1 Introduction

Milk fat secretion and milk fatty acid (FA) composition are of great interest with regard to human nutrition. Apart from their contribution to dairy products’ sensorial quality and to the amount of dietary energy, different lipid and FA compounds (short- and medium-chain saturated, branched, mono- and polyunsaturated, cis and trans, conjugated FA, etc.) present in ruminant milk fat are indeed potentially positive or negative factors for the health of consumers [1–3].

Dairy products provide indeed 25–60% of the overall saturated fat consumption in Europe, which makes them, since decades, a target of dieticians’ criticism due to the negative effects of excessive consumption of saturated FA on human health [4]. The image of saturated FA should, however, be weighed by the fact that C_12–C_16 saturated FA are thought to be atherogenic only when consumed in excessive amounts, that 18:0 has no atherogenic effect and that saturated fat could even be protective when compared to a low-fat, high-carbohydrate diet [5, 6]. The allegedly atherogenic effect of certain trans monounsaturated FA (MUFA) [7] has not been confirmed for vaccenic acid (11-18:1), the main isomer present in milk [8, 9]. The intake of some trans isomers of 18:2 seems to be particularly harmful, although further research is needed to discriminate between industrial and ruminant isomer profiles [10]. In other respects, it has been shown in humans that the consumption of milk fat [11] could sometimes decrease cardiovascular and/or metabolic syndrome risk factors. Branched-chain FA, such as iso-15:0, anteiso-15:0 and iso-16:0, have been shown to present anti-cancer activity in human breast cancer cell models [12]. The experience of the Mediterranean diet increased the interest in oleic acid (c9-18:1) intake [13]. The interest in increasing the n-3/n-6 ratio of polyunsaturated FA (PUFA) has been confirmed [14]. During the last decade, it has been shown in animal models and/or human cell line cultures that c911-CLA, the main natural isomer of conjugated linoleic acids (CLA), and possibly t9t11-CLA, exhibit several interesting features, especially for the prevention of certain forms of cancer [3, 15–17]. Finally, any evaluation of the effect of dairy products on human health is to be considered in relation to the combined effects of milk FA profile, amount of dairy fat intake, its duration, and interactions between different milk FA as well as between these FA and the rest of the diet (macro- and micronutrients from vegetables, fruits, oils, meat, fish, soft drinks, wine, etc.).

Keywords: Diet composition, biohydrogenation intermediates, mammary metabolism, fatty acid desaturation, milk fatty acids.
Whatever the future conclusions of human nutrition studies on the specific effect of the different milk FA, and their interaction with the basal diet, there is a great challenge for scientists working on ruminants to be able to modulate the milk FA composition. The ruminant milk FA composition is linked to intrinsic (animal species, breed, genotype, pregnancy and lactation stages) or extrinsic (environmental) factors \[18, 19\]. In a given animal species, the effects linked to breed or genotype are significant but restricted and they can only be achieved over long terms. The effect of the lactation stage on milk fat content and FA composition is noticeable and mainly linked to body fat mobilization in early lactation \[20\], but it only lasts a few weeks each year. Seasonal effects are very large and mainly due to changes in feeding factors.

Nutrition therefore constitutes a natural and economical way for farmers to markedly and rapidly modulate the milk FA composition. The largest changes can be obtained either by changing the forages in the diets of ruminants, particularly pasture, or by adding plant or marine lipid supplements to the diet \(\text{e.g.} [19, 21–23]\).

The following review pays particular attention to recent studies on the impact of different diets on the main FA classes of interest for human nutrition (saturated and cis MUFA, trans MUFA and PUFA, including CLA) in bovine and caprine milk fat. The goat responds very differently to dietary factors than the cow, both in milk fat secretion and in some aspects of milk FA composition \[24, 25\], which is of interest for consumers of caprine dairy products and provides an interesting model for mechanistic studies on milk fat secretion and composition. After a short presentation of the digestive and metabolic background, this review presents data on the effects on milk FA of forage sources (Section 3) and lipid supplements (Section 4), as well as forage-concentrate-lipid interactions and persistency of milk FA responses (Section 5).

2 Digestion and metabolism of dietary FA

The FA present in forages, cereals, and oil seeds are mainly 18-carbon PUFA \(18:2n-6\) and \(18:3n-3\), whereas some oil seeds are rich in MUFA (mainly \(c9-18:1\)) and marine products (fish oil, algae, etc.) are rich in long-chain PUFA [mainly \(20:5n-3\) (EPA) and \(22:6n-3\) (DHA)]. These dietary FA are extensively metabolized and biohydrogenated in the rumen, resulting not only in the production of \(18:0\) but also a wide range of isomers of PUFA and MUFA, especially trans and conjugated FA \[26, 27\] (Fig. 1). These intermediates of ruminal biohydrogenation (RBH) vary largely with changes in diet composition, as demonstrated by their appearance in milk fat under various conditions \(\text{e.g.} [28–31]\). Apart from being absorbed in the gut and directly secreted into milk, some RBH intermediates are transformed by body tissues, especially by the mammary gland (Fig. 2) where the \(\Delta-9\) desaturase \(\text{[stearoyl-CoA desaturase (SCD)]}\) acts by adding a \(c9\)-double bond on different FA \[27, 32\], which partly reverses the effect of RBH and decreases the saturation level and the melting point of milk fat \[33\]. Furthermore, RBH intermediates act as regulators or disruptors of mammary lipogenesis, which results in changes in the amount of secreted milk fat but also in milk FA composition \(\text{e.g.} [34]\), Shingfield and Griinari, this issue), including short- and medium-chain de novo synthesized FA (Fig. 2). Finally, rumen-escaped dietary PUFA and \(18:0\) produced in the rumen can be seen as residual precursors and the end-product of RBH, respectively. Thus, RBH modifies yields and/or interacts with all milk FA and plays a crucial role in the interaction between ruminant diet and mammary FA synthesis and secretion.

Fig. 1. Main putative RBH pathways. When cis or trans configurations are not mentioned, it means that the various cis-cis, cis-trans and trans-trans configurations could exist. Thick arrows represent the major pathways \[26\]; thin arrows represent other putative pathways, as suggested by increases in the corresponding isomers during \textit{in vitro} incubations of pure linoleic and linolenic acids \[46\]; dotted arrows represent RBH pathways including unknown 18:3 isomer intermediates. Not all putative FA are mentioned, and the numerous interconversions among 18:1 isomers \[47, 48\] are not represented.
2.1 RBH and lipid digestion

The disappearance of 18:3n-3 and 18:2n-6 in the rumen averages 93 and 85%, respectively (review [35]). The extent of RBH depends to a low extent on the amount or the nature of the lipids in the diet, except when they are protected against microbial attack [36]. Besides FA trapping in vegetable cells, the main factor in the variation of RBH is the percentage of concentrate in the diet. Diets containing more than 70% concentrates strongly reduce RBH [37–39]. This is probably due to a low pH, which has been shown to limit the rate of lipolysis [40] and, for linoleic acid, the isomerization and the second reduction, leading to the accumulation of vaccenic acid [41].

Although Harfoot and Hazlewood [26] described only a limited number of pathways that result in a small number of isomers, it has long been known [42] that rumen digesta and bacteria contain a wide variety of cis- and trans-18:1 isomers (Fig. 1). Several isomers of CLA were also described. Specific isomers can be associated to dietary PUFA: 18:3n-3 metabolism results in the production of a series of intermediates (mainly c9t11c15:18:3, f9t12t15:18:3, t11c15:18:2, t11t13-CLA, t11c13-CLA, t11-18:1, t13-18:1) whereas 18:2n-6 metabolism results in the production of 8,10CLA, 9,11-CLA, t10c12;CLA and mainly t10-18:1. This has been shown in vivo with lipid supply from linseed or sunflower [39, 43, 44], and more specifically in vitro with pure FA [45, 46]. Using labeled c9-18:1 and t9-18:1, it has been shown that these FA may be converted into a large number of 18:1 isomers [47, 48]. Besides the nature of dietary FA, the diet composition is a major determinant of the composition of RBH intermediates. A high proportion of concentrates together with an 18:2n-6 supply results in a shift of t11-18:1 to t10-18:1 in the rumen [39, 49].

Dietary polyunsaturated 20- and 22-carbon FA as EPA and DHA are extensively biohydrogenated and result in a large number of unsaturated FA and a small number of saturated FA [50, 51]. In addition, these FA inhibit the RBH of 18-carbon (C18) PUFA [52, 53].

CLA are present in very small amounts in digestive contents (less than 0.5% of total FA). This low concentration is due to rapid biohydrogenation, and c9t11-CLA always represent less than half the total CLA. The t10c12 isomer is generally either undetectable or present in very small amounts (e.g. [39, 49]).

2.2 Duodenum-plasma-milk relationships

Most FA reaching the duodenum are unesterified. They are absorbed in the intestine and can be Δ-9 desaturated in the enterocyte [54], but only to a limited extent. They are then esterified in the enterocyte and exported to the other organs as chylomicrons and very-low-density lipoproteins. When duodenum and plasma total FA profiles are compared, the essential and non-essential FA differ: proportions of 18:2n-6 and 18:3n-3 are largely higher in plasma and their proportions are only remotely linked to their amount in the duodenum [55]. As a consequence, non-essential FA such as trans-18:1 and several 18:2 isomers have lower proportions in plasma FA than their proportions in duodenum FA, but there are close linear relationships between plasma and duodenal proportions. The profile of total plasma FA reflects a mean of several lipid classes differing substantially in their FA profile and metabolism. Thus, FA profiles of triacylglycerols and free FA (the main sources of plasma FA for the mammary gland) are richer in 18:0 and DHA are extensively biohydrogenated and result in a large number of unsaturated FA and a small number of saturated FA [50, 51]. In addition, these FA inhibit the RBH of 18-carbon (C18) PUFA [52, 53].
When calculating the transfer efficiencies of \textit{trans}-18:1 and non-conjugated PUFA from duodenum to milk in Figs. 3 and 4, mean values are similar among the different FA. However, they differ largely between diets, with high values (62–71\%) when milk fat content is regular (33–34 g/kg, with the two high-forage diets) and low values (24–44\%) when milk fat content is low (22–27 g/kg, with the five low-forage diets). This could be explained [55] by the fact that the mammary 18-carbon FA uptake and secretion was limited when \textit{de novo} FA synthesis was decreased by milk fat-depressing diets (see Sections 3–5). Nevertheless, when one compares the profiles of duodenum and milk 18-carbon FA, the relations are very close for all FA, both essential and non-essential [55]. As an example, there is a close linear relationship between the proportion of t11-18:1 in the duodenum and its proportion in milk 18-carbon FA, with a slope of 0.73 (Fig. 5). The proportion of c9t11-CLA in milk is also closely related to the proportion of t11-18:1 in the duodenum, illustrating the post-ruminal origin of most c9t11-CLA, \textit{i.e.} from mammary desaturation of t11-18:1 of dietary origin [27, 51, 55, 59]. The ratio of the two slopes equals 0.44, which is close to the 0.38–0.43 c9t11-CLA/t11-18:1 ratio in cow milk [60–62]. The similarity of the C\textsubscript{18} profiles between duodenum and milk is also exemplified in Figs. 3 and 4. The pattern of the duodenal flows of \textit{trans}-18:1 differs according to dietary factors (source of added lipids in this case [39, 43]), \textit{i.e.} mainly t11-1 and t13-016-18:1 for linseed oil supplementation \textit{versus} mainly t10- and t11-18:1 for sunflower oil- and fish oil-supplemented diets. These differences are closely reflected in the pattern of milk FA secretion. The similarity between duodenum and milk FA patterns also applies to PUFA, except c9t11-CLA which is largely synthesized in the mammary gland from t11-18:1 uptake (Figs. 3–5). In the comparison between duodenum and milk FA, the differential behavior between essential and non-essential FA observed for plasma does not exist: for a similar forage/concentrate ratio, secretion of 18:2n-6, t11c15-18:2 and 18:3n-3 in milk is proportional to duodenal flow [55]. The different behaviors of essential FA somewhat confuse the similarity between plasma and duodenal FA profiles, and thus in particular the putative use of plasma profiles as a predictor of duodenal profiles. This does not

<table>
<thead>
<tr>
<th>FA</th>
<th>Mean proportion in plasma (x)</th>
<th>Mean proportion in milk (y)</th>
<th>Milk/plasma ratio (y/x)</th>
<th>Equation</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c9t11-CLA</td>
<td>0.22</td>
<td>1.70</td>
<td>7.7</td>
<td>( y = 6.69x + 0.21 )</td>
<td>0.81</td>
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<tr>
<td>t10-18:1</td>
<td>0.52</td>
<td>2.58</td>
<td>5.0</td>
<td>( y = 4.95x + 0.01 )</td>
<td>0.93</td>
</tr>
<tr>
<td>c9t13-18:2</td>
<td>0.12</td>
<td>0.46</td>
<td>3.8</td>
<td>( y = 2.98x + 0.09 )</td>
<td>0.61</td>
</tr>
<tr>
<td>t11c15-18:2</td>
<td>0.37</td>
<td>1.28</td>
<td>3.5</td>
<td>( y = 2.90x + 0.20 )</td>
<td>0.96</td>
</tr>
<tr>
<td>t11-18:1</td>
<td>1.99</td>
<td>3.73</td>
<td>1.9</td>
<td>( y = 1.50x + 0.74 )</td>
<td>0.86</td>
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<tr>
<td>t13-14-18:1</td>
<td>0.78</td>
<td>1.34</td>
<td>1.7</td>
<td>( y = 2.48x - 0.58 )</td>
<td>0.89</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>9.33</td>
<td>1.00</td>
<td>0.11</td>
<td>( y = 0.06x + 0.40 )</td>
<td>0.38</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>42.10</td>
<td>2.22</td>
<td>0.05</td>
<td>( y = 0.08x - 1.19 )</td>
<td>0.48</td>
</tr>
<tr>
<td>Goat</td>
<td></td>
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</tr>
<tr>
<td>c9t11-CLA</td>
<td>0.79</td>
<td>2.34</td>
<td>3.0</td>
<td>( y = 2.42x + 0.44 )</td>
<td>0.83</td>
</tr>
<tr>
<td>c9t13-18:2</td>
<td>0.23</td>
<td>0.41</td>
<td>2.0</td>
<td>( y = 1.48x + 0.07 )</td>
<td>0.72</td>
</tr>
<tr>
<td>t11c15-18:2</td>
<td>0.53</td>
<td>1.35</td>
<td>2.8</td>
<td>( y = 2.18x + 0.20 )</td>
<td>0.87</td>
</tr>
<tr>
<td>t11-18:1</td>
<td>4.28</td>
<td>5.69</td>
<td>1.3</td>
<td>( y = 1.26x + 0.29 )</td>
<td>0.91</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>6.00</td>
<td>1.28</td>
<td>0.22</td>
<td>( y = 0.23x - 0.13 )</td>
<td>0.89</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>24.65</td>
<td>2.07</td>
<td>0.09</td>
<td>( y = 0.09x - 0.07 )</td>
<td>0.55</td>
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</tbody>
</table>
Fig. 3. Duodenal flows and milk secretion of main trans-18:1 in dairy cows (g/day) (adapted from [29, 39, 43, 59]). Experiment 1: F = forage (grass hay); linseed oil at 3% diet DM. Experiment 2: diet contained 35% forage (grass hay).

Fig. 4. Duodenal flow and milk secretion of main 18:2 isomers and 18:3 n-3 in dairy cows (g/day) (adapted from [29, 39, 43, 59]). Experiment 1: F = forage (grass hay); linseed oil at 3% diet DM. Experiment 2: diet contained 35% forage (grass hay).
increase due to an alternative pathway for RBH of 18:2 n-

blob and the triacylglycerols are secreted as milk fat glo-

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Increasing due to an alternative pathway for RBH of 18:2 n-

denoted, which decrease de novo either from dietary FA absorption or from body fat mobili-

These different lipogenic pathways are regulated in part by long-chain FA (including MUFA and PUFA) originating either from dietary FA absorption or from body fat mobilization, which decrease de novo FA synthesis [20, 69, 70]. Furthermore, a crucial role has been proposed recently for trans FA in milk fat depression ([34], Shingfield and Grinari, this issue). These FA are linked to alterations in PUFA RBH, especially t10-18:1 and t10c12-CLA which increase due to an alternative pathway for RBH of 18:2n-6, when rumen pH decreases. A curvilinear relationship between milk fat depression and small increases in milk t10c12-CLA was indeed observed in some studies [30, 34, 71, 73] but not in others [29, 72]. An inhibitory effect of t10c12-CLA on milk fat synthesis and mammary lipogenic gene expression was demonstrated by duodenal infusion in dairy cows [74]. However, in nutritional studies, the levels of t10c12-CLA in the rumen, duodenal fluid or milk always remained very low compared to the levels used in infusion studies, whereas t10-18:1 levels were much higher [29, 34, 39, 75, 76]. Furthermore, curvilinear relationships occurred between milk t10-18:1 and fat yield responses (e.g. [29, 30], Shingfield and Grinari, this issue).

Nevertheless, postruminal infusion of 43 g t10-18:1/day/cow over 4 days had no effect on milk fat synthesis [77]. Although in some trials the duodenal flow or milk concentration of t10-18:1 can be more than twice (Fig. 3) or 16 times higher [30], respectively, than in the study by Lock et al. [77], it is likely that the formation of t10-18:1 and t10c12-CLA is accompanied by the formation of other RBH intermediates that could also inhibit (or co-inhibit with t10c12-CLA) milk fat synthesis. Indeed, in nutritional studies, several trans-18:1 and 18:2 isomers vary simulta-

neously and exhibit high negative correlations with milk fat content and secretion (e.g. [29–31, 78]). Furthermore, five different CLA isomers have been post-ruminally infused into cows (review Shingfield and Grinari, this issue). Among them, only c10r12-CLA [79] and 9c11-CLA [80] reduced milk fat synthesis. However, the relative efficiency of infused 9c11-CLA was lower than that of t10c12-CLA [80], which is in agreement with regression lines observed between milk fat secretion and the concentration of these isomers in milk fat in cow feeding trials [30, 31].

Another mechanism linked to RBH putatively regulating milk fat depression is the decrease in 18:0 availability for mammary c9-18:1 synthesis that occurs when PUFA-rich oils, in particular marine oils, inhibit the last step(s) of trans-18:1 isomer hydrogenation ([29, 31, 81], Shingfield and Grinari, this issue).

In the goat, nearly all types of lipid supplements added to a large variety of basal diets induce a sharp increase in milk fat content [24, 25], in contrast to the cow. This is likely due to a lower ruminal yield of t10-18:1 and associated isomers (see Section 5) combined with the fact that the mammary lipogenesis seems much less responsive to post-ruminally infused t10c12-CLA [82]. In other respects, the effects of dietary lipids on mammary SCD gene expression differ markedly between cows and goats (review [68]). These differences could be partly explained by factors linked to the diet (e.g. level of starch) and the nature and presentation of the lipid supplements, but also to species differences observed in milk C18-9 desa-

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3 Effects of forage source on milk FA

3.1 Cow milk

3.1.1 Effects of pasture

Fresh grass dry matter (DM) contains 1–3% FA, with the highest values during spring and autumn, and about 50–75% of these FA as 18:3 n-3 [83, 84]. Compared to mixed winter diets, pasture increases milk fat concentrations of 18:0 (+2 g/100 g FA), 18:1 (+8), 18:3 (+1.0) and CLA (+0.6) and decreases 10:0–16:0 (–13) (reviews [21, 85]).

This has been confirmed and defined over the last 5 years. Milk fat c9-18:1 is higher on pasture than with high-forage winter diets (22–24 vs. 15–18 g/100 g FA, Fig 6), despite the grass being low in c9-18:1 [86]. This result could be explained by the higher 18:3n-3 intake on pasture, resulting, after RBH, in higher 18:0 absorption and mammary Δ9 desaturation. From six direct comparisons between grass pasture and mixed winter diets (review [23]), it can be calculated that pasture significantly increases (p <0.05) milk fat concentrations of 18:3n-3 and CLA (+0.5 and +0.9 g/100 g FA, respectively). Other studies showed that pasture, compared to either maize silage [87] or total mixed rations ([88–91] and review [92]), increases r11-18:1, c9t11-CLA and 18:3n-3 (and sometimes 15:0, 17:0 and c9-18:1) and decreases 16:0 (and sometimes 8:0–14:0 and 18:2n-6). Inconsistent results were observed across studies on 4:0, 6:0 and 18:0.

When the pasture percentage increases in the total diet, linear increases in 18:3n-3, r11-18:1 and c9t11-CLA and decreases in 10:0–16:0 are observed. Thus, when pasture increased from 33 to 100% of the diet, 18:3n-3 and c9t11-CLA each increased from 0.8–0.9 to 2.0–2.2 g/100 g FA [93] or from 0.4 to 0.7 (18:3n-3) and 0.5 to 1.6 (c9, r11-CLA) [94]. The very consistent effects of pasture on 18:3n-3, r11-18:1 and c9t11-CLA are related to the high content of 18:3n-3 in most pastures, which is partly biohydrogenated into r11-18:1 and partly absorbed intact in the gut and secreted into milk. Consistent with this, pasture milk is richer in r11c15-18:2 (an intermediate of RBH of 18:3n-3) than winter milk (0.5–0.8 vs. <0.1 g/100 g FA [84]). However, pasture milk 18:3n-3 and c9t11-CLA are frequently lower than 0.7 and 1.1 g/100 g FA, respectively ([86, 90, 95] and reviews [21, 23, 85]). This is likely due to the decrease in FA and 18:3n-3 contents in mature compared to young growing grass [23], in agreement with the observation that milk concentrations of 18:0, c9-18:1, r11-18:1, c9t11-CLA and 18:3n-3 are much higher at 3 wk than 6 wk after turning out to pasture ([86] and Fig. 6). However, temporal adaptation in rumen or mammary metabolism could also occur (see Section 5) and contribute to such changes.

Dewhurst et al. [23] reviewed six direct comparisons between milk produced during the summer, either on alpine pastures or in lowland conditions (either on pasture or with conserved forages and concentrates). It can be calculated from this review and from Lucas et al. [96] that alpine milk is significantly richer in c9-18:1 (+3.8 g/100 g FA), r10+11-18:1 (+2.6), CLA (+1.3) and 18:3n-3 (+0.8), and poorer in 12:0 (–0.9), 14:0 (–1.9) and 16:0 (–6.0) (Tab. 2). Furthermore, c9t11-CLA and 18:3n-3 decreased in a field survey in the order: alpine pasture > permanent grassland pasture of first use > second use > temporary pasture > grass silage > hay > maize silage [96]. Quick changes (a few days) were observed after either turning out to pasture or transition from fresh grass to a silage diet [21, 97].

High c9-18:1 and 18:3n-3 concentrations in alpine milk are observed despite the fact that there is not always a high content of these two FA in the alpine pastures. Leiber et al. [98] suggested that this may be related to other botanical components that could reduce RBH and/

![Fig. 6. Effects of forage source on oleic, rumenic and linolenic acid concentrations (g/100 g total FA) of cow milk fat (adapted from [86]). CB, concentrate-based diet. High-forage diets: MS, maize silage; H, hay; NG, natural grassland; P, pasture at 3 or 6 (■) weeks after beginning of pasture; RG, rye-grass; S, silage.](image-url)
Grass or grass silage and that ensiling produces a decrease in grass FA and release of free FA from grass acylglycerols [103]. Several studies in the 1970s also showed high milk 18:3n-3 concentrations when cows were fed hay (review [21]) and this was confirmed by a meta-analysis comparing milks from five hay-fed to ten grass silage-fed cow groups, having 0.99 vs. 0.43 g 18:3/100 g FA (p < 0.001) (F. Glasser, A. Ferlay, Y. Chilliard, unpublished). Furthermore, when hay is dried in the barn in order to limit wilting losses, its 18:3n-3 concentration is high and enables the yield of milks with similar or higher 18:3n-3 concentrations than pasture and higher t11-18:1 and c9t11-CLA concentrations than grass silage (Fig. 6). A direct comparison of wrapped haylage (51% DM) with grass silage (39% DM) made from the same grassland showed only marginal changes in milk FA composition [104]. Silage prepared from semi-natural grassland resulted in slightly higher milk concentrations of t11-18:1, c9t11-CLA, 18:2n-6 and 18:3n-3 compared with botanical species-poor, intensively managed grassland [105]. Overall, any differences in FA profiles between milks coming from either hay- or grass silage-based diets would be of limited magnitude.

### 3.1.2.2 Legume silage

It can be calculated from the review of Dewhurst et al. [23] that, in six direct comparisons with grass silage (four trials using red clover and two white clover), legume silage increases milk 18:2n-6 and 18:3n-3 by 0.4 and 0.6 g/100 g FA, respectively. These authors explain that this result may be due to legumes being rich in these PUFA (white clover especially in 18:3n-3); the transfer efficiency of 18:3n-3 from diet to milk being higher with red clover compared to grass silage (9 vs. 4.5%); passage rate through the rumen being higher for white clover; and red clover having a much lower lipolysis in the rumen than grass due to its polyphenol oxidase activity.

Milk from organic farming is richer in 18:3n-3 than milk from conventional farming, whereas a difference in c9t11-CLA is less consistently observed [28, 106, 107]. This high concentration in 18:3n-3 is very likely related to the larger use of legume plants in organic farming. Furthermore, in one study, organic milk was richer in several trans-18:1 isomers (mainly t13-18:1, but not t10-18:1) and in several minor CLA isomers [28]. This observation deserves further research on a larger scale to be confirmed and to understand its putative causes.

### 3.1.2.3 Maize silage

Maize silage (which generally contains 30–40% grain) is rich in 18:2n-6 and c9-18:1, and poor in 18:3n-3. This may explain why feeding maize silage (six trials with >60%
maize silage) compared to grass silage (five trials with >58% grass silage) sharply increased the milk \( n-6/n-3 \) ratio; however, 18:0 and total 18:1 did not change (review [21]). This was confirmed by direct comparisons which, moreover, showed no change in \( c11\text{-18:1 and } c9\text{-t11-CLA} \) [30]. Part of these decreasing or increasing isomers are RBH intermediates of 18:3
\( n-3 \) or 18:2
\( n-6 \), respectively. Compared to a hay-based diet, a diet rich in concentrate (Tab. 3) or low-concentrate (Fig. 6) diets, and likely result from dietary FA differences.

### 3.1.3 Effect of forage/concentrate ratio

The effect of increasing the concentrate percentage in the diet on milk fat content is dependent on the range of increase: when the concentrate does not exceed 50–60% of the diet, the milk fat content does not vary largely, whereas a strong decrease is generally observed above 60% [22, 108, 109]. The effects on milk FA composition also largely differ according to the range of concentrate percentage variation: in a trial on pasture with concentrate increasing from 3 to 35% [110, 111], the main effects were to increase milk fat concentrations of 4:0-14:0, \( \text{trans-18:1 isomers (except } c11\text{-18:1) and } 18:2\text{-n-6, and to decrease } c9\text{-18:1, } c11\text{-18:1, } c9\text{t11-CLA and } 18:3\text{n-3. By contrast, in a trial using grass hay with concentrate increasing from 36 to 66% [29], the main effects were to increase milk fat concentrations of all } \text{trans-18:1 isomers (particularly } c10\text{-18:1), } c9\text{t11-CLA and } 18:2\text{-n-6, and to decrease } 14:0\text{-18:0, and } 18:0 \) (Tab. 4). Similar trends were observed in other studies, either in the low range of concentrate percentages with diets based on grass or legume silages [112] or in the high range with diets based on maize silage and alfalfa haylage [38, 49]. In other respects, increasing the dietary concentrate percentage from 20 to 70% (or increasing maize silage) decreased milk odd- and branched-chain FA, with a clear linear decrease in the ratio of odd-chain iso-FA to anteiso-FA (review [113]).

Thus, it seems that increasing the concentrate intake in the low range favors both milk 18:2n-6 and mammary \textit{de novo} FA synthesis despite a shift in RBH towards more \textit{trans}-18:1 isomers, at the expense of 18:3n-3 and \textit{c9t11-}

### Tab. 3. Effect of forage source, and source and amount of oil supplement on milk yield, fat content and FA composition (g/100 g total FA) (A. Ferlay, Y. Chilliard, unpublished data).

<table>
<thead>
<tr>
<th>Forage Source</th>
<th>Oil</th>
<th>Amount [% DM]</th>
<th>Milk Yield [g/kg]</th>
<th>Fat Content [g/kg]</th>
<th>4:0-8:0</th>
<th>10:0-14:0</th>
<th>16:0</th>
<th>18:0</th>
<th>c9-18:1</th>
<th>c10-11:1</th>
<th>18:2</th>
<th>18:3</th>
<th>c9t11-18:1</th>
</tr>
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<tbody>
<tr>
<td>Maize silage</td>
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<tr>
<td>S0</td>
<td>1.5</td>
<td>29.2</td>
<td>35.2</td>
<td>8.3</td>
<td>27.1</td>
<td>17.1</td>
<td>25.4</td>
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<td>4.0</td>
<td>2.1</td>
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<tr>
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<td>30.2</td>
<td>31.9</td>
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<td>14.6</td>
<td>21.2</td>
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<td>0.01</td>
<td>0.10</td>
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<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
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<td>0.01</td>
</tr>
</tbody>
</table>

\[1\] 20 cows were used in two replicated 5 × 5 Latin Square designs with 3-week periods.
\[2\] SO, sunflower oil (18:2n-6 rich); LO, linseed oil (18:3n-3 rich).
\[3\] ALA, alpha-linolenic acid (18:3n-3); LA, linoleic acid (18:2n-6).
\[4\] Diet containing 60% maize silage, 5% grass hay and 35% concentrates.
\[5\] Diet containing 47% grass silage, 13% grass hay and 40% concentrates.
\[6\] Significant effect at \( p < 0.01 \), 0.05 or 0.10. Interactions were significant (\( p < 0.10 \)) for milk yield (oil-amount), fat content (forage-amount), 18:0, \( t10+11-18:1 \), 18:2n-6, 18:3n-3 (forage-oil), 18:2n-6 and ALA/LO (oil-amount) and 18:3n-3 and ALA/LO (forage-amount).
Tab. 4. Effect of increasing concentrate intake on milk FA composition in cows receiving either pasture or hay/concentrate diets.†

<table>
<thead>
<tr>
<th>Basal diet</th>
<th>Pasture</th>
<th>Grass hay</th>
<th>Pasture</th>
<th>Grass hay</th>
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<tr>
<td>Concentrate [% DM]</td>
<td>LC</td>
<td>MC1 (MC-LC)</td>
<td>MC2</td>
<td>HC (HC-MC)</td>
</tr>
<tr>
<td>Milk fat content [%]</td>
<td>3.8</td>
<td>3.3</td>
<td>(+0.5)</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Milk FA [g/100 g]

| 4:0–12:0 | 9.0 | 10.9 | (+1.9) | 14.8 | 15.9 | (+0.7) |
| 14:0 | 8.0 | 9.4 | (+1.4) | 12.1 | 11.6 | (–0.5) |
| 16:0 | 24.3 | 24.3 | (0) | 29.4 | 25.7 | (–3.7) |
| 18:0 | 12.0 | 12.7 | (+0.7) | 7.0 | 6.2 | (+0.8) |
| c9:18:1 | 29.8 | 26.9 | (–2.9) | 15.3 | 14.9 | (+0.4) |
| f6+7+8:18:1 | 0.31 | 0.40 | (+0.09) | 0.19 | 0.40 | (+0.21) |
| f9:18:1 | 0.31 | 0.38 | (+0.07) | 0.14 | 0.23 | (+0.09) |
| f10:18:1 | 0.90 | 1.18 | (+0.28) | 0.28 | 1.66 | (+1.38) |
| f11:18:1 | 3.58 | 2.85 | (–0.73) | 1.12 | 1.32 | (+0.20) |
| f12:18:1 | 0.47 | 0.55 | (+0.08) | 0.20 | 0.34 | (+0.14) |
| 18:2n-6 | 2.22 | 3.16 | (+0.94) | 1.61 | 2.48 | (+0.87) |
| 18:3n-3 | 1.17 | 0.77 | (–0.40) | 0.78 | 0.76 | (–0.02) |
| c911-CLA | 1.36 | 1.24 | (–0.12) | 0.62 | 0.81 | (+0.19) |

† LC, MC, HC = low-, medium-, high-concentrate diet.  ‡ High allowance of grass pasture (adapted from Bargo et al. [110, 111]).  § Adapted from Loo et al. [29].

CLA. On the other hand, increasing the concentrate in the high range clearly orients RBH to the t10-pathway, resulting in milk fat depression and a milk rich in trans-FA and 18:2n-6 (in agreement with observed duodenal flows [39, 49] and Figs. 3, 4) and poor in 14:0–18:0. However, increasing the concentrate in the high range did not result in similar responses when it was added to a diet where unsaturated FA were replaced by saturated ones [114], showing that important interactions exist between the effects of the starch, fiber and lipid components of the cow diet (see Section 5).

The starch source itself may also change RBH and milk FA composition. Thus, simply replacing wheat (rapidly degradable starch) with potatoes (slowly degradable starch) (both at 30% of the diet) increased ruminal pH and milk concentrations of 4:0–16:0 and decreased c9-18:1 and trans-18:1 (mainly t10-18:1, –1.5 g/100 g FA) [115].

3.2 Goat milk

Grazing mountain spring pasture, compared to winter diets (alfalfa hay, straw and concentrates), increased goat milk fat 18:3n-3 (+0.5 g/100 g FA) without changing c911-CLA [116]. Zero-grazing fresh rye-grass, compared to a rye-grass hay-based diet, did not change 18:3n-3 and c911-CLA and increased c9-18:1 (Tab. 5), whereas small but significant decreases were observed for 11:0–14:0, c9-14:1, 16:1, c9-17:1 and odd- and branched-chain FA [117].

Compared to rye-grass hay, alfalfa hay decreased 14:0, c9-14:1, 16:1 and odd- and branched-chain FA and increased 20:0–24:00, 18:2n-6, 18:3n-3 (+0.3 g/100 g FA), EPA and the 18:3n-3/18:2n-6 ratio ([117] and Tab. 5, trial B). Dehydrated alfalfa, richer in lipids and 18:3n-3, increased milk fat 18:0, t11-18:1 and 18:3n-3, compared to alfalfa hay [118].

Increasing the concentrate percentage in the diet (alfalfa hay-based diets) from 32–33 to 56–67 decreased 16:0, 18:3n-3 (–0.3 g/100 g FA) and the 18:3n-3/18:2n-6 ratio, and increased 10:0–14:0, 18:2n-6, t11-18:1, c911-CLA and other trans FA ([19] and Tab. 6). Similar trends were observed when comparing total mixed rations containing 55–60 vs. 30–35% forage [118, 119]. Compared to alfalfa hay, maize silage decreased 16:0, odd- and branched-chain FA, 18:2n-6, 18:3n-3 (–0.3 g/100 g FA) and the 18:3n-3/18:2n-6 ratio, and increased 18:0 ([120] and Tab. 5, trial A).

Altogether, the differences observed in goat milk FA due to the forage source and the forage/concentrate ratio are consistent with the results in cows. These effects are, however, of small magnitude, especially when compared to the effects of lipid supplementation (see Sections 4 and 5). The main effect was a consistently higher milk 18:3n-3 concentration with good-quality alfalfa hay-based diets, or on pasture, while increasing concentrates or maize silage had the opposite effect.

4 Effects of lipid supplements on milk FA

Feeding lipid supplements to dairy ruminants has been used widely for decades by researchers and, to some extent, by farmers to modify dairy performance and energy metabolism (reviews [22, 121]) and/or milk FA composition (reviews [4, 18, 25, 33]). Both the source and presentation form of the lipids influence their effects. Attempts to change the proportion of one category of FA often induce changes in other FA, which may be considered positive or negative for consumer health. Thus, diets that decrease milk saturated FA and increase PUFA and/or CLA generally result in higher energy metabolism (reviews [22, 121]) and/or milk FA composition (reviews [4, 18, 25, 33]).
Tab. 5. Effects of forage source and plant oils rich in either c9-18:1, 18:2n-6 or 18:3n-3 on goat dairy performances and milk FA composition (adapted from [19, 25, 117, 120, 169, 181, 182]).

<table>
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<tr>
<th>N. goats</th>
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<th>Trial B</th>
<th>Trial C</th>
<th>Trial D</th>
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<td>MS</td>
<td>AH</td>
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<td>Forages</td>
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<td>38</td>
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<td>12:0 [%]</td>
<td>0.24</td>
<td>2.20</td>
<td>2.97</td>
<td>0.07</td>
<td>0.70</td>
<td>0.29</td>
<td>0.16</td>
<td>0.13</td>
<td>0.95</td>
<td>0.33</td>
<td>0.15</td>
<td>1.34</td>
<td>1.24</td>
<td>0.15</td>
<td>0.50</td>
<td>0.33</td>
<td>0.44</td>
<td>3.23</td>
<td>1.56</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:0 [%]</td>
<td>0.32</td>
<td>0.17</td>
<td>0.69</td>
<td>0.60</td>
<td>0.42</td>
<td>1.37</td>
<td>0.74</td>
<td>0.46</td>
<td>0.26</td>
<td>0.89</td>
<td>0.39</td>
<td>0.19</td>
<td>0.96</td>
<td>1.04</td>
<td>0.57</td>
<td>1.15</td>
<td>0.19</td>
<td>0.15</td>
<td>0.69</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALA/LA</td>
<td>0.16</td>
<td>0.12</td>
<td>0.45</td>
<td>0.20</td>
<td>0.27</td>
<td>0.79</td>
<td>0.37</td>
<td>0.20</td>
<td>0.20</td>
<td>0.69</td>
<td>0.22</td>
<td>0.15</td>
<td>0.66</td>
<td>0.49</td>
<td>0.26</td>
<td>0.83</td>
<td>0.08</td>
<td>0.05</td>
<td>0.36</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes:
- Trials A and B were on six and seven groups of goats, respectively, with a treatment period of 5 wk. Trials C and D were 3 × 3 Latin Square design with 3-week periods.
- MS, maize silage; AH, alfalfa hay; RH, rye-grass hay; FR, fresh rye-grass; NH, natural grassland hay.
- –, Control; OSO, oleic sunflower oil; LO, linseed oil; SO, sunflower oil (130 g oil/day).
- Means within a trial with different letters differ at p < 0.05.
- # Ether extract % diet DM.
- †† Concentrate, including lipids.
- ††† E.E. % DM.
- §§ Fatty acids as g/100 g total FA.
- Others trans: trans 18:1 and 18:2 (conjugated and non-conjugated), except t11-18:1 and c9t11-CLA, but including t10-18:1.
- ALA, alpha-linolenic acid (18:3n-3); LA, linoleic acid (18:2n-6).
### Tab. 6. Effects of concentrate, plant oils, extruded oilseeds and vitamin E on goat dairy performances and milk FA composition (adapted from [19, 25, 183-188]).

<table>
<thead>
<tr>
<th>N. goats</th>
<th>Trial E</th>
<th>Trial F</th>
<th>Trial G</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 High</td>
<td>12 Medium</td>
<td>12 High</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 High</td>
<td>12 Medium</td>
</tr>
<tr>
<td>N. goats</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Forage/concentrate ratio</td>
<td>High</td>
<td>Medium</td>
<td>High</td>
</tr>
<tr>
<td>Lipid suppl.</td>
<td>–</td>
<td>LO</td>
<td>LO</td>
</tr>
<tr>
<td>E.E. [% DM]</td>
<td>2.1</td>
<td>3.0</td>
<td>6.1</td>
</tr>
<tr>
<td>Starch [% DM]</td>
<td>13.8</td>
<td>34.7</td>
<td>45.7</td>
</tr>
<tr>
<td>Milk fat [g/kg]</td>
<td>120-160</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:0</td>
<td>6.3</td>
<td>6.1</td>
<td>9.7</td>
</tr>
<tr>
<td>18:2</td>
<td>14.9</td>
<td>14.4</td>
<td>15.0</td>
</tr>
<tr>
<td>18:3</td>
<td>0.54</td>
<td>1.27</td>
<td>7.78</td>
</tr>
<tr>
<td>Other trans</td>
<td>0.30</td>
<td>0.70</td>
<td>3.05</td>
</tr>
<tr>
<td>10-18:1</td>
<td>0.6</td>
<td>1.3</td>
<td>4.7</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>0.12</td>
<td>0.33</td>
<td>0.43</td>
</tr>
<tr>
<td>ALA/ALA</td>
<td>0.28</td>
<td>0.11</td>
<td>0.91</td>
</tr>
</tbody>
</table>

1 Trials E and F were on seven groups of goats, with a treatment period of 5 wk. Trial G was a 3 x 3 Latin Square design with 3-week periods.

2 Forage/concentrate ratio; trial E: alfalfa hay; trial F: alfalfa hay + maize silage, 0.35 kg DM/day; trial G: natural grassland hay; LowS, R = low forage + slowly or rapidly degradable starch, respectively.

3 Control; LO, linseed oil; SO, sunflower oil; EL, extruded linseed (70) and wheat (30); ELS, extruded linseeds (40), sunflower seeds (30) and wheat (30); 130 or 180 g oil/day in trials 1, 3 or 2, respectively; E, vitamin E (1250 IU/day).

4 a,b,c,d,e means within a trial with different letters differ at p < 0.05.

5 Ether extract % diet DM.

6 Forage, including lipids.

7 E, vitamin E (1250 IU/day).

8 Fatty acids as g/100 g total FA.

9 Others trans: trans 18:1 and 18:2 (conjugated and non-conjugated), except t11-18:1 and c9t11-CLA, but including t10-18:1.

10 Other trans: means within a trial with different letters differ at p < 0.05.

11 Forage, including lipids.

12 Ether extract % diet DM.
4.1 Cow milk

4.1.1 Saturated FA and oleic acid

The potential to decrease medium-chain saturated FA (10:0–16:0) is great. For example, with hay-based diets, these four FA represented 56% of cow milk fat and decreased to 29% after linseed oil (5% of diet DM) supplementation [30]. Conversely, if lipid supplements are rich in medium-chain FA, these could be increased. Such is the case with palm oil calcium salts, which increase palmitic acid concentration (+2.1 g/100 g FA, for 762 g/day mean supplementation over six trials; [123, 124] and review [125]). Furthermore, intake of 476 g/day of palm oil FA (containing 87% 16:0) increased milk 16:0 concentration by 8.4 g/100 g FA [126].

Contrary to medium-chain FA, milk short-chain FA concentrations (4:0, 6:0 and, to a lesser extent, 8:0) are not changed, either by body lipid mobilization [20] or plant or marine oil duodenal infusions (review [33]). That specificity is probably due to the fact that these FA are partly synthesized by metabolic pathways not dependent on acetyl-CoA carboxylase [127]. However, 6:0 and 8:0 are slightly reduced by lipid supplementation in the diet (review [19]), probably because trans and conjugated FA coming from RBH are more potent than other FA in inhibiting lipogenic pathways.

Odd- and branched-chain FA concentrations are decreased by 18:2n-6 or 18:3n-3 supplementation, whereas the opposite effect is observed with fish oil supplementation, which decreased even-chain iso-FA but markedly increased iso-17:0, likely due to specific effects on fiber ruminal digestion (review [113]).

Stearic acid secretion in milk can be increased either by dietary 18:0 intake or by supplementation of 18-carbon unsaturated FA [29, 99, 128] because they are in large part hydrogenated to 18:0 in the rumen [39, 43]. Similarly, oleic acid secretion can be increased either through its direct gut absorption and mammary secretion or mainly (ca. 80% [55]) from its RBH followed by mammary desaturation of 18:0. Another way to increase milk 9-18:1 secretion is the distribution of oleamides [36, 129, 130].

Tallow supplementation (rich in 16:0, 18:0 and c9-18:1) sharply increases milk fat 18:0 and 18:1 and reduces 10:0–14:0 ([131] and review [21]), although few data are available on its putative effects on trans-18:1 [132]. However, most of these supplements have a bad image in Europe since the BSE crisis and the banning of animal protein sources for feeding ruminants.

When feeding unprotected vegetable oils or seeds containing high levels of c9-18:1, 18:2n-6 or 18:3n-3, the proportions of both 18:0 and c9-18:1 are increased in milk. For example, cow’s milk c9-18:1 concentration was increased 1.18–1.35 times in a dose-dependent way when adding either linseed or sunflower oil to the diet (Tab. 3). Positive and variable responses of c9-18:1 or cis-18:1 were also observed when feeding rapeseed oil (1.33–1.67 times), rapeseeds (1.92 times), high-oleic sunflower oil (1.27 times), soybean oil (1.22 times), extruded soybeans (1.17 times), linseed oil (1.26–1.80 times) or linseeds (1.22 times) (reviews [4, 23]).

By contrast, fish oil intake does not clearly change 4:0–16:0 but sharply decreases milk 18:0 and c9-18:1 concentrations (reviews [4, 21]) due to the inhibition of the last step of RBH which results in a high production of ruminal and milk t11-18:1 at the expense of 18:0.

4.1.2 Polyunsaturated FA

Essential PUFA are not synthesized by tissues in ruminants, so their concentration in milk is closely related to the quantities absorbed in the intestine and hence to dietary PUFA intake and to the proportion of that escaping RBH (see Section 2.1).

4.1.2.1 Linoleic acid (n-6 series)

In the absence of supplementary lipids, the proportion of 18:2n-6 in milk FA is between 2 and 3%. When rations are supplemented with 18:2n-6-rich seeds or oils like soybean or sunflower, this proportion rarely exceeds control values by more than 1.5% (reviews [19, 23] and Tab. 3). However, higher increases were observed using either extruded soybeans (+1.9 g/100 g FA [133]), micronized soybeans (+2.4 g/100 g FA [134]), butylsoyamide (+2.7 g/100 g FA [135]), or roasted soybeans (+3.0 g/100 g FA [136]).

It has often been suggested that giving lipids in the form of seeds rather than oil would limit RBH because seed hulls would restrict bacterial access to lipids. However, rapeseed hulls appear to have a less protective effect than soybean or sunflower hulls on milk fat content and 18:2n-6 percentage in cows fed these respective seeds [4, 19]. Furthermore, when added to a maize silage diet, crushed raw soybean or rapeseeds decreased milk fat content (−10 g/kg) and yield of 4:0–16:0 to the same extent as their respective oils given in free form with oil meals [137]. Additional research is necessary to confirm these trends in a larger number of direct comparisons between oil and seeds.

Direct comparison showed that extruded soybean increased less 18:2n-6 than raw or micronized soybean
Lipid supplements can be protected from RBH by encapsulating them in a tanned-protein layer. Amounts of 15–20% of 18:2n-6 in milk FA have been reached with encapsulated soybean, rapeseed, cotton, safflower or sunflower oil supplements [142]. The limitations of such a dietary practice are linked to the processing costs and to the controversial use of formaldehyde. Other so-called lipid protection techniques, such as FA salts, do not prevent PUFA hydrogenation or the negative effect of rapeseed oil on milk fat content [143] and review [19] because the salts are dissociated in the rumen as the pH decreases. Lastly, it is worth remembering that increasing the 18:2n-6 proportion in dairy products is not a target in itself, insofar as improving the nutritional value of these products preferably requires increasing the 18:3n-3/18:2n-6 ratio.

### 4.1.2.2 Linolenic acid and long-chain n-3 FA

Apart from grass and some legumes, linseed is the only oilseed easily available in Europe which provides very high 18:3n-3 levels, representing more than 50% of FA. Rapeseed contains ca. 7% of 18:3n-3, some of which is probably secreted in milk. However, as noted with 18:2n-6, rapeseed oil or seed addition does not significantly increase milk 18:3n-3 (reviews [4, 19]). Soybean lipids contain ca. 8% of 18:3n-3 and enables milk 18:3 to be increased (+0.6–0.7 g/100 g FA) when roasted [136] or micronized [134].

Few trials have been conducted, where cows’ diets were supplemented with linseed oil or raw seeds. Among six older studies, two found no increase and four found increases in milk 18:3n-3 in the range of +0.3 to +0.8 g/100 g FA (review [19]). Among seven other studies, the same range (0.3–0.9) of increase was observed [29, 30, 62, 99, 128, 134, 144]. Formaldehyde treatment of linseeds did not increase milk 18:3n-3 more than raw seeds did [145, 146]. Furthermore, the intake of 200–460 g/day of 18:3n-3 from extruded linseeds [128, 144, 147, 148] or extruded rapeseeds + linseeds [149] increased the cow milk fat 18:3n-3 percentage by 0.3–0.9 g/100 g FA, i.e. in the range observed above with unprocessed linseeds or oil and lower than what could be obtained with grazing high-quality pastures (see Section 3.1.1.).

The variability of the published results requires new studies that discriminate between the effects of oil and seeds and those of seed processing in particular. However, the potential to increase milk 18:3n-3 seems to be limited even when high doses of supplementation are used. Thus, when the linseed oil amount was increased from zero to 1.5 and 3.0% of diet DM, the 18:3n-3 response in milk was not increased proportionally to the oil intake (Tab. 3), and increasing xylose-treated, whole cracked linseeds from 8 to 21% of the diet only slightly increased milk 18:3n-3 (+0.5 g/100 g FA) [150].

The secretion of long-chain FA of the n-3 series (EPA and DHA) may be increased when marine oils (fish or algae) are added to cow rations. The transfer efficiency from diet to milk, however, is low (2.6% for EPA and 4.1% for DHA in 16 groups of cows; review [21]) because of high RBH and of preferential incorporation into plasma phospholipids and cholesterol esters [57]. The increase in EPA and DHA concentrations in milk FA is therefore minimal when fish oil is added to the cow ration and rarely exceeds 0.5% of total FA (reviews [4, 21]). Higher transfer efficiencies, have been noted during the post-rumen infusion of fish oil (16–33%; review [21]) or using tuna oil-soybean protected from RBH by formaldehyde treatment (18–32% [151]). EPA and DHA concentrations in cow milk FA tended to decrease after linseed oil supplementation, although milk 18:3n-3 concentration increased [29], which confirms that 18:3n-3 is not a significant source of milk EPA and DHA in cattle.

### 4.1.3 Trans FA and CLA

The diets that influence the milk f11-18:1 and CLA are mainly: (1) diets providing lipid precursors (18:2n-6 or 18:3n-3) for f11-18:1 formation in the rumen and (2) diets that modify the microbial activity associated with PUFA hydrogenation in the rumen [27]. Combinations of these various factors induce wide variations of milk CLA and trans-18:1 concentrations, and strong interactions occur between forages, starchy concentrates and lipid supplements (see Section 5 and [114]).

Vegetable oils rich in 18:2n-6 (sunflower, soybean) markedly increase milk c9f11-CLA content, up to ca. 2 g/100 g FA above controls. This effect is linear as increasing amounts of soybean oil (review [33]) or sunflower oil (Tab. 3) are added to the diet (up to at least 4% oil of diet DM). Adding calcium salts of rapeseed oil or soybean (which was the more efficient) to the ration increased milk c9f11-CLA concentration [152]. This confirms that calcium salts of PUFA are largely hydrogenated to trans FA.

Increasing linseed oil (18:3 rich) intake from 1.5 to 3.0% of the diet increased milk f11-18:1 and c9f11-CLA con-
centration more than linearly, almost as much as with the same doses of sunflower oil (18:2 rich) (Tab. 3). This result is in agreement with a trial comparing linseed and safflower oils [62] but differs from another where soybean oil increased much more milk t11-18:1 and c9t11-CLA than linseed oil did [153]. However, it could be that in the latter trial an unusually large amount of t11-18:1 was produced by RBH of soybean oil PUFA at the expense of 18:0, which did not increase in milk, contrary to results reviewed by Dewhurst et al. [23] with soybean oil.

Increasing the intake of either 18:2 n-6 or 18:3 n-3 or both (substituted to diet c9-18:1) showed that their combined effect on milk c9t11-CLA (10.3 g/100 g FA) was slightly higher than the separate effects (1 for each PUFA) [154]. In sum, vegetable oils increase milk trans-18:1 and c9t11-CLA more than extruded seeds, which in turn increase it more than raw seeds ([138, 144, 152, 155] and review [19]).

For a given incorporation level to the ration, fish oils are more effective at increasing the CLA concentration than vegetable oils. Thus, c9t11-CLA proportions increased from 0.2–0.6% with the control diet to 1.5–2.7% in most studies with diets supplemented with 200–300 g/day fish oil (reviews [4, 21]). It is likely that the EPA and DHA of these oils, or their RBH products, indirectly but sharply increased the t11-18:1 concentration in the rumen (Fig. 3) by inhibiting the reduction of this FA into 18:0. That would explain why the combination of sunflower and fish oil strongly increased the milk t11-18:1 and c9t11-CLA content [156]. However, an extruded soybean-fish oil mixture increased these FA more than fish oil alone in Holstein but not in Brown Swiss cows [157].

Besides its major effects on t11-18:1 and c9t11-CLA, plant oil supplementation modifies to some extent the profile of other trans and conjugated isomers differently according to the oil FA profile. Thus, a high level of c9-18:1 intake in particular increases milk f6+7+8-18:1 and t7c9-CLA [61, 99]; 18:2n-6 intake increases milk f6+7+8-, f9-, f10-18:1, t12-18:1 and t10f12-, t9f11-, t8t10-, t7t9-, t10c12-, t8c10-, t7c9-CLA [10, 29, 30, 59, 99]; and 18:3n-3 intake increases milk c15-, t13+14-, t15-, t16-18:1, c9t12-, c9t13-, t11c15-18:2, and t9f11-, t12f14-, t11f13-, c12f14-, t11f13-, c11f13-CLA [10, 29, 30, 99] (Figs. 3, 4, 7), as well as conjugated 18:3 [144, 158]. These relationships can be explained, at least in part, by the RBH pathways from Fig. 1, combined with mammary SCD activity on trans-18:1 isomers, especially t7-, t12- and t13-18:1. In other respects, fish oil intake increases milk c11-, f6+7+8-, f9-, t10-, t12-, t13+14-18:1 and t11c15-18:2 [51, 59, 81] (Figs. 3, 4).

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4.2 Goat milk

The goat does not show any milk fat depression after adding plant oil PUFA to high-concentrate diets [24, 25], contrary to the cow (reviews [19, 23, 34]). It is thus of interest to know to what extent the response to plant oils of the milk FA profile could differ between these two ruminant species.

4.2.1 Saturated FA and oleic acid

As in cows, the potential to decrease milk medium-chain saturated FA (10:0–16:0) in goats is very high. For example, with hay-based diets, these four FA represented 59% of goat milk fat and fell to 38% after linseed oil supplementation or to 33% if vitamin E was added with linseed oil [19]. The concentration of milk saturated FA that are considered to have the highest atherogenic potential (12:0, 14:0 and 16:0 [159]) was 43–50 g/100 g FA in milk from goats receiving 11 control diets and decreased to 26–35 g/100 g FA in 25 lipid-supplemented diets (Tabs. 5, 6). As with cows, goat milk short-chain FA concentrations (4:0, 6:0 and, to a lesser extent, 8:0) are either unchanged or only slightly reduced by dietary lipid supplementation or body lipid mobilization [24].

In goats, in a diet comparison combining different forages, concentrate percentages and lipid sources, it appears that high milk concentrations of 18:0 and c9-18:1 (13–17% and 23–29 % of total FA, respectively) were obtained either with unprotected high-oleic sunflower oil (Tab. 5) or with whole raw oilseeds, in the rank lupin > soybean > linseed > sunflower seeds > rapeseeds [19, 119, 160, 161]. Extruded soybean also increased milk 18:0 and c9-18:1 [162].

However, the goat milk c9-18:1 percentage did not or only slightly increase after linseed or sunflower oil, or extruded seeds, supplementation, although 18:0 was substantially increased (Tabs. 5, 6). Thus, the c9-18:1 response differs between goat and cow. It can indeed be observed that in cows the c9-18:1/18:0 ratio is not markedly decreased or increased by sunflower, soybean or linseed oil supplementation (–0.06 to –0.17, Tab. 3; +0.03 [163]; +0.24 [164]; +0.15 to –0.42 [30]; +0.04 to –0.29 [62]; –0.16 to –0.35 [153]; –0.06 in a meta-analysis of 17 trials, F. Glasser, L. Bernard, A. Ferlay, Y. Chilliard, unpublished). In goats, by contrast, this ratio decreased markedly in the rank high-oleic sunflower oil (–0.60, n = 4 treatment groups) < linoleic-rich sunflower oil (–0.77, n = 4) < linseed oil (–0.88, n = 10) < extruded seeds (–1.09, n = 2) (Tabs. 5, 6). This suggests that the desaturation ratio of 18:0 decreases more with diets that increase the availability of PUFA and/or trans FA (Tabs. 5, 6) for the goat mammary gland, since these FA are putative inhibitors of the SCD [68, 165]. Further research is needed to understand if the difference between goat and cow is due to differences in RBH or in mammary SCD regulation. Available data suggest indeed that mammary SCD could be more sensitive to PUFA-rich diets in goats than in cows (review [68]). Vitamin E, when added to goat linseed oil-supplemented diets, further decreased this desaturation ratio while simultaneously further increasing milk trans-18:1 and 18:2 ([19] and Tab. 6). Finally, the efficiency of high-oleic sunflower oil to increase milk c9-18:1 could be due to the combination of greatly increasing 18:0 availability (Tab. 5) and increasing duodenal flow of rumen-escaped c9-18:1, without increasing largely trans FA having inhibitory effects on mammary SCD.

The case of lupin seeds is interesting since this seed, rich in c9-18:1 and 18:2n-6, is the only one which did not decrease the 18:0 desaturation ratio and did not increase (or even decreased) goat milk PUFA and t11-18:1 percentages [24], suggesting that lupin unsaturated FA were totally hydrogenated despite being consumed as crude whole seed.

4.2.2 Polyunsaturated FA

4.2.2.1 Linoleic acid

In the absence of supplementary lipids, the proportion of 18:2n-6 in goat milk FA is between 2 and 3%. When rations are supplemented with 18:2n-6-rich seeds or oils like soybean or sunflower, that proportion rarely exceeds control values by more than 1.5% ([160, 162] and Tabs. 5, 6). Comparing the effects of sunflower oil and seeds in goats revealed that seed 18:2n-6 was, paradoxically, more strongly hydrogenated to 18:0 than oil 18:2n-6, the latter being more recovered either intact or in the form of t11-18:1 and c9t11-CLA in milk [24]. It may therefore be supposed that the release of seed lipids was slow, which enhanced their total hydrogenation. A similar observation was made with 18:2n-6-rich lupin seed, which strongly increased milk 18:0 and c9-18:1 while reducing 18:2n-6 and c9t11-CLA. This may explain why raw oilseeds are more efficient at increasing milk 18:0 and c9-18:1 than free oils (see Section 4.2.1).

The addition of linseed oil to goat’s diet decreased the milk 18:2n-6 percentage while it increased the 18:3n-3 percentage. Opposite responses between these two PUFA were also observed when sunflower oil (18:2n-6 rich) was added (Tab. 5). Besides illustrating that the different PUFA are not secreted independently from each other, it should be noted that such a substitution seems to be less marked in cows (Tab. 3) than in goats.
4.2.2.2 Linolenic acid and long-chain n-3 FA

Few trials have been conducted where goats’ diets were supplemented with linseed oil or seeds. The milk 18:3n-3 response suggests that 18:3n-3 from whole crude linseeds was more widely hydrogenated to 18:0 than 18:3n-3 from free oil [24], similar to what was described above for sunflower 18:2n-6. The response to extruded linseeds (+3.6% oil in hay-based diet DM) was high in the goat, where 18:3n-3 increased more (+1.9 g/100 g FA) than after linseed oil supplementation (+0.9 g/100 g FA) (Tab. 6). The response to extruded linseed fat-rich cake (equivalent to 1.5% oil addition to a “dry totally mixed” diet) was, however, not so high (+0.5 g/100 g FA) [166]. Formaldehyde treatment of whole linseed increased the goat milk 18:3n-3 concentration more than untreated seed (+1.1 vs. +0.6 g/100 g FA), but not beyond the effect of a corresponding dose of unprotected oil (+1.3) [24, 165]. The goat milk 18:3n-3/18:2n-6 ratio was sharply increased by linseed oil (130 g/day) and, more markedly, by extruded linseed supplementation (Tabs. 5, 6). Altogether, the milk 18:3n-3 concentration responded more to treated or untreated linseeds or linseed oil supplementation in the goat than in the cow (review [19], Tabs. 3, 5, 6 and Section 4.1.2.2). This could be related to the fact that the 18:3n-3 milk/plasma ratio in the goat was twice that in the cow and that the correlation between milk and plasma 18:3n-3 was much higher in the goat (Tab. 1).

There are few studies in goats concerning the effects of dietary marine oils in order to increase milk EPA and DHA. The transfer efficiency from diet to milk was low (4–5%) for these two FA from non-protected oils because of high RBH and was increased to some extent with protected oils [167, 168].

4.2.3 Trans FA and CLA

The dietary factors that influence the milk c9,11-18:1 and CLA concentration are basically the same in goats and cows. There is a strong linear correlation between milk c9,11-CLA and c9,11-18:1 concentrations among a wide variety of diets in goats (slope = 0.40 [24]) as in cows (slope 0.38–0.43 [60–62]). However, in 36 diets studied in goats (Tabs. 5, 6), the c9,11-CLA/c9,11-18:1 ratio was 0.6–0.7 for control diets compared to 0.3–0.5 for lipid-supplemented diets. With combinations of five different forages with either no oil addition or c9-18:1-, 18:2n-6- or 18:3n-3-rich oils, a considerable range of c9,11-CLA was observed: from 0.3 to 5.1% of total FA. The main factor of variation was the nature of oil, with sunflower (18:2n-6 rich) > linseed (18:3n-3 rich) > high-oleic sunflower (c9-18:1 rich) > no oil addition. The response to c9-18:1-rich oil, albeit much less than a similar amount of PUFA-rich oils, is consistent with a possible c9-18:1 isomerization into c11-18:1 in the rumen [47] or could be due to an inhibition of the last step in hydrogenation of dietary PUFA. The responses were lower with extruded linseeds or sunflower seeds than with the same doses of oils (Tab. 6).

Few data are available on the influence of feeding plant oils on the various 18:1 and 18:2 isomers in goat milk. c9f11-CLA is the most important CLA isomer and its concentration is the most variable because of the importance of its mammary synthesis by SCD. In addition, this enzyme probably synthesizes t7c9-CLA [66]. This isomer is not well separated in studies using only GLC for analyzing the milk FA profile; preliminary results suggest, however, that it is increased in goats fed high-oleic sunflower oil [169], in agreement with data regarding cows (see Section 4.1.3). Linseed oil increases goat milk c9c11- and/or t11c13-CLA as well as c9f13-18:2 and t11c15-18:2 [19], whereas t10c12-CLA generally remains at trace levels in goats [25, 119].

5 Effects of forage-concentrate-lipid interactions on milk FA and persistency of the responses

The changes in milk FA composition that are obtained by lipid supplementation of ruminant diets are linked to the lipid source (animal fat, plant or marine oil), and to their presentation form, technological treatment and amount included in the diet (see Section 4). However, the responses are also largely dependent on both the forage source and the diet forage/concentrate ratio (reviews [19, 23]).

5.1 Cow milk

Linseed oil supplementation interacts significantly with the diet forage/concentrate ratio on the milk concentration of several FA: The oil effect was higher on t10-18:1, t11c15-18:2 and 18:3n-3 when added to a high-concentrate diet, and was higher on 18:0 and c9-18:1 (increases) and 16:0 (decrease) when added to a high-forage diet [29]. Although factorial interaction was not studied, changing concentrate percentage and soybean oil intake together strongly increased milk fat concentrations of t10-18:1, t7c9- and t10c12-CLA, mainly at the expense of 4:0–16:0, t11-18:1, t13±14-18:1 and c9f11-CLA [75]. Indirect comparison suggests that sunflower oil could be more efficient than soybean oil to increase milk t10-18:1 when added to similar high-concentrate diets [30, 75], whereas direct comparison showed that linseed oil is less efficient than sunflower oil is ([59] and Fig. 3).
In a study on linseed and sunflower oils (Tab. 3), milk 18:0 and c9-18:1 concentrations increased even more when adding plant oils to a grass silage compared to a maize silage diet (+3.4 vs. 1.9 and 4.7 vs. 2.8 g/100 g FA, respectively) whereas the opposite was true for c9t11-CLA (+0.7 vs. +1.3 g/100 g FA, respectively), t10+11-18:1 and PUFA (e.g. +0.3 vs. +0.45 g/100 g FA, respectively, for the 18:2n-6 response to sunflower oil) (Tab. 3). Thus, RBH seems to be less complete with maize silage, likely due either to a lower ruminal pH or changes in microbial population with this diet. Furthermore, there was a greater decrease in milk fat content when oils were added to a maize than a grass silage-based diet (Tab. 3), in agreement with previous observations on maize silage diets supplemented either with soybean or rapeseeds, which decreased the milk 4:0–16:0 yield without changing total 18:1 [137], or with tallow, which decreased cell wall (NDF) digestion [170] and milk fat content [171] and increased milk t10-18:1 concentration [132].

The effect of the forage source was also observed with fish + sunflower oil-supplemented diets: Replacing grass silage with maize silage increased 12:0, 14:0, t10-18:1, c9c11-CLA, EPA and DHA and decreased 18:0, c9-18:1, t15-18:1, t16-18:1, trans-18:2 and 18:3n-3, while increasing the concentrate level increased milk t10-18:1, c9c11- and t10c12-CLA, other CLA having a trans-11 double bond and 18:3n-3, with few interactions with the forage source [78]. In cows receiving oil-rich rapeseed cake, replacing grass silage with maize silage decreased milk fat content, increased milk fat concentrations of c9-18:1, t10-18:1 (+4.0 g/100 g FA), c9t11- and t10c12-CLA, 18:2n-6 and decreased 4:0–16:0, 18:0, 18:3n-3, and the effects were more marked for t10-18:1 and t10c12-CLA when the dietary concentrate and/or starch percentage was higher [71]. Again, RBH seems to be less complete with maize silage.

In most of the studies reported above, a common feature is an increase in milk t10-18:1 with either high-concentrate or maize silage diets supplemented with PUFA-rich oils. In studies reviewed by Bauman and Griinari ([34]) and Shingfield and Griinari (this issue), low-fiber, high-starch diets supplemented with PUFA-rich plant oil sharply reduce mammary lipid secretion and strongly increase the proportions of milk t10-18:1 and, to a certain extent, of t10c12-CLA. It is therefore possible that t10c12-CLA results from RBH modifications induced by low-fiber diets and is one of the precursors of the t10-18:1 yield in the rumen. It is worth noting that, under such conditions, milk r11-18:1 and c9t11-CLA syntheses only increased slightly (trans-11 to trans-10 shift) by comparison with what happened with high-fiber diets supplemented with oil (when t11-18:1 is the major intermediate of PUFA RBH) [114].

Furthermore, it was recently observed that the milk FA response to plant and/or marine oil supplementation is time dependent (Tab. 7), probably reflecting RBH and/or metabolic adaptations. Bauman et al. [172] and Dhiman et al. [173], with respect to maize silage-containing diets, first reported that the milk c9t11-CLA response could be transient, peaking during the second week after beginning lipid supplementation. It was subsequently observed that the c9t11-CLA response to lipid supplementation was higher with a diet containing 60% hay than 70% maize silage, and that its decrease after 3 wk of supplementation was accompanied by an increase in the milk fat t10-18:1 percentage, which was more marked with maize silage, whatever the source of plant or marine oil [174] and Tab. 7. The maximum c9t11-CLA transient response was observed at 2–3 wk after beginning lipid supplementation in studies using mixed diets containing 25% maize silage and 50% concentrates [157, 175], but at 5–6 days with diets higher in maize silage and/or concentrations ([30, 31], Tab. 7, Fig. 7). With the addition of oil to diets rich in maize silage and/or concentrates, a large decrease in milk fat content and increase in milk fat t10-18:1 percentage (up to 4.5–18.6 g/100 g FA) were observed concurrent with the c9t11-CLA decrease [30, 31, 174] as well as increases in milk-specific trans FA, including t6+7+8-18:1, t10c12- and t9c11-CLA [30, 31].

By contrast, when diets were based on grass silage [176], hay [30], or legume silage and hay [62], the milk fat c9t11-CLA response to oil supplements was stable at a medium level for 14 wk [176], at a high level for at least 3 wk ([30], Fig. 7) or at a very high level for at least 8 wk ([62], Tab. 7). At the same time, t10-18:1 remained lower than 0.7 [30] or 1.4 g/100 g FA [62].

Adding dietary vitamin E has been shown to avoid the trans-11 to trans-10 shift in cows receiving a maize silage-based diet supplemented with extruded linseeds and linseed oil; however, this effect was observed only if vitamin E was added at the start of linseed supplementation but not if it was added once the trans-11 to trans-10 shift had occurred [177]. No significant effect of vitamin E addition on milk t10-18:1 concentration was observed in cows receiving a legume silage- and hay-based diet supplemented with safflower oil; however, a trend to increase t10-18:1 was observed in the absence of monensin, whereas the stimulatory effect of monensin on milk t10-18:1 was abolished by vitamin E [62]. The addition of monensin together with a high level of safflower oil (6% of the diet) maximized the milk c9t11-CLA concentration (5.1 g/100 g FA) due to the simultaneous increases in both

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Tab. 7. Persistency of cow milk trans-18:1 and CLA isomers after lipid supplementation to hay-, grass silage- or maize silage-based diets.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Fatty acids (g/100g total FA)</th>
<th>Duration [days]</th>
<th>Isomer</th>
<th>Basal</th>
<th>First wave (achieved at day)</th>
<th>Final</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS(50)</td>
<td></td>
<td>98</td>
<td>c9t11-CLA 0.5</td>
<td>1.1 (28–49)</td>
<td>1.0 [176]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GH (64)</td>
<td></td>
<td>21</td>
<td>c9t11-CLA 0.5</td>
<td>2.9 (18)</td>
<td>2.7 [30]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BS (26) + AS (21) + AH (13)</td>
<td></td>
<td>56</td>
<td>c9t11-CLA 0.5</td>
<td>4.0 (14)</td>
<td>4.3 [62]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BS (26) + AS (21) + AH (13)</td>
<td></td>
<td>56</td>
<td>c9t11-CLA 0.5</td>
<td>2.7 (14)</td>
<td>2.9 [62]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BS (26) + AS (21) + AH (13)</td>
<td></td>
<td>20</td>
<td>c9t11-CLA 0.4</td>
<td>3.4–5.9 (7–13)</td>
<td>nr [174]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS (25) + AH (25)</td>
<td></td>
<td>28</td>
<td>c9t11-CLA 0.6</td>
<td>1.6 (14)</td>
<td>1.3 [157]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS (25) + AH (25)</td>
<td></td>
<td>70</td>
<td>c9t11-CLA 0.8</td>
<td>1.4 (21)</td>
<td>1.7 [175]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS (25) + AH (25)</td>
<td></td>
<td>20</td>
<td>c9t11-CLA 0.4</td>
<td>3.2–5.1 (7–13)</td>
<td>nr [174]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS (25) + AH (25)</td>
<td></td>
<td>20</td>
<td>c9t11-CLA 0.7</td>
<td>8.3–11.8 (13)</td>
<td>nr [174]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS (25) + AH (25)</td>
<td></td>
<td>21</td>
<td>c9t11-CLA 0.5</td>
<td>2.2 (6)</td>
<td>1.0 [30]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS (65)</td>
<td></td>
<td>28</td>
<td>c9t11-CLA 0.9</td>
<td>5.4 (5)</td>
<td>2.2 [31]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS (27)</td>
<td></td>
<td>21</td>
<td>c9t11-CLA 0.6</td>
<td>1.9 (6)</td>
<td>1.1 [30]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>t11-18:1</td>
<td>1.0</td>
<td>1.9 (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>t10-18:1</td>
<td>3.0</td>
<td>18.6 (18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>t10c12-CLA</td>
<td>0.01</td>
<td>0.05 (9)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 AH, GH = alfalfa or grass hay; AS, BS, GS, MS = alfalfa, barley, grass or maize silage (respectively). The figures indicate percentages in diet DM.
2 Concentrate percentage in diet DM.
3 ESB, FM, FO, LO, RO, SFO, SO = extruded soybean, fish meal, fish oil, linseed oil, rapeseed oil, safflower oil, sunflower oil (respectively). The figures indicate percentages of related oils in diet DM.
4 nr = not reported.

The effect of monensin in cultures of ruminal microorganisms was to increase t10-18:1 formation, interacting with PUFA addition and grain source [178].

After adding linseed oil to a hay-based diet, milk 18:3n-3 increased slightly until day 6 and then returned to the baseline at day 9 [30] and unpublished data). This suggests that, although there was no trans-11 to trans-10...

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shift under these conditions (see above), the rumen microflora needed a few days before adapting to PUFA-rich oil feeding. Furthermore, adding fish oil and sunflower oil to a maize silage-based diet increased milk EPA and DHA to a maximum at 5 days, after which the apparent transfer efficiency from diet to milk decreased from ca. 5% to less than 1.5% [31], thus suggesting that time-dependent adaptation in RBH of long-chain n-3 FA also occurred with diets favoring the trans-11 to trans-10 shift.

5.2 Goat milk

Forage-concentrate-lipid interactions also occur in goats. Thus, dietary linseed oil increased more milk 18:3n-3 when given to goats receiving hay-based diets than diets either rich in concentrates (Tab. 6, trials E and F) or based on maize silage (Tab. 5, trial A). This interaction is the opposite of what was observed in cows (Tab. 3) and deserves further research. Furthermore, the milk c9-18:1 response to high-oleic sunflower oil supplementation was higher with hay- than with maize silage-based diet, whereas the 18:0 response was lower (Tab. 5, trial A), in agreement with the hypothesis that diets increasing the yield of trans FA could inhibit mammary SCD (see Section 4.2.1).

For a given oil supplementation, the response of c9t11-CLA to oil strongly interacts with forage source. Thus, the response to sunflower oil was highest with maize silage (trial D vs. trial C) and lowest with high-concentrate diet (68%, trial F), whereas the response to linseed oil was lower with maize silage than with either hay or fresh grass (Tabs. 5, 6). While the milk c9t11-CLA response to linseed oil supplementation did not change when the dietary concentrate increased from 30 to 54%, either in the presence or absence of vitamin E supplementation, it decreased with a high-concentrate (69%) diet (Tab. 6, trials E and F). This suggests that a minimal range of 55–65% concentrate in the diet is needed to decrease the milk c9t11-CLA response to plant oils, and that the best responses are allowed by association of sunflower oil with either maize silage-based (Tab. 5, trial D) or hay-rich diets (Fig. 8).

Tab. 8. Mid-term effects1 of different diets and/or supplements on milk FA composition (g/100 g total FA) in dairy cows and goats (see text for the origin of data).

<table>
<thead>
<tr>
<th>Diets/supplements</th>
<th>Pasture</th>
<th>High concentrate</th>
<th>18:2-rich oil</th>
<th>Linseed oil</th>
<th>High concentrate + plant oil</th>
<th>Fish oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:0–16:0</td>
<td>–</td>
<td>+/–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+/–</td>
</tr>
<tr>
<td>18:0</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+/0</td>
<td>–</td>
</tr>
<tr>
<td>c9–18:1</td>
<td>+ +</td>
<td>–/0</td>
<td>+ (0/+)*</td>
<td>+ (0)*</td>
<td>–/0</td>
<td>–</td>
</tr>
<tr>
<td>r11–18:1</td>
<td>+ +</td>
<td>–/0</td>
<td>+ +</td>
<td>+</td>
<td>0/+ (++)*</td>
<td>+ +</td>
</tr>
<tr>
<td>r10–18:1</td>
<td>0</td>
<td>+ (0)*</td>
<td>+/0</td>
<td>+/0</td>
<td>++ (+)</td>
<td>+</td>
</tr>
<tr>
<td>c9t11-CLA</td>
<td>+ +</td>
<td>0</td>
<td>++/+ + + + +</td>
<td>+ (+)</td>
<td>++ + (+ +++)</td>
<td>+ +</td>
</tr>
<tr>
<td>Other trans</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++ (+)</td>
<td>+</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>0 (−)*</td>
<td>0/0</td>
<td>0</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>+</td>
<td>−/0</td>
<td>0 (−)*</td>
<td>+ (+)*</td>
<td>0/0</td>
<td>0</td>
</tr>
<tr>
<td>EPA + DHA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>−</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

1 Effects after at least 3 wk on the diet, compared to a winter medium-concentrate diet, based on grass hay or silage.

2 Isomers of 18:1 and 18:2 (conjugated and non-conjugated).

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Data in cows showed that milk r11-18:1 and c9t11-CLA responses to lipid supplementation are transient with some diets (see Section 5.1). Such is not the case in goats: even with high-concentrate diets supplemented with PUFA-rich oils, the response of c9t11-CLA was maximal 2 wk after the beginning of oil supplementation and then remained stable at very high levels (Fig. 8) despite the t10-18:1 percentage increasing 5–8 times above control values in diets without dietary oils (Tab. 6). Furthermore, adding sunflower oil and fish oil to goat diet increased milk c9t11-CLA up to 10 g/100 g FA, i.e. among the highest values ever recorded in ruminant milk [179]. Finally, high milk CLA levels were observed after 9–10 wk of lipid supplementation, without a decrease from what was observed in the same goats after 5 wk (Fig. 9). This confirms that the goat is a very good responder and that its milk c9t11-CLA response is stable for at least 2.5 months. This stability may also be related to the stability of t10-18:1, which always remained at levels <3.5 g/100 g FA (Tabs. 5, 6), much lower than those reported in cows receiving similar diets (see Section 5.1), thus contributing to the lack of milk fat depression in goats receiving high-concentrate diets with PUFA-rich oils (see Section 2.3). A very stable response was also observed for other milk FA, including 18:3 n-3 (Fig. 9), which could be related to the good response of the goat for this PUFA (see Section 4.1.2.2).

It should be stressed, however, that the achievement of high levels of goat milk c9t11-CLA (>2% of total FA) with oil supplements is accompanied by high levels not only of r11-18:1 (6–13%) but also of other trans isomers of 18:1 and conjugated or non-conjugated 18:2 (3–6% with grass-based diets, 9–11% with maize silage diets and, for a given forage, linseed oil > high-oleic sunflower oil > sunflower oil; Tabs. 5, 6).

6 Conclusion

There is a considerable potential to modify milk FA composition by changing cow or goat feeding conditions (Tab. 8). RBH, combined with mammary lipogenic and Δ-9 desaturation pathways, considerably modifies the profile of dietary FA and thus milk composition. Pasture has major effects to decrease saturated FA and increase several FA considered as favorable for human health (c9-18:1, 18:3n-3 and c9t11-CLA), compared to winter diets, especially those based on maize silage and concentrates. Large variations are, however, putatively linked to grass vegetation stage and/or quality, deserving further research. Plant lipid supplements, especially linseed, have effects similar to pasture although they simultaneously increase several trans isomers of 18:1 and conjugated or non-conjugated 18:2, especially when added to maize silage or concentrate-rich diets. The goat seems to respond better for milk 18:3n-3 and c9t11-CLA, sometimes less for c9-18:1, and to be less prone to the RBH trans-11 to trans-10 shift, which has been shown to be time-dependent in the cow. Further research is needed to better manage RBH and favor the mammary secretion of either 18:0 + c9-18:1 or r11-18:1 + c9t11-CLA. The respective physiological roles of most milk trans FA have not been studied to date. However, encouraging results...
came recently from animal [8, 9] or human studies [10, 180] using dairy products modified by changing ruminant nutrition, which need confirmation, together with the evaluation of effects on dairy product sensorial quality, before recommending a larger use of lipid supplements and how to combine them with the different feeding systems used by dairy farmers.

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Feeding factors, rumen biohydrogenation and milk fatty acids


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