***
	P	-0.14	0.22**	0.17**	0.25***	-0.05	0.07	0.17	0.55***	1	0.27***	0.48***	0.16***	0.16***
	Mg	0.07	0.20***	0.13***	0.16***	0.04	0.22	0.14***	0.22***	0.13	1	0.53***	0.61***	0.18***
	S	-0.29***	0.47***	0.43***	0.74***	-0.40***	0.57***	0.26***	0.58***	0.52***	0.08	1	-0.03	0.29***
Minerals, mg/kg	K	0.37***	-0.35***	-0.13***	-0.34***	-0.08***	-0.20	-0.08	0.03	0.22***	-0.33***	1	0.01	0.30***
	Na	-0.08	0.29***	0.11***	0.23***	-0.11	0.17	0.17	0.11	0.73***	0.21***	-0.06	1	0.37***
	Cl	-0.00	0.12**	0.02	0.02	0.26***	0.10**	0.12***	0.10	0.07	0.19***	0.06	0.38***	1

*** Correlation is significant at $P < 0.05$.

3.3. Non-genetic effects on mineral profile

Factors affecting mineral concentration in Comisana and Massese breeds are shown in Table 2. Parity was important for Mg, Na, K and Cl in the Comisana, whereas in Massese, it influenced the levels of all minerals except Na. The effect of DIM was important on all minerals in Comisana except K, conversely, in Massese breed, DIM had a significant impact only on K levels. Similarly, previous studies have shown that mineral concentration in cows (Petrera et al., 2016; Stocco et al., 2019; Toscano et al., 2023), donkeys (Malacarne et al., 2019), goats (Pan et al., 2024) and sheep (Casoli et al., 1989; Polychroniadou & Vafopoulou, 1985) are influenced by parity order and DIM. For example, in Karagouniki and Serron breeds ewes, Mg concentrations increased after the seventh and tenth weeks of lactation, while Na content increased slowly throughout lactation and the K concentrations decreased after the fourteenth week (Polychroniadou & Vafopoulou, 1985). Also in goats, it was reported that the concentration of Ca, Na, Mg, K in milk from Laoshan breed decreased from 1 to 150 days postpartum, followed by an increase between 150 and 240 days (Pan et al., 2024).

The MY was important for P, Na and Cl in the Comisana breed, whereas it affected all the minerals in the Massese breed (Table 2). In both breeds, Pearson's correlations between MY and minerals were negative (Table 3), except for K and Mg in Massese. A study on Holstein cows found that higher Na and Mg levels were associated with lower milk yield, while Ca, K, and Na positively correlated with fat content in higher-yielding cows (Nozad et al., 2012). However, these authors noted that mineral content is not stable across different milking times and cows. In the present study, the distinct physiological adaptations between the Comisana and Massese breeds likely influence how these ewes utilize and excrete minerals during lactation.

Also milk gross composition was found to be important for Ca, Mg, and Na in both breeds and for S and Cl in Comisana and Massese, respectively. Pearson's correlations were generally higher in the Comisana (Table 3), suggesting a breed-specific metabolic pathway. In Holstein cows, similar relationships between Ca, Mg, P, S, and fat content have been reported (Toscano et al., 2023).

Casein content significantly impacted the levels of Ca, P, S and Na in the Comisana, and affected almost all minerals in the Massese, except K and Cl. Pearson's correlations were all positive suggesting a synergistic effect due to the structural properties of casein micelles. These micelles are complexes composed of casein proteins and calcium phosphate, with sulfur also incorporated into their structure through sulfur-containing amino acids such as cysteine and methionine (Lucey & Horne, 2009). Furthermore, Na plays a role in stabilizing casein micelles, helping them remain suspended in milk (Farrell et al., 2006).

Lactose is the least variable component in milk (Miglior et al., 2017). In the present study, it was important for all the minerals except for Ca and P in the Comisana breed, and for Ca, S, K and Cl in the Massese breed (Table 2). Negative correlations were observed with Ca, Mg, and S, while positive correlations were noted with Na, K, and Cl (Table 3). The different importance of lactose on these minerals between the two breeds further highlights that each breed may prioritise different minerals based on their physiological needs and lactation demands. Notably, lactose, K, Na and Cl which collectively play a crucial role in regulating osmotic pressure, showed positive correlations. Toscano et al. (2023) reported negative associations between lactose and Na and K, and positive with P and S in Holstein cows.

As aforementioned, milk pH acts as an important quality indicator and can reflect udder health status (Fox et al., 2017). Monitoring milk pH, along with other traits like minerals, can provide valuable insights into the overall health of the udder and help identify early potential health issues. In the present study, pH influenced S levels in the Comisana breed and all minerals in the Massese breed (Table 2). Pearson's correlations between milk pH and minerals were all positive, except for K (Table 3).

The SCS was a significant factor for P, Mg, Na, and Cl in the Comisana

breed, and P, Na, K, and Cl in the Massese breed (Table 2). Pearson's correlations were all positive in the Comisana, while in the Massese only K showed a negative correlation (−0.20). High SCS levels typically lead to a decrease in several minerals due to mastitis-related stress, except for Na and Cl, which tend to increase. In dairy cattle, Libera et al. (2021) reported increased Cl and decreased P, K and Mg in milk with high SCS, also as a consequence of altered milk pH. Our findings highlight the complexity of how udder health or, more specifically, the level of SCS affects mineral balance.

3.4. Genetic effects and heritability of milk yield and quality

A central question in animal food production is whether the variation of a particular trait is mainly determined by non-genetic or genetic effects, or by a combination of both. The extent to which a trait is influenced by genetic effects determines its potential for permanent improvement through genetic selection. Accurate estimation of genetic parameters depends on the quality and structure of the data, on appropriate statistical methodologies, and on the comprehensive inclusion of relevant confounding factors in the statistical models (Meyer, 2008; Thompson et al., 2005; Weigel et al., 2017). In the present study, genetic parameters for MY, composition and mineral content were estimated using models optimized for the available data structure. Despite the moderate sample size, heritability estimates for MY, milk quality and mineral profile were consistent with those reported in the literature for dairy livestock species, including sheep.

The estimates of variances components and heritability for MY, milk quality and mineral profile for Comisana and Massese breeds are shown in Table 4 (pH was excluded because it did not meet convergence). Heritability for MY was 0.14 and 0.29 for Comisana and Massese, respectively. These values were consistent with previous findings in dairy sheep. For example, the heritability estimates for MY in the Valle del Belice breed was 0.10 (Sutera et al., 2021), 0.21 in the Laxta breed (Legarra & Ugarte, 2001), and 0.24 in the Churra breed (El-Saied et al., 1999). However, the estimates found in this study for the Comisana ewes were lower than those observed in the Greek Chios (0.35) and Lacauene (0.34) breeds (Barillet, 2007; Ligda et al., 2000).

As regards milk fat content, heritability was moderate in both Comisana (0.22) and Massese (0.15) breeds, aligning with values reported in the literature for dairy sheep, which range from 0.06 in the Valachian breed (Oravcová et al., 2005) to 0.12 in various local breeds (Oravcová et al., 2005; Puledra et al., 2017; Sutera et al., 2021).

Heritability estimates for protein percentage differed substantially between Comisana (0.10) and Massese (0.34). However, the value observed in Comisana was similar to those reported for Valle del Belice (0.15; Sutera et al., 2021) and Sarda (0.13; Puledra et al., 2017) ewes. In comparison, heritability estimates for protein fractions are generally higher in dairy cattle, ranging from 0.28 to 0.58 (Ghavi Hossein-Zadeh, 2022, 2024), and show greater variability in goats, from 0.03 to 0.38 (Mucha et al., 2022). Contrary to what occurred with protein, heritability estimates for casein were similar between breeds (0.30 and 0.28 for the Massese and the Comisana, respectively), comparable to estimates from Sarda (0.28) and Churra (0.21) ewes (Bittante et al., 2017; Othmane et al., 2002). These similarities suggest a consistent genetic influence on casein content, despite differences in total milk protein heritability across breeds. This variation could potentially be attributed to differences in the influence of specific protein fractions or the impact of varying environmental conditions on overall protein content.

The heritabilities for lactose were similar between breeds and moderate, with 0.31 for Comisana and 0.29 for Massese. These estimates are slightly higher than those obtained by Selvaggi et al. (2017) using a sire model for Comisana (0.14), Sarda (0.17) and Lecce (0.18), but lower than those reported by Pazzola et al. (2018) for Sarda (0.41). Carta et al. (2023) reported estimates of 0.10 for intra flock heritability for this trait in Sarda ewes.

Heritability estimates for SCS were 0.08 in the Comisana and 0.28 for the Massese breeds. Similar estimates found in Comisana have been reported for Churra sheep (0.11; Othmane et al., 2002) and for Saanen (0.05) and Alpine goats (0.02; Biffani et al., 2020). In contrast, Marshall et al. (2024) reported a heritability of 0.24 for Saanen goats, and Rupp et al. (2011) found a heritability of 0.20 for Alpine goats. These differences may be attributed to variations in breeding strategies, environmental factors, and management practices across different studies and populations.

3.5. Genetic effects and heritability of mineral profile

Despite the importance of mineral composition for the dairy industry, limited research has been conducted on the genetic parameters of milk mineral content in dairy sheep. Indeed, several studies have evaluated mineral content and its heritability across dairy cattle (Buitenhuis et al., 2015; Patel et al., 2018; Stocco et al., 2019; Visentin et al., 2019). Heritability estimates for mineral profile in dairy cattle vary widely, depending on sample size (Buitenhuis et al., 2015; Zaalberg

Table 4

Estimates of additive variance ($\hat{\sigma}_a^2$), sampling variance ($\hat{\sigma}_s^2$), residual variance ($\hat{\sigma}_r^2$), contribution of sampling variance ($\frac{\hat{\sigma}_s^2}{\hat{\sigma}_f^2}$), and heritability (h^2) for milk yield, composition and minerals from Comisana and Massese ewes.

	Comisana					Massese				
	$\hat{\sigma}_a^2$	$\hat{\sigma}_s^2$	$\hat{\sigma}_r^2$	$\frac{\hat{\sigma}_s^2}{\hat{\sigma}_f^2}$	$h^2 + SE$	$\hat{\sigma}_a^2$	$\hat{\sigma}_s^2$	$\hat{\sigma}_r^2$	$\frac{\hat{\sigma}_s^2}{\hat{\sigma}_f^2}$	h^2
<i>Production, kg/sampling</i>										
Milk yield	2.4977	2.7079	15.229	0.1325	0.14 ± 0.10	3.0481	1.9225	5.2136	0.1887	0.29 ± 0.14
<i>Composition</i>										
Fat, %	199.07	166.140	532.81	0.1850	0.22 ± 0.11	137.56	104.77	672.32	0.1145	0.15 ± 0.10
Protein, %	5.1926	44.628	24.988	0.5965	0.10 ± 0.06	73.656	23.572	118.7	0.1091	0.34 ± 0.14
Casein, %	38.68	40.06	48.17	0.3156	0.30 ± 0.11	37.234	36.96	54.901	0.2863	0.28 ± 0.14
Lactose, %	20.203	27.21	15.99	0.4291	0.31 ± 0.10	17.863	19.94	23.311	0.3262	0.29 ± 0.13
SCS ¹	0.350	0.506	3.883	0.1068	0.081 ± 0.06	1.6474	*	4.054	*	0.28 ± 0.12
<i>Minerals, mg/kg</i>										
Ca	18,523	6750.1	21,578	0.1440	0.45 ± 0.11	26,602	5707.9	32,468	0.088	0.41 ± 0.17
P	17,607	3392.3	14,297	0.0961	0.49 ± 0.12	8176.4	7290.9	32,416	0.1522	0.17 ± 0.11
Mg	171.09	1614.7	484.36	0.7112	0.25 ± 0.12	95.452	1820.3	480.22	0.7597	0.16 ± 0.11
S	561.97	*	1072	*	0.34 ± 0.10	362.24	704.66	1507.3	0.2737	0.14 ± 0.11
K	5249.1	*	7811.3	*	0.40 ± 0.11	2581.1	1188.9	5354.9	0.1302	0.28 ± 0.12
Na	2581.6	55,674	6993.3	0.8532	0.29 ± 0.12	2021.5	408,910	11,683	0.9664	0.14 ± 0.14
Cl	3610.2	737.78	4202.5	0.0628	0.42 ± 0.10	2110.8	3172	7066.9	0.2568	0.17 ± 0.12

¹ SCS = $\log_2(\text{somatic cell count} \times 10^{-5}) + 3$.

et al., 2021), phenotyping methodology and genetic variations between breeds or populations (Buitenhuis et al., 2015; Ghavi Hossein-Zadeh, 2022; Toffanin et al., 2015; Visentin et al., 2019). For instance, van Hulzen et al. (2009) using minerals records estimated by inductively coupled plasma-atomic emission spectrometry (ICP-AES) from 1,860 Holstein Friesian, found high intra-herd heritability of 0.57, 0.67 and 0.46 for Ca, P, and K, respectively. When using minerals predicted from mid-infrared spectra (MIR), Sanchez et al. (2021) found heritability estimates for Ca, P, and K in Montbéliarde which averaged around 0.55, while Toffanin et al. (2015), reported lower estimates of heritability for Ca (0.10) and P (0.12) in Holstein-Friesian cows.

In the present study, the method employed for mineral quantification is particularly noteworthy as it can be used in routine practice offering greater precision than predicted quantification methods such as MIR. Heritability estimates for mineral profile varied between Comisana and Massese breeds (Table 4), but overall, were higher than milk traits. Within minerals and between breeds, Comisana showed moderate heritability estimates for P, K, S, and Cl compared to Massese, except for similar estimates of calcium (Ca) heritability (0.45 in Comisana and 0.41 in Massese). This may be due to the genetic control of Ca homeostasis (e.g., genes involved in calcium absorption and bone mineralization). In contrast, the heritabilities for P (0.49 vs 0.17), Cl (0.42 vs 0.17), S (0.34 vs 0.14), and K (0.40 vs 0.18) were markedly higher in Comisana than in Massese, reflecting breed-specific genetic regulation and possible differences in environmental and management factors. Similarly, Zaalberg et al. (2021) reported lower heritability estimates for Ca (0.19 and 0.21 in Danish Jersey and Holstein) and Na (0.13 and 0.17 in Danish Jersey and Holstein) using predicted milk minerals records of approximately 300 cows.

Heritability estimates for Mg and Na were moderate in both breeds, 0.25 and 0.29 in Comisana, and 0.16 and 0.14 in Massese, respectively. These results are lower than estimates reported in dairy cattle (e.g., Mg heritability of 0.60; Sanchez et al., 2021). Visentin et al. (2019) and Costa et al. (2019), using a larger dataset and MIR predicted minerals, reported heritability estimates for Na of 0.32 and 0.37, respectively. High Mg content in milk has been linked to increased coagulation rates but reduced cheese yield, while Na content influences ionic strength (Gaucheron, 2011) and can indicate mammary gland health (Stocco et al., 2021). Heritabilities for S and Cl were higher in Comisana (0.34 and 0.42) compared to Massese (0.14 and 0.17).

Overall, within trait, Comisana and Massese had different estimates in terms of genetic parameters. These results are somewhat expected because of a) the trait genetic architecture within breeds, b) the fitted models and c) the data structure. For example, selection objectives differ between breeds and choosing to select for one trait over another can have varying impacts on genetically correlated traits (e.g., MY has a genetic correlation with K which is 3 times larger in Massese than in Comisana, 0.37 vs 0.10). The stepwise approach used to identify the best model to estimate genetic parameter eventually selected different models within breeds. For example, models used to analyse SCS in Massese and S and K in Comisana did not include the random effect of sampling day. In such a situation the variance due to sampling day is partially absorbed into the additive effect, eventually inflating heritability. As a matter of fact, SCS heritability in Comisana (0.08) was one-third of the SCS heritability in Massese (0.28).

4. Conclusions

This study focused on characterizing milk composition and mineral content in Comisana and Massese sheep breeds to understand their genetic and non-genetic background. Overall, the magnitude of the genetic component is not negligible, especially for mineral contents, which are essential for both nutritional and technological aspects. Potential use of these traits in animal breeding programs could be considered, particularly because, for instance, Ca and P significantly influence milk coagulation, curd firming, syneresis, and cheese yield. Moreover, the use of

WDXRF for the analysis of mineral profile offer the possibility to collect these traits on many samples facilitating their integration in genetic evaluations and breeding strategies. Complementing these programs with the appropriate management practices could further improve milk properties in the short term.

CRedit authorship contribution statement

Johanna Ramirez-Diaz: Writing – original draft, Formal analysis. **Arianna Manunza:** Writing – review & editing. **Giorgia Stocco:** Writing – review & editing, Writing – original draft. **Alessio Negro:** Writing – review & editing, Resources. **Claudio Cipolat-Gotet:** Writing – review & editing, Data curation. **Stefano Biffani:** Writing – review & editing, Supervision, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2025.145483>.

Data availability

Data will be made available on request.

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