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Invited Review

Feeding strategies to design the fatty acid profile of sheep milk and cheese

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ABSTRACT - The majority of sheep milk produced in the world is transformed into cheese. Feeding is a major factor affecting the quality of sheep milk and, therefore, of sheep cheese. Because fat is the main compound of cheese, this review gives an update on the effects of feeding and nutrition on milk fat content and deeply discusses feeding strategies aimed at increasing the levels of healthy fatty acids (FA), such as conjugated linoleic acid and omega-3 FA, in milk and cheese in the human diet. In addition, the use of alternative feed resources such as by-products, aromatic plants, and phenolic compounds in the sheep diet and their effects on milk and cheese FA composition are also discussed. Among feeding strategies, grazing and the use of supplements rich in oils seem to be the best and the cheapest strategies to improve the nutritional value of the fatty acid profile in sheep cheese.

Key Words: by-products, cheese, CLA, dairy sheep, milk quality, nutrition

Introduction

The production of whole fresh sheep milk in the world accounts for approximately 1.4% of the global milk yield (9,584 MTON/year as average for 2007-2011; FAOSTAT, 2013). In many countries, sheep milk and milk products (e.g., yogurt and cheese) are produced using unspecialized breeds and are consumed locally, whereas in many Mediterranean (e.g., Italy, Spain, France, Portugal, Greece, Turkey, Israel) and East European countries (especially Bulgaria and Romania) sheep milk is often produced by specialized dairy breeds, and sheep milk products, especially cheese, are commercialized internationally. Taking into account the high quality of sheep milk, characterized by low allergenic activity and high concentration of nutraceutical compounds, and the relatively high price of sheep cheese, there is an interesting potential worldwide market for this industry, with a growing interest in various countries such as U.S., Brazil, and China. Because a lot of sheep milk is transformed into yogurt and cheese, milk quality is evaluated mainly in terms of its technological and coagulation properties, which depend primarily on fat and protein contents and on the somatic cell count (SCC). However, the increasing attention of consumers to the nutritional and health aspects of food has recently shifted the focus of dairy sheep producers towards the achievement of an appropriate milk lipid composition. Furthermore, more and more consumers are demanding dairy products with a special flavor associated with the territory where the animals live.

Dairy sheep breeds are usually raised using pasture as the main source of feedstuffs during lactation, even though indoor feeding is common in some countries, such as Spain and Israel. Due to the variability of pasture availability and quality, hay, silages and concentrates are commonly and largely used even in pasture-based systems, especially in flocks with high milk production (Molle et al., 2008).

The chemical composition of milk, in terms of fat content and its fatty acid (FA) composition, depends on dietary (composition and availability), animal (breed, lactation stage, body condition) and environmental (especially cold and heat stress) factors. Dietary factors that affect milk fat and protein content and cheese yield have been reviewed previously in detail by our research group (Pulina et al., 2006).

This review provides a specific update on studies regarding feeding strategies useful to design the fat content and its FA profile in sheep milk and cheese, including the use of alternative sources, such as by-products, aromatic plants and phenolic compounds.

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Milk fat content

Fat is present in milk as globules of different sizes where the core of triglycerides is enclosed in a triple-layer membrane. The number and diameter of fat globules in milk depends on physiological, environmental, and genetic factors (Martini et al., 2004)

More than 98% of lipids in ovine milk, as in the milk from other ruminant species, consist of triacylglycerols, composed of glycerol and three FA with different carbon chain lengths. The FA needed by the epithelial cells of the mammary gland for FA synthesis and secretion in milk are provided by plasma uptake or *de novo* synthesis. The *de novo* synthesis FA in milk originate from acetate and beta-hidroxybutyrate produced by rumen fermentation. These volatile FA are the main carbon sources for the secretory cells of the mammary gland involved in the *de novo* synthesis of short-chain FA (C4:0-C14:0) and a portion of C16:0. Differently, the remaining part of C16:0 and almost all of the long-chain FA C18:0-C22:0) in milk come from lipids circulating in blood, originated from absorption in the small intestine or mobilization of adipose tissue. In reality, the FA with a chain length varying from 14 to 18 carbons might be further modified in the mammary gland through the activity of desaturase enzymes. Moreover, the milk fat contains also odd and branched-chain fatty acids (OBCFA) derived mostly from the intestinal uptake of lipids from the membrane of bacteria leaving the rumen (Vlaeminck et al., 2006).

Fat concentration in milk can be changed by diet, especially by factors affecting rumen fermentation. Indeed, the acetate:propionate concentration ratio in the rumen fluid plays a fundamental role in the synthesis of milk lipids in ruminant species. The content and source of neutral detergent fiber (NDF) and non-fibrous carbohydrates (NFC; i.e., sugars, starch, soluble fiber) in the diet influence markedly the volatile fatty acid profile in the rumen. For optimal milk production and rumen function, Cannas et al. (2002) and Cannas (2004) recommend that the levels of NDF in the diet of dairy sheep should not be lower than 33 g of NDF/100 g of dry matter (DM), with an optimal range of NDF from 45 to 33 g/100 g of DM and of NFC from 28 to 38 g/100 g of DM as 6.5% fat corrected milk production goes from less than 0.5 kg/d to above 1.7 kg/d. The source of NDF and NFC might affect milk yield and fat concentration. By replacing NDF from coastcross hay with a highly fermentable NDF source like soybean hulls at increasing percentages (33, 67 and 100%), Araujo et al. (2008) found that milk yield and milk-fat yield increased with a quadratic effect, with maximum values at 67% of substitution rate, whereas milk fat concentration was not affected, suggesting that the fiber of soybean hulls stimulated milk fat synthesis more than the fiber of coastcross hay. Various studies have investigated the substitution of part of the dietary starch with highly digestible NDF from soybean hulls or beet pulps. In a study conducted in Brazil on Santa Inês ewes during the first three months of lactation, Gentil et al. (2011) substituted 20 or 40% of corn meal, in a diet that had 38% of this starchy feed, with soybean hulls (inclusion of 0, 7.6 and 15.1 g/100 g of DM). Milk yield and milk fat content were not affected by these treatments. In contrast, various experiments have shown that when NFC, mostly starch, from cereal components (ground corn and wheat grain; i.e., control diet) were replaced by NDF from soybean hulls or beet pulps during the second half of the lactation, ewes had a marked increase in milk and milk-fat yield (Cannas et al., 1998; Zenou and Miron, 2005; Cannas et al., 2013), and, surprisingly, also an increase in milk fat concentration, which was statistically significant for Cannas et al. (1998) and Zenou and Miron (2005) and only numerical for Cannas et al. (2013). These results suggest a marked ability of these by-products, rich in digestible fiber, to stimulate milk fat synthesis.

Milk fat content and composition can be also strongly affected by the energy balance (EB) of ewes, especially in early lactation. When the EB is negative, milk fat concentration and its concentration in long-chain-preformed FA increase due to the uptake of non-esterified FA derived from body fat mobilization from the mammary gland (Bocquier and Caja, 2001; Cannas and Avondo, 2002; Pulina et al., 2006).

The diet supplementation of dairy ewes with rumen-bypass fat, such as soaps of FA, can be a useful feeding strategy to increase milk fat concentration. In general, Ca salts of palm oil increased milk yield and milk fat percentage, at dietary levels ranging between 90 and 200 g/d, but tended to depress milk and fat yield at higher doses (Pulina et al., 2006). Milk fat content did not decrease when Ca salts of polyunsaturated fatty acid (PUFA) from tuna oil (Kitessa et al., 2003) or Ca soap of olive oil (Antongiovanni et al., 2002; Dobarganes García et al., 2005) were fed to ewes. The effect of linseed on milk fat content is controversial. In some studies linseed increased the milk fat content in mid-lactating goats (Nudda et al., 2013) and sheep (Caroprese et al., 2011), whereas in others extruded linseed supplementation to early lactating ewes (<30 days in milk, DIM) raised with their suckling lambs caused milk fat depression (Gómez-Cortés et al., 2014; Nudda et al., 2013).

Feeding strategies to manipulate the fatty acid profile of milk and cheese

The enrichment of sheep milk and cheese with FA having healthy properties, especially vaccenic acid (VA; C18:1 t11,), c9,t11 CLA, also named rumenic acid (RA), and α-linolenic acid (ALA; C18:3 n3), has acquired considerable relevance as a consequence of the encouraging results of human studies. For example, in clinically healthy subjects, the consumption of 200 g/week for 10 weeks of a sheep cheese naturally enriched with VA and RA (3.26 and 1.56 g/100 lipids, respectively) rather than a regular cheese with lower VA and RA concentrations (0.4 and 0.19 g/100 lipids, respectively) caused significant positive biochemical changes of atherosclerotic markers (Sofi et al., 2010). A recent study on hypercholesterolemic subjects showed that, when compared with a control cheese (1.5 g/100 g of fat), the intake of sheep cheese naturally enriched with conjugated linoleic acid (CLA; 2.5 g/100 g of fat) remarkably decreased the plasma concentrations of the endocannabinoid anandamide and decreased the LDL-cholesterol level by 7% (Pintus et al., 2013). This kind of cheese is currently available in the Italian market.

In dairy ewes, numerous studies have shown that the diet is the most important factor influencing the milk fatty acid profile and healthy FA content, which can be increased several-fold by dietary means. Fortunately, the milk processing does not change substantially its FA profile; therefore the FA concentrations in the fat of dairy products are primarily dependent on the fatty acid content of the unprocessed raw milk (Nudda et al., 2005). The major variation in milk FA composition has been reached by varying the amount and type of forages, especially pasture, in the sheep diet or by adding vegetable or marine oils to the diet, because these factors influence the process of biohydrogenation of dietary unsaturated fatty acids in the rumen.

Effects of pasture-based diets

Green pasture is an excellent source of ALA and is one of the most effective feeds in shifting the milk FA composition towards a healthy spectrum (Nudda et al., 2003; Gómez-Cortés et al., 2009a). The marked effects of pasture on the milk fat content of ALA, VA and CLA are related to the high content of ALA in green pasture, which is partly biohydrogenated into VA in the rumen and then secreted into milk and partially converted into c9,t11 CLA in the mammary tissue by the action of stearoyl-CoA desaturase. The milk FA composition (Table 1) was improved by increasing pasture availability (Nudda et al., 2003) or intake (de Renobales et al., 2012) or by including specific forage species in the pasture (Addis et al., 2005; Atti et al., 2006). As pasture intake increased, the milk content of ALA (R^2 = 0.69) and CLA (R^2 = 0.79) increased

Table 1 - Effects of pasture and forage-based diets on content of conjugated linoleic acid (c9,t11 CLA), vaccenic acid (VA; C18:1 t11), linoleic acid (LA; C18:2 n6) and α-linolenic acid (ALA; C18:3 n3) in sheep milk (g/100 g of FA)

Diet	c9,t11 CLA	C18:1 t11	C18:2 n6	C18:3 n3	References
High pasture	2.15	3.74	3.22	0.88	Nudda et al. (2003)
Low pasture	0.84	1.32	2.31	0.62	
HC-winter	2.35	4.00	2.74	1.62	Addis et al. (2005)
RY-winter	1.20	2.08	1.59	1.47	
BM-winter	2.30	3.24	2.35	2.19	
Sulla-winter	1.25	3.18	1.58	2.98	
HC-spring	2.33	3.13	3.48	1.26	Addis et al. (2005)
RY-spring	1.43	2.52	1.54	1.44	
BM-spring	1.65	2.25	2.82	1.84	
Sulla-spring	1.12	2.36	1.75	3.15	
Corn silage	0.82	4.94	4.97	0.42	Reynolds et al. (2006)
Alfalfa pellet	0.17	2.04	3.43	1.05	
Corn silage	0.64	2.13	3.00	0.26	Reynolds et al. (2006)
Alfalfa haylage	0.71	1.63	2.12	0.65	
Pasture	1.62	3.88	1.72	1.07	Gómez-Cortés et al. (2009a)
Pasture + oat grain	0.78	1.59	1.60	0.59	
No pasture	0.87	1.95	2.67	0.33	
No pasture	0.45	1.08	3.81	1.09	de Renobales et al. (2012)
0.49PI[4] + 0.9 AH	0.92	4.98	4.38	1.54	
1.09PI + 0.6 AH	0.96	3.25	3.42	1.57	
1.16PI + 0.3 AH	1.41	3.06	3.67	2.29	

HC - *Hedysarium coronarium*; RY - ryegrass; BM - Bermuda grass; PI - pasture intake (kg DM/d); AH - alfalfa hay (kg DM/d).

(de Renobales et al., 2012). In Sardinia (Italy), where most dairy sheep are fed on pasture, the concentrations of RA, VA and ALA in milk fat were the highest in late winter-early spring, when grass availability was the highest, and decreased as lactation progressed and pasture availability and quality decreased (Nudda et al., 2005).

Pasture plant species can have a marked effect on the milk FA profile. Legume-based pastures were associated with high levels of CLA and ALA and with low levels of saturated FA compared with ryegrass pasture (Addis et al., 2005). In addition, the intake of crown daisy (*Chrysanthemum coronarium* L.) favored the presence of high amounts of CLA in milk fat, and intake of sulla *(Hedysarum coronarium* L.) forage increased markedly the content of ALA in milk, reaching 3 g/100 g of fat (Addis et al., 2005).

When green pasture is not available, the forage source and physical form can change the content of healthy FA in milk fat. In fact, Reynolds et al. (2006) reported that the c9,t11 CLA concentration was higher and ALA was lower in milk of sheep fed corn silage than in that of sheep fed alfalfa pellets. When corn silage was replaced by alfalfa haylage, there was also an overall positive effect on FA profile, with an increase in ALA and c9,t11 CLA content in milk (Table 1).

Cabiddu et al. (2006) conducted an experiment on dairy ewes fed restricted concentrate and fresh forage from one of four species (crown daisy, *Chrysanthemum coronarium* L.; burr medic, *Medicago polymorpha* L.; annual ryegrass, *Lolium rigidum* Gaudin; and sulla, *Hedysarum coronarum* L.) *ad libitum*, during winter (i.e., February-March), and evaluated individual daily intake of ALA by the ewes, daily milk yield, fat concentration in milk and fatty acid composition of cheese. As expected, forage species had a strong effect on the fatty acid composition of cheese, as a consequence of the different feed fatty acid composition and intake in the different experimental groups. Based on data of Cabiddu et al. (2006), we estimated the daily production of VA and c9,t11 CLA in milk, assuming that the FA composition of cheese reflects that of raw milk, and obtained a linear relationship between the estimated total daily intake of ALA and the estimated daily amount of VA and c9,t11 CLA in milk (Figure 1). These results confirm that in lactating ewes green grass is a valuable source of ALA, which can be used as a tool to improve the amount of healthy fat compounds in ovine milk and cheese.

This implies that in dairy-sheep farming systems where grass is the basic constituent of diet, milk fat can have relevant amounts of healthy fatty acids, which can be affected by seasonal pasture availability and composition.

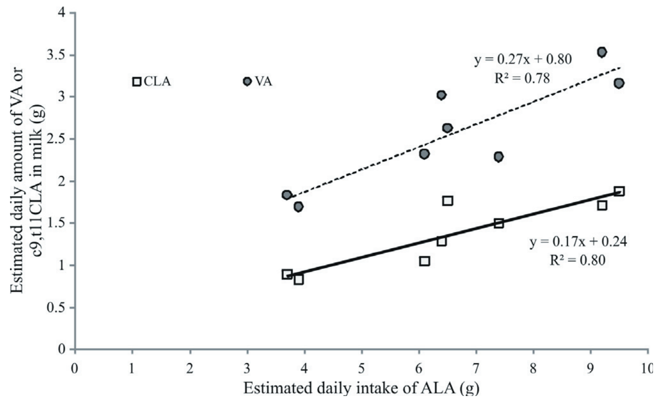


Figure 1 - Relationship between estimated daily amount (g) of c9,t11 conjugated linoleic acid (CLA) and vaccenic acid (VA) in milk and daily intake (g) of alfa-linolenic acid (ALA) in experimental groups of ewes feeding diet based on data from Cabiddu et al. (2006).

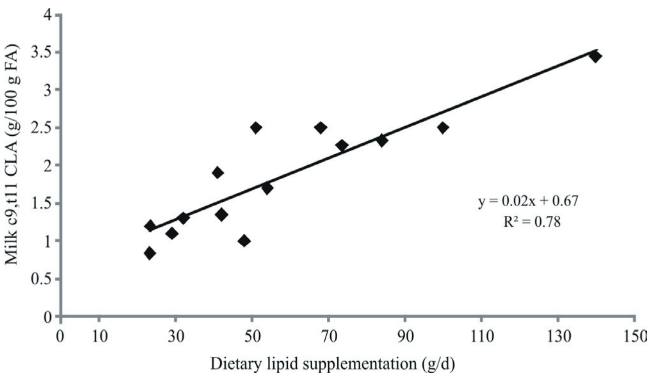
Effects of vegetable-oil supplementation

The addition of vegetable oils in the diet of dairy sheep is a valuable tool to enhance the dietary energy content and might influence the fatty acid composition of milk fat. This feeding strategy is particularly useful when the diet of ewes has a poor content and composition of fatty acids, i.e., when stored forages are used.

The CLA content in sheep milk is affected by the fat content, type of predominant fat and physical form of the dietary fat supplement.

When pooling data from different experiments (Luna et al., 2005; Zhang et al., 2006a,b; Gómez-Cortés et al., 2008, 2009a,b; Bodas et al., 2010; Mele et al., 2006, 2007, 2011), a positive linear relationship ($R^2 = 0.78$) was found between the amount of supplemented dietary fat rich in linoleic and linolenic acids and the CLA content in milk (Figure 2). In particular, a milk CLA content higher than 2 g/100 g fat but lower than 3 g/100 g fat was reached when dietary lipid intake ranged between approximately 50 and 100 g/d, whereas a milk CLA concentration higher than 3 g/100 g fat (i.e., 3.44 g/100 g fat) was reached with a very high dose of soybean oil (140 g/d) associated with a high-concentrate diet. This high value of CLA could be the consequence of a lower rumen biohydrogenation of linoleic acid and a higher rumen and duodenal flow of biohydrogenation intermediates, especially trans 11 C18:1 (Kucuk et al., 2004).

Linseed, soybeans, safflower and sunflower are the most commonly used sources of unsaturated plant lipids to enhance CLA and unsaturated FA content in milk fat, but their effects depend on the dose used (Table 2). Linseed supplementation increased the content of VA, c9,t11 CLA and PUFA n-3 without negative impact on milk yield and composition (Luna et al., 2008; Gómez-Cortés et al., 2009b; Bodas et al., 2010; Mele et al., 2011). Supplementation with soybean oil (Bodas et al., 2010; Gómez-Cortés et al., 2011b) was more effective than linseed oil (Zhang et al., 2006b) or sunflower oil (Castro et al., 2009) in increasing the milk CLA content in sheep milk and cheese. This suggests that ruminal biohydrogenation of unsaturated FA was more complete in ewes fed linseed than in those fed soybean and therefore the amount of VA and CLA flowing through the duodenum was higher in sheep fed diets supplemented with oils rich in linoleic acid than in sheep fed oils rich in linolenic acid, as already postulated for dairy cows (Lock and Garnsworthy, 2002; Bu et al., 2007). In addition, C18:2 c9, t11 CLA in milk originates partially from the ruminal biohydrogenation of C18:2 as an intermediate rumen product and mainly (from 64 to 98%) from endogenous synthesis from VA in the mammary gland by Delta-9-desaturase activity (Griinari et al., 2000; Piperova et al., 2002). The higher effectiveness of soybean oil than sunflower oil in increasing CLA needs to be confirmed, considering that both oils have a similar content of linoleic acid, which is their predominant fatty acid. Vegetable fat supplements are usually more effective in the form of free oil than in the form of seeds at increasing the milk CLA content. For example, 70 g of free linseed oil (Bodas et al., 2010)



Each point corresponds to a mean value of treatment.

Figure 2 - Relationship between dietary fat supplementation and sheep milk CLA content based on data from different experiments (Luna et al., 2005; Zhang et al., 2006a,b; Gómez-Cortés et al., 2008, 2009a,b; Bodas et al., 2010; Mele et al., 2006, 2007, 2011).

Table 2 - Effects of lipid supplementation on content of conjugated linoleic acid (c9,t11 CLA), vaccenic acid (VA; C18:1 t11), linoleic acid (LA; C18:2 n6) and α-linolenic acid (ALA; C18:3 n3) in sheep milk (g/100 g of total FA)

Diet	Dose, g/d	c9,t11 CLA	C18:1 t11	C18:2 n6	C18:3 n3	References
Alfalfa pellet	0	0.17	2.04	3.43	1.05	Reynolds et al. (2006)
+ SO-MO	162	0.27	6.61	3.85	0.99	
Silage	0	0.82	4.94	4.97	0.42	
+ SO-MO	147	0.90	9.29	4.63	0.53	
Control	0	1.0	0.9	2.3	0.9	Zhang et al. (2006a)
Linseed	210	1.5	1.5	3.5	1.8	
Sunflower seed	182	2.3	1.5	4.1	1.4	
Linseed oil	23	1.2	3	2.3	1.6	Zhang et al. (2006b)
	32	1.3	3.8	2.5	1.7	
	41	1.9	4.2	2.5	2	
Control	0	0.55	0.88	2.71	0.32	Castro et al. (2009)
Sunflower oil	28	0.61	1.20	3.04	0.42	
HIDROPALM[1]	28	0.63	0.92	2.92	0.43	
Palm oil	63	0.39	0.78	1.99	0.52	Bodas et al. (2010)
Olive oil	63	0.91	2.08	1.51	0.36	
Soybean oil	63	2.58	6.52	3.17	0.53	
Linseed oil	63	1.59	4.27	1.76	1.07	
Soybean oil	0	0.83	1.88	2.49	0.38	Gómez-Cortés et al. (2011b)
	17	1.39	3.87	2.63	0.31	
	34	2.40	6.94	2.87	0.29	
	51	2.95	8.50	2.95	0.28	
Control	0	0.53	1.07	6.58	0.34	Maia et al. (2011)
Canola oil	3%	1.29	3.45	6.91	0.30	
Sunflower oil	3%	1.26	3.16	7.00	0.30	
Castor oil	3%	0.54	1.10	7.68	0.31	
Control	70	0.37	0.85	2.40	0.31	Gómez-Cortés et al. (2014)
Extruded linseed	70	0.89	3.03	1.81	0.94	

SO - sunflower oil; MO - marine oil.
[1] HIDROPALM (NOREL, SA, Madrid, Spain).

was more effective in increasing c9,t11 CLA than 70 g of fat from extruded linseed (Gómez-Cortés et al., 2014). This is likely because when oil is inside intact seeds it is released gradually whereas when it is given as free oil it is immediately available in the rumen. In addition, based on studies performed in dairy cows (Chilliard et al., 2009), processed dietary lipid sources such as extruded, rolled, micronized, or roasted seeds are normally more effective at increasing milk CLA content than raw seeds, probably because raw seeds disperse their oil content in the rumen more slowly and less completely than processed seeds, with a lower impact on the rumen environment (Mughetti et al., 2007; Doreau et al., 2009).

Effects of marine oil sources

Among all types of fat source supplements, marine oil resulted in the highest CLA, VA and omega-3 FA concentration in sheep milk (Table 3). In fact, protected fish oil supplemented at 30 and 45 g/d caused a marked increase in the CLA content in sheep milk (Mozzon et al., 2002). Despite being poor in CLA precursors, marine oil is effective in increasing milk VA and CLA through the inhibition of the reduction of VA to 18:0 by rumen bacteria.

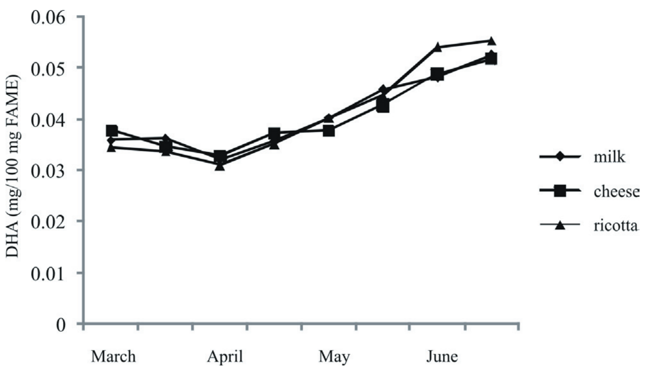
Supplementation of rumen-protected marine oil, which is rich in eicosapentaenoic acid (EPA; C20:5 n3) and docosahexaenoic acid (DHA; C22:6 n3), was able to noticeably increase the concentration of these long-chain FA in milk fat compared with that of milk from untreated sheep (Mozzon et al., 2002; Kitessa et al., 2003; Ferreira et al., 2011), even if the carry-over from feed to milk was only 20% (Mozzon et al., 2002; Kitessa et al., 2003). Nevertheless, the transfer of EPA and DHA from the diet into milk was markedly higher than that reported for dairy cows (see review of Lock and Bauman, 2004) and dairy goats (Kitessa et al., 2001). Unfortunately, the enhancement of PUFA omega-3 long-chain fatty acids (LC-PUFA) such as EPA and DHA in milk by the diet is difficult because of the high level of biohydrogenation of these FA in the rumen, which has been estimated to be 72% for DHA in sheep (Wachira et al., 2000). Another reason for this extensive biohydrogenation is the fact that these LC-PUFA are not transported with the plasma lipid fractions (TG and NEFA), which are the main mammary sources of FA uptake (Lock and Bauman, 2004).

In lactating ewes, there was a linear increase in milk EPA (from 4 to 21 g/kg) and DHA (from 43 to 124 g/kg) content as the dose of algae in the diet increased (23.5, 47 and 94 g/d), whereas those FA were not detected in the milk from control ewes (Papadopoulos et al., 2002). In addition, the evaluated dairy products, i.e., feta cheese and yogurt, had a similar polyunsaturated FA profile to that of the EPA- and DHA-enriched milk (Papadopoulos et al., 2002), evidencing that the milk processing did not alter the concentration of this LC-FA of nutritional interest. This is also supported by our data on DHA content in milk, cheese and ricotta sampled from two processing plants in Northern Sardinia from March to June 2004 (Figure 3), which evidenced that the DHA in dairy products reflects the FA composition of raw milk used for cheese making.

Table 3 - Effects of marine source supplement on content of conjugated linoleic acid (c9,t11 CLA), α-linolenic acid (ALA; C18:3 n3) and long-chain fatty acids, eicosapentaenoic acid (EPA; C20:5 n3) and docosahexaenoic acid (C22:6 n3) in sheep milk

Diet	c9,t11 CLA	C18:3 n3	C20:5 n3	C22:6 n3	References
Control	0.68	0.39	0.04	0.00	Mozzon et al. (2002)
30 g/d fish oil	1.67	0.46	0.10	0.20	
45 g/d fish oil	2.87	0.42	0.17	0.36	
Control	nr	nr	0.04	0.04	Kitessa et al. (2003)
6 g/d docosahexaenoic acid	nr	nr	0.47	1.90	
Control	0.44	0.53	0.10	0.03	Toral et al. (2010)
25 g sunflower oil + 0 g marine algae	1.23	0.41	0.07	0.02	
25 g sunflower oil + 8 g marine algae	2.78	0.37	0.10	0.17	
25 g sunflower oil + 16 g marine algae	2.58	0.36	0.13	0.46	
25 g sunflower oil + 24 g marine algae	3.22	0.34	0.15	0.57	
0% fish oil + 4% soybean oil[1]	1.35	0.26	nd	nd	Ferreira et al. (2011)
0.25% fish oil + 3.75% soybean oil	1.49	0.26	0.03	0.02	
0.50% fish oil + 3.50% soybean oil	1.65	0.26	0.03	0.03	
0.75% fish oil + 3.25% soybean oil	1.45	0.24	0.04	0.03	
25 g fish oil/kg DM	2.42	0.49	0.04	0.05	Bichi et al. (2013)
25 g soybean oil + 8 g marine algae/kg DM	3.99	0.48	0.06	0.38	

nd - not detected; nr - not reported.
[1] Diet supplemented with 4% of fat



DHA - docosahexaenoic acid; FAME - fatty acid methyl esters.

Figure 3 - Seasonal evolution of DHA (mg/100 mg FAME) in Sarda sheep milk, cheese and ricotta sampled every two weeks from March to June 2004 in two milk-processing plants located in North Sardinia, Italy (our data).

Effects of the forage-to-concentrate ratio and of the types carbohydrates

The forage-to-concentrate (F:C) ratio in the diet seems to be less efficient in changing the CLA content in dairy-sheep milk (Antongiovanni et al., 2004; Gómez-Cortés et al., 2011a) than in dairy-cow milk (Nudda et al., 2007). In fact, dairy sheep fed diets containing 75:25 and 60:40 forage (hay)-to-concentrate ratios had little change in milk VA and CLA concentrations (Antongiovanni et al., 2004). In addition, diets with increasing F:C ratios (30:70, 50:50, and 70:30) decreased VA and ALA concentration, but did not influence c9,t11CLA in sheep-milk fat (Gómez-Cortés et al., 2011a). When dairy ewes were fed diets with high NFC instead of low NFC (430 *vs.* 280 g/kg of DM) the content of VA and c9,t11 CLA in milk increased (Nudda et al., 2004), indicating that when the NFC in the diet increases, the biohydrogenation of unsaturated FA in the rumen probably slows down and a higher amount of unsaturated FA escapes the rumen reaching the mammary gland. This also suggests that the lower sensitivity of dairy sheep, compared with cattle, to variations in the forage-to-concentrate ratio is because dairy sheep usually have higher passage rate and thus lower starch fermentation and higher escape of concentrates from the rumen than dairy cows (Cannas et al., 2003).

Effects of tannin extracts and tannin-rich forage

The utilization of tannin-rich feeds or extracts has gained interest to modulate rumen ammonia production (Deaville et al., 2010; Theodoridou et al., 2010) or methane (Liu et al., 2011), to reduce methane emissions, or to limit intestinal parasites (Houdijk et al., 2012). Because tannins certainly affect ruminal fermentation, they might also alter milk and cheese FA composition (Vasta et al., 2008).

In this regard, Cabiddu et al. (2009) reported that grazing *Hedysarum coronarium*, a tannin-rich forage, enhanced the content of ALA but decreased that of VA and RA in sheep milk and cheese. The addition of a commercial mixture of quebracho (reddish brown wood trees of the genus *Schinopsis*, especially *S. lorentzii*) and chestnut tannin extracts (10 g/kg of DM per day) to a diet containing sunflower oil was not efficient in enhancing VA and CLA in sheep milk (Toral et al., 2011). In another study, two doses (20 and 40 g/d per ewe) of quebracho and chestnut commercial tannin extract had no effects on milk yield and composition (Castañares et al., 2011). In a recent study on the addition of quebracho tannin extract (20 g/d per ewe) to a diet containing soybean oil, it was confirmed that this source of polyphenols was not able to improve the milk FA composition in sheep milk (Toral et al., 2013). In an experiment conducted by Pulina et al. (2010), the inclusion of a commercial extract of chestnut tannins in the diet of sheep grazing on pasture caused an accumulation of linoleic acid (LA;C18:2 n6) and ALA in the rumen. Therefore, all these findings show that tannins are not effective in increasing VA and CLA in milk fat, probably because of the lowering effects of tannins on the rumen microbial activity and on the rumen biohydrogenation process.

Effects of olive by-products

In the review by Molina-Alcaide and Yáñez-Ruiz (2008) on the utilization of olive by-products, it was evidenced that the inclusion of by-products from olive trees and olive oil extraction in the diets offered to sheep caused a decrease in short- and medium-chain FA and a marked increase in long-chain FA in milk, mainly due to the increase in oleic acid in milk fat. The inclusion of olive leaves and grape marc increased the concentration of PUFA n3, VA and CLA content in sheep milk fat (Tsiplakou and Zervas, 2008), but the high levels of Cu in olive leaves could restrict the use of this by-product in sheep feeding (Molina-Alcaide and Yáñez-Ruiz, 2008).

Essential oils

Recently, the use of essential oils (EO) in lactating ruminants has increased because of their antimicrobial properties.

Few studies have evaluated the effects of dietary supplementation of commercial complexes, plant extracts or unprocessed leaves containing EO on sheep milk yield and composition. In a study of Giannenas et al. (2011), a commercial EO complex (Crina), containing thymol, eugenol, vanillin, guaiacol, and limonene as main components, was used at three dosages. Milk yield was not significantly affected at the lowest dosage (0.075 g/d per head of EO complex) but markedly and progressively increased above this dosage (+20% and +35% for 0.15 and 0.217 g/d per head of EO complex, respectively). Interestingly, in this study there was also a marked reduction of somatic cell count for all dosages considered, suggesting that this was one of the causes of the increase in milk yield. Despite the marked increase in milk yield, milk fat and protein concentrations were not reduced. In a study conducted by Chiofalo et al. (2012), a natural extract of *Rosmarinus officinalis* L., composed of rosmarinic acid, carnasol, and carnosic acid, supplied at the dose of 0.6 g/ewe per day did not change milk yield and reduced fat content, whereas at the dose of 1.2 g/ewe per day it increased milk yield and lactose content, but reduced protein content by 1.6 g/kg of milk.

In lactating Sarda dairy ewes, leaves of different aromatic plants (*Melissa officinalis* L., *Ocimum basilicum* L. and *Thymus vulgaris* L.) were used as source of essential oils and were tested at three dosages (50, 125 and 200 g/d, DM basis) by Manca et al. (2012). Milk yield was not affected, whereas milk fat concentration was the highest for *Melissa officinalis* L. in comparison with the other two plants. All milk FA groups were affected by treatments, except for the trans FA and the branched-chain FA (BCFA) (Table 4; Manca et al., 2012). As the dose of aromatic plant increased, the BCFA increased, suggesting that these plants favor microbial rumen activity, and PUFA n3 and the sum of CLA isomers also increased, suggesting a reduction of the biohydrogenation process, considering that these FA are intermediates of this process.

These results are in agreement with studies carried out in goats. In lactating Damascus goats, Kholif et al. (2012) found that supplementation with garlic oil, cinnamon oil or ginger oil increased unsaturated FA and c9,t11 CLA, and that supplementation with cinnamon oil also increased C18:3 n3. Miri et al. (2013) reported that cumin (*Cuminum cyminum* L) seed extract fed at two doses (12.7 and 25.3 g/kg DM) to lactating goats caused an increase in PUFA and CLA in the milk. Boutoial et al. (2013) supplemented extracts of rosemary leaves (*Rosmarinus officinalis* spp.) to lactating Murciano-Granadina goats and observed that PUFA increased as the dosage (unclearly defined in the paper) of extracts increased.

Interestingly, the studies in dairy cattle do not support the effects observed in small ruminants. Indeed, Benchaar et al. (2007) found on dairy cattle that the profile of milk fatty acids of cows was not influenced by the supplementation with 750 mg per day of a mixture of EO compounds. Similarly, Hristov et al. (2013) did not observe variations in milk fatty acids when supplementing the diet of lactating cows with three dosages of *Origanum vulgare* leaves.

Therefore, it appears that in the few studies available on dairy cows milk fatty acids were not affected by the supply of EO, whereas in all studies on lactating ewes and goats an increase in the unsaturation of FA and of CLA was observed, suggesting that EO can reduce the biohydrogenation process, potentially improving the nutraceutical value of the milk. This difference related to animal species could be a result of the high feed rumen passage rate of small ruminants, in comparison with that of large ruminants, which could limit the ability or the need of rumen bacteria to complete the biohydrogenation process.

Table 4 - Effects of plant, period and dose on proportions of sheep-milk fatty acids (g/100 g of FAME)[1]

Item	Fatty acids, g/100 g of FAME						
	Σ SFA	Σ MUFA	Σ BCFA	Σ PUFA n3	Σ PUFA n6	Σ TFA	Σ CLA
Plant species							
Melissa officinalis	77.45a	18.15b	2.71	0.89b	2.06b	1.26	0.86b
Ocimum basilicum	76.42ab	18.94ab	2.68	0.98b	2.11b	1.11	0.84b
Thymus vulgaris	75.79b	19.17a	2.72	1.04a	2.42a	1.18	0.93a
P for plant	0.001	0.03	NS	0.001	0.001	NS	0.02
Dose, g/d per ewe							
200	76.67	18.36	2.89a	1.10a	2.24	1.30	0.99a
125	76.13	19.09	2.78ab	1.01ab	2.19	1.28	0.94ab
50	76.45	18.87	2.60b	0.93bc	2.24	1.03	0.89bc
0	76.97	18.68	2.54b	0.85c	2.13	1.12	0.82c
P for dose	NS	NS	0.001	0.001	NS	NS	0.001

FAME - fatty acid methyl esters; SFA - short-chain fatty acids; MUFA - monounsaturated fatty acids; BCFA - odd branched-chain fatty acids; PUFA - polyunsaturated fatty acids; TFA - trans fatty acids; CLA - conjugated linoleic acid.
a,b,c - Within a column, means without a common letter differ (P<0.05).
[1] Manca et al. (2012).

Conclusions

Feeding is the main strategy to improve the quality of sheep milk. This review points out the noticeable ability of the diet composition to modify the fat content and fatty acid profile in sheep milk. Pasture is the main and the cheapest tool to improve the milk fatty acid profile as long as the diet is well-formulated to reach the desired milk nutritional goals. Interestingly, the fiber source and level can be important factors influencing the effect of different fat supplements on fatty acid, such as vaccenic acid, conjugated linoleic acid and polyunsaturated fatty acids of the n3 series, with positive effects on human health. Tannins are not effective in increasing vaccenic acid and conjugated linoleic acid in sheep milk fat. More studies are needed to evaluate the effects of a large number of agroindustrial by-products from different origin and with different content of biochemical compounds on milk and cheese fat quality, in order to promote their use in dairy sheep nutrition in a sustainable and profitable manner. Recent findings indicate that essential oils can improve the fatty acid composition of milk, by interfering with the biohydrogenation process of fatty acids.

From a practical point of view, enough information is currently available to adopt feeding strategies aimed at improving the nutraceutical properties of sheep milk and cheese, as demonstrated by the presence in the market of Pecorino cheese naturally enriched with conjugated linoleic acid. Thus, in the near future it might be possible to design dairy products with a desirable spectrum of fatty acids.

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