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

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Complete List of Authors:	<p>Paschino, Pietro; Università degli Studi di Sassari, Dipartimento di Medicina Veterinaria</p> <p>Stocco, Giorgia; Università degli Studi di Parma, Dipartimento di Scienze Medico-Veterinarie</p> <p>Dettori, Maria Luisa; Università degli Studi di Sassari, Dipartimento di Medicina Veterinaria; Università degli Studi di Sassari,</p> <p>Pazzola, Michele; University of Sassari, Department of Animal Biology</p> <p>Marongiu, Maria Laura; Università degli Studi di Sassari</p> <p>Pilo, Carlo Emanuele; Università degli Studi di Sassari</p> <p>Cipolat Gotet, Claudio; Università degli Studi di Parma Dipartimento di Scienze Medico-Veterinarie; Università degli Studi di Parma,</p> <p>Vacca, Giuseppe Massimo; Università degli Studi di Sassari, Dipartimento di Biologia Animale; Università degli Studi di Sassari,</p>
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INTERPRETIVE SUMMARY

Characterization of milk composition, coagulation properties and cheese-making ability of goats reared in extensive farms. *By Paschino et al. page 000.* Farming of local goat breeds is an important source of livelihood in many areas of the world. The aim of this study was to characterize milk composition, coagulation properties and cheese-making ability of Sarda goat breed. Lactation period and parity were important sources of variation for milk yield and composition. Milk energy level influenced daily milk and cheese productions, while geographical area was more significant for milk composition and coagulation properties. 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.

TECHNOLOGICAL QUALITY OF SARDA GOAT MILK

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

Pietro Paschino*, Giorgia Stocco‡, Maria L. Dettori*, Michele Pazzola*, Maria L. Marongiu*, Carlo E. Pilo*, Claudio Cipolat-Gotet^{1‡}, and Giuseppe M. Vacca*

*Department of Veterinary Medicine, University of Sassari, via Vienna 2, 07100 Sassari, Italy

‡Department of Veterinary Science, University of Parma, Via del Taglio 10, 43126 Parma, Italy

¹Corresponding author: claudio.cipolatgotet@unipr.it

ABSTRACT

The aims of this study were to explore the variability of milk composition, coagulation properties, and cheese-making traits of Sarda goat breed, and to investigate the effects of animal and farm factors, and the geographical 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 coagulation properties (MCP), modeled curd-firming over time (CF_t) parameters, and cheese-making (%CY: cheese yield; %REC: recovery of nutrients; dCY: daily CY) traits. Farms were classified in two categories based on milk energy level (ME; high and 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 ME level, geographical area, days in milk and parity, and the random effect of farm within ME level and geographical area. Data about MCP, CF_t and cheese-making process were analyzed using the same model, with the inclusion of the animal effect and pendulum of the lactodynamograph instrument, allowing the measure of repeatability of these traits. Results showed that animal had higher 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 cells and bacterial count) and less cheese, but with higher recovery of fat and protein in the curd, compared with the 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 ME level was significant for daily productions of milk and cheese, coagulation time, and recovery of protein in the curd, which were better in high-ME farms. As regards to geographical area, milk composition and percentage cheese yield were superior in the Central-East area, while daily milk and cheese productions, and MCP were better in the South-West. This result was explainable by the crossbreeding phenomenon of Sarda goats with Maltese breed bucks occurred with greater intensity in the South-West compared to the Central-East

47 area of the island. The results provided by this study could be of great interest for the goat dairy
48 sector. Indeed, methods described in the present study could find a possible applicability for other
49 farming methods, goat breeds and geographical areas. The collection of a wide range of phenotypes
50 at individual animal level are fundamental for the characterization of local populations and can be
51 used to guarantee breed conservation, persistence of traditional farming system, and increase the
52 farmers' profit.

53

54 **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 those areas (i.e., Northern Brazil, the Caribbean, Mediterranean and Middle East 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 labelled with connotation of origin (i.e., 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 multi-purpose local goat breeds and their genetic potential. Preservation of local breeds is fundamental, as they are essential for the animal biodiversity (Di Trana et al., 2015) and they 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 to the production of functional foods (Čermák et al., 2013); the traditional extensive farming system can be integrated with the agro tourism activity, or with the organic farming, which is still growing in the European market (Dubeuf et al., 2011). The high potential of local breeds worldwide is reported in several studies aimed at characterizing milk composition and cheese-making aptitude of indigenous vs. cosmopolitan goat breeds. Those studies have evidenced that, despite the lower milk production, local breeds are characterized by higher milk fat, protein and total solids contents (Kouniba et al., 2007), curd firmness, cheese yield (%CY) and recovery of nutrients in the curd (%REC) (Vacca et al., 2018a; 2018b). The differences among breeds are explainable not only by the 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 (DAD-IS, 2014). Sarda is characterized by a skillful

grazing behavior and is perfectly adapted to the semiarid environment. In Sardinia, those goats are reared in traditional and extensive farming system methods, with grazing free pasture of the Mediterranean scrubland. The Sarda breed is also characterized by 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 (i.e., Saanen, Murciano-Grandina, Maltese) over the years, with the purpose to increase milk production, led to the recombination of the original genetic characteristics of the Sarda breed. This phenomenon occurred with greater intensity in South-West areas of Sardinia (Vacca et al., 2016). At research level, only few previous studies have examined milk production and quality, coagulation and cheese-making ability from Sarda goat breed, in most of the cases, analyzing a low number of samples. Given that the characterization of local breeds' productions (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, a large survey was carried out on the Sarda goat breed. The present study aimed to: 1) quantify the contribution of the animal and farm effects (within milk energy level and geographical area) on milk composition, coagulation properties, and cheese-making traits; 2) study the effect of high or low class of milk energy production at farm level (defined according to the milk net energy daily yielded by the goats); 3) assess the effect of the geographical area; and 4) characterize the effect 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, in order to keep a good variability of the dataset. 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; 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 (100-200) or large (> 200). As regards to the altitude, farms were grouped into those located in the plain (< 200 m above sea level, asl), in the hills (200-500 m asl), and in the mountains (> 500 m asl). As reported in Table 1, the majority of farms in the Central-East of the island were located in the mountains (8/11), and animals were hand-milked (10/11), while in the South-West farms were mostly located in the hills and plains (9/10), with mechanical milking (7/10). We also used the daily milk energy output (**dMEO**) of goats to define farms into 2 classes of milk energy (**ME**) levels. The net energy content (**NE_L**) of milk was calculated using the equation proposed by the NRC (2001):

$$NE_L (Mcal/kg) = 0.0929 \times fat, \% + 0.0547 \times protein, \% + 0.0395 \times lactose, \%$$

Then **NE_L** (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 an ANOVA (GLM procedure; SAS Institute Inc., Cary, NC) to obtain the LSMeans for the selected farms after correcting for DIM and parity of the goats. After ranking the dMEO LSMeans of the 21 farms, they were categorized into high ME level (high-ME: n = 10, average dMEO = 5.05 MJ/d) and low ME level (low-ME: n = 11, average dMEO = 2.91 MJ/d) based on the median value (3.91 MJ/d).

127 ***Analysis of Milk Composition***

Individual samples (200 mL/goat) were collected in sterile sample containers during the morning milking (one 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, while 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 (i.e., swollen, hot udder) or disease were not sampled. Milk fat, protein, lactose, total solids, pH and NaCl were achieved using a MilkoScan FT6000 milk analyzer (Foss Electric A/S, Hillerød, Denmark) calibrated according to FIL-IDF references (ISO 9622:2013 - IDF 141:2013 for fat, protein, lactose, pH and NaCl; ISO 6731:2010 - IDF 21:2010a for total solids). Somatic cell count (SCC) were determined by Fossomatic 5000 somatic cell counter (Foss Electric A/S, Hillerød, Denmark) and transformed into the logarithmic [$\log_2(\text{SCC} \times 10^{-5}) + 3$] somatic cell score (SCS), as reported by Shook (1993). Total bacterial count (TBC) was measured by using a BactoScan FC150 analyzer (Foss Electric A/S, Hillerød, Denmark) and transformed into the logarithmic bacterial count [$\text{LBC} = \log_{10}(\text{total bacterial count}/1,000)$] according to ISO 21187:2004 – IDF 196:2004. Milk yield was recorded for each goat by using the recorder jar in the case of mechanical milking, and by using 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 milking of the same day of samples collection.

The 9-MilCA

The 9-mL milk cheese-making assessment (**9-MilCA**) 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 double per each individual milk sample (two replicates of 9 mL), for a total of 1,140 observations. This procedure allowed to record the single-point traditional MCP [rennet coagulation time (**RCT**, min), defined as the time interval between rennet addition and gelation; curd-firming time (**k₂₀**, min), as the time between gelation and the attainment of curd firmness of 20 mm; curd firmness at 30 min after rennet addition (**a₃₀**, mm)] and the %CY traits, as %CY_{CURD}, %CY_{SOLIDS} and %CY_{WATER}, calculated as the ratio of the weight of fresh curd, curd dry matter and water retained in curd, respectively, to the weight of the milk processed, and multiplied by 100. The recorded nutrients 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, and multiplied by 100. Daily cheese yields (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 to exploit all these values allowed 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:

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

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 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 the SAS (SAS Institute Inc., Cary, NC, version 9.4). 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 a MIXED procedure (SAS Institute Inc., Cary, NC, version 9.4), according to the following model:

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

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

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

199 Orthogonal polynomial contrasts were estimated between the LSMs of classes of days in milk
 200 (linear, quadratic and cubic relationships), to observe possible significant patterns in the tested milk
 201 traits. The four degrees of freedom of parity effect were used to test the following contrasts: 1) 1st vs
 202 2nd; 2) 1st vs $\geq 2^{nd}$; 3) 2nd vs $\geq 3^{rd}$; and 4) 3rd vs $\geq 4^{th}$.

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

206

207

RESULTS

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

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

216 Table 4 reports least square means of milk and coagulation traits according to milk energy
 217 level and geographical area. The effect of ME level was significant with higher daily productions of
 218 milk and cheese, better coagulation time RCT_{eq} and greater %REC_{PROTEIN} from goats reared in high-
 219 ME farms. With regard to the effect of the geographical area, goats reared in South-West area showed
 220 higher dMY and NaCl, lower concentrations of milk fat, protein, lactose and Total Solids. The k_{20}
 221 was shorter and a_{30} was higher in milk from goats reared in the South-West area.

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

230 The effect of parity and orthogonal contrasts are reported in Table 5. Goats at first parity had
 231 lower milk production compared with multiparous goats. Goats at second parity had slightly lower
 232 milk production compared with goats at third and higher parities. Milk from younger goats was

characterized by higher fat, lactose, and Total Solids contents, and lower NaCl, SCS and LBC, compared with the older ones. The LBC was lower in goats at first and second parities.

DISCUSSION

Effects of Animal and Farms, 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), while curd firmness values reported by 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 a delayed coagulation time but higher curd firmness values, and overall high milk protein and casein contents are associated 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), higher %CY (Guo et al., 2004), %REC traits and daily cheese productions (Pazzola et al., 2019). The animal incidence on the total variability of milk and cheese traits supports that Sarda goats are characterized by a large genetic component (i.e., casein polymorphism), even larger when compared to 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 a 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 (i.e., curd firmness at 60 min, potential

curd firmness, syneresis rate). However, although MCP are highly influenced by instrument (mechanical or optical devices; Cipolat-Gotet et al., 2012) and conditions of analysis (i.e., type and concentration of rennet, milk temperature, 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 from bovine milk (Ferragina et al., 2017). In this study, the individual goat repeatability of RCT and k_{20} was higher than that of dairy cows, but for a_{30} it was lower (Stocco et al., 2017). Moreover, if 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, 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 the maximum consistence of the curd is usually attained within 30 min from rennet addition, and after 30 min in 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 six breeds of goats. These results evidenced that 9-MilCA 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, by using instruments commonly used in many laboratories for the evaluation of MCP. The concomitant use of the modeling of the renneting data allows to a more detailed description of the coagulation process. All these phenotypes are of interest for the dairy sector, and the possibility to collect them at individual level could fasten 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 production, composition, coagulation traits and cheese-making ability of Sarda goat milk. The average values of milk energy output (dMEO, MJ/d) of both ME levels were significantly lower than those observable in bovine herds (where this factor was expressed as herd productivity level, **HP**; 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 remind that all the 21 farms were characterized by the extensive management system, and the ME 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 two ME levels.

In previous studies, goat milk composition was slightly influenced by the type of farming system (Van Quackebecke et al., 1996), and the effect of the 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 kg of milk (high producing goats: >1.5 milk kg/d). Inglingstad et al. (2014) studied the effect of the 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 from those from dairy cows, in which the effect of HP level was larger (Stocco et al., 2017; Stocco et al., 2018b). The faster coagulation and the higher %REC_{PROTEIN} in milk samples from goats reared in high-ME farms could be related to the protein and casein genetic variants, rather than their overall quantities, as no differences in milk composition have been observed between ME farm levels. Two recent studies have accurately evidenced that, also in goats, composition of milk can directly influence coagulation and cheese-making ability (Stocco et al., 2018a; Pazzola et al., 2019), but there are also some genes of particular interest (whey protein genes), especially in the Sarda breed, that influence the renneting properties (Dettori et al., 2015a;

2015b). Probably, those genes have also an important influence on cheese yields and on the efficiency of recovery of nutrients in the curd, but this aspect is still under investigation.

312

313 *Effect of Geographical Area*

314 The idea to test the effect of geographical area was linked to the crossbreeding phenomenon
315 happened with greater intensity in the South-West area of the island (Vacca et al., 2016), where
316 farmers were used to cross their goats with the Maltese (Italian breed from Sicily) bucks, as previously
317 mentioned. This has led to the recombination of the original genetic traits of both Sarda and Maltese
318 goats. Several breeds have been imported in order to improve the productive performances of the
319 animals, and the best results have been obtained with Maltese bucks, combining both the high milk
320 yield and suitability for grazing the extensive Sardinian scrublands (Usai et al., 2006). Crossing
321 autochthonous goats with specialized dairy breeds ensures high milk production, although imported
322 animals are scarcely adaptable to local climate conditions (Lu and Miller, 2019). However, when
323 farmers rear high-yielding goats imported from areas geographically similar to those of local breeds,
324 traits from both types are successfully absorbed from the crossbred animals (i.e., Majorera goats;
325 Dickson et al., 1991; Capote et al., 2004). On the other hand, if the nutritional requirements for milk
326 production are not satisfied, the use of high-yielding goats becomes ineffective (Capote, 2002). A
327 recent study has evidenced that coagulation, curd-firming and syneresis traits are better in Maltese
328 goats compared to Alpine breeds (Pazzola et al., 2018), although the composition of milk from
329 Maltese is much more similar to that from Alpine goats and neatly worse when compared with the
330 Sarda. Indeed, milk from the Sarda is characterized by superior quality and technological aptitude
331 compared to other dairy goat breeds (i.e., Saanen, Camosciata delle Alpi, Murciano-Granadina,
332 Maltese; Vacca et al., 2018a). But the higher %CY and %REC traits from Sarda breed can only
333 partially counterbalance the higher MY characterizing Maltese goats (Vacca et al., 2018b). Thus, the
334 increase of milk production was the main objective of farmers who crossed their Sardinian goats with
335 Maltese bucks, even though description of 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 (in %) 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; 2019b), but this could not concurrently explain the higher curd firmness value. Possibly, differences in the proportion of milk protein fractions, in the frequency of their genetic variants, or both (due to the crossbreeding recombination), could explain the difference in the a_{30} trait between the two areas. It is also possible that these differences in milk characteristics result from the different altitudes in which farms were located, and therefore to the different pasture composition. In fact, many farms in the Central-East area were located in the mountains, where trees prevail over shrubs.

Goats reared in the two areas produced the same %CY, but with different solids and water proportion: %CY_{SOLIDS} was higher value in 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} similar between areas). High water content in the curd increases %CY values, but an excess of water could be not 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 Days in Milk

Many studies in the literature have investigated the effect of goat days in milk 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-non-fat and Total Solids from Alpine goats were higher in the first 30 DIM, then they 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 a marked daily variations of goat SCC. In the present study, the possibility to divide lactation period into many classes allowed to 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 can become 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 four 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, respectively.

In dairy cows both traditional MCP and modeled CF_t parameters are strongly influenced by DIM, contrarily 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., 2018b), and also in sheep it is observed the same pattern for these traits (Cipolat-Gotet et al., 2016b). However, besides the differences among species, it is important to remind that the different division of DIM in classes among studies could led to very different results, especially within species.

Effect of Parity

No information is available on the effect of many orders of parity on coagulation and cheese-making traits of goats. In opposite, 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 order of parity. Mioč et al. (2008) did not observe significant changes across parities for milk fat and lactose, but only a decreased protein content moving from the 1st to the $\geq 5^{\text{th}}$ parity. Zeng and Escobar (1995) did not found any significant effect of parity on goat milk composition,

SCC and bacterial count, but only on milk yield. That difference could be attributable to the low number of parities considered (from 1st to 3rd parity). On the contrary, results by Lôbo et al. (2017), regarding the effect 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 1st to the 7th parity. Those authors have 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 of 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 to the older ones. This finding could be explained by the higher fat content and lower SCS and LBC in milk from the first compared to the latter (Stocco et al., 2018a). Among %CY traits, only %CY_{SOLIDS} was affected by parity, with higher values in goats at 1st and 2nd parity compared with goats at $\geq 3^{\text{rd}}$ parity. 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 were higher in younger goats, except for %REC_{SOLIDS}, while the lowest %REC values were recorded from goats with $\geq 5^{\text{th}}$ parity. 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 the 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. Results evidenced that the individual animal played a much more important role compared with the farm for the variability of coagulation, %CY (except for %CY_{WATER}), %REC and dCY traits. The animal repeatability for both traditional MCP and CF_t parameters was high and higher than other species.

414 The effect of milk energy level was limited to higher daily productions of milk and cheese, better
415 coagulation time, and higher %REC_{PROTEIN} from goats reared in high MEL-farms. The few
416 differences found between the two milk energy levels could be in part derived from the different MY
417 produced by the goats, as the farming system was similar among farms. The effect of geographical
418 area confirmed the presence of the unplanned crossbreeding, because of the higher MY in goats reared
419 in the South-West area, where Sarda goats have been crossed with Maltese bucks. However, despite
420 the lower MY, the quality of milk produced from the pure Sarda breed (Central-East area) was better
421 than the crossbred goats (South-West area). Days in milk were a valuable source of variation, in
422 particular for milk composition, that in part reflected fluctuations in %CY traits. Parity was much
423 more important in determining the differences between the primiparous vs. the multiparous goats, in
424 terms of lower production (milk and cheese) and better milk composition. However, because small
425 differences were detected for coagulation and %CY traits across parities, the technological quality of
426 milk from Sarda goats is maintained also in old animals. The recovery of fat and protein were higher
427 from younger goats, and this suggested the little better curd composition from younger goats
428 compared to that obtained from older animals. The characterization of Sarda breed provided by this
429 study could be a key point for its preservation and enhancement, and could be of great interest for the
430 goat dairy sector. Finally, the approach, methods and statistics used for the present study could also
431 find a possible applicability for other farming methods, goat breeds and geographical areas.

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For Peer Review

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TABLES AND FIGURES

607

608 **Table 1.** Characteristics of sampled farms (N = 21).

	Geographical 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 (\leq 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, no. of farms:		
Mechanical	1	7
Hand-milked	10	3

609 ¹asl = above sea level.

Table 2. 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.

	Mean	SD	Percentile ¹	
			P5	P95
MY ² , kg/d	1.10	0.51	0.42	2.20
<i>Milk composition</i>				
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
Total Solids, %	14.83	1.53	12.50	17.50
NaCl ³	245	50	170	327
pH	6.72	0.10	6.55	6.88
SCS ⁴	6.63	1.80	3.63	9.49
LBC ⁵	1.75	0.87	0.30	3.27
<i>Traditional MCP⁶</i>				
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
<i>Modeled CF_t parameters⁷</i>				
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
<i>Cheese yields, %</i>				
%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
<i>Nutrients recovery, %</i>				
%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
<i>Daily production traits, kg /d</i>				
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

¹Percentile = 5th and 95th percentiles, which indicate the upper and lower 5% limits in the 2-tailed distribution of data; ²MY = milk yield; ³NaCl = Sodium Chloride; ⁴SCS = log₂ (SCC × 10⁻⁵) + 3; ⁵logarithmic bacterial count (LBC) = log₁₀ (total bacterial count/1,000); ⁶RCT = 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; ⁷RCT_{eq} = rennet coagulation time estimated by CF_t modeling; k_{CF} = curd firming instant rate constant; CF_P = asymptotic potential curd firmness.

Table 3. Analysis of variance 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) and 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.

	Fixed effects (F -value and significance)				Random effects (% on total variance)			RMSE ²	Repeatability ³ , %
	DIM	Parity	ME ¹	Area	Farm(ME, Area)	Animal	Pendulum		
MY ⁴ , kg/d	7.6***	15.2***	25.9***	8.1*	30	-	-	0.32	-
<i>Milk composition</i>									
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	-
Total Solids, %	7.3***	9.5***	0.5	8.0*	41	-	-	1.20	-
NaCl ⁵	7.4***	21.0***	0.0	17.5***	37	-	-	37.70	-
pH	3.6**	7.9***	0.7	0.3	38	-	-	0.08	-
SCS ⁶	3.3**	23.5***	0.8	3.5	29	-	-	1.53	-
LBC ⁷	1.8	6.0***	2.0	0.1	60	-	-	0.54	-
<i>Traditional MCP⁸</i>									
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
<i>Modeled CF_t parameters⁹</i>									
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
<i>Cheese yields, %</i>									
%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

<i>Nutrients recovery, %</i>									
%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
<i>Daily production traits, kg /d</i>									
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

¹ME = milk energy level; ²RMSE = root mean square error; ³Repeatability, % = $\frac{\sigma^2_{Farm(HP * Area)} + \sigma^2_{Animal} + \sigma^2_{Pendulum}}{\sigma^2_{Farm(HP * Area)} + \sigma^2_{Animal} + \sigma^2_{Pendulum} + \sigma_e^2} \times 100$; ⁴MY = milk yield; ⁵NaCl = Sodium Chloride; ⁶SCS = $\log_2 (SCC \times 10^{-5}) + 3$; ⁷logarithmic bacterial count (LBC) = $\log_{10} (\text{total bacterial count}/1,000)$; ⁸RCT = 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; ⁹RCT_{eq} = rennet coagulation time estimated by CF_t modeling; k_{CF} = curd firming instant rate constant; CF_p = asymptotic potential curd firmness;

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

Table 4. Least square means of Milk Energy (ME) level and of geographical 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. Bold numbers were used to evidence significant differences for $P < 0.05$, $P < 0.01$, and $P < 0.001$, as reported in Table 3.

	ME Level		Geographical area	
	High	Low	Central-East	South-West
MY ¹ , kg/d	1.29	0.80	0.90	1.18
<i>Milk composition</i>				
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
Total Solids, %	15.33	15.01	15.80	14.54
NaCl ²	233	231	205	260
pH	6.70	6.73	6.71	6.73
SCS ³	6.50	6.10	5.88	6.72
LBC ⁴	1.51	1.92	1.68	1.76
<i>Traditional MCP⁵</i>				
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
<i>Modeled CF_t parameters⁶</i>				
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
<i>Cheese yields, %</i>				
%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
<i>Nutrients recovery, %</i>				
%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
<i>Daily production traits, kg /d</i>				
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; ²NaCl = Sodium Chloride; ³SCS = $\log_2 (\text{SCC} \times 10^{-5}) + 3$; ⁴logarithmic bacterial count (LBC) = $\log_{10} (\text{total bacterial count}/1,000)$; 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.

Table 5. Least square 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.

	Parity, LSMeans					Contrasts, <i>F</i> -value and significance			
	1 st	2 nd	3 rd	4 th	≥5 th	1 st vs 2 nd	1 st vs ≥ 2 nd	2 nd vs ≥ 3 rd	3 rd vs ≥ 4 th
MY ¹ , kd/d	0.86	1.03	1.11	1.11	1.11	25.9***	57.9***	8.2**	0.0
<i>Milk composition</i>									
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
Total Solids, %	15.56	15.32	15.14	15.07	14.77	4.2*	20.7***	10.9**	4.0*
NaCl ²	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 ³	5.51	5.90	6.61	6.50	7.00	6.2*	51.6***	41.2***	1.2
LBC ⁴	1.59	1.62	1.81	1.75	1.81	0.3	10.4**	14.4***	0.4
<i>Traditional MCP⁵</i>									
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
<i>Modeled CF_t parameters⁶</i>									
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
<i>Cheese yields, %</i>									
%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

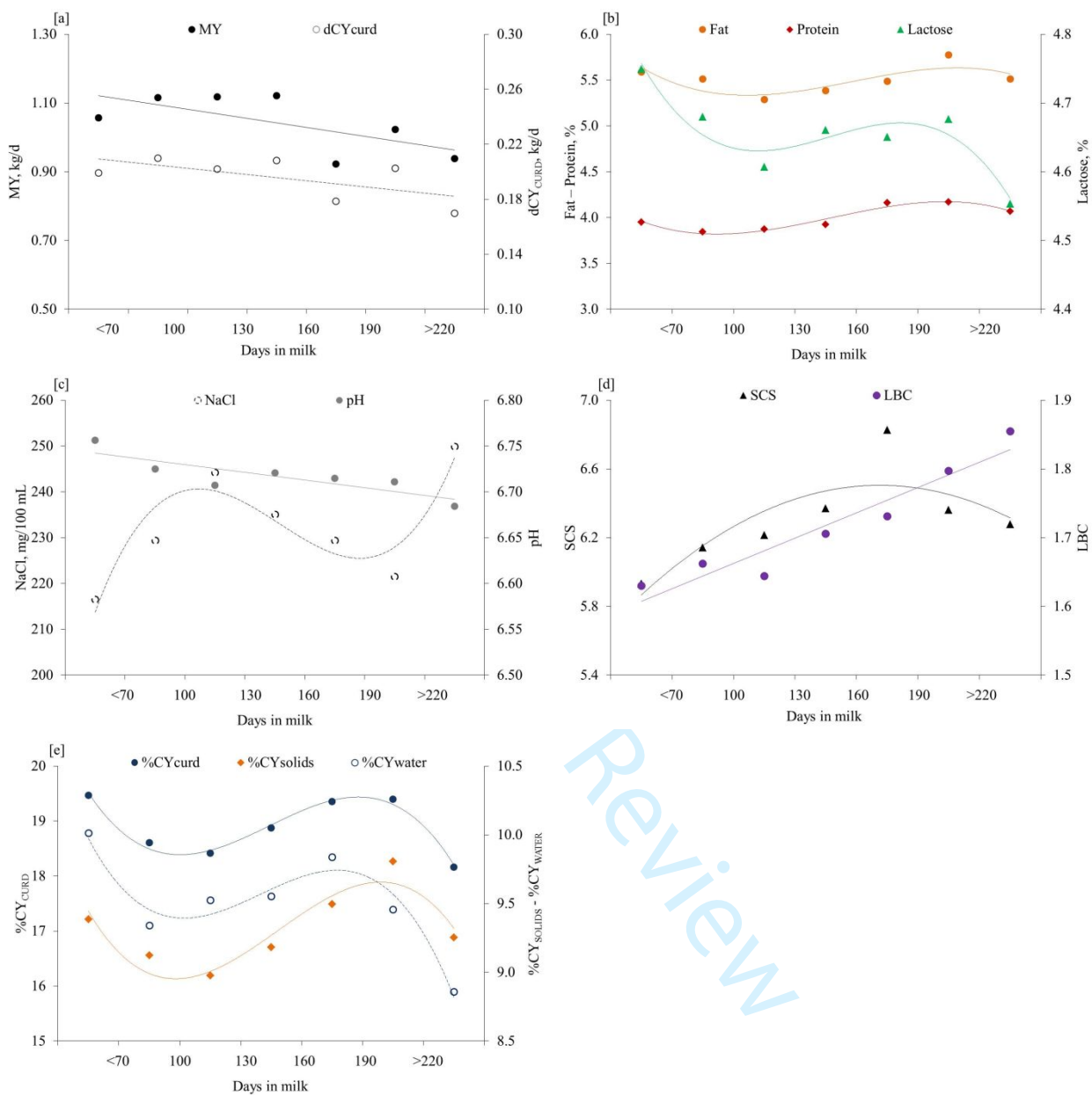
%CY _{WATER}	9.63	9.62	9.51	9.63	9.16	0.0	0.6	1.1	0.4
<i>Nutrients recovery, %</i>									
%REC _{FAT}	85.89	85.76	84.86	85.07	84.66	0.1	3.0	4.5*	0.0
%REC _{PROTEIN}	82.44	82.07	81.43	81.03	81.04	1.4	13.8***	12.2***	2.0
%REC _{SOLIDS}	61.34	60.98	60.84	60.51	59.82	0.5	3.3	2.1	2.4
<i>Daily production traits, kg /d</i>									
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; ²NaCl = Sodium Chloride; ³SCS = $\log_2 (\text{SCC} \times 10^{-5}) + 3$; ⁴logarithmic bacterial count (LBC) = $\log_{10} (\text{total bacterial count}/1,000)$;

⁵RCT = 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; ⁶RCT_{eq} = rennet coagulation time estimated by CF_t modeling; k_{CF} = curd firming instant rate constant; CF_p = asymptotic potential curd firmness;

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

Figure 1.



619 **Figure 1.** Effect of days in milk on MY, dCY [a], fat, protein and lactose [b], NaCl and pH [c], SCS
620 and LBC [d], and %CY traits [e] of goats.

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