Milk quality of Assaf ewes raised in Central Italy (Tuscany)

F. SALARI[a], I. ALTOMONTE[a], M. D'AURIA[b], M. MARTINI[a,c]

[a] Department of Veterinary Sciences, University of Pisa - Viale delle Piagge, 2 - 56124 Pisa
[b] Graduate in Science and Technology of Animal Production
[c] Interdepartmental Research Center "Nutraceuticals and Food for Health" - Via del Borghetto, 80 56124 Pisa, Italy

SUMMARY

Introduction - The demand from outside Italy for Italian cheeses made from sheep milk has recently increased. Due to the agricultural productivity crisis, thousands of dairy farms have closed down. In the farms that have remained, there has been a tendency to increase the average milk production. Thus, in Italy there has been a recent increase in the importation of foreign high-yielding sheep breeds.

Aim - This study focuses on the Assaf breed imported from Spain, which is being used to replace traditional breeds. The milk yield and quality from the Assaf sheep were monitored during lactation.

Materials and methods - Ten pluriparous Assaf ewes underwent milk sampling. Milk quality, fatty acid composition and coagulation properties were analysed from day 20 to day 170 of lactation.

Results and discussion - The Assaf sheep showed a high production capacity (about 1.3±0.50 litres / day, on the average) but lower percentage of fat and protein than Assaf reared in other Mediterranean Countries and than local breeds. The coagulation properties (k20 and a30) were better at the end of lactation (k20=1.37 min, a30=36.51 mm). In this phase, the increase in saturated fatty acids and medium chain fatty acids, the decrease in monounsaturated, and saturated: unsaturated fatty acid ratio, indicated a poorer milk nutritional quality in this period.

Conclusions - We confirmed the high productivity of the Assaf sheep. The nutritional quality of its milk fat seems better at the beginning of lactation when less saturated, and more monounsaturated fatty acids are present in the milk. Furthermore, higher protein and better coagulation properties of the milk were observed at the end of lactation.

KEY WORDS

Assaf, milk quality, coagulation parameters, fatty acids.

INTRODUCTION

Recently, the demand in foreign markets, particularly in developing countries and Asia, for Italian cheeses made with sheep milk has been increasing. In Italy thousands of dairy farms have closed down and the average farm size has tended to increase. Nevertheless, Italian sheep milk production has decreased, with a 12% drop from 2008 to 2011[1] and thus, milk production is currently insufficient to cover the market demand. This trend has been made worse by the seasonality of sheep milk production, which strongly limits the availability of small ruminant milk at certain times of the year.

Sardinia and Tuscany, which have a great potential for sheep farming, have been affected by the crisis resulting in economic damage to dairy farms that fail to guarantee markets with sheep milk products.

In the last few years there has been an increase in the importation of foreign high-yielding sheep breeds in an attempt to increase the average milk production per head and to meet the increasing market demand; particularly Assaf and Lacaune are imported. The Assaf is a cross between Awassi and East Friesian sheep[2] that was introduced in Spain from Israel in the late 1970s, due to their high production potential.

The average milk yield during conventional lactation in Assaf and Lacaune breeds is higher than the average milk yield in Italian breeds[3]. However, unlike the native breeds, the Assaf and Lacaune need intensive farming systems to maximize their productive potential, together with a greater commitment by farmers in terms of farming technologies, health care and expenditure.

Since sheep milk is used mainly for cheese making, monitoring the milk composition of foreign breeds is fundamental for farmers. Milk quality is also important due to the fact that the price milk commands in terms of purchase by factories is based on the milk protein and fat percentages. In addition, fatty acid content of milk affects the nutritional value of dairy products[4]. The evaluation of the milk nutraceutical quality helps to identify products with better health characteristics and is particularly important for consumers and gives added value to the products that can thus be sold for a higher price on the market.

We thus focused on the Assaf breed that was imported from Spain in order to replace the local Massese breed, which is traditionally farmed in a semi-intensive system based on natural pasture. In this study, the milk yield and quality from the Assaf sheep were monitored during lactation.

Autore per la corrispondenza:
Federica Salari (federica.salari@unipi.it).

MATERIALS AND METHODS

Sampling, data collection and milk analysis

Ten pluriparous Assaf ewes underwent milk sampling from March to September 2014, starting from day 20 of lactation until day 170.

The pregnant animals were imported from Spain, intensively reared in a farm of Central Italy (Tuscany). The ewes were kept indoors and routinely milked twice a day after lamb weaning (20th day of lactation).

After a period of adaptation, the ewes were fed a daily diet according to the requirements for dairy sheep that was consisted of mixed hay (1.7 kg), corn meal (0.4 kg), barley meal (0.2 kg) and soybean meal 44% (0.1 kg; Table 1).

During the sampling period, the data on the milk yield of each animal were collected and a sample of milk was taken, cooled at 4 °C and taken to the laboratory.

A total of 420 analyses were carried out on each milk sample for the following parameters:

- Fat, protein, lactose, dry matter by an infrared-based milk analyzer (Milko-Scan™, Italian Foss Electric, Padova, Italy).
- Somatic cell count (SCC) by the fluoro-opto-electronic method (Fossomatic 360, Padova, Italy).
- Milk fatty acid analysis after fat extraction according to Rose-Gottlieb[5] and metilation according to Christie[6]. The acid profile was analyzed by a Perkin Elmer Auto System gas chromatograph equipped with a flame ionization detector, a capillary column (60 m x 0.25 mm, Factor Four Varian) and the carrier gas was helium (He). The injection and the detector temperature was set at 270 °C and 300 °C, respectively. The oven was set as follows:
  - Level 1, 50 °C for 2 min; Level 2, from 50 to 180 °C at a rate of 2 °C min^{-1} and held for 20 min; Level 3, from 180 to 200 °C at a rate of 1 °C min^{-1} and held for 15 min; Level 4, from 200 to 220 °C at a rate of 1 °C min^{-1} and held for 30 min. A standard fatty acid mixture was used to calibrate and identify individual peaks according to their retention times.
- Milk Coagulation Properties: the analysis was performed with a Formagraph (Italian Foss Electric, Padova, Italy) as described by Martini et al.[7]. Briefly, the samples were analyzed at 35 °C using 0.2 ml of liquid rennet at 8% Hansen aqueous solution. The recorded parameters were as follows: rennet coagulation time (r) in minutes from adding the rennet to the beginning of coagulation; curd firming time (k20) in minutes required for the curd to be cut and the width of the diagram to be 20 mm; curd firmness (a30): curd strength (mm) measured at 30 minutes from adding the rennet.
- Milk fat globules: A direct method[4] was used to determine the diameter (µm) and the number of fat globules per mL of milk in each fresh sample stained with Acridine Orange by a fluorescence microscope (Leica Ortomat Microsystem, Milan, Italy) equipped with a camera (TiEsseLab, Milan, Italy) and TS view 2.0 image software.

Statistical analysis

Data on milk quality, fatty acid composition and coagulation properties underwent statistical analysis using a model for repeated measurements considering days in milk (DIM) as a fixed effect (<30, 30-50, 50-80, 80-110, 110-140, 140-170 DIM) and the individual as a random effect. The statistical analysis was carried out using JMP software[8].

RESULTS

The average daily milk yield per head was 1.30±0.500 litres / day. The lactation peak was detected between 30 and 50 days (Table 2), with an average yield of 2.14 litres per day, after which the milk yield declined consistently by up to 1.15 litres / per day. However, the ewes also maintained the high milk yield in last period monitored.

The average dry matter content was just over 15% (15.61 ± 1.150) and the average fat and protein were 5.10 ± 0.844 and 4.80 ± 0.641 respectively. After the peak of lactation, percentage of dry matter showed an increasing trend, mainly related to the higher fat and protein contents, whereas no significant changes were found in lactose levels (4.73 ± 1.198). Also the somatic cell count (SCC) did not change significantly.

As far as concern milk coagulation properties, k20 showed a tendency to decrease after the lactation peak and the curd firmness (a30) improved after 110 days.

Regarding milk fat quality (Table 3) the average diameter of the milk fat globules was 5.09 ± 1.205 µm and the maximum monounsaturated fatty acids (MUFA) values and the lowest saturated fatty acids (SFA) levels were recorded during the first 30 days of lactation. Moreover short chain fatty acids (SCFA) were significantly higher in the central phase of lactation, between 30 and 110 days, and medium chain fatty acids (MCFA), significantly increased in the last lactation phase (140-170

Table 1 - Chemical composition of feed in the sheep diet and estimation of nutritional values (forage unit for milk production) (MkFU).

		Mixed hay 1,700 g	Corn meal 400 g	Barley meal 200 g	Soybean meal 44% 100 g
DM	g	1518.10	344.00	172.00	88.00
Proteins	g on DM	122.96	35.70	19.95	42.50
Lipids		36.34	12.73	3.44	0.97
Fibre		531.30	8.84	8.77	7.39
Ash		121.44	4.47	3.96	6.28
NDF		994.29	35.70	29.93	13.46
ADF		639.08	11.90	11.01	9.59
Ca		10.63	0.03	0.10	0.30
P		2.36	1.05	0.71	0.65
MkFU	on DM	0.85	0.43	0.20	0.10

DM: dry matter; NDF: neutral detergent fiber; ADF: acid detergent fibre, MkFU: milk forage Units.

Table 2 - Daily milk yield, milk chemical composition, somatic cell count (SCC), and coagulation parameters of Assaf sheep milk during lactation.

		DIM						SEM
		<30	30-50	50-80	80-110	110-140	140-170	
Milk	litres	1.86AB	2.14A	1.17B	1.37B	1.03B	1.04B	0.329
DM	%	15.19AB	14.97B	15.96AB	15.24AB	16.22A	16.46A	0.892
Fat	%	5.02AB	4.65B	5.46AB	4.59B	5.67A	5.46AB	0.589
Protein	%	4.49b	4.75b	4.64b	4.73b	4.80b	5.37a	0.542
Lactose	%	4.79	4.77	4.74	4.74	4.72	4.65	0.197
SCC	Number of cells *1000	578.83	477.11	417.37	359.00	462.20	438.47	530.827
Log_{10} SCC		5.35	5.35	5.48	5.18	5.25	5.42	5.328
r	min	13.29A	13.18A	9.28B	9.29B	10.02AB	10.53AB	2.130
k20	min	2.1a	1.86a	1.80a	1.77a	1.54ab	1.37b	0.405
a30	mm	32.26AB	30.36AB	28.69B	27.52B	31.69AB	36.51A	4.503

DM: Dry matter; SCC: somatic cell count; r: rennet coagulation time; k20: curd firming time; a30: curd firmness.
A, B: $P \leq 0.01$
a, b: $P \leq 0.05$

Table 3 - Classes of fatty acids and fat globule characteristics in Assaf milk during lactation.

		DIM						SEM
		<30	30-50	50-80	80-110	110-140	140-170	
SCFA (≤C10)	%	13.21C	18.27A	17.67A	17.15A	13.43C	15.63B	1.410
MCFA (≥C11≤C17)	%	43.93C	51.73B	48.16B	51.10B	48.23B	55.71A	2.301
LCFA (≥C18)	%	42.85A	29.99D	34.17B	31.72C	37.35B	28.67D	2.617
SFA	%	67.91B	77.39A	74.95A	76.73A	74.52A	77.21A	2.131
MUFA	%	28.56A	18.57C	20.13BC	18.63C	21.01B	18.09C	1.965
PUFA	%	4.53	4.04	4.92	4.63	4.47	4.69	0.754
UFA/SFA		0.50A	0.29C	0.33BC	0.30C	0.34B	0.30C	0.040
n3/n6		0.17	0.23	0.16	0.23	0.26	0.24	0.076
Average Diameter	micron	5.29	4.96	5.66	5.11	4.63	4.93	1.082
Number of globules	number $*10^{10}$ per ml	0.88	1.03	0.93	0.73	1.19	0.83	0.477

A, D: $P \leq 0.01$
SCFA: short chain fatty acids; MCFA: medium chain fatty acids; LCFA: long chain fatty acids; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UFA: unsaturated fatty acids.

days), during which the lowest values of long chain fatty acids (LCFA) were also registered. At end of lactation, an increase in the saturated: unsaturated ratio was also recorded.

DISCUSSION

The average daily milk yield, fat and protein percentages were lower than those reported in other studies on the same breed reared in Spain[9], these differences may reflect changes in the production conditions.

Furthermore the average fat and protein percentages were in the ranges, reported by Milán et al.[10] for Assaf and Awassi crossbreed milk.

Milk yield was higher than the local Sarda and Massese breeds, which on average produce about one litre of milk per day[11,12].

The increasing percentage of dry matter after the peak of lactation was reported also in other studies[13], while relatively constant lactose content during lactation has also been found by Kuchtík et al.[14].

The average log_{10} SCC (5.30 ± 0.551) was similar to the findings of Milán et al.[12]. Although there is currently no EU Regulation on SCC limits in sheep milk, the values ≤ 1000 x 10^3 cells / ml are widely considered as acceptable in milk for cheesemaking[15]. However, SCC > 500 x 10^3 cells / ml are generally considered as being associated with a decrease in milk yield.

Milk coagulation properties are important technological parameters as they influence cheese manufacturing, yields and ripening[16]. Coagulation properties vary with the breed in relation to the physical and chemical characteristics of their milk, especially linked to total protein and casein content[7,17].

The length of r after the peak of lactation was similar to the

mean value reported by Martins et al.[17] who reported lower milk clotting properties for the milk of Assaf sheep when compared with Portuguese traditional breeds.

In contrast, k20 was generally lower than that reported for the same breed[17]. Curd firmness (a30) was greater at the end of lactation (140-170 DIM) compared to the central phase (80-110 and 110-140 DIM) corresponding to the increasing percentage of dry matter and protein. The increment of these components could be partially explained by the decrease in milk yield that normally occurs at this stage in the lactation curve. In the milk of extensively reared Massese sheep higher values of a30 have been reported by Pugliese et al.[18], these authors found in general a better cheese-making aptitude during the first 2-3 months postpartum, and a worsening thereafter.

Sheep cheese is enjoyed in terms of its taste and flavor, however, it is commonly considered as potentially negative for human health because of its high SFA content.

In general, a more favorable fatty acid profile for human health would present less SFA and more MUFA and polyunsaturated fatty acids (PUFA). The MUFA content in the milk analysed was similar to the findings reported for the same breed by Dervishi et al., 2015[9] but the average of SFA were higher, whereas PUFA were lower.

The trend of MUFA and SFA indicates a better nutritional quality of the milk at the beginning of lactation, which was also found by Martini et al. in sheep[21].

Nevertheless, for nutritional effects on human health as a whole, the overall FA profile should be considered[3]. In fact, although they are saturated, SCFA have been shown to have positive effects on human health. Conversely, some MCFA are known for their hypercholesterolemic action[19].

In addition, the higher SCFA in the central phase of lactation, is probably linked to an increase in endogenous fat synthesis as observed and hypothesised by Lock and Bauman[20] in cows during the intermediate stages of lactation.

At the end of lactation, the increase in SFA and MCFA, the decrease in MUFA, and in the saturated: unsaturated ratio, indicated a deterioration in milk nutritional quality.

An universally recommended omega 3/ omega 6 ratio for a healthy diet has not been set by the scientific community, furthermore the intake of omega 6 in western diets is excessive[21]. Ruminant milk is not a rich source of omega 3, but in general, dairy sheep that predominantly graze have higher omega 3 concentrations in milk. A higher quality and availability of grass, the introduction of flax, *Carthamus tinctorius* and sunflower, *Helianthus annuus*[22] or fish oil[23] in the sheep's diet can improve omega 3 concentrations in milk fat.

The milk of Assaf sheep had milk fat globules of a larger diameter compared to the literature on Italian sheep breeds[24], in line with literature reports that breed is a genetic variability factor that affects the size and number of globules[4].

CONCLUSIONS

Our work has confirmed the high productivity of the Assaf. The nutritional quality of its milk fat is better at the beginning of lactation when less saturated, and more monounsaturated fatty acids are found in the milk. Furthermore, higher protein and better coagulation properties were found at the end of lactation.

References

1. Annuario Statistico Italiano (2014) Capitolo 13. In: Agricoltura Istat, Ed. Istituto nazionale di statistica, Roma ISBN.
2. Pollott G.E., Gootwine E. (2004) Reproductive performance and milk production of Assaf sheep in an intensive management system. J Dairy Sci, 87: 3690-3703.
3. Carta A., Casu S., Salaris S. (2009) Current state of genetic improvement in dairy sheep. J Dairy Sci, 92: 5814-5833.
4. Martini M., Salari F., Altomonte I. (2016) The Macrostructure of Milk Lipids: The Fat Globules, Crit Rev Food Sci Nutr, 56: 1209-1221.
5. A.O.A.C. (1996) Official Methods of Analysis. Association of Official Agricultural Chemists. Washington, D.C., Chapter 33.
6. Christie W.W. (Ed.) (1982) Lipid analysis. Chromatografic and spectroscopic analysis of lipids: general principles. In: Oxford: Pergamon Press. 3: 25-49.
7. Martini M., Mele M., Scolozzi C., Salari F. (2008) Cheese making aptitude and the chemical and nutritional characteristics of milk from Massese ewes. Ital J Anim Sci, 7: 419-437.
8. J.M.P. (2002) User's guide ver.5.0, S.A.S. Institute Inc., Cary (NC), U.S.A.
9. Dervishi E., Serrano M., Joy M., Sarto P., Somera A., González-Calvo L., Berzal-Herranz B., Molino F., Martinez-Royo A., Calvo J.H. (2015) Structural characterisation of the acyl CoA: diacylglycerol acyltransferase 1 (DGAT1) gene and association studies with milk traits in Assaf sheep breed. Small Rumi Res, 131: 78-84.
10. Milán M.J., Caja G., González-González R., Fernández-Pérez A.M., Such X. (2011) Structure and performance of Awassi and Assaf dairy sheep farms in northwestern Spain. J Dairy Sci, 94: 771-784.
11. Nudda A., Mele M., Battacone G., Usai M.G., Macciotta N.P.P. (2003) Comparison of conjugated linoleic acid (CLA) content in milk of ewes and goats with the same dietary regimen. It J Anim Sci, 2 (1S): 515-517.
12. Martini M., Liponi G.B., Salari F. (2010) Effect of forage: concentrate ratio on the quality of ewe's milk, especially on milk fat globules characteristics and fatty acids composition. J Dairy Res, 77: 239-244.
13. Oravcova M., Margetín M., Tan in V. (2015). Daily milk yield, and milk fat and protein content, Mljekarstvo 65, 48-56.
14. Kuchtík J., Šustová K., Urban T., Zapletal D. (2008) Effect of the stage of lactation on milk composition, its properties and the quality of rennet curdling in East Friesian ewes. Czech J Anim. Sci, 53: 55-63.
15. Riggio V., Maizon D.O., Portolano B., Bovenhuis H., van Arendonk J.A.M. (2009) Effect of somatic cell count level on functional longevity in Valle del Belice dairy sheep assessed using survival analysis. J Dairy Sci, 92: 6160-6166.
16. Bencini R. (2002). Factors affecting the clotting properties of sheep milk. Journal of the Science of Food and Agriculture. 85, 705-719.
17. Martins A.P.L., Belo A.T., Vasconcelos M.M., Fontes A.L., Pereira E.A., Belo C. (2009) Characterisation of production system of Niza cheese (PDO): effect of sheep breed on milk composition and coagulation properties. In: Pacheco F., Ed., Morand-Fehr P. Ed. Changes in sheep and goat farming systems at the beginning of the 21st century: research, tools, methods and initiatives in favour of a sustainable development Zaragoza: CIHEAM / DRAP-Norte / FAO Options Méditerranéennes: Série A. Séminaires Méditerranéens; 91: 221-225.
18. Pugliese C., Acciaioli A., Rapaccini S., Parisi G., Franci O. (1999) Evolution of chemical composition, somatic cell count and renneting properties of the milk of Massese ewes. Small Rumin Res, 35: 71-80.
19. Fattore E., Fanelli R. (2013) Palm oil and palmitic acid: a review on cardiovascular effects and carcinogenicity. Int J Food Sci Nutr, 64: 648-659.
20. Lock A.L., Bauman D.E. (2004) Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. Lipids, 39: 1197-1206.
21. A.P. Simopoulos (2006) Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases. Biomed Pharmacother, 60: 502-507.
22. Nudda A., Pulina G. (2014) Influenza dell'alimentazione sul miglioramento delle caratteristiche nutraceutiche dei prodotti ovini (latte, carne). Large Anim Rev, 4 (1S): 20-22.
23. Kitessa S.M., Peake D., Bencini R., Williams A.J. (2003). Fish oil metabolism in ruminants. III. Transfer of omega-3 polyunsaturated fatty acids (PUFA) from tuna oil into sheep's milk. Animal Feed Science & Technology. 108, 1-14.
24. Martini M., Salari F., Scolozzi C., Bianchi L., Pauselli M., Rossetti E., Verità P. (2006) Morphometric characteristics of sheep milk fat globules (part I): influence of genetic type and phase of lactation. In: 14 Congreso International de la Federacòn Mediterrànea da Sanidad y Producciòn de Ruminantes. Lugo, Santiago de Compostela, Espana. 667-671.