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Research Article

Effects of Season on Ovine Milk Composition

Paola Scano^{1*}, Ignazio Ibba², Mattia Casula³, Marino Contu² and Pierluigi Caboni³

¹Dipartimento di Scienze Chimiche e Geologiche, Università di Cagliari, SS 554 km 4.5, 09042 Monserrato, Cagliari, Italy

²Associazione Regionale Allevatori Sardegna, Laboratorio Analisi Latte, Loc. Palloni. Nuraxinieddu. 09170 Oristano. Italy

³Dipartimento di Scienze della Vita e dell'Ambiente, Università di Cagliari, via Ospedale 72, 09124 Cagliari, Italy

Abstract

In the Mediterranean area, the seasonality of sheep milk production and quality heavily affects the dairy industry profitability. This research paper is aimed to study the changes of ovine milk composition in different months of the year. The composition of more than 126000 samples of bulk milk from grazing Sarda breed sheep, bred in Sardinia (Italy), collected in 2017 from January to July was studied. Milk main traits, physical-chemical parameters, and fatty acid composition were measured, and data analyzed by descriptive statistics, mean comparison test and multivariate statistical analysis. An increase of proteins and fat was observed in summer, accompanied by a decrease of proteins/fat ratio. Milk urea showed higher levels in winter-spring (January-April). Rumenic acid, trans-vaccenic acid and linolenic acid decreased in late spring-summer (May-July). Somatic cell levels were found not correlated with the bacterial count, and their trend suggests a dependence to milk dilution. NaCl content compensated the lower levels of lactose in the summer season. Based on a big data set, results highlighted the main differences in bulk milk composition, over a long period of the year. Differences can be attributed to traditional system of breeding.

Keywords: Milk composition; Milk urea; Ovine milk seasonality; Rumenic acid; Sheep's milk

Introduction

Sheep milk is known to have high concentrations of proteins, fat, minerals, and vitamins, and thus a high nutritional value when compared to milks of other domestic species. Ovine milk can be used

*Corresponding author: Paola Scano, Dipartimento di Scienze Chimiche e Geologiche, Università di Cagliari, SS 554 km 4.5, 09042 Monserrato, Cagliari, Italy, Tel: +39 0706754391; E-mail: scano@unica.it

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to produce different cheese varieties, yogurt, and ricotta containing prebiotic ingredients and/or probiotic bacteria, which are the major categories in the functional food market. Nowadays, ovine dairy products have gained market size due to the product's quality, high yield, and nutritional value [1].

Sardinia (Italy) is one of the dominant European regions for sheep dairy production with more than 3.5 million sheep (3.7% of the EU total in 2009) which produce more than 300,000 MT of milk corresponding to about 4% of total world production [2]. Approximately the 90% of this milk is processed into different types of cheese. Sardinia boasts a wide range of high quality ripened sheep cheese, namely Pecorino Romano, Pecorino Sardo and Fiore Sardo, recognized with the Protected Designation of Origin (PDO) under the European law. However, the cheese production is characterized by a great discontinuity due to the low milk production and often low milk technological quality during summer time. This fact is related to the seasonality of the Sardinian ovine breeding system, with the lactation curve of sheep that follows the evolutionary curve of grazing. The lactation curve is characterized by a maximum peak in the spring season, in correspondence of the maximum availability of grass, followed by a gradual decrease of milk yield until the summer season when the ewes dry-off [3]. In Sardinia, 75% of the sheep milk is produced between January and May [4]. Several variables (e.g., stage of lactation, diet, temperature and season) contribute to this trend. Indeed, on average, in the traditional systems, the milk produced in the summer often is produced by ewes at the end of lactation, fed on stubble and with insufficient supplements, and exposed to heat stress [4]. Milk production and cheese making are therefore done during the winter- spring period. This production system, characterized by variations in the technological characteristics of milk, has important implications for the dairy industry. Specifically, in summer, low milk production and low milk technological quality have a repercussion on cheese yields, on the efficiency of cheese dairy industries and on the market sector, with a reduction in cash flow and lack of fresh (not ripened) dairy products.

There is also a strong relationship between sheep nutrition and milk quality which is mainly identifiable in its technological and coagulation properties. Indeed, these properties are determined by milk fat and protein concentration, Somatic Cell Content (SCC) and other parameters. Data on ovine milk composition and physical-chemical characteristics changes during the production season is essential for successful development of dairy sheep products as well as for the marketing of the products [5]. In this work, we studied seasonal changes of ovine milk compositional characteristics by descriptive statistics and multivariate statistical analysis, during the production period i.e. from January to July.

Materials and Methods

Sampling

A total of 126259 samples of ovine bulk milk from 375 different breeders located in 375 municipalities covering the whole Sardinian

territory, were analyzed. Samples were collected from January to July in 2017, within the International Committee for Animal Recording (ICAR) program. In 2016, Sardinia average temperatures were 10, 11, 12, 14, 17, 21, 24 and 25 °C, for January, February, March, April, May, June and July respectively. The max-min temperatures were: 14-5, 15-7, 16-7, 18-9, 22-12, 26-16, 29-19 °C, from January to July respectively. Samples were obtained from the Sardinian association of sheep breeders (ARA Sardegna). Milk samples were collected following the standard ISO 707:2008 [6].

Milk composition

Fat, proteins, caseins, lactose (expressed as g/100 mL of milk), and urea (expressed as mg/100 mL of milk) content, freezing point (expressed as degrees Horvet), and pH values were obtained with a Milkoscan FT6000 (Foss, Denmark), following the procedure ISO 9622:2013 [7]. Fatty Acids (FA) were measured using mid-infrared spectrometry-based prediction models as previously reported [8]. Spectra were acquired by using a Spectrometer Milkoscan FT6000 (Foss, Denmark). Samples underwent SCC evaluation procedure, according to ISO 13366-2:2006 [9]. Bacterial count (BC, expressed as CFU/mL) was based on the standard flow cytometric method with BactoScan (Foss Analytical, Hillerød, Denmark).

Statistical analysis

Univariate data analysis was performed to give valuable descriptive information on the data, as the mean, the Standard Deviation (SD) and coefficient of variation % (CV%). SCC and BC original values were also log-transformed. The null hypothesis "means are not significantly different" was tested by the One-way Analysis of Variance (ANOVA) and mean differences between groups (months) were tested for statistically significance by using the Tukey post-hoc comparison test at p<0.0001. Linear correlations between two variables were calculated using the Pearson correlation coefficient. Principal Component Analysis (PCA) was performed with SIMCA-P+ (version 14.1, Umetrics, Umeå, Sweden) software, while for the other statistical analysis the software Origin Pro 2016 (Origin Lab corporation, Northampton, MA, USA) was used.

Results and Discussion

At first a PCA was performed on the whole data set, the resulting score plot (Figure 1A) clearly showed that, along the PC1, samples are displaced according with the month of collection, going from January (light circles, left side) to July (dark squares, right side). The loading plot (Figure 1B) indicated that summer samples were characterized by high levels of fat and proteins, while winter samples by lactose, rumenic acid (18:2 *cis-9 trans-*11), *trans-*vaccenic acid (18:1 *trans-*11) and linolenic acid (18:3 *cis-9*,12,15).

As reported in table 1, over the 7 months here examined, milk fat showed a mean value of 6.6 ± 0.9 g/100 mL of milk, with a minimum in April and a maximum level in July. Proteins showed mean value of 5.6 ± 0.4 g/100 mL, a minimum in January and a maximum value in July. From the minimum to the maximum values, fat showed an increase up to 36% and proteins of 10%. In this regard, it is worth to underline that the CV values were approximately 13-10% and < 10% for fat and proteins, respectively (Table1), indicating a higher variability of fat content with respect to proteins. Caseins, the main components of milk proteins (approximately the 78%) followed the same trend of proteins. As shown in figure 2, milk protein and fat

levels followed the same trend, having almost comparable values in the period of January-May and a statistically significant increase in June and July. This in accordance with previous literature data reported on ovine milk from sheep bred in the Mediterranean area reporting that in the winter-spring period, corresponding to the higher milk yield, there were lower levels of milk fat and protein content [4]. The trend of the protein-to-fat ratio is reported in figure 2. According with previous literature data, at the end of lactation (May-July), proteins did not rise proportionally to the increase of fat content, therefore the protein/fat ratio falls progressively. In this period, a progressive decrease in milk yield and a corresponding increase in milk total solids content were observed. Although the seasonal increase in milk total solids (proteins and fat in particular) resulted in a significant increase of cheese yield [10,11], the decrease of the milk protein/fat ratio leads to significant changes in the composition of the obtained cheese [12,13]. Addis et al., [14], comparing the physico-chemical characteristics of PDO Pecorino Romano cheeses produced in several dairies located in Sardinia, observed that those produced in June contained less proteins, more fat and salt than cheese produced in the spring period. Pecorino Romano PDO cheese produced during the summer season (May-July) achieved more slowly the suitable structure for "grating" use, the main commercial destination of this cheese [3]. Fat, proteins and salt content markedly affect rheological and sensory characteristics of cheese, especially hard cheese [15].

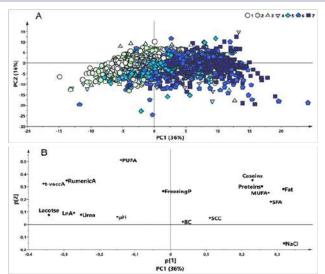


Figure 1: PCA of milk data set (126259 samples X 15 parameters). A) Score plot: 1 January, 2 February, 3 March, 4April, 5 May, 6 June, 7 July; B) Loading lactose. SFA (saturated fatty acids), MUFA (unsaturated fatty acids), PUFA (poly unsaturated fatty acids), t-vacc (*trans*-vaccenic acid), LnA (linolenic acid), rumenicA (rumenic acid).

A parameter strictly connected with the quality of ewe's diet is the milk urea content. Determining milk urea levels was found a useful management tool to evaluate the metabolism and intake of proteins while minimizing potential negative effects on animals [16]. Milk urea content (Figure 2) was higher in January-March, with a decrease (11.5%) in summer (June-July). It has been reported that in Sardinia and in other Mediterranean areas the ovine milk produced during late winter-spring seasons is characterized by lower levels of fat and protein due to the higher milk yield and by an increase of milk urea that impairs the milk technological properties in terms of lower cheese yield [17].

	January (n=18369)			February (n=19604)			March (n=19915)			April (n=19947)			May (n=19918)			June (n=19193)			July (n=9312)			Total (n= 126242)		
	Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%
Fat	6.3ª	0.8	13	6.3ab	0.8	13	6.3b	0.8	13	6.1°	0.7	11	6.6 ^d	0.8	11	7.4°	0.8	10	7.6 ^r	0.7	10	6.6	0.9	14
Prot	5.4ª	0.3	6	5.5b	0.3	6	5.6°	0.3	5	5.5 ^d	0.3	5	5.6°	0.3	6	5.8e	0.4	7	6.0 ^r	0.5	8	5.6	0.4	7
Case	4.2ª	0.3	7	4.2ª	0.3	7	4.4 ^b	0.3	7	4.3°	0.2	5	4.3 ^d	0.3	6	4.5°	0.3	8	4.6 ^r	0.4	8	4.3	0.3	7
Pr/Ft	0.9 ^a	0.1	13	0.9ª	01	15	0.94	0.1	12	0.9ª	0.1	11	0.85°	0.09	10	0.79 ^{ab}	0.07	9	0.80 ^b	0.06	8	0.9	0.11	13
Lact	5.0 ^a	0.1	2	4.9b	0.1	2	4.9°	0.1	3	4.9 ^b	0.1	3	4.8 ^d	0.2	3	4.5°	0.2	5	4.2 ^r	0.4	8	4.8	0.3	6
Urea	40°	9	22	43 ^b	9	20	40°	8	21	39°	9	22	36 ^d	8	24	26°	9	35	24 ^r	10	41	36	11	30
NaCl	122ª	18	14	129 ^b	18	14	131°	18	13	135 ^d	18	13	144°	18	12	173 ^r	29	17	2108	44	20	144	33	23
FrP	0.582acd	0.009	2	0.582b	0.009	2	0.582acd	0.009	2	0.583acdf	0.009	2	0.583°	0.009	1.5	0.58 ^{df}	0.01	2	0.588	0.01	2	0.58	0.01	2
pH	6.8a	0.1	2	6.7a	0.1	2	6.7b	0.1	2	6.7 ^{cd}	0.1	2	6.7 ^e	0.1	1.5	6.7e	0.1	2	6.72 ^d	0.10	2	6.7	0.1	2
SCC	1353a	1047	77	1330bc	1114	84	1323bc	1156	87	1264abc	1127	89	1357 ^d	1213	89	1567°	1324	85	1809 ^r	1617	89	1396	1202	86
BC	1666	3805		1706	3830		1383	3380		1426	3501		1268	3233		1364	3346		1296	3243		1453	3502	
SFA	3.9 ^a	0.6	14	3.9a	0.5	13	4.06b	0.53	13	4.04 ^b	0.48	12	4.35°	0.52	12	4.47 ^d	0.56	13	4.54°	0.55	12	4.2	0.6	14
MUFA	1.5a	0.3	22	1.4 ^b	0.3	22	1.39°	0.31	23	1.23 ^d	0.24	20	1.50 ^e	0.34	23	2.05 ^r	0.41	20	2.248	0.41	19	1.6	0.5	29
PUFA	0.39a	0.05	12	0.36b	0.04	12	0.35°	0.05	13	0.32 ^d	0.04	14	0.31°	0.04	12	0.31°	0.04	14	0.30 ^f	0.04	14	0.33	0.05	16
TV-A	3.6a	0.7	20	3.3b	0.7	22	3.2°	0.8	25	2.7 ^d	0.8	27	2.0°	0.7	38	1.3 ^r	0.8	60	0.98	0.7	73	2.5	1.1	46
RumA	1.5ª	0.3	20	1.5 ^b	0.3	21	1.4°	0.3	23	1.2 ^d	0.3	26	0.9°	0.3	30	0.8 ^r	0.3	43	0.78	0.3	47	1.2	0.4	37
LnA	1.0 ^a	0.3	24	1.2 ^b	0.3	21	1.0°	0.3	25	1.0°	0.3	25	0.9 ^d	0.3	30	0.6e	0.3	40	0.7 ^r	0.3	40	0.9	0.3	34

Table 1: Descriptive statistics and means comparison of ovine milk composition parameters over 7 months. Fat, Prot (proteins), Lac (lactose), Case (caseins), NaCl (g/100 mL of milk); Urea (mg/100 mL of milk); FrP (freezing point, -Horvet); SCC (log 10³ cell/mL of milk); BC (Bacterial count, CFU/mL); SFA (saturated fatty acids), MUFA (monounsaturated fatty acids; PUFA (poly unsaturated fatty acids) (g/100 mL of milk); TV-A (trans-vaccenic acid), RumA (rumenic acid), LnA (linolenic acid) (g/100 g of milk fat). Different letters in the same row indicate that the difference of the means is significant at the 0.001 level.

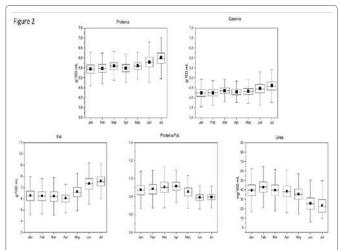


Figure 2: Box and whisker plots of fat, proteins, caseins, proteins/fat ratio and urea.

This fact has been related to the characteristics of the pasture with young sward poor in fiber and rich in highly fermentable proteins and/or non-protein nitrogen. High levels of dietary protein can cause animal health and reproduction issues, as well as the impairment of the milk characteristics and technological properties [16-18]. In addition, an increase of milk urea determines an increase of environmental urinary nitrogen excretion and it is associated with a worsening of animal welfare condition and different ruminant's pathologies such as fertility, subclinical mastitis and foot problems [19].

From January to May, lactose contents were comparable, in summer (June and July), lactose levels dropped of 16.6% (Figure 3) and within month, lactose showed a low CV% values. In summer, the increasing temperatures and solar radiation are generally related to a decreased of food intake [20]. The exposure of the animals to higher

environmental temperature stimulates the peripheral thermal receptors to transmit suppressive nerve impulses to the appetite centre in the hypothalamus and thereby causing a decrease in feed intake. The decrease in feed intake could be the adaptive mechanism to produce less body heat [21]. Since in lactating animals most of the blood glucose is used by the breast for the lactose synthesis, it could be speculated, according to Hamzaoui et al., [22], that the reduction in lactose secretion is a strategy to maintain stable glycaemia. Milk is isotonic with blood, and lactose is responsible for 50% of the osmotic pressure of milk. Thus, milk with a low level of lactose has a high level of inorganic salts or other compounds to maintain the osmotic pressure [23]. To keep the osmotic equilibrium, the decrease of the osmolyte lactose was found strictly correlated (r = -0.89) with the increased levels of NaCl (Figure 3).

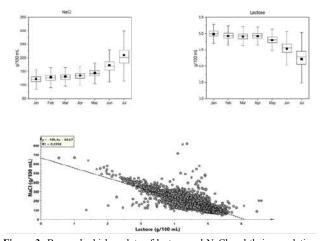


Figure 3: Box and whisker plots of lactose and NaCl and their correlations plot.

As reported in Table 1, the freezing point values, expressed as negative Horvet degrees, and pH had very low variability among months of production. The overall trend of SCC was similar to that observed for fat and proteins, with higher values in June and July, suggesting that milk dilution can play a determinant role for SCC values. As reported in Table 1, the mean SCC levels, although with high SDs, were higher than 1300×10^3 cell/100 mL of milk set as unofficial threshold, indeed, SCC physiological levels in ovine milk are still the subject of controversy [24], without an official threshold for mastitis diagnosis. The SCC value has a strong economic impact on sheep breeders as payment of milk depends on this value [25]. For what concerns the performance of high SCC milk in the production of cheese, no relevant differences between high and low SCC milk for ovine cheese manufacturing were observed [26,27].

The high SCC level found during summer is not accompanied by an increase of BC values, that, indeed, showed a fluctuating trend, suggesting that somatic cells can be mainly produced from the epithelium rather that from udder infection. However, the counting method used so far does not allow differentiation of the milk flora (lactic, psychotropic and pathogenic bacteria) [25].

We also studied the FA composition of milk and results are reported in Table 1 and depicted in figure 4. Going from January to July, the FA composition changed; there was an increase of saturate FA (SFA) and mono unsaturated FA (MUFA), and a decrease of Poly Unsaturated FA (PUFA). The reduced pasture quality after spring and the decrease of PUFA content as grass matured might, in part, explain the decrease of these FA in milk of grazing sheep. The same trend was observed by Nudda et al., [28]. Furthermore, from March to July, a reduction of the trans-vaccenic acid levels, the Conjugated Linoleic Acid (CLA) isomer rumenic acid, and the linolenic acid was observable. Among ruminants, sheep milk fat has the highest levels of CLA and trans-vaccenic acid. It has been reported that rumenic acid has important health effects, including antiobesity, anticarcinogenic, antidiabetic, antioxidant, and immunoregulatory effects [1]. The production of the trans-vaccenic acid and the rumenic acid is due to the ruminal biohydrogenation of linoleic acid, combined with mammary lipogenic and Δ-9 desaturation, these pathways considerably modifying the profile of dietary FA [29]. The measured rumenic acid and trans-vaccenic acid contents were found strongly positively correlated (r = 0.89) which is consistent with a predominant origin of rumenic acid in the mammary gland from trans-vaccenic acid via Δ9-desaturase [30]. The pasture plays a pivotal role in affecting the levels of trans-vaccenic acid, rumenic acid and also of α -linolenic acid. It is well established that the concentration of rumenic acid is higher in milk of grazing ruminants compared to those animals on diets based on conserved forages [28]. Natural pasture in the Mediterranean basin positively contributes to the rumenic acid content of sheep milk and cheese [28], changes in ewe's diet due to seasonal variation can modify its concentration in milk.

Conclusion

Results of this work indicated that milk characteristics of sheep bred in Sardinia heavily depend on the breeding system, climate and pasture. In future works data will be collected for more than one year to assess the annual variability of ovine milk produced in Sardinia.

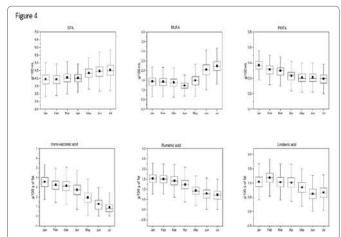


Figure 4: Box and whisker plots of SFA (saturated fatty acids), MUFA (monounsaturated fatty acids; PUFA (poly unsaturated fatty acids); trans-vaccenic acid; rumenic acid; linolenic acid.

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