Olive oil calcium soaps and rumen protected methionine in the diet of lactating ewes: effect on milk quality

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ABSTRACT

Eight Massese ewes were fed 4 diets with alfalfa hay as the forage (73% on the DM basis): 1) control diet (C); 2) diet C supplemented with olive oil calcium soaps, 50 g/d (L); 3) diet C supplemented with protected methionine, 5 g/d (M) or 4) plus both soaps and methionine (ML); the experimenthal design was a 4x4 Latin square with 2 replicates per diet. During the experimental periods, lasting one week each, the ewes were milked twice daily (8:00 a.m. and 7:00 p.m.). Milk yield was not affected by diet quality, but milk fat percentage and 6.5% fat corrected milk yield were higher in diets L, M and ML with respect to diet C ($P<0.05$). Milk protein content was depressed and blood urea increased following the Ca soap diet alone or with protected methionine. Diet M worsened ($P<0.05$) Rennet clotting time (r) and curd firmness after 30 minutes (A_{30}). Saturated fatty acids $C_{10:0}$, $C_{12:0}$, $C_{14:0}$ and $C_{16:0}$ were depressed in milk fat with the Ca soap supplemented diet, some of them significantly. $C_{18:1}$ increased ($P<0.05$) with diet L only, whereas the association of Ca salts and methionine in diet ML significantly affected the linoleic acid and CLA content.
It is concluded that the use of olive oil fatty acids as a protected fat source seems to improve the milk fatty acid characteristics towards a safer pattern, but the presence of this type of Ca salts in the diet appears to worsen the metabolic utilisation of amino acids.

Key words: Ewe milk, Olive oil calcium salts, Protected methionine, Fatty acids.

RIASSUNTO

SAPONI DI CALCIO DELL'OLIO D'OLIVA E METIONINA RUMINO-PROTETTA NELLA DIETA DI PECORE IN LATTAZIONE: EFFETTI SULLA QUALITÀ DEL LATTE

Otto pecore di razza Massese sono state alimentate, utilizzando un disegno sperimentale a quadrato latino 4x4 con due replicazioni per cella, con quattro diete a base di fieno di erba medica (73% della s.s.) e mangime concentrato: 1) dieta controllo (C); 2) dieta C integrata con 50 g/d di saponi di calcio dell'olio d'oliva (L); 3) dieta C integrata con 5 g/d di metionina protetta (M); 4) dieta C integrata con 50 g/d di saponi di calcio dell'olio d'oliva e 5 g/d di metionina protetta (ML). La produzione di latte non è stata influenzata dalla dieta, mentre la produzione corretta al 6.5% di grasso e la percentuale di grasso del latte sono aumentate in tutti i casi rispetto alla dieta C, a prescindere dal tipo di integrazione ($P<0.05$). La percentuale di proteina è diminuita nel caso dell'integrazione con saponi di calcio sia somministrati da soli sia insieme alla metionina protetta; in concomitanza si è osservato un aumento del contenuto di urea nel sangue. I parametri relativi al tempo di coagulazione e alla consistenza della cagliata dopo 30 minuti sono peggiorati nel caso della som-

ministrazione con metionina protetta. Gli acidi grassi saturi a media catena hanno subito una diminuzione in conseguenza dell'aggiunta di sali di calcio alla dieta. Gli acidi octadecenoici sono aumentati (P<0.05) con la somministrazione della dieta L, mentre l'acido linoleico e i CLA sono aumentati significativamente con la dieta ML. In conclusione, l'uso di sali di calcio dell'olio d'oliva come fonte grassata nell'alimentazione delle pecore da latte può migliorare la frazione acidica del grasso del latte verso una composizione più salubre, tuttavia sembra influenzare negativamente l'utilizzazione metabolica degli aminoacidi.

Parole chiave: *Pecore da latte, Saponi di calcio dell'olio d'oliva, Metionina protetta, Acidi grassi.*

Introduction

Fats may be added to the diet of lactating ruminants in order to meet the high energy requirements of animals during the critical phase of early lactation, to increase milk fat content and to change the fatty acid profile of milk fat (Palmquist and Jenkins, 1980). However, the achievement of these goals is affected by several factors such as i) fat digestibility, ii) change in feed intake, digestibility and utilisation of the rest of the feed components as a consequence of the added fat and iii) type of fat. Unprotected fats have negative effects on the metabolism of cellulolytic bacteria involved in fiber digestibility and, thus, decrease milk fat percentage and yield as fat intake increases (Jenkins, 1993). Moreover, the fiber digestibility is usually lower when the added fat is rich in polyunsaturated fatty acids (PUFA).

Increasing the amount of fat supply to 5-6% of total DM results in higher milk fat content (Palmquist e Jenkins, 1980). Diets including a higher amount of fat, especially if rich in PUFA, and poor in fiber result in lower milk fat, as a result of the negative effects i) on the metabolism of cellulolytic bacteria involved in fiber digestibility; ii) on the mammary synthesis of short chain fatty acids, and iii) on mammary uptake of blood lipids (Palmquist, 1994). Technological treatments may be effective in protecting some unsaturated dietary lipids, in order to avoid the above-mentioned undesirable negative effects on fiber digestibility and to obtain a milk fatty acid composition more recommendable for human health (Ney, 1991; Havel, 1997). Calcium salts (CS) should protect fatty acids against ruminal biohydrogenation; however, contrasting results have been reported according to the different degree of unsaturation of the fatty acids (Ferlay *et al.*, 1992). The resistance of calcium soaps to dissociation and biohydrogenation may also depend, in fact, on the degree of fatty acid unsaturation and thus, on the degree of protection (Chilliard *et al.*, 1993). The supplementation with calcium soaps increased milk fat content, altered milk fatty acid composition, and lowered total nitrogen content in sheep (Rossi *et al.*, 1991; Casals *et al.*, 1999). Rumen protected amino acids are recommended to better balance energy and nitrogen in the metabolism of animals, to improve both milk fat and long chain fatty acid (LCFA) content and to decrease the unsaturated/saturated fatty acid ratio (Kalscheuer *et al.*, 1997; Rotunno *et al.,* 1998; Santos *et al.*, 1998).

The aim of this work was to study the effect of supplementing the diet of lactating ewes with calcium salts from a highly unsaturated fat source obtained from olive oil in association with or without rumen-protected methionine, on digestibility, intake, milk production and composition.

Material and methods

Animals

From October till December, eight Massese ewes at their third lambing, with an average live weight of 54.6±3.7 kg, after weaning (25-30 days post-partum), were kept in individual boxes with a peat bedding, in order to make it possible to control and measure their individual feed intakes and milk yields. Peat was chosen to avoid possible uncontrolled intakes of dry matter from the bedding. The animals were weighed and blood samples drawn from the jugular vein before and after each experimental period,

always at the same time, after the morning meal and the morning milking. The urea, triacylglycerol and glucose blood contents were assayed by spectrophotometric analysis using specific commercial kits (UREA-S, TG-MPR2 and PERIDOCHROM GLUCOSE, Roche Diagnostics GmbH, Mannheim, Germany).

Diets

The basal diet, used as the control diet (diet C), consisted of alfalfa hay and a pelleted mixed feed made of maize (60% DM), soybean meal (40% DM), minerals and vitamins as the concentrate. The forage/concentrate ratio was approximately 73/27 on the DM basis. In fact, the amount of concentrate was adjusted to milk yield each week (600 g concentrate/kg milk) and the forage was offered *ad libitum*. The basal diet was supplemented with 50 g/head per day (2.6 % on DM intake) Liposal (r) (calcium salts of free fatty acids obtained from olive oil) in diet L, with 5 g/head per day (0.26% on DM intake) Methioby® (rumen protected methionine) in diet M, with both Liposal and Methio-by (50 and 5 g/head per day, respectively) in diet ML. The average content of fatty acids of Liposal® as a percentage of total fatty acids is reported in Table 1. Diet C was formulated to meet the ewes' requirements (INRA, 1987) as closely as possible devoid of fat and amino acid supplementation. Hay and concentrates were fed separately twice daily. The most important average composition characteristics of the 4 diets, as consumed by the ewes, are reported in Table 2. Crude protein, ether extract, crude fiber, ash, calcium and phosphorus were analysed according to AOAC methods (1990); fiber fractions after Goering and Van Soest (1970) and acid insoluble ash after van Keulen and Young (1977). The metabolizable energy (ME), net energy for lactation (NE_l), milk forage units (UFL) and proteins digestible in the intestine (PDIN and PDIE) figures were calculated according to Vermorel *et al.* (1987) and Verité *et al.* (1978). The 4 diets were in any case comparable to each other, with the only exceptions for ether extract (roughly doubled in diets with Liposal) and for ME and NE_l.

Table 1. Chemical and fatty acid composition of Liposal®.

Chemical composition (% DM):	
Dry Matter	97
Crude Fat	93
Ca	7
Fatty acid composition (% total fatty acid):	
$C_{8:0}$	0.97
$C_{10:0}$	0.95
$C_{12:0}$	8.40
$C_{14:0}$	8.16
$C_{16:0}$	20.13
$C_{16:1}$	0.54
$C_{18:0}$	7.95
$C_{18:1}$	34.48
$C_{18:2}$	17.64
$C_{18:3}$	0.78

Diet apparent digestibility was determined for each treatment; faecal samples were collected every day from each ewe throughout the experimental periods. Individual mixed samples of faeces were analysed with the same methods used for feeds. Dietary digestibility coefficients were estimated by using acid insoluble ash as an internal marker (Van Keulen and Young, 1977).

Experimental design

A 4x4 Latin square experimental design with two replicates per diet was used as the most suitable for 8 animals. Each experimental period lasted one week and was preceded by an adaptation period of 10 days, meant to allow the rumen microbes to get accustomed to the new diet.

Milk yield and milk sample analyses

The animals were milked twice daily (at 8:00 a.m. and 7:00 p.m.) and individual milk samples taken in the morning and in the afternoon were pooled in a single sample. Milk samples were analysed for fat, proteins and lactose by infrared analysis (Milkoscan 133 B; Italian Foss Electric,

Table 2. Chemical composition and nutritive value of the diets (DM basis).

		Diet C	Diet L	Diet M	Diet ML
Crude protein	g/kg	187.8	182.1	190.0	183.7
Ether extract	"	20.3	44.7	20.5	44.0
Crude fibre	"	262.2	255.9	264.0	257.7
Ash	"	92.5	92.3	92.6	92.4
Ca	"	11.5	12.7	11.4	12.7
P	"	3.5	3.4	3.5	3.3
NDF	"	447.5	436.2	448.7	436.8
ADF	"	301.2	295.7	302.7	298.1
ADL	"	70.1	68.5	70.4	69.1
Acid insoluble ash	"	5.1	5.0	5.0	4.8
ME	MJ/kg	9.2	9.5	9.2	9.6
NE_l	"	5.4	5.7	5.4	5.6
UFL*	kg^{-1a}	0.76	0.80	0.76	0.79
PDIN[b]	g/kg	126	122	128	124
PDIE[c]	"	114	111	113	110

[a] *Milk Forage Units, converted from NE_l, MJ/kg.*

[b] *Digestible Protein in the Intestine units, with nitrogen as the limiting factor (INRA, 1988).*

[c] *Digestible Protein in the Intestine units, with energy as the limiting factor (INRA, 1988).*

Diet C = Control; L = C + Liposal® ; M = C + Methio-by® ; ML = C + Liposal® + Methio-by®.

Padua, Italy); somatic cells count (SCC), and bacterial count (BC) were checked by means of a Fossomatic 215 Cell Counter (Foss Electric, DK-3400 Hillerod, Denmaerk) and standard microbic count in Plate Count Agar; (time of incubation 48h at 32°C) respectively. The SCC data were transformed in linear score according to Wiggans and Shook (1987). Rennet clotting time (r), rate of curd firming (K_{20}), curd firmness after 30 and 45 minutes (A_{30} and A_{45}) and pH were also measured (ASPA, 1995; Formagraph apparatus, Delacroix-Buchet *et al.*, 1994) at the end of each experimental period. The fatty acids of milk fat were analysed by gas chromatography as the methyl ester derivatives, after trans-esterification with sodium methoxide (Christie, 1982). The gas chromatographic apparatus DANI (Milan, Italy) was equipped with a FID detector and a capillary column (OMEGAWAX 30 m length, 0.25 μm thickness, 0.32 mm i.d.) with nitrogen as the carrier gas (flow 1 ml/min and split ratio 1:30). The temperature programming was 100°C for 2 min, then up to

Table 3. Dry matter intakes and apparent digestibility coefficient of the experimental diets.

		Diet C	Diet L	Diet M	Diet ML	SE
Dry matter intake	g/d	1858	1903	1878	1943	23.22
Organic matter digestibility	%	62.86	62.67	62.63	62.01	0.54
Crude protein digestibility	"	68.94	66.60	68.43	67.62	0.65
NDF digestibility	"	46.72	47.11	46.71	45.36	0.78
ADF digestibility	"	34.41	35.73	34.19	35.17	0.98

Diet C = Control; L = C + Liposal®; M = C + Methio-by®; ML = C + Liposal® + Methio-by®.

Table 4. Milk yield, milk composition and clotting traits.

		Diet C	Diet L	Diet M	Diet ML	SE
Milk yield	(g/d)	919	913	940	897	27.7
Fat corrected milk	g/d (6.5%)	1002.4[a]	1051.6[b]	1075.4[b]	1061.9[b]	36.87
Milk fat	%	7.43[a]	8.06[b]	7.98[a,b]	8.39[b]	0.54
Milk proteins	"	6.99[a]	6.65[b]	6.83[a]	6.67[b]	0.09
Milk lactose	"	4.63	4.63	4.69	4.66	0.18
SCC, linear score		4.51	4.46	4.84	4.52	0.39
pH		6.52	6.50	6.53	6.52	0.04
r, min:sec		20:30[a]	22:15[b]	25:30[b]	21:00[a]	5.95
K_{20}, min:sec		01:30	01:30	01:45	01:30	0.29
A_{30}, mm		42.42[a]	47.49[c]	33.12[b]	43.91[a]	14.76
A_{45}, mm		54.54	57.12	53.50	60.00	9.67

[a,b] means within a row lacking common small superscript letters differ (P<0.05).
Diet C = Control; L = C + Liposal® ; M = C + Methio-by® ; ML = C + Liposal® + Methio-by®.

215°C at a rate of 15°C/min, 215°C for 3 minutes, up to 245°C at the rate of 10°C/min and finally 245°C for 20 min. The temperatures of the injector and detector were 270°C and 300°C, respectively. Heneicosanoic acid methyl ester was introduced as the internal standard.

Statistical analysis

Data were subjected to ANOVA in a replicate Latin square design using a linear model with the fixed effects of i) diet; ii) replicate; iii) period within replicate and iv) ewe within replicate. Linear contrasts were used to compare diet C vs. diets L, M and ML (SAS, 1999).

Results and discussion

DM intakes (about 1.9 kg/head per day) and digestibility percentages didn't significantly differ between diets (Table 3), thus the differences among data related to milk yield and quality (Table 4), when present, should be attributed not to the quantity but to the quality of the diets. Milk yield was not affected by the presence of either Ca salts or protected methionine in the diet, but milk fat percentage (8%, 7% and 13% more fat vs. control diet with $p<0.05$, respectively for Ca salts, methionine and both supplements, indicating a kind of associative effect) and fat corrected milk were. As far as somatic cell count was concerned, the diet did not significantly affect the parameter.

Blood glucose, triglycerides and urea values are reported in Table 5. In agreement with Chilliard (1993), fat supplementation had no consistent effects on circulating glucose. The only haematic parameter which appeared to be affected by the diet quality was blood urea, which was appreciably higher when Ca soaps ($p<0.05$) were added to the diet. The surplus of blood urea could be related to the fall in milk protein observed when fat was supplied (Table 4). Decreases in milk protein content when calcium soap is included in the diet have been reported in ewes (Casals *et al.*, 1999; Horton *et al.*, 1992; Rossi *et al.*, 1991; Kovessy *et al.*, 1987), cows (Kowalski *et al.*, 1999; Chilliard *et al.*, 1993), but not in goats (Baldi *et al.*, 1992). The decreased milk protein concentration with fat supplementation may be related to a combination of factors such as: i) dilution effects, when milk yield was increased; ii) reduction of microbial protein synthesis, when dry matter intake (DMI) was depressed (Palmquist and Jenkins, 1980); or changes in glucose metabolism, as suggested by Smith *et al.* (1978). In our experiment, neither milk yield nor DMI varied, thus our results seem

Table 5. Haematic parameters (mg/10 ml).

	Diet C	Diet L	Diet M	Diet ML	SE
Glucose	66.54	63.83	62.50	65.85	2.06
Triglycerides	34.97	27.43	33.28	29.77	2.07
Urea	66.47[a]	82.49[b]	67.06[a]	71.61[a]	3.25

[a,b] means within a row lacking common small superscript letters differ (P<0.05).
Diet C = Control; L = C + Liposal® ; M = C + Methio-by® ; ML = C + Liposal® + Methio-by®.

to be in agreement with the third hypothesis. Also, Cant *et al.* (1993 a; b) indicated that the observed drop in milk protein content was due to an energy-dependent reduction in mammary gland blood flow, that resulted in a reduction of the availability of amino acids at the mammary gland. Moreover, Dunkley (1977) suggested that the effect on milk protein concentration was due to a decrease of the casein fraction. In this study, clotting parameters did not seem to confirm this hypothesis. Diet L and ML, in fact, had Rennet properties similar to those of the control diet. The addition of methionine alone worsened milk quality, namely the Rennet clotting time (r value, table 4). The inclusion of protected methionine in the diet, therefore, did not improve the technological properties of ewe milk. Previous researches showed that milk protein depression with fat added diets may be avoided by providing either methionine or lysine (Christensen *et al.*, 1994; Canale *et al.*, 1990; Chow *et al.*, 1990), but in this case the protected methionine included in diet ML did not change the milk protein content in comparison with diet L. As expected, milk fat content was positively affected by Ca soap in the diet L and ML, but also diet M resulted in a slight increase of milk fat content.

Short chain fatty acids (SCFA) did not vary among the diets, while medium and long chain (MCFA and LCFA, respectively) did (Table 6). The inclusion of Ca soaps alone and especially when associated with methionine ($P<0.05$) resulted in lower levels of MCFA, namely C_{12}, C_{14} and C_{16} (Table 7). Diet with methionine alone was in any case comparable with diet C. Also total saturated fatty acids decreased as a consequence of the Ca soap supply as well (Table 6). As expected, the higher contents of LCFA and MUFA for diet L and

Table 6. Milk fatty acid classes (% of total fatty acids).

	Diet C	Diet L	Diet M	Diet ML	SE
SCFA	6.64	6.62	6.64	7.08	0.12
MCFA	60.19[a]	53.19[b]	59.78	52.63[b]	0.15
LCFA	33.17[a]	40.18[b]	33.58[a]	40.28[b]	0.26
SFA	54.98[a]	51.45[b]	55.68[a]	52.06[b]	0.35
MUFA	21.39[a]	26.08[b]	21.40[a]	25.38[b]	0.59
PUFA	5.21	5.17	5.01	5.78	0.10
SFA/UFA	2.07[a]	1.65[b]	2.11[a]	1.67[b]	0.15

[a,b] means within a row lacking common small superscript letters differ (P<0.05).
Diet C = Control; L = C + Liposal®; M = C + Methio-by®; ML = C + Liposal® + Methio-by®.
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.

Table 7. Acidic composition of milk fat (% of total fatty acids).

Fatty acids	Diet C	Diet L	Diet M	Diet ML	SE
$C_{6:0}$	3.78	3.69	3.69	4.38	0.20
$C_{8:0}$	2.86	2.93	2.95	2.70	0.27
$C_{10:0}$	10.34	9.35	10.01	8.40	0.81
$C_{12:0}$	6.11[a]	5.27[b]	6.05[a]	4.93[b]	0.40
$C_{14:0}$	12.63[a]	11.24[b]	12.67[a]	11.35[b]	0.58
$C_{14:1}$	0.32	0.26	0.23	0.32	0.03
$C_{15:0}$	1.30	1.14	1.10	1.08	0.08
$C_{16:0}$	27.27[a]	23.90[b]	27.63[a]	24.62[b]	1.01
$C_{16:1}$	1.34	1.28	1.28	1.23	0.05
$C_{17:0}$	0.89	0.76	0.81	0.70	0.05
$C_{18:0}$	7.68[a]	9.90[b]	8.24[a]	10.08[b]	0.29
$C_{18:1}$ *(cis + trans)*	19.74[a]	24.54[b]	19.88[a]	23.82[b]	0.98
$C_{18:2}$ n-6	2.93[a]	2.82[a,b]	2.97[a]	2.69[b]	0.10
Total CLA	1.11[a]	1.24[a,b]	0.85[a]	1.86[b]	0.08
$C_{18:3}$ n-3	1.16	1.11	1.19	1.23	0.07
$C_{20:0}$	0.55	0.57	0.45	0.61	0.08

[a,b] means within a row lacking common small superscript letters differ (P<0.05).
Diet C = Control; L = C + Liposal® ; M = C + Methio-by® ; ML = C + Liposal® + Methio-by®.
CLA = conjugated linoleic acid.

ML were due to the increase of $C_{18:1}$ fatty acid in milk fat. Since the gas-chromatographic analysis with a 30 m length column does not make it possible to distinguish the *cis* and *trans* isomers of $C_{18:1}$, the sum of all geometrical and positional isomers of this group of fatty acids is reported (Table 7). However, previous research showed an increase of total $C_{18:1}$ *trans* isomers as a result of the inclusion of Ca soaps of olive oil in diet of dairy cows, while oleic acid did not vary (Secchiari *et al.*, 2001). The higher content of $C_{18:0}$ (stearic acid) for diet L and ML was probably due to the rumen biohydrogenation process of oleic acid included in Ca soaps. Ca soaps, in fact, are not completely protected against ruminal biohydrogenation as reported by Chilliard *et al.* (1993), especially when unsaturated fatty acids are included. The total conjugated linoleic acid (CLA) content was higher when ewes were fed diet L and particularly diet ML. In this case, we may note that the highest content of CLA in milk fat corresponds to the lowest level of linoleic acid ($C_{18:2}$, n-6), that is the ruminal precursor of CLA (Parodi, 1999). On the contrary, linolenic acid content ($C_{18:3}$, n-3) did not vary among diets. Diet L and ML made it possible to obtain safer characteristics of milk fatty acid composition as reflected by lower SFA/UFA ratio, lower MCFA and higher CLA contents (Tables 6 and 7).

Conclusions

Supplementing diets with Ca salts of natural fats is a common practice in ruminant feeding as a means to increase the energy potential of the diet during the most critical phase of lactation. The use of olive oil fatty acids as the fat seemed to upgrade the fatty acid characteristics of milk towards a safer composition. Unfortunately, the presence of this kind of Ca salts in the diet appears to affect the metabolic utilisation of amino acids and, probably, of C_{18} PUFA in an undesired way. In our study, the addition of rumen-protected methionine did not seem to improve the metabolic balance towards a better utilisation of amino acids.

Rennet clotting time and curd firmness after 30 minutes worsened slightly with methionine, but not with soaps or with a combination of the two. The control diet resulted the best and ML diet was not different from diet C.

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