



Cheese making aptitude and the chemical and nutritional characteristics of milk from Massese ewes

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ABSTRACT

The purpose of this study was to determine the effects of season, locality and the different altitudes at which farms are located, on the physico-chemical composition, morphometric characteristics of fat globules, fatty acid composition and cheese making aptitude of milk of Massese ewe's raised in 11 flocks from two provinces of north-west Tuscany (Massa Carrara and Lucca).

The winter lactation shows higher percentages of casein, lactose and not fat dry matter ($P \leq 0.01$); curd firming time (k_{20}) is significantly lower and there is a greater curd firmness (a_{30}) ($P \leq 0.01$); while in the summer there is a higher percentage of lipids ($P \leq 0.01$). The effect of the season significantly influences ($P \leq 0.01$) the size of the fat globules and impacted significantly on the fatty acids composition of the milk. In the hills the milk has a higher percentage of dry matter, protein, casein, fat, phosphorous and not fat dry matter ($P \leq 0.01$), whereas it has a lower percentage of lactose and calcium ($P \leq 0.05$). The Somatic Cell Count (SCC) and the Total Bacterial Count (TBC) are statistically greater on the plains ($P \leq 0.01$), while milk produced in the hills shows higher quantity of α -linolenic acid and lower saturated fatty acids ($P \leq 0.05$).

In the two typical rearing areas for Massese ewes we found differences amongst dry matter, fat, phosphorous and SCC, higher ($P \leq 0.01$) in the province of Massa Carrara that also had the best rheological parameters, but we found the highest cheese yield ($P \leq 0.05$) in the province of Lucca where there are also the greatest weight loss ($P \leq 0.01$).

The milks produced in the winter season and in hilly areas present the best physico-chemical and nutritional characteristics. However, we found that the technological side should be improved by diversifying cheese-making techniques in relation to the characteristics of milk. In fact, currently these techniques do not fully exploit the potential to transform those milks with the best qualitative characteristics.

Key words: Milk quality, Massese sheep, Season, Altitude, Locality.

RIASSUNTO

CARATTERISTICHE CHIMICO-NUTRIZIONALI ED ATTITUDINE ALLA TRASFORMAZIONE DEL LATTE DI PECORA MASSESE

Scopo dello studio è stato quello di verificare gli effetti della stagione di parto, dell'altimetria e della localizzazione degli allevamenti, sulla composizione chimico-fisica, le caratteristiche morfometriche dei globuli

di grasso, il profilo acidico e l'attitudine alla caseificazione del latte di pecore Massesi proveniente da 11 allevamenti di due provincie della Toscana nord-occidentale.

Nella lattazione invernale sono stati rilevati maggiori percentuali di caseina, lattosio e sostanza secca magra ($P \leq 0,01$), il minore tempo di formazione del coagulo (k_{20}) e la maggiore consistenza (a_{30}) ($P \leq 0,01$), mentre in estate si evidenzia una superiore percentuale di lipidi ($P \leq 0,01$). L'effetto della stagione di parto influenza significativamente sia la dimensione dei globuli di grasso ($P \leq 0,01$) sia la composizione acidica del latte.

Il latte di collina è caratterizzato da maggiori percentuali di sostanza secca, proteine, caseina, grasso, fosforo e sostanza secca magra ($P \leq 0,01$) e da minori contenuti di lattosio e calcio ($P \leq 0,05$). Il contenuto di cellule somatiche e la carica batterica sono superiori in pianura ($P \leq 0,01$) mentre il latte prodotto in collina presenta una quantità più elevata di acido α -linolenico ed un minor contenuto di acidi grassi saturi ($P \leq 0,05$).

Nelle due aree tipiche di allevamento della pecora Massese sono state rilevate differenze per sostanza secca, grasso, fosforo e cellule somatiche ($P \leq 0,01$), superiori nella provincia di Massa Carrara. A tale provincia si associano inoltre migliori parametri reologici, mentre, le superiori rese in formaggio ($P \leq 0,05$) sono state rinvenute nella provincia di Lucca, che presenta anche i maggiori cali di stagionatura ($P \leq 0,01$).

I lattati prodotti nella stagione invernale e nelle aree di collina oltre ad avere caratteristiche fisico-chimiche e nutrizionali migliori, risultano più idonei per la trasformazione, tuttavia la valorizzazione di questo aspetto, dovrebbe prevedere una diversificazione della tecnica di caseificazione, in relazione alle diverse tipologie di latte, per sfruttare al meglio le potenzialità di trasformazione di lattati con le migliori caratteristiche qualitative.

Parole chiave: Qualità del latte, Pecora Massese, Stagione di parto, Altimetria, Area di allevamento.

Introduction

Interest in the nutrient composition of ewe's milk has been growing due to the nutritional value of milk in the human diet. It is well known that the composition of milk and cheese is influenced by many factors related to species and breed, stage of lactation, dietary composition, season, locality, environment and climate (Pulina *et al.*, 2006; Tsiplakou *et al.*, 2006; Morand-Fehr *et al.*, 2007).

Sheep's milk and cheese have interesting characteristics in terms of their specific composition of protein, fat and fatty acids. Their quality is linked to the historical and traditional production methods, but also to marketing and consumer requirements. Various factors are involved including farming system with predominantly extensive grazing, specific transformation technologies and the processes of cheese making and ripening. About 95% of sheep's milk is transformed into dairy products whose

quality is affected by the region and the location where they are produced (Boyazoglu and Morand-Fehr, 2001).

The main production areas of ewes in Italy are approximately 30% in Central Italy and 52% in the islands.

The Massese breed is one of the most important Italian dairy sheep, and is located above all in north-west Tuscany, comprising approximately 175,000 heads and producing 200-300 kg of milk per lactation (Pugliese *et al.*, 1999). The usually semi-extensive breeding technique has a strong influence on milk quanti-qualitative production, as also reported by Macciotta *et al.* (1999). Massese sheep have been the focus of biological and functional studies (Franci *et al.*, 1999; Martini *et al.*, 2004b) that have contributed our knowledge of the productive potentialities of this breed and its morphology (Martini *et al.*, 1993, 1997). In relation to the morphological characteristics, the almost exclusive use of internal replacement has led to dif-

ferent morphological types which depend on the province where the animals originate, the altitude where they are bred, and the farming techniques.

The aim of this study was to determine the effects of season, locality and the different altitudes at which farms are located, on the physico-chemical composition, morphometric characteristics of fat globules, fatty acid composition and cheese making aptitude of milk Massese ewe's raised in north-west Tuscany.

Material and methods

Animals and sampling

The study involved the collection and the analysis of bulk milk from Massese flocks reared in two provinces in north-west Tuscany: Lucca and Massa Carrara. The farms were identified on the basis of information supplied by Breeder Association in relation to altitude: lowland and hill (up to 500 m above sea level) and to the size of the flocks (the farms with less than 50 subjects were excluded).

Within every class of altitude and province a random selection was made from the available farms.

The study was carried out on 11 flocks in the intermediate phase of the two seasonal lactations (autumn-winter; spring-summer) referred to as the autumn and spring lambing.

The animals were reared according to the semi-extensive system, involving the use of natural pastures integrated with grass hay and small amounts of concentrate feed (never above 300 g/day), given to the animals at the time of milking.

The bulk milk samples for quantitative and qualitative analysis were taken from the morning milking. In each lactation season one bulk milk sample from each flock was collected twice during a one-month pe-

riod at a distance of 15 days, for a total of 44 samples and each sampling was carried out in duplicate. No preservatives were added.

Milk analysis

Bulk milk samples were analyzed for: dry matter, protein, fat, lactose by infrared analysis (Milkoscan, Italian Foss Electric, Padova, Italy); density, freezing point, pH, casein, ash, phosphorus and calcium (AOAC, 1990), somatic cell count (SCC) (Fossomatic) and total bacterial count (TBC, plate count agar, 30 °C, 72 h), non-fat dry matter was calculated to be the difference between dry matter and fat content.

Morphometric analysis of milk fat globules

Morphometric analysis of the fat globules (number of fat globules mL⁻¹ of milk and diameter) was performed following the method of Scolozzi *et al.* (2003). In short: fresh milk, diluted 1:100 with distilled water was added to an Acridine Orange solution 0.1% in a phosphate buffer pH 6.8 and placed in a Burker chamber for observation using a fluorescence microscope and the Quantimet 500 image analyzer system (Leica Ortomat Microsystem, S.P.A., Milano, Italy).

This method is simple to perform and is used to characterize native fat globules in fresh milk without handling the milk any more than necessary. Moreover this method allows us to directly measure the diameter of every single, visible, milk fat globule with the image analyzer system.

Milk fatty acids composition

An aliquot of the milk samples was stored at -20 °C until the fat was extracted for fatty acid analysis. Milk fat extraction was performed according to Rose-Gottlieb's method (AOAC, 1990) modified by Secchiari *et al.* (2003). Short chain fatty acid composition (SCFA, from C4 to C8) was determined according to Molkentin and Precht (2000) on a

second aliquot of the same sample. For this, 25 mg of lipid extract were trans-esterified with 0.2 mL of methanol KOH 2N. Fatty acid methyl esters (FAME) were dissolved in hexane containing methyl valerate as the internal standard. The butyric acid content was calculated using a regression curve based on five response factors obtained by increasing the C4:0:C5:0 ratio (the ratio varied from 0.15:0.40 mg·mL⁻¹ to 1.20:0.40 mg·mL⁻¹). Fatty acids from C6 to C8 were quantified using methyl valerate as the internal standard. The SCFA content was determined using a ThermoQuest (Milan, Italy) gas-chromatograph equipped with an FID and a high polar fused silica capillary column (Chrompack CP-Sil 88 Varian, Middelburg, the Netherlands; 100 m × 0.25 mm internal diameter; film thickness 0.20 µm), initially at temperature of 40 °C for 4 min, which was then increased to 180 °C at 5 °C·min⁻¹, at which temperature it was held for 35 min.

Methyl esters of medium and long chain fatty acids were prepared by the alkali catalysed trans-methylation procedure of Christie (1982), with nonadecanoic acid methyl ester (Sigma Chemical Co., St. Louis, MO, USA) as the internal standard. The composition of fatty acid with a chain higher than eight atoms of carbon was determined by gas chromatography using a ThermoQuest (Milan, Italy) gas-chromatograph apparatus equipped with an FID and a high polar fused silica capillary column (Chrompack CP-Sil 88 Varian, Middelburg, Netherlands; 100 m × 0.25 i.d.; film thickness 0.20 µm) according to the method described in Mele *et al.* (2006). Helium was used as the carrier gas at a flow of 1 mL·min⁻¹. The split ratio was 1:100. An aliquot of the sample was injected under the following GC conditions: the oven temperature was programmed at 120 °C and held for 1 min, then increased to 180 °C at a rate of 5 °C·min⁻¹, held for 18 min, increased to 200 °C

at 2 °C·min⁻¹, held for 1 min, increased to 230 °C at a rate of 2 °C·min⁻¹ and held for 19 min. The injector temperature was set at 270 °C, whereas the detector temperature was set at 300 °C. All the results concerning the milk fatty acid composition are expressed as g per 100 g of fat.

Lactodynamographic analysis

The analysis of the milk's rheological parameters was performed without pH standardization by Formagraph (Italian Foss Electric, Padova, Italy); samples were tested at 35 °C using 0.2 mL of a 8% aqueous solution of Hansen rennet for 10 mL of milk.

The following parameters were recorded: r=clotting time: the time (min) from the addition of rennet to the beginning of coagulation; k₂₀=curd firming time: the time (min) needed until the curd is firm enough to be cut, i.e, the width of the diagram equals 20 mm; a₃₀=curd firmness (mm): measured 30 minutes after the addition of rennet.

Cheese making procedure, yield and ripening

At the time of each milk sampling cycle, in each farm about 70 kg of milk were used to produce a two-month old cheese (Caciotta) and whey cheese (Ricotta). Milk was heated at 38 °C and coagulated by the addition of 30 g (1:17,000) of lamb rennet (Fabre S.R.L., LC, Italy) per 100 L of milk. After 20-25 min, the curd was cut into small pieces (5 mm) and allowed to stand for 2 min. Subsequently the curd was transferred into circular moulds allowing the whey to dry off. The curd was maintained for 2 h at 25 °C and, during this time, the moulds were turned upside down five times. Manual dry salting was carried out by spreading salt over the curd. The cheese-making process was repeated for each cycle under the same conditions, using different dairymen. No starter culture was added.

The ricotta was obtained by heating the whey derived from the cheese production to 85 °C. This was done to get the whey proteins to rise and coagulate. The curd was then skimmed off manually and transferred into moulds, allowing the residual whey to drain off at a refrigerating temperature.

The cheese yield after 24 hours and the weight of the fresh ricotta were calculated from the total milk produced as g cheese/100 g milk and expressed as percentage. About 12 caciotta loaves per cheese production cycle were obtained. To estimate the decrease in weight of the cheeses during ripening, two of the caciotta loaves, which weighed approximately 1kg, were selected at random from each cheese production cycle, for a total of 88 whole cheeses.

The 88 selected caciotta loaves were ripened for two months at 8-10 °C and 80-85% relative moisture; the decrease in weight of each caciotta was determined as a percentage of the total weight of the cheese after 10 (fresh), 30 (semi-seasoned) and 60 (seasoned) days of ripening: these are the typical times for local commerce depending on market demands.

Statistical analysis

The frequency distribution of total counted and measured milk fat globules was evaluated according to their size: diameters of the fat globules were divided into ten classes of 1µm widths (from 0 µm to >9 µm). For each milk sample, the percentages of fat globules within each size class were calculated. All ten classes were represented in all the milk samples evaluated, therefore each milk sample was characterised by a different percentage of fat globules, for each diameter size class.

Subsequently the ten classes were grouped into three size categories of fat globules: small globules (SG) with a diameter <2 µm, medium-sized globules (MG)

with a diameter from 2 to 5 µm, and large globules (LG) with a diameter > 5 µm.

Means and standard deviations of the physico-chemical, hygienic, rheological parameters of milk yield in cheese and ricotta and weight loss of cheese were calculated.

The differences between the factors of variability for all the estimated parameters were tested with the following model: $y_{ijklm} = \mu + P_i + A_j[P_i] + B_k + S_l + BS_{kl} + PB_{ik} + PS_{il} + \varepsilon_{ijklm}$ where: y_{ijklm} =parameters considered; μ =overall mean; P_i =fixed effect of the i^{th} locality ($i=1, 2$); $A_j[P_i]$ = fixed effect of the j^{th} farm ($i=1, \dots, 11$), within the i^{th} locality; B_k =fixed effect of the k^{th} altitude ($k=1, 2$); S_l = fixed effect of the l^{th} season ($l=1, 2$); BS_{kl} =interaction of the altitude k and season l ; PB_{ik} =interaction of the locality i and altitude k ; PS_{il} =interaction of the locality i and season l ; ε_{ijklm} =casual residual effect.

The statistical analysis was performed using JMP software, vers. 5.0 per PC, of the SAS Institute (2002).

Results and discussion

Physico-chemical, hygienic and technological characteristics of the milk

Average values and standard deviations of Massese milk's qualitative parameters are reported in Tables 1, 2 and 3. The means of the milk yield during the sampling period were equal to 0.71 L/head \pm 0.189.

Most of the parameters reported in Table 1 fall within the average range of values for Massese sheep raised according to extensive techniques (Pugliese *et al.*, 1997; Secchiari *et al.*, 2002); the exceptions being the values regarding SCC content which are lower than those reported in the literature concerning milk sheep farms in Central-Southern Italy (Rosati *et al.*, 2004) and which lie however, in the range reported from Bergonier *et al.* (2003) for the ovine species.

The mean value of TBC of milk is slightly

Table 1. Physico-chemical and hygienic characteristics of milk.

		mean	SD
Dry matter	%	17.51	0.946
Fat	"	6.44	0.853
Protein	"	5.71	0.421
Casein	"	4.73	0.696
Lactose	"	4.49	0.230
Non fat dry matter	"	11.06	0.461
Ash	"	0.90	0.059
Ca	g/100g	0.21	0.057
P	"	0.22	0.071
Casein/Fat		0.75	0.144
Freezing point	°C	-0.543	0.001
Density	g/mL	1.040	0.003
TBC	CFU*1000/mL	667.88	1020.609
SCC	cell*1000/mL	1133.22	530.500

TBC: Total Bacterial Count; SCC: Somatic Cell Count.

Table 2. Morphometric characteristics of milk fat globules and fatty acid composition.

		mean	SD
Globules/mL	no.x10 ⁹	2.44	1.19
Average diameter	µm	5.06	0.435
Small globules	%	0.32	0.460
Medium globules	"	43.51	11.764
Large globules	"	56.17	12.083
SCFA	g/100g fat	14.06	2.686
MCFA	"	39.21	2.985
LCFA	"	35.16	3.488
SFA	"	63.54	4.634
MUFA	"	22.91	2.566
PUFA	"	4.30	0.686
INS/SAT	"	0.43	0.066

higher than that of 500,000 indicated by 853/2004 EU regulation for products derived from raw milk, but it is within the limits of 1,500,000 for thermally-treated milk. In any case, the variability related to this parameter highlights the differences due to the different farms.

Evaluating the crioscopic point is a quick and simple method that is used to verify the addition of water in milk. The 853/2004 EU regulation only impose a tolerance limit on bovine milk, which is equal to $-520 \text{ m } ^\circ\text{C}$. The average value that we found, which was $-542 \text{ m } ^\circ\text{C}$, is higher than the average reported in the literature for Massese sheep (Profumo *et al.*, 2004), Valle del Belice (Scatassa, 2000), and Sarda (Cannas *et al.*, 2000); in this last study the authors gave a value of $-550 \text{ m } ^\circ\text{C}$ as the upper limit above which it is advisable to carry out another determination.

Studies on cows and sheep (Politis and Ng-Kwai-Hang, 1988; Sinclair *et al.*, 2007)

Table 3. Rheological characteristics of milk, cheese and *ricotta* yields and weight loss of cheese during ripening.

		mean	SD
pH		6.71	0.065
Clotting time r	min	12.99	5.418
Curd firming time k_{20}	"	1.23	0.452
Curd firmness a_{30}	mm	35.07	8.478
Cheese yield	%	19.95	2.890
<i>Ricotta</i> yield	"	11.61	2.916
Weight loss at 10 days	"	10.86	4.427
Weight loss at 30 days	"	20.66	6.313
Weight loss at 60 days	"	27.39	8.032

show that incorporation between casein and fat during the coagulation of the milk is maximum when the casein:fat ratio (C/F) is as close as possible to 0.7. In our study the average ratio was equivalent to 0.75, which is higher than the average reported by Jaeggi (2005) and by Wendorff (2003) in ewe milk.

The average number of globules per milliliter of milk and the average diameter of the fat globules shown in Table 2 are in the range found on individual milk for Massese (Martini *et al.*, 2004a; Cecchi *et al.*, 2005). Analogous values were also found for Comisana and Sarda sheep (Martini *et al.*, 2006), but they are different from those found by Mehaia (1995) for milk from the same species. It goes underline that, while to our methods allow us to measure the diameter of every single, visible, milk fat globule, other methods use the refractive index in order to carry out an indirect analysis of the standard parameters of milk fat globules using software.

The average values of the composition of fatty acids in milk (Table 2) are in agreement with those in the literature for other Italian dairy sheep (Antongiovanni *et al.*, 2002; Martini *et al.*, 2004b).

The mean of curd firmness (a_{30}), reported in Table 3, is in the lowest variability values (31.63 to 60.61) of Massese (Giulioti *et al.*, 1998; Martini *et al.*, 2000). On the other hand, the clotting times (r) show lower values than the mean values of Massese ewe milk (Cecchi *et al.*, 1997).

The cheese yields of Massese ewe's milk (Table 3) show an average value higher than milk from Sarda (Pirisi *et al.*, 1999) and Sicilian ewes (Todaro *et al.*, 2001), and the average weight loss of cheeses after 60 days is about three times higher than that found after 10 days.

Effect of lactation season on milk traits

The production season significantly affects part of the chemical composition of the milk (Table 4), as found by Jaeggi *et al.* (2005) in sheep milk and by Smit *et al.* (2000) in cow milk.

In winter lactation not only is there a higher density of milk but also higher percentages ($P \leq 0.01$) of casein, lactose and not fat dry matter. Similar results are reported by Sevi *et al.* (2004) and by Jaeggi *et al.* (2005), the latter with regard to casein content. The highest levels of casein were observed in the autumn for farms in the

Table 4. Effect of the season on the physico-chemical and hygienic characteristics of milk.

		Spring-summer		Autumn-winter	
		mean	SE	mean	SE
Dry matter	%	17.68	0.090	17.53	0.090
Fat	"	6.80 ^A	0.077	6.24 ^B	0.077
Protein	"	5.73	0.048	5.76	0.048
Casein	"	4.46 ^B	0.070	5.06 ^A	0.070
Lactose	"	4.41 ^B	0.017	4.56 ^A	0.017
Non fat dry matter	"	10.83 ^B	0.037	11.35 ^A	0.037
Ash	"	0.90	0.011	0.90	0.011
Ca	g/100g	0.20	0.010	0.21	0.007
P	"	0.15	0.010	0.26	0.007
Casein / Fat		0.66 ^B	0.009	0.82 ^A	0.009
Freezing point	°C	-0.540 ^B	1.736	-0.547 ^A	1.736
Density	g/mL	1.03 ^B	0.000	1.04 ^A	0.000
TBC	CFU*1000/mL	439.42	134.481	796.58	134.481
Log SCC	cell*1000/mL	3.02	0.022	2.99	0.022

A, B: $P \leq 0.01$.

province of Massa Carrara (season x locality interaction $P \leq 0.01$). In the summer, there is a higher percentage of lipids ($P \leq 0.01$), as reported by Carta *et al.* (1995) for the Sarda breed. This trend is most evident for farms in the hills where the percentage of fat in the summer season reaches its highest levels (altimetry x season interaction $P \leq 0.05$). The higher percentage of fat may be due to an effect of concentration linked to the lower quantity of milk produced (average daily production S 1.28 vs W 1.54 L/head) as reviewed by Mele *et al.* (2005).

In the winter the casein:fat ratio (C/F) increases and moves away considerably, with respect to the summer, from the optimal value of 0.7.

The values relating to the percentage of calcium and phosphorous and to the content of the SCC during the two sampling peri-

ods do not show significant differences but do have a trend that is similar to the one described for the same parameters by Sevi *et al.* (2004), who report that the content of phosphorous is higher in winter while the somatic cells count is higher in summer.

The significant increase in the crioscopic point ($P \leq 0.01$) in the summer was also noted by Cannas *et al.* (2000) for Sarda ewe milk.

The effect of the season (Table 5) significantly influences ($P \leq 0.01$) the size of the fat globules and there is an increase in the average diameter in the summer season. A relationship between the percentage of fat in milk and the average size of the globules has been found in cows by Martini *et al.* (2003) and Wiking *et al.* (2003).

As expected, the effect of the production season impacted significantly on the profile of the fatty acids of the milk reported in

Table 5. Effect of the season on the morphometric characteristics of milk fat globules and fatty acid composition.

		Spring-summer		Autumn-winter	
		mean	SE	mean	SE
Globules/mL	no.x10 ⁹	2.37	0.214	2.56	0.214
Average diameter	µm	5.27 ^A	0.060	4.85 ^B	0.060
Small globules	%	0.23 ^b	0.067	0.44 ^a	0.067
Medium globules	"	38.59 ^B	1.750	48.47 ^A	1.750
Large globules	"	61.18	1.782	51.09	1.782
SCFA	g/100g fat	13.07	0.904	15.06	0.904
MCFA	"	37.37 ^B	0.768	41.06 ^A	0.768
LCFA	"	37.76	1.048	37.18	1.048
SFA	"	60.61 ^B	1.134	66.48 ^A	1.134
MUFA	"	23.07	0.776	22.75	0.776
PUFA	"	4.53	0.160	4.07	0.160
INS/SAT	"	0.46	0.020	0.41	0.020
C13:0 ¹	"	0.06 ^B	0.003	0.09 ^A	0.003
C14:0 ¹	"	8.73 ^b	0.282	10.03 ^a	0.282
C18:1 trans11 ¹	"	2.80 ^A	0.108	1.57 ^B	0.108
C18:2 n 6 cis ¹	"	1.51 ^b	0.083	1.90 ^a	0.083
C18:3 n 3 ¹	"	1.10 ^a	0.068	0.84 ^b	0.068
CLA ¹	"	1.47 ^A	0.065	0.90 ^b	0.065

¹ In Table are reported only significant fatty acids.

A, B: $P \leq 0.01$; a, b: $P \leq 0.05$.

Table 5. For Massese, the partial deseasoning of births which is created as a result of the longer periods of mating, means that a significant number of ewes lambed when grassy turfs are growing again. The milk produced by sheep that lambed in the spring thus has characteristics that can be directly related to the presence of high quantities of fresh forage in their diet, as highlighted in previous works on the composition of ovine milk (Secchiari *et al.*, 2001; Nudda *et al.*, 2005) and bovine milk (Bargo *et al.*, 2005). The effect of the production season leads to a higher content of α -linolenic acid (LNA)

(C18:3 n3) in the summer season milk ($P \leq 0.05$), given that this is the main fatty acid in pasture grass. The ingestion of LNA is associated with the increase in vaccenic acid in milk (C18:1 trans-11), since vaccenic acid represents an intermediate in the process of biohydrogenation of the LNA in the rumen (Harfoot and Hazlewood, 1988).

The accumulation of vaccenic acid in turn led to an increase in the content of rumenic acid (cis 9, trans 11 CLA), of which, as is well known, more than 80% derives from the desaturation of the vaccenic acid in the mammary gland by the delta-9 desaturase

enzyme (Griinari *et al.*, 2000). In fact, the levels of vaccenic acid and rumenic acid found in milk produced by ewes that lambed in the summer is comparable with those reported in the literature for ewes fed with fresh forage (Secchiari *et al.*, 2001). On the other hand, the milk produced by the ewes that lambed in the winter, has a higher content of saturated fatty acids (SFA) and in particular of myristic acid (C14:0), whose hypercholesterol characteristics are well known (Keys *et al.*, 1965). Higher content of saturated fatty acids (SFA) is due to the greater use of conserved forage and concentrate, used at that time of year as a result of the lower availability of fresh grass and the shorter time spent grazing. In fact, the consumption of fresh forage increases the quantity of polyunsaturated fatty acids both in ovine and bovine milk (Molle *et al.*, 2004; Couvreur *et al.*, 2006).

Table 6 shows that the pH value of milk is significantly more acidic ($P \leq 0.05$) in winter milk. It is known that a low pH value has a positive impact on the rheological parameters (Duranti and Casoli, 1991; Mar-

tini *et al.*, 1999). In agreement with Rapacini *et al.* (1996) winter milk has a value of curd firming time (k_{20}) that is significantly lower ($P \leq 0.01$) and a greater curd firmness (a_{30}) ($P \leq 0.01$).

As observed by Jaeggi *et al.* (2005), despite the fact that little is known about the effect of the season on the yield of cheese, generally as the temperature rises during the summer the cheese yield from ovine milk decrease. However, many studies on cheese yield are conducted with ovine breeds such as Sarda, for which there is traditionally a concentration of lambing during the winter. Thus the arrival of summer coincides with the final stage of lactation, and this prevents the disjunction of the effect of the lactation phase with the phase of the productive season. In our study we did not observe any modifications in the cheese yield between the two seasons (Table 6), however ricotta yields were significantly higher in winter ($P \leq 0.05$).

Effect of altitude on milk traits

In accordance with Macciotta *et al.* (1999),

Table 6. Effect of the season on the rheological characteristics of milk, cheese and ricotta yields and weight loss of cheese during ripening.

		Spring-summer		Autumn-winter	
		mean	SE	mean	SE
pH		6.72 ^a	0.009	6.69 ^b	0.012
Clotting time r	min	11.87	0.665	11.39	0.858
Curd firming time k_{20}	"	1.41 ^A	0.054	1.13 ^B	0.067
Curd firmness a_{30}	mm	35.36 ^B	0.623	38.51 ^A	0.805
Cheese yield	%	19.88	0.453	20.02	0.444
Ricotta yield	"	10.82 ^b	0.538	12.40 ^a	0.538
Weight loss at 10 days	"	11.98	0.899	9.81	0.873
Weight loss at 30 days	"	22.18	1.078	19.23	1.045
Weight loss at 60 days	"	26.06	1.217	25.81	1.182

A, B: $P < 0.01$; a, b: $P < 0.05$.

who report that the environmental influence is enhanced by the different altitudes at which farms are located, we revealed significant differences between milk from the plains and milk from the hills in terms of chemical and hygienic quality (Table 7). In the hills the milk has a higher percentage ($P \leq 0.01$) of dry matter, protein, casein, fat, phosphorous and not fat dry matter, whereas it has a lower percentage of lactose and calcium ($P \leq 0.05$). In addition, the casein/fat ratio ($P \leq 0.01$) is optimal for cheese-making. The content of SCC and the TBC are statistically greater on the plains ($P \leq 0.01$) according to *Rassu et al.* (1993). These authors show that the altitude where rearing takes place seems to affect the total bacterial count, which is lower in the mountains probably due to the inhibitory effect induced by low temperatures. Interestingly, milk

produced by farms located in the hills in the province of Massa Carrara, where this breed originates from, has the best composition characteristics in terms of the content of protein, casein, lactose and TBC (altimetry interaction x locality, $P \leq 0.01$). The bacterial count showed values under 100,000 CFU/mL, which is significantly lower than requested by the health rules.

There were no differences between the two altimetric zones either in terms of the morphometry of the fat globules (Table 8) or the lactodynamographic parameters and for the cheese yield (Table 9). The pH values, the ricotta yields and the weight loss of cheeses after 30 and 60 days of ripening show higher values ($P \leq 0.01$) in the plains (Table 9).

The composition of the fatty acids of milk was also affected by the altitude of the farm

Table 7. Effect of the altitude on the physico-chemical and hygienic characteristics of milk.

		Lowland		Hill	
		mean	SE	mean	SE
Dry matter	%	16.91 ^B	0.085	18.29 ^A	0.095
Fat	"	5.94 ^B	0.070	7.10 ^A	0.081
Protein	"	5.55 ^B	0.045	5.95 ^A	0.050
Casein	"	4.57 ^B	0.066	4.95 ^A	0.074
Lactose	"	4.51 ^a	0.016	4.45 ^b	0.018
Non fat dry matter	"	10.92 ^B	0.035	11.26 ^A	0.039
Ash	"	0.89	0.010	0.91	0.012
Ca	g/100g	0.22 ^a	0.008	0.19 ^b	0.009
P	"	0.19 ^b	0.783	0.22 ^a	0.009
Casein / Fat		0.78 ^A	0.009	0.70 ^B	0.010
Freezing point	°C	-0.544	1.637	-0.542	1.830
Density	g/mL	1.04	0.000	1.03	0.000
TBC	CFU*1000/mL	3.06 ^A	0.021	2.95 ^B	0.021
Log SCC	cell*1000/mL	963.29 ^A	126.790	272.71 ^B	141.760

A, B: $P \leq 0.01$; a, b: $P \leq 0.05$.

Table 8. Effect of the altitude on the morphometric characteristics of milk fat globules and fatty acid composition.

		Lowland		Hill	
		mean	SE	mean	SE
Globules/mL	no.x10 ⁹	2.20	0.202	2.74	0.226
Average diameter	µm	5.03	0.057	5.10	0.063
Small globules	%	0.34	0.063	0.29	0.070
Medium globules	"	44.56	1.650	42.25	1.844
Large globules	"	55.10	1.680	57.46	1.880
SCFA	g/100g fat	14.84	0.852	13.13	0.953
MCFA	"	40.02	0.724	38.25	0.809
LCFA	"	37.36	0.988	37.61	1.105
SFA	"	65.17 ^a	1.069	61.60 ^b	1.195
MUFA	"	22.93	0.731	22.89	0.818
PUFA	"	4.13 ^b	0.151	4.50 ^a	0.169
INS/SAT	"	0.42	0.019	0.45	0.021
C14:1 ¹	"	0.15 ^B	0.022	0.24 ^A	0.024
C14:0 iso ¹	"	0.10 ^b	0.008	0.14 ^a	0.009
C17:0 ¹	"	0.59 ^b	0.037	0.72 ^a	0.041
C18:1 trans 10 ¹	"	0.15 ^a	0.010	0.11 ^b	0.012
C18:1 trans 12 ¹	"	0.21 ^a	0.022	0.14 ^b	0.025
C18:3 n 3 ¹	"	0.88 ^b	0.064	1.07 ^a	0.072
C20:4 n 6 ¹	"	0.10 ^b	0.006	0.12 ^a	0.007
C21:0 ¹	"	0.07 ^b	0.006	0.10 ^a	0.007

¹In Table are reported only significant fatty acids.

A, B: $P \leq 0.01$; a, b: $P \leq 0.05$.

locations above all in terms of polyunsaturated fatty acid content (Table 8). In fact, milk produced in the hills would seem to have a higher quantity of LNA and lower SFA. This confirms data from Collomb *et al.* (2001) in relation to milk produced by bovines fed on plain and hill pastures. The higher quantity of LNA in milk produced by sheep in the hills is probably due to longer grazing periods compared to those on the plain, and also to the higher fresh forage to concentrate ratios in their diet. Farms in

the hills have a longer period of vegetation of the grass in their pastures which means a longer contribution of LNA for animals fed by grazing.

On the other hand, the fact that farmers on the plain have less pasture available means that they have to integrate more concentrate into the diet. This fact might be one of the reasons for the greater content of C18:1 *trans* 10 and C18:1 *trans* 12 in milk from farms on the plains, since increasing amount of concentrate in the ration led to

Table 9. Effect of the altitude on the rheological characteristics of milk, cheese and *ricotta* yields and weight loss of cheese during ripening.

		Lowland		Hill	
		mean	SE	mean	SE
pH		6.72 ^a	0.009	6.69 ^b	0.013
Clotting time r	min	12.09	0.627	11.17	0.886
Curd firming time k_{20}	"	1.27	0.051	1.27	0.056
Curd firmness a_{30}	mm	37.81	0.588	36.06	0.831
Cheese yield	%	20.20	0.422	19.61	0.476
<i>Ricotta</i> yield	"	13.63 ^A	0.508	9.18 ^B	0.568
Weight loss at 10 days	"	11.24	0.834	10.35	0.942
Weight loss at 30 days	"	22.58 ^A	0.997	18.11 ^B	1.126
Weight loss at 60 days	"	30.20 ^A	1.128	23.64 ^B	1.275

A, B: $P \leq 0.01$; a, b: $P \leq 0.05$.

Table 10. Effect of the locality on the physico-chemical and hygienic characteristics of milk.

		LUCCA		MASSA CARRARA	
		mean	SE	mean	SE
Dry matter	%	17.35 ^B	0.085	17.85 ^A	0.094
Fat	"	6.20 ^B	0.070	6.84 ^A	0.081
Protein	"	5.70	0.045	5.79	0.050
Casein	"	4.82	0.066	4.70	0.074
Lactose	"	4.60 ^A	0.016	4.37 ^B	0.018
Non fat dry matter	"	11.16 ^a	0.035	11.025 ^b	0.039
Ash	"	0.89	0.010	0.91	0.903
Ca	g/100g	0.22 ^a	0.008	0.19 ^b	0.009
P	"	0.19 ^B	0.008	0.23 ^A	0.009
Casein / Fat		0.80 ^A	0.009	0.69 ^B	0.009
Freezing point	°C	-0.543	1.637	-0.543	1.830
Density	g/mL	1.04 ^A	0.000	1.03 ^B	0.000
TBC	cfu*1000/mL	2.92 ^B	0.021	3.11 ^A	0.024
Log SCC	cell*1000/mL	525.75	126.790	710.25	141.760

A, B: $P \leq 0.01$; a, b: $P \leq 0.05$.

Table 11. Effect of the locality on the morphometric characteristics of milk fat globules and fatty acid composition.

		LUCCA		MASSA CARRARA	
		mean	SE	mean	SE
Globules/mL	no.x10 ⁹	2.59	0.202	2.27	0.226
Average diameter	µm	4.98 ^b	0.057	5.16 ^a	0.063
Small globules	%	0.33	0.063	0.31	0.070
Medium globules	"	45.88 ^a	1.650	40.66 ^b	1.844
Large globules	"	53.79 ^b	1.680	59.03 ^a	1.879
SCFA	g/100g fat	14.38	0.852	13.69	0.953
MCFA	"	38.62	0.724	39.92	0.809
LCFA	"	36.47	0.988	38.68	1.105
SFA	"	62.82	1.069	64.41	1.195
MUFA	"	22.48	0.731	23.42	0.818
PUFA	"	4.17	0.151	4.45	0.169
INS/SAT	"	0.43	0.019	0.43	0.021
C14:1 ¹	"	0.16 ^b	0.022	0.23 ^a	0.024
C16:1 ¹	"	1.02 ^a	0.042	0.85 ^b	0.047
C18:1 cis11 ¹	"	0.24 ^a	0.011	0.20 ^b	0.012
C18:1 trans11 ¹	"	2.08 ^b	0.102	2.31 ^a	0.114
C18:3n3 ¹	"	0.88 ^b	0.064	1.08 ^a	0.072
C20:1 ¹	"	0.02 ^b	0.007	0.04 ^a	0.008
C21:0 ¹	"	0.07 ^B	0.006	0.1 ^A	0.007

¹ In Table are reported only significant fatty acids.

A, B: $P \leq 0.01$; a, b: $P \leq 0.05$.

accumulate these two fatty acids in the rumen (Loor *et al.*, 2004). This trend in the fatty acid composition of milk is even more evident in the milk produced by hill farms in the province of Massa Carrara (altimetry interaction x locality, $P \leq 0.01$) for which we found higher values of LNA (1.36 g/100 g lipids), CLA (1.49 g/100 g lipids), vaccenic acid (2.99 g/100 g lipids), and PUFA (5.17 g/100 g lipids).

Effect of locality on milk traits

The rearing location may lead to quali-

tative differences in the milk in relation to both the productive characteristics of the location and to small differences in local traditions. The effect of location was also found by Smit *et al.* (2000) for cows for most of the physical and chemical parameters of the milk.

In the two typical rearing areas for Massese ewes we found significantly statistical differences amongst many of the chemical parameters examined (Table 10). In fact, quantities of dry matter, fat, phosphorous and SCC were higher ($P \leq 0.01$) in

Table 12. Effect of the locality on the rheological characteristics of milk, cheese and *ricotta* yields and weight loss of cheese during ripening.

		LUCCA		MASSA CARRARA	
		mean	SE	mean	SE
pH		6.73 ^A	0.009	6.67 ^B	0.013
Clotting time r	min	12.84 ^a	0.627	10.42 ^b	0.886
Curd firming time k ₂₀	"	1.35	0.051	1.19	0.069
Curd firmness a ₃₀	mm	35.78 ^b	0.588	38.10 ^a	0.831
Cheese yield	%	20.76 ^a	0.474	19.12 ^b	0.425
<i>Ricotta</i> yield	"	12.10	0.663	10.87	0.595
Weight loss at 10 days	"	13.55 ^A	0.936	8.55 ^B	0.840
Weight loss at 30 days	"	24.25 ^A	1.120	17.08 ^B	1.005
Weight loss at 60 days	"	31.26 ^A	1.267	23.15 ^B	1.137

A, B: $P \leq 0.01$; a, b: $P \leq 0.05$.

the province of Massa Carrara, whereas in the province of Lucca, lactose, casein/fat ratio, density ($P \leq 0.01$), not fat dry matter and calcium ($P \leq 0.05$) were on average higher.

With regard to the morphometry of the fat globules (Table 11) we confirmed the relationship already found for the season effect between the greater percentage of fat and the highest average diameter found in the province of Massa Carrara.

The effect of the rearing location on the composition of the fatty acids of milk only had significant results on some fatty acids (Table 11). This general homogeneity between the compositions of the fatty acids in milk produced in the two provinces was expected because feeding and rearing techniques (which are the two most important factors of variation for the fatty acid composition of milk) tend to differ in relation to the altitude of the farms rather than the geographical location. The Massa Carrara province had the best rheological parameters connected to the lowest pH value ($P \leq 0.01$), a shorter clotting time (r), and a greater curd firmness (a₃₀) ($P \leq 0.05$)

(Table 12).

We found the highest cheese yield (Table 12) in the province of Lucca ($P \leq 0.05$), where there are also the greatest weight loss ($P \leq 0.01$). This may be due to a better retention of water inside the curd, itself a consequence of the smaller average diameter of the fat globules since the membrane of small-sized globules tends to retain more water (Michalski *et al.*, 2003) and it would seem to favor the production of cheeses that, at 24h, lose less whey and contain more moisture than those products with milk rich in large fat globules (Wiking *et al.*, 2004; Martini *et al.*, 2008).

Effect of the farms

With regard to the farms variability, we found significant differences ($P \leq 0.01$) for all the physico-chemical and hygienic parameters, with the exception of the percentage of protein, ash and density. The number of fat globules/mL was also statistically different among farms ($P \leq 0.05$), as were k₂₀ and a₃₀ ($P \leq 0.05$), the cheese yield and the ricotta yield ($P \leq 0.05$).

Conclusions

The Massese is one of the dairy sheep that is most commonly found in central Italy. However, there has been no detailed description of the quality of the milk produced in terms of the main factors of environmental variation on a representative sample of the population, including the area from which the breed originated. Given that the semi-extensive system is the most commonly used for rearing this breed, the composition of Massese ewe's milk is subject to natural qualitative variations linked to the milking season, altitude and location, and to how the farm is managed. This gives rise to differences in nutritional values, in the organoleptic characteristics and in presamic coagulation. In turn, this affects the ability to transform and also the cheese yields, and consequently the homogeneity of the dairy products. An efficient assessment of these products must

include comprehensive knowledge of the variability that the main environmental factors have on the characteristics of the milk itself. This means that the productivity of the farms can be optimized through suitable feeding and rearing strategies.

The milks produced in the winter season and in hilly areas present the best physico-chemical and nutritional characteristics. However, we found that the technological side should be improved by diversifying cheese-making techniques in relation to the characteristics of milk. In fact, currently these techniques do not fully exploit the potential to transform those milks with the best qualitative characteristics.

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