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Abstract: Traditionally, in Mediterranean areas the goat population was composed of autochthonous breeds with strong milk production seasonality. In the last decades, high productive alpine breeds were introduced together with more widespread out-of-season milk production practices. This study is a large-scale survey on the seasonal variations of the main compositional characteristics of goat milk obtained from Sarda and Saanen breeds reared on the Mediterranean island of Sardinia (Italy). Analysis of data indicated that milk from the Sarda breed was significantly richer, at p < 0.001, in protein, fat, and lactose, and had a lower urea mean content than Saanen. Throughout the year, fluctuations of mean contents of the milk parameters were similar for the two groups of goats, indicating that, besides genetic intrinsic differences, climate and herbage growth influenced the Sarda as well as the Saanen goats. During the summer, milk from Saanen showed a marked drop in fat and protein contents, with 21% of samples showing a fat-to-protein ratio <1. No significant differences were found for the somatic cell count; however, the Sarda breed showed a higher bacterial count, suggesting improper milk handling and/or storage equipment more frequently encountered in extensive and semi-extensive farm systems.

Keywords: goat milk seasonality; goat milk reversion syndrome; Sarda goat; Saanen goat; goat milk urea; goat farming systems

# 1. Introduction

The domestic goat, due to its adaptability to harsh environmental conditions, is the most geographically widespread livestock species. Goats are mostly reared in smallholder farming systems widely distributed throughout the world, with a prevalence in developing countries where they cover an important economic, environmental, and sociological role. Goat milk is used for cheesemaking and as 'drinking milk' in human nutrition and it is an alternative to cow milk because of its chemical composition, digestibility and low allergenicity [1], infant formulae based on goat milk are marketed and in 2012 the EFSA (European Food Safety Authority, Parma, Italy) approved the use of goat milk as a protein source for milk formulae [2]. Currently, goat milk production is mainly concentrated in Asia (52.7%) and Africa (25.7%); Europe (16.6%) and America (4.9%) contribute less to world production [3]. Although the goat population is mostly concentrated in arid areas and low-income countries, dairy goat farms are also found in high-income and technologically advanced countries. The highest production of milk per goat is in Europe, which produces 16.6% of the world's goat milk, with only 4.3% of the goat population, thanks to the high specialization in the goat dairy sector of France, Spain, Greece, and others [4]. In Italy there are 1,065,000 head of goats, with approximately 25% reared on the Mediterranean island of Sardinia [5]. Traditionally, in Sardinia, goats are reared mainly for milk production [6,7]. The most numerous breeds are the autochthonous Sarda and its crossbreeding with the Mediterranean Maltese breed [6]. The Sarda is one of the most numerous and most important breeds in the Mediterranean basin [6]. Goats are medium-small sized, sturdy, frugal, and fully adapted to the exploitation of low-potential



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rural areas and wastelands [6,8]. Adult Sarda goats kid mainly in late autumn or early winter and primiparous goats in early spring. Drying off occurs in summer for both adult and primiparous females [6,7]. In the mid-1980s, the Saanen breed was introduced in Sardinia, especially although not exclusively, in the lowland areas, in farms equipped with irrigation and adequate facilities for breeding in stables [8]. The Saanen is a cosmopolitan breed originating in Switzerland, with high milk yields and which adapts very well to intensive and semi-intensive farming systems. Saanen is one of the most productive dairy breeds used for producing milk for direct human consumption and can be considered the Holstein of dairy goats [8]. Compared to the Saanen, the Sarda breed has a lower milk yield, but milk is richer in macro-constituents and has superior qualitative and technological characteristics [9,10]. The aim of this study was to analyze and compare, over the seasons, the chemical physical composition of milk samples from the autochthonous Sarda breed (and crossbreeds) and the imported Saanen goats reared in Sardinia.

#### 2. Materials and Methods

Samples. A total of 7068 samples of bulk goat milk were collected from October 2016 to September 2020 from 174 farms located in 91 municipalities distributed in different regions of Sardinia (Italy). A total of 3783 samples were obtained from Sarda and Sarda-Maltese breeds (hereafter named Sarda) and 3285 from Saanen breed. The goat farming system varied greatly, ranging from semi-extensive to semi-intensive [11]. The Sarda goats were reared in 113 farms, where they were allowed to graze natural pasture during the day and were stabled at night. The Saanen were reared at 61 farms, where animals were kept prevalently in housing systems.

Milk composition. Milk analyses were performed by the Sardinian Regional Breeders Association (ARAS) laboratory using a Milkoscan FT6000 (Foss, Hillerød, Denmark) that employs the Fourier transform infrared spectroscopy (FTIR). Fat, protein, lactose, and NaCl (expressed as g/100 mL of milk), urea (mg/100 mL of milk) freezing point (- Horvet), and pH values were obtained following the procedure ISO 9622:2013. The saturated, monounsaturated, and polyunsaturated fatty acid contents (expressed as g/100 mL of milk) were estimated using mid-infrared spectrometry-based prediction models by a Milkoscan FT6000 (Foss, Hillerød, Denmark) and subsequently normalized to 100. Somatic cell count (SCC) for each sample was estimated as outlined in the ISO 13366-2:2006 procedure (ISO 13366-2 IDF 148-2 2006) by a Fossomatic 5000 (Foss, Hillerød, Denmark). Total bacterial count (TBC, expressed as CFU/mL) was based on the standard flow cytometric method with BactoScan (Foss Analytical, Hillerød, Denmark).

Statistics. To obtain descriptive information on the data and to calculate the means and their standard deviations, we performed univariate analysis. When the two or more groups of samples were compared, the null hypothesis ("the means are not significantly different among the groups of samples") was tested by the one-way analysis of variance (ANOVA) technique. A two-way ANOVA was used to test the interaction between data set variables (year, farm, and month). Multiple comparison of the means was performed using the post-hoc Tukey comparison test. Statistical analyses were performed with the software OriginPro2022 (OriginLab, Northampton, MA, USA). Pairwise linear correlations between variables were measured by the Pearson correlation coefficient.

#### 3. Results

A total of 7068 bulk milk samples were examined. They were collected for four years. Number of samples per months for the two breeds are plotted in Figure 1.

As can be seen, although the total number of samples for the two groups of goats was very similar, distribution over the months changes substantially. The number of milk samples of the autochthonous breeding group (Sarda breed) is much higher from March to August with a drop in autumn. For the Saanen, the number of milk samples did not undergo great variations during the year. Due to the large number of farms involved in this survey, parity, and season of kidding as well as days of lactation were not recorded.



For all the studied parameters, means, standard deviation (SD), and minima and maxima values are reported in Table 1.

Figure 1. Number of collected milk samples per month over four years.

Table 1. Descriptive statistics and means comparison of goat milk parameters collected for four years.

	Sarda ( <i>n</i> = 3783)					р			
	Mean	SD	Min	Max	Mean	SD	Min	Max	
Fat	4.71	0.92	2.00	7.79	3.92	0.79	2.26	7.71	***
Protein	3.79	0.47	2.40	9.40	3.50	0.44	2.25	7.40	***
Fat/Protein	1.24	0.21	0.61	2.22	1.12	0.16	0.54	2.15	***
Lactose	4.64	0.26	2.49	6.48	4.54	0.22	2.60	5.44	***
Urea	35	9	10	76	39	9	10	74	***
NaCl	241	43	121	478	269	39	119	539	***
Freezing point	0.57	0.01	0.40	0.75	0.57	0.01	0.41	0.73	***
pH	6.77	0.09	4.83	7.12	6.74	0.07	5.58	7.04	***
ŜCC	1823	1781	4	20,600	1681	1551	7	24,100	*
TBC	1219	3163	2	17,000	762	2223	2	17,000	***
log <sub>10</sub> (SCC)	6.08	0.47	3.60	7.31	6.11	0.34	3.85	7.38	*
log <sub>10</sub> (TBC)	5.15	0.93	3.60	7.31	5.05	0.80	0.30	7.23	***
Fatty aci	ds (%)								
SFA	74.5	1.5	72.3	76.9	72.9	1.2	71.6	75.3	***
MUFA	21.5	1.5	19.0	23.6	22.7	1.1	20.4	24.1	***
PUFA	4.06	0.11	3.80	4.20	4.42	0.11	4.21	4.58	***

Fat, Protein, Lactose, NaCl (g/100 mL); Urea (mg/100 mL); Freezing point (-Horvet); Somatic cells count (SCC  $\times$  10<sup>3</sup> cells/mL); Total bacterial count (TBC  $\times$ 10<sup>3</sup> cfu/mL). \* p < 0.05; \*\*\* p < 0.001.

Effects of different variables (year, month, and farm) on mean differences of the milk main components (fat, protein, lactose, and urea, as reported in Table 1) are reported in Table S1. Results for Sarda and Saanen breeds indicate that means for all the studied parameters significantly differ (p < 0.001) during the months and among farms. This is not surprising for the month that the milk samples were collected in. On the other hand, differences among farms can suggest different farm systems as well as a possible influence of microclimate of the area. Moreover, interactions between variables are significant (p < 0.001). As for year of milk production, we can notice that the urea mean content showed no significant differences for the Sarda goat group, as well as the fat and protein mean content for the Saanen (p > 0.05). Interactions between breed and year were not significant for fat, protein, and urea.

The pairwise linear correlation coefficients were calculated for the main parameters and results reported in Table 2.

	Fat	Protein	Fat/Protei	n Lactose	SCC	TBC	Urea	FreezingP	pН	NaCl
Fat	1.00	0.65	0.81	0.25	0.15	0.06	-0.23	0.10	0.24	-0.52
Protein		1.00	0.09	0.11	0.29	0.10	-0.18	0.24	0.24	-0.33
Fat/Protein			1.00	0.23	-0.01	0.01	-0.16	-0.06	0.11	-0.42
Lactose				1.00	-0.34	-0.02	0.01	0.42	0.35	-0.81
SCC					1.00	0.12	-0.20	0.03	-0.09	0.25
TBC						1.00	-0.11	0.06	-0.30	0.01
Urea							1.00	0.09	-0.01	0.08
FreezingP								1.00	0.07	0.02
pН									1.00	-0.34
NaCl										1.00

**Table 2.** Correlation matrix between main milk components for Sarda and Saanen milk samples. Values >0.60 are in bold.

The fat and protein mean contents over the months for the four years of collection are reported in Figure 2.



**Figure 2.** Monthly mean contents of: (**a**) fat; (**b**) protein; (**c**) fat-to-protein ratio; (**d**) box and whisker plots of fat-to-protein ratio (boxes indicate the mean and SD, whiskers the lower and upper quartile, the green line indicates the value of one). Data are reported for the Sarda (blue line and diamonds) and Saanen (red line and squares) groups of milk samples.

The fat content and the type of fatty acids are among the most variable components of milk both from a qualitative and quantitative point of view. Fat and fatty acid contents depend on the composition of the diet, the stage of lactation, seasonality, and genotype. The

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average fat content for 4 years has been calculated as  $4.71 \pm 0.92$  and  $3.92 \pm 0.79$  g/100 mL of milk for the Sarda and the Saanen breed, respectively (Table 1). The difference between the means was statistically significant at p < 0.001. Over the months, the milk fat content (Figure 2a) showed a similar trend for both goat groups, with higher values in late autumn/winter (November-February) and lower values in summer (between June and August). For the Sarda group, the maximum fat value was recorded in November and the minimum in June (5.10  $\pm$  0.89 vs. 4.55  $\pm$  0.83 g/100 mL, respectively, *p* < 0.001), with a decrease of approximately 11%. For the Saanen, the maximum fat value was recorded in December and the minimum in July (4.67  $\pm$  0.81 and 3.40  $\pm$  0.52 g/100 mL, respectively, p < 0.001) with a decrease of approximately 28%. The difference between the highest fat content values of the two groups (5.10  $\pm$  0.89 and 4.67  $\pm$  0.81 g/100 mL, *p* < 0.001, for Sarda and Saanen, respectively) is approximately 0.43 g/100 mL. As for the lowest values (4.55  $\pm$  0.83 and 3.40  $\pm$  0.52 g/100 mL, *p* < 0.001, for Sarda and Saanen, respectively) the difference is approximately 1.15 g/100 mL. While in the cold months the average values of milk fat are comparable for the two groups of goats, in the hottest months a more marked decrease in the fat content can be observed in the Saanen.

The mean protein content over 12 months for 4 years has been calculated as  $3.79 \pm 0.47$  and  $3.50 \pm 0.44$  g/100 mL of milk (p < 0.001), for the Sarda and the Saanen breed, respectively. The protein mean contents (Figure 2b) show a similar trend to that of the fat content (Figure 2a). Fat and protein contents are positively correlated with a coefficient = 0.65 (Table 2). The highest protein levels were recorded in autumn, while the lowest were in summer. For both groups, the maximum was in November ( $4.29 \pm 0.93$  and  $4.09 \pm 0.39$  g/100 mL, for Sarda and Saanen, respectively, p > 0.001) and the minimum in June ( $3.67 \pm 0.40$  and  $3.19 \pm 0.19$  g/100 mL, for Sarda and Saanen, respectively, p < 0.001). For the Sarda goats, the difference between minimum and maximum mean values was approximately 15%; for the Saanen group, the decrease of the protein content during summer was 22%. While in the cold months, the average values of protein in milk are comparable for the two groups of goats, reaching similar values in January, in the summer, a more marked decrease in the protein content can be observed for the Saanen.

It is interesting to note that the mean milk fat and protein contents of Saanen goats did not significantly differ among the years of collection, indicating that their overall contents are less variable than for Sarda goats (Table S1).

The ratio fat-to-protein contents was also calculated, this parameter is widely used to assess milk composition quality, values <1 indicate that the fat percentage goes below milk protein percentage and fall in the range of the reversion syndrome. The trend of the mean content of fat-to-protein ratio over the months is reported in Figure 2c, while overall descriptive statistics are depicted in Figure 2d. This parameter is positively correlated to the fat content with a correlation coefficient = 0.81 (Table 2). Milk from Saanen was more prone to fall in the inverse syndrome (21% of samples had values <1 vs. the 7% of the Sarda), particularly in summer where a notable drop of fat content has been registered.

The mean lactose contents, reported in Table 1, over 12 months for 4 years has been calculated as  $4.64 \pm 0.26$  and  $4.54 \pm 0.22$  g/100 mL of milk, p < 0.001, for the Sarda and the Saanen breed, respectively. As shown in Figure 3a, the lactose had, over the months, a different trend than protein and fat, with maxima values in February–March ( $4.84 \pm 0.18$  and  $4.75 \pm 0.16$  g/100 mL, for Sarda and Saanen breeds, respectively) and a sharp content drop up until the late summer with minima mean values in August–September ( $4.38 \pm 0.19$  and  $4.36 \pm 0.14$  g/100 mL, for Sarda and Saanen breeds, respectively). For each group, the differences from maximum and minimum values were statistically significant at p < 0.001. Comparing the two groups of goats, while the difference between the maxima values was statistically significant (p < 0.001), during late summer no significant differences (p > 0.001) can be registered in the lactose mean contents. Years of collection had a marked effect on the lactose content of both groups (Table S1).



**Figure 3.** Monthly mean contents of: (**a**) lactose; (**b**) urea. Data are reported for the Sarda (blue line and diamonds) and Saanen (red line and squares) groups of milk samples.

The urea mean content over 12 months for 4 years has been calculated as  $35 \pm 9$  and  $39 \pm 9 \text{ mg}/100 \text{ mL}$  of milk, for the Sarda and the Saanen breed, respectively, p < 0.001 (Table 1). For both groups, during the year the urea mean content showed a complex trend (Figure 3b), with higher content in spring and autumn and minima in summer and winter. In the Sarda group, the maximum mean value of urea was recorded in May ( $37 \pm 9 \text{ mg}/100 \text{ mL}$ ) and the minimum in July ( $32 \pm 10 \text{ mg}/100 \text{ mL}$ ), with a decrease of about 10%; the difference between the means of the maximum and minimum is statistically significant p < 0.001. For the Saanen, the maximum mean values were recorded in May and the minimum in December ( $41 \pm 9 \text{ and } 36 \pm 9 \text{ mg}/100 \text{ mL}$ , respectively, p < 0.001), with a decrease of about 12%. Differences between the highest and lowest mean values of the two groups of goats are statistically significant (p < 0.001). The total mean urea content is quite stable among years for the Sarda goats, while differences exist for the Saanen (p < 0.01).

The fatty acid content was recorded as a percentage of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). The data are reported in Table 1 expressed as mean and SD and shown in Figure 4.

The Sarda breed showed higher SFA and lower unsaturated FA contents than the Saanen breed. Over the months, the trend of SFA and MUFA contents was similar for the two groups of goats (Figure 4). SFA content was higher in March (77  $\pm$  4 and 73  $\pm$  14, for Sarda and Saanen, respectively, *p* < 0.001) and lower in August (72  $\pm$  4 and 72  $\pm$  3, for Sarda and Saanen, respectively, *p* > 0.001); on the contrary, MUFA showed higher values in August (24  $\pm$  4 and 24  $\pm$  3 for Sarda and Saanen, respectively, *p* > 0.001); on the contrary, MUFA showed higher values in August (19  $\pm$  4 and 23  $\pm$  16 for Sarda and Saanen, respectively, *p* < 0.001). These results indicate that, while a significant SFA and MUFA content difference between the breeds can be observed in winter, in summer both goat groups showed similar values. The PUFA content follows a peculiar trend for both breeds (Figure 4), with higher values in spring and autumn and lower in summer and winter, showing, particularly for the Sarda goats, some similarities with the urea content (Figure 3).

The total mean values of milk somatic cell count (SCC) for the two breeds were 1215 and  $1278 \times 10^3$  cells/mL for Sarda and Saanen, respectively (p > 0.001). Over the months, the two groups of milk samples showed the same trend, with lower values in March and April and a rising in their mean contents in summer till winter (Figure 5).



**Figure 4.** Monthly calculated mean % contents of: (**a**) saturated fatty acids (SFA); (**b**) unsaturated fatty acids (MUFA); (**c**) polyunsaturated fatty acids (PUFA). Data are reported for the Sarda (blue line and diamonds) and Saanen (red line and squares) groups of milk samples.



**Figure 5.** Monthly mean contents of: (**a**) log 10 SCC (decadic logarithm of somatic cell count); (**b**) log 10 TBC (decadic logarithm of total bacterial count). Data are reported for the Sarda (blue line and diamonds) and Saanen (red line and squares) groups of milk samples.

The total bacterial count (TBC) mean content was higher in the milk of the Sarda goats with respect to Saanen ( $1219 \times 10^3$  and  $762 \times 10^3$  cfu m/L, for Sarda and Saanen, respectively, *p* < 0.001). Over the months, for both groups, the TBC contents showed lower values in spring–summer and maxima in winter (Figure 5).

# 4. Discussion

In Sardinia, goat farming was traditionally concentrated in hilly or mountainous marginal areas, where goats, mainly of the autochthonous Sarda breed, were able to exploit these areas covered by ligneous vegetation and unsuitable for tillage or for raising other domestic herbivore species. Most of the farms were under extensive management [11]. Out-of-season breeding was not widespread and in some cases the practice of manual milking survived. More recently alpine breeds, such as the Saanen, were introduced in semi-intensive farming systems [11,12]. Saanen goats, compared to the autochthonous breeds, have relatively higher productive and reproductive performances but lower technological properties in cheese manufacturing [10]. Vacca et al. [10] comparing Alpine vs. Mediterranean breeds reared in Sardinia, registered a mean yield of 2.79 and 0.95 Kg/d, for Saanen and Sarda, respectively.

# 4.1. Out-of-Season Milk Production

Traditionally, goat milk production in the Mediterranean basin followed a strong seasonal pattern, with the peak being in the spring, a marked reduction in early summer and nil or low availability of milk from August to October–November [13]. However, with the rigorous application of specific protocols, it was possible to manage small ruminant reproduction throughout the year and maintain continuous milk production. In our data the availability of milk in all twelve months of the year (Figure 1) indicates an out-of-season milk production for both groups, although more marked for the Saanen.

### 4.2. Fat and Protein Content and Reverse Syndrome

In agreement with the literature, the Sarda goats showed higher fat and protein milk content than the Saanen [10]; however, we detected a mean fat content significantly lower than that reported by Vacca et al. [10] (i.e., 4.71 vs. 5.33, respectively). As already observed for commingled goat milk collected from different breeds [14], over the months, the fat and protein content showed a marked seasonality with lower mean contents in summer. The milk composition differences over the seasons can be due to genetic and environmental factors, such as dietary factors, in particular the characteristics of feeds

and the feeding techniques, housing factors, and the climatic conditions of the area where the animals are reared [15,16] The observation that the drop in the fat and protein mean contents from winter to summer was more marked for the Saanen (28 and 22% for fat and protein, respectively) than for the Sarda (11 and 15% for fat and protein, respectively) is in accordance with previous observations on the adaptability to the hot Mediterranean summer season of this alpine breed, which is prone to heat stress, strongly affecting the quality of milk [17]. A better adaptability of native breeds to the climatic conditions of the Mediterranean areas was reported [15]. Compared to Sarda breed, a higher percent of Saanen milk samples showed values of fat-to-protein ratio below the threshold of 1 (7 and 21% of milk samples, for Sarda and Saanen, respectively). This trend indicates that this alpine breed is more prone to a reversion syndrome, particularly in summer; a ratio of crude protein to fat content >1 during the summer months was already observed for commingled goat milk collected from different breeds [14]. The reversion of milk fat to protein contents can be genetically determined or it can occur in high concentrate diets [18]. These observations suggest that in hot months production of milk requires particular control of the rearing environment and diet in goats subjected to heat stress. The reduction in milk fat content with respect to protein affects the physical and chemical properties of cheese as well as the fat content in cheese itself, since it is responsible for the amount of milk fat that remains in the curd. Under the same production conditions and the same technological procedure, cheeses from milk with higher fat content have a finer, softer consistency, while the cheeses obtained from milk with lower fat content have a tough consistency [19].

#### 4.3. Lactose Content and Heat Stress

In accordance with Vacca et al. [10], the Sarda breed had a higher lactose content than the Saanen. Over the months, the lactose content showed the same trends for the two breeds, with the highest contents in the winter months and lower similar values in summer. The results recorded in the present study, regarding the seasonal variation of the lactose content, are in accordance with the findings of Dobranic et al. [20] who analyzed bulk cow milk samples and found that the lactose content of cow's milk undergoes marked seasonal variation, with higher levels during winter and lower levels during summer. As well as for protein and fat, milk lactose content can also be related to external temperature and sunlight exposure [15,17]. Hot climate can affect animal welfare, especially in the Mediterranean regions, where in summer, temperatures and humidity increase considerably. Exposure of animals to elevated ambient temperature stimulates peripheral thermal receptors to transmit suppressive nerve impulses to the appetite center in the hypothalamus, thereby causing a decrease in food intake. Decrease in food intake may be an adaptive mechanism for producing less body heat. However, while concentrations of plasma glucose do not change during heat exposure, mammary glucose uptake tends to decrease, consequently lowering the lactose synthesis [21]. Since in lactating animals a fraction of the blood glucose is used by the mammary gland for lactose synthesis, it could be hypothesized that the reduction in lactose secretion is a strategy for maintaining stable blood glucose during heat stress [22]. In summer, the autochthonous Sarda breed as well as the imported Saanen showed a marked drop in the lactose content, suggesting that these two breeds implemented the same strategy to defend themselves from the hot climate. Milk is isotonic with blood and lactose responsible for 50% of the osmotic pressure of milk. Therefore, milk with a low level of lactose to maintain isotonic osmotic pressure with the blood has a high level of inorganic salts or other osmolytes. This observation is confirmed by the opposite trends of lactose and NaCl contents observed over the months for both breeds (Table 2, correlation coefficient = -0.81).

## 4.4. Urea, PUFA Content and Pasture

The Saanen goats showed a higher milk urea content than the Sarda breed ( $35 \pm 9$  and  $39 \pm 9 \text{ mg}/100 \text{ mL}$ , for Sarda and Saanen, respectively, *p* < 0.001). While the optimal concentration of urea in cow's milk is known and is often used to assess the balance of

energy and proteins in diets, in goat milk the optimal urea level threshold is not defined. Numerous studies were conducted on cow milk urea content while there are only a few for goat milk due to its less economic importance and its greater diversity, in terms of ways of breeding and keeping goats [23]. Bendelja Ljoljić et al. [23] suggested that a milk urea concentration between 40 and 45 mg/100 mL can be a good indicator of a balanced diet for Alpine goats. In a study conducted with Saanen goats fed with different percentages of crude protein in the diet, the average milk urea concentration ranged from 42.12 to 49.61 mg/100 mL [16]. To the best of our knowledge, no data are present in the literature for Mediterranean breeds, included the Sarda. The main determinants of urea formation in milk are the amount of crude protein intake and the ratio between protein and energy proportion in diet [24]. Goat diet with an excessive amount of crude protein and energy unbalanced diet causes the excess of nitrogenous substances in rumen along with the release of ammonia and rise in concentration of urea in blood and milk, which adversely affects the production, milk coagulation properties, environmental pollution, and reproductive capabilities of goats. It has been assessed that the crude protein content in the diet explains most of the total variance in the milk urea mean content across the seasons [23,25]. Sardinia has a typical Mediterranean climate with most rainfall concentrated between October and March, and the available herbage changes considerably throughout the seasons both in terms of biomass and quality. There are usually two peaks of herbage growth, rich in crude protein. There is a minor peak between October and December after the first autumn rains, but the main peak (about three-quarters of the total herbage yield) occurs between March and June when the herbage starts to mature and dry out [11]. The trend of herbage growth, with two seasonal peaks, and therefore availability of high-quality crude protein, is well reproduced by the seasonal variation of milk urea contents detected for Sarda and Saanen breeds. While this observation has an obvious explanation for the autochthonous breed which has free access to the grassland, this is less straightforward for the Saanen kept in housing systems, suggesting that the Saanen raised in Sardinia are mostly fed with fresh forage ("cut and carry"). If the observed seasonal trend can be determined by the crude protein intake from the grassland, the total mean urea content difference between Sarda and Saanen goats is due to other factors such as breed intrinsic differences or unbalanced diet supplements. In our data, the seasonality of herbal growth not only determined the trend of the milk urea content across months, but also the PUFA, in fact fresh grass is not only rich in crude protein but also in PUFA [26]. While the PUFA trend across the months is strongly influenced by seasonality of herbage growth, the MUFA and SFA follow different trends. In summer, we observed an increase in MUFA, accompanied by a decrease in SFA, probably due to fat mobilization. This trend was already observed in the milk of goats [27] and other ruminants [28].

## 4.5. Freezing Point

The freezing point value is generally recognized as the least variable property of milk and the best single index of "added water." The lactose and chloride concentration of milk account for about 75% of the freezing point depression, and one tends to compensate for the changes in concentration of the other [29]. Most of the variability in the freezing point value has been attributed to changes in the concentration of the nonchloride ash fraction of milk; therefore, milk having a major content of protein, fat, and lactose showed overall lower freezing point values [29]. Accordingly, our data show that milk of the Sarda breed has a lower freezing point than the Saanen. Evidence exists that the freezing point value of milk varies with season, feed, ambient temperature, grazing, carbohydrate content of the ration, breed, time of milking, access to water, weather, morning or evening milking, and the time interval between feeding and milking [30]. Many of these factors are interrelated, probably causing the observed fluctuating trends of the mean freezing point values over the months (data not shown).

## 4.6. Somatic Cell Count and Total Bacterial Count

Comparing the two breeds, the difference of the total mean SCC score was not statistically significant. Over the months, the two groups of milk samples showed the same trend. In dairy cows, milk SCC is used as an indicator for udder health status and hygienic quality of milk, while in goat milk a limit, as a proven indicator for udder health status, has not yet been established [31]. According to Paape et al. [32], the SCC in clinically healthy goats range from 270 to  $2000 \times 10^3$  cells/mL and Raynal-Ljutovac et al. [33] stated that the cell count threshold established for goat milk for payment system in France, as in other countries such as the USA, is  $1000 \times 10^3$  cells/mL [34]. While the European legislation fixes a limit for SCC in bovine milk (Regulation EC, 2004), no limit for goat milk has been defined; however, our milk samples exhibited mean contents within the range indicated by Paape et al. [32] for healthy goats.

In milk, the TBC expresses the amount of microbial contamination. Bacteria in milk can originate from the mammary gland, the udder and teat skin, reflecting the possible presence of mastitis, or can be due to cleaning procedures, milk handling and storage. The European Union legislation has set a threshold for total BC in the bulk milk of small ruminants equal to  $1500 \times 10^3$  colony-forming units (cfu) m/L for milk that undergo thermal processing and  $500 \times 10^3$  cfu m/L for milk that would be used for direct consumption [35]. Total bacterial count mean content was higher in the milk of the Sarda breed with respect to Saanen ( $1219 \times 10^3$  and  $762 \times 10^3$  cfu m/L, for Sarda and Saanen, respectively, *p* < 0.001). These latter values are below the threshold indicated by the EU for milk which undergoes thermal treatment. A study, which compared the TBC values of native Mediterranean breeds vs. imported breeds, ascribed the higher TBC values found in the former, rather than to genetics, to other factors such as hand-milking, improper milk handling, and storage equipment [35]. These problems are more frequently encountered in extensive and semi-extensive farm systems [35] and may be the cause of the higher values found in the Sarda breed samples.

## 5. Conclusions

Overall results of this work indicated that milk composition of Sarda and Saanen breeds reared in the same territory exhibited a strong seasonality and dependence on climate and pasture from grassland. During summer, milk from the alpine breed showed a significant drop of fat and protein contents, with a high proportion of samples with reverse syndrome, probably due to the Mediterranean climate. This suggests that to also obtain high-quality milk in the hot months, a more controlled farming systems should be designed for the Saanen breed. Moreover, to lower the TBC, more control and good practices can be suggested to the Sarda farming systems.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/dairy3030038/s1 Table S1: Two-way ANOVA effects of different variables (year, month, farm) and their interactions on mean differences of the milk main components (fat, protein, lactose, and urea, as reported in Table 1) for Sarda and Saanen milk samples.

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