Phenotypic and genetic relationships between indicators of the mammary gland health status and milk composition, coagulation, and curd firming in dairy sheep

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ABSTRACT

The present study investigated the effect of somatic cell count, lactose, and pH on sheep milk composition, coagulation properties (MCP), and curd firming (CF) parameters. Individual milk samples were collected from 1,114 Sarda ewes reared in 23 farms. Milk composition, somatic cell count, single point MCP (rennet coagulation time, RCT; curd firming time, k_{20}; and curd firmness, a_{30}, a_{45}, and a_{60}), and CF model parameters were achieved. Phenotypic traits were statistically analyzed using a mixed model to estimate the effects of the different levels of milk somatic cell score (SCS), lactose, and pH, respectively. Additive genetic, herd, and residual correlations among these 3 traits, and with milk composition, MCP and CF parameters, were inferred using a Bayesian approach. From a phenotypic point of view, higher SCS levels caused a delayed gelification of milk. Lactose concentration and pH were significant for many milk quality traits, with a very intense effect on both coagulation times and curd firming. These traits (RCT, RCT estimated using the curd firming over time equation, and k_{20}) showed an unfavorable increase of about 20% from the highest to the lowest level of lactose. Milk samples with pH values lower than 6.56 versus higher than 6.78 were characterized by an increase of RCT (from 6.00 to 14.3 min) and k_{20} (from 1.65 to 2.65 min) and a decrease of all the 3 curd firmness traits. From a genetic point of view, the marginal posterior distribution of heritability estimates evidenced a large and exploitable variability for all 3 phenotypes. The mean intra-farm heritability estimates were 0.173 for SCS, 0.418 for lactose content, and 0.206 for pH. Lactose (favorably), and SCS and pH (unfavorably), at phenotypic and genetic levels, were correlated mainly with RCT and RCT estimated using the curd firming over time equation and scarcely with the other curd firming traits. The SCS, lactose, and pH were significantly correlated with each other's. In conclusion, results reported in the present study suggest that SCS, pH, and lactose affect, contemporarily and independently, milk quality and MCP. These phenotypes, easily available during milk recording schemes measured by infrared spectra prediction, could be used as potential indicators traits for improving cheese-making ability of ovine milk.

Key words: sheep, milk coagulation, somatic cell count, lactose, pH

INTRODUCTION

Although sheep milk production is limited compared with cow milk production, being 2% of total bovine milk yield (Ramos and Juarez, 2011), in many areas dairy ewes and their related production represent an important sector of agricultural activities. This occurrence is particularly evident for southern European countries, where small ruminants are traditionally linked to the territories and cultures (Boyazoglu and Morand-Fehr, 2001).

Given that sheep milk is almost completely processed into cheese, quality of raw milk is a fundamental feature to predict the final cheese yield. Composition and the measured coagulation properties are suitable at the laboratory and dairy industry level to assess the suitability of milk for cheese production (Bencini, 2002; Pazzola et al., 2014). Other aspects affecting milk quality are represented by hygienic traits. The negative influence of udder inflammatory condition on milk and cheese quality is a well-documented topic in dairy cows

(Viguier et al., 2009) and sheep (Albenzio et al., 2004; Leitner et al., 2016).

Several markers for mastitis detection have been proposed both at the farm and dairy-plant level. Somatic cell count is the most used method (Hogeveen, 2011; Kelly et al., 2011). Somatic cells in milk are mainly represented by cells of the immune system and epithelial cells, and the difference between 2 or more consecutive counts are used to estimate inflammatory states and distinguish infected udders, as a positive correlation is present between udder inflammatory response and SCC (Schukken et al., 2003).

In the European Union, the criteria for hygienic production of sheep milk are reported in Regulation 853/2004 (EU, 2004), but no limit has been fixed for SCC. In the United States, sheep milk must meet standards set by the Ordinance of Pasteurized Milk of the Food and Drug Administration (US PMO, 2007), and content of somatic cells must be lower than 750,000/mL. Even with the differences between legal documents dealing with sheep milk SCC, the individual measurement of SCC is a useful tool to reveal subclinical mastitis also in dairy sheep farming (Berthelot et al., 2006; Boyazoglu and Morand-Fehr, 2001; Riggio and Portolano, 2015).

Many parameters other than SCC are proposed as diagnostic tools for mastitis (Hogeveen, 2011; Kelly et al., 2011; Jensen et al., 2016). In the past, lactose and pH changes have been used to estimate udder inflammatory processes (Vanlandingham et al., 1941). Mastitis causes damage to the barrier between blood and milk, the consequent change of fluid flow, and the interrelated variation of both lactose and pH (Poulsen et al., 2015). Recently, the decrease of lactose percentage has been regularly recognized for the detection of mastitis in cattle (Auldist et al., 1995; Gonçalves et al., 2016). Several studies have also estimated the genetic correlations between these milk traits and coagulation properties in both dairy cattle (Bittante et al., 2012) and sheep (Othmane et al., 2002; Puledda et al., 2017).

Up to date, no study has been conducted to simultaneously investigate, at the field level, the effect of milk quality markers on a complete range of ovine milk quality and technological traits. Therefore, the aims of the present study were (1) to investigate the effect of SCC, lactose, and pH on sheep milk composition, coagulation properties (**MCP**), and curd firming (**CF**) parameters; (2) to assess the relevance of the aforementioned indicator traits of mammary gland status in explaining the variation of technological traits by estimating the additive genetic correlations between SCC, lactose, and pH, and milk composition, MCP, and CF parameters.

MATERIALS AND METHODS

Animals and Milk Sampling

The present study is based on data recorded from 1,114 Sarda ewes reared in 23 different commercial farms located in Sardinia, Italy. Animals and farms are described in Pazzola et al. (2014). To obtain a representative sample of health animals, ewes were submitted to clinical examinations by veterinarians with experience in the field of small ruminant practice. Ewes showing clinical mastitis, and any other evident disease, were discarded, whereas healthy ewes were retained and sampled. This sampling strategy allowed to focus our study on associations between milk parameters and deviating levels of SCS, lactose, and pH.

Sampled ewes ranged in number from 22 to 89 per each farm; they were between 2 and 7 mo after parturition, and from first to seventh parity.

Ewes from each farm were individually sampled on a single day. Milk samples were collected in 200-mL disposable sterile plastic containers during the afternoon milking and refrigerated at 4°C. Daily milk yield (morning plus evening milking) was recorded on the same day of collection.

Analysis of Milk Traits and Coagulation Properties

Milk samples were analyzed within 24 h after collection for milk composition [fat, protein, casein, lactose, and pH; casein number (%) calculated as the ratio between casein and protein contents], SCC, and single point MCP (rennet coagulation time, **RCT**, as the interval between rennet addition and gel formation; curd firming time, k_{20}, as the interval between gel formation and a curd firmness of 20 mm; and curd firmness a_{30}, a_{45}, and a_{60}, 30, 45, and 60 min after rennet addition, respectively). Lactose and pH were measured using a MilkoScan FT6000 milk analyzer (Foss Electric A/S); SCC with a Fossomatic 5000 somatic cell counter (Foss Electric A/S); the others traits were measured using the methods reported in Pazzola et al. (2014). The model parameters of curd-firming [curd firmness at an infinite time (C_{FP}, measured in mm), curd-firming instant rate constant (k_{CF}, $\% \times min^{-1}$), rennet coagulation time from the result of modeling (RCT_{eq}, min), syneresis instant rate constant (k_{SR}, $\% \times min^{-1}$) that tends to reduce curd firming over time (CF_t) beyond a maximum curd firmness (CF_{max}, mm) after a given time interval (t_{max}, min)] were measured as reported in Vacca et al. (2015). The analysis of both the traditional MCP and parameters of CF_t modeling was performed

to achieve a deeper description of milk coagulation traits: the former are more common and the latter are more informative of the complete pattern recorded by the lactodynamograph (Bittante et al., 2012; Vacca et al., 2015).

As regards MCP, RCT was labeled as missing for values higher than 30 min (n = 5) and for not-coagulating samples (RCT >60 min, n = 10); k_{20} was labeled as missing for values higher than 5 min (n = 19); values of curd firmness were missing at 0 mm (a_{30}: n = 15; a_{45}: n = 10; a_{60}: n = 7). To normalize the distribution, SCC was transformed in the logarithmic score SCS [$\log_2(SCC \times 10^{-5}) + 3$]; Ali and Shook (1980).

Statistical Analysis

Phenotypic Analysis. Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) according to the following linear model:

$$y_{ijklmnopqr} = \mu + DIM_i + parity_j + MUCM_k + dMY_l$$
$$+ \text{flock size}_m + \text{farm(flock size)}_{nm} + SCS_o$$
$$+ \text{lactose}_p + pH_q + e_{ijklmnopqr}, \qquad [1]$$

where $y_{ijklmnopqr}$ is the observed trait; μ is the overall intercept of the model; DIM_i is the fixed effect of the ith class of DIM (i = 1 to 8; class 1: 61 to 80 d, class 2: 81 to 100 d, class 3: 101 to 120 d; class 4: 121 to 140 d; class 5: 141 to 160 d; class 6: 161 to 180 d; class 7: 181 to 200 d; class 8: >200 d); $parity_j$ is the fixed effect of the jth parity of the ewes (j = 1 to 5 or more); $MUCM_k$ is the fixed effect of the kth measuring unit of the coagulation meter (k = 1 to 10); dMY_l is the fixed effect of lth class of single test-day milk yield (l = 1 to 7: class 1 <0.55; class 2: 0.55 to 0.96; class 3: 0.97 to 1.38; class 4: 1.39 to 1.79; class 5: 1.80 to 2.21; class 6: 2.22 to 2.64; class 7 >2.64); flock size$_m$ is the fixed effect of mth flock dimension (m = 1 to 3; class 1: <300 ewes; class 2: 300 to 600 ewes; class 3: >600 ewes); farm(flock size)$_{nm}$ is the random effect of the farm/test day nested within flock size; SCS_o is the fixed effect of oth class of milk SCS (o = 1 to 7: class 1 <2.07; class 2: 2.07 to 3.12; class 3: 3.13 to 4.18; class 4: 4.19 to 5.25; class 5: 5.26 to 6.31; class 6: 6.32 to 7.37; class 7 >7.37); lactose$_p$ is the fixed effect of pth class of milk lactose content (p = 1 to 7: class 1 <4.47; class 2: 4.47 to 4.61; class 3: 4.62 to 4.75; class 4: 4.76 to 4.89; class 5: 4.90 to 5.03; class 6: 5.04 to 5.17; class 7 >5.17); pH_q is the fixed effect of qth class of milk pH (q = 1 to 7: class 1 <6.56; class 2: 6.56 to 6.61; class 3: 6.62 to 6.65; class 4: 6.66 to 6.69; class 5: 6.70 to 6.73; class 6: 6.74 to 6.78; class 7 >6.78)

and $e_{ijklmnopqr}$ is the random residual. Each of the 7 classes of SCS, lactose, and pH were designed on the basis of distribution of the variables: each single class explained 0.5 standard deviation of the variable; the fourth was centered on the mean value; and the first and the seventh represented the tails of the distribution. The farm/test day nested within flock size and residuals were assumed to be independently and normally distributed with a mean of zero and variance σ_F^2 and σ_e^2. Percentage of variance explained by the farm was calculated by dividing the corresponding component of variance by the total variance.

Polynomial contrasts (linear, quadratic, and cubic component) were estimated to look at the effect of SCS, lactose, and pH on milk yield, quality, MCP, and CF.

Genetic Analysis. Nongenetic effects previously described were taken into account in estimating the genetic parameters between the 3 indicators of the mammary gland health status (i.e., SCC, lactose, and pH) and milk composition, MCP, and CF parameters. Briefly, the model accounted for the effects of farm-date (23 levels), DIM (8 levels), parity of the ewes (1 to 5 or more), and the measuring unit of the coagulation meter (**MUCM**; 10 levels, based on the position of the individual pendulum).

Bivariate sire models were fitted to estimate (co)variance components for the traits of concern. The model assumed was

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z}_1\mathbf{f} + \mathbf{Z}_2\mathbf{s} + \mathbf{e}, \qquad [2]$$

where $\mathbf{y}$ is a vector of records for traits 1 and 2; $\mathbf{X}$, $\mathbf{Z}_1$, and $\mathbf{Z}_2$ are appropriate incidence matrices for systematic effects in $\mathbf{b}$, farm/date effects in $\mathbf{f}$, and sire genetic effects in $\mathbf{s}$, respectively; and $\mathbf{e}$ is a vector of random residuals. (Co)variance components and related parameters were estimated using a Bayesian approach and Markov-chain Monte Carlo methods (Sorensen and Gianola, 2002). All traits were taken as continuous variables, and their values were assumed to be sampled from the following multivariate normal (**MVN**) distribution:

$$p(\mathbf{y}|\mathbf{b}, \mathbf{f}, \mathbf{s}, \mathbf{R}) \sim MVN(\mathbf{Xb} + \mathbf{Z}_1\mathbf{f} + \mathbf{Z}_2\mathbf{s}, \mathbf{I} \otimes \mathbf{R}), \qquad [3]$$

where $\mathbf{y}$, $\mathbf{b}$, $\mathbf{f}$, $\mathbf{s}$, $\mathbf{X}$, $\mathbf{Z}_1$, and $\mathbf{Z}_2$ are as defined above; $\mathbf{R}$ is a 2 × 2 matrix of residual (co)variances; and $\mathbf{I}$ is an identity matrix of appropriate order. The data were properly ordered within the vectors, and vectors $\mathbf{s}$ and $\mathbf{f}$ contained the effects of both traits individual by individual.

In a Bayesian setting, we assumed

$$p\left(\mathbf{s} \mid \mathbf{G}\right) \sim MVN\left(0, \mathbf{A} \otimes \mathbf{G}\right)$$

and

$$p\left(\mathbf{f} \mid \mathbf{F}\right) \sim MVN\left(0, \mathbf{I} \otimes \mathbf{F}\right),$$

where $\mathbf{G}$ is a 2×2 sire (co)variance matrix, $\mathbf{A}$ is the additive genetic relationship matrix among sires, $\mathbf{F}$ is a 2×2 (co)variance matrix for farm/date effects, and $\mathbf{I}$ is the identity matrix of the same order as the number of levels of farm/date effects. Flat priors were assumed for the effects in $\mathbf{b}$, as well as for $\mathbf{G}$, $\mathbf{F}$, and $\mathbf{R}$. Marginal posterior distributions of all unknowns were estimated using the Gibbs sampling algorithm. The TM program (http://snp.toulouse.inra.fr/~alegarra) was used for all Gibbs sampling procedures. The lengths of the chain and the burn-in period were assessed by visual inspection of trace plots, and by the diagnostic tests described by Geweke (1992) and Gelman and Rubin (1992). After some preliminary analysis, chains of 850,000 samples were used, with a burn-in period of 50,000. Subsequently, 1 in every 200 successive samples was retained. The mean was used as point estimate of parameters of concern. For the phenotypic, genetic, farm, and residual correlations, besides the mean of each marginal posterior distribution, we also estimated the probability of each mean being greater than 0 when the mean is positive, or lower than 0 when the mean is negative (P). We considered all estimates with P greater than 90% as "relevant" correlations. A more detailed description of these features can be found in Blasco (2005).

Intra-farm heritability (h^2) was computed as

$$h^2 = \frac{4\sigma_s^2}{\sigma_s^2 + \sigma_e^2},$$

where σ_s^2 and σ_e^2 are sire and residual variances, respectively.

The proportion of the total variance caused by farm-date (h_f) was computed as

$$h_f = \frac{\sigma_f^2}{\sigma_s^2 + \sigma_f^2 + \sigma_e^2}.$$

Here, σ_s^2, σ_f^2, and σ_e^2 were additive sire, farm-date, and residual variances, respectively.

The additive genetic correlations (r_a) were computed as

$$r_a = \frac{\sigma_{s1,s2}}{\sigma_{s1} \cdot \sigma_{s2}},$$

where $\sigma_{s1,s2}$ was the sire additive sire covariance between traits 1 and 2; and σ_{s1} and σ_{s2} were the sire genetic standard deviations for traits 1 and 2, respectively.

The phenotypic correlations (r_p) were computed as

$$r_p = \frac{\sigma_{p1,p2}}{\sigma_{p1} \cdot \sigma_{p2}},$$

where $\sigma_{p1,p2}$ was the phenotypic covariance between traits 1 and 2; and σ_{p1} and σ_{p2} were the phenotypic standard deviations for traits 1 and 2, respectively. The phenotypic variance and covariance were computed by summing the 3 random effects (co)variance components.

The farm-date correlations (r_f) were computed as

$$r_f = \frac{\sigma_{f1,f2}}{\sigma_{f1} \cdot \sigma_{f2}},$$

where $\sigma_{f1,f2}$ was the farm-date covariance between traits 1 and 2, and σ_{f1} and σ_{f2} were the farm-date standard deviations for traits 1 and 2, respectively.

The residual correlations (r_e) were computed as

$$r_e = \frac{\sigma_{e1,e2}}{\sigma_{e1} \cdot \sigma_{e2}},$$

where $\sigma_{e1,e2}$ was the residual covariance between traits 1 and 2, and σ_{e1} and σ_{e2} were the residual standard deviations for traits 1 and 2, respectively.

RESULTS AND DISCUSSION

Descriptive Statistics and Source of Variation of Milk Quality and Technological Properties

Table 1 summarizes the descriptive statistics of milk yield, composition, and coagulation traits, and the results from the linear model [1]. The effects of flock size, individual farm within flock size, stage of lactation, parity, daily milk yield, and the MUCM (individual pendulum of the Formagraph instrument) on milk traits are reported and discussed in 2 previous papers using data sets interrelated to the one of the present study (Pazzola et al., 2014; Vacca et al., 2015).

The average value of daily milk yield was in agreement with recent papers on the same sheep breed (Sitzia et al., 2015; Puledda et al., 2017) but higher

Table 1. Descriptive statistics (mean and SD) and results from the linear model for yield, components, and coagulation traits of milk from Sarda sheep (n = 960) with F-value and significance for fixed effects and the proportion of variance (in percentage) explained by the farm effect[1]

Trait	Mean	SD	Flock size	Farm	DIM	Parity	dMY	SCS	Lactose	pH	MUCM	RMSE
Daily milk yield, g/d	1,594	833	0.1	71	14.8***	16.4***	—	0.7	1.0	1.0	—	412
Milk quality trait												
Fat, %	6.4	1.1	1.2	32	6.5***	1.0	6.3***	2.2*	9.7***	1.0	—	0.80
Protein, %	5.4	0.6	1.8	36	12.3***	1.3	5.7***	1.6	12.0***	1.1	—	0.44
Fat:protein ratio	1.2	0.2	2.6	39	1.8	1.5	2.0	2.5*	2.1*	1.4	—	0.13
Casein, %	4.2	0.5	1.9	34	12.2***	1.1	6.0***	1.8	8.1***	1.1	—	0.37
Casein number, %	77.9	1.8	0.6	25	5.7***	1.1	5.2***	1.4	7.2***	4.7***	—	0.90
Single point MCP[2]												
RCT, min	8.7	4.1	0.3	28	1.1	1.2	1.3	8.3***	18.0***	78.7***	2.0*	2.27
k_{20}, min	1.9	0.5	2.1	17	1.3	0.8	1.9	2.1*	3.3**	45.0***	9.3***	0.37
a_{30}, mm	50.2	11.7	3.6	45	0.8	2.2	3.2**	0.6	2.2*	10.7***	15.1***	7.96
a_{45}, mm	45.9	14.6	4.3*	39	1.6	1.9	2.0	1.0	3.5**	3.4**	14.6***	10.25
a_{60}, mm	42.1	16.3	3.4	32	0.9	1.3	1.1	1.0	2.0	3.0**	14.7***	12.17
CF_t parameter[3]												
RCT_{eq}, min	9.8	4.7	0.3	27	0.7	0.8	0.8	7.1***	17.9***	80.2***	2.0*	2.47
k_{CF}, %/min	26.3	9.4	2.7	31	1.4	1.7	1.6	1.5	4.0***	11.1***	4.9***	6.43
k_{SR}, %/min	1.0	0.7	3.0	39	1.2	3.0*	2.0	0.7	2.4*	1.0	7.4***	0.47
CF_P, mm	62.5	9.2	4.7*	38	0.6	2.9*	1.1	0.4	1.7	19.6***	24.7***	6.19
CF_t derived trait[4]												
CF_{max}, mm	54.4	9.3	4.7*	38	0.6	2.9*	1.1	0.4	1.7	19.6***	24.7***	6.19
t_{max}, min	28.0	9.8	3.7*	24	0.7	0.6	0.3	1.2	4.8***	2.7*	3.7***	7.80

[1]DIM = days in milk at the stage of lactation; dMY = daily milk yield; MUCM = measuring unit of the coagulation meter (individual pendulum 1 to 10); RMSE = root mean squared error.

[2]MCP = milk coagulation properties; RCT = rennet coagulation time of samples coagulating within 60 min of enzyme addition; k_{20} = curd-firming time of samples reaching 20 mm of firmness within 60 min from enzyme addition; a_{30} = curd firmness at 30 min after enzyme addition; a_{45} = curd firmness at 45 min after enzyme addition; a_{60} = curd firmness at 60 min after enzyme addition.

[3]RCT_{eq} = rennet coagulation time estimated using the curd firming over time (CF_t) equation; k_{CF} = curd firming instant rate constant; k_{SR} = syneresis instant rate constant; CF_P = asymptotic potential curd firmness.

[4]CF_{max} = maximum curd firmness attained within 45 min; t_{max} = time at attainment of CF_{max}.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

than those published in older ones (Macciotta et al., 1999; Pulina et al., 2005). This result may be indicative of a fast increasing of milk yield in the Sarda sheep throughout the last decades, which is a general feature of genetic schemes in dairy sheep breeds (Carta et al., 2009). On the other hand, the negative effects of a fast increasing of milk yield are well known in dairy farming. The increase in milk yield per cow has been proven as one of the causes for increased incidence of mastitis and reproductive disorders, because of the unfavorable genetic correlation between those traits (Koeck et al., 2014). Fat, protein, casein, lactose concentration, and pH were in line with data recorded in previous studies on sheep milk (Ramos and Juarez, 2011), and specifically, on the Sarda breed (Pulina et al., 2005; Manca et al., 2016).

In the present study, we decided to include simultaneously all the 3 mastitis indicators in the analysis model to assess the effect of each single trait (SCS, lactose, and pH) corrected (independently) for the other indicators. In a preliminary model, we also tested these indicators one at a time with the other fixed effects (DIM, parity, MUCM, dMY, and flock size; data not reported in the study). Results obtained with the 2 different models (indicators included together in the model vs. each indicator at a time) were similar in terms of the significance of SCS, lactose, and pH effects (F-values and significance slightly lower when tested together), and the least squares means patterns of coagulation traits were absolutely similar. Given that each of 3 indicator were correlated with each other, it was not unexpected that in the preliminary model with one trait at a time, the significance level for each one was greater than in the final combined analysis.

Effect of SCS on Milk Quality and Technological Properties

Values of SCC of the data set (mean values and SD 1,251 ± 2,991 × 1,000 cells/mL, data not shown in tables) were characterized by a very large range of variation (Pazzola et al., 2014). In agreement with the review by Riggio and Portolano (2015), a value of SCC up to one million per mL is a normal finding in milk from healthy ewes. In particular, 291 out of 1,114 samples of our study, 26%, were higher than the limit of 750,000/mL reported in the US PMO (2007). Mean values and standard deviation of the logarithmic value, SCS, was 4.71 ± 2,13, data not shown in tables.

The effect of SCS was significant for some of the measured traits, and specifically for fat content and fat:protein ratio at $P < 0.05$, as well as for RCT and RCT$_{eq}$ at $P < 0.001$ (Table 1). According to SCS classes, the

values for fat content and fat:protein ratio followed a nonlinear quadratic trend (Supplemental Table S1; https://doi.org/10.3168/jds.2017-13975). This finding is not in agreement with other studies on dairy species, but the diversity of the protocols used, SCC levels, and statistics should be noted. Albenzio et al. (2004), in Comisana ewes, and Bobbo et al. (2016), in dairy cows, find that the most significant effect of SCC is on protein and lactose content, but not on fat content. Those authors also report a general inconsistency of results among the studies dealing with the effect of SCC on milk composition, and speculate that the inflammatory process associated with the high levels of SCC could affect the mammary cells during biosynthesis of both protein and fat fractions.

Among the coagulation single point traditional MCP and CF traits, the effect of SCS significantly affected RCT and RCT$_{eq}$ (Table 1), but not a$_{30}$, a$_{45}$, and a$_{60}$. This is in accordance with the finding that curd firmness in the sheep species is almost independent of coagulation time (Pazzola et al., 2014). Despite the significant curvilinear pattern (quadratic contrast) for RCT and RCT$_{eq}$ (Supplemental Table S1; https://doi.org/10.3168/jds.2017-13975), the highest mean values were recorded for milk samples with SCS higher than 7.37. The delayed milk coagulation is in agreement with previous studies. With regard to dairy cows, Bobbo et al. (2016) have recently shown that SCC have a nonlinear effect on milk technological traits. With regard to sheep, Albenzio et al. (2004), in an investigation focused on single point MCP up to 30 min, reported a more evident increase of coagulation time with high SCC, especially in the final stages of lactation. Also, Rovai et al. (2015) reported that RCT of sheep milk is even doubled for infected vs. uninfected udder. In addition, the study by Vacca et al. (2015) showed that milk samples classified as not coagulated, on the basis of curd-firming modeling, are characterized by the highest value of SCS, which is about 2-fold compared with coagulated samples. The influence of SCC on rennet coagulation times and the CF$_t$ models' parameters are shown in Figure 1a. This summarizes that when SCC was very high (>7.37 SCS), the time after rennet addition to gelification was delayed. On the other hand, the slightly higher CF$_t$ values shown after the reaching of the maximum value of curd firmness (CF$_{max}$) of the samples belonging to the lowest SCS class (<2.07) were not significantly different from the other classes. The effect of SCC on milk coagulation, also in animals without clinical signs of mastitis, is attributable to the proteolytic effect of some enzymes [e.g., plasmin, responsible for the augmented degradation of caseins (Poulsen et al., 2015)].
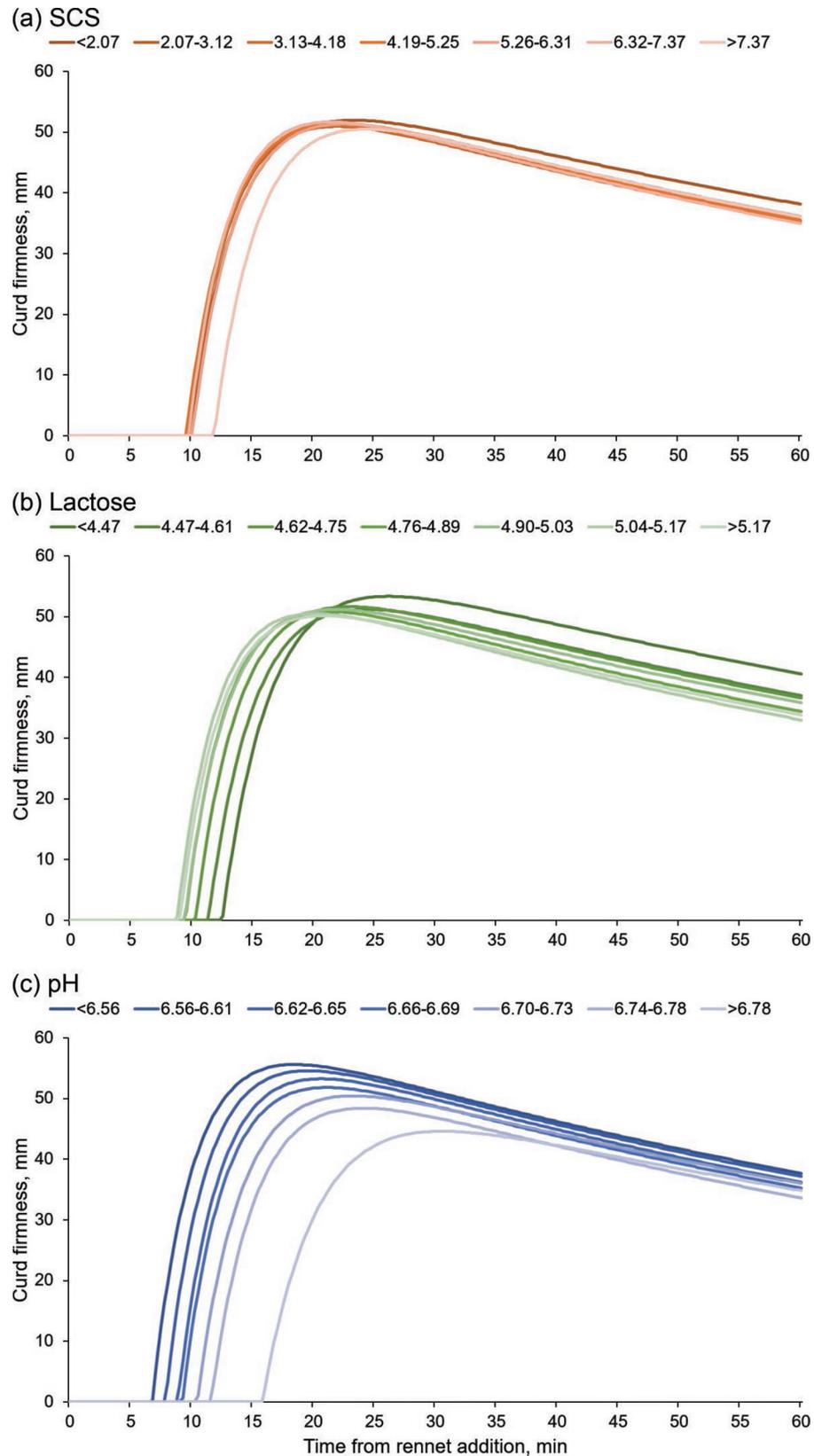
Figure 1. Pattern of curd firmness after rennet addition [curd firming over time (CF_t) modeling] of milk samples according to SCS (a), lactose concentration (b), and pH (c) effects. Color version available online.

Effect of Lactose on Milk Quality and Technological Properties

Mean values and standard deviation for lactose were 4.81 ± 0.28 g/100 mL (data not shown in tables). Lactose concentration was significant for almost all the measured milk traits, with high values of significance (Table 1). In particular, fat, protein, and casein content, and fat:protein ratio were characterized by a linear decrease with increasing percentage of lactose classes, whereas casein number was the opposite. Coagulation times and CF were shorter up to about 20% from the lowest to the highest class of lactose content: RCT from 11.4 to 8.2, RCT_{eq} from 12.5 to 9.0 and k_{20} from 2.08 to 1.94 min. Classes of lactose higher than 4.76% were characterized by a general linear decrease of curd firmness values, specifically a_{30} and a_{45}. Analysis of modeling of curd firmness summarized all these findings. Indeed, contrasts revealed a linear trend with the lowest lactose classes characterized by the decrease of k_{CF} and k_{SR} and the increase of CF_p, CF_{max} and t_{max} (Supplemental Table S2; https://doi.org/10.3168/jds .2017-13975). These results led to the generalized delay of coagulation, the increase of the maximum CF, and the slowdown of syneresis processes for the samples belonging to the lowest classes of lactose, as clearly shown in Figure 1b. The valid mechanisms to explain the association between lactose and MCP is still to be clarified (Poulsen et al., 2015). In accordance with the role of lactose as an osmotic regulator in milk demonstrated by those last authors, and the negative association between lactose and protein in our study (the lower the lactose, the higher the protein content; Table 3), we can speculate that the real mechanism was fairly linked to the modification of percentage and composition of minerals and proteins.

Results of the present study showed that lactose concentration lower than 4.61% should be considered as an alarm because of the deterioration of technological traits of milk gelification and are consistent with other authors who consider the reduction of lactose content as a potential indicator of subclinical mastitis. Bianchi et al. (2004), in research performed on Sarda ewes, recorded a decrease of lactose from 4.80 to 4.51 g/100 mL in healthy versus infected udder. Also, Vivar-Quintana et al. (2006) detected that in Spanish sheep the concentration of lactose is significantly lower with the increase of SCC. Manca et al. (2016) have recently demonstrated a marked negative correlation between lactose and SCS. Many authors have tried to explain the uniformity of results throughout the different papers and the reliability of lactose as a marker for udder health. Munro et al. (1984), and more recently, Vivar-Quintana et al. (2006), speculated that mammary epithelial cell damage due to the inflammatory process can reflect on the lactose content and that a decreased blood flow to the udder can reduce the availability of glucose for the synthesis of lactose. Albenzio et al. (2004) suggested that the lower content of lactose in milk samples with high SCC is attributable to a partial replacement of lactose with other osmotically active components such as the chlorides.

Effect of pH on Milk Quality and Technological Properties

Mean values and standard deviation for pH were 6.67 ± 0.09 (data not shown in tables). The pH had a high significant effect almost exclusively on coagulation traits, except k_{SR} (Table 1). All significant trends were of the linear type. Starting from the first pH class (milk samples with pH lower than 6.56) to the last (higher than 6.78), the increase of single point RCT (from 6 to 14.3 min), k_{20} (from 1.65 to 2.65 min), and the decrease of the 3 curd firmness a_{30}, a_{45}, and a_{60} were recorded (Supplemental Table S3; https://doi.org/10.3168/jds .2017-13975). Analysis of the models' parameters also revealed the concurrent increase of the estimated RCT_{eq} and t_{max}, and the decrease of k_{CF}, CF_p, and CF_{max} (Supplemental Table S3), with an intense worsening of the arched line summarizing coagulation and syneresis process, mainly for the samples with pH higher than 6.78 (Figure 1c).

Among the milk traits, pH is often indicated as a marker of udder inflammation for both cattle (Kelly et al., 2011) and sheep species (Albenzio et al., 2004). The results reported in the present study are in agreement with previous data available in the literature. The pH is one of the most important parameters influencing the stability of casein micelles and thus the milk coagulation (Bencini, 2002; Pirisi et al., 2007). Milk samples with pH tending to basicity are characterized by longer clotting times. Bittante et al. (2017), analyzing the phenotypic correlation among the milk traits of a data set related to the one of the present study, recently highlighted a negative effect of high pH on coagulation traits, especially coagulation times, but not curd syneresis.

Heritability of SCS, Lactose, and pH and Correlations with Milk Quality and Technological Properties

The heritability estimates of ovine milk SCS, lactose, and pH according to a Bayesian approach is shown in Table 2. A marked uncertainty was demonstrated for all 3 parameters. The mean value of intra-farm heritability estimates for SCS was 0.173. The first estimates found in literature are usually much lower. The value

Table 2. Estimates of sire variance $\left(\sigma_s^2\right)$ farm-date variance $\left(\sigma_f^2\right)$ residual variance $\left(\sigma_e^2\right)$ intra-farm heritability, and farm-date variance as proportions of total variance (h_f) for SCS, lactose, and pH[1]

Trait	σ_s^2		σ_f^2		σ_e^2		h^2		h_f
	Mean	PSD	Mean	PSD	Mean	PSD	Mean	PSD	
SCS	0.158	0.08	1.105	0.43	3.461	0.15	0.173	0.08	
Lactose, %	0.007	<0.01	0.021	<0.01	0.063	<0.01	0.418	0.11	0.227
pH	0.0003	<0.01	0.003	<0.01	0.007	<0.01	0.206	0.11	0.275

[1]Mean = mean of the marginal posterior density of the parameter; PSD = posterior standard deviation.

for Spanish Churra ewes is 0.04 (Baro et al., 1994), but as reviewed by Carta et al. (2009) on various breeds, the large majority of estimates ranges in the interval between 0.10 and 0.20. de la Fuente et al. (2011) found a heritability for SCS at 0.09 in primiparous and 0.13 in multiparous Churra ewes. Also, Riggio et al. (2010), on Valle del Belice sheep, estimated a heritability value at 0.09. The same authors found that the heritability value of SCS was only 0.03 in ewes with positive milk bacterial count and 0.10 for ewes with negative bacterial count, with the genetic correlation between the 2 traits being 0.62. Also, Puledda et al. (2017), on the Sarda breed, reported very low values of heritability, particularly for SCS at 0.03.

The phenotypic, genetic, farm, and residual correlations between SCS and the other milk yield, quality, and technological traits are summarized in Table 3. Correlations of SCS with milk yield were low (−0.259 to +0.130) as were correlations with fat and protein content (+0.058 to +0.335), which were positive and consequently unfavorable.

The information in the literature on genetic correlations between SCS and other milk traits is not exhaustive. el-Saied et al. (1998) showed small phenotypic and genetic correlations between SCS and milk yield and protein content. As reviewed by Carta et al. (2009), phenotypic and genetic relationships between SCS and milk yield and composition are generally low and not consistent among different studies.

In our study, SCS was much more positively (unfavorably) correlated with RCT and RCT_{eq} with estimates between +0.449 (farm correlation between SCS and RCT) and +0.616 (genetic correlation between SCS and RCT). Correlations between SCS and the other traditional MCP were much lower, with the remarkable exception of the genetic unfavorable correlations with k_{20} (+0.588) and a_{30} (−0.468). Also, for CF_t equation parameters and derived traits, the correlations with SCS were generally low, with the remarkable exception of the positive (+0.521) genetic correlation with the time required to achieve the maximum curd firmness (t_{max}). Puledda et al. (2017), on the same sheep breed, obtained similar results for SCS phenotypic correlations

with RCT and k_{20}, whereas genetic correlations were very different, being very small and negative (−0.14 between SCS and RCT) and very large and negative (−0.72 between SCS and k_{20}).

With regard to the other 2 traits, the mean value of intra-farm heritability of lactose content and pH of ovine milk were 0.418 and 0.206, respectively (Table 2). Due to the limited data available in the literature, the only comparison could be done with the value of 0.16 recorded for pH by Puledda et al. (2017) on the same breed, which is similar to our estimate.

The phenotypic, genetic, farm, and residual correlations between lactose and pH, and the other milk traits are reported in Table 3. Similarly to the results evidenced for SCS, correlations with milk yield were generally moderate (−0.277 to +0.247) with the exception of the larger correlation between lactose content and milk yield at the farm level (+0.430). Lactose showed negative correlations with fat, protein, and casein contents of milk (−0.082 to −0.475), whereas pH was not correlated with milk composition, except for the positive genetic correlation with protein and casein. Also, lactose (favorably) and pH (unfavorably) were much more correlated at the phenotypic, genetic, farm, and residual levels, with RCT, RCT_{eq}, k_{20}, and t_{max} (only genetically for pH), and less with the other CF traits.

Analysis performed on the effects of SCS, lactose, and pH clearly evidenced that these 3 milk traits showed very high values of correlation with each other's (negatively for lactose with SCS and lactose with pH, positively for SCS with pH; Table 3). Multivariate factors analyses of milk traits have been carried out in different dairy species, and in all of the cases an udder health latent explanatory variable was proposed. In the bovine species, Macciotta et al. (2012) reported an udder health factor (which is the fourth explanatory factor of that data set, explaining 10% of total variance), mainly based on lactose (coefficient +0.818) and SCS (−0.651), but not on milk pH. In the Sarda goat, Vacca et al. (2016) found a hygiene factor (second, 19% of total variance) based on SCS (−0.83), lactose (+0.71), and also log bacterial count (−0.70), but not on milk pH. In the Girgentana goat, Todaro et al. (2005) reported that a

Table 3. Features of the marginal posterior density of phenotypic (r_p), additive genetic (r_a), farm/date (r_f), and residual (r_e) correlations between SCS, lactose, and pH, and single test-day milk yield, composition, traditional milk coagulation properties (MCP), curd firming (CF_t) model parameters, and maximum curd firmness traits[1]

Trait	SCS				Lactose, %				Milk pH			
	r_p	r_a	r_f	r_e	r_p	r_a	r_f	r_e	r_p	r_a	r_f	r_e
Milk yield, kg/d	−0.163	0.130	−0.259	−0.134	0.247	−0.098	0.430	0.159	−0.184	−0.221	−0.277	−0.119
Milk composition												
Fat, %	0.108	0.058	0.07	0.129	−0.325	−0.351	−0.475	−0.247	0.028	−0.009	0.084	−0.002
Protein, %	0.291	0.176	0.335	0.280	−0.304	−0.360	−0.160	−0.374	0.063	0.417	0.012	0.067
Fat:protein ratio	−0.072	−0.148	−0.132	−0.039	−0.152	−0.127	−0.391	−0.022	−0.005	−0.415	0.101	−0.046
Casein, %	0.247	0.152	0.311	0.224	−0.233	−0.306	−0.082	−0.304	0.030	0.373	0.005	0.022
Casein number, %	−0.129	−0.252	0.017	−0.164	0.280	0.452	0.543	0.213	−0.337	0.173	−0.042	−0.439
Subclinical mastitis indicator												
Lactose, %	−0.490	**−0.888**	−0.453	−0.473					—	—	—	—
pH	—	—	—	—	−0.395	−0.266	−0.649	−0.288				
SCS	—	—	—	—	—	—	—	—	0.351	**0.436**	0.334	0.354
Single point MCP[2]												
RCT, min	0.478	**0.616**	0.449	0.480	−0.512	**−0.470**	−0.527	−0.512	0.690	**0.759**	0.551	0.745
k_{20}, min	0.267	**0.588**	0.101	0.308	−0.288	**−0.571**	−0.274	−0.277	0.578	**0.696**	0.531	0.613
a_{30}, mm	−0.035	**−0.468**	0.044	−0.066	0.088	0.031	0.238	0.002	−0.344	−0.266	−0.343	−0.363
a_{45}, mm	0.035	−0.123	0.137	−0.017	0.024	−0.163	0.201	−0.072	−0.248	0.000	−0.326	−0.216
a_{60}, mm	0.017	0.063	0.132	−0.042	0.039	−0.038	0.164	−0.019	−0.252	0.061	−0.339	−0.219
CF_t parameter[3]												
RCT_{eq}, min	0.480	**0.537**	0.492	0.474	−0.559	**−0.498**	−0.599	−0.553	0.694	**0.637**	0.561	0.753
k_{CF}, %/min	−0.173	−0.139	−0.166	−0.179	0.108	0.295	−0.112	0.212	−0.232	**−0.796**	0.048	−0.362
k_{SR}, %/min	−0.077	0.301	−0.080	−0.893	0.010	0.048	−0.196	0.126	0.112	−0.147	0.348	−0.024
CF_P, mm	−0.064	−0.304	0.045	−0.121	0.151	0.205	0.297	0.067	−0.428	−0.385	−0.405	−0.461
Maximum CF[4]												
CF_{max}, mm	−0.074	−0.319	0.044	−0.135	0.148	0.160	0.281	0.077	−0.434	−0.363	−0.398	−0.473
t_{max}, min	0.154	**0.521**	0.203	0.118	−0.083	−0.322	0.199	−0.206	0.013	**0.757**	−0.097	0.032

[1]Mean of the marginal posterior density of the correlation; bold indicates additive genetic correlations with ≥90% of posterior probability accumulated above 0 (positive estimates) or below 0 (negative estimates).

[2]RCT = rennet coagulation time of samples coagulating within 60 min of enzyme addition; k_{20} = curd-firming time of samples reaching 20 mm of firmness within 60 min from enzyme addition; a_{30} = curd firmness at 30 min after enzyme addition; a_{45} = curd firmness at 45 min after enzyme addition; a_{60} = curd firmness at 60 min after enzyme addition.

[3]RCT_{eq} = rennet coagulation time estimated using the CF_t equation; k_{CF} = curd firming instant rate constant; k_{SR} = syneresis instant rate constant; CF_P = asymptotic potential curd firmness.

[4]CF_{max} = maximum curd firmness attained within 45 min; t_{max} = time at attainment of CF_{max}.

factor called slow milks (first, 51% of total variance) is associated with milk pH (+0.852) and RCT (+0.562). Manca et al. (2016) proposed, for the Sarda sheep, an udder health factor (second, 19% of total variance) that is mainly based on lactose content (+0.87), milk NaCl (−0.86), milk freezing point (+0.64), and SCS (−0.42), but not pH. In that last study, both milk freezing point and NaCl content were highly correlated with lactose content. It worth noting that those authors obtained a very high value of heritability estimate of this latent explanatory variable (0.378), similar to our heritability estimate of lactose.

CONCLUSIONS

Evidence of phenotypic and genetic relationships between predictive markers of udder health and milk traits is limited for the sheep species. In the present study, from a phenotypic point of view, the effect of SCS, lactose, and pH were significant on many of the milk traits. High levels of somatic cells caused a general delay, and consequently, the worsening of milk coagulation. Similar effects were recorded for lactose concentration and pH, which caused a linear decrease of curd firmness values and a predicted decreasing quality of milk processing. The heritability estimates showed very high values for SCS, whereas for lactose and pH we recorded median values in line with data available in the literature. The SCS and lactose, and to a lesser extent pH, were closely related among each other, especially from the genetic point of view, and all together draw a picture of udder health that has a strong relationship with milk technological traits. Therefore, these traits could be used as indicators to prevent mastitis and improve cheese-making ability of ovine milk at both the management and genetic levels.

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