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A. Cabiddu*, G. Carta, G. Molle*, M. Decandia*, M. Addis*, G. Piredda*, A. Delogu*, A. Pirisi*, V. Lai***, V. Cera***, L. Taras***, C. Lallai** and S. Banni******
*Istituto Zootecnico e Caseario per la Sardegna,
Strada Sassari-Fertilia km 18,600, 07040 Olmedo, Italy
**Caseificio I.C.A., 09041 Dolianova, Italy
***Associazione Regionale Allevatori della Sardegna, Via Cavalcanti 8, 09128 Cagliari, Italy
****Dipartimento di Biologia Sperimentale, Università degli Studi di Cagliari,
Cittadella Universitaria di Monserrato, 09042 Cagliari, Italy

SUMMARY – Conjugated linoleic acid (CLA) is a naturally occurring anti-carcinogen compound found in dairy products. A survey was carried out during the years 2000-2002 in South Sardinia, on a total of 15 dairy sheep farms characterised by different pedological conditions, pasture composition and supplementation levels. Samples of bulk milk and cheese, produced in the same factory, were collected monthly, during the milking period, and fatty acid composition, inclusive of CLA was determined. Data of feeding management were also recorded every month and hand-plucked samples of main forage species on offer were taken. The level of CLA in milk was high (18.46 ± 0.93 mg/g of fat), when the flocks grazed pastures at vegetative phase while it dropped down to 13.34 ± 1.67 mg/g of fat during pasture reproductive phase. The same trend was found in the content of precursors like linolenic acid in some grazed species that markedly declined (about 50%) in the reproductive phase. The level of supplementation tended to negatively affect the milk CLA content. Similar effects of pasture maturity and supplementation levels were shown on the CLA content of manufactured cheeses. In conclusion, the level of CLA in milk and cheese was positively related with the amount of sheep requirements covered by the grazed herbage.

Key words: Conjugated linoleic acid, nutrition, grazing, dairy sheep.

RESUME – *"Relation entre le système d'alimentation et la teneur en acide linoléique conjugué dans le lait et le fromage des brebis". L'acide linoléique conjugué (CLA) est un composant naturel, ayant des effets anticancérigènes et que l'on trouve dans les produits laitiers (lait et fromages). Au cours des année 2000-2002 nous avons effectué, dans le sud de la Sardaigne, un suivi sur 15 élevages de brebis laitières qui se caractérisaient par des différences dans les conditions pédologiques, la composition botanique des pâturages et le niveau de supplémentation. On a prélevé chaque mois des échantillons de lait de mélange et de fromages produits dans la même fromagerie pendant la période de lactation et procédé aux analyses de la composition des acides gras, CLA compris. Des données relatives aux régimes alimentaires ont également été enregistrées chaque mois sur chaque élevage via l'analyse d'échantillons de différents fourrages pâturés. Nous avons constaté un niveau de CLA élevé (18,46 ± 0,93 mg/g de matière grasse) dans le lait lorsque les animaux pâturent dans des prairies au stade végétatif. Par contre, quand l'herbe atteint le stade reproductif, le niveau de CLA baisse jusqu'à 13,34 ± 1,67 mg/g de matière grasse. Le même phénomène est observé avec un déclin marqué de presque 50% du niveau des précurseurs comme l'acide linolénique dans l'herbe en phase reproductive. Par ailleurs, le niveau de supplémentation semble être corrélé de façon négative avec le niveau de CLA du lait. Une situation analogue sur la dynamique du niveau de CLA a été observée sur les fromages. En conclusion, on peut dire que le niveau de CLA dans le lait et dans les fromages est corrélé de façon positive avec une bonne disponibilité de l'herbe verte dans la ration des animaux.*

Mots-clés : *Acide linoléique conjugué, nutrition, pâturage, brebis laitières.*

Introduction

Medical studies have recently proven that milk and dairy products have a potent role in preventing some diseases. In particular, there has been a surge of interest in conjugated linoleic acid (CLA) in man's diet because of increasing evidence, based largely on animal studies, suggesting potential benefits of CLA on man's health (Ip *et al.*, 1994). The CLA occurs naturally in many foodstuffs, the main dietary sources being dairy products and other food derived from ruminants. The CLA is a

collective term used to denote one or more positional and geometric isomers of linoleic acids (*cis*-9, *cis*-12 – 18:2). Recent studies have demonstrated that the CLA reduces mammary tumor incidence (Ip *et al*., 1999). Its anticarcinogenic properties have been found *in vivo*, in several experimental animals and *in vitro* in human tumor cell cultures. The most isomer has been found to be *cis*-9, *trans*-11 octadecadienoic acid, the only one that can be incorporated in membrane phospholipids. The concentration of CLA in milk and dairy products, usually ranges from 4 to 20 mg/g fat (Prandini *et al*., 2001). Specific factors that cause this variation have not been investigated extensively, although it is clear that dietary factors are of major importance (Griinari and Bauman, 1999). Recent studies indicated that CLA level in sheep milk and cheese is affected by concentrate supplementation level, forage species, and forage physiological stage (Delogu *et al*., 2000; Cabiddu *et al*., 2001, 2003; Addis *et al*., 2002; Piredda *et al*., 2002). The aim of this study was to survey the concentration of CLA and its precursor in dairy sheep milk and cheese along the whole lactation.

Materials and methods

The survey was carried out during years 2000-2002 on a total of 15 dairy sheep farms, located in South Sardinia. These farms were characterised by different pedological conditions, pasture botanical composition and supplementation levels. Samples of bulk milk from each dairy sheep farm were collected every 21 days. Samples of milk and cheese produced in the same factory, were collected monthly, during the milking period, and fatty acid composition, inclusive of CLA (*cis*-9, *trans*-11) was determined (Christie, 1989). Data of feeding management (time on the pasture, phenological phase of pasture, amount of hay and concentrate in sheep diet) and milk yield were also recorded every month on the sampling day. Hand-plucked samples of main grazing forage species on offer were occasionally taken to measure dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), (Martillotti *et al*., 1987) and fatty acid composition (Christie, 1989; Chin *et al*., 1992). Estimated herbage DM intake (DMIH) was calculated by differences between requirements (maintenance + lactation) and allowances from supplements. All data were analysed by GLM with sampling month, botanical species or pasture phenological phase as fixed effects. Treatments means were separated by *t* test for multiple comparisons.

Results and discussions

The more frequently grazed forage species are listed in Table 1. The chemical composition of these species confirmed that the phenological phase plays an essential role in modulating nutritional characteristics. The proportion of ether extract (EE) decreased passing from vegetative to reproductive stage. Also herbage fatty acids composition was affected by the phenological phase, mainly in Italian ryegrass. In this species linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) acid levels decreased in reproductive phase, whereas in sulla only the linolenic acid showed a reduction (–30%). Milk yield and fat (Table 2) showed the typical trend for Sarda breed (Carta *et al*., 1995). The monthly pattern of milk CLA during the survey (Fig. 1) was characterised by a maximum level (25.49 mg/g of fat) in March, when the amount of green herbage in the diet was about 74% (Fig. 1). In the following months the level of CLA decreased, despite the increase of the herbage proportion in the diet. This probably related to the lower levels of CLA precursors in mature pasture, particularly in the grasses (Table 1). The content of vaccenic ($C_{18:1\ trans\text{-}11}$) and linolenic acids in milk showed a similar trend to that of CLA (Fig. 1). As a matter of fact, linear relationships were found between vaccenic acid and CLA (R^2 = 0.87, $P < 0.0001$) and between linolenic acid and CLA ($R^2 = 0.48$, $P < 0.0001$; Fig. 2). Linoleic was higher than linolenic acid; in particular their profiles were specular (Fig. 1). The level of CLA in milk fat was also negatively correlated with the level of supplementation ($r = –0.43$, $P < 0.001$). The herbaceous species affected milk fat CLA concentration; in fact, when sheep grazed legumes or grasses the level of CLA were 20.04 and 11.64 mg/g of fat, respectively ($P < 0.05$; Fig. 3). The CLA in milk fat was substantially increased when sheep consumed diets containing herbaceous species during active growth phase as compared with mature forages (18.46 vs 13.34 mg/g of fat, $P < 0.05$) for growth and reproductive stages, respectively; Fig. 4).

Forage species and phenological phase did not affect statistically milk linoleic acid content, whereas linolenic acid was higher when sheep grazed legumes than grasses ($P < 0.0001$, Fig. 3).

The CLA, vaccenic, linoleic and linolenic acids in cheese (Table 3) showed similar trends to those reported for milk.

Table 1. Chemical composition of forage species grazed by lactating sheep

Forages species	Stage	N	DM (%)	% DM			Linoleic acid (g/kg DM)	Linolenic acid (g/kg DM)
				CP	NDF	EE		
Grasses								
Italian ryegrass	Growth	3	17.57	25.79	44.88	4.31	1.71	11.97
	Reproductive	1	37.72	7.40	56.57	2.12	0.91	0.99
Barley	Reproductive	1	18.92	17.86	46.93	3.70	1.94	8.646
Barley + oats	Growth	1	19.27	20.55	38.66	4.26	1.80	13.91
Barley + oats + Italian ryegrass	Growth	1	15.05	22.26	49.24	4.39	2.08	11.12
Ryegrass + oats + barley	Reproductive	1	19.29	18.40	46.66	3.94	1.12	5.95
Legumes								
Sulla	Growth	5	15.26	25.46	33.97	3.02	1.70	14.00
	Reproductive	1	17.67	23.37	34.47	2.83	1.72	10.17
Berseem clover	Growth	7	19.42	26.82	35.87	3.47	2.86	12.39
Subterranean clover	Reproductive	1	18.95	19.79	39.84	3.85	2.42	7.47
Natural pasture	Reproductive	1	25.88	11.28	46.25	2.51	1.70	3.56

Table 2. Milk yield and fat proportion

	Milk yield (kg/head/d)	Fat (%)
January	1.24[ab]	5.11[c]
February	1.37[a]	5.81[b]
March	1.40[a]	5.15[c]
April	1.33[ab]	5.28[c]
May	1.19[b]	5.79[b]
June	0.71[c]	6.06[b]
July	0.46[d]	6.92[a]
N	*147*	*132*
Means	*1.14*	*5.61*
SEM	*0.03*	*0.08*
P<	*0.0001*	*0.0001*

[a,b,c,d]Means in column with different letter differ at P < 0.05.

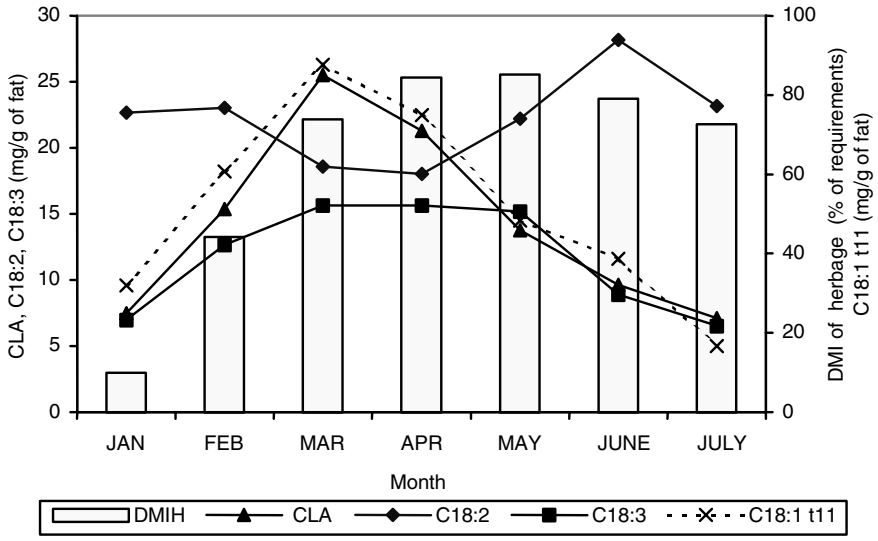

Fig. 1. Seasonal variation of estimated DM herbage intake (DMIH), as proportion of total diet, linoleic, linolenic, vaccenic and conjugated linoleic acids in sheep milk.

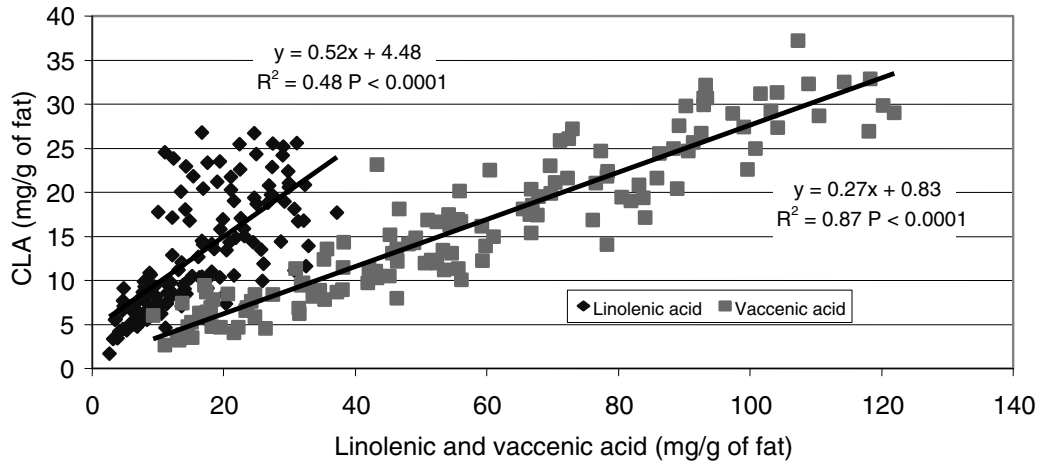

Fig. 2. Relations between vaccenic, or linolenic acids with CLA content in milk.

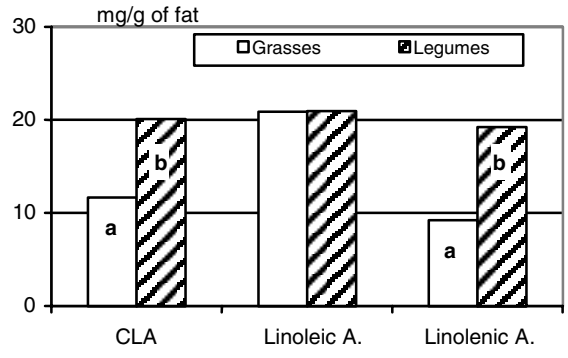

Fig. 3. Level of CLA, linoleic and linolenic acids in milk from sheep grazing legumes or grasses.

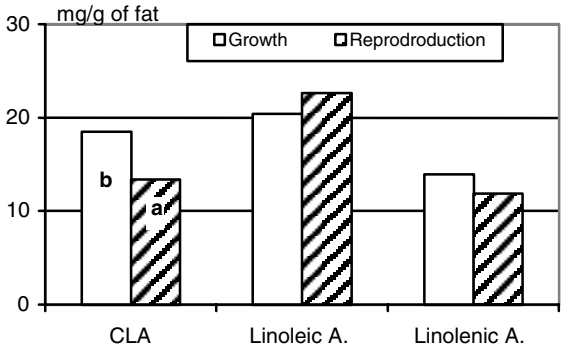

Fig. 4. Level of CLA, linoleic and linolenic acid in milk from sheep grazing herbaceous species at different phenological stages.

Table 3. Fatty acids composition of cheese at 24 h

	CLA (mg/g fat)	Vaccenic acid (mg/g fat)	Linoleic acid (mg/g fat)	Linolenic acid (mg/g fat)
January	–	–	–	–
February	16.06[ab]	69.12[ab]	22.35	11.78
March	21.38[a]	82.08[a]	20.64	16.36
April	18.41[ab]	56.46[ab]	21.93	15.58
May	16.50[ab]	49.79[ab]	23.88	16.13
June	–	–	–	–
July	8.04[b]	22.67[b]	27.35	7.32
N	*132*	*132*	*132*	*132*
Means	*13.28*	*57.65*	*21.28*	*13.01*
SEM	*0.74*	*2.59*	*0.43*	*0.56*
P<	*0.0001*	*0.0001*	*0.0001*	*0.0001*

[a,b]Mean in column with different letter differ at P < 0.05.

Conclusion

The results of the present study show that also in commercial farms and factories the content of CLA and its precursors in sheep milk and cheese varies significantly with the amount of green herbage in the diet, depending on the herbaceous species and their phenological stages. In particular, the maximum level of CLA was found when sheep graze legumes or when the pasture is actively growing.

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