

Dietary lipids and forages interactions on cow and goat milk fatty acid composition and sensory properties

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Abstract – This review summarises the known effects of dietary factors on bovine and caprine milk fatty acid composition, as well as the regulation of cow and goat mammary lipid secretion. Special attention is given to fatty acids that could play a role for human health, such as saturated fatty acids, oleic acid, n-6- or n-3-C18 to C22 polyunsaturated fatty acids, *trans* isomers of C18:1 and C18:2, and isomers of conjugated linoleic acid (CLA). The main dietary factors taken into account are the nature of forages, including pasture, the forage:concentrate ratio and diet starch content, and the supplementation of dairy rations with crude or processed vegetable oils or oilseeds, and vitamin E. A particular emphasis is given to studies on interactions between these dietary factors, which show that there is a considerable plasticity of ruminant milk fatty acid composition. Despite the existence of several studies on the effects of dietary factors on the sensorial quality of milk and dairy products, there is a need to evaluate more deeply how the different feeding strategies could change the nutritional, sensorial and technological aspects of milk fat quality.

dairy cow / dairy goat / forages / oilseeds / milk / fatty acids / human health

1. INTRODUCTION

Dairy products provide 25 to 35% of the overall saturated fat consumed by man, which makes them the preferential target of dieticians' criticisms [1]. The deleterious reputation of saturated fatty acids (FA) should however be weighted with the fact that stearic acid has no atherogenic effect. The allegedly atherogenic effect of certain *trans* mono-unsaturated fatty acids has not been confirmed as regards the main isomer present in milk, i.e. vaccenic (*trans*11-18:1). The interest of increasing the n-3/n-6 ratio of polyunsaturated fatty acids (PUFA) has been confirmed. Lastly, the properties of conjugated linoleic acid (CLA), whose main iso-

mer, ruminic acid (*cis*9,*trans*11-18:2), exhibits interesting features, as demonstrated in animal models, are now better known, for the prevention of certain forms of cancer in particular.

These new facts underline the interest of modulating the milk FA composition. Mammals' milk FA composition is linked to intrinsic (animal breed, genotype, lactation and pregnancy stages) or extrinsic (environmental) factors. In a given animal species, the effects linked to breed or genotype are significant but restricted [2–6] and they can only be achieved in the mid-term or through interaction with constraints inherent in other criteria. The lactation stage effect is marked and mainly linked to lipid

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Table I. Effects of dietary lipid supplementation on cow's milk yield and composition.

Dietary lipids	N (A) ¹	Milk yield (kg·d ⁻¹) ²	Protein content (g·kg ⁻¹)	Fat content (g·kg ⁻¹)	Fat yield (g·d ⁻¹)
Animal fat (AF) ³	22 (688)	+0.5	-0.6*	-1.4	-18
Encapsulated AF ³	26 (941)	+1.0*	-1.8*	+4.0*	+143*
Saturated FA ³	10 (644)	+1.7*	-0.6*	+0.5	+58*
Palm oil Ca-salts ³	29 (593)	+0.9*	-1.2*	+0.4	+47*
Rapeseed oil (RO) ⁴	5 (742)	-1.9*	0.0	-5.9*	-215*
RO Ca-salts ⁴	11 (562)	+0.5	-2.0*	-3.9*	-94*
Rapeseeds ^{4,5}	11 (927)	+0.8	-0.3*	-3.1*	-83*
Heated rapeseeds ⁴	10 (531)	+0.1	-0.5	-1.1*	+39
Sunflower oil ⁴	5 (459)	+1.0	-1.1*	-3.4*	-57*
Sunflower seeds ^{4,5}	8 (503)	+0.7	+0.4	-1.2	+9
Soybean oil ⁴	34 (529)	+0.3	-0.8*	-3.3*	-63*
Soybeans ⁵	18 (517)	-0.7	-0.8*	+1.3*	-2
Extruded soybeans ⁴	16 (544)	+2.7*	-1.1*	-1.9*	+29
Heated soybeans ⁴	14 (692)	+1.7*	-1.1*	0.0	+49*
Linseed oil ⁴	10 (475)	+1.3*	-0.9*	-1.8	-7
Linseeds ^{4,5}	8 (686)	-0.4	-0.5	+0.3	+3
Encapsulated vegetable oils ³	26 (693)	0.0	-0.8	+6.4*	+120*
Marine oils ³	27 (305)	+0.2	-1.2*	-9.1*	-208*

¹ Number of lipid-supplemented groups (amount of lipids, g·d⁻¹).

² Effects expressed as "treated group-control group", * $P < 0.05$.

³ From [20].

⁴ Review by A. Ferlay and Y. Chilliard, unpublished.

⁵ Whole, rolled or ground.

store mobilisation in early lactation [7], but it only lasts a few weeks each year. In contrast, seasonal effects are quantitatively very important. Although certain effects of temperature or photoperiod can be evidenced, it is the variations of ruminant nutrition which determine most of the seasonal variations of milk FA composition. The effects of cheese-making technology on FA composition are minimal in relation to those of feeding [8].

Nutrition therefore constitutes a natural and economical way for farmers to sharply and rapidly modulate milk FA composition, in particular by adding lipid supplements to the diet. The consequences of these practices on dairy cows' milk fat and protein

synthesis and contents are now well known: a tendency to increase milk production (with saturated lipids and soybean in particular), slight but almost systematic decrease in protein contents, limited variations of the fat content except with rapeseed oil and especially with fish oil, which induce sharp decreases, and encapsulated lipids that strongly increase it (Tab. I). In contrast, nearly all types of lipid supplements induce a sharp increase in goat milk fat content without modifying milk yield or protein content [7]. After a reminder of the mammary lipogenesis metabolic pathways, this article successively analyses the impacts of dairy cows' and goats' diets on the main fatty acid

classes: saturated and *cis* mono-unsaturated, polyunsaturated and lastly CLA and *trans* mono-unsaturated. Then the effects of nutrition on the sensory characteristics of dairy products will be briefly evoked, as they may partly be due to post-milking lipolysis of milk fat (short-chain fatty acid release) or to polyunsaturated fatty acid oxidation.

2. METABOLIC PATHWAYS NUTRIENT FLUXES INVOLVED IN MILK FAT SYNTHESIS

2.1. Mammary lipogenesis

Milk fatty acids have a dual origin: they are either taken up from plasma lipoproteins (60% of the fatty acids secreted in milk, [9]) or they are synthesized *de novo* in the mammary gland from acetate and 3-hydroxybutyrate. The main metabolic pathway involves two key enzymes: acetyl-CoA carboxylase (ACC) and fatty acid synthetase (FAS). FAS synthesizes FAs that feature up to 16 carbon atoms. The cellular and molecular factors that regulate the chain length of the fatty acids synthesized, within the same species or between the various ruminants species, have yet to be identified. As an example, the factors that determine the specificities of goat milk, which contains more 8- and especially 10-carbon-atom fatty acids [10], are very little-known.

Pre-formed fatty acids are transported in plasma as non-esterified fatty acids (NEFA) or as triglyceride-rich lipoproteins. Lipoprotein lipase (LPL) permits triglyceride hydrolysis and FA uptake by the mammary gland. The amount of triglyceride uptake is generally well-correlated to its plasma concentration. Furthermore, the mammary gland uses plasma NEFA released by adipose tissue as a source of long-chain FA for milk lipid synthesis. The FAs stored as triglycerides in ruminant adipose tissue mainly are 16:0, 18:0 and *cis*9-18:1. For this reason, lipid mobilization, which occurs in early lactation and/or when the energy balance is negative, induces a sharp increase in stearic and oleic acids in milk [7].

The mammary gland cannot convert C16:0 into C18:0 by extending the carbon chain. Moreover, totally differentiated secretory mammary cells exhibit high delta-9 desaturase activity, which converts stearic acid into oleic acid (*cis*9-18:1) and so contributes more than 50% of all the oleic acid secreted in milk [11, 12]. In addition, approximately 30% of the vaccenic acid (*trans*11-18:1) originated in the rumen can be desaturated to form rumenic acid (*cis*9,*trans*11-18:2), the main isomer of CLA in milk [13]. Other minor conjugated or non conjugated isomers of C18:2 are probably synthesized by delta-9 desaturation of other *trans*-18:1 isomers [14, 15].

These various metabolic pathways (*de novo* synthesis, uptake from plasma, desaturation) contribute to the formation of a pool of FAs that are used for triglyceride formation (97 to 98% of milk total lipids) through esterification on glycerol. FA asymmetrical distribution on the glycerol molecule influences the physical properties of milk fat and the digestibility of certain FAs. That peculiarity adds up to other characteristics of the mammary metabolism (short-chain FA synthesis, long-chain FA desaturation) towards lowering the melting point of milk fat and thus reducing the effects of rumen hydrogenation. The effects of dietary factors on the triglyceride molecular structure are still little-known.

2.2. Lipid transformations in the rumen

In the rumen lipids undergo high-intensity metabolism linked to microbial activity. Dietary FAs are strongly hydrogenated. Linoleic acid (*cis*9,*cis*12-18:2) is isomerised into rumenic acid (*cis*9,*trans*11-18:2), then the latter is hydrogenated into vaccenic acid (*trans*11-18:1) and eventually into stearic acid (C18:0). Linolenic acid induces a larger number of intermediaries, including vaccenic acid, but rumenic acid production does not seem to occur. In fact, biochemical pathways are much more complex. No less than twelve 18-carbon, mono-unsaturated isomers can be found in the rumen

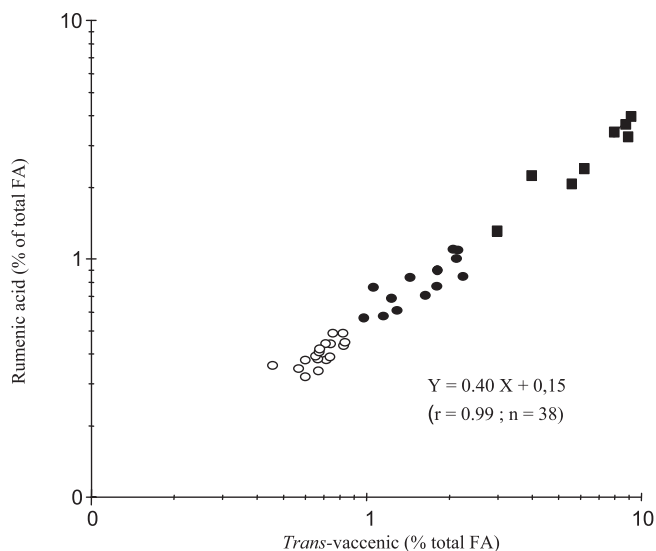


Figure 1. Relationship between the contents of trans-vaccenic and rumenic acids in goat milk. Each point is the mean of values from 7 to 16 goats (N = 401 milks from 38 experimental groups) (adapted from [7]). ○ Hay-based diets (either without lipid supplementation or with untreated lupin seeds or soybeans). ● Hay-based diets (either without lipid supplementation or supplemented with high-oleic sunflower oil or untreated linseeds or sunflower seeds) or corn silage-based diets (either without lipid supplementation or with high-oleic sunflower oil). ■ Hay or corn silage-based diets, supplemented with either linseed oil or sunflower oil.

medium [16] and many CLA isomers have been identified [17].

Indeed, the terms CLA (Conjugated Linoleic Acid) gather a large number of geometric and position isomers (all with conjugated double bonds) of linoleic acid. The hydrogenation of *trans*-18:1 classically constitutes the limiting step for the full hydrogenation of unsaturated C18, and *trans*-C18:1 frequently occurs in the rumen, contrarily to CLA [13]. A small proportion of the CLA produced by rumen PUFA hydrogenation is nonetheless absorbed in the intestine, taken up by the mammary gland and secreted in milk. However, rumenic acid synthesis mainly occurs (probably more than 75%) in the udder, in proportion to the amount of vaccenic acid formed in the rumen (Fig. 1 and [18]).

The quantitative and qualitative balance of rumen metabolism has been described by Doreau and Ferlay [19]. Hydrogenation in

the rumen averages 80% for linoleic acid and 92% for linolenic acid. The hydrogenation rate is mainly dependent on the proportion of concentrate in the diet. When it exceeds 70%, hydrogenation only averages 50 and 65% for these two FAs. That phenomenon is related to a collapse in rumen pH.

3. CONTROLLING THE FA COMPOSITION OF MILK LIPIDS BY THE NUTRITION OF DAIRY ANIMALS

3.1. Saturated fatty acids and oleic acid

Most fatty acids originating from de novo lipogenesis are saturated (C4:0 to C16:0), because mammary delta-9 desaturase is only weakly active on fatty acids with less than 18 carbon atoms. Long-chain FAs (at least 18 carbon atoms) are powerful inhibitors of

acetyl-CoA carboxylase and of de novo lipogenesis in mammary cells. This effect is more marked when FAs have a long chain, are more unsaturated and contain more *trans* double bonds (review by Chilliard et al. [9]).

When the bioavailability of C18 FAs increases (as a result of either increased dietary intake or body lipid mobilization), C8:0 to C16:0 FA secretion decreases, and their concentration (Tabs. II–V) decreases even more through dilution in a larger quantity of long-chain FAs. Dietary FAs also have an indirect negative effect on mammary de novo lipogenesis because of the decreased intake of fermentable organic matter and hence production of volatile FAs, and of the lower acetate/propionate ratio in the rumen. These modifications indeed reduce acetate and 3-hydroxybutyrate bioavailability to mammary lipogenesis. The potential to decrease medium-chain saturated FAs (C10 to C16:0) is very large. For example, with hay-based diets, these FAs represented 59% of goat milk fat and decreased to 38% after linseed oil supplementation, or to 33% if vitamin E was added with linseed oil (Tab. V).

Pasture intake has effects close to those of 18-carbon-atom FA supplementation. Indeed, compared to grass silage-based diets, milk myristic and palmitic acid concentrations are reduced (review by Chilliard et al. [20]). Conversely, if lipid supplements contain mainly medium-chain fatty acids, these will be increased. Such is the case with palm oil calcium salts, which increase palmitic acid concentration (+ 21 mg·g⁻¹ for 770 g·d⁻¹ mean supplementation over six trials). Such an effect is not beneficial to milk nutritional quality.

Contrary to medium-chain FAs, short-chain FA concentrations (C4:0, C6:0 and C8:0 to a lesser extent) are classically either unchanged or only slightly reduced by increased lipid supplementation in the diet (Tabs. II–IV) or body lipid mobilization. That specificity is probably due to the fact that those fatty acids are partly synthesized by metabolic pathways not dependent on

acetyl-CoA carboxylase [21, 22]. The stability of butyric acid concentration in milk is interesting because that FA has beneficial effects on human health [23].

Stearic acid secretion in milk can be increased either by dietary stearic acid intake or by supplementation of C18 unsaturated FAs because they are in part hydrogenated into stearic acid in the rumen. The same applies to oleic acid either through its direct secretion or from its synthesis through the action of mammary desaturase on stearic acid. It is recommended to increase the *cis*9-18:1/18:0 ratio to reduce butter firmness and improve its nutritional quality, so as to reduce atherogenous risks in man [1]. That ratio is regulated by the respective bioavailabilities of the two FAs, by mammary desaturase activity and by the modulating factors of that activity (PUFA or *trans* FAs availability in particular).

Among the dietary factors which alter the secretion of stearic and oleic acids in milk, tallow supplementation (rich in C16:0, C18:0 and *cis*9-18:1) has been extensively explored (review by Chilliard et al. [20]). It appears that tallow favourably modifies the nutritional quality of milk FAs by sharply reducing their atherogenicity index [24]. However, those supplements are now banned in Europe, in application of the principle of precaution with regard to the theoretical risks of prionic contamination.

Other ways to increase milk oleic acid secretion include the distribution of oleamides [25] or properly protected oleic-acid-rich vegetable oils or seeds (oleic sunflower, rapeseed). When giving unprotected vegetable oils or seeds containing high levels of oleic, linoleic or linolenic acids, the absorbed fluxes of dietary oleic acid which escapes ruminal hydrogenation can be increased, although the main response is an increase in the stearic acid produced in the rumen, which is then transformed in part into oleic acid in the udder. Thus, the proportion of stearic acid and, sometimes, oleic acid are increased in milk (Tabs. II–V). For example, cow's milk oleic acid was multiplied by

Table II. Effects of supplementation with oilseeds on milk yield, fat content and yield, and fatty acid composition in dairy cow¹.

Supplement	Linseed		Sunflower (18:2-rich)		Soybean			Rapeseed	
	Oil ²	Seed ³	Oil ²	Seed ⁴	Oil ⁵	Raw ⁶	Extruded ⁷	Oil ⁸	Seed ³
Dietary lipids (%)	+3	+2.5	+3	+4.2	+2.3	+2.8	+1.8	+2.1	+2.3
Milk yield (kg·d ⁻¹)	+1.5	-1.7	+2.2	-2.5	+1.5	-0.3	-0.2	+0.8	-0.9
Milk fat content (g·kg ⁻¹)	-3.3	+0.3	-4.8	-7.1	-0.9	-2.1	+0.6	-2.8	-1.9
Milk fat yield (g·d ⁻¹)	-47	+30	-57	-259	+10	-40	+10	-70	-30
Milk fatty acids (% of total FA)									
C4:0	-0.1	nd ⁹	-0.2	-1.2	+0.5	+0.3	+0.1	+0.4	nd
C6 to C8:0	-0.8	-0.6	-0.9	-2.1	-0.4	-0.2	-0.4	-1.1	-0.9
C10 to C14:0	-4.6	-3.2	-5.3	-8.6	-5.2	-3.7	-3.6	-2.7	-3.9
C16:0	-9.6	-3.2	-9.9	-8.7	-5.1	-7.5	-5.4	-10.7	-4.0
C18:0	+3.0	+3.8	+3.3	+4.1	+3.4	+0.8	+1.7	+4.0	+3.3
C18:1	+7.1	+4.0	+9.2	+15.8	+6.3	+7.9	+4.6	+13.6	+5.7
C18:1 c9	+4.0	nd	+5.2	(+9.7) ¹⁰	nd	nd	+2.7	+10.6	nd
C18:1 t11	+3.1	-0.4	+4.0	(+7.4) ¹⁰	nd	nd	+1.4	+3.0	+0.02
C18:2 c9 c12	-0.1	-0.7	+0.4	+1.3	-1.0	+0.9	+1.9	-0.1	-0.8
C18:3 c9 c12 c15	+0.2	+0.8	-0.1	+0.3	-0.04	-0.1	+0.3	+0.3	+0.02
C18:2 c9 t11	+1.2	-0.2	+1.6	nd	(+1.7) ¹¹	(-0.02) ¹¹	+0.5	+0.6	+0.01

¹ Difference between fat supplemented and control groups.

² A. Ferlay and Y. Chilliard, unpublished data, 47.5 or 59.8% silage, 12.9 or 4.7% grass hay, 36.7 or 32.4% concentrate, and 3% of oil for diets based on grass or maize silage, respectively.

³ [91] 43.9% alfalfa silage, 11.8% barley silage, 8.3% ground linseeds (3.3% oil) or rapeseeds (3.4% oil), and 36.0% concentrate.

⁴ [92] 40% maize silage, 15% alfalfa hay, 19% concentrate, and 21% of rolled sunflower seeds (9.8% oil).

⁵ [93] 30% alfalfa silage, 20% maize silage, 47.7% concentrate, and 2.3% oil.

⁶ [94] 42% dehydrated alfalfa pellets and long alfalfa hay, 16% maize silage, 14.7% ground raw soybeans (2.9% oil), 27.3% concentrate.

⁷ [95] 25% alfalfa silage, 25% maize silage, 39.4% concentrate, and 10.6% extruded soybeans (2.1% oil).

⁸ [96] 17.5% alfalfa haylage, 30.7% maize silage, 48.5% concentrate, and 3.3% oil.

⁹ Nd, not determined.

¹⁰ All *cis*-18:1 or *trans*-18:1 isomers.

¹¹ [97] 34% alfalfa silage, 17% maize silage, 45.4 or 31.0% concentrate, and 3.6% soybean oil or 18% raw cracked soybeans (3.6% oil), for diets supplemented with oil or soybeans.

Table III. Milk yield and composition in goats fed a low forage diet¹, supplemented or not with oils or whole crude oilseeds during 11 weeks² (7 goats per group) (adapted from [7]).

Diet	Control	Linseed oil	Linseeds	Sunflower oil	Sunflower seeds	Lupine seeds	Soybeans
Milk yield ³ (kg·d ⁻¹)	2.86	3.12	2.91	3.15	3.11	3.16	3.37
Fat content (g·kg ⁻¹)	25.5 ^a	28.6 ^b	31.5 ^b	30.7 ^b	31.3 ^b	29.2 ^b	29.6 ^b
Fat yield (g·d ⁻¹)	72 ^a	90 ^b	93 ^b	94 ^b	95 ^b	92 ^b	97 ^b
Fatty acids (w% of total FA)							
C4 + C6 + C8	7.9 ^b	7.5 ^{ab}	7.8 ^b	7.7 ^b	6.9 ^a	7.4 ^{ab}	7.1 ^{ab}
C10 + C12 + C14	24.9 ^b	16.3 ^a	18.0 ^a	17.3 ^a	16.9 ^a	18.6 ^a	16.9 ^a
C16:0	25.8 ^c	16.9 ^a	19.0 ^b	18.2 ^{ab}	18.7 ^b	19.4 ^b	19.6 ^b
C18:0	9.0 ^a	13.8 ^b	15.2 ^{bc}	13.0 ^b	15.9 ^c	13.5 ^b	16.6 ^c
C18:1 t11	0.95 ^a	2.92 ^{bc}	1.28 ^a	3.94 ^c	2.30 ^b	0.66 ^a	0.85 ^a
C18:1 c9	19.1 ^a	22.6 ^{ab}	24.7 ^{bcd}	20.8 ^a	23.9 ^{bc}	27.4 ^d	26.3 ^{cd}
C18:2 c9 c12	2.2 ^c	2.2 ^{bc}	1.9 ^b	3.4 ^e	3.0 ^d	1.6 ^a	3.3 ^{de}
C18:3 c9 c12 c15	0.41 ^a	1.68 ^d	1.24 ^c	0.49 ^a	0.51 ^b	0.63 ^b	0.40 ^a
C18:2 c9 t11	0.56 ^b	1.38 ^d	0.60 ^b	2.28 ^e	0.84 ^c	0.28 ^a	0.40 ^{ab}
AI ⁴	2.92 ^c	1.21 ^a	1.61 ^b	1.36 ^a	1.48 ^{ab}	1.72 ^b	1.52 ^{ab}
Desaturation index ⁵	0.69 ^c	0.63 ^b	0.62 ^{ab}	0.61 ^{ab}	0.59 ^a	0.67 ^c	0.61 ^{ab}

¹ Natural grassland hay (30%) and concentrates with or without oils or oilseeds (70%).

² 3.4 ± 0.6% added lipid in DM intake (supplemented-control).

³ Data in same row with similar superscript letters do not differ at $P < 0.05$ level.

⁴ Atherogenicity index, (C12 + 4 C14 + C16):(sum of unsaturated FA).

⁵ C18:1c9:(C18:0 + C18:1c9).

1.18 to 1.34 when adding either sunflower or linseed oil to the diet, and these responses were dose-dependent, and more marked with grass silage than maize silage diet (Fig. 2). Grazing is another way to increase stearic and oleic acid concentrations in cow milk. Oleic acid percentage in milk fat can increase from 14–18% with lipid-poor winter diets to 22–24% at pasture, which provides PUFA that can be hydrogenated in the rumen [26].

In goats, when comparing 26 diets combining different forages, concentrate percentages and lipid sources (Fig. 3), it appears that the highest milk oleic percentages (more than 24% of total FA) are obtained either with unprotected high-oleic sunflower oil (and more with alfalfa hay or rye-grass than with maize silage) or with oilseeds, in the rank lupin > soybean > linseed > sunflower. Beside the direct absorption of die-

tary oleic acid, these results arise also from the absorption of stearic acid yielded from oleic acid in the rumen, and then its delta 9-desaturation in the mammary gland. It can be observed that the *cis*9-18:1/18:0 ratio is decreased by lipid supplements: more markedly by oilseeds than oil, and more markedly by PUFA-rich oils than high-oleic oil (Fig. 4 and Tab. V). This ratio is further decreased when vitamin E is added to the diet (Tab. V). All together, these results suggest that the desaturation ratio of stearic acid in the mammary gland is decreased by diets which increase the availability of either PUFAs or *trans*-FAs (see Tabs. III–V), as these FAs are putative inhibitors of the delta-9-desaturase [27–29].

The case of lupine seeds is interesting because this seed, rich in 18:1 and 18:2, is the only one which did not decrease the

Table IV. Interactions between forage nature and vegetable oil supplementation (5–6 % of diet DM) on goat milk yield and composition (adapted from [81])¹.

Forage	Maize silage			Alfalfa hay		
	C ²	LO	OSO	C ³	LO	OSO
Milk yield (kg·d ⁻¹)	3.62	3.95	3.61	3.65	3.61	3.54
Fat content (g·kg ⁻¹)	33.4 ^b	33.4 ^b	36.3 ^{bc}	29.7 ^a	36.9 ^c	35.1 ^{bc}
Fat yield (g·d ⁻¹)	121 ^{ab}	129 ^a	132 ^a	108 ^b	134 ^a	123 ^{ab}
Fatty acids (w% of total FA)						
C4:0	2.2 ^{ab}	2.8 ^d	2.6 ^{cd}	2.2 ^{ab}	2.4 ^{bc}	2.2 ^{ab}
C6:0	2.5 ^{bc}	2.6 ^c	2.4 ^{bc}	2.3 ^b	2.1 ^{ab}	2.1 ^{ab}
C8:0	2.8 ^d	2.6 ^{cd}	2.4 ^{bcd}	2.4 ^b	2.0 ^a	2.0 ^{ab}
C10:0	10.0 ^c	8.1 ^{ab}	7.4 ^a	8.8 ^b	6.1 ^a	6.5 ^a
C12:0	4.7 ^b	3.0 ^a	3.0 ^a	4.6 ^b	2.7 ^a	2.9 ^a
C14:0	11.7 ^c	8.2 ^b	8.4 ^b	12.2 ^c	7.6 ^a	8.4 ^b
C16:0	28.8 ^b	18.5 ^a	18.7 ^a	31.1 ^c	18.2 ^a	17.8 ^a
C18:0	7.5 ^b	9.5 ^c	13.8 ^b	6.0 ^a	10.8 ^d	12.7 ^e
C18:1 t10	0.24 ^a	2.97 ^c	2.20 ^c	0.07 ^a	0.29 ^a	0.70 ^b
C18:1 t11	1.18 ^{ab}	6.18 ^c	1.88 ^{ab}	0.45 ^a	8.80 ^d	2.12 ^b
C18:1 c9	15.7 ^{ab}	14.2 ^a	23.5 ^c	16.6 ^b	15.6 ^{ab}	27.7 ^d
C18:2 c9c12	2.0 ^d	1.5 ^b	1.4 ^a	2.3 ^e	1.7 ^c	1.6 ^b
C18:3 c9c12c15	0.32 ^b	0.69 ^d	0.17 ^a	0.60 ^d	1.37 ^e	0.42 ^c
C18:2 c9 t11	0.59 ^{ab}	2.42 ^c	0.80 ^{ab}	0.33 ^a	3.22 ^d	1.02 ^b
Desaturation index ⁴	0.67 ^c	0.61 ^{ab}	0.63 ^b	0.73 ^d	0.59 ^a	0.68 ^c

¹ C, LO, OSO = control, linseed oil, oleic sunflower oil, respectively; twelve goats per group, except hay-control group ($n = 10$); results obtained after 5 weeks of lipid supplementation.

² Including 48% of concentrates.

³ Including 44% of concentrates.

⁴ 18:1c9:(C18:0 + C18:1c9); data in same row with similar superscript letters do not differ at $P < 0.05$ level.

desaturation ratio (Fig. 4B), and which did not increase (or even decreased) goat milk PUFAs and vaccenic acid (Tab. III), suggesting that its unsaturated FAs were totally hydrogenated, despite the fact it was consumed as crude whole seed. Conversely, either linseed oil or extruded linseeds supplementations decreased strongly the desaturation ratio, simultaneously to the high increases in both 18:3n-3 and *trans*-FAs percentages in milk fat (Fig. 4 and Tab. V).

3.2. Polyunsaturated fatty acids

PUFAs are not synthesized by tissues in ruminants, therefore their concentration in

milk is closely related to the quantities absorbed in the intestine, hence the quantities leaving the rumen. Those quantities may be increased by dietary PUFA intake and by factors which decrease rumen hydrogenation, such as fatty acid trapping in vegetable cells, high forage/concentrate ratio or the implementation of PUFA-rich oil encapsulation techniques.

3.2.1. Linoleic acid (*n-6 series*)

With most non lipid-added diets, the proportion of linoleic acid in milk fatty acids is classically between 2 and 3%. When rations are supplemented with linoleic acid-rich seeds

Table V. Effects of linseed oil, vitamin E or extruded linseed supplementations on milk fatty acid composition in goats receiving during 5 weeks diets with either high or low forage:concentrate ratio¹.

Supplement ²	No supplement		Linseed oil (LO)		LO + vitamin E		ELS ³	Statistical effects (<i>P</i> <)			
	F:C ⁴	High	Low	High	Low	High		Low	High	F:C (<i>n</i> = 72)	LO (<i>n</i> = 48)
Milk yield (kg·d ⁻¹)		4.26 ^a	4.39 ^{ab}	4.25 ^a	4.28 ^a	4.19 ^a	4.74 ^b	4.26 ^a	0.08	NS	NS
Fat content (g·kg ⁻¹)		28.1 ^a	27.0 ^a	33.2 ^b	33.3 ^b	34.9 ^b	34.8 ^b	35.4 ^b	NS	0.001	NS
Fat yield (g·d ⁻¹)		119 ^a	120 ^a	140 ^{ab}	141 ^b	149 ^{bc}	166 ^c	150 ^{bc}	NS	0.001	0.07
Milk fatty acids (% of total FA)											
C10+C12+C14		26.8 ^e	29.2 ^f	19.0 ^{bc}	22.7 ^d	16.1 ^a	20.2 ^c	18.8 ^b	0.003	0.001	0.001
C16:0		31.9 ^d	27.4 ^c	18.7 ^b	19.8 ^b	16.9 ^a	18.8 ^b	17.2 ^a	NS	0.001 ⁷	0.001
C18:0		6.3 ^a	6.1 ^a	9.8 ^c	8.6 ^b	10.9 ^d	9.3 ^{bc}	11.3 ^d	0.04	0.001	0.001
C18:1 t10		0.12 ^a	0.33 ^{ab}	0.43 ^{ab}	1.00 ^c	0.57 ^b	1.06 ^c	0.59 ^b	0.005	0.001 ⁷	NS
C18:1 t11		0.54 ^a	1.27 ^a	7.78 ^c	7.36 ^{bc}	9.52 ^d	8.15 ^c	6.48 ^b	NS	0.001 ⁷	0.005
C18:1 c9		14.9 ^b	14.4 ^{ab}	15.0 ^b	13.3 ^a	14.9 ^b	13.3 ^a	14.6 ^b	0.001	NS	NS
C18:2 c9 c13		0.10 ^a	0.22 ^b	0.50 ^{de}	0.41 ^c	0.54 ^{ef}	0.48 ^d	0.59 ^f	NS	0.001 ⁷	0.002
C18:2 c9 c12		2.4 ^c	2.8 ^d	1.8 ^a	1.8 ^{ab}	1.8 ^{ab}	1.8 ^a	2.0 ^b	NS	0.001 ⁷	NS
C18:2 t11 c15		0.01 ^a	0.01 ^a	1.96 ^b	1.82 ^b	2.22 ^c	2.32 ^c	1.83 ^b	NS	0.001	0.004
C18:3 c9 c12 c15		0.78 ^b	0.43 ^a	1.69 ^d	1.08 ^c	1.74 ^d	1.19 ^c	2.66 ^e	0.001	0.001 ⁷	NS
C18:2 c9 t11		0.30 ^a	0.70 ^a	3.05 ^c	3.33 ^c	3.25 ^c	3.08 ^c	2.09 ^b	NS	0.001	NS
C18:2 c9c11+t11c13		0.04 ^a	0.03 ^a	0.87 ^d	0.57 ^{bc}	1.13 ^e	0.76 ^{cd}	0.40 ^b	0.09	0.001 ⁷	0.005
Sum of <i>trans</i> FA ⁵		1.42 ^a	3.30 ^b	15.57 ^d	15.67 ^d	18.35 ^e	16.78 ^d	13.51 ^c	NS	0.001 ⁷	0.003
Desaturation index ⁶		0.70 ^c	0.71 ^c	0.60 ^b	0.61 ^b	0.58 ^a	0.58 ^{ab}	0.56 ^a	NS	0.001	0.008

¹ 7 groups of 12 goats. Adapted from [98–100].² Linseed oil at 4.4% of diet DM; vitamin E at 1250 IU/d/goat.³ Extruded linseeds: extruded mixture (70/30) of linseeds and wheat (i.e. 4.3% linseed oil in diet DM).⁴ Forage: concentrate ratio. High = 70% alfalfa hay, 8% starch; Low = 46% alfalfa hay, 29% starch.⁵ C18:1 or C18:2 with at least one *trans* double bond.⁶ C18:1c9:(C18:0 + C18:1c9).⁷ F:C – LO interaction (*P* < 0.05).⁸ No interaction with F:C (in presence of LO).a,b,c,d,e,f Data in same row with similar superscript letters do not differ at *P* < 0.05 level.

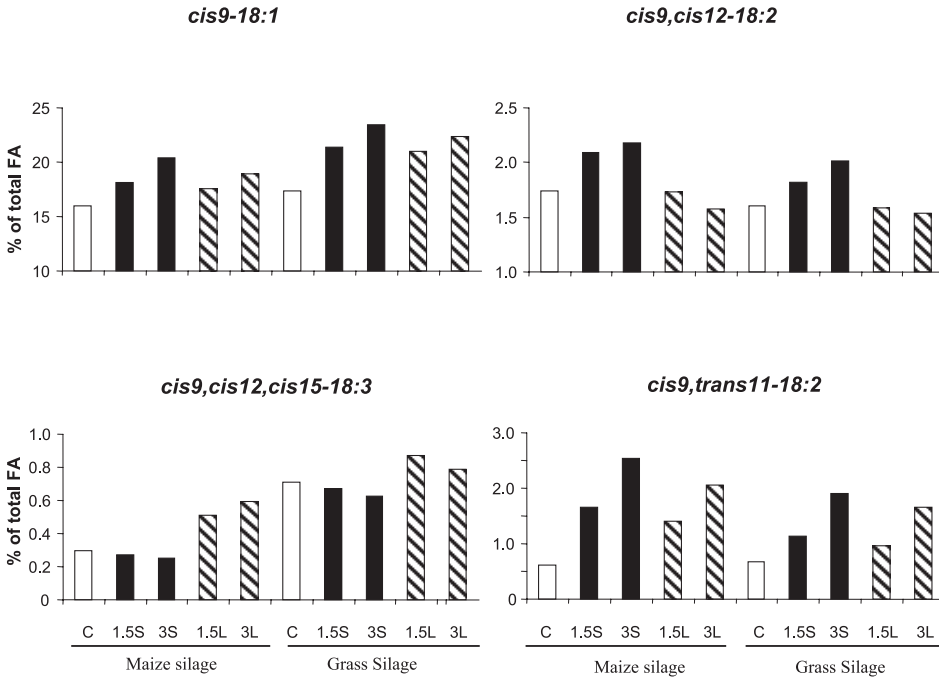


Figure 2. Effect of nature of forage, and nature and dose of oil supplement on milk percentages of *cis9-18:1*, *cis9,cis12-18:2*, *cis9,cis12,cis15-18:3* and *cis9,trans11-18:2* in dairy cows (A. Ferlay and Y. Chilliard, unpublished data). Abbreviations used: C, control (no oil); 1.5S or 3S, diet supplemented with 1.5 or 3% of sunflower oil (18:2-rich); 1.5L or 3L, diet supplemented with 1.5 or 3% of linseed oil (18:3-rich) (20 cows were used in 2 replicated 5 × 5 Latin Square designs with 3-week periods; maize or grass silage diets contained 13 or 5% grass hay, and 40 or 35% concentrates, respectively). Milk fat content ($\text{g}\cdot\text{kg}^{-1}$) was 39, 35, 32, 37, 34, 37, 37, 35, 37, 36 for the 10 groups, as presented from left to right.

or oils like soybean or sunflower, that proportion rarely exceeds control values by more than 1.5% (Tabs. II and III).

It has often been suggested that giving lipids in the form of seeds rather than oil would limit rumen hydrogenation because seed sheaths would restrict bacterial access to lipids. For example, with soybean, it would limit the reduction of the acetate/propionate ratio in rumen volatile fatty acids [30] such a reduction often being generated by unsaturated lipid supplementation. Furthermore, raw or processed soybean at low doses, or sunflower seed at large doses do not reduce largely cow milk lipid secretion (Tab. I) and increase linoleic acid concentration to some extent (Tab. II). Further-

more, direct comparison showed that extruded soybean increased less C18:2n-6 than raw soybean [31], probably because extrusion enhanced oil release from vegetable structures, enhancing their gradual hydrogenation. However, rapeseed sheaths appear to have a less protective effect than soybean or sunflower ones on milk fat content (Tab. I) and C18:2n-6 percentage (Tab. II). Additional research is necessary to confirm such trends because there are few direct comparisons between oil and seeds. Comparing sunflower oil and seeds in goats revealed that seed C18:2 was, paradoxically, more strongly hydrogenated to stearic acid than oil C18:2, found either intact or in the form of *trans* FA and CLA in milk (Tab. III). It

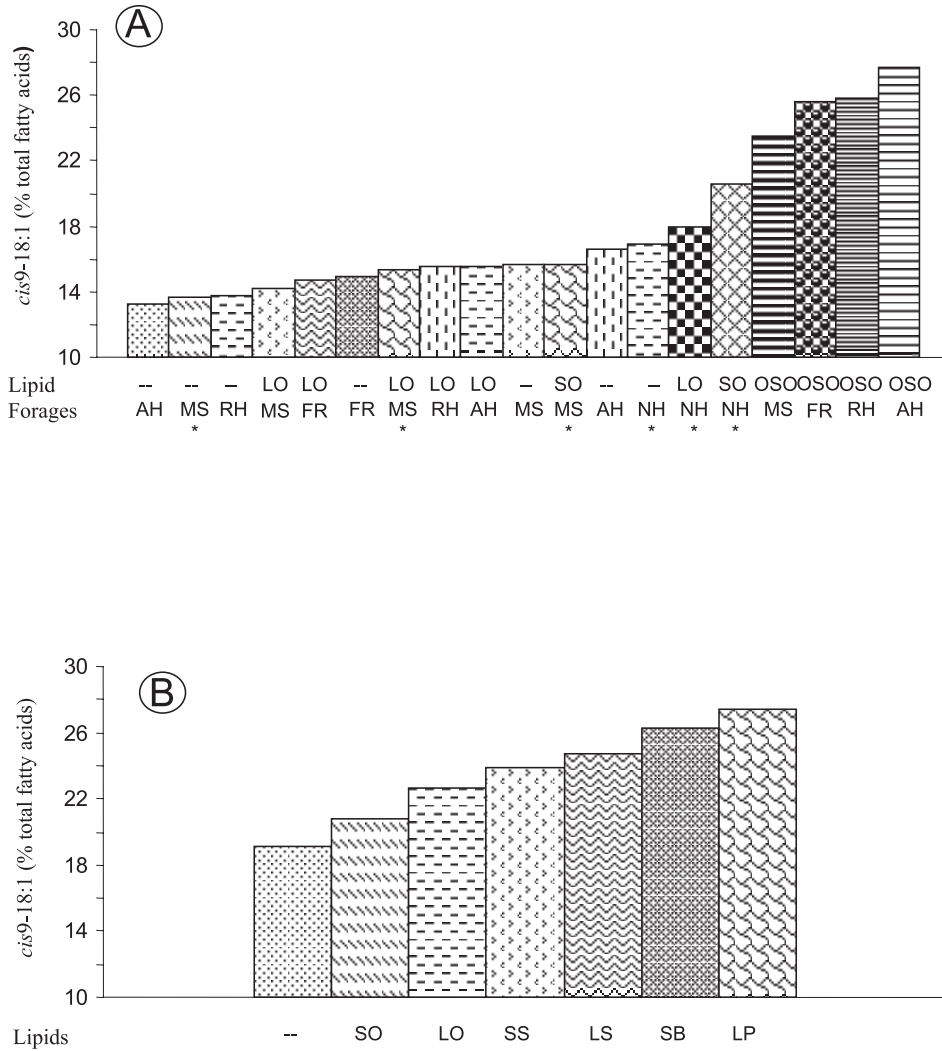


Figure 3. Forage-oil interactions on milk fat oleic acid percentage. **(A)** in goats receiving medium-concentrate (45–60% of diet DM) diets supplemented, or not, with oil (5–6% of diet DM) during 3(*)–5 weeks (10–14 goats per group; 181 goats) (adapted from [7, 15, 81, 82, 83, 108], **(B)** in goats receiving high-concentrate (70% of diet DM) diet supplemented, or not, with oil or whole crude oilseeds (3.4% of oil in diet DM) during 11 weeks (7 goats per group, 49 goats) (adapted from [7]). Forages: AH, alfalfa hay; FR, fresh green rye-grass; NH, natural grassland hay; MS, maize silage; RH, rye-grass hay. Lipids: --, control diet (without oil addition); LO, linseed oil; LP, lupine seeds; LS, linseeds; OSO, high-oleic sunflower oil; SB, soybeans; SO, sunflower oil; SS, sunflower seeds.

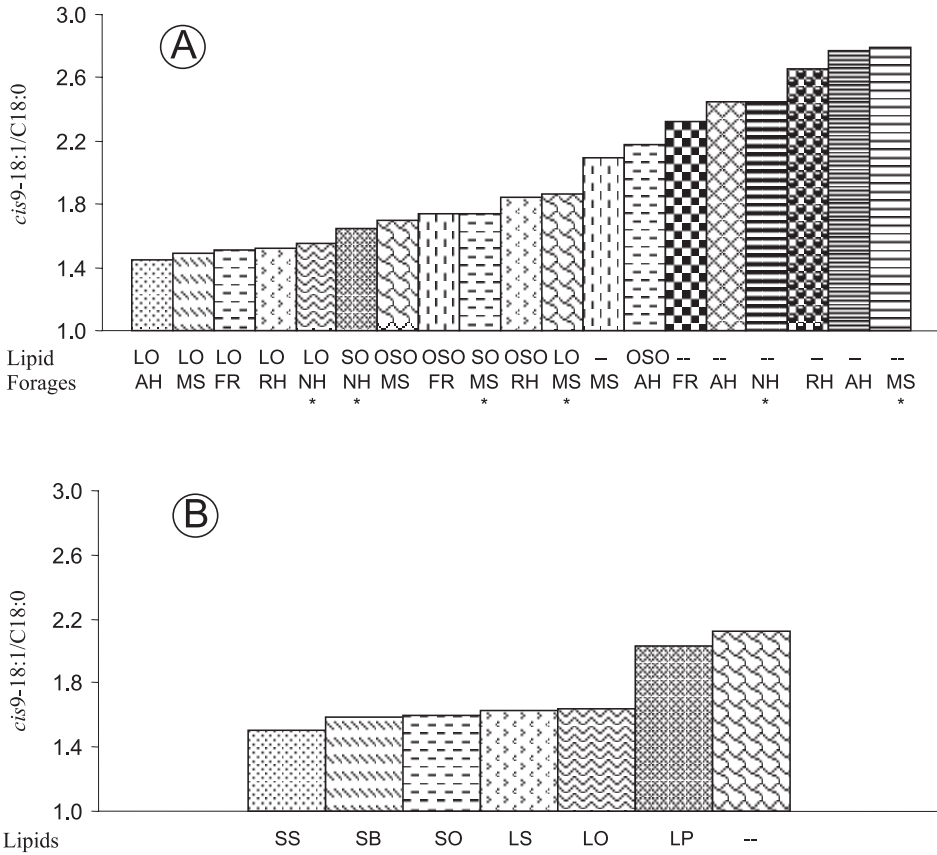


Figure 4. Forage-oil interactions on milk fat oleic:stearic ratio. (A) in goats receiving medium-concentrate (45–60% of diet DM) diets supplemented, or not, with oil (5–6% of diet DM) during 3(*)–5 weeks (10–14 goats per group; 181 goats) (adapted from [7, 15, 81, 82, 83, 108]), (B) in goats receiving high-concentrate (70% of diet DM) diet supplemented, or not, with oil or whole crude oilseeds (3.4% of oil in diet DM) during 11 weeks (7 goats per group, 49 goats) (adapted from [7]). Forages: AH, alfalfa hay ; FR, fresh green rye-grass; NH, natural grassland hay; MS, maize silage; RH, rye-grass hay. Lipids: --, control diet (without oil addition); LO, linseed oil; LP, lupine seeds; LS, linseeds; OSO, high-oleic sunflower oil; SB, soybeans; SO, sunflower oil; SS, sunflower seeds.

may therefore be supposed that the slow release of seed lipids enhances their total hydrogenation, at least in goats. A similar observation was made with C18:2-rich lupin seed, which strongly increased stearic and oleic acids while reducing milk C18:2n-6 and CLA (Tab. III).

Lipid supplements can be protected from rumen degradation by their encapsulation

in a tanned-protein layer. Fifteen to 20% proportions of linoleic acid in milk fatty acids have been reached with encapsulated soybean, rapeseed, cotton, safflower or sunflower oil supplements [32]. The limitations of such a dietary practice is linked to the processing cost and to the controversial use of formaldehyde. Other lipid protection techniques, such as FA salts, do not prevent

polyunsaturated FA hydrogenation [33, 34] or the negative effect of rapeseed oil on milk fat content (Tab. I), because the salts are dissociated in the rumen as the pH decreases.

The addition of linseed oil (18:3-rich) to cow's or goat's diet decreased milk linoleic acid percentage, probably because it increased linolenic percentage (Fig. 2 and Tabs. IV and V). Opposite responses between these 2 PUFAs were also observed when sunflower oil (18:2-rich) was added (Fig. 2). This illustrates that the different PUFAs are not secreted independently from each others. Lastly, it is worth reminding that increasing the linoleic acid proportion in dairy products is not a target in itself, insofar as improving the nutritional value of those products first requires to increase the linolenic/linoleic ratio.

3.2.2. *Linolenic acid and n-3 fatty acids*

Fresh green grass is the main source of alpha-linolenic acid, which explains why milk produced from grass-based diets contain more C18:3 n-3 than maize-based or concentrate-rich ones ([20, 26], Fig. 2 and Tabs. IV and V). However, hay making considerably reduces linolenic acid concentration in forage, as a result from concomitant decreases in FA and linolenic acid concentrations, whereas silage-related modifications are of lower extent [35]. It is mainly in the Spring and in the Autumn that FA contents and C18:3 n-3 concentrations are the highest in grass [36]. That explains the sharp increase in milk linolenic acid induced by turning out to pasture, which can be as high as 2.5% of total FAs [37, 38]. However, concentrations not exceeding 1% have sometimes been noted in milk from pasture-fed cows [6, 39], probably when vegetation stage advances.

Apart from forage, only linseed provides very high linolenic acid levels, representing more than 50% of FAs. For example, a cow eating 20 kg DM per day on a spring or autumn sward may ingest up to 400 g lino-

lenic acid per day, a similar amount to that provided by a winter ration with 3.7% linseed oil (or 12% linseed) addition. Rapeseed contains a significant amount of linolenic acid, some of which is probably secreted in milk. However, as noted with C18:2, rapeseed oil or seed addition does not increase milk C18:3 any significantly (Tab. II).

Few trials have been conducted, where cows' diets were supplemented with linseed oil or seeds. Kennelly [40] noted an increase in milk linolenic acid concentration by 6 mg·g⁻¹ of total FAs. Other authors found either no increase [41] or increases in the range 3–8 mg·g⁻¹ ([42–44], Tab. II). Response to linseed oil, however, is stronger with a high-concentrate diet (9 mg·g⁻¹) than with a high-hay diet (3 mg·g⁻¹) ([14] and Loor et al. unpublished). The variability of the results obtained requires new studies by discriminating between the effects of oil and seeds, in particular. Table II results nonetheless suggest that ground linseeds do not increase milk C18:3 much more than linseed oil. The reverse has even been observed in goats, where C18:3 from whole crude linseeds was more widely hydrogenated to C18:0 than C18:3 from free oil (Tab. III), as previously observed with sunflower C18:2. In other respects, linseed oil C18:3 seems to be less hydrogenated when given to goats receiving a hay-based than either a maize silage-based diet (Tab. IV) or a medium-concentrate + hay diet (Tab. V).

The intake of 200–400 g·d⁻¹ of linolenic acid from extruded rapeseeds and/or linseeds increased cow milk fat linolenic acid percentage by 3–6 mg·g⁻¹ [45, 46], i.e. in the range observed with unprocessed linseeds or oil. The response to extruded linseeds seems to be however different in the goat (Tab. V), where linolenic acid increased more (+19 mg·g⁻¹) than after linseed oil supplementation (+9 mg·g⁻¹). This high response of the goat milk 18:3 to extruded linseeds confirms previous results suggested by a comparison between extruded rapeseeds and linseeds [47].

As with linoleic acid-rich oil supplements, linseed oil protection by encapsulation is the only way to warrant very high linolenic concentration in milk. For instance, a 64 mg·g⁻¹ of linolenic acid in cow milk fat has been achieved by supplementing 410 g protected linseed oil per day [48]. Mere formaldehyde treatment of linseed increased goat milk C18:3 concentration more than untanned seed (+11 vs. +6 mg·g⁻¹, [7], Chilliard et al. unpublished) but not beyond the effect of a similar dose of unprotected oil (+13 mg·g⁻¹, Tab. III). However, formaldehyde treated linseeds did not increase [49] or very slightly increased [44] cow milk 18:3n-3 when compared to untreated seeds, thus confirming that cow is less responsive than goat.

One main factor that may restrict the use of linseeds in dairy cows' diet is its highly negative effect on vegetable wall digestion, combined with a sharp decrease in protozoa count and a trend of the VFA curve towards propionate [30]. However, all the digestive data were collected from sheep at maintenance receiving linseed oil. On the contrary, in productive dairy cattle zootechnical results indicate that linseed lipid supplementation does not induce any noticeable reduction of the energy and dairy value of the rations (Tabs. I and II); it has been recently verified also that the digestibility of maize silage-based (Ferlay et al., unpublished) or hay-based [50] rations in dairy cows was not affected by linseed oil supplementation.

The secretion of long-chain FAs of the n-3 series (C20:5 or EPA, and C22:6 or DHA, respectively) may be increased when marine oils (fish or algae) are added to cows' rations (review by Chilliard et al. [20]). The effectiveness of the transfer from diet to milk, however, is low (2.6% for EPA and 4.1% for DHA) because of high rumen hydrogenation, with EPA in particular. Higher transfer effectiveness between 16 and 33%, have been noted during post-rumen infusion of fish oil. The increase in EPA + DHA concentrations in milk FAs is therefore mini-

mal when fish oil is added to the cows' ration and rarely exceeds 0.5% of total FAs.

3.3. *Trans* fatty acids and conjugated linoleic acid (CLA)

3.3.1. *Effects on mammary lipogenesis*

Low dose marine oil supplementation induces a sharp decrease in milk fat content and secretion (Tab. I). That phenomenon could be linked to *trans* FA synthesis in the rumen and their inhibitory effect on mammary lipogenesis [20]. Milk fat content is otherwise sharply decreased by low-fiber and high-starch diets and/or by the administration of unprotected, unsaturated vegetable oils. The involvement of *trans*-18:1 in that decrease has long been suggested [51]. Griinari et al. [52] later showed the importance of the interaction of starch, fibre and PUFA contents in the diet.

The results in Table II also show that linseeds and rapeseeds reduce cow's mammary lipid secretion, and increase percentage of *trans*-18:1 in milk fat, less than their oil counterparts. It thus appears that hydrogenation is more complete (although it may sometimes involve a lower proportion of added PUFAs) when lipids are provided in seeds, probably because their gradual release does not affect the ecosystem of the rumen as much as oil supplementation in two meals per day (see also [53]). Direct oil/seed comparisons in goat (Tab. III) have confirmed those hypotheses, which are also in agreement with the decrease in the yield of *trans*-18:1, that has been observed when oil supplementation to dairy cow was broken down in 24 meals compared to 2 meals per day [54]. However, when a very large amount of sunflower seeds was added to a maize silage-rich diet, cow milk fat yield decreased and *trans*-18:1 percentage increased largely (Tab. II).

Nevertheless, in certain studies, an increase in the proportion of total *trans*-18:1 in milk was not accompanied by a lower milk fat yield. This has been better understood since

the studies conducted by Piperova et al. [55], and other studies reviewed by Bauman and Griinari [56], which showed that low fiber diets supplemented with PUFA-rich plant oil sharply reduced mammary lipid secretion and strongly increased the proportions of *trans*10-18:1 and to a certain extent, of *trans*10,*cis*12-18:2. It is therefore possible that *trans*10,*cis*12-18:2 results from rumen biohydrogenation modifications induced by low-fiber diets and is one of the precursors of the *trans*10-18:1 yielded in the rumen. It is worth noting that under such conditions, vaccenic and rumenic acid syntheses only increased slightly by comparison with what happened with high-fiber diets supplemented with oil [57] or with low-fiber diets supplemented with oil and vitamin E [58].

Various CLA isomers (*cis*9,*trans*11 and *trans*10,*cis*12 in particular) were infused in the duodenum of dairy cows for three to five day periods. At low-dose (1.25 to 5 g·d⁻¹) *trans*10,*cis*12-18:2 greatly reduced lipid (-7 to 36%) and short (C4:0 to C8:0)- or medium- (C10 to C16:0) chain FA secretions [29, 59, 60]. A decrease in long-chain (> C16) FA secretion was also noted during infusion of *trans*10,*cis*12-18:2, suggesting reduced uptake of circulating FAs. The C18:1/C18:0 ratio decreased in parallel, suggesting reduced delta-9 desaturase activity [29, 61].

Those new results revived the theory of fatty acid biohydrogenation as the central mechanism of milk fat depression with certain diets [56, 62]. However, in some trials, ration supplementation with fish oil greatly reduced milk fat yield without any noticeable increase in *trans*10,*cis*12-18:2 while increased rumenic acid accounted for 96% of the sharp increase in CLA [63, 64]. Likewise, a low-fiber ration supplemented with linseed oil greatly reduced mammary lipid secretion without increasing *trans*10,*cis*12-18:2, neither in the duodenum [17] nor in milk [14]. *Trans*10,*cis*12-CLA therefore is not the only factor susceptible to induce milk fat depression. Other C18:1, C18:2 or C18:3 isomers produced in the rumen and/or in the

udder might be involved ([14, 56, 57] and Loor et al., unpublished results). So, many questions are still to be unravelled, regarding the respective effects of these various isomers on the successive steps of mammary lipid synthesis. The transfer rate from duodenum to milk of long-chain FAs of dietary (PUFAs) or ruminal (*trans*-FAs) origin was lower with high-concentrate diets [18], suggesting that steps other than de novo FA synthesis and FA desaturation are altered with such diets.

Some of the decrease in lipid secretion under high-concentrate diets could also result from a propionate or glucose effect. However, duodenal infusion of 1.2 kg·d⁻¹ glucose or its propionate equivalent only reduced lipid secretion by 5 to 8%. Propionate altered the FA profile only slightly whereas glucose reduced short-chain FA (C4 to C8) and, more markedly, C18-FA secretion [65]. As the 2 infusion treatments decreased to the same extent the plasma acetate and β-OH-butyrate concentrations [65], these changes cannot totally explain the different responses in milk FA profile.

If the milk fat depression could be related to the concentrations of certain *trans* FA in cows, the situation is less clear in goats. In that species, milk fat content and yield are not reduced, but are almost always increased by vegetable oil supplementation [7], even with low-fiber (Tabs. III and V) or maize silage-based (Tab. IV) diets. Milk fat content positive response to lipid supplementation was however lower when goats were given maize silage, reflecting either the high milk fat content ensured by this basal diet, or a negative effect of the *trans*10-18:1 increase which was specifically induced by the maize silage-oil interaction, thus limiting the positive effect of oils on milk fat content and on CLA secretion which was otherwise observed with the alfalfa diet (Tab. IV). This was confirmed by the analysis of results from 19 diets studied on 181 goats, with combinations of 5 different forages without or with supplementation by 3 different oils (Fig. 5A). In the range of the

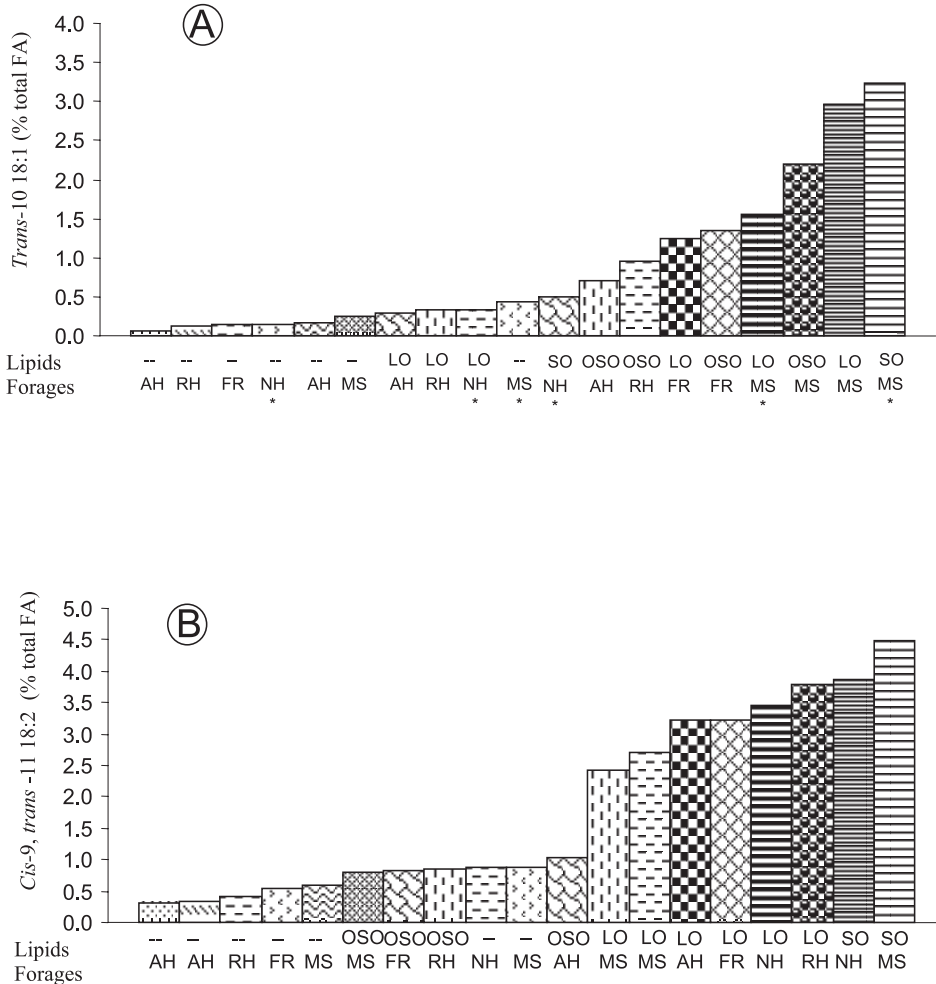


Figure 5. Forage-oil interactions on milk fat percentages of *trans*10-18:1 (A) and *cis*9, *trans*11-18:2 (B), in goats receiving medium-concentrate (45–60% of diet DM) diets supplemented, or not, with oil (5–6% of diet DM) during 3(*)-5 weeks (10–14 goats per group; 181 goats) (adapted from [7, 15, 81, 82, 83, 108]). Forages: AH, alfalfa hay; FR, fresh green rye-grass; NH, natural grassland hay; MS, maize silage; RH, rye-grass hay. Lipids: --, control diet (without oil addition); LO, linseed oil; OSO, high-oleic sunflower oil; SO, sunflower oil.

concentrate percentages (45 to 60%) of the diets that were studied, high concentrations of *trans*10-18:1 (1.2 to 3.2%) were always observed with either maize silage or fresh green rye-grass diets supplemented with oleic-, linoleic- or linolenic-rich oil. For hay-based diets, the highest values were observed

with high-oleic sunflower oil supplementation, consistently with a possible *cis*9-18:1 isomerisation into *trans*10-18:1 in the rumen [66].

In a goat trial using either green rye-grass or rye-grass or alfalfa hay as forage, and linseed oil or high oleic sunflower oil

supplements to induce large variations in *trans* isomers [67], there was no correlation between the milk fat content and the proportions of the various *trans*-18:1 or CLA isomers (including *trans*10-isomers), contrary to what was observed in dairy cows (see above). However, goat milk fat content was negatively correlated with several saturated and mono-unsaturated C14 to C16 FAs and n-6 PUFAs, and positively with stearic acid [67], which confirmed that this substrate is a major regulating factor of mammary lipid secretion in that species, as suggested in earlier studies with lipid-poor diets [68]. Contrary to what was observed in the cow [58], vitamin E supplementation to goats receiving linseed oil did not interact with forage: concentrate ratio, and did not change either milk fat content or *trans*10-18:1 percentage, although it increased the other *trans*-FAs and the 18:0, and decreased the C10-C16:0 percentages and the 18:0 desaturation ratio (Tab. V). Thus vitamin E tended to increase further the main effects of linseed oil addition to goat diet.

3.3.2. Dietary variation factors

The dietary factors that influence the milk CLA and *trans*11-18:1 composition are

included in two main categories: (1) diets providing lipid precursors (C18:2 or C18:3) for CLA and/or *trans*-18:1 formation in the rumen, (2) diets that modify the microbial activity associated with PUFA hydrogenation in the rumen. Combinations of these various factors induce wide variations of milk CLA and *trans*-18:1 concentrations (up to 4% rumenic acid and 10% vaccenic acid, Fig. 1), and strong interactions occur between forages, starchy concentrates and lipid supplements ([52], Figs. 2 and 5B, and Tabs. IV and V).

The proportions of *trans*-18:1 and CLA in cow's milk produced from maize silage-based diets (more than 60% of the ration) are small (1.1 to 2.2% and 0.4 to 0.6%, respectively) ([20] and Fig. 2). CLA concentration in the milk of dairy cows switching from winter diet to young, natural meadow grass increases sharply [26, 69]. Nevertheless, the milk CLA proportions measured in cows at pasture are variable (0.5 to 1.7%) (Tab. VI). Milk CLA concentration increases with green grass availability [38, 70] and is further increased by lipid supplements (Tab. VI and [71]). In other respects, the observed concentrations are higher in the Spring and in the Autumn than

Table VI. Effects of pasture with or without lipid supplements on milk fat CLA in dairy cows (review by Chilliard et al. [101]).

Winter diet	Milk fat CLA (% of total FA)		Treatment duration	References
	Pasture	Pasture + Lipid supplement		
0.3	1.3	–	–	[102]
0.3	0.6	–	4 mo	[103]
0.4	1.2	–	3 wk	[104]
0.5	1.1	–	4 wk	[41]
0.4	0.7	–	4 mo	[105]
0.4	1.1/1.4	–	3 mo	[106]
–	0.5	0.5/0.8	8 wk	[70]
–	1.7	2.5/2.2	3 wk	[39]
–	0.8	1.3/1.8	6 wk	[107]
0.3	–	1.3	4 wk	[5]
0.6	1.7	–	3 wk	[26]
0.6	0.8	–	6 wk	[26]

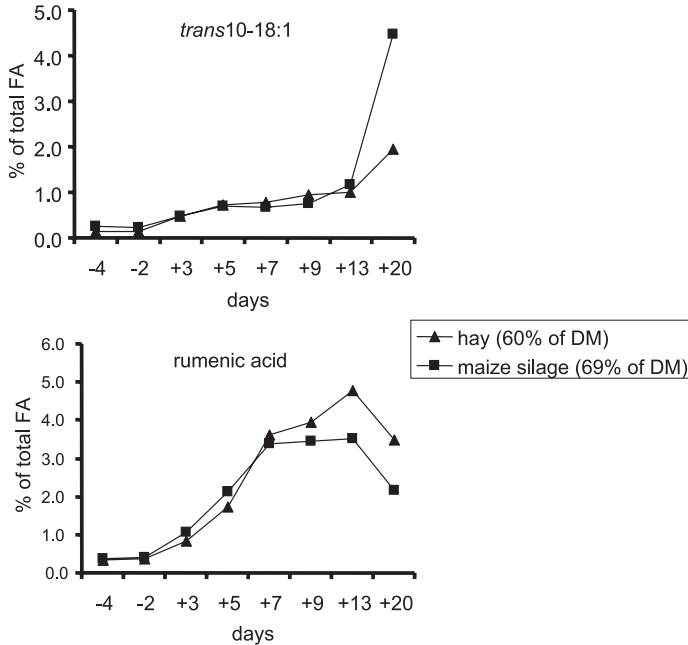


Figure 6. Effect of nature of forage on the kinetics of percentages of cow milk *trans*10-18:1 and rumenic acid after the addition of oil into the diet (adapted from [80]).

in Summer. Young grass high C18:3 concentration and low fiber content probably combine to increase CLA and *trans*-18:1 production. Also, the particular botanical composition of natural highland meadows seems to promote high milk CLA concentrations (up to 2.4%, [72]), whereas a botanical composition effect of cultivated swards appears to be low [73]. Comparisons in cows (Figs. 2 and 6) suggest that the milk rumenic acid response to lipid supplementation differs between forages, with hay > maize silage > grass silage. Further studies are needed to confirm and explain these interactions.

C18:2-rich vegetable oils (sunflower, soybean) highly increase milk rumenic acid content. This effect is linear as increasing amounts of soybean oil are added to the diet (up to at least 4% of diet DM) ([9] and Fig. 2). Adding rapeseed oil calcium salts to the ration increased also milk rumenic acid

concentration. This confirmed that calcium salts of PUFAs are partially hydrogenated. Overall, vegetable oils increase milk rumenic acid more than extruded seeds, which in turn increase it more than raw seeds ([74], Tabs. III and V). This effect is therefore more or less marked as PUFAs from free oil, extruded seeds or raw seeds disrupt rumen metabolism more or less intensively, consistently with the respective effects of oils or seeds on milk *trans*11-18:1 plus *cis*9,*trans*11-18:2 (Tabs. II–V and [31, 75]).

Increasing linseed oil (C18:3-rich) intake increased linearly milk rumenic acid concentration, almost as much as sunflower oil (C18:2-rich) when a grass silage based diet was used, and a little bit less when added to a maize silage diet (Fig. 2). That could be explained by a sharp increase in rumen transformation of C18:3 to *trans*11-18:1, which would be later taken up by the udder and desaturated by delta-9 desaturase to yield

rumenic acid. Surprisingly, addition of a very small level of linseeds ($21\text{g}\cdot\text{d}^{-1}$, i.e. 8 g oil) and $21\text{g}\cdot\text{d}^{-1}$ of trace element mixture increased significantly cows' milk rumenic and linolenic acids [76], and it remains to see if the effect was due either to linseeds or to trace elements.

Fish oils are more effective than vegetable oils, when equally added to the ration, at increasing the CLA concentration. So, CLA proportions increased from 0.2–0.6% with the control diet to 1.5–2.7% with diets supplemented with fish oil ($200\text{--}300\text{g}\cdot\text{d}^{-1}$, [20, 24]). It is likely that the PUFAs (EPA and DHA) of those oils increase *trans*11-18:1 concentration in the rumen, probably through inhibiting the reduction of that FA into stearic acid. That would explain why the combination of vegetable oils and fish oil strongly increased milk CLA content [77, 78].

Rumenic acid secretion in milk is correlated to the duodenal flow of *trans*11-18:1 [18]. Furthermore, there is a strong linear correlation between milk rumenic acid and *trans* 11-18:1 concentrations under a wide variety of diets, either in goats (Fig. 1) or cows. However, the milk rumenic acid/*trans*11-18:1 ratio could be decreased with fish oil supplementation [24]. In this case, the very high concentration of *trans*11-18:1 may exceed the desaturation capacity of the mammary gland, or fish-oil specific FAs (EPA, DHA or intermediate hydrogenation compounds) may inhibit delta-9 desaturase activity.

Previous data [79] suggested that the milk rumenic acid response to lipid supplementation could be transient, with a maximum during the second week after the beginning of lipid supplementation. We confirmed this recently [80] and observed that the rumenic acid response to lipid supplementation was higher with hay diet than maize silage diet, and that the decrease after 3 weeks of supplementation was accompanied by an increase in milk fat *trans*10-18:1 percentage, that was more marked with maize silage (Fig. 6). This confirms the the-

ory proposed by Bauman and Griinari [56] and Bauman et al [57] on the *trans*10-18:1 pathway which would decrease the yield of ruminal vaccenic acid and its availability for rumenic acid synthesis in the mammary gland. This raises also the question of the sustainability of high CLA responses in dairy cattle, and further studies are needed on interactions between dietary fiber, starch, fatty acids and other components.

No data are available on the short-term kinetics of CLA response in goat milk. However the high CLA levels obtained after 5 weeks of lipid supplementation (Fig. 5B and Tabs. IV and V) have been confirmed in the same goats after 9–10 weeks of supplementation [81]. This shows that goat species is a very good responder and that its milk rumenic acid response is stable during at least 2–3 months. Indeed, goat milk rumenic acid varies largely according to feeding factors. The 19 diets studied in Figure 5B, with combinations of 5 different forages with either no oil addition or 18:1-, 18:2- or 18:3-rich oils, yielded a range of rumenic acid from 0.3 to 4.5% of total FAs. The main factor of variation was the nature of oil with sunflower (18:2-rich) \geq linseed (18:3-rich) \gg oleic sunflower (18:1-rich) $>$ no oil addition. The response to oleic acid-rich oil, albeit much less than similar amount of either linseed or sunflower oil, is consistent with a possible *cis*9-18:1 isomerisation into *trans*11-18:1 in the rumen [66] or could be due an inhibition of the last step of hydrogenation of dietary PUFA. For a given oil supplementation, the response to oil interacted strongly with the nature of forage. Thus the response to sunflower oil was highest with maize silage, whereas the response to linseed oil was lower with maize silage than with either hays or fresh grass (Tab. IV and Fig. 5B). However, milk rumenic acid response to linseed oil supplementation was not changed when diet concentrate (or starch) percentage increased from 30% (or 8%) to 54% (or 29%, respectively) (Tab. V), and this was not changed by vitamin E supplementation.

Few data are available on the influence of feeding on the various milk CLA isomers. Ruminic acid (*cis*9,*trans*11-CLA) is classically the one whose concentration is the most variable because of the importance of its mammary synthesis by delta-9 desaturase. In addition, this enzyme synthesizes *trans*7,*cis*9-CLA, quantitatively the second isomer present in milk. That isomer is increased in cow by low-fiber diets supplemented with soybean oil [55] and probably in goats by high-oleic sunflower oil supplementation [82]. Low-fiber diets increase *cis*11,*trans*13- and *cis*9,*cis*11-CLA isomers, whereas linseed oil increases *cis*9,*cis*11-, *trans*11,*cis*13- and *trans*11,*trans*13-CLA, as well as *trans*13-18:1, *cis*9,*trans*13-18:2 and *trans*11,*cis*15-18:2 ([14, 15] and Tab. V). It should be stressed that the obtention of a high level of ruminic acid (3–4% of total FAs) is accompanied by high levels not only of vaccenic acid (8–10%) but also of other *trans*-isomers of C18:1 and conjugated or non-conjugated C18:2 (5–10%) and C18:3 (Tab. V, and [14, 81, 83]). The respective physiological roles of these various isomers and their possible nutritional interest to man have not been studied to date.

4. EFFECTS OF FEEDING FACTORS ON THE SENSORY QUALITY OF DAIRY PRODUCTS

Before recommending dietary modifications to breeders to modify milk FA composition, it has to be ascertained that such practices would not be detrimental to the sensory quality of dairy products. Cow milk sensory analysis was performed by two comparisons (triangular tasting tests) of crude, unskimmed milk from groups of cows receiving different diets [84]. Differences exist between milk produced by grazing cows and others obtained from cows fed hay-based or high-concentrate diets. Also, milk samples from cows fed grass silage were identified as different from milk derived from hay-based or maize silage diets. Sup-

plementing maize silage-based diets with 3% sunflower oil led to moderate but noticeable sensory differences, whereas the distinction is no longer possible with grass silage-based diets. Addition of 5% linseed oil to a maize silage-based diet was easily identified. Adding 2.5% fish oil induced sensory defects.

Cheese or butter sensory qualities are defined by their aspect and texture, in addition to their organoleptic properties (flavour). Certain FAs exert a specific effect on the hardness and spreadability of butter. Increased palmitic acid concentration combined with a decrease in short-chain FAs lead to lower spreadability. The 16:0/*cis*9-18:1 ratio is the most accurate indicator of butter firmness [85]. So, pasture grazing led to more spreadable butter with a reduced 16:0/*cis*9-18:1 ratio [86]. Also, butter derived from hay-based diets was rated as less firm and with less flavour than butter produced from a maize silage-based diet, in parallel with an increase in mono- and poly-unsaturated FAs with the hay-based diet [87].

With regard to cheese, the type of pasture induces a modification of milk fatty acid composition, which affects cheese texture. Unsaturated fatty acid-rich milk produces less firm Abondance cheeses [85]. Cantal-type cheeses produced with milk derived from high-concentrate diets are firmer than those obtained from natural grass, probably because of their higher C18:0 and lower PUFA concentrations. Pasture led to more “animal” and less “bitter” and less “sour” odour, such differences being less marked with pasteurized milk [88]. Hay, compared with maize silage, led to Emmental cheeses containing more mono- and poly-unsaturated FAs but less firm and piquant in taste, with a less pronounced “bone-fruit” flavour [87].

Other experiments have also shown the effects of forage and lipid supplements and their interactions on goat cheese flavour [89, 90]. Linseed oil or sunflower oil supplementation (5–6% of the ration) reduces the “goaty” taste in milk or fresh cheese, linked to the lower secretion of lipase and

reduced post-milking lipolysis [7]. Also, more bitter, piquant, oxidized or fishy flavours may occur, especially with the hay + linseed oil combination which maximizes milk C18:3 concentration (Tab. IV).

5. CONCLUSION

Feeding factors make it possible to vary milk FA composition in many ways. Recent advances in the knowledge of FA synthesis mechanisms [digestion and metabolism] and their putative physiological effects in human consumers have significantly boosted ongoing research and potential applications. As regards ruminant nutrition, the aim is to better understand the effects of using grass-based diets, new combinations of feedstuffs in concentrates, and oil seed technology and processing. However, very few direct comparisons have been made between the main types of basal diets (different types of forages, starchy concentrates, etc.) combined with various lipid supplements (oils, seeds, technological processing and lipid dose added to the basal diet). So, the trends reported in this paper need to be confirmed and specified. However, it is clear that the plasticity of milk fat composition is very large, according to numerous interactions between forage-concentrates-oils-minerals-vitamins, time after dietary changes, as well as ruminant species [7, 109], on almost all major and minor FAs, including several *trans* isomers of C18:1, C18:2 and C18:3.

Insofar as human nutritional recommendations may still vary in the coming years, and as the putative effect of a large number of specific FAs (e.g. *trans* isomers of C18:1, C18:2, C18:3) on human health are not yet known, animal nutritionists have to keep exploring the response patterns of major and minor milk fatty acids and to model their synthesis mechanisms. At the same time, the side effects of the various dietary practices on health safety (presence of antinutritional factors, variations of nutrients with pro-oxidant effects, etc.), on technological

and sensory quality as well as on the image of dairy products need to be better assessed.

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