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Estimates of heritability and genetic correlations for milk coagulation properties and individual laboratory cheese yield in Sarda ewes

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Short title: Genetic parameters for clotting properties of milk

Abstract

Objective of this study was to estimate genetic parameters of milk coagulation properties (**MCPs**) and individual laboratory cheese yield (**ILYC**) in a sample of 1 018 Sarda breed ewes farmed in 47 flocks. Rennet coagulation time (**RCT**), curd firming time (**k_{20}**) and curd firmness (**a_{30}**) were measured using Formagraph instrument (Foss, Hillerød, Denmark) whereas ILYC were determined by a micro manufacturing protocol. About 10% of the milk samples did not coagulate within 30 minutes and 13% had zero value for k_{20}. The average ILCY was 36%. (Co)variance components of considered traits were estimated by fitting both single- and multiple-trait animal models. Flock-test date explained from 13% to 28% of the phenotypic variance for MCPs and 26% for ILCY, respectively. The largest value of heritability was estimated for RCT (0.23±0.10) whereas it was about 0.15 for the other traits. Negative genetic correlations between RCT and a_{30} (–0.80±0.12), a_{30} and k_{20} (–0.91±0.09), and a_{30} and ILCY (–0.67±0.08) were observed. Interesting genetic correlations between MCPs and milk composition ($r_G > 0.40$) were estimated for pH, NaCl and Casein. Results of the present study suggest to use only one out of three MCPs to measure milk renneting ability, due to the highly genetic correlations among them. Moreover negative correlations between ILCY and MCPs suggest a great care when using these methods to estimate cheese yield from small milk samples.

Keywords: clotting properties, rennet, dairy sheep, genetic parameters, variance components.

Implication

The estimation of genetic parameters is the first and essential step to select for coagulation traits and cheese yield in dairy sheep. The aim of this paper is to fill a gap in comparison to what happens in dairy cattle; indeed heritability estimates for clotting properties are missing for small ruminants. Since the sheep milk is almost totally destined for cheese making, the estimation of heritability of coagulation traits may enable future scenarios of selection for such traits, with potential implications on selection schemes and milk payment tables.

Introduction

The Italian dairy sheep stock consists of about 5.5 million ewes. The largest breed is the Sarda with more than 3 million sheep (BDN, 2014). The Sarda breed accounts for about 43% of the national total ovine stock and about 4% of EU sheep stock (Eurostat, 2014). The total milk production of the Sarda is about 300 000 t of milk per year (about 4% of total world production, FAOSTAT, 2014). The breeding programme currently involves a breeding nucleus of 212 941 milk recorded sheep farmed in 1 032 flocks, and the commercial population (ICAR, 2014). Since the beginning of the program, the total milk yield per lactation has been the main selective goal of Sarda sheep (Sanna *et al.,* 1997; Carta *et al.,* 2009). Recording for fat and protein percentage on first lambing ewes started in 1998. The milk is totally transformed in cheese with a production of 50-60 000 tons per year of three Protected Designation Origin products, mostly destined for export (Furesi *et al.,* 2013). Thus the milk cheese making ability could be a breeding goal of great interest for this breed.

Pecorino Romano cheese yield could be predicted from bulk milk composition using suitable equations (Pirisi *et al.,* 1994). However, predictions on individual milk are less accurate due to the variability of milk solids, thus individual laboratory cheese yield (ILCY) has been proposed as an indicator of potential cheese yield in individual ovine milk samples (Othmane *et al.,* 2002). ILCY shows low heritability and a positive genetic correlation with milk composition and negative with milk yield in Spanish Churra sheep (Othmane *et al.,* 2002).

Other indicators of milk cheese making ability, extensively studied in cattle, are milk coagulation properties (**MCPs**) (Aleandri *et al.,* 1989; Ikonen *et al.,* 1999; De Marchi *et al.,* 2008; Bonfatti *et al.,* 2014). They are usually defined by three parameters: rennet coagulation time (**RCT**, min), curd firming time ($\mathbf{k_{20}}$, min) and curd firmness ($\mathbf{a_{30}}$, mm), commonly measured by using either mechanical or optical devices (Bittante *et al.,* 2012). Several studies have reported that an appreciable proportion of the MCPs variation in cow milk is of additive genetic nature. A recent review by Bittante *et al.,* (2012) reported moderate values of heritability (about 0.26) for RCT and a_{30}, whilst few study report reliable estimates for k_{20}. In general, MCPs exhibit moderate to high genetic correlations with pH and somatic cell count, and very low to null with milk traits, respectively (Bittante *et al.,* 2012).

Few studies have been carried out on MCPs in small ruminants, especially in sheep. In particular effects of environmental factors, feeding, breed, parity and lactation stage on MCPs, milk composition and laboratory cheese yield, have been investigated (Jaramillo *et al.,* 2008; Bittante *et al.,* 2014; Pazzola *et al.,* 2014). In some researches, novel milk coagulation and syneresis parameters, estimated by a nonlinear modelling of the entire curd-firming process were used (Vacca *et al.,* 2015). Finally, Relationships between MCPs, sanitary status of the mammary gland were also investigated in sheep (Rovai *et al.,* 2015). Analysis of environmental factors affecting MCPs and ILCY and the estimation of their genetic parameters for MCPs and ILCY are essential steps for planning their improvement by means of selection. Aim of this study was to estimate heritability of MCPs, ILCY and their phenotypic and genetic correlations with milk yield and composition in Sarda dairy ewes.

Material and methods

Animals, milk sample collection and laboratory analysis

The study involved 1 018 Sarda ewes from 47 flocks located in the four historical provinces of Sardinia. Pedigree and milk recording information were supplied by the national association of small ruminant breeders (ASSONAPA, Rome, Italy). The pedigree file included more than 1.8 million animal records. Dairy ewes were offspring of 499 rams; other details about the structure of the population are given in Table 1. Individual milk samples (100 ml) were collected in the mid-late lactation (from 45 to 249 days in milk, average = 156±37.4 days) from April to July 2014 by the provincial association of breeders (APA). Milk samples were added with preservatives (bronopol, 62,5 µl/100 ml). Analyses were carried out within the 24 h after sampling and the milk samples were kept refrigerated during transportation from the farms to the laboratory.

The milk samples were split into two subsamples of 50 ml each and analysed in order to determine composition and cheese making attitude of milk, respectively. Standard milk analysis were performed at the milk lab of the Regional Association of Animal Breeds of Sardinia (ARA, Oristano, Italy). Milk composition was spectroscopically determined by MilkoScan^{TM} (Foss Electric, Denmark). Somatic cell count (SCC) was also determined using the Fossomatic^{TM} (Foss Electric, Denmark). MCPs were measured by using a Formagraph Instrument (Foss Electric A/S, Hillerød, Denmark). Briefly, 10 mL of each individual sample were heated to 35° C before the addition of 200 µL of rennet solution (Hansen Naturen 215, with 80 ± 5% and chymosin 20 ± 5 % pepsin, PacovisAmrein AG, Bern, Switzerland) diluted to 0.8% in distilled water. This analysis ended within 30 min after rennet addition and produced a lactodinamographic path as reported by Bittante *et al.,* (2012). RCT is the time between rennet addition and the start of the milk coagulation,

k_{20} is the time at which the typical oscillation graph reaches the width of 20 mm, and a_{30} is the width of the graph at 30 min after rennet addition. ILCY was determined according to a modified method of Othmane *et al.,* (2002), further details of the methodology used are provided in Manca *et al.,* (2016). The predicted pecorino cheese yield (**PPCY**) was also calculated using the equation proposed by Pirisi *et al.,* (1994): PPCY = 1.747 x protein% + 1.272 x fat%.

Statistical Analysis

Thirteen traits were analysed: RCT, k_{20}, a_{30}, ILCY, PPCY, milk yield (**MY**), fat percentage (**FP**), protein percentage (**PP**), casein percentage (**CSN**), conjugated linolenic acid percentage (**CLA**), pH, NaCl, Somatic cell score (**SCS**). Non-coagulating samples were eliminated, as well as the missing records for the other traits. Since the k_{20} parameter presented a skewed distribution, a log transformation was also applied on this trait. (Co)variance components were estimated by using Restricted Maximum Likelihood (**REML**) methodology implemented in VCE v. 6.0 software (Groeneveld *et al.,* 2010). Both a Single- (**ST**) and multiple-trait (**MT**) animal models were fitted. The raw data included 1 018 animals with phenotypes. Ancestors were extracted from the pedigree file considering up to the fourth previous generation. A total of 5 234 animals were included in the numerator relationship matrix (**A**). ST and MT analysis were carried out on MCPs using the following linear mixed model

$y_{ijklmno} = \quad + \quad + \quad + \quad + \quad + \quad +$ [1].

In the ST model, for each trait $y_{ijklmno}$ is a single measure for the i-th individual; μ is the overall mean; *PAR* is the fixed effect of *j*-th parity with 6 levels (j = 1 to 5 and PAR≥ 6); *LM* is the fixed effect of *k*-th lambing month with 6 levels (k = October to March); *DIM* is the fixed effect *l*-th class of days in milk with 6 levels (l=1: from 45-80d, l=2: 81-120d, l=3: 121-150d, l=4: 151-180d, l=5: 181-210, l=6: >210d); *RP* is the fixed effect of the *m*-th position of the milk samples in the rack of Formagraph, (m = 1 to 10); *ftd* is the cross-classified random effect of the n-th combination flock-test date (n = 1 to 70 levels) with $ftd_n \sim N(0,\mathbf{I}\ {}^{2}_{ftd})$, where $\mathbf{I}$ and σ^2_{ftd} were the identity matrix and the variance associated to flock-test date, respectively; a_o is the random genetic effect for the *o*-th animal (o = 1 to 5 234 levels) with $a_o \sim \quad {}^{2}_{a})$ and $e_{ijklmno}$ is the random residual term with $e_{ijklmno} \sim N(0,\mathbf{I}\ {}^{2}_{e})$ where σ^2_a and σ^2_e are the additive genetic and residual variance, respectively. Genetic parameters of ILCY, milk yield and composition traits were estimated with a mixed linear model that had the same structure of eq. [1], but that did not included the effect of the rack position.

In the MT animal model $\mathbf{y}_i$ represented the vector of dependent variables for the i-*th* individual, whereas the fixed and random effects were the same as eq. [1]. Two different MT analyses were carried out: i) a five-traits animal model, including the 3 MCPs, ILCY and PPCY, that was aimed at estimating genetic correlations among coagulation properties and cheese yields; ii) a series of bi-variate analyses for ILCY and each of the MCPs with all the remaining variables was performed to evaluate the genetic correlations among abovementioned properties and milk yield and composition. For both MT animal models the (co)variances for random effects were assumed to follow a multivariate normal distribution and they were modelled as $[\mathbf{a}_1 \; \dots \; \mathbf{a}_n]' \sim N(0, \mathbf{A} \otimes \mathbf{G})$, $\left[\mathbf{ftd}_1 \; \dots \; \mathbf{ftd}_n\right]' \sim N(0, \mathbf{I} \quad \mathbf{F})$ and $\left[\mathbf{e}_1 \; \dots \; \mathbf{e}_n\right]' N(0, \mathbf{I} \otimes \mathbf{R})$, where: **A** and **I** have previously been defined; *n* was the number of traits analysed; **G**, **F** and **R** were the *n* by *n* genetic additive, flock-test-date and residual covariance matrices, respectively (the element $\sigma^2_{i,j(i=j)}$ in the diagonal are the variance and the off-diagonal $\sigma_{i,j(i \neq j)}$covariance between trait *i* and trait *j*). For each trait, both for ST and MT analyses, heritability was computed as $h^2 = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_{ftd} + \sigma^2_e}$, for each analysed trait. Moreover, phenotypic and genetic correlations among MCPs and all the other traits have been computed.

Results

Descriptive statistics

Fat, protein and casein percentages (Table 2) were similar to those observed in individual data either in Sarda (Pazzola *et al.,* 2014; Manca *et al.,* 2016) and in other Italian breeds, such as Valle del Belice (Cappio-Borlino *et al.,* 1997), Massese (Martini *et al.,* 2008) and alpine breeds (Bittante *et al.,* 2014). Milk solid were lower than those reported for Spanish breeds (Othmane *et al.,* 2002; Jaramillo *et al.,* 2008). The average values of the test-day records for CLA (1.26±0.57) was lower than values reported for Sarda and Massese breeds (Nudda *et al.,* 2005; Martini *et al.,* 2008). The pH exhibited the smallest variability whereas somatic cell count varied accordingly to the breed average (Pazzola *et al.,* 2014).

About 6% (n=64) of the samples did not coagulate within 30 min (**NC** samples thereafter), and at the same time they did not present any values for a_{30} and k_{20}. The samples with RCT>29 min and A_{30}< 1 mm, about 9% (n=90) and 10% (n=101) respectively, were discarded. Also 129 samples (about 13%) that did not reach a curd firmness of 20 mm were excluded from the analysis (Figure 1). The percentage of NC samples within each DIM class tended to increase along the lactation. However, when the NC samples are referred to the total number of samples, largest values were observed in the central DIM classes. The same trend is observed for the percentage of missing k_{20} records (Figure 2).

The a_{30} and k_{20} parameters presented a more skewed distributions compared to RCT and ILCY (Figure 1). The average values for RCT were lower than those reported by Pazzola et *al.* (2014) and Vacca et *al.* (2015), whereas k_{20} and a_{30} varied accordingly to the values reported in literature for dairy sheep (Table 2). Average ILYC measured in the current study was 36.2±9.3%, whereas predicted cheese yield using the equation was equal 17.3±2.4% (Table 2).

A not negligible fraction of the phenotypic variance can be ascribed to the differences between flocks. The flock-test date effect explained a quota of phenotypic variation ranging from 13% to 33% for the three milk coagulation parameters and cheese yield (Table 3). The largest percentage of variance explained by flock-test date was recorded for MY (56%), followed by FP (50%), pH (30%), PP and CSN (20%). Moreover, for the last two traits the flock variance matched those of MCPs.

Heritability, phenotypic and genetic correlations of coagulation traits

Heritability estimates (Table 3) were moderate for RCT ($h^2 = 0.23$) and lower for a_{30} and k_{20} ($h^2 = 0.15$). The estimate for ILCY ($h^2 = 0.16$) has doubled the values by Othmane *et al.,* (2002) heritability for PPCY was of the same magnitude of ILCY. Values for other milk traits ranged from 0.03 for SCS to 0.28 of NaCl content. Intermediate values were observed for MY (0.08), FP (0.10), PP (0.13) and CSN percentages (0.15) (Table 3).

Moderate to high phenotypic correlations were observed among coagulation traits (Table 4). The curd firmness at 30 min showed a negative correlation with RCT and k_{20}. Conversely, k_{20} was positively associated with RCT ($r_P = 0.84$). ILCY and PPCY presented moderate to low positive correlations with RCT-k_{20} and negative with a_{30}, respectively. Genetic correlations between MCPs were large and negative, those involving a_{30}, positive (between RCT and k_{20}) respectively. The largest value was for the correlation between k_{20} and a_{30} ($r_A = –0.91$). Genetic correlations for a_{30} and RCT are in the range of variability of MCPs as recently reviewed by Bittante *et al.,* (2012). Unexpected results were the negative genetic correlation between ILCY and a_{30} as well as the positive correlations between ILCY, RCT ($r_A=0.55$) and k_{20} ($r_A=0.64$). Conversely, PPCY was positively associated with a_{30} ($r_A=0.22$) and negatively with k_{20} ($r_A=–0.19$) even if the magnitude of these estimates were lower than those involving ILCY and with larger standard errors. PPCY and ILCY were moderately associated ($r_A=0.47$). Heritabilities of MCPs and ILCY estimated by the MT animal model were very close to those obtained with the ST model, with lower standard errors though (data not shown).

Phenotypic and genetic correlations between milk coagulation and milk quality traits

Phenotypic correlations among the three MCPs and milk traits were negligible apart from those involving SCS, pH and NaCl (Table 5). In particular, pH was negatively and moderately correlated with a_{30} and pH (r_P=–0.42) and positively and strongly with RCT, respectively. NaCl and SCS were moderately correlated with three MCPs. Moreover, RCT only was weakly associated to PP and CSN percentages. Phenotypic correlation of ILCY (PPCY) with fat and protein percentage were r_P=0.46 (r_P=0.91) and r_P=0.37 (r_P=0.72), respectively.

The majority of genetic correlations among MCPs and milk production and composition variables were close to zero (MY, FP with RCT) or presented very large standard errors (FP, PP, CS, SCS with a_{30}). Of interest are those between pH and RCT (r_A= 0.68) and pH and a_{30} (r_A = –0.83). RCT was also moderately correlated to casein (r_A= 0.44), but unexpectedly close to zero genetic association (with large standard errors though) were found among protein and casein and a_{30}. Moreover, both RCT and k_{20} showed a positive genetic association with NaCl, whereas, no reliable associations were found with functional compound like CLA. As expected, ILCY was positively correlated with milk composition (FP, PP and CSN) and negatively correlated with MY. High trivial genetic correlation were observed among fat, protein and casein and PPCY, and although of reduced magnitude when compared to ILCY, a negative correlation between PPCY and MY was observed.

Discussion

In general, studies on milk rheological properties are characterised by a relevant variability of results. Moreover, several variables affecting clotting properties have been identified so far (Bittante *et al.,* 2012). In the present study, some milk samples did not coagulate within the reference time of 30 minutes. The percentage of non-coagulating milks was larger than in previous studies on Sarda and Alpine breeds (Bittante *et al.,* 2014; Pazzola *et al.,* 2014), but smaller than the value (24%) observed on Sarda bulk milk (Giangolini *et al.,* 2004). The result of the present study are similar to those observed in dairy cattle where the proportion of samples that did not coagulate and those with missing k_{20} are on average 19% and 33% across studies, respectively (Bittante *et al.,* 2012).

According to some authors, the milk coagulation process should be faster in ovine than bovine milk (Bittante *et al.,* 2012; Pazzola *et al.,* 2014). The average RCT found in the present study does not confirm this hypothesis. It was twofold the values measured in Sarda and Alpine (Pazzola *et al.,* 2014; Bittante *et al.,* 2014). On the other hand, it is in agreement with results obtained in other studies on Sarda (Pirisi *et al.,* 2000; Mele *et al.,* 2006) Massese (Pugliese *et al.,* 2000; Martini *et al.,* 2008) and Spanish (Jaramillo *et al.,* 2008; Rovai *et al.,* 2015) sheep breeds. Average values and distributions of k_{20} and a_{30} are in agreement with a previous report on Sarda ewes (Pazzola *et al.,* 2014).

Average ILYC measured in the current study was similar to those estimated by Jaramillo *et al.,* (2008) but 10% higher than previous finding on Churra sheep (Othmane *et al.,* 2002). Anyhow, the actual cheese yield is clearly overestimated by the use of ILCY. Whereas the PPCY were in accordance to the average Pecorino Romano cheese yield (Pirisi *et al.,* 2002) and it was moderately correlated with ILCY. The overestimation of cheese yield could be ascribed to the method of micro-manufacturing used (Othmane *et al.,* 2002; Bonfatti *et al.,* 2014) [see later in the discussion] .

Diifferently from what is observed in dairy cattle, the flock environment exerted a significant role. Compared to previous works on Sarda (Pazzola *et al.,* 2014; Vacca *et al.,* 2015) the proportion of variance explained by flock-test day was similar for k_{20}, but slightly lower for RCT and a_{30}. The fraction of variance explained by flock for MCPs was dramatically higher in comparison with studies on cattle (Ikonen *et al.,* 2004; Tyrisevä *et al.,* 2004; Vallas *et al.,* 2010), probably due to the peculiarities of sheep farming.

Heritability of milk coagulation, composition and cheese yield traits.

For some traits a significant quota of phenotypic variance was additive genetic, in other cases the majority of the variation was of environmental nature. Estimates of heritability for RCT were moderate and just in few case presented small standard errors. The comparison can be made only with dairy cattle data due to the lack of information for sheep in the literature. Values obtained in the present study confirmed reports for dairy cattle (Ikonen *et al.,* 1999; Tyrisevä *et al.,* 2004; Cassandro *et al.,* 2008). However, RCT heritability was below the findings of Ikonen *et al.,* (2004) and Vallas *et al.,* (2010). In the case of a_{30} the heritability was of the same extent of other studies (Cassandro *et al.,* 2008; Cecchinato *et al.,* 2011) but sensibly lower than Ikonen *et al.,* (1999; 2004) and Tyrisevä *et al.,* (2004). The k_{20} parameter had a similar heritability of a_{30} but few reports have been found on heritability of k_{20} in literature (Bittante *et al.,* 2012).

The heritability estimate of ILCY was double in magnitude compared to values reported by Othmane *et al.,* (2002) whose estimates derived from a sample of similar size, even if with ~7,500 test-day records of sheep milk over two generations. The cheese yield equation-predicted on the basis of fat and protein percentage has an heritability of the same magnitude of ILCY. Heritabilities for milk composition traits were from low to intermediate. Values for fat and protein were markedly lower than those reported for Sarda sheep (Sanna *et al.,* 1997). The use of one test day per animal and the reduced sample size in comparison to other works, may at least partially justify these differences. However, values observed in the present study were close to those reported by Othmane *et al.,* (2002) and they were in the range of variability observed for dairy sheep (Oravcová *et al.,* 2005).

Phenotypic and genetic correlations between milk coagulation traits and cheese yield

The knowledge of genetic associations among coagulation, milk yield and quality traits is essential when exploring the possibility to select in favour of one of the MCPs traits. The overall phenotypic correlation pattern of MCPs confirmed what observed in Sarda and Churra breeds (Nudda *et al.,* 2001; Jaramillo *et al.,* 2008), whilst partially disagree with the results of Pazzola *et al.,* (2014). The latter authors found a low negative correlation between RCT and a_{30} (–0.15) indicating a substantial phenotypic independency between these two traits. In the present paper, moderate phenotypic and high negative genetic correlations were obtained between these two traits, respectively. This result is similar to previous reports in dairy cattle (Cassandro *et al.,* 2008, Ikonen *et al.,* 2004; Bittante *et al.,* 2012). Indeed, if milk takes less time to coagulate, then more time is available for the process of curd firming. Since the repeatability of RCT is quite higher (Bittante *et al.,* 2012), this means that also in sheep one measure of RCT is enough to predict both traits.

Furthermore, the strong negative phenotypic correlation between k_{20} and curd firmness at 30 min was expected, due to the positive association of k_{20} with RCT. Similar relationships were previously found in Sarda sheep (Pazzola *et al.,* 2014). Additive genetic variance in common between these two traits (k_{20}, a_{30}) have been scarcely investigated in dairy cattle, due to the higher percentage of missing values for k_{20} parameter.

Negative correlation between ILCY and a_{30} (either phenotypic or genetic) and positive correlations between ILCY and RCT-k_{20} were unexpected. Conversely, PPCY presented a weak positive genetic association with a_{30} and negative with k_{20}. The possible explanation for this correlation pattern can be formulated considering two conflicting aspects. The first, is the interaction between predicted cheese yield and a_{30} as function of the fat percentage (Aleandri *et al.,* 1989). These authors found that the predicted cheese yield was positively associated with a_{30} with low fat milk, and negatively associated to a_{30} with high fat cow milk. Hence, the high fat level of ovine milk, compared to cow milk, could partially explain our results. A second issue is represented to the method used for measuring cheese yield. Indeed micro-cheese factoring can produce biased estimation of actual cheese yield, due to the small amount of milk used. This fact is also confirmed by the overestimation of cheese yield, found also in other works (Othmane *et al.,* 2002; Jaramillo *et al.,* 2008). Moreover, Bonfatti *et al.,* (2014) found that cow milk with short RCT and high a_{30} did not exhibit higher cheese yield in model cheeses, being the cheese yield variation in their experiment more likely associated to variation in milk fat and protein percentages. The modest genetic correlation between the cheese yield predicted by regression (PPCY) and a_{30} seem to suggest this second hypothesis, even if further investigations are needed to clarify the relationship between ILCY and MCPs in sheep milk.

Phenotypic and genetic correlation among milk coagulation, milk yield and composition

The study of the genetic associations between MCPs and milk yield and chemical composition is crucial for evaluate proper selection strategies. The phenotypic correlations between RCT and protein and casein percentages found in the present study were in agreement with results on sheep (Jaramillo *et al.,* 2008; Nudda *et al.,* 2001) and in cattle (Bittante *et al.,* 2012). The worsening of the coagulation properties of sheep milk (>RCT and <a_{30}) with increased somatic cell count is documented in literature (Pirisi *et al.,* 2000; Nudda *et al.,* 2001; Raynal-Ljutovac *et al.,* 2007). An increased somatic cell count can be the also the result of intramammary inflammatory process (Rovai *et al.,* 2005). However in sheep, high somatic cell count in milk can be often unrelated with pathological conditions, differently from cow. Several factors (breed, parity, stage of lactation, type of birth, estrus, diurnal) affect SCC variation in sheep milk (Raynal-Ljutovac *et al.,* 2007). Increased RCT and k_{20} and reduced a_{30} with increasing pH were previously reported in sheep milk (Bencini *et al.,* 2002; Pirisi *et al.,* 2000). Finally, low to moderate phenotypic correlation were observed among individual cheese yield and milk traits. The highest association was between ILCY and fat percentage, and it was half of the correlation found by Jaramillo *et al.,* (2008) but agreed with the results of Othmane *et al.,* (2002).

Interesting genetic correlations have been estimated between pH, casein, NaCl and RCT-a_{30}. For the pH, similar values have been reported for dairy cattle (Ikonen *et al.,* 2004; Cassandro *et al.,* 2008; Vallas *et al.,* 2010; Cecchinato *et al.,* 2011). Moderate correlations between RCT and casein percentage and no association among protein, casein percentages and a_{30} were also reported by Ikonen *et al.,* (2004). On the other hand, results of the present study were opposite to what found by some other authors (Cassandro *et al.,* 2008; Cecchinato *et al.,* 2011). A suggestive negative association between CLA with rennet properties might confirm what found by Bittante *et al.,* (2014) in milk of sheep supplemented with rumen-protected conjugated fatty acid source.

Moderate to high positive genetic correlation were found between NaCl, a_{30}-k_{20}. Conversely unreliable negative genetic correlation were found between SCS and RCT, differently from what observed by other authors in cow milk (Ikonen *et al.,* 2004; Cassandro *et al.,* 2008; Cecchinato *et al.,* 2011). A very high genetic correlation among NaCl and SCS (0.98±0.31) was found in the present study. This is an interesting results because suggest the possibility to use indirect indicators of udder status (different from SCS) linked to the rennet properties. As far as cheese yield concern, the positive genetic correlation among milk composition and ILCY and negative with milk yield confirm what found by Othmane *et al.,* (2002).

Conclusions

This study provided estimates of genetic parameters for milk coagulation properties of sheep milk of Sarda Breed. From the selective point of view, a not negligible proportion of phenotypic variance was additive genetic, and the heritability estimated for MCPs were in agreement with those found in cow milk for MCPs. Genetic correlations found in the present study suggest the chance to use only one of the rennet parameter, since they are highly genetic correlated, however negative correlation between ILCY and favourable rennet properties suggests to be careful in the use of this methods to predict cheese yield from small milk samples.

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Table 1 *Descriptive statistics for animals and flocks structure of Sarda population involved in this study.*

Items	n.[1]	average	sd	min	max
Flock size	-	95.5	57.1	13	233
Number of Lactation	1 016	4.0	2.3	1	12
Age (Month)	1 016	48.1	27.2	12	133
Ewes per ram	499	2.1	1.7	1	15
Rams per flock	47	16.9	5.8	6	33

[1] For the number of lactation and age n. is the number of records; for the last two items, n. is the number of rams and flocks, respectively.

Table 2 *Number of records, overall mean, standard deviation, minimum (Min), Maximum (Max) and coefficient of variation for sheep milk yield and composition, milk coagulation properties and individual laboratory cheese yield.*

Trait[1]	n.[2]	Mean	SD	Min	Max	CV (%)
Milk yield and composition						
MY (L/day)	1 005	1.72	0.42	0.61	3.30	24.4
FP (g/100 mL)	998	6.06	1.35	2.55	12.00	22.3
PP (g/100 mL)	998	5.47	0.61	3.86	8.76	11.2
CSN (g/100 mL)	998	4.25	0.50	2.91	6.89	11.8
CLA (g/ 100g FAME)	908	1.26	0.57	0.00	3.04	45.2
SCS	994	4.68	2.33	0.06	11.13	49.8
pH (U)	1 002	6.58	0.14	5.65	7.36	2.1
NaCl (mg/100 mL)	998	146.88	45.16	64.30	551.70	30.7
Cheese-related traits						
RCT (min)	1 008	15.18	4.29	2.37	30.07	28.3
k_{20} (min)	879	1.75	0.74	0.50	7.00	42.3
logk_{20}	879	1.99	0.16	1.48	2.62	8.1
a_{30} (mm)	990	52.63	16.08	0.98	107.80	30.6
ILCY (%,w/v)	1 017	36.24	9.33	4.67	80.14	25.7
PPCY (%,w/v)	998	17.28	2.43	11.71	29.55	14.1

[1] MY = test day milk yield; FP = fat; PP = protein; CSN = casein; CLA = Conjugated linoleic Acid, FAME=fatty acid methyl esters; SCS = somatic cell score, $\log_2[(SCC \mu l^{-1}/100)+3]$; RCT = rennet coagulation time; k_{20} = curd firming time; Logk_{20} = $\log_{10}$ of curd firming time in sec; a_{30} = curd firmness; ILCY= individual laboratory cheese yield; PPCY=Predicted Pecorino Cheese Yield,U=pH unit.

[2] Number of samples used to compute descriptive statistics

Table 3 *Estimates of genetic (σ_a^2) and environmental variance (σ_{ftd}^2, σ_e^2), heritability (h^2) and percentage of variance explained by flock-test-day (r^2_{ftd}) and standard errors (SE) for sheep milk coagulation properties, individual cheese yield, milk production and composition traits.*

Trait[1]	n.[2]	σ_a^2	σ_{ftd}^2	σ_e^2	h^2	*(SE)*	r^2_{ftd}	*(SE)*
Cheese-related traits								
RCT (min)	908	5.83	3.32	16.17	0.23	(0.10)	0.13	(0.03)
*log*k$_{20}$	879	0.004	0.006	0.016	0.15	(0.11)	0.23	(0.04)
a$_{30}$ (mm)	907	27.74	54.92	110.49	0.14	(0.10)	0.28	(0.05)
ILCY (%,w/v)	1 017	12.45	20.40	46.46	0.16	(0.09)	0.26	(0.05)
PPCY (%,w/v)	998	0.66	1.55	2.46	0.14	(0.09)	0.33	(0.05)
Milk yield and Composition								
MY (L/day)	1 005	0.013	0.09	0.06	0.08	(0.05)	0.55	(0.04)
FP (g/100 mL)	998	0.16	0.76	0.71	0.10	(0.07)	0.47	(0.05)
PP (g/100 mL)	998	0.04	0.06	0.20	0.13	(0.10)	0.20	(0.04)
CSN (g/100 mL)	998	0.03	0.04	0.13	0.15	(0.11)	0.20	(0.04)
CLA (g/100g FAME)	908	0.02	0.14	0.12	0.09	(0.06)	0.50	(0.05)
SCS	994	0.16	0.72	4.18	0.03	(0.07)	0.14	(0.03)
pH (U)	1 002	0.003	0.006	0.01	0.16	(0.08)	0.30	(0.05)
NaCl (mg/100 mL)	998	543.1	289.1	1104.7	0.28	(0.13)	0.15	(0.03)

[1] RCT = rennet coagulation time; logk$_{20}$ = log$_{10}$ of curd firming rate; a$_{30}$ = curd firmness; ILCY= individual laboratory cheese yield; PPCY=Predicted Pecorino Cheese Yield; MY = test day milk yield; FP = fat; PP = protein; CSN = casein; CLA = Conjugated linoleic Acid, FAME=fatty acid methyl esters; SCS = somatic cell score log$_2$(SCCμl^{-1}/100+3), U=pH unit

[2] Number of samples used to estimate variance components.

Table 4 *Phenotypic correlation (below the diagonal) and genetic correlation (above the diagonal) between sheep milk traits and milk coagulation properties estimated with a 5-trait animal model (standard error in brackets).*

Trait [1]	RCT	*log*k_{20}	a_{30}	ILCY	PPCY
RCT (min)		$0.84_{(0.09)}$	$–0.80_{(0.12)}$	$0.55_{(0.15)}$	$0.08_{(0.21)}$
logk_{20} (min)	0.79		$–0.91_{(0.09)}$	$0.64_{(0.11)}$	$–0.19_{(0.16)}$
a_{30} (mm)	–0.60	–0.76		$–0.67_{(0.08)}$	$0.22_{(0.17)}$
ILCY (%,w/v)	0.41	0.32	–0.34		$0.47_{(0.18)}$
PPCY (%,w/v)	0.23	0.07	–0.13	0.51	

[1] RCT = rennet coagulation time; k_{20} = curd firming rate; a_{30} = curd firmness; ILCY= individual laboratory cheese yield; PPCY=Predicted Pecorino Cheese Yield.

Table 5 *Phenotypic (r_P) and genetic correlation (r_G) among coagulation traits analyzed with bi-variate animal model in combination with sheep milk yield and composition*

	r_P					r_G				
Trait[1]	RCT	*log*k_{20}	a_{30}	ILCY	PPCY	RCT	*log*k_{20}	a_{30}	ILCY	PPCY
MY (L/day)	–0.09	0.07	–0.04	–0.09	–0.15	0.03 (0.39)	0.04 (0.48)	0.27 (0.46)	–0.88 (0.42)	–0.60 (0.46)
FP (g/100mL)	0.09	0.03	–0.12	0.46	0.91	–0.02 (0.38)	–0.34 (0.42)	0.32 (0.18)	0.45 (0.31)	0.93 (0.06)
PP (g/100mL)	0.30	–0.04	0.03	0.37	0.72	0.41 (0.32)	–0.42 (0.42)	0.09 (0.37)	0.75 (0.27)	0.85 (0.13)
CSN (g/100mL)	0.29	–0.06	0.05	0.38	0.73	0.44 (0.11)	–0.43 (0.45)	0.00 (0.48)	0.65 (0.27)	0.84 (0.13)
CLA (g/100g FAME)	–0.01	–0.05	0.14	–0.25	–0.37	–0.27 (0.38)	–0.46 (0.46)	0.27 (0.40)	–0.32 (0.34)	–0.33 (0.36)
SCS (U)	0.45	0.35	–0.30	0.35	0.29	–0.14 (0.92)	–0.72 (1.35)	0.11 (0.77)	0.58 (0.61)	0.29 (0.93)
pH (U)	0.70	0.55	–0.42	0.18	–0.28	0.68 (0.19)	0.44 (0.34)	–0.83 (0.21)	0.58 (0.42)	–0.21 (0.53)
NaCl (mg/100mL)	0.45	0.44	0.35	0.08	0.09	0.52 (0.27)	0.68 (0.32)	0.05 (0.51)	0.87 (0.65)	0.24 (0.51)

MY = test day milk yield; FP = test day fat percentage; PP = test day protein percentage; CSN = test day casein percentage; CLA = Conjugated linoleic Acid, FAME=fatty acid methyl esters; SCS = somatic cell score $\log_2[(\mathrm{SCC}\mu l^{-1}/100)+3]$, RCT = rennet coagulation time; k_{20} = curd firming rate; a_{30} = curd firmness; ILCY= individual laboratory cheese yield; PPCY=Predicted Pecorino Cheese Yield.

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Figure 1 Frequency distribution of the three milk coagulation properties and the Individual laboratory cheese yield (ILCY) in Sarda sheep, for the raw data (before data editing). The very first (a_{30}) e the last two bars (RCT) represent those samples that have been discarded from the analysis.

Figure 2 Percentage of not coagulating (NC) or missing k_{20} samples (NoK20), percentage of not coagulating samples (NC_byDIM) and missing k_{20} (NoK20_byDIM) within each class of DIM.