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Effects of olive oil calcium soaps and phase of lactation on the fatty acid composition in the milk of Massese ewes

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ABSTRACT

A basic diet of hay and concentrate, the latter supplemented with olive oil calcium soap (7% as fed) was given to 11 Massese ewes (group S) for a 3-month period, while a basic diet without the supplement was given to 11 control Massese ewes (group C) during the same period. Fatty acid composition of milk produced from the 5th to the 16th week post-partum was evaluated monthly.

In group S, the fatty acids C6:0, C8:0, C10:0, C12:0, C14:0, C14:1, C18:3n-6 and C20:3n-3 (P<0.01) decreased significantly and C16:0, C18:1, total CLA, C20:0, C20:1, C20:2, C20:5n-3 (EPA), C23:0 (P<0.01) and C18:2 (P<0.05) increased with respect to the control group C. This trend lead to a decrease in short-chain fatty acids (P<0.05), an increase in unsaturated fatty acids (P<0.01), particularly MUFA, and an improved UFA/SFA ratio in group S (P<0.01). The lactation phase also significantly influenced fatty acid composition: the unsaturated/saturated fatty acids ratio was higher at the end of the lactation period.

It can be concluded that the addition of protected olive oil fats to the diet of lactating ewes may improve fatty acid milk composition. In addition, the final phase of production positively affects milk quality relative to human health.

Key words: Ewe's milk, Olive oil calcium soap, Phase of lactation, Fatty acids.

RIASSUNTO

EFFETTO DEI SAPONI DI CALCIO DELL'OLIO DI OLIVA E DELLA FASE DI LATTAZIONE SULLA COMPOSIZIONE ACIDICA DEL LATTE DI PECORE MASSESI

Undici pecore di razza Massese sono state alimentate per tre mesi con una dieta a base di fieno e concentrato con aggiunta di saponi di calcio dell'olio d'oliva (7% sul t.q.)(gruppo S). La stessa dieta di base, senza il supplemento, è stata fornita per lo stesso periodo ad altre 11 pecore Massesi di controllo (gruppo C). La composizione acidica del latte prodotto dalla 5° alla 16° settimana dopo il parto è stata valutata mensilmente.

Nel latte del gruppo S sono diminuiti significativamente gli acidi grassi C6:0, C8:0, C10:0, C12:0, C14:0, C14:1, C18:3n-6 e C20:3n-3 (P<0,01) ed aumentati il C16:0, il C18:1, il CLA totale, il C20:0, il C20:1, il C20:2, il C20:5n-3 (EPA), il C23:0 (P<0,01) ed il C18:2 (P<0,05) rispetto al gruppo C: questo andamento ha determinato una diminuzione degli acidi grassi a corta catena (P<0,05), un aumento degli acidi grassi insaturi, specialmente dei MUFA (P<0,01) ed un miglioramento del rapporto acidi grassi INS/SAT nel gruppo S (P<0,01). Anche il periodo di lattazione ha influito significativamente sulla composizione acidica, portando ad un più alto valore il rapporto tra acidi grassi INS/SAT, alla fine della lattazione.

Si può concludere che l'utilizzazione di olii protetti nella dieta di pecore in lattazione può modificare positivamente la composizione acidica del latte; inoltre la fase finale della produzione influisce positivamente sulla qualità del latte relativamente alla salute umana.

Key words: *Latte ovino, Saponi di calcio dell'olio di oliva, Fase di lattazione, Acidi grassi.*

Introduction

The nutritional quality of animal products is a very important parameter, especially with regard to the links between food and health, a vital research field today. Modifying feeding practices for dairy animals aims to increase the amount of unsaturated fatty acids in milk fat. The international medical-scientific world now holds saturated fatty acids partially responsible for some diseases, especially those of a cardio-vascular nature (Ney, 1991, Trevisi *et al.*, 1992; Strata, 2000) and ewe's milk is particularly rich in these fatty acids.

Some important milk fatty acids, C20:5 n-3 (EPA), C22:6 n-3 (DHA), geometric and positional isomers of C18:2 (CLA), - supplies of which are limited in the human diet (Galli, 1999) - and the increase in the unsaturated/saturated fatty acids ratio have provoked considerable interest (O'Donnel, 1989; Grummer, 1991; Bertoni and Trevisi, 1999). They have been tested regarding the use of animal nutritional strategies as possible agents in human preventive medicine.

Although several Authors have shown that fat supplement to the diet of ruminants affect milk yield and composition (Storry, 1970; Storry *et al.*, 1980; Banks *et al.*, 1990; Palmquist *et al.*, 1993; Perez Alba *et al.*, 1997; Rotunno *et al.*, 1998; Antongiovanni *et al.*, 2002; Chilliard *et al.*, 2002), contradictory results still exist due to the complexity of factors involved in the synthesis and control of fatty acids in milk.

Few data are available on the quality of fatty acids in ewe's milk. The aim of this study was to con-

Table 1. Composition of concentrate (% as fed).

	Group C	Group S
Corn grain	34	34
Soybean meal solv. ext.(44% CP)	17	17
Wheat middlings (4.5% CF)	4.65	4.65
Oat	14	14
Sunflower seed meal (28% CP)	13	13
Soft wheat coarse bran	6.50	6.50
Limestone	1.85	1.85
Calcium diphosphate	0.65	0.65
Common salt	0.65	0.65
Sodium bicarbonate	0.20	0.20
Mineral vitamine premix	0.50	0.50
Carob fruit	4	-
Lignum bisulfite	1.50	-
Bentonite	1.50	-
Liposal ®	-	7

Group C: Control; Group S: Experimental.
CP: crude protein; CF: crude fiber.

Table 2. Chemical composition of concentrate and hay (% on DM basis).

	Group C	Group S	Alfalfa hay
Dry matter (DM)	91.07	91.89	92.64
Crude protein	20.40	19.37	17.96
Ether extract	2.69	4.19	3.63
Crude fibre	7.85	7.71	38.08
N-Free Extract	61.26	62.28	33.94
Ash	7.80	6.45	6.39
NDF	17.23	17.1	51.15
ADF	11	10.86	46.10
Cellulose	7.86	8.30	33.62
Hemicellulose	6.23	6.24	5.05
Lignin	2.49	1.78	11.93

tribute to knowledge of the fatty acid composition of ewe's milk when animals are fed a supplement of olive oil calcium soap (Liposal ®). The influence of the lactation phase on milk was also considered.

Material and methods

Test project

The trial was carried out on individual milk from twenty-two multiparous Massese ewes that had experienced at least three lambings. All the ewes delivered during the same week and participated in the study once lambs were weaned (about 30 days post-partum).

Ewes were subdivided into two homogeneous groups of 11 subjects each and subjected to the following treatments: control group (C) (with a live weight of 57.18 ± 9.60 kg and a milk yield of 0.91 ± 0.42 kg, respectively) was fed a diet based on alfalfa hay given *ad libitum* plus 1.5 kg of concentrates per day, while the experimental group (S) (with a live weight of 60.91 ± 10.60 kg and a milk yield of 1.18 ± 0.36 kg, respectively) was supplemented with 7% (as fed) of olive oil calcium soap (Liposal ®).

The analytical composition of the concentrates (Table 1), the chemical composition of the diets (Table 2) and the fatty acid profile of the concentrates (Table 3) have also been reported.

The chemical composition of the diet was determined according to AOAC methods (1984) and to the procedures described by Goering and Van Soest (1970).

After a 10-day adaptation period to the diets, milk samples were collected during the morning milking, every four weeks from the 5th to the 16th week post-partum and subjected to fatty acid analysis.

Chemical analysis of the milk

The chemical and rheological compositions of milk have been previously reported (Martini *et al.*, 2000).

Table 3. Fatty acid composition of the concentrate (%).

Fatty acids	Diet C	Diet S
C8:0		0.058
C10:0	0.093	0.163
C12:0	1.034	3.208
C14:0	1.456	2.387
C16:0	23.632	22.981
C16:1	0.301	1.721
C18:0	8.080	11.019
C18:1	28.969	34.207
C18:2	33.668	21.354
C18:3	1.624	1.601
other	0.290	1.227

Least detectable value 0.05 %

Table 4. Fatty acid composition of milk (%): diet effect.

	Group C		Group S	
Samples number	11		11	
Fatty acids	mean	SE	mean	SE
C4:0	4.08	0.569	4.80	0.335
C6:0	3.94[B]	0.191	3.23[A]	0.163
C8:0	4.52[B]	0.144	2.96[A]	0.148
C10:0	12.28[B]	0.325	7.57[A]	0.320
C12:0	8.77[B]	0.275	6.06[A]	0.215
C14:0	14.70[b]	0.390	13.39[a]	0.340
C14:1	0.36[B]	0.013	0.23[A]	0.014
C15:0	1.19	0.045	1.07	0.056
C16:0	25.82[A]	1.261	30.23[B]	0.905
C16:1	2.04	0.414	2.19	0.191
C18:0	3.45	0.277	4.31	0.534
C18:1 (cis and trans)	12.53[A]	0.451	16.45[B]	0.801
C18:2n-6	3.59[a]	0.145	4.10[b]	0.168
C18:3n-6	0.13[B]	0.011	0.08[A]	0.008
C18:3n-3	0.67	0.034	0.71	0.044
Total CLA	0.73[A]	0.036	1.07[B]	0.046
C20:0	0.22[A]	0.016	0.40[B]	0.019
C20:1	0.08[A]	0.010	0.20[B]	0.014
C20:2	0.06[A]	0.011	0.08[B]	0.006
C20:3	0.13	0.014	0.17	0.012
C20:3n-3	0.26[B]	0.021	0.19[A]	0.009
C20:4n-6	0.00	0.003	0.00	0.002
C20:5n-3 (EPA)	0.12[A]	0.010	0.16[B]	0.011
C22:3n-6	0.02	0.005	0.02	0.006
C23:0	0.04[A]	0.005	0.07[B]	0.006
C24:0	0.15	0.011	0.14	0.011
C22:6n-3 (DHA)	0.07	0.007	0.09	0.009

Different letters on the same row correspond to significant differences (Capital letters: P<0.01; lower case letters: P<0.05).

The acidic composition of the milk was determined using a Perkin Elmer Auto System gas chromatography fitted with Omega Wax 320 capillary column, FID type detector, and helium as gas carrier, according to Secchiari *et al.*, 2001.

A mixture of fatty acids standards (Sigma-Aldrich) was used for the calibration and identification of single peaks according to the relative retention times.

Since the gas chromatographic analysis with a 30-m length column did not allow the separation of *cis* and *trans* isomers, the sum of positional and geometrical isomers of C18:1 and C18:2 (CLA) was evaluated.

Statistical analysis

The results were analyzed by means of the following mathematical model:

$y_{ijk} = \mu + \alpha_i + \delta_{ij} + \beta_k + (\alpha\beta)_{ik} + \varepsilon_{ijk}$

μ = general average;

Table 5. Fatty acids in milk (%) by group of fatty acids: diet effect.

	Group C		Group S	
Samples number	11		11	
FA groups	mean	SE	mean	SE
SCFA	27.59[b]	1.181	21.96[a]	1.203
MCFA	49.87	1.365	50.61	1.449
LCFA	22.54	0.807	27.42	0.793
MUFA	15.01[A]	0.670	19.07[B]	0.810
PUFA	5.80[A]	0.200	6.68[B]	0.245
SFA	79.18[B]	0.756	74.25[A]	0.710
UFA	20.81[A]	0,756	25.75[B]	0.710
UFA/SFA	0.27[A]	0.013	0.35[B]	0.013

FA: fatty acids; SCFA: short-chain FA (C4-C10); MCFA: medium-chain FA (C12-C16); LCFA: long-chain FA (C>18); MUFA: monounsaturated FA; PUFA: polyunsaturated FA; SFA: saturated FA (C4-C24); UFA: unsaturated FA (C14:1-C24:1). Differents letters on the same row correspond to significant difference (Capital letters: P<0.01; lower case letters: P<0.05).

α_i = effect of the i-th treatment (i = 1,…2);

δ_{ij} = effect of the subject j within the treatment group;

β_k = effect of the k-th sampling time (k = 1,....4);

$(\alpha\beta)_{ik}$ = effect of the interaction;

ε_{ijk} = casual residual effect.

Statistical analysis has been carried out with JMP software, vers. 3.1.6.2., S.A.S. Institute (1996).

Results and discussion

The treatment-sampling time interaction was not statistically significant.

Dietary influence

In accordance with previous research carried out on both sheep and cattle by other Authors (Schauff e Clark, 1992; Schauff *et al.*, 1992; Salimei *et al.*, 1992; Chilliard *et al.*, 1993; Palmquist *et al.*,1993; Perez Alba *et al.*, 1997; Rotunno *et al.*, 1998), diet S led to a statistically significant drop in fatty acids from C6:0 to C14:1 (Table 4); on the other hand, although palmitic acid (C16:0) resulted higher (P<0.01) in group S, according to Rotunno *et al.* (1998) and compared with similar tests on sheep by other researchers that used protective fat supplements (Grummer, 1991; Schauff *et al.*, 1992; Chillard *et al.*, 1993; Todaro *et al.*, 1997), this did not occur in the study of Antongiovanni *et al.* (2002).

Stearic acid (C18:0) appeared to be higher in group S; C18:1, with a preferential uptake from the mammary gland (Grummer, 1991) and the C18:2 resulted statistically higher in group S, in accordance with similar trials carried out in ruminants (Shauff *et al.*, 1992; Trevisi *et al.*, 1992; Perez Alba *et al.*, 1997). On the other hand in group S, a statistically significant decrease was recorded in the C18:3 n-6 (GLA) and C20:3 n-3 (P≤0.01).

Total CLA (geometric and positional isomers of C18:2) percentage was statistically higher in group S (P≤0.01); the same trend was observed for the C20:0, C20:1, C20:2 and C20:5n-3 (EPA).

The decrease in the C6:0, C8:0 and C10:0 in group S is not positive because these are considered hypocholesterolemic fatty acids by Jensen *et al.* (1990), Grummer, (1991) and Ney (1991); on the contrary, a drop in the C12:0 and C14:0 is positive

Table 6. Fatty acid composition of milk (%): effect of stage of lactation.

	Sampling 1		Sampling 2		Sampling 3		Sampling 4	
Samples number	22		22		22		22	
Fatty acids	average	SE	average	SE	average	SE	average	SE
C4:0	4.61	0.353	5.09	1.089	4.03	0.313	4.04	0.544
C6:0	3.86	0.215	3.78	0.354	3.27	0.185	3.37	0.252
C8:0	3.99	0.268	4.01	0.265	3.45	0.278	3.39	0.252
C10:0	9.84	0.561	10.02	0.631	9.46	0.668	10.19	0.941
C12:0	7.49	0.451	7.69	0.433	7.56	0.453	6.73	0.501
C14:0	13.34[a]	0.508	13.87	0.521	15.27[b]	0.474	13.62[ac]	0.562
C14:1	0.33	0.028	0.28	0.024	0.29	0.024	0.27	0.015
C15:0	1.29[B]	0.084	1.20[B]	0.063	1.20[B]	0.042	0.78[A]	0.033
C16:0	27.37	1.284	29.07	2.175	29.31	1.418	26.42	1.380
C16:1	2.08	0.382	1.61	0.106	1.95	0.110	2,90	0.842
C18:0	5.10[BCb]	0.730	3.56[ABa]	0.739	2.54[A]	0.371	4.43[B]	0.332
C18:1 (cis and trans)	13.45[A]	0.836	12.44[Aa]	0.705	14.15[Ab]	0.587	18.49[B]	1.400
C18:2n-6	4.13[B]	0.240	4.15[B]	0.219	4.11B	0.197	2.94[A]	0.127
C18:3n-6	0.14[B]	0.016	0.09[A]	0.015	0.09[A]	0.015	0.10	0.011
C18:3n-3	0.73[B]	0.048	0.84[B]	0.053	0.74[B]	0.048	0.43[A]	0.023
Total CLA	0.83[B]	0.071	0.89[B]	0.043	1.09[A]	0.086	0.80[B]	0.057
C20:0	0.34[B]	0.028	0.35[B]	0.027	0.36[B]	0.035	0.19[A]	0.021
C20:1	0.14	0.025	0.12	0.018	0.14	0.025	0.17	0.017
C20:2	0.07[a]	0.015	0.06	0.010	0.05[A]	0.007	0.10[Bb]	0.014
C20:3	0.17	0.015	0.18	0.026	0.13	0.010	0.13	0.019
C20:3n-3	0.19[A]	0.011	0.27[BCa]	0.036	0.27[Ca]	0.019	0.19[ABb]	0.010
C20:5n-3 (EPA)	0.17[B]	0.019	0.15	0.016	0.14	0.012	0.11[A]	0.007
C22:3n-6	0.01[A]	0.006	---	0.001	0.05[B]	0.009	---	0.000
C23:0	0.06	0.010	0.06	0.009	0.06	0.006	0.05	0.011
C24:0	0.17[B]	0.014	0.15[B]	0.015	0.17[B]	0.013	0.08[A]	0.012
C22:6n-3 (DHA)	0.08[Aab]	0.011	0.07[ABb]	0.008	0.12[Bc]	0.013	0.05[Aa]	0.008

Different letters on the same row correspond to significant differences (Capital letters: P<0.01; lower case letters: P<0.05).

because they are hypercholesterolemic fatty acids (Jensen *et al.*, 1990; Grummer, 1991; Ney, 1991; Trevisi *et al.*, 1992). Palmitic acid content (C16:0), higher in group S, is less cholesterolemic than lauric (C12:0) plus myristic acid (C14:0) (Ney, 1991). A positive effect is caused by the greater percentage of the C18:1 and C18:2 in group S, since they reduce the cholesterol level in the blood and contribute to the prevention of cardiovascular diseases (Jensen *et al.*, 1990; Hornstra, 1999).

The drop in C18:3 n-6 (GLA) in group S may be considered a beneficial factor for human health (Besler and Grimble 1993 a,b; Galli, 1999; Zambon *et al.*, 1999).

Bauman *et al.* (2001), Chilliard *et al.* (2001) and Kelsey *et al.* (2003) have shown that diet affects milk CLA; in our research as well, total conjugated linoleic acid (CLA) was statistically higher ($P \leq 0.01$) in the experimental diet. Recently many researchers have focused their attention on

Table 7. Groups of fatty acids in milk (%): effect of stage of lactation.

	Sampling 1		Sampling 2		Sampling 3		Sampling 4	
Samples number	22		22		22		22	
FA groups	average	SE	average	SE	average	SE	average	SE
SCFA	22.30^{A}	0.856	22.90^{A}	1.584	20.21^{A}	1.113	34.27^{B}	1.708
MCFA	51.91^{B}	1.205	53.71^{B}	1.978	55.59^{B}	1.253	39.21^{A}	1.033
LCFA	25.79	1.078	23.39	1.205	24.20	0.947	26.52	1.658
MUFA	16.01^{Aab}	0.844	14.44^{Aa}	0.774	16.53^{Ab}	0.632	21.83^{B}	1.571
PUFA	6.52^{B}	0.337	6.71^{B}	0.286	6.79^{B}	0.308	4.85^{A}	0.179
SFA	77.47	0.863	78.84^{B}	0.902	76.68^{b}	0.814	73.31^{Aa}	1.709
UFA	22.53	0.863	21.16^{A}	0.902	23.32^{a}	0.814	26.68^{Bb}	1.709
UFA/SFA	0.29^{A}	0.013	0.27^{A}	0.014	0.31	0.014	0.38^{B}	0.032

FA: fatty acids; SCFA: short-chain FA (C4-C10); MCFA: medium-chain FA (C12-C16); LCFA: long-chain FA (C≥ 18); MUFA, monounsaturated FA; PUFA: polyunsaturated FA; SFA, saturated FA (C4-C24); UFA: unsaturated FA (C14:1-C24:1). Different letters on the same row correspond to significant differences (Capital letters: P<0.01; lower case letters: P<0.05).

diene linoleic acid, as it has shown important nutritional and health properties (Pariza *et al.*, 1985; Ha *et al.*, 1990; Shulz *et al.*, 1992 a,b; Lee *et al.*, 1994; Belury, 1995; Parodi, 1996; Banni *et al.*, 1999; Parodi, 1999; Secchiari *et al.*, 2003). Despite its low concentration, the significant increase (P<0,01) in group S in eicosapentadienoic acid (EPA) exerts a positive effect on human health as a result of its anti-inflammatory and antitumoral action (Galli, 1999).

Olive oil soaps supplementation determined a statistical decrease in short chain fatty acids in group S (P<0.05; Table 5) and a slight increase in long chain ones, in accordance with the results of other Authors (Pulina *et al.*, 1990; Shauff *et al.*, 1992; Shauff and Clark, 1992; Sklan, 1992; Rotunno *et al.*, 1998). The increase (P<0.01) in unsaturated fatty acids and particularly of MUFA in milk from group S has potential benefits for human health (Secchiari *et al.*, 2003). The dietary addition of Ca soap statistically decreased the total saturated fatty acids (SFA) content in milk, while the unsaturated (UFA) increased (P<0.01), as observed by Rotunno *et al.* (1998) and Antongiovanni *et al.* (2002). Hence, the unsaturated/saturated fatty acids ratio significantly increased, thus contributing to the prevention of cardiovascular diseases (Muscio and Cianci, 1992).

Influence of the phase of lactation

Few data regarding the influence of the lactation phase on the fatty acid composition of ruminant milk are available (Jensen *et al.*, 1990; Grummer, 1991; Palmquist *et al.*, 1993; Casoli *et al.*, 1994; Martini *et al.*, 1998).

The acidic composition of milk in relation to the phase of lactation is shown in Table 6.

Among fatty acids with statistically significant differences, C18:1 showed an increase during the last three periods observed, while C20:5n-3 (EPA) decreased between the first and the last period. The C14:0, the total CLA, the C20:3n-3, the C22:3n-6, and the C22:6n-3 (DHA) increased at the third sampling; an opposite trend was noted for the C18:0. At the final sampling, a decrease in C15:0, the C18:2, the C18:3 n-3, C20:0 and C24:0 was observed, while the C20:2 increased.

The fatty acids classified by group are shown in Table 7. Short chain fatty acids increased significantly (P<0.01) at the end of lactation, with a detrimental effect (P<0.01) on the medium-chain fatty acids. At the fourth sampling the ratio UFA/SFA was also higher (P<0.01) and the MUFA increased to the detriment of the PUFA (P<0.01): this trend is desirable since it reduces the possibility of fatty acid oxidation (Ulbricht and Soutgate, 1991).

Conclusions

In conclusion, we can confirm that for dairy ewes, feeding practices may by used to modify the fatty acid profile of milk. In particular, the inclusion of olive oil calcium soap in the diet of ewes improves the unsaturated/saturated ratio (as recommended by the WHO for human health), as well as total CLA and EPA contents. Also, the phase of lactation significantly and positively influences milk fatty acid composition, favouring the improvement of the UFA/SFA ratio at the end of lactation.

Considering that ewe's milk is used for cheese making, we intend to carry out further research in order to evaluate possible modifications of fatty acid composition of cheese compared with that of milk.

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