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Phenotypic factors affecting coagulation properties of milk from Sarda ewes

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ABSTRACT

In this study, milk-coagulation properties (MCP) were characterized in the Sarda sheep breed. Milk composition and MCP [rennet-coagulation time (RCT), curd-firming time [time to reach a curd firmness of 20 mm (k_{20})], and curd firmness (a_{30}), (a_{45}), and (a_{60})] were obtained extending the lactodynamographic analysis from 30 to 60 min from a population of 1,121 ewes from 23 different farms. Managerial characteristics of farms and parity, individual daily milk yields and stage of lactation of ewes were recorded. Data were analyzed using a mixed-model procedure with fixed effects of days in milk, parity, daily milk yield, and flock size and the random effect of the flock/test day nested within flock size. Sampled farms were classified as small (<300 ewes) and medium (300 to 600 ewes), and these were kept by family operations, or as large (>600 ewes), often operated through hired workers. Daily milk yield was, on average, 1.58 ± 0.79 L/d and variability for this trait was very high. The average content of fat, protein, and casein was respectively 6.41, 5.39, and 4.20%. The class of flock size had a significant effect only on curd firmness, whereas days in milk affected RCT and k_{20}. The flock test day, parity, and daily milk yield were important sources of variation for all MCP. The mean value of RCT (8.6 min) and the low occurrence of non-coagulating samples (0.44%) confirmed the excellent coagulation ability of sheep milk compared with cattle milk. A more rapid coagulation was observed in mid-lactating, primiparous, and high-yielding ewes. The k_{20} was usually reached in less than 2 min after gelation, with the most favorable values at mid lactation. The mean value of curd firmness 30 min after rennet addition (a_{30}) was, on average, 50 mm and decreased to 46 and 42 mm respectively after 45 (a_{45}) and 60 min (a_{60}). The decreasing value of curd-firmness traits was likely to be caused by curd syneresis and whey expulsion. The correlation between RCT and a_{30} was much lower than in dairy cows and about null for a_{45} and a_{60}. This means that curd firmness in dairy ewes is almost independent of gelation time and this can provide specific information for this species. In conclusion, this study showed that milk from Sarda sheep is characterized by an earlier gelation, a faster increase in curd firmness with time, and greater curd firmness after 30 min compared with dairy cows. Furthermore, correlations between MCP in sheep are much lower than in bovines and some of the assumptions and interpretations related to cows cannot be applied to sheep.
Key words: sheep milk, coagulation property, Sarda sheep breed, flock characteristic

INTRODUCTION

Milk-coagulation properties (**MCP**) are generally used in the dairy industry to assess the suitability of milk for cheese making and predict yield and technological characteristics of cheese. Since the 1970s, many techniques have been investigated and both mechanical and infrared-based instruments have been assembled to obtain MCP (Cipolat-Gotet et al., 2012). One of the most commonly used mechanical instruments is the Formagraph (Foss Electric A/S, Hillerød, Denmark). It is able to provide a diagram that graphically illustrates the sequences of formation and development of curd after the addition of rennet (usually for 30 min) and it allows the measurement of 3 MCP: rennet-coagulation time (**RCT**; measured in min), curd-firming time [time to reach a curd firmness of 20 mm ($\mathbf{k_{20}}$); min], and curd-firmness ($\mathbf{a_{30}}$; mm) traits (McMahon and Brown, 1982). Milk-coagulation properties have been extensively studied in dairy cows and the effects of the breed, milk genetic variants, and measurement methods as well as repeatability, heritability, and genetic correlations with milk quality traits have recently been reviewed by Bittante et al. (2012).

Milk-coagulation properties of milk from small ruminants, and particularly from sheep, are much less known. Milk production from dairy sheep farming is of great economic importance, especially in the countries of Southern Europe and the Mediterranean basin in

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general (Vacca et al., 2010; Ramos and Juarez, 2011). The European Union produced about 2.8 million tonnes of ewe milk in 2011, representing 29% of the whole world ovine milk production (FAOSTAT, 2013). This milk is mainly produced by local breeds, such as in Sardinia, a region of Italy in the center of the Mediterranean Sea, where it has been estimated that 5% of the whole world ewe's milk is produced by its indigenous breed, the Sarda sheep (Vacca et al., 2008).

The Sarda is a dairy specialized breed, well adapted to the difficult environment of the island of Sardinia (Macciotta et al., 1999) where farming is characterized by a large variation in the application of semi-extensive and semi-intensive methods (Carta et al., 2009). Nowadays, the Sarda is the top-ranked sheep breed in Italy, with an estimated total population size of about 3 million head (Carta et al., 2009), among which 220,000 animals in 1,000 farms are officially recorded (ICAR, 2013). Milk from Sarda ewes is almost completely used to produce 3 protected designation of origin cheeses, according to the rules of the European Union: Pecorino Romano (EU, 2009), Pecorino Sardo, and Fiore Sardo (EU, 1996). In contrast to cattle, MCP are scarcely used as a tool to characterize milk for protected designation of origin dairy products and for milk payment scales (Bittante et al., 2011a,b).

To our knowledge, no study has yet investigated ovine MCP under field conditions and on the basis of a large sampled population. Hence, the objectives of this paper were to (1) characterize traditional MCP of the Sarda sheep breed obtained using the Formagraph instrument (RCT, k_{20}, and a_{30}); (2) analyze curd firmness after the standard interval of 30 min [i.e., at 45 (**a_{45}**) and 60 min (**a_{60}**)] by extending the duration of the analysis to 60 min; (3) investigate the effect of management, feeding, and farming system on MCP, using the flock size as a statistical indicator; and (4) investigate individual effects, such as parity, stage of lactation, and daily milk yield, on a large population on MCP.

MATERIALS AND METHODS

Animals and Milk Sampling

The study involved 1,121 ewes reared in 23 different farms, evenly distributed over the whole island of Sardinia, Italy. Farms were chosen among those officially registered in the flock book of the Sarda breed and were generally managed following the common semi-extensive and semi-intensive methods as described by Carta et al. (2009) and Carcangiu et al. (2011). In brief, lactating ewes were pasture fed, with a commercial concentrate supplementation given during the milkings, which were performed by manually operated milking machines twice per day (often at 0600 and at 1600 h); reproduction was based on natural mating or, rarely, on AI, and lambs were milk fed by their dams and weaned when they were about 1 mo old.

A report regarding general characteristics of sampled flocks, such as geographical location, flock size, and working environment description, management, and feeding conditions is summarized in Table 1. On the basis of management and feeding characteristics, farms were classified in 3 categories: (1) traditional semi-extensive farming with free-ranging pasture, (2) presence of cultivated grasslands and rotational pasture and control of the lambing season, or (3) modern semi-intensive farming with investments made in new buildings and facilities, use of TMR, and advice provided by consultants and experts in animal feeding. Scores were assigned by one skilled technician to minimize error of subjective criteria.

Groups of ewes ranging in size from 32 to 82 were sampled per flock (1 sampling day for each flock). Ewes presenting clinical symptoms of disease, those within 60 d after parturition (often feeding their lambs), and those after 7 mo of lactation were excluded. The sampled ewes were selected to represent the different levels of parities, DIM, and daily milk yield. The selected ewes were daughters of 120 different sires (with a minimum of 4 and a maximum of 40 daughters per sire).

During the afternoon milking, individual milk samples were collected from each ewe in 200-mL disposable sterile plastic containers and, on the same day, daily milk yield (morning plus evening milking) was recorded. Milk samples were kept at 4°C and analyzed within 24 h after collection.

Analysis of Milk Traits and Coagulation Properties

The chemical composition (fat, protein, casein, lactose, urea, and pH) of individual milk samples was analyzed using a MilkoScan FT6000 milk analyzer (Foss Electric A/S), casein number (%) was calculated as the ratio between casein and protein contents, and SCC was determined with a Fossomatic 5000 somatic cell counter (Foss Electric A/S) and total bacterial count (**TBC**) with a BactoScan FC150 analyzer (Foss Electric A/S) according to International Dairy Federation methods [IDF (2006) and IDF (2000), respectively]. Both SCC and TBC were logarithmically transformed to normalize the distribution: SCC to SCS, as proposed by Ali and Shook (1980), and TBC to logarithmic bacterial count [TBC = $\log_{10}$ (total bacterial count/1,000)].

Measures of MCP were obtained using a Formagraph (Foss Italia S.P.A., Padova, Italy). For each individual sample, 10 mL was heated to 35°C before the addition of 200 μL of rennet solution [Hansen Naturen Plus 215

Table 1. Characteristics of sampled farms according to flock size

Item[1]	Flock size		
	Small (<300 ewes)	Medium (300–600 ewes)	Large (>600 ewes)
Farms, no.	8	7	8
Ewes/farm, no. (mean ± SD)	204 ± 47	439 ± 85	894 ± 255
Sampled ewes, no.	335	361	425
Geographical area, no. of farms			
Plains (<200 m asl)	1	1	6
Hills (200–500 m asl)	4	5	1
Mountain (>500 m asl)	3	1	1
Altitude, m asl (mean ± SD)	371 ± 174	307 ± 172	181 ± 203
Family-operated farms, no.	8	6	2
Type of management and feeding, no. of farms			
Traditional[2]	5	1	0
Intermediate[3]	2	6	2
Modern[4]	1	0	6

[1]asl = above sea level.

[2]Semi-extensive farming with free-ranging pasture in natural grasslands.

[3]Presence of cultivated grasslands and rotational pasture and control of the lambing season.

[4]Semi-intensive farming, investments in new buildings and facilities, use of TMR, and assistance by consultants and experts in animal feeding.

(Pacovis Amrein AG, Bern, Switzerland), with 80 ± 5% chymosin and 20 ± 5% pepsin; 215 international milk clotting units (**IMCU**)/mL; diluted to 1.2% (wt/vol) in distilled water to achieve 0.0513 IMCU/milk mL]. This analysis continued for 60 min after rennet addition and the following coagulation traits were recorded: RCT (min), as the period from rennet addition to gel formation (bifurcation of the line); k_{20} (min), as the interval between gel formation and the time at which the width of the graph attains 20 mm; and curd firmness (mm), defined by the distance between the 2 arms at 30 (a_{30}), 45 (a_{45}), and 60 min (a_{60}) after rennet addition. In milk samples that did not coagulate (n = 5) or coagulated more than 30 min (n = 10) after rennet addition, RCT was considered to be missing, whereas k_{20} was labeled as missing when it occurred after 5 min (n = 10) as well as when milk samples reached a curd firmness of 20 mm later than 60 min after rennet addition (n = 9). Curd firmness was considered as a missing observation when the result was 0 (a_{30}: n = 15; a_{45}: n = 10; a_{60}: n = 7).

Statistical Analysis

Preliminary analyses showed that because the number of flocks sampled (23) and the classification criteria of individual flocks (flock size, geographical area, type of operation, and type of farm on the basis of management and feeding characteristics) were highly correlated, only one of the above factors could be included in the model to avoid multicollinearity problems. The flock size was found to be the factor producing the better model fitting according to the Akaike information criterion (Akaike, 1969; Agresti, 2002).

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

$$y_{ijklmno} = \mu + \text{Flock size}_m + \text{Flock(Flock size)}_{n:m} + \text{DIM}_i + \text{parity}_j + \text{MUCM}_k + \text{MY}_l + e_{ijklmno}, \quad [1]$$

where $y_{ijklmno}$ is the observed trait; μ is the overall intercept of the model; Flock size_m is the fixed effect of the mth flock dimension, as a statistical indicator of management, feeding, and farming system ($i = 1$ to 3; class 1: <300 ewes; class 2: 300 to 600 ewes; class 3: >600 ewes); Flock(Flock $\text{size})_{n:m}$ is the random effect of the flock test day nested within flock size; 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 ($k =$ individual pendulum 1 to 10); MY_l is the fixed effect of lth class of single test-day milk yield ($l = 1$ to 7; class 1: <0.60; class 2: 0.60 to 0.99; class 3: 0.99 to 1.38; class 4: 1.39 to 1.77; class 5: 1.78 to 2.17; class 6: 2.18 to 2.57; class 7: >2.57); and $e_{ijklmno}$ is the random residual. The flock 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, respectively. The Flock(Flock $\text{size})_{n:m}$ was used as the error term to test the significance of flock size_m, whereas all the other fixed effects were tested on residual variance.

Table 2. Descriptive statistics of milk yield and composition of Sarda ewes

Item	No.	Mean	SD	P5[1]	P95[1]
Parity, no.	1,121	2.30	1.36	1	5
DIM, d	1,120	140	42	78	205
Daily milk yield, kg/d	1,111	1.58	0.79	0.33	3.27
Fat, %	1,107	6.41	1.10	4.71	8.40
Protein, %	1,109	5.39	0.62	4.54	6.58
Casein, %	1,107	4.20	0.51	3.49	5.16
Casein number, %	1,100	77.87	1.28	75.76	79.84
Lactose, %	1,106	4.82	0.28	4.31	5.24
Urea, mg/100 mL	1,105	31.81	11.08	13.80	50.80
pH	885	6.67	0.08	6.54	6.82
SCS,[2] U	1,120	4.71	2.13	2.04	9.23
TBC,[3] U	1,039	2.53	0.96	1.04	4.23

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

[2]SCS = $\log_2$ (SCC × 10^{-5}) + 3.

[3]Total bacterial count (TBC) = $\log_{10}$ (total bacterial count/1,000).

The percentage of variance explained by the flock was calculated by dividing the corresponding component of variance by the total variance. Relationships among the different MCP traits as well as among residuals of the same traits were investigated.

Finally, contrasts were estimated between least squares means of MCP for the effect of (1) DIM: (a) linear, (b) quadratic, and (c) cubic component; (2) parity: (a) primiparous vs. pluriparous, (b) second parity vs. third to fifth parity, (c) third parity vs. fourth or fifth parity, and (d) fourth parity vs. fifth parity; and (3) daily milk production: (a) linear, (b) quadratic, and (c) cubic component.

RESULTS

Characteristics of Sampled Farms and Individual Milk Samples

The main features of Sardinian farms sampled for this study are summarized in Table 1. About one-third of farms were classified as small (<300 ewes), one-third as medium (300 to 600 ewes), and the remaining one-third as large (>600 ewes).

Small flocks had an average number of 204 dairy ewes and were distributed almost entirely in the hills and mountains of the island (the average altitude was 371 m above sea level). All small flocks were kept by family operations and used traditional management and feeding techniques.

The flocks of medium size had, on average, 439 dairy ewes and were distributed almost exclusively in the hilly areas of the island (307 m above sea level). They were mainly family operated and used traditional techniques innovated by the use of rotational pasture on cultivated grasslands.

Last, the large flocks had an average of 894 heads of dairy ewes; they were mainly located in the plains of the island and were often operated by hired workers, and used modern facilities and feeding techniques. Descriptive statistics of sampled ewes and milk samples are shown in Table 2.

Daily milk yield was, on average, 1.58 L/d, but variability for this trait was very high. The average content of fat, protein. and casein was 6.41, 5.39, and 4.20%, respectively; the variability for fat was almost double that of protein and casein content. The casein number, which is the percentage ratio between casein and protein contents, was on average 77.87%, with low variability. Low variability existed for lactose content and pH and a comparatively high variability for urea, SCS, and TBC.

The distribution of data according to the different MCP is shown in Figure 1, in addition to their mean values and standard deviations and also coefficients of kurtosis and skewness. Both time intervals (RCT and k_{20}) were leptokurtic and right skewed, whereas the 3 measures of curd firmness moved from the leptokurtic and left-skewed distribution recorded 30 min after rennet addition to the distribution more close to a Gaussian feature recorded after 1 h.

The correlation coefficients between the different MCP are presented in Table 3. Correlations estimated using the raw data were very similar to those calculated between the residuals obtained after correcting the raw data for the flock/test day, parity, DIM, and milk yield of ewes according to model [1]. It was also apparent that the correlations between the 2 time intervals and among the 3 measures of curd firmness were all positive and showed high values, whereas those between the time intervals and the curd-firmness measures were negative or close to zero.

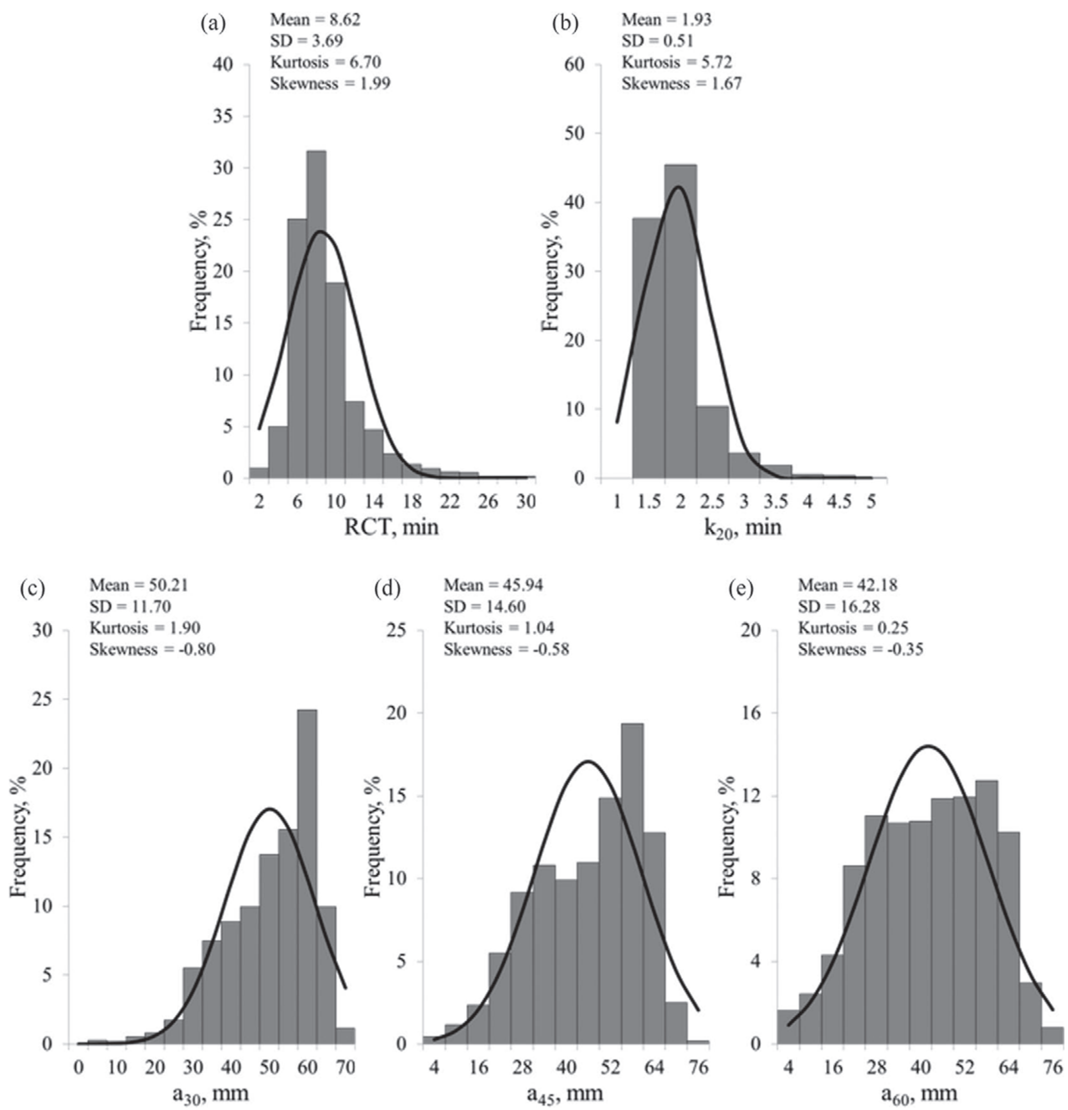

Figure 1. Distribution, mean, SD, kurtosis, and skewness of (a) rennet coagulation time (RCT), (b) curd-firming time (k_{20}), and curd firmness measured (c) 30 min (a_{30}), (d) 45 min (a_{45}), and (e) 60 min (a_{60}) after rennet addition to milk samples of 1,121 Sarda ewes (skewness and kurtosis were estimated using model residuals).

Table 3. Pearson product-moment correlations between coagulation traits above the diagonal and between their residuals (according to the model including Flock/test day, parity, DIM, and milk yield of ewes) below the diagonal

	Coagulation time,[1] min		Curd firmness,[2] mm		
Item	RCT	k_{20}	a_{30}	a_{45}	a_{60}
RCT	—	0.65***	−0.15***	0.02^{NS}	$−0.01^{NS}$
k_{20}	0.69***	—	−0.50***	−0.31***	−0.27***
a_{30}	−0.23***	−0.45***	—	0.87***	0.76***
a_{45}	0.03^{NS}	−0.20***	0.79***	—	0.92***
a_{60}	0.01^{NS}	−0.16***	0.63***	0.88***	—

[1]RCT = rennet-coagulation time; k_{20} = curd-firming time (time to reach a curd firmness of 20 mm).

[2]a_{30} = 30 min after rennet addition; a_{45} = 45 min after rennet addition; a_{60} = 60 min after rennet addition.

$***P < 0.001$.

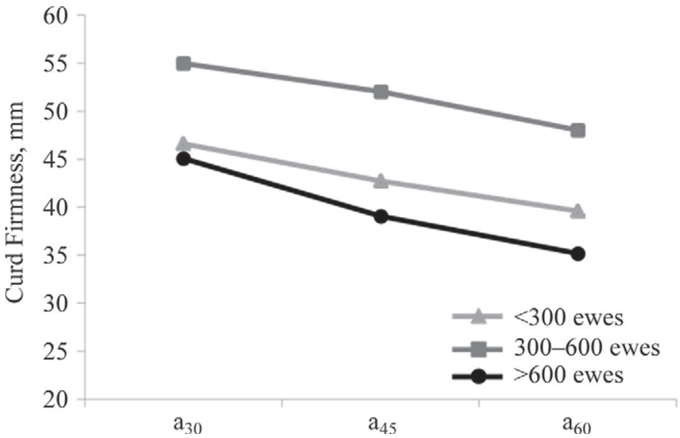

Figure 2. Effect of flock size on curd firmness of Sarda ewes (the contrasts between large and small flock size were not significant, whereas the contrasts between medium and the average of small and large flock size were significant for all curd firmness values). a_{30}, a_{45}, and a_{60} = curd firmness measured 30, 45, and 60 min after rennet addition, respectively.

Factors Affecting Milk Coagulation Properties

The results of statistical analyses carried out on MCP data of milk samples from Sarda ewes are shown in Table 4. The class of flock size, which was tested as the variability among flocks within class, had a significant effect only on curd-firmness measures (Figure 2).

The flock test day was an important source of variation for all MCP (Table 4), varying from 20 to 21% of total variance in the case of the time intervals (RCT and k_{20}) to almost the double that value (39%) for a_{30}. Days in milk affected the 2 time intervals and not the measures of curd firmness (Table 4). Both time intervals showed a curvilinear response (significant quadratic contrast) to DIM (Table 5). Indeed, both traits showed the most favorable results at mid lactation (121 to 140 DIM), when the shorter k_{20} resulted in a rapid increase in curd firmness during the first stages of the monitored period of lactation (also the linear contrast was significant). The linear contrast of DIM was significant also for a_{30} and a_{45}, showing a tendency for an increase in curd firmness during lactation.

Parity was, in general, a significant source of variation for only RCT (Table 4), but the orthogonal contrast between primiparous and multiparous ewes reached statistical significance also for k_{20} and a_{30} (Table 6). On the contrary, none of the contrasts between different parities of multiparous ewes was significant.

Also daily milk yield affected the 2 time intervals and especially the a_{30} (Table 4). Rennet coagulation time showed a linear improvement (reduction) with an increase in daily milk yield (Table 7). An improvement of k_{20} (reduction) and a_{30} (increase) was also recorded for high-yielding ewes, but for both traits, the improvement was more evident moving from low to intermediate milk yield than from intermediate to high levels (linear and quadratic response).

The individual position of milk samples within the 10 rack positions of the instrument was also statistically significant for all MCP except RCT (Table 4). Similar results have been obtained for dairy cows (Ikonen et al., 2004; Tyrisevä et al., 2004; Cipolat-Gotet et al., 2012) and confirmed the low reproducibility of amplitude measurements of the different pendulums. As a result, position of the pendulums should be considered when MCP data are statistically analyzed.

DISCUSSION

RCT

Bittante et al. (2012), in a review on modeling and genetics of MCP, report that, with the exception of

Table 4. Results from ANOVA (*F*-value and significance) for milk coagulation properties of Sarda ewes

		Coagulation time,[1] min		Curd firmness,[2] mm		
Item	df	RCT	k_{20}	a_{30}	a_{45}	a_{60}
Flock size, *F*-value	2	0.54^{NS}	0.40^{NS}	4.08*	4.77*	4.41*
Flock/test day[3]	20	0.21	0.20	0.39	0.35	0.28
DIM, *F*-value	7	3.35**	2.92**	1.38^{NS}	1.33^{NS}	0.81^{NS}
Parity, *F*-value	4	2.40*	2.03^{NS}	2.05^{NS}	0.78^{NS}	0.49^{NS}
dMY,[4] *F*-value	6	2.14*	2.62*	4.62***	1.89^{NS}	1.59^{NS}
MUCM,[5] *F*-value	9	1.27^{NS}	8.65***	16.77***	15.13***	14.45***
RMSE[6]	1,048–1,056	3.31	0.46	8.71	11.12	13.04

[1]RCT = rennet-coagulation time; k_{20} = curd-firming time (time to reach a curd firmness of 20 mm).

[2]a_{30} = 30 min after rennet addition; a_{45} = 45 min after rennet addition; a_{60} = 60 min after rennet addition.

[3]Flock/test day effect expressed as the proportion of the variance explained by flock/test date within flock size on the total variance.

[4]Daily milk yield.

[5]Measuring unit of the coagulation meter (individual pendulum 1 to 10).

[6]Root means square error.

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

Table 5. Least squares means of milk coagulation properties of Sarda ewes across classes of days in milk

Item	Ewes, no.	Coagulation time,[1] min		Curd firmness,[2] mm		
		RCT	k_{20}	a_{30}	a_{45}	a_{60}
DIM						
≤80 d	71	9.54	2.16	46.63	41.50	38.27
81–100 d	131	8.81	2.08	47.85	43.18	40.60
101–120 d	231	8.88	2.02	48.83	44.48	40.79
121–140 d	149	8.08	1.89	49.24	45.58	41.78
141–160 d	134	8.75	1.95	49.64	45.12	41.27
161–180 d	155	8.87	1.91	50.89	46.88	43.15
181–200 d	159	9.62	1.93	49.40	45.28	41.00
>200 d	76	10.28	2.05	48.97	45.03	40.79
Contrast, *P*-value						
Linear		NS	**	*	*	NS
Quadratic		**	*	NS	NS	NS
Cubic		NS	NS	NS	NS	NS

[1]RCT = rennet-coagulation time; k_{20} = curd-firming time (time to reach a curd firmness of 20 mm).
[2]a_{30} = 30 min after rennet addition; a_{45} = 45 min after rennet addition; a_{60} = 60 min after rennet addition.
*$P < 0.05$; **$P < 0.01$.

Table 6. Least squares means of milk coagulation properties of Sarda ewes across parities

Item	Ewes, no.	Coagulation time,[1] min		Curd firmness,[2] mm		
		RCT	k_{20}	a_{30}	a_{45}	a_{60}
Parity (P)						
1	459	8.49	1.91	50.25	45.90	41.97
2	214	8.97	1.99	47.80	44.26	40.89
3	207	9.12	2.03	48.78	44.56	41.45
4	116	9.81	2.05	48.70	44.72	40.71
≥5	115	9.14	2.02	49.12	43.72	39.77
Contrast, *P*-value						
P1 vs. P2 + P3 + P4 + P5		*	**	*	NS	NS
P2 vs. P3 + P4 + P5		NS	NS	NS	NS	NS
P3 vs. P4 + P5		NS	NS	NS	NS	NS
P4 vs. P5		NS	NS	NS	NS	NS

[1]RCT = rennet-coagulation time; k_{20} = curd-firming time (time to reach a curd firmness of 20 mm).
[2]a_{30} = 30 min after rennet addition; a_{45} = 45 min after rennet addition; a_{60} = 60 min after rennet addition.
*$P < 0.05$; **$P < 0.01$.

Table 7. Least squares means of milk coagulation properties of Sarda ewes across classes of daily milk yield (dMY)

Item	Ewes, no.	Coagulation time,[1] min		Curd firmness,[2] mm		
		RCT	k_{20}	a_{30}	a_{45}	a_{60}
dMY, kg/d						
<0.60	80	10.36	2.31	43.14	40.88	37.39
0.60–0.98	142	9.94	2.02	49.14	44.66	40.44
0.99–1.38	265	8.98	2.00	48.03	43.38	39.05
1.39–1.77	280	8.86	1.94	49.85	45.50	41.07
1.78–2.17	125	8.52	1.93	52.43	47.26	43.39
2.18–2.57	75	8.53	1.88	49.38	44.79	42.02
>2.57	144	8.53	1.92	50.54	45.96	43.33
Contrast, *P*-value						
Linear		**	***	**	NS	*
Quadratic		NS	**	**	NS	NS
Cubic		NS	NS	NS	NS	NS

[1]RCT = rennet-coagulation time; k_{20} = curd-firming time (time to reach a curd firmness of 20 mm).
[2]a_{30} = 30 min after rennet addition; a_{45} = 45 min after rennet addition; a_{60} = 60 min after rennet addition.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

the 2 oldest papers cited, RCT values of cow milk are between 10 and 20 min. The average RCT obtained in the present study (8.6 min) confirmed that sheep milk coagulated much faster than cow milk. Different physicochemical properties, such as larger casein micelles and higher levels of calcium and other minerals per casein weight, may explain this (Park, 2007; Park et al., 2007). This high variability depends on the different breeds but mainly on the experimental conditions. Differences in milk temperature, acidification, and rennet addition cause large differences in MCP in bovine (Okigbo et al., 1985; Mishra et al., 2005) and ovine milk (Ould Eleya et al., 1995; Bencini, 2002; Barłowska et al., 2011).

In cattle, the occurrence of noncoagulating milk samples (**NC**) is one of the main problems of MCP. Noncoagulating samples are those samples that do not coagulate within 30 min from rennet addition to milk. They have been very frequently evidenced for both individual and bulk milk samples in cattle (Ikonen et al., 1999), water buffaloes (Cecchinato et al., 2012), sheep (Pazzola et al., 2013; Bittante et al. 2014), and goats (Devold et al., 2011; Pazzola et al., 2012, 2014). The presence of NC samples also affects the actual meaning of average values of MCP and the possibility to estimate breeding values of animals (Ikonen et al., 2004; Cecchinato et al., 2011). Because of this concern, it has been proposed to extend the duration of conventional MCP tests to periods longer than 30 min for bovine milk (Bittante et al., 2013). In the present study, the incidence of NC samples was only 0.44%, confirming the excellent coagulation ability of the Sarda sheep.

As in cattle, ovine MCP are strongly affected by experimental conditions. Using the same instrument, temperature, and rennet type and concentration as in the present study but in a different laboratory and environment, Bittante et al. (2014) obtained an average RCT of 6.50 min for milk samples from autochthonous Alpine ewes fed an indoor diet and a value of 9.44 for those receiving the same diet supplemented with CLA. In the present study, the average RCT of Sarda ewes fed pasture and concentrates was between the aforementioned values for the Alpine breeds. Abilleira et al., (2010), using a different instrument, lower temperature (32 vs. 35°C), and lower rennet concentration (0.0439 vs. 0.0512 IMCU/mL) for bulk milk samples from Latxa ewes, obtained average RCT of about 12 min at the beginning of lactation with indoor feeding and 13 to 15 min in pasture-fed ewes during mid lactation.

The variability in RCT due to the flock effect was moderate (21% of total variance) and slightly higher than the herd effect for dairy cows (Ikonen et al., 2004; Tyrisevä et al., 2004; Cecchinato et al., 2013). Moreover, the effect of flock size was not significant, despite the concurrent differences in environmental conditions and feeding and management characteristics associated with the flock size of Sardinian farms (Table 1). Similar results have been found in dairy cows, as the effect of management practice and, in particular, of feeding, was not very important in relation to RCT variability (Tyrisevä et al., 2004; Butler et al., 2010).

Among the causes of variation related to RCT in an individual, DIM was the most important in Sarda ewes, confirming previous results reported in dairy cows (Ikonen et al., 2004; Cipolat-Gotet et al., 2012) and Guirra and Manchega ewes (Jaramillo et al., 2008), which consistently show an increase of RCT during lactation. In contrast to those studies, milk from Sarda ewes was characterized by a decrease in RCT during early lactation and an increase in the last stages, so that the shortest coagulation time was registered for milk samples collected from mid-lactation ewes.

In Sarda ewes, coagulation occurs slightly earlier in the first lactation compared with subsequent lactations, confirming the results observed in cows (Cipolat-Gotet et al., 2012), but not those obtained from Guirra and Manchega ewes by Jaramillo et al. (2008) with different instruments and experimental conditions.

Also, daily milk yield level significantly affected RCT of Sarda ewe milk because a decrease in coagulation time was observed in milk samples collected from high-yielding compared with low-yielding animals (Table 6). According to the review of Bittante et al. (2012), the phenotypic correlations between RCT and milk yield are moderate or null in dairy cows and more frequently negative (favorable) than positive.

k_{20}

Milk produced by Sarda ewes reached, on average, curd firmness of 20 mm (k_{20}) less than 2 min after gelation; 20 mm is usually considered to be the target value for curd cutting. This interval is very short compared with bovine milk, in which k_{20} is from 5 to 15 min (Bittante et al., 2012). This trait is seldom reported in large surveys of cattle because a significant proportion of dairy cows produce milk that does not reach the k_{20} within 30 min of the addition of rennet, which is the standard duration of the lactodynamographic test. The combined effect of rapid gelation and a fast curd-firming process meant that the majority of sheep milk samples reached the k_{20} within 30 min after rennet addition and, thus, produce a k_{20} value associated with the sample. For dairy cows, the incidence of samples without a k_{20} value within 30 min varies from a minimum of 8% in Finnish Friesians (Ikonen et al., 1997) to a maximum of 32% in German Friesians (Oloffs et al., 1992) and 33% in Finnish Ayrshires (Ikonen et al., 1999). Due to its low average value, the distribution of k_{20} data was far from normal in ewes of the present study (Figure 1), whereas it is much more close to a Gaussian distribution in cows (Cipolat-Gotet et al., 2012).

In addition, k_{20} of Sarda ewes' milk was affected by DIM and daily milk yield and it showed a curvilinear trend during lactation, similarly to dairy cows (Cipolat-Gotet et al., 2012). But in the case of sheep, milk with the most favorable k_{20} was produced at mid lactation, whereas in bovine milk, it occurs at the early or late stages of lactation. The rate of gel firming, measured with different instruments and experimental conditions, has shown a decreasing trend during lactation both in Lacaune (Pellegrini et al., 1997) and in Guirra and Manchega ewes (Jaramillo et al., 2008). The k_{20} of Sarda sheep showed a curvilinear trend in relation to daily milk yield, with a shortening (improvement) of this trait moving from low-yielding to mid-yielding sheep and a more stable pattern afterward. In Lacaune sheep (Pellegrini et al., 1997), the gel-firming rate was slightly higher in high-yielding than in low-yielding ewes. In dairy cows, the phenotypic correlation between k_{20} and milk yield was not significant (Ikonen et al., 1997; Cipolat-Gotet et al., 2012).

Curd Firmness

The a_{30} of milk samples from Sarda ewes was, on average, 50 mm. The average a_{45} and a_{60} values decreased to 46 and 42 mm, respectively, whereas the standard deviation increased. Values of curd firmness, measured with the same technique from Alpine sheep breeds (Bittante et al., 2014), are about 5 to 10 mm higher than those from Sarda ewes. The results obtained from sheep milk reported here are generally similar to those from autochthonous goat breeds from Sardinia (Pazzola et al., 2011) and Ethiopia (Mestawet et al., 2013) but higher than those obtained from buffaloes (Cecchinato et al., 2012) and especially from dairy cows (Cipolat-Gotet et al., 2012) when a similar quantity of rennet is used.

In the case of dairy cows, the informative value of a_{30} beyond that of RCT is not very great, because the 2 traits are highly correlated. Compared with sheep, in the bovine species, late gelation and slow curd firming make the a_{30} strictly dependent on both these traits: the time available for curd firming (30 min minus the RCT) is about 1- to 2-fold the k_{20}, and a_{30} is about 1- to 2-fold 20 mm. Reviewing the results of 9 large studies, Bittante et al. (2012) have found that the phenotypic correlations between a_{30} and RCT are, on average, $-81 \pm 10\%$ and that the corresponding genetic correlations are even higher ($-0.92 \pm 0.05\%$).

In the case of the ovine species, the scenario is completely different from cows. For Sarda ewes reported in the present paper, the time available for curd firming in a standard 30-min test (30 min − 8.62 min = 21.38 min) was much longer: on average, about 11-fold the value of k_{20} (21.38 min/1.93 min = 11.1), whereas the a_{30} was not much greater: about 2.5-fold 20 mm (50.21 mm/20 mm = 2.51). This explains the correlation between RCT and a_{30} in Sarda sheep, which was much lower than in dairy cows (the value of the Pearson correlation between the raw data was −15% and the one calculated on residues of the statistical model used was −23%; Table 3) and about null for a_{45} and a_{60}. This means that curd firmness in dairy ewes is almost independent of gelation time and that it can hold specific important information that should be better studied.

In Sarda ewes, the decreasing values of average a_{30}, a_{45}, and a_{60} (50.21, 45.94, and 42.18 mm, respectively) provides evidence that curd firmness was not continuously increasing toward an asymptotical value, as is often assumed (Bittante, 2011), but apparently decreases. This was interpreted by Bittante et al. (2013) as the effect of curd syneresis causing whey expulsion, as has been recently demonstrated in Alpine sheep breeds (Bittante et al., 2014). The syneresis process is different in ovine and bovine species and it is affected by several technological features (Calvo and Balcones, 2000). Regarding dairy sheep, the pattern of curd firmness depending on time should be better studied to achieve new insights on this species.

Curd-firmness traits of milk from the Sarda breed were significantly influenced by the effect of class of flock size. Moreover, the variability of these traits among the different flocks was much greater than that observed for RCT and k_{20}. This last characteristic is not in agreement with results found in different bovine dairy breeds (Tyrisevä et al., 2003; Ikonen et al., 2004), which are characterized by a less significant effect of herd.

In contrast to RCT and k_{20}, the sources of variation due to the effects of the individual animal (DIM, parity, and milk yield) slightly affect curd-firmness traits. In particular, Sarda ewes of the present study showed an increase in curd firmness for high-yielding animals and in late stages of lactation, similar to what was observed in the Latxa sheep breed (Abilleira et al., 2010).

CONCLUSIONS

Milk coagulation properties were studied for the first time on a large number of dairy ewes. Compared with bovine milk, sheep milk was characterized by an earlier gelation, a much more rapid increase in curd firmness with time, and a higher a_{30}. It was demonstrated that the correlations between MCP in the ovine and, in particular, in the Sarda sheep breed are much lower than in bovine species and that some of the assumptions and interpretations developed on the latter species cannot be applied to sheep. The effects of Sardinian flock size, as an indicator of management, feeding, and farming system, and individual flock on MCP were quantified as well as the main individual (parity, DIM, and daily milk yield) and technical (cuvette position) causes of variation. It was also evidenced that, in dairy ewes, the gelation, curd-firming, and syneresis processes need further analysis and that the corresponding parameters have to be better defined in relation to environment, management, feeding, genetics of animals, and processing characteristics of milk.

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