Characterization of milk composition, coagulation properties, and cheese-making ability of goats reared in extensive farms

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ABSTRACT

The aims of this study were to explore the variability of milk composition, coagulation properties, and cheese-making traits of the Sarda goat breed, and to investigate the effects of animal and farm factors, and the geographic area (Central-East vs. South-West) of an insular region of Italy, Sardinia. A total of 570 Sarda goats reared in 21 farms were milk-sampled during morning milking. Individual milk samples were analyzed for composition, traditional milk coagulation properties (MCP), modeled curd-firming over time parameters (CF_t), and cheese-making traits (cheese yield, %CY; recovery of nutrients, %REC; daily cheese yield, dCY). Farms were classified into 2 categories based on milk energy level (MEL; high or low), defined according to the average net energy of milk daily produced by the lactating goats. Milk yield and composition were analyzed using a mixed model including the fixed effects of MEL, geographic area, days in milk, and parity, and the random effect of farm within MEL and geographic area. Data about MCP, CF_t , and the cheese-making process were analyzed using the same model, with the inclusion of the effects of animal and pendulum of the lactodynamograph instrument, allowing the measure of repeatability of these traits. Results showed that animal had greater influence on coagulation and cheese-making traits compared with farm effect. Days in milk influenced milk composition, whose changes partly reflected the modifications of %CY traits. Moreover, large differences were observed between primiparous and multiparous goats: primiparous goats produced less milk of better quality (higher fat, lower somatic cell and bacterial counts) and less cheese, but with higher recovery of fat and protein in the curd, compared with multiparous goats. The repeatability was very high, for both coagulation (84.0 to 98.8%) and cheese-making

traits (89.7 to 99.9%). The effect of MEL was significant for daily productions of milk and cheese, coagulation time, and recovery of protein in the curd, which were better in high-MEL farms. As regards geographic area, milk composition and percentage cheese yield were superior in the Central-East area, whereas daily milk and cheese production and MCP were better in the South-West. This result was explainable by the phenomenon of crossbreeding Sarda goats with Maltese bucks, which occurred with greater intensity in the South-West than in the Central-East area of the island. The results provided by this study could be of great interest for the goat dairy sector. Indeed, the methods described in the present study could be applicable for other farming methods, goat breeds, and geographic areas. The collection of a wide range of phenotypes at individual animal level is fundamental for the characterization of local populations and can be used to guarantee breed conservation and the persistence of traditional farming systems, and to increase farmers' profit.

Key words: goat milk, coagulation, cheese-making, cheese yield

INTRODUCTION

Goat farming is an important sector of agriculture in many areas of the world, representing one of the main sources of livelihood (Lu and Miller, 2019). In some of these areas (northern Brazil, the Caribbean, and the Mediterranean and Middle Eastern regions of Europe), goat breeding is part of cultural heritage (Di Trana et al., 2015) and is based on the use of autochthonous breeds (Boyazoglu et al., 2005; Sponenberg et al., 2019), whose products are usually labeled with connotation of origin (e.g., Ibores, Chabichou du Poitou, Formaggella di Luinese). It is known that autochthonous goats perform better than cosmopolitan goat breeds in harsh environments, because of their greater physiological and anatomical adaptation (Silanikove, 2000). In this respect, it is strategic to improve knowledge and support the multipurpose local goat breeds and their

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genetic potential. Preservation of local breeds is fundamental, as they are essential for biodiversity (Di Trana et al., 2015) and can be a valuable source of economic income for dairy farmers. For example, a specific local breed can be closely related to a product (dairy or meat) with particular organoleptic characteristics (Di Trana et al., 2015); autochthonous breeds reared on the traditional extensive farming system can be associated with the production of functional foods (Čermák et al., 2013); and the traditional extensive farming system can be integrated with agro-tourism activity or with organic farming, which is still growing in the European market (Dubeuf, 2011). The high potential of local breeds worldwide is reported in several studies aimed at characterizing milk composition and cheese-making aptitude of indigenous compared with cosmopolitan goat breeds. Those studies have evidenced that, despite lower milk production, local breeds are characterized by higher contents of milk fat, protein, and total solids (Kouniba et al., 2007), curd firmness, cheese yield (%CY), and recovery of nutrients in the curd (%REC; Vacca et al., 2018a,b). The differences among breeds are explainable not only by different milk composition but also by genetic factors, in particular milk protein variants (Damián et al., 2008; Pazzola et al., 2014a).

Among autochthonous goat breeds reared in Sardinia, the Sarda is the most prevalent, with 29,000 animals recorded in the official herd book (FAO, 2014). The Sarda is characterized by skillful grazing behavior and is perfectly adapted to the semiarid environment of the island. In Sardinia, those goats are reared in traditional and extensive farming system methods, with free grazing pasture of the Mediterranean scrubland. The Sarda breed is also characterized by the excellent qualitative and technological characteristics of its milk and by very favorable genetic features (Pazzola et al., 2017). However, the repeated and unsupervised importation of dairy-specialized goat bucks (e.g., Saanen, Murciano-Grandina, Maltese) over the years, with the purpose of increasing milk production, has led to recombination of the original genetic characteristics of the Sarda breed. This phenomenon occurred with greater intensity in the South-West region of Sardinia (Vacca et al., 2016). At research level, only a few previous studies have examined milk production and quality, coagulation properties, and cheese-making ability from the Sarda goat breed, in most cases analyzing a low number of samples. Given that the characterization of local breeds' production (i.e., milk to cheese) represents one of the most important strategies for their conservation and a useful marketing instrument to raise public awareness about the importance of their preservation, we performed a large survey of the Sarda goat breed. The present study aimed to (1) quantify the contribution of animal and

farm effects, within milk energy level and geographic area, on milk composition, coagulation properties, and cheese-making traits; (2) study the effect of high or low classification of milk energy production at farm level, defined according to the milk net energy daily yielded by the goats; (3) assess the effect of geographic area; and (4) characterize the effects of different stages of lactation and parity.

MATERIALS AND METHODS

Farm Characteristics and Milk Sampling

This study involved 570 Sarda goats reared in 21 farms located in the Central and South-East areas of Sardinia. Animals were selected among those officially registered in the flock books and enrolled in the milk recording system of provincial associations of goat breeders, on the basis of DIM and parity, to keep a good variability of the data set. Characteristics of sampled farms are reported in Table 1. Goat farming system included the extensive techniques described by Usai et al. (2006): mating was of natural type without estrus synchronization; kidding was concentrated from November to March; and adult goats were allowed to graze during the morning on natural pastures composed of the most common plants and bush of the Mediterranean area (Vacca et al., 2010). Farms were grouped as small (<100 lactating goats), medium (100 to 200 lactating goats), or large (>200 lactating goats). As regards altitude, farms were grouped into those located in the plain (<200 m above sea level), in the hills (200 to 500 m above sea level), or in the mountains (>500 m above sea level). As reported in Table 1, the majority of farms in the Central-East of the island were located in the mountains (8 out of 11), and animals were hand-milked (10 of 11), whereas in the South-West, farms were mostly located in the hills and plains (9 of 10), with mechanical milking (7 of 10). We also used the daily milk energy output (dMEO) of goats to define farms into 2 classes of milk energy levels (MEL). The net energy content of milk was calculated using the equation proposed by the NRC (2001):

$$\begin{aligned} \text{Net energy (Mcal/kg)} = & 0.0929 \times \text{fat, \%} + 0.0547 \\ & \times \text{protein, \%} + 0.0395 \times \text{lactose, \%}. \end{aligned}$$

Then net energy (energy of 1 kg of milk) was converted to megajoules per kilogram and multiplied by the daily milk yield (dMY, kg/d) of each goat (MJ/d) to obtain the individual dMEO. Individual dMEO data were tested using ANOVA (GLM procedure, SAS version 9.4; SAS Institute Inc., Cary, NC) to obtain the least squares means (LSM) for the selected farms after cor-

Table 1. Characteristics of sampled farms (n = 21)

Item	Geographic area	
	Central-East	South-West
Farms, no.	11	10
Goats, no.	194	376
Milk energy level, no. of farms		
High (>3.91 MJ/d)	5	5
Low (≤3.91 MJ/d)	6	5
Flock size, no. of farms		
Small (<100 goats)	2	3
Medium (100–200 goats)	7	3
Large (>200 goats)	2	4
Altitude, no. of farms		
Plain (<200 m asl ¹)	2	4
Hill (200–500 m asl)	1	5
Mountain (>500 m asl)	8	1
Milking system, no. of farms		
Mechanical	1	7
Hand milked	10	3

¹asl = above sea level.

recting for DIM and parity of the goats. After ranking the dMEO LSM of the 21 farms, they were categorized into high (n = 10, average dMEO = 5.05 MJ/d) or low MEL (n = 11, average dMEO = 2.91 MJ/d) based on the median value (3.91 MJ/d).

Analysis of Milk Composition

Individual samples (200 mL/goat) were collected in sterile sample containers during the morning milking (1 sampling day for each farm) and stored at 4°C immediately after collection. In mechanical milking systems, milk was sampled from the recorder jar under each stall, and in the case of hand-milked systems, milk was collected from stainless steel graduated pails. Milk samples were analyzed within 24 h after collection. Clinical examinations were performed at the farms by veterinarians, and goats showing any clinical symptoms of mastitis (e.g., swollen, hot udder) or disease were not sampled. Milk fat, protein, lactose, total solids, pH, and NaCl were calculated using a MilkoScan FT6000 milk analyzer (Foss Electric A/S, Hillerød, Denmark), calibrated according to FIL-IDF references (ISO-IDF, 2013, for fat, protein, lactose, pH, and NaCl; ISO-IDF, 2010, for total solids). Somatic cell counts were determined using a Fossomatic 5000 somatic cell counter (Foss Electric) and transformed into the logarithmic [$\log_2(\text{SCC} \times 10^{-5}) + 3$] SCS, as reported by Shook (1993). Total bacterial count was measured using a BactoScan FC150 analyzer (Foss Electric) and transformed into the logarithmic bacterial count [$\text{LBC} = \log_{10}(\text{total bacterial count}/1,000)$] according to ISO-IDF (2004). Milk yield (MY) was recorded for each goat using the recorder jar in the case of mechanical milking and the

stainless steel graduated pails in the case of manual milking. Daily milk yield was then calculated as the total yield of morning plus evening milkings of the same day of sample collection.

The 9-mL Milk Cheese-Making Assessment

The 9-mL milk cheese-making assessment proposed and described in detail by Cipolat-Gotet et al. (2016a) was used to obtain traditional milk coagulation properties (MCP), %CY, and %REC traits. The procedure was performed in duplicate per each individual milk sample (2 replicates of 9 mL), for a total of 1,140 observations. This procedure allowed recording of the single-point traditional MCP [rennet coagulation time (RCT, min), defined as the time interval between rennet addition and gelation; curd-firming time (k_{20} , min), the time between gelation and the attainment of curd firmness of 20 mm; and curd firmness at 30 min after rennet addition (a_{30} , mm)]; and %CY traits, %CY_{CURD}, %CY_{SOLIDS}, and %CY_{WATER}, calculated as the ratio of the weights of fresh curd, curd DM, and water retained in curd, respectively, to the weight of the milk processed, multiplied by 100. The recorded nutrient recovery traits were: %REC_{PROTEIN}, %REC_{FAT}, and %REC_{SOLIDS}, calculated as the ratio of the weight of the curd components (protein, fat, and total solids, respectively) to the weight of the same milk component, multiplied by 100. Daily cheese yield (dCY) traits (dCY_{CURD}, dCY_{SOLIDS}, and dCY_{WATER}, kg/d) were calculated by multiplying %CY (%CY_{CURD}, %CY_{SOLIDS}, and %CY_{WATER}, respectively) by the individual dMY of goats.

Modeling of Curd Firmness

During the lactodynamographic analysis, the Formagraph recorded every 15 s the width (mm) of the oscillatory graph of the pendula immersed in each milk sample. Consequently, 120 curd firmness measures were recorded for each milk sample during 30-min analysis. The possibility of exploiting all these values allowed us to appropriately model the evolution of the coagulation process of each sample and to make the lactodynamographic analysis more informative. We used a 3-parameter model (Bittante, 2011) as follows:

$$CF_t = CF_P \times \left(1 - e^{-k_{CF}(t - RCT_{eq})} \right),$$

where CF_t is curd firmness at time t (mm); CF_P is the asymptotical potential value of CF at an infinite time in absence of syneresis (mm); k_{CF} is the curd-firming

instant rate constant (%/min); and RCT_{eq} (min) is RCT estimated by the CF_t equation on the basis of all data points.

Statistical Analysis

For each milk sample, a curvilinear regression was fit to the 120 CF_t values available for each sample using the nonlinear procedure (PROC NLIN) of SAS (SAS Institute Inc.). The parameters of each individual equation were estimated employing the Marquardt iterative method (350 iterations and 10^{-5} level of convergence).

Traditional MCP, CF_t parameters, and cheese-making traits were analyzed using the MIXED procedure of SAS, according to the following model:

$$y_{mnopqrst} = \mu + DIM_m + Parity_n + Area_o + MEL_p + Farm(MEL_p, Area_o)_q + Animal_r + Pendulum_s + e_{mnopqrst}, \quad [M1]$$

where $y_{mnopqrst}$ is the observed trait (RCT , k_{20} , a_{30} ; RCT_{eq} , k_{CF} , CF_P ; %CY, %REC, and dCY traits); μ is the overall population mean; DIM_m is the fixed effect of the m th class of days in milk [$m = 1$ to 7; class 1: ≤ 70 d (126 samples); class 2: 71 to 100 d (242 samples); class 3: 101 to 130 d (121 samples); class 4: 131 to 160 d (128 samples); class 5: 161 to 190 d (186 samples); class 6: 191 to 220 d (164 samples); class 7: > 220 d (82 samples)]; $Parity_n$ is the fixed effect of the n th parity [$n = 1$ to 5; class 1: first parity (186 samples); class 2: second (270 samples); class 3: third (226 samples); class 4: fourth (162 samples); class 5: $\geq$ fifth (296 samples)]; $Area_o$ is the fixed effect of the o th class of area [$o = 1$ to 2; class 1: Central-East; class 2: South-West]; MEL_p is the fixed effect of the p th class of milk energy level [$p = 1$ to 2; class 1: high MEL (> 3.91 MJ/d); class 2: low MEL (≤ 3.91 MJ/d)]; $Farm(MEL_p, Area_o)_q$ is the random effect of the q th farm ($q = 1$ to 21) within the p th class of MEL and the o th class of area; $Animal_r$ is the random effect of the r th animal ($r = 1$ to 570); $Pendulum_s$ is the random effect of the s th pendulum of the lactodynamograph instrument ($s = 1$ to 8); and $e_{mnopqrst}$ is the random residual $\sim N(0, \sigma_e^2)$, where σ_e^2 is the residual variance.

Milk composition was analyzed using a modification of model [M1], without inclusion of the random effect of animal and pendulum of the instrument, named model [M2].

Orthogonal polynomial contrasts were estimated between the LSM of classes of DIM (linear, quadratic, and cubic relationships) to observe possible significant patterns in the tested milk traits. The 4 degrees of

freedom of parity effect were used to test the following contrasts: (1) first versus second; (2) first versus second or greater; (3) second versus third or greater; and (4) third versus fourth or greater.

Repeatability (expressed in percentage) for MCP, CF_t parameters, and cheese-making traits was calculated as the ratio of the sum of the variances of the random effects [Farm(MEL, Area), animal, pendulum] to the sum of the variances of the random effects and the random residual.

RESULTS

Descriptive statistics of milk yield, composition, traditional coagulation properties (MCP), modeled curd-firming over time (CF_t) parameters, and cheese-making traits of individual goat milk samples are reported in Table 2.

We performed ANOVA for fixed effects, variance of random effects, and repeatability of MCP, CF_t parameters, and cheese-making traits, as reported in Table 3. As regards the random effects, farm variance was generally lower than 50%, excluding LBC. Animal variance was larger than that of farm on coagulation (from 47 to 85%), %CY (except for %CY_{WATER}), %REC (from 68 to 75%), and dCY traits (from 61 to 73%). Percentages of repeatability were between 84.0 (a_{30}) and 99.9% (%CY_{SOLIDS}).

Table 4 reports LSM of milk and coagulation traits according to milk energy level and geographic area. The effect of MEL was significant, with higher daily productions of milk and cheese, better coagulation time RCT_{eq} , and greater %REC_{PROTEIN} from goats reared in high-MEL farms. With regard to the effect of the geographic area, goats reared in the South-West area showed higher dMY and NaCl, and lower concentrations of milk fat, protein, lactose, and total solids. The k_{20} was shorter and a_{30} was higher in milk from goats reared in the South-West area.

Figure 1 summarizes the effect of DIM. Daily milk yield and daily cheese yield decreased across DIM (Figure 1a); fat and protein contents had a tendency to increase, and lactose decreased, all with cubic trends (Figure 1b); NaCl increased with a cubic trend, with a peak between 161 and 190 d (Figure 1d). Coagulation traits were not affected by DIM, except for CF_P , which showed a quadratic trend, with the lowest values between 131 and 160 d (data not shown); cheese yield traits changed across lactation with cubic trends (Figure 1e); %REC traits were not affected by DIM, except %REC_{SOLIDS}, with the lowest value between 101 and 130 d (data not shown).

The effect of parity and orthogonal contrasts are reported in Table 5. Goats at first parity had lower milk

Table 2. Descriptive statistics of milk yield, composition, traditional coagulation properties (MCP), and modeled curd-firming over time (CF_t) parameters and cheese-making traits of individual goat milk samples

Item	Mean	SD	Percentile ¹	
			P5	P95
MY, ² kg/d	1.10	0.51	0.42	2.20
Milk composition				
Fat, %	5.28	1.22	3.41	7.47
Protein, %	3.93	0.48	3.19	4.81
Lactose, %	4.61	0.27	4.15	5.04
TS, %	14.83	1.53	12.50	17.50
NaCl	245	50	170	327
pH	6.72	0.10	6.55	6.88
SCS ³	6.63	1.80	3.63	9.49
LBC ⁴	1.75	0.87	0.30	3.27
Traditional MCP ⁵				
RCT, min	13.37	4.55	8.15	23.30
k ₂₀ , min	3.61	1.19	2.15	6.30
a ₃₀ , mm	37.14	9.90	18.42	51.04
Modeled CF_t parameters ⁶				
RCT _{eq} , min	13.60	3.90	8.88	22.07
k _{CF} , %/min	21.21	6.92	10.22	33.67
CF _P , mm	42.22	10.25	25.12	58.52
Cheese yields (CY), %				
%CY _{CURD}	19.04	3.12	14.57	24.68
%CY _{SOLIDS}	9.09	1.56	6.77	11.64
%CY _{WATER}	9.78	2.08	6.86	13.96
Nutrient recovery (REC), %				
%REC _{FAT}	85.34	4.34	76.89	91.67
%REC _{PROTEIN}	81.19	2.71	76.25	85.25
%REC _{SOLIDS}	60.43	4.28	52.76	67.01
Daily production traits, ⁷ kg/d				
dCY _{CURD}	0.208	0.096	0.079	0.392
dCY _{SOLIDS}	0.098	0.043	0.041	0.182
dCY _{WATER}	0.107	0.050	0.039	0.206

¹P5 and P95 = 5th and 95th percentiles, which indicate the upper and lower 5% limits in the 2-tailed distribution of data.

²MY = milk yield.

³SCS = $\log_2(\text{SCC} \times 10^{-5}) + 3$.

⁴LBC = logarithmic bacterial count: $\log_{10}(\text{total bacterial count}/1,000)$.

⁵RCT = measured rennet gelation time; k₂₀ = time interval between gelation and attainment of curd firmness of 20 mm; a₃₀ = curd firmness 30 min after rennet addition.

⁶RCT_{eq} = rennet coagulation time estimated by CF_t modeling; k_{CF} = curd firming instant rate constant; CF_P = asymptotic potential curd firmness.

⁷dCY = daily cheese yield.

production compared with multiparous goats. Goats at second parity had slightly lower milk production compared with goats at third and higher parities. Compared with older goats, milk from younger ones was characterized by higher fat, lactose, and total solids contents, and lower NaCl, SCS, and LBC. The LBC was lower in goats at first and second parities.

DISCUSSION

Effects of Animal and Farm, and Repeatability of Coagulation and Cheese-Making Traits

To the best of our knowledge, this is the first study presenting a detailed description of dairy production

performance (from milk to cheese) of a local goat (Sarda) breed, so we could only partially compare our results with those available from the literature. On average, MY and percentages of milk fat and protein were similar to data reported by Pazzola et al. (2017), whereas curd firmness values reported in this study are slightly lower, and %CY_{CURD} is higher.

It is acknowledged that a variable proportion of the animal effect on coagulation traits is due to genetic aspects (i.e., casein polymorphism, whey protein genes; Damián et al., 2008; Dettori et al., 2015a) and composition of milk (Vacca et al., 2018a). Large amounts of α_{S1} -casein (Clark and Sherbon, 2000) are related to delayed coagulation times but higher curd firmness values, and overall high milk protein and casein contents are associ-

Table 3. ANOVA for fixed effects (F -value and significance) and variance of random effects (expressed as percentage variance on the total variance) for daily milk yield, composition, traditional coagulation properties (MCP), modeled curd-firming over time (CF_t) parameters, and cheese-making traits, and repeatability of traditional MCP, modeled CF_t parameters, and cheese-making traits

Item	Fixed effect (<i>F</i> -value and significance)			Random effect (% of total variance)					
	DIM	Parity	MEL ³	Area	Farm (MEL, Area)	Animal	Pendulum	RMSE ¹	Repeatability, ² %
MY, ⁴ kg/d	7.6***	15.2***	25.9***	8.1*	30	—	—	0.32	—
Milk composition									
Fat, %	3.0**	8.5***	2.1	5.1*	44	—	—	0.95	—
Protein, %	14.6***	2.5*	2.0	3.7	35	—	—	0.38	—
Lactose, %	5.1***	11.4***	1.6	9.6**	27	—	—	0.22	—
TS, %	7.3***	9.5***	0.5	8.0*	41	—	—	1.20	—
NaCl	7.4***	21.0***	0.0	17.5***	37	—	—	37.70	—
pH	3.6**	7.9***	0.7	0.3	38	—	—	0.08	—
SCS ⁵	3.3**	23.5***	0.8	3.5	29	—	—	1.53	—
LBC ⁶	1.8	6.0***	2.0	0.1	60	—	—	0.54	—
Traditional MCP ⁷									
RCT, min	0.6	1.2	2.4	3.0	24	72	0.46	0.84	96.5
k ₂₀ , min	1.9	0.4	1.4	12.8**	24	57	4.56	0.50	85.5
a ₃₀ , mm	1.3	2.5*	0.1	19.0**	6	75	3.17	3.95	84.0
Modeled CF _t parameters ⁸									
RCT _{eq} , min	0.6	0.3	5.1*	1.0	13	85	0.39	0.43	98.8
k _{CF} , %/min	0.3	0.5	3.7	2.3	15	75	0.14	2.13	90.7
CF _P , mm	1.9	2.1	1.3	1.1	42	47	3.55	3.25	91.9
Cheese yields (CY), %									
%CY _{CURD}	2.3*	3.5**	0.0	0.3	41	52	0.03	0.79	93.3
%CY _{SOLIDS}	2.9**	3.3*	0.7	5.8*	36	64	0.00	0.06	99.9
%CY _{WATER}	1.9	1.7	0.1	1.0	47	42	0.02	0.70	89.7
Nutrients recovery (REC), %									
%REC _{FAT}	1.8	1.8	3.5	0.2	25	75	0.00	0.25	99.6
%REC _{PROTEIN}	0.4	5.9***	5.1*	2.7	28	70	0.01	0.29	98.8
%REC _{SOLIDS}	2.5*	2.3	0.5	1.7	31	68	0.02	0.39	99.2
Daily production traits, ⁹ kg/d									
dCY _{CURD}	2.7*	4.1**	24.8***	5.5*	30	68	0.00	0.01	98.3
dCY _{SOLIDS}	2.7*	4.6**	36.7***	2.4	26	73	0.00	0.00	99.9
dCY _{WATER}	2.6*	4.0**	14.3***	6.6*	36	61	0.02	0.01	96.8

¹RMSE = root mean square error.

²Repeatability, % = $\frac{\sigma_{\text{Farm(MEL} \times \text{Area)}}^2 + \sigma_{\text{Animal}}^2 + \sigma_{\text{Pendulum}}^2}{\sigma_{\text{Farm(MEL} \times \text{Area)}}^2 + \sigma_{\text{Animal}}^2 + \sigma_{\text{Pendulum}}^2 + \sigma_e^2} \times 100$, where $\sigma_{\text{Farm(MEL} \times \text{Area)}}^2$, σ_{Animal}^2 , $\sigma_{\text{Pendulum}}^2$, and σ_e^2 are the Farm(MEL × Area), animal, pendulum, and residual variances, respectively.

³MEL = milk energy level.

⁴MY = milk yield.

⁵SCS = $\log_2(\text{SCC} \times 10^{-5}) + 3$.

⁶LBC = logarithmic bacterial count: $\log_{10}(\text{total bacterial count}/1,000)$.

⁷RCT = measured rennet gelation time; k₂₀ = time interval between gelation and attainment of curd firmness of 20 mm; a₃₀ = curd firmness 30 min after rennet addition.

⁸RCT_{eq} = rennet coagulation time estimated by CF_t modeling; k_{CF} = curd firming instant rate constant; CF_P = asymptotic potential curd firmness.

⁹dCY = daily cheese yield.

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

Table 4. Least squares means of milk energy level (MEL) and geographic area for milk yield, composition, traditional coagulation properties (MCP), modeled curd-firming over time (CF_t) parameters, and cheese-making traits of individual goat milk samples

Item	MEL		Geographic area	
	High	Low	Central-East	South-West
MY, ¹ kg/d	1.29	0.80	0.90	1.18
Milk composition				
Fat, %	5.78	5.24	5.93	5.08
Protein, %	3.91	4.09	4.12	3.88
Lactose, %	4.62	4.69	4.75	4.56
TS, %	15.33	15.01	15.80	14.54
NaCl	233	231	205	260
pH	6.70	6.73	6.71	6.73
SCS ²	6.50	6.10	5.88	6.72
LBC ³	1.51	1.92	1.68	1.76
Traditional MCP ⁴				
RCT, min	12.93	14.55	14.64	12.83
k ₂₀ , min	4.63	4.15	5.24	3.54
a ₃₀ , mm	31.07	31.56	25.13	37.50
Modeled CF_t parameters ⁵				
RCT _{eq} , min	12.94	14.56	14.12	13.38
k _{CF} , %/min	22.38	19.83	20.08	22.13
CF _p , mm	41.39	45.14	45.04	41.49
Cheese yield (CY), %				
%CY _{CURD}	18.95	18.85	19.13	18.66
%CY _{SOLIDS}	9.50	9.14	9.84	8.80
%CY _{WATER}	9.44	9.59	9.18	9.84
Nutrient recovery (REC), %				
%REC _{FAT}	84.33	86.17	85.46	85.03
%REC _{PROTEIN}	82.34	80.87	82.15	81.06
%REC _{SOLIDS}	61.08	60.32	61.41	59.99
Daily production traits, ⁶ kg/d				
dCY _{CURD}	0.244	0.148	0.173	0.218
dCY _{SOLIDS}	0.120	0.071	0.089	0.102
dCY _{WATER}	0.122	0.078	0.084	0.115

¹MY = milk yield.²SCS = $\log_2(\text{SCC} \times 10^{-5}) + 3$.³LBC = logarithmic bacterial count: $\log_{10}(\text{total bacterial count}/1,000)$.⁴RCT = measured rennet gelation time; k₂₀ = time interval between gelation and attainment of curd firmness of 20 mm; a₃₀ = curd firmness 30 min after rennet addition.⁵RCT_{eq} = rennet coagulation time estimated by CF_t modeling; k_{CF} = curd firming instant rate constant; CF_p = asymptotic potential curd firmness.⁶dCY = daily cheese yield.

ated with high %CY and %REC traits (Pazzola et al., 2019). Moreover, high milk fat is generally associated with better coagulation properties (Clark and Sherbon, 2000) and higher %CY (Guo et al., 2004), %REC traits, and daily cheese production (Pazzola et al., 2019). The animal effect on the total variability of milk and cheese traits supports that Sarda goats are characterized by a large genetic component (casein polymorphism), even larger than that of other goat breeds (Moioli et al., 2007). However, this aspect needs to be better explored if the preservation of the Sarda breed is considered.

With regard to repeatability, we compared our results with some data from bovines and ovines, as this is the first study reporting individual repeatability values for coagulation traits in goat milk. Bovine MCP are characterized by lower instrumental repeatability and

reproducibility than milk composition traits (Tyrisevä et al., 2003; Dal Zotto et al., 2008), especially for those properties recorded in the last part of the lactodynamographic analysis (curd firmness at 60 min, potential curd firmness, and syneresis rate). However, although MCP are highly influenced by instrument (mechanical or optical devices; Cipolat-Gotet et al., 2012) and conditions of analysis (type and concentration of rennet, milk temperature, and acidification; Nájera et al., 2003), the individual cow repeatability can be considered high (Bittante et al., 2012). Repeatability values of MCP from sheep are almost similar to those of bovine milk (Ferragina et al., 2017). In this study, the individual goat repeatability of RCT and k₂₀ was higher than that of dairy cows, but for a₃₀ it was lower (Stocco et al., 2017). Moreover, among species (Ferragina et

al., 2017; Stocco et al., 2017) the repeatability of modeled CF_t parameters is generally higher compared with that of traditional MCP, and in goats this difference was even greater. The very different coagulation patterns among species provided by the modeling of data

obtained during the lactodynamographic test (Pazzola et al., 2018) could explain why repeatability values of these traits are also different: in sheep and goat milk maximum consistency of the curd is usually attained within 30 min of rennet addition, and after 30 min in

Figure 1. Effects of DIM on (a) milk yield (MY) and daily cheese yield (dCY); (b) fat, protein, and lactose; (c) NaCl and pH; (d) SCS and logarithmic bacterial count (LBC); and (e) cheese yield traits (%CY) of Sarda goats.

cows. Consequently, a_{30} values are usually measured in the ascending phase of the curd-firming curve when bovine milk is analyzed, and in the descending phase when milk from small ruminants is tested. The high individual repeatability of cheese-making traits was expected, but it was even higher compared with that reported in Vacca et al. (2018a) for 6 breeds of goats. These results evidenced that the 9-mL milk cheese-making assessment can be a powerful tool, particularly compelling for the characterization of the cheese-making ability of milk from local populations. Indeed, it allows the rapid, cheap, and partly automated analysis of several samples per day, using instruments commonly used in many laboratories for the evaluation of MCP.

The concomitant use of the modeling of the renneting data allows a more detailed description of the coagulation process. All these phenotypes are of interest for the dairy sector, and the possibility of collecting them at the individual level could speed the improvement and favor the conservation of the genetic resources of local goat populations.

Effect of Milk Energy Level

To our knowledge, no previous studies have investigated the effect of milk energy at farm level on the production, composition, coagulation traits, and cheese-making ability of Sarda goat milk. The average values

Table 5. Least squares means of classes of parity and their orthogonal contrasts (F -value and significance) for milk yield, composition, traditional coagulation properties (MCP), modeled curd-firming over time (CF_t) parameters, and cheese-making traits of individual goat milk samples

Item	Parity, LSM					Contrast, F -value and significance			
	1st	2nd	3rd	4th	≥5th	1st vs. 2nd	1st vs. ≥2nd	2nd vs. ≥3rd	3rd vs. ≥4th
MY, ¹ kd/d	0.86	1.03	1.11	1.11	1.11	25.9***	57.9***	8.2**	0.0
Milk composition									
Fat, %	5.82	5.57	5.53	5.41	5.21	6.9**	20.9***	5.5*	6.3*
Protein, %	3.99	4.00	3.97	4.07	3.95	0.0	0.0	0.0	1.4
Lactose, %	4.73	4.70	4.62	4.63	4.58	2.4	23.8***	21.1***	0.6
TS, %	15.56	15.32	15.14	15.07	14.77	4.2*	20.7***	10.9**	4.0*
NaCl	213	224	237	239	249	7.5**	49.5***	33.6***	4.9*
pH	6.73	6.74	6.72	6.71	6.69	1.1	3.4	20.8***	5.5*
SCS ²	5.51	5.90	6.61	6.50	7.00	6.2*	51.6***	41.2***	1.2
LBC ³	1.59	1.62	1.81	1.75	1.81	0.3	10.4**	14.4***	0.4
Traditional MCP ⁴									
RCT, min	13.13	13.35	14.00	14.38	13.83	0.2	2.3	2.6	0.1
k_{20} , min	4.38	4.35	4.41	4.27	4.53	0.0	0.0	0.2	0.0
a_{30} , mm	33.40	32.44	30.17	31.59	28.98	0.5	5.1*	3.6	0.0
Modeled CF_t parameters ⁵									
RCT _{eq} , min	13.51	13.82	13.91	13.97	13.55	0.4	0.5	0.0	0.1
k_{CF} , %/min	20.99	21.11	21.86	20.49	21.08	0.0	0.0	0.0	1.8
CF_P , mm	44.66	43.76	41.52	43.97	42.41	0.6	2.8	1.4	2.6
Cheese yields (CY), %									
%CY _{CURD}	19.34	19.15	18.95	18.91	18.15	0.4	3.6	3.3	2.2
%CY _{SOLIDS}	9.60	9.43	9.29	9.34	8.93	0.9	5.0*	2.9	1.0
%CY _{WATER}	9.63	9.62	9.51	9.63	9.16	0.0	0.6	1.1	0.4
Nutrient recovery (REC), %									
%REC _{FAT}	85.89	85.76	84.86	85.07	84.66	0.1	3.0	4.5*	0.0
%REC _{PROTEIN}	82.44	82.07	81.43	81.03	81.04	1.4	13.8***	12.2***	2.0
%REC _{SOLIDS}	61.34	60.98	60.84	60.51	59.82	0.5	3.3	2.1	2.4
Daily production traits, ⁶ kg/d									
dCY _{CURD}	0.173	0.194	0.210	0.206	0.196	5.3*	12.4***	1.8	1.3
dCY _{SOLIDS}	0.084	0.095	0.102	0.101	0.097	6.8**	15.6***	2.3	0.6
dCY _{WATER}	0.087	0.099	0.106	0.107	0.100	5.5*	12.8***	2.0	0.4

¹MY = milk yield.

²SCS = $\log_2(\text{SCC} \times 10^{-5}) + 3$.

³LBC = logarithmic bacterial count: $\log_{10}(\text{total bacterial count}/1,000)$.

⁴RCT = measured rennet gelation time; k_{20} = time interval between gelation and attainment of curd firmness of 20 mm; a_{30} = curd firmness 30 min after rennet addition.

⁵RCT_{eq} = rennet coagulation time estimated by CF_t modeling; k_{CF} = curd firming instant rate constant; CF_P = asymptotic potential curd firmness.

⁶dCY = daily cheese yield.

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

of milk energy output (dMEO, MJ/d) of both MEL were significantly lower than those observable in bovine herds (where this factor was expressed as herd productivity level; Stocco et al., 2017), but this was expected, considering the different species and the environment in which the goats are reared. It is important to remember that all the 21 farms studied here were characterized by the extensive management system, and the MEL classification allowed for a balanced division of Central-East and South-West farms. Even though dMEO is based on daily production of fat, protein, and lactose, no difference in milk composition was observed between the 2 MEL categories.

In previous studies, goat milk composition was slightly influenced by the type of farming system (Van Quackebeke et al., 1996), and the effect of feeding type has not been extensively studied in goats. However, Min et al. (2005) reported that dMY, milk fat, protein, and lactose were the lowest for goats grazing only on mixed vegetative forages (no concentrate) compared with those fed with 0.33 or 0.66 kg of concentrate per kilogram of milk (high-producing goats: >1.5 kg of milk/d). Inglingstad et al. (2014) studied the effect of diet (cultivated vs. rangeland pasture, and high vs. low hay quality) on goat milk composition and coagulation properties and evidenced that, in general, milk from goats on pasture was richer in protein and casein compared with milk from goats fed with hay, so that RCT was significantly shorter in milk from goats fed with hay.

Results of the present study are different than those from dairy cows, in which the effect of herd productivity level was larger (Stocco et al., 2017, 2018a). The faster coagulation and the higher %REC_{PROTEIN} in milk samples from goats reared in high-MEL farms could be related to the protein and casein genetic variants, rather than to their overall quantities, as no differences in milk composition have been observed between MEL farm categories. Two recent studies have accurately evidenced that the composition of goat milk can directly influence coagulation and cheese-making ability (Stocco et al., 2018b; Pazzola et al., 2019), but we also find some genes of particular interest (whey protein genes), especially in the Sarda breed, that influence renneting properties (Dettori et al., 2015a,b). Probably, these genes also have an important influence on cheese yields and on the efficiency of recovery of nutrients in curd, but this is still under investigation.

Effect of Geographic Area

The idea to test the effect of geographic area was linked to the crossbreeding phenomenon that has oc-

curred with greater intensity in the South-West area of the island (Vacca et al., 2016), where farmers have frequently crossed their goats with Maltese bucks (an Italian breed from Sicily), as previously mentioned. This has led to recombination of the original genetic traits of both Sarda and Maltese goats. Several breeds have been imported to improve the animals' productive performance, and the best results have been obtained with Maltese bucks, combining their high MY and suitability for grazing the extensive Sardinian scrublands (Usai et al., 2006). Crossing autochthonous goats with specialized dairy breeds ensures high milk production, although imported animals are seldom adaptable to local climate conditions (Lu and Miller, 2019). However, when farmers rear high-yielding goats imported from areas geographically similar to those of local breeds, traits from both types are successfully absorbed from the crossbred animals (e.g., Majorera goats; Capote, 2017). On the other hand, if the nutritional requirements for milk production are not satisfied, the use of high-yielding goats becomes ineffective (Capote, 2002). A recent study has evidenced that coagulation, curd-firming, and syneresis traits are better in Maltese goats compared with Alpine breeds (Pazzola et al., 2018), although the composition of milk from Maltese is much more similar to that from Alpine goats and much worse compared with the Sarda. Indeed, milk from the Sarda is characterized by superior quality and technological aptitude compared with other dairy goat breeds (e.g., Saanen, Camosciata delle Alpi, Murciano-Granadina, Maltese; Vacca et al., 2018a). But the higher %CY and %REC traits from the Sarda breed can only partially counterbalance the higher MY characterizing Maltese goats (Vacca et al., 2018b). Thus, increase of milk production was the main objective of farmers who crossed their Sardinian goats with Maltese bucks, even though descriptions of the characteristics of milk quality, coagulation process, and cheese-making ability of these animals have not been reported yet. In bovines it is acknowledged that crossbred cows produce lower quantities (in kg) of milk, fat, and protein but higher concentrations (%) of these components in milk, compared with purebred cows. Thus, generally, crossbreeding is considered a viable method for improving the technological properties of milk, particularly the coagulation time and curd-firming process, but also other important traits related to fertility and longevity (Malchiodi et al., 2014). The shorter k_{20} recorded in goats from the South-West area could be related to the lower content of fat and lactose in milk (Stocco et al., 2019a,b), but this could not concurrently explain the higher curd firmness value. Possibly, differences in the proportions of milk protein fractions, in the frequency

of their genetic variants, or both (due to crossbreeding recombination), could explain the difference in the a_{30} trait between the 2 areas. It is also possible that these differences in milk characteristics result from the different altitudes at which farms were located and, therefore, to different pasture compositions. In fact, many farms in the Central-East area were located in the mountains, where trees prevail over shrubs.

Goats reared in the 2 areas produced the same %CY but with different proportions of solids and water: %CY_{SOLIDS} was greater in the Central-East area (perhaps deriving from the higher total solids in milk). Daily fresh cheese yield was higher in the South-West area, but it was higher because of the higher value of water retained in the curd (dCY_{WATER}) and not because of the higher total solids (dCY_{SOLIDS} were similar between areas). High water content in the curd increases %CY values, but an excess of water could not be considered a positive finding, as, generally, high moisture content leads to a worsening of cheese quality during ripening, with a depreciation of the final product (Martin et al., 1997).

Effect of DIM

Many studies in the literature have investigated the effects of goat DIM on composition (Zeng et al., 1997; Fekadu et al., 2005; Idamokoro et al., 2017), but less is known about the effect on coagulation and cheese-making traits. Zeng et al. (1997) reported that concentrations of milk fat, protein, solids-nonfat, and total solids from Alpine goats were higher in the first 30 DIM and then declined slightly and remained constant until drying off. Those authors also reported that daily milk production decreased gradually, in agreement with our results, together with the daily cheese production. Zeng et al. (1997) observed marked daily variations of goat SCC. In the present study, the possibility of dividing the lactation period into many classes allowed a better representation of the trends of each component, although they were sometimes erratic. The division into early, middle, and late lactation can lead to curvilinear or cubic trends becoming linear, as evidenced by Mioč et al. (2008) and Idamokoro et al. (2017) for goat milk composition. However, Mestawet et al. (2012), studying the variation of milk composition during early, middle, and late lactation of 4 goat breeds in Ethiopia, reported quadratic trends for fat, protein, and total solids, with the lowest values at middle lactation and a peak in late lactation.

In dairy cows, both traditional MCP and modeled CF_t parameters are strongly influenced by DIM, contrary to the results of the present study, and this could

be explained by the much more linear trend of each milk component in cows (Stocco et al., 2017). As a consequence of this, bovine cheese-making traits (especially %CY traits) greatly change (linear increase) during lactation (Stocco et al., 2018a), and in sheep the same pattern for these traits has also been observed (Cipolat-Gotet et al., 2016b). However, besides the differences among species, it is important to remember that the different division of DIM in classes among studies could lead to very different results, especially within species.

Effect of Parity

No information is available on the effects of many orders of parity on the coagulation and cheese-making traits of goats. Rather, some studies are focused on the variability of milk composition (Mioč et al., 2008; Lôbo et al., 2017), SCC, and bacterial count (Zeng and Escobar, 1995) of goats with different orders of parity. Mioč et al. (2008) did not observe significant changes across parities for milk fat and lactose, but found a decreased protein content moving from the first to the fifth or greater parity. Zeng and Escobar (1995) did not find any significant effect of parity on goat milk composition, SCC, and bacterial count, but only on MY. That difference could be attributable to the low number of parities considered (from first to third parity). On the contrary, results by Lôbo et al. (2017), regarding the effects of 7 orders of parity of dairy goats in Brazil, have evidenced the decrease of milk components across parities and, in agreement with our results, the increase of SCS from the first to the seventh parity. Those authors suggested that the increase of somatic cells could be related to the growing susceptibility of the goats to mastitis (especially of subclinical type) over the different lactations. That result could be further supported by the greater difference in LBC found in the present study between younger (lower) and older goats (higher). The differences of milk composition found among parities were not followed by great differences in coagulation traits, as only higher curd firmness (a_{30}) was observed in younger goats compared with the older ones. This finding could be explained by the higher fat content and lower SCS and LBC in milk from the first compared with the latter (Stocco et al., 2018b). Among %CY traits, only %CY_{SOLIDS} was affected by parity, with higher values in goats at first and second parities compared with goats at the third parity or greater. This result could be attributable to the different composition of milk in younger goats, as it is acknowledged that milk composition affects %CY and %REC traits (Pazzola et al., 2019). Indeed, recovery of nutrients was higher in younger goats, except for %REC_{SOLIDS},

whereas the lowest %REC values were recorded from goats in the fifth parity or greater. As expected, daily cheese productions were lower in younger goats, and this was mainly due to the lower MY. In sheep, younger ewes are characterized by better MCP, compared with older ones, and by higher %REC_{SOLIDS} (Cipolat-Gotet et al., 2016b).

CONCLUSIONS

This study provided a detailed characterization of the Sarda goat breed in terms of milk quality, technological characteristics, cheese yield, and milk nutrients recovery in the curd. The favorable qualitative and technological characteristics of milk from Sarda suggests that this breed has a high potential for cheese production that needs to be preserved. The characterization of the Sarda breed provided by this study could be a key point for its preservation and enhancement, and could be of great interest for the goat dairy sector. The collection of a wide range of phenotypes at the individual animal level is fundamental for the characterization of local populations and can be used to guarantee breed conservation, persistence of traditional farming system, and increase the farmers' profit.

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