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Nutritional approaches to improve the fatty acid profile of milk fat in sheep and goats

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Abstract. Sheep and goat milk are sources of bioactive compounds with health-promoting properties. The demand for sheep and goat milk has grown worldwide over the years, because of both the increased request for cheese and other traditional dairy products, and the success of sheep and goat milk as component of infant formulas, drinking milk and nutraceutical product. Animal feeding is one of the main factors affecting the quality of fat in sheep and goat milk and, therefore, in the derived dairy products. In particular, nutrition can readily alter milk fat concentration and fatty acid (FA) profile. This review compared the effects of sheep and goats feeding strategies on milk fat composition, with focus on FA with potential healthy properties, such as conjugated linoleic acid (CLA) and polyunsaturated fatty acids (PUFA) of the omega3 family. The nutritional strategies to design the FA profile of sheep and goat dairy products based on grazing and use of vegetable oils and the different nutritional responses of the two species are reviewed and discussed.

Keywords. Dairy sheep and goats – Milk fatty acid composition – Nutrition – CLA – Omega-3 – Alpha linolenic acid – Odd and branched chain fatty acids.

Approches nutritionnelles pour améliorer le profil en acides gras de la matière grasse du lait chez les ovins et caprins

Résumé. Le lait des ovins et caprins est une source de composés bioactifs possédant des propriétés favorables à la santé. La demande de lait de brebis et de chèvres a augmenté à l'échelle mondiale au fil des années, en raison à la fois de la plus grande consommation de fromage et autres produits laitiers traditionnels, et du succès du lait ovin et caprin comme composante de formulations pour nouveaux-nés, de lait à boire et de produits nutraceutiques. L'alimentation animale est l'un des principaux facteurs qui influencent la qualité de la matière grasse du lait des ovins et caprins et, par conséquent, des produits laitiers dérivés. En particulier, la nutrition peut facilement altérer la concentration de matière grasse et le profil en acides gras (AC). Cette révision a comparé les effets des stratégies d'alimentation des ovins et caprins sur la composition de la matière grasse du lait, en se focalisant sur les AC ayant des propriétés potentielles de santé, tels que l'acide linoléique conjugué (CLA) et les acides gras poly-insaturés (PUFA) de la famille oméga3. Dans cet article sont examinées et discutées les stratégies nutritionnelles visant à moduler le profil en AC des produits laitiers des ovins et caprins en se basant sur le pâturage et l'utilisation d'huiles végétales, ainsi que les différentes réponses nutritionnelles de ces deux espèces.

Mots-clés. Brebis et chèvres laitières – Composition en acides gras du lait – Nutrition – CLA – Omega-3 – Acide alpha-linolénique – Acides gras ramifiés impairs.

I – Introduction

In Mediterranean countries, sheep milk is primarily used for the production of cheese, whereas goat milk is also largely used to produce yogurt and drinking milk. Goat and sheep milk is also suitable for formula and follow-on formula for infants (Directive 2006/141/EC) who cannot use breast milk (Grant *et al.*, 2005). In New Zealand, infant formulas for babies from birth until three years of age have been developed and their production is markedly increasing.

Milk and dairy products of small ruminants have a high nutritional value and are a considerable source of high-quality dietary proteins and fats. For the latter, there is an increasing research finalized to improve its nutritional quality by enhancing the content of fatty acids (FA) putatively associated with benefits on human health, such as branched-chain fatty acids (BCFA), ruminant trans-fatty acids (R-TFA), especially cis9, trans11-conjugated linoleic acid (c9,t11CLA) also known as rumenic acid (RA) and trans11-18:1 vaccenic acid (VA), and α -linolenic acid (C18:3n3; ALA). More details on these effects are reported in next section. The high concentration of these types of FA in milk and dairy products of small ruminants, usually higher than that found in cow milk, is of particular interest (Prandini *et al.*, 2011; Lobos-Ortega *et al.*, 2012).

Dietary regimen can greatly affect the amount of these biologically active molecules in sheep and goat milk fat (Chilliard *et al.*, 2003; Nudda *et al.*, 2014; Ferlay *et al.*, 2017). Several studies highlighted the positive and marked role of pasture-rich diets in enhancing the concentration of healthy FA in goats (Tudisco *et al.*, 2010; Nudda *et al.*, 2007; Tsiplakou *et al.*, 2006) and sheep (Nudda *et al.*, 2014). Another strategy to increase the concentration of beneficial FA in milk is to supplement the diet of dairy sheep (Nudda *et al.*, 2014) and goats (Chilliard *et al.*, 2003; Nudda *et al.*, 2006; 2008; 2013) with sources of unsaturated plant lipids. In addition, an interaction effect between alpha-s1-casein gene polymorphism and diet on goat milk FA composition has also been reported (Valenti *et al.*, 2010; Chilliard *et al.*, 2013).

Due to the importance of the feeding regimen of milk FA, this review provides the following: a) a specific update on the studies regarding feeding strategies able to modify the healthy FA content in sheep and goat milk and dairy products, with focus on the use of pasture and vegetable oil sources, and b) an evaluation of the differences between sheep and goats regarding the ability of feeding strategies to modify their milk FA composition.

II – Beneficial effects of fatty acids

Dairy products are the major source of RA and VA, although small amounts of RA isomers can be endogenously synthesized in humans (Adlof *et al.*, 2000). RA and VA originate from the incomplete biohydrogenation of PUFA by microorganisms in the rumen (Kepler *et al.*, 1966), and VA is also the main precursor of CLA in the mammary gland, by the action of D9 desaturase. Several animal and human cell line studies found that CLA, especially the isomer c9t11 CLA (RA), exerts many biological effects (Yang *et al.*, 2015; Ferlay *et al.*, 2017). Positive biological effects have also been ascribed to VA (Jacome-Sosa *et al.*, 2016; Krogager *et al.*, 2015). Moreover, RA has acquired significant relevance because of the encouraging positive results obtained by using dairy products, naturally rich in this FA, in human trials. In healthy subjects, the intake of 200 grams per week of a sheep cheese naturally enriched with VA and RA (3.26 and 1.56 g/100 lipids, respectively), when compared with a regular cheese (0.4 VA and 0.19 RA g/100 lipids, respectively), for ten weeks reduced significantly inflammatory substances, such as interleukin-6, interleukin-8 and tumour necrosis factor- α , and improved some hemorheological parameters (Sofi *et al.*, 2010). In hypercholesterolemic subjects, the intake of sheep cheese naturally enriched with RA and ALA unexpectedly decreased the plasma concentrations of the endocannabinoid anandamide by 40% and the LDL-cholesterol level by 7% (Pintus *et al.*, 2013), compared to the intake of a control cheese. Milk is also a source of n-3 FA, with ALA being the most abundant in dairy products. Research in humans and laboratory animals reported beneficial effects of ALA in the prevention of cardiovascular (Del Gobbo *et al.*, 2016; Barbeau *et al.*, 2017; Ganguly *et al.*, 2017) and other (Yamagishi *et al.*, 2017) diseases. Several biological effects of RA against enteropathy (Bergamo *et al.*, 2016), atherosclerosis (Bachmair *et al.*, 2012), cancer (Wang *et al.*, 2013; Lu *et al.*, 2015), and inflammations (Mollica *et al.*, 2014; Penedo *et al.*, 2013) have been evidenced in multiple observational studies in animals and in *in vitro* experiments. Ruminant products are also an important source of BCFA, FA with at least one methyl branch along the carbon chain (Ran-Ressler *et al.*, 2014), which

were associated with increased expression of anti-inflammatory cytokines in an animal model (Ran-Ressler *et al.*, 2014). In ruminant products, there are also odd-chain FA (**OCFA**), of which the pentadecanoic (15:0) and heptadecanoic (17:0) acids have generated interest among scientists recently because of their inverse association with risk of type 2 diabetes and cardio-vascular diseases in humans (Pfeuffer and Jaudszus, 2016).

III – Dietary effects on healthy fatty acids in sheep and goat milk

1. Effect of pasture and forage-based diets

Fresh pasture is an excellent source of ALA and is one of the most effective feeds in shifting milk FA composition towards a healthy spectrum in sheep (Albenzio *et al.*, 2016; Nudda *et al.*, 2014) and goats (D'Urso *et al.*, 2008; Renna *et al.*, 2012). In particular, fresh grass grazed directly or cut and supplemented in the diet has a marked effect on VA and RA content in milk fat, maybe because green forage has a high content of ALA, which is partly biohydrogenated into VA in the rumen and partially converted into RA in the mammary tissue by the action of delta-9 desaturase. Differences between these species in responses to pasture-based diets have been hypothesized, because the RA content is normally higher in sheep milk than in goat and cow milk on pasture (Jahreis *et al.*, 1999). A survey that evaluated the FA profile of sheep and goat cheese produced during the year (Nudda *et al.*, 2008) evidenced that the content of RA in goat cheese was lower than that observed in sheep milk and did not change significantly during the months of production, whereas the content of RA in sheep cheese was the highest in March and April in correspondence of the highest pasture availability and quality (Fig. 1).

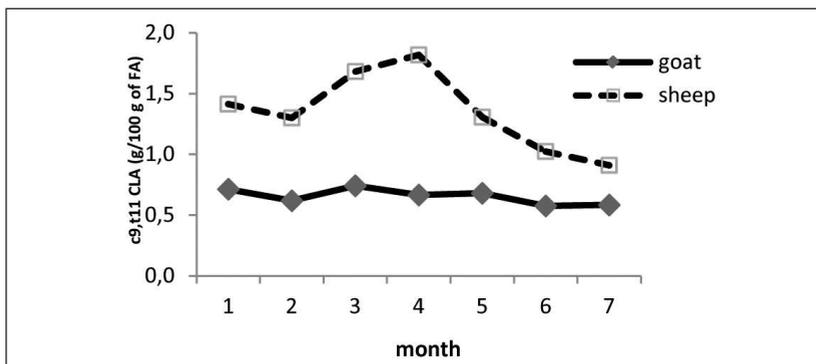


Fig. 1. Temporal evolution of c9,t11 CLA in ovine and caprine cheese produced in Sardinia from January (Month 1) to July (Month 7) (from Nudda *et al.*, 2008).

The seasonal evolution of RA in milk and cheese of goats (Nudda *et al.*, 2007) and sheep (Nudda *et al.*, 2005), sampled from March to June in different processing plants located in Sardinia (Italy; Fig. 2), clearly evidenced a different pattern of this FA concentration between the two species. A pattern similar to that reported in Figure 1 has been observed in a survey carried out by Tsiplakou *et al.* (2006) from January to June, under semi-extensive sheep/goat farming systems in Greece. Data of these two species are difficult to compare due to the different experimental conditions in which the surveys were carried out. However, our experiment (Nudda *et al.*, 2003) with goats and sheep maintained in the same environmental conditions and fed the same mixed pasture of *Trifolium* sp. and *Lolium* sp. confirmed that the RA content was significantly higher in ewes than goats (+230%) in April, when the diet was composed mainly by pasture ($P < 0.01$).

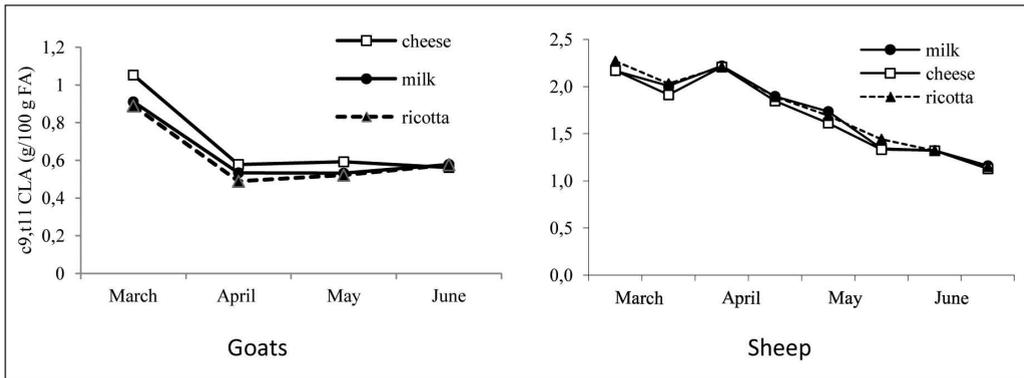


Fig. 2. Seasonal evolution of ruminic acid (c9,t11 CLA, mg/100 mg FA) in milk, cheese and ricotta of goats (adapted from Nudda *et al.*, 2007) and sheep (adapted from Nudda *et al.*, 2005).

This species difference was markedly reduced in May, when pasture availability and quality decreased and the proportion of concentrate in the diet increased (Fig. 3). The lack of change in RA content between April and May in goat milk is difficult to explain.

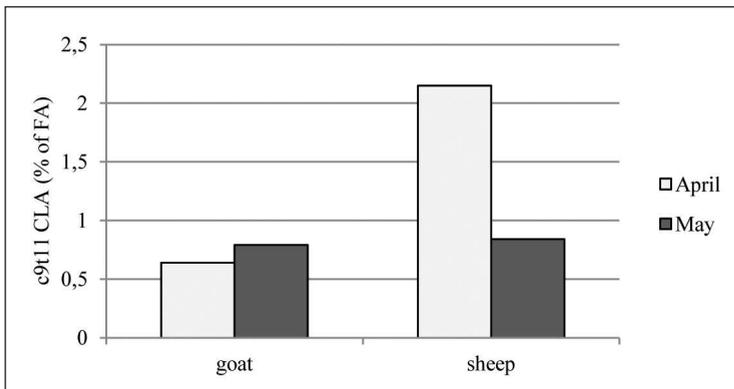


Fig. 3. c9,t11 CLA content of caprine and sheep milk fat, with both species grazing the same pasture during April (high pasture availability) and May (dry pasture). Data from Nudda *et al.* (2003).

Differences between sheep and goats could be related to differences in feeding behavior (e.g. total intake, selectivity and timing) during grazing. Interestingly, in two different studies, as pasture intake increased, the content of RA increased both in sheep (de Renobales *et al.*, 2012) and goats (Renna *et al.*, 2012), but the extent of the response was higher in sheep than goats. Therefore, daily pasture intake explained only partially the increase in CLA content in milk. Addis *et al.* (2005) observed that PUFA level in milk was higher in ewes grazing pure legumes and grass-legume mixtures than in those grazing pure grass pastures. This could be a consequence of the higher content of PUFA in legumes compared to grasses (Cabiddu *et al.*, 2005) with an obviously higher daily intake of PUFA. This indirectly suggests that a possible mechanism explaining the lower RA content in milk of goats fed on pasture is their aversion to legumes, which are richer in CLA precursors. Indeed, goats had a markedly lower preference for legumes compared to grasses when fed on pasture (Fedele *et al.*, 1993) and showed an aversion to legume flavor and a preference for

grass flavor in short-term cafeteria trials (De Rosa *et al.*, 2002). Other possible mechanisms that could explain the species differences in milk RA are related to the fact that goats have more frequent and smaller meals compared to sheep (Abijaoudé *et al.*, 2000) and thus possibly a more regular pattern in rumen pH and feed outflow and more complete rumen bio-hydrogenation of dietary unsaturated FA. The two mechanisms we hypothesized above have not been demonstrated and certainly need solid experimental evidence to be confirmed.

2. Effects of vegetable oil supplementation

The supplementation of vegetable oils to the diet of dairy sheep and goats is a valuable tool to enhance dietary energy content and can influence the FA composition of milk fat. This feeding strategy is particularly useful when the diet has a low content of unsaturated FA, e.g., when hay or silage is the main forage source. The amount of RA in sheep and goat milk is mainly affected by the amount, type and physical form of fat added to the diet (Albenzio *et al.*, 2016; Chilliard *et al.*, 2003; Gómez-Cortés *et al.*, 2011; Shi *et al.*, 2015). Linseed, soybeans, safflower, sunflower, and rapeseed are the main lipid supplements used to increase the content of RA and unsaturated FA in milk (Albenzio *et al.*, 2016; Nudda *et al.*, 2014; Sanz Sampelayo *et al.*, 2007;). Linseed is the most widely used supplement to improve the content of the healthy FA in the milk of both species, being very rich in ALA. The average extent of the increase in RA and VA using linseed is greater, on average, for sheep than goats (Table 1). The opposite pattern is observed in ALA concentration, which is greater in goat milk than in sheep milk. The reasons for these different responses between sheep and goats have not been deeply investigated, but, based on the different response of milk FA composition to this lipid source, it seems that in goats the ALA biohydrogenation process occurs very slowly, compared to sheep, leading to higher ALA rumen outflow towards the mammary gland.

The effect of dietary supplementation of soybean oil and sunflower oil in sheep and goats is reported in Table 2. The concentrations of VA and RA increased with sunflower and soybean oil supplementation in both species. The average increment of these FA was the greatest for sunflower oil in goats (+258% for CLA, +223% for VA), and for soybean oil in sheep (+350% for CLA, +596% for VA)(Table 2). The reasons for this species difference are unclear, given the similar FA composition of these two oil sources. The greater increase in VA and RA for sunflower oil than soybean oil has also been recently evidenced in rumen fluid in an *in vitro* study (Roy *et al.*, 2017). However, inter-species differences in responses to lipid supplements could be related to an interaction between basal diet composition and PUFA in the diet, which are important determinants of the extent of ruminal biohydrogenation and the formation of specific biohydrogenation intermediate products that could affect affects the expression of lipogenic genes involved in FA uptake (LPL), de novo synthesis (ACACA and FASN) and delta-9 desaturation (SCD1) in mammary tissue (Bernard *et al.*, 2009; Chilliard *et al.*, 2014). As said before, goats tend to have more frequent and smaller meals compared to sheep (Abijaoudé *et al.*, 2000) and thus, possibly, a more regular pattern of rumen pH and feed outflow. However, dairy goats are usually fed diets with much higher doses of concentrates and starch than dairy sheep and this can lead to marked modifications of the rumen environment. To clarify this issue, specific experiments in which the same basal diets and supplementations are given to the two species should be designed.

Other lipid sources, such as as safflower (Shi *et al.*, 2015), pomegranate (Emami *et al.*, 2016) and marine oils (Sanz Sampelayo *et al.*, 2007), are effective in increasing milk CLA content in goats and sheep (Albenzio *et al.*, 2016; Nudda *et al.*, 2014). Moreover, the dietary inclusion of lipid sources in combination with tannins could be another practical tool for increasing the unsaturated FA content in milk of goats (Abo-Donia *et al.*, 2017) and sheep (Buccioni *et al.*, 2015, 2017).

Table 1. Effects of dietary linseed supplementation on the fatty acid profile of sheep and goat milk. Effects are expressed as the differences in percentage (%) of change between fat-supplemented group and non-supplemented control group

	Dose	Form	c9,t11 CLA	C18:1 t11	C18:2n6	C18:3 n3	Reference
goats	200 g/d	EL	60	87	3	99	Nudda <i>et al.</i> , 2006
	160 g/d	EL	112	148	13	132	Nudda <i>et al.</i> , 2008
	200 g/d	EL (+pasture)	54	100	1	145	Nudda <i>et al.</i> , 2013
	25g/kgDM	Oil	100	26	12	98	Emami <i>et al.</i> , 2016
	360+40g/d	EL	103	187	5	288	Bernard <i>et al.</i> , 2016
	3.4% DMI	oil	133	190	0	325	Chilliard <i>et al.</i> , 2003
	55-61 g/d	oil	256	399	-29	137	Bernard <i>et al.</i> , 2009
SHEEP	210 g/d	EL	50	67	52	100	Zhang <i>et al.</i> , 2006a
	200 g/d	EL	213	294	11	153	Correddu <i>et al.</i> , 2016
	100 g/d	EL (+pasture)	28	42	245	48	Cabiddu <i>et al.</i> , 2017
	63 g/d	oil	308	447	-12	106	Bodas <i>et al.</i> , 2010
	41 g/d	oil	90	367	9	122	Zhang <i>et al.</i> , 2006b
	210 g/d	EL	198	207	0	250	Mele <i>et al.</i> , 2011

EL = extruded linseed; FO = fish oil; DMI = dry matter intake.

Table 2. Effects of dietary soybean and sunflower fat supplementation on the fatty acid profile of sheep and goat milk. Effects are expressed as the differences in percentage (%) of change between fat-supplemented group and non-supplemented control group

	Form	c9,t11 CLA	C18:1 t11	C18:2 n6	C18:3 n3	Reference
goats	SO	199	214	14	-10	Bouattour <i>et al.</i> , 2008
	SO+FO	609	466	34	13	Tsiplakou and Zervas, 2013
	SW	-33	-10	-50	-25	Chilliard <i>et al.</i> , 2003
	SUN	283	290	55	25	Chilliard <i>et al.</i> , 2003
	SUN-oil	590	792	31	-47	Ollier <i>et al.</i> , 2009
	SUN-oil	384	562	13	-33	Bernard <i>et al.</i> , 2009
SHEEP	SO	316	809	15	-38	Mele <i>et al.</i> , 2006
	SO	562	736	59	2	Bodas <i>et al.</i> , 2010
	SO	171	242	13	-23	Gómez-Cortés <i>et al.</i> , 2011
	SUN-seeds	130	67	78	56	Zhang <i>et al.</i> , 2006b
	SUN-oil	138	195	6	-12	Maia <i>et al.</i> , 2011
	SUN-oil	11	36	12	31	Castro <i>et al.</i> , 2009

SO = soybean oil; SW = whole soybean; SUN = sunflower.

IV – Conclusions

Based on the literature, it appears that the FA composition of the milk of sheep and goats can be largely modified and improved by feeding, with an increase in the concentration of beneficial FA. This modification can be induced both on pasture-fed and indoor-fed sheep and goats. Sheep seem to respond to the utilization of pasture with a larger increase in beneficial FA compared to goats, possibly because of the higher preference for legumes of the former. The utilization of vegetable oils as dietary supplements, in sheep and goats fed basal diets made of hay or silage, can also positively change the FA composition of milk. The responses to supplementation with various types of vegetable oils differ substantially between the two small ruminant species, with higher production of beneficial FA when linseed or soybean oil are used in sheep, and when sunflower oil is used in goats. It is not

clear if these differences depend on intrinsic species differences (e.g. feeding behavior or metabolism) or by the type of diets most commonly used in the two species. Clearly, more research is needed to determine all the factors affecting the extent and type of FA change in sheep and goat milk.

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