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Review article

**Ruminant milk fat plasticity:
nutritional control of saturated, polyunsaturated,
trans and conjugated fatty acids**

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Abstract — After a brief survey of metabolic pathways and nutrient fluxes involved in mammary lipogenesis, this review summarises the known effects of diet on ruminant milk fat composition. Special attention is given to fatty acids that could play a positive role for human health, such as butyric acid, oleic acid, C18 to C22 polyunsaturated fatty acids and conjugated linoleic acid (CLA). The efficiency of the transfer of C18:2, C18:3, C20:5, C22:5 and C22:6, from the duodenum to the milk, is reviewed. The main dietary factors taken into account are the nature of forages, including pasture, and the supplementation of dairy rations with protected or unprotected vegetable or fish oils. Dose-response curves of milk CLA are reviewed for different fat supplements, as well as the non-linear relationship between milk CLA and *trans* C18:1. The potential of dietary factors to increase the mean CLA content in cow milk fat is about 300% above basal values. There is, however, a need to evaluate how the different feeding strategies could change the other aspects of milk fat quality.

ruminant / nutrition / milk / fatty acids / human health

Résumé — **Plasticité de la matière grasse du lait de ruminant : contrôle nutritionnel des acides gras saturés, polyinsaturés, *trans* et conjugués.** Après un bref rappel des voies métaboliques et des flux de nutriments qui concourent à la lipogenèse mammaire, cette revue est consacrée aux principaux effets des facteurs alimentaires sur la composition des lipides du lait de ruminant. Un intérêt particulier est porté aux acides gras qui peuvent avoir des effets positifs sur la santé humaine, tels que les acides butyrique, oléique, linoléique conjugué (CLA) et les acides gras polyinsaturés, de 18 à 22 atomes de carbone. L'efficacité du transfert des C18:2, C18:3, C20:5, C22:5 et C22:6, du duodénum au lait est estimée à partir des données de la bibliographie. Les principaux facteurs alimentaires considérés sont la nature des fourrages, dont l'herbe pâturée, et la supplémentation des rations pour vaches laitières avec des huiles végétales ou de poisson, protégées ou non. L'augmentation potentielle

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de la teneur en CLA de la matière grasse du lait de vache par les facteurs alimentaires est d'environ 300 % au-dessus du niveau de base. Des courbes dose-réponse du CLA du lait sont établies à partir des différentes publications sur les effets de suppléments lipidiques, ainsi que la relation non linéaire qui existe entre le CLA et le C18:1 *trans* du lait. Il reste toutefois à évaluer les conséquences des différentes stratégies alimentaires sur les autres aspects de la qualité du lait.

ruminant / nutrition / lait / acides gras / santé humaine

1. INTRODUCTION

Ruminant fat is an important part of the human diet in many countries, particularly bovine milk fat which represents up to 75% of the total consumption of fat from ruminant animals. Although dairy products (which have a very low cholesterol content) provide only 15–25% of the total fat in the human diet, they provide about 25–35% of the total saturated fat [46, 116].

The regulation of the lipid composition of ruminant meat (adipose tissue and muscle) has been reviewed in several recent papers [45, 65, 146]. Milk fatty acid (FA) composition has a number of effects on milk quality, including aspects such as its physical properties (e.g. melting point and hardness of butter, crystallisation and fractionation of milk fat) as well as its nutritional properties (e.g. potential effects of specific FA on human health). FA composition also affects the organoleptic properties of milk (due to factors such as the effect of free short-chain FA and oxidative changes in FA). The aim of this review is to summarise the known effects of diet on ruminant milk fat synthesis and composition, since controlling milk fat quality at the farm level is achieved most rapidly, reversibly and efficiently by controlled changes in the diet of dairy cows. In this review, particular emphasis will be laid on FA having a potential antiatherogenic or anticarcinogenic role, such as butyric acid, oleic acid, polyunsaturated FA (especially n-3 FA) and conjugated linoleic acid (CLA). The FA which have a potential negative effect on human health, such as saturated

(lauric, myristic and palmitic acids) and *trans* FA, or part of them [95, 111, 122, 143] will also be reviewed.

2. METABOLIC PATHWAYS AND NUTRIENT FLUXES INVOLVED IN MILK FAT SYNTHESIS

2.1. Mammary lipid synthesis

Milk fat is synthesised either from FA which are taken up from the blood (ca. 60%) or by de novo synthesis in the mammary gland (ca. 40%) (Fig. 1). FA are synthesised de novo from acetate and 3-hydroxybutyrate (3-HB), the latter contributing to ca. 15% of the carbon content. The main metabolic pathway involves two key enzymes, acetyl-CoA carboxylase (ACC) and FA synthase (FAS). ACC catalyses the formation of malonyl-CoA from acetate, and FAS catalyses condensation cycles of malonyl-CoA with either acetyl-CoA or butyryl-CoA, which arise from acetate or 3-HB metabolism respectively (review by Barber et al. [9]). The chain-termination reaction produces C14:0 and predominantly C16:0, and is catalysed in most tissues and species by the intrinsic thioesterase activity of FAS, termed thioesterase I. In the mammary gland of rodent species, the chain length of medium-chain FA (C8:0 to C12:0) is controlled by the activity of a second thioesterase, thioesterase II. In contrast to rodents, ruminant mammary gland FAS exhibits a transacylase with both loading and releasing activity for acyl chains from two to twelve carbon chain length [96]. Cellular and

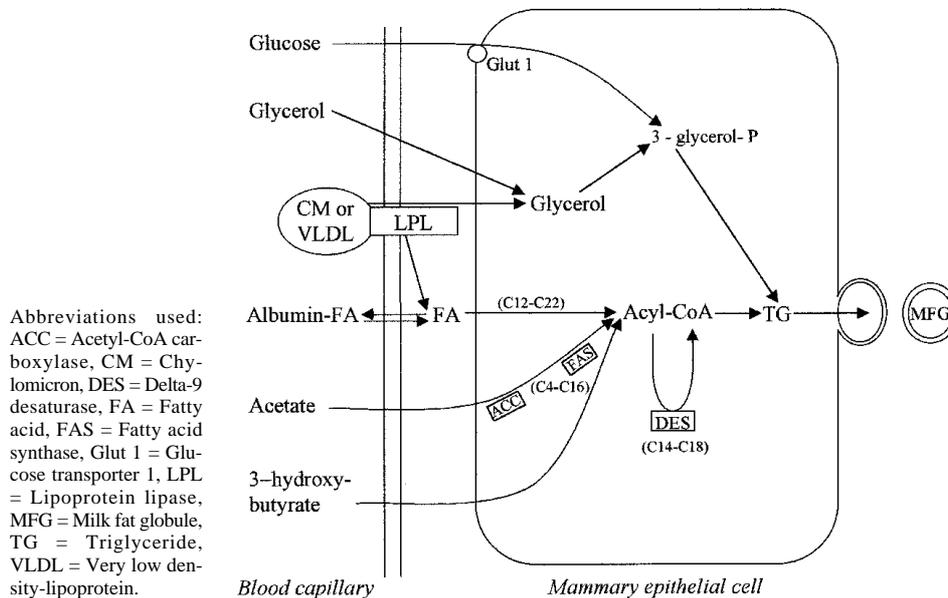


Figure 1. Milk fat synthesis and secretion in ruminants.

molecular factors that regulate the different patterns of milk short- and medium-chain FA between and within ruminant species have not yet been identified.

In goat milk, physiological variations in the percentages of C4:0 to C8:0 FA are not well related to those of C10:0 to C16:0 FA [22, 128]. This could be related to the occurrence in ruminant mammary glands of metabolic pathways that do not involve malonyl-CoA [13, 120], although this hypothesis remains to be confirmed. Preformed FA taken up by the mammary gland arise either from plasma non-esterified FA (NEFA) or from triglyceride-rich lipoproteins (chylomicra and very low density lipoproteins, VLDL). NEFA uptake is directly related to their plasma concentration, which is itself mainly related to body fat mobilisation [29]. The ability of mammary tissue to utilise triglyceride FA from plasma lipoproteins is determined by the activity of the enzyme lipoprotein lipase

(LPL). The cellular and molecular regulation of mammary LPL synthesis and activity is not yet fully understood in rodent species, despite numerous old and recent studies (review by Barber et al. [9]). However, as in rodents, LPL activity is high in the lactating mammary gland of ruminants (e.g. Chilliard et al. [30]). Consequently, the mammary gland uptake of triglycerides is generally well correlated to their plasma concentration [62], except in the higher range of concentrations (above ca. 0.4 mM [5]) when LPL activity could become limiting.

Contrary to other ruminant tissues, the lactating mammary gland is not able to convert C16:0 to C18:0 by chain elongation [112]. However, fully differentiated mammary secretory cells express a high delta-9 desaturase activity, which converts stearic acid to oleic acid (*cis*-9 C18:1) [94]. About 40% of the stearic acid taken up by the gland is desaturated, thus contributing to more

than 50% of the oleic acid that is secreted into milk fat [16, 57]. Furthermore, vaccenic acid (*trans*-11 C18:1) formed in the rumen and absorbed from the intestine can be desaturated to form rumenic acid, i.e. *cis*-9, *trans*-11 C18:2. This is the major isomer of conjugated linoleic acid (CLA) in ruminant milk [69]. The delta-9 desaturase activity could be inhibited by polyunsaturated FA [132] as well as by cyclopropenoic FA (e.g. from cottonseed [148]).

FA synthesis, uptake and desaturation contribute to a pool of FA available for esterification on glycerol to mainly form triglycerides, which comprise 97–98% of the total milk lipids. The asymmetric distribution of FA on the glycerol molecule, e.g. the preferential esterification of short-chain FA and oleic acid on the *sn*-3 position, influences physical properties of milk fat by decreasing its melting point [121]. Thus, despite the fact that ruminants mainly absorb saturated long-chain FA from the intestine (see below), they express several metabolic peculiarities to decrease the melting point of their body lipids, and especially milk lipids. These peculiarities include the desaturation of long-chain FA (by intestinal, adipose and mammary tissue), the synthesis of short- and medium-chain FA, the lack of chain elongation, and the uneven esterification pattern of the various FA molecules in mammary secretory cells.

2.2. Rumen metabolism

2.2.1. The production of volatile fatty acids in the rumen

The microbial population of the rumen is able to degrade and ferment dietary carbohydrates and proteins, to produce volatile FA (VFA). The most important of these are acetate, propionate and butyrate. Acetate and butyrate are precursors of milk short- and medium-chain FA (see above). Butyrate is first converted to 3-hydroxybutyrate, mainly in the rumen wall (e.g. Nozière et al. [115]).

The main factors which increase propionate production, which may limit the flux of milk fat precursors towards the mammary gland, have been recently reviewed [54]. The most efficient ways of increasing propionate production are, firstly, to increase the proportion of concentrate in the diet, especially if the concentrate is composed of rapidly rumen degradable cereals. Secondly, the intake of dietary lipids may be increased, in particular by vegetable or fish oils, and thirdly by the use of ionophore antibiotics.

The equilibrium between acetate and butyrate also depends on the diet. Decreasing the proportion of fibre in the diet results in a lower acetate: butyrate ratio [86], but the butyrate proportion remains generally lower than 10%. The means of increasing rumen butyrate are not numerous, and are related to an active protozoal population which is often linked to a high content of soluble sugars in the diet. Diets rich in beets have been shown to strongly increase butyrate, up to 30% of total VFA [140]. Indeed, beets are rich in soluble sugars and can be incorporated in ruminant diets in significant amounts. Diets that are rich in slowly degradable starch (such as diets with a high maize content) or diets that are based on rye-grass (which is rich in soluble sugars) may result in butyrate comprising between 15 and 20% of VFA [86].

2.2.2. The fate of dietary fatty acids in the rumen

The rumen is the site of an intense microbial lipid metabolism. Lipolysis of dietary glycolipids, phospholipids and triglycerides leads to free FA which are hydrogenated to a large extent. The main biochemical mechanisms which operate in the rumen have been described by Harfoot and Hazlewood [75], and the result of this metabolism in terms of flows of absorbable FA has been detailed by Doreau and Ferlay [52] and Doreau and Chilliard [50]. Briefly, polyunsaturated FA (PUFA) are first isomerised

then hydrogenated. For example, linoleic acid (*cis*-9, *cis*-12 C18:2) is isomerised to conjugated linoleic acid (CLA) (*cis*-9, *trans*-11 C18:2) and then hydrogenated firstly to *trans*vaccenic acid (*trans*-11 C18:1) and then to stearic acid (C18:0). In fact, the biochemical pathways are numerous and dependent on the microbial ecosystem. A large amount of 18-carbon monounsaturated FA is found in duodenal contents [15]. Among *trans* monounsaturated FA (TMUFA), the *trans*-11 isomer (*trans*vaccenic acid) is the main one, but a total of 12 *trans* isomers have been found, some of these being specific to particular bacterial populations [89]. Their appearance in rumen contents is not instantaneous following addition of a substrate such as linolenic acid [92], suggesting a cascade of chemical reactions where several types of bacteria could intervene. These TMUFA isomers arise from several isomers of CLA [58] and from several conjugated C18:3 isomers produced from dietary α - and γ -linolenic acids [75]. It has also been suggested [69] that double bond migration may occur.

Ruminal biohydrogenation, defined as the disappearance of linoleic and linolenic acids between the mouth and duodenum, is extensive in most cases: on average 80 and 92% for linoleic and linolenic acids respectively [52]. The less extensive hydrogenation of linoleic acid could be explained in part by an incorporation of this acid into bacteria [12], but it is also probably due to a different biochemical pathway of hydrogenation [75]. Biohydrogenation is not clearly related to the amount or origin of dietary lipids. The extent of ruminal biohydrogenation mainly depends on the type of diet. When concentrates comprise more than 70% of the diet, biohydrogenation is on average only 50 and 65% for linoleic and linolenic acid, respectively. This has been shown to be due to a drop in pH, limiting at first lipolysis, and thus hydrogenation which occurs only on free FA [139]. A large amount of dietary linoleic acid and a decrease in the

rate of hydrogenation are the two main factors which contribute to an increase in the concentration of the intermediate compounds CLA and TMUFA [114].

Biohydrogenation also occurs on 20- and 22-carbon FA. The disappearance of C20:5 (EPA) and C22:6 (DHA) is extensive, but generally these FA do not become fully saturated. Instead, numerous intermediate compounds are produced [51]. It has been suggested that large amounts of dietary EPA and DHA could reduce the extent of their hydrogenation *in vitro* or *in vivo* during short-term (3 days) experiments [73]. Due to an unknown mechanism, the supply of EPA and/or DHA increases the ruminal production of TMUFA [145] and probably of CLA, both probably originating from the hydrogenation of dietary C18 PUFA.

Since the 1970's, different attempts have been made to protect lipids against biohydrogenation. The first approach was the encapsulation of lipids by formaldehyde-treated proteins [3]. Although the degree of protection is sometimes uncertain, this technique is to date the only one which results in large amounts of PUFA escaping ruminal degradation. In addition, TMUFA and CLA production is reduced. Besides its cost, the main drawback of this technique is the use of formaldehyde, which is questionable now for animal production due to its potential impact on the image of the products. Among the other techniques which have been investigated, the use of the calcium salts of FA is the most popular. The ability of calcium salts to prevent interactions between FA and microbes has been widely demonstrated for palm oil FA. However, palm oil has a high degree of saturation. Calcium salts of unsaturated FA are dissociated in the rumen due to their low pKa, so that the FA are then extensively hydrogenated [60]. A more recent technique is the formation of fatty acyl amides. This is being developed but the protection against biohydrogenation is probably not complete [78].

2.3. Adipose tissue metabolism

The mammary gland uses plasma NEFA released from adipose tissue as a source of long-chain FA for milk fat synthesis. The FA stored as triglycerides in ruminant adipose tissue are mainly C16:0, C18:0 and *cis*-9 C18:1, and to a much lesser extent C14:0, *cis*-9 C16:1, C17:0, *trans*-11 C18:1 and minor FAs, with large variations between adipose tissue sites [10]. The FA composition of adipose tissue is also dependent on feeding conditions (see Introduction).

The availability of plasma NEFA for the mammary gland is highly correlated to body fat mobilisation, which occurs during early lactation and/or when energy balance is negative [23]. The amount and nature of dietary fat are also involved in the regulation of body fat mobilisation [21]. The FA composition of plasma NEFA, although poorly understood in lactating cows, is probably dependent on the nutritional history of the cow (previous FA storage), as well as on the order in which the different adipose tissue sites, and the different FA within each adipose tissue site, are mobilised.

3. MANIPULATION OF MILK FATTY ACID COMPOSITION AND SECRETION BY DIETARY FACTORS

3.1. Decreasing saturated fatty acids

3.1.1. Short- and medium-chain fatty acids

Most of the FA arising from de novo synthesis are saturated (C4:0 to C16:0), because the delta-9 desaturase has very low activity with FA shorter than 18 carbon chain length, although a small proportion of C14:0 and C16:0 is desaturated to C14:1 and C16:1 [107]. Long-chain FA (with 16 or more carbon atoms) are potent inhibitors of mammary FA synthesis, through a direct

inhibitory effect on ACC activity [9]. Thus, when long-chain FA are available either from the diet, or from body fat mobilisation, there is a decrease in the percentage of medium-chain FA (C8:0 to C14:0 or C16:0) in milk fat (e.g. Chilliard et al. [30, 31]). This is due to both a higher secretion of long-chain FA from the blood (dilution effect), and a lower de novo synthesis of FA. A third factor which could accentuate this change is the reduced availability of acetate and 3-HB for mammary FA synthesis. This would be brought about by the decreased voluntary feed intake caused by an increased dietary fat intake (review by Chilliard [21]), as well as the decreased acetate: propionate ratio in the rumen brought about by the increased intake of unprotected, unsaturated FA (review by Doreau et al. [54]). The upshot of this is that the prediction of the effect of dietary fat is complex, owing to the nature (FA composition), the presentation (oilseeds, protected or unprotected oils) and the amount of dietary fat, together with its interactions with forages and/or concentrates in the basal diet, since dietary environment affects ruminal biohydrogenation. Some examples are given in Table I. A duodenal infusion of rapeseed oil increased C18:1, C18:2 and C18:3, at the expense of mainly C14:0 and C16:0. Protected soya oil feeding mainly increased C18:0, C18:1 and C18:2, at the expense of FA from C10:0 to C16:0, whereas unprotected soya oil added to the diet mainly increased C18:1 (due largely to *trans* isomers [8]) at the expense of FA from C6:0 to C16:0. Calcium salts of palm oil slightly increased C16:0, C18:0 and C18:1, at the expense of FA from C10:0 to C14:0.

Several observations may be made from these results. Firstly, the proportion of butyric acid in milk fat has never been significantly decreased (and even tended to be slightly increased) by feeding fat (see also [57, 120, 121]). Secondly, the proportion of short-chain FA with six and eight carbon chain lengths decreased only when unprotected oil affects rumen function (see also

Table I. Effects of vegetable oil supplementation on milk fatty acid composition (g per 100 g fat).

	Rapeseed oil duodenal infusion ¹		Encapsulated soya oil ²		Unprotected soya oil ³		Calcium salts of palm oil ⁴	
	Control	+630 g·d ⁻¹	Control	+630 g·d ⁻¹	Control	+600 g·d ⁻¹	Control	+650 g·d ⁻¹
C4:0	3.0	3.1	1.4	1.7	3.1	2.8	2.9	3.3
C6:0	2.2	2.3	1.8	1.5	2.7	1.7	2.0	2.5
C8:0	2.1	2.3	1.0	0.8	1.7	1.3	2.0	1.8
C10:0	4.4	4.1	3.3	2.1	4.0	1.9	4.2	3.4
C12:0	4.8	4.7	4.0	2.2	4.5	2.3	5.1	3.8
C14:0	12.2	10.8	12.2	7.1	12.9	8.7	12.0	10.4
C16:0	33.7	26.2	29.0	17.5	32.2	23.9	31.6	33.3
C18:0	7.6	7.7	13.4	15.3	11.2	12.9	6.5	7.3
C18:1	18.1	23.3	24.6	32.8	23.6	38.9	19.6	23.0
C18:2	1.9	6.4	7.6	16.2	2.2	3.2	2.8	2.8
C18:3	0.2	2.8	1.9	3.0	–	–	0.2	0.2
Milk fat (g·d ⁻¹)	976	1 047	930	1 064	782	706	1 065	1 152

¹ [25], Chilliard and Doreau, unpublished results, ² [108], ³ [129], ⁴ [33].

[31, 33]). Furthermore, the proportion of C4:0, C6:0 and C8:0-FA was hardly changed by the addition of protected fat or by the duodenal infusion of fat (see also [41, 55]). This is in agreement with the observation that body fat mobilisation increased C18:0 and C18:1 in milk fat at the expense of C10:0 to C16:0-FA without significantly decreasing the C4:0 to C8:0-FA [22, 30, 43, 135]. These last mentioned FA are synthesised in part by non-malonyl CoA mechanisms, i.e. not involving ACC. This could also be related to the fact that a duodenal infusion of fish oil sharply decreased palmitic secretion with few effects on the secretion of C4:0 to C14:0-FA, contrasting with the dramatic effect of an infusion of fish oil into the rumen on these last-mentioned FA (Fig. 2). Thirdly, the proportion of palmitic acid is always decreased (except when present in large amounts in the fat supplement from palm oil). The consistency in the proportion of butyric acid is interesting, particularly because of the putative

positive effects of butyric acid on human health [122].

The relative efficiency of the different long-chain FA to inhibit mammary FA synthesis seems to differ between monogastric and ruminant species, but also according to experimental conditions in vivo or in vitro [9]. Inhibition tends to be higher when the number of carbon atoms and/or the degree of unsaturation increases. Furthermore, the *trans* isomers of C18:1 and CLA could be very potent inhibitors of fat synthesis (see below). Besides these mechanisms, a decreased availability of acetate and butyrate (or 3-HB) due to changes in the rumen bacterial population and changes in rumen VFA production could contribute to the large decrease in mammary short- and medium-chain FA synthesis observed when diets are fed which depress milk fat content (see below). Furthermore, a large decrease in the percentage of butyric acid in milk fat was observed with high concentrate diets, although ruminal butyric acid percentage

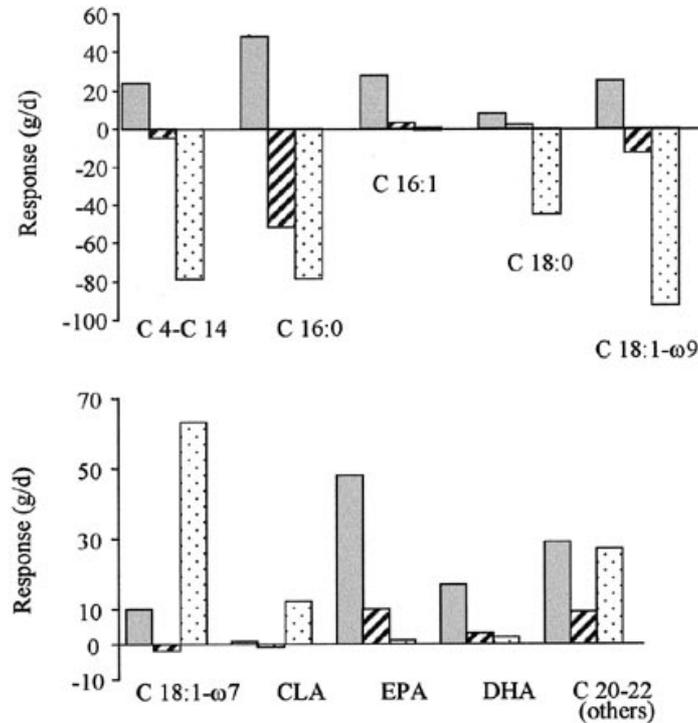


Figure 2. Effects of duodenal (D) or ruminal (R) infusion of fish oil on the secretion response (treated-control) of milk fatty acids (FA) in the dairy cow (from [27, 35]). (■) infused fish oil FA, (▨) secretion response to D, (▤) secretion response to R.

did not change [124]. Propionate infusion into the rumen decreased the yield of all FA from C4 to C16, and more markedly C4 to C8-FA. In contrast, glucose infusion into the duodenum decreased the yield of C4 to C8-FA and C14 to C18-FA, but not C10 and C12 [76]. The difference between the 2 treatments could be related to the fact that propionate infusion decreased acetate and butyrate concentrations in the rumen, whereas glucose probably increased the use of acetate, 3-HB and long-chain FA by adipose tissue.

3.1.2. Long-chain fatty acids

A decrease in the C16:0 to C18:0 ratio should be beneficial to human health [113]. As described in the previous section, C16:0

content in milk fat can be decreased by all factors which inhibit FA synthesis, as well as by feeding supplements poor in palmitic acid, such as rapeseed or sunflower [71]. Conversely, the addition of calcium salts of palm oil ($770 \text{ g}\cdot\text{d}^{-1}$ in 6 trials) increased the C16:0 content of milk fat by $21 \text{ mg}\cdot\text{g}^{-1}$ [33].

Another goal for human health and dairy technology (to increase fat fluidity) could be to decrease the C18:0: *cis*-9 C18:1 (oleic acid) ratio. Feeding oleamide [79] or efficiently protected oils or seeds rich in oleic acid (e.g. soya and rapeseed) is a means to achieve this goal (Tab. I). However, feeding fat supplements rich in stearic acid, such as tallow or hydrogenated FA, do not increase the C18:0 to C18:1 ratio [7] because a large part of stearic acid is desaturated to oleic acid by the mammary gland. Feeding

unprotected oils increases both *cis* and *trans* C18:1 isomers [8] which arise from ruminal metabolism (*trans* C18:1) and from mammary desaturation of C18:0 produced in the rumen. The milk C18:0 to C18:1 ratio is sharply increased by feeding protected cotton seeds to goats [72] likely due to inhibition of mammary delta-9 desaturase by cyclopropenoic FA from cotton seeds.

3.2. Increasing polyunsaturated fatty acids

Polyunsaturated FA are not synthesised by ruminant tissues, so that their concentration in milk is dependent on the amount which flows out of the rumen. This amount can be increased by the use of feeds rich in PUFA and by factors which decrease their

biohydrogenation, including natural protection by plant structures. The transfer rate of PUFA from the duodenum to milk can be calculated (Figs. 3 and 4) using techniques of abomasal or duodenal infusions, which simulate high flows of duodenal FA.

3.2.1. Linoleic acid

The use of large amounts of encapsulated safflower oil produced milk fat rich in linoleic acid (up to 35% of milk fat). Feeding encapsulated rapeseed, sunflower, soybean or cotton seed oil resulted in the proportion of linoleic acid in total fat being between 15 and 20% [72, 98, 109]. Besides these changes in the proportions of FA, the structure of milk triglycerides can be modified with a decrease in the proportion of C16:0 and an increase in C18:1 esterified

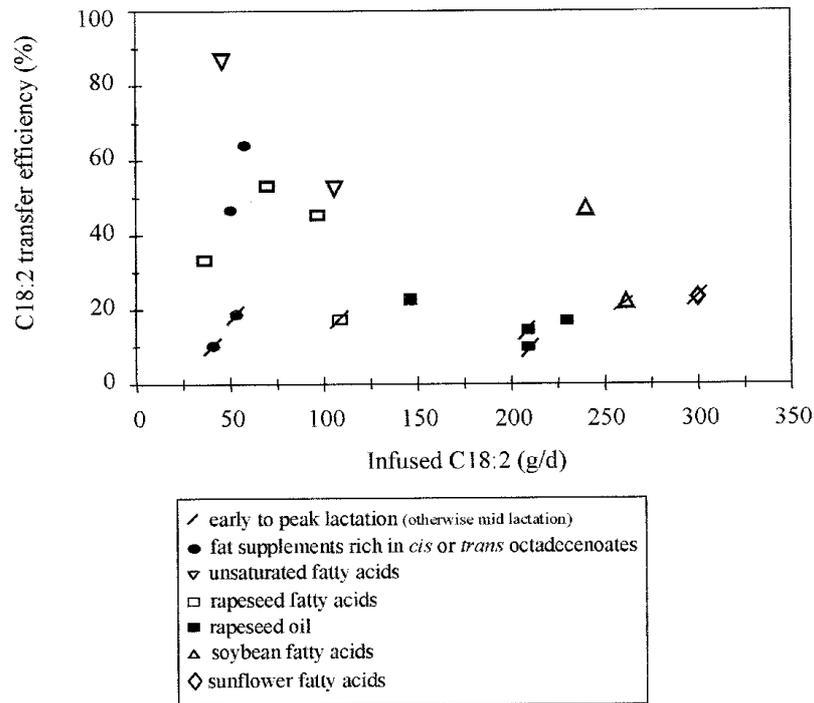


Figure 3. Transfer efficiency [milk C18:2 (treated-control) / infused C18:2] into milk of linolenic acid infused into the abomasum or the duodenum of the dairy cow (from [32, 41, 55, 63, 100, 118, 125, 141]).

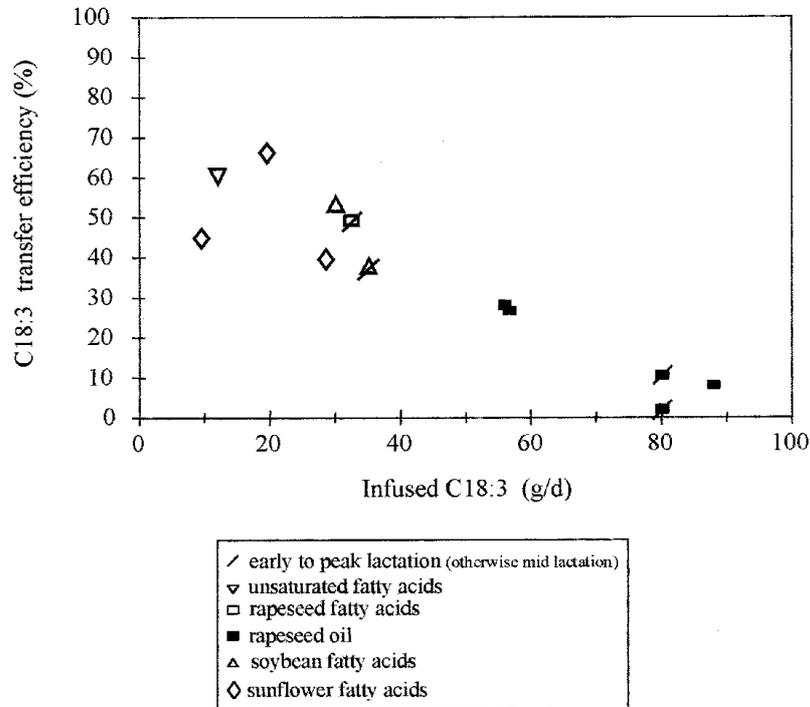


Figure 4. Transfer efficiency [milk C18:3 (treated-control) / infused C18:3] into milk of linolenic acid infused into the abomasum or the duodenum of the dairy cow (from [32, 41, 55, 100, 118]).

at the *sn*-2 position of glycerol [82]. This would have potential consequences on the physical properties of milk fat. The transfer efficiency into milk of linoleic acid infused into the duodenum is however highly variable and ranges from 10 to 90% (Fig. 3). These variations are not clearly related to either the amount of linoleic acid infused, the plant origin of the FA, its state (free vs. esterified), or the stage of lactation of the cow. However the lowest efficiencies (less than 30%) were all observed when large amounts of FA were infused, and when cows were in early to peak lactation. This effect of lactation stage is puzzling, since the flows of FA are driven primarily towards the mammary gland during early lactation [23].

With most diets based on forages and without lipid supplements, the percentage of linoleic acid in milk fat is between 2 and 3%. With diets enriched with unprotected oils or seeds from soyabean or sunflower, the percentage of linoleic acid in milk fat does not increase above 4%, and the increase compared with the control diet is less than 1.5% (e.g. Tab. I). As an exception, Wu et al. [147] increased linoleic acid in milk fat by almost 3%, by adding a mixture of cottonseed and safflower oil (4% of diet DM) to the diet. In some experiments the use of raw soyabeans instead of soyabean oil resulted in an increase by 1% in the proportion of linoleic acid in milk fat [134]. A partial protective effect of the seed is likely. Calcium salts of rapeseed FA or soybean oil do not increase the proportion of milk

linoleic acid [38, 56, 59, 97], because the salts dissociate in the rumen when pH falls. This is then followed by an extensive ruminal hydrogenation. The presentation of soyabean FA as butylsoyamide results in an increase in the linoleic acid concentration in milk fat by 2% without an increase in *trans* C18:1 [80], supporting the hypothesis that fatty acyl amides escape in part from ruminal biohydrogenation.

The incorporation of dietary linoleic acid in milk is less than that in beef. This may be due to a specific use of this acid for muscle phospholipids, where it can reach 20–30% of total FA in extreme cases, whereas triglycerides of the adipose tissue, which is mostly the uneaten part of bovine carcasses, are not enriched in linoleic acid with the same priority [45]. However, there is a significant storage of linoleic acid in adipose tissue triglycerides when the availability of this FA increases, as shown by its 71% increase in perirenal adipose tissue of mid lactation cows after a 5-week duodenal infusion (1 kg·d⁻¹) of rapeseed oil [32].

3.2.2. Linolenic acid

As with linoleic acid, the incorporation of linolenic acid in milk fat can be very high and reach more than 20% of total milk FA. This occurs when unusual amounts (20% of the diet) of linseed oil coated with formaldehyde-treated proteins are fed [109].

With more reasonable supplementation of protected linseed oil (410 g·d⁻¹) the proportion of linolenic acid in milk fat reaches 6.4% [67]. These results suggest a high transfer efficiency from the duodenum to milk, as shown in Figure 4, especially when small amounts (less than 40 g·d⁻¹) of C18:3 are infused into the intestine (the transfer efficiencies ranged from 35 to 70%).

Grass is the main source of C18:3. Hay making greatly decreases the concentration of C18:3, due to a decrease in FA content, and to a decrease in C18:3 percentage in FA. As a consequence, the amount of C18:3 absorbed, although limited, is much higher for fresh grass than for hay or concentrate diets, despite a lower percentage of hydrogenation of concentrate diets (Tab. II). The percentage of C18:3 in milk may therefore be four times higher following turn out to pasture than it was before (2.4 vs. 0.7% in a trial by Decaen and Ghadaki [44]). Since the fat percentage in grass and the percentage of linolenic acid in grass fat are higher in early spring and in late autumn than at other times [11] and also dependent on grass varieties, it is understandable that pasture does not often increase the percentage of linolenic acid in milk. In studies reported by Lawless et al. [101] and Kelly et al. [91], the concentration of linolenic acid in milk fat remained less than 1%.

Among the non-forage feedstuffs, linseed is the only one which has significant

Table II. Ruminal hydrogenation and absorption of linolenic acid (C18:3) in sheep receiving three diets (Bauchart D. and Poncet C., unpublished data).

Diet	Fresh grass	Hay:concentrate (75:25)	Hay:concentrate (30:70)
FA intake (g per 100 g DM)	2.38	0.88	0.88
C18:3 intake (g·d ⁻¹)	14.0	0.85	0.46
C18:3 intake (% total FA)	56.2	8.8	4.5
C18:3 hydrogenation (%)	95.9	92.9	87.0
C18:3 absorbed (g·d ⁻¹)	0.49	0.04	0.05
C18:3 absorbed (% FA)	3.1	0.3	0.3

amounts of C18:3, which represents more than 50% of FA. Very few studies have analysed the effect of linseed or linseed oil on milk fat composition. Using linseed, Kenney [93] in two experiments and Mansbridge et al. [106] increased the proportion of linolenic acid by up to 1.5% of total FA. A significant increase was also found by Brunshwig et al. [19] with an expeller linseed meal. On the contrary, the incorporation of 5.3% linseed oil in the diet resulted in less than 0.5% linolenic acid in milk fat [90]. Contradictory results have been obtained with calcium salts of linseed oil. Chouinard et al. [38] did not observe an increase in milk C18:2 and C18:3 after 4 weeks of supplementation, whereas Brzoska et al. [20] did, after 3 weeks, using a mixed commercial product. The trend to a higher proportion of linolenic acid in milk when linseeds are used compared with linseed oil could be due to a partial protection by whole linseed against biohydrogenation due to the localisation of oil in the seed or meal. However, no direct comparisons between whole linseeds, linseed oil and calcium salts are available.

A major factor which limits the use of linseed is its well-known negative effect on fibre digestion [18], due to a large reduction in the protozoal population [53] accom-

panied by a shift in rumen VFA towards propionate production [85]. The potential positive effects on milk fat composition are therefore largely outweighed by the decrease in the energy value of the diet.

3.2.3. EPA and DHA

An increase in C20 and C22 n-3 FA can be achieved by the use of fish oil. Fish oil is rich in EPA and DHA, their relative proportion being highly dependent on the type of fish and, to a lesser degree, on environmental conditions [1]. Only other marine organisms (algae, plankton) are also rich in EPA and DHA [66]. Fish oil also results in a strong decrease in total fat secretion, which can be an objective in European countries, due to fat production quotas [26, 54].

The transfer efficiency into milk of EPA and DHA from fish oil infused ($270 \text{ g}\cdot\text{d}^{-1}$) into the duodenum was 20 and 18%, respectively, thus allowing an increase in the EPA + DHA concentration in milk fat up to 2 g per 100 g FA (Fig. 2 and Tab. III). When fish oil is unprotected, the transfer efficiency of EPA and DHA in milk fat is much lower (e.g. 3 and 11%, respectively, in Fig. 2), due to the ruminal hydrogenation of a large part of these FA [51] combined to a relatively low transfer efficiency of total C20 and C22

Table III. Response¹ and apparent transfer efficiency² into milk of C20:5, C22:5 and C22:6 n – 3 from fish oil given in the diet or infused into the duodenum (DU) of the cow.

Treatment	Control	Fish oil (FO) in the diet			FO in the DU
		180 ⁴	270 ⁵	360 ⁴	
Amount ($\text{g}\cdot\text{d}^{-1}$)	0 ³				270 ⁵
C 20:5 (EPA)	0.04–0.05	0.14 (1%)	0.28 (3%)	0.37 (2%)	1.31 (20%)
C 22:5 (DPA)	0.04–0.06	0.16 (12%)	0.32 (39%)	0.30 (15%)	0.41 (71%)
C 22:6 (DHA)	0.03–0.06	0.53 (21%)	0.37 (11%)	0.63 (11%)	0.42 (18%)

¹ Concentration in milk fat (g per 100 g). ²In brackets: milk FA response (treated – control)% fish oil FA. ³ Fifteen cows. ⁴ Nine cows [34]. ⁵ Six cows [27].

FA in milk (32% for 270 g·d⁻¹ fish oil infused into the rumen, Fig. 2). These lower transfer rates of EPA and DHA could also be explained in part by the fact that these FA are concentrated in cholesterol ester and phospholipid fractions of plasma [117], which are only weakly used by the mammary gland for lipid synthesis. Thus, the EPA + DHA concentrations in milk fat remain generally lower than 1.0% (Tab. III and [73, 104, 105, 117]).

The response to fish oil supplementation of milk C22:5 (DPA) is very different from those of EPA and DHA (Tab. III). When fish oil was infused into the duodenum, the apparent transfer efficiency of C22:5 was 71%, compared to 18–20% for DHA and EPA. This suggests that C22:5 is very efficiently used by the mammary gland, although EPA and DHA are probably spared for other purposes in the animal body. When fish oil was added to the diet, the mean transfer efficiencies were 2, 22 and 14% for EPA, C 22:5 and DHA, respectively. A high transfer efficiency of C22:5 was also observed by Offer et al. [117], i.e. 30% with fish oil (250 g·d⁻¹) added to the diet.

The differences in the ratios of these 3 FA between oil, duodenum and milk (Tab. IV) could be helpful to understand what happens. When oil is infused into the rumen, duodenal ratios suggest that C 22:5 is much less hydrogenated (in relative terms) than DHA and EPA, and that DHA is much

less hydrogenated than EPA. Another possibility could be that some EPA is elongated to C 22:5 by rumen microbial metabolism. The ratios then remain similar between the duodenum and milk (Tab. IV), indicating that all 3 FA are used with similar efficiencies after intestinal absorption. When oil is directly infused into the duodenum, the ratios in the duodenum are, as expected, similar to the ratios in the oil. The changes in the ratios between the duodenum and milk confirm that, in this case, C 22:5 is 3 to 4 times more efficiently secreted than EPA and DHA, probably because the mammary clearance of this particular FA increases when its availability increases.

Fish oil increases EPA and DHA in muscle in beef cattle [130]. However, their incorporation occurs in the muscle phospholipid fraction but not in muscle triglycerides or subcutaneous adipose tissue [4]. The small amount of EPA and DHA which escapes rumen hydrogenation is therefore preferentially directed towards muscle phospholipids, rather than carcass or milk triglycerides.

Despite this positive effect of fish oil on the amount of EPA and DHA in milk and meat, this feeding practice will probably be of limited interest. Indeed fish oil tends to decrease milk flavour [99]. Curiously, this negative effect has not been shown in bovine meat [146], but this remains to be confirmed.

Table IV. Changes in the ratios between EPA, C22:5 n – 3 (DPA) and DHA from the diet to the duodenum and the milk in cows receiving fish oil (270 g·d⁻¹) into the rumen or the duodenum¹.

Treatment	Fish oil	Ruminal infusion		Duodenal infusion	
		Duodenum	Milk	Duodenum	Milk
DPA: EPA	0.08	1.61	1.14	0.13	0.31
DPA: DHA	0.23	1.04	0.86	0.24	0.98
DHA: EPA	0.35	1.55	1.32	0.55	0.32

¹ Data from 6 cows ([27, 51]; Chilliard and Doreau, unpublished data).

3.3. Increasing conjugated linoleic acid and controlling *trans* monoenes

Very intensive research has been conducted on *trans* FA and CLA during the last five years. The reasons are, firstly, the recently acquired knowledge about the putative beneficial effects of CLA isomers on human health, including the prevention of cancer, atherogenesis, lipid peroxidation and obesity, and the modulation of immune function [122, 133]. Secondly, the controversy on the putative negative effect of the specific isomers of *trans* C18:1 occurring in either dairy products or margarines, respectively [143, 144]. Thirdly, the new knowledge and hypotheses on the central role of *trans* isomers of C18:1, and possibly of some isomers of CLA, on the milk fat depressing effects of specific diets [69].

3.3.1. Conjugated linoleic acid

CLA is an intermediate in the rumen hydrogenation of linoleic acid, whereas *trans*-11 C18:1 (vaccenic acid) is a common intermediate in the biohydrogenation of linoleic and α - and γ -linolenic acids (see above). Since the reduction of *trans* C18:1 is generally rate limiting for the complete hydrogenation of unsaturated C18-FA, there is often a ruminal accumulation of *trans*

C18:1, but rarely CLA, as suggested by the low ratio (1:40) of CLA to *trans* C18:1 in a trial cited by Griinari and Bauman [69]. Since this ratio was much higher (1:3) in milk fat of the same cows, it was suggested that a major portion of milk CLA is synthesised by the mammary gland. Similarly, since milk CLA was greatly increased with diets low in C18:2, such as pasture feeding [91, 101, 133] or those which included fish oil supplements (Fig. 5) it may be suggested that there could be CLA synthesis by tissues.

This hypothesis has been discussed in detail by Griinari and Bauman [69], reviewing experimental data in vivo and in vitro in different animal species. They suggested that ruminant mammary (and adipose) cells are able to synthesise *cis*-9, *trans*-11 CLA from *trans*-11 C18:1, and other CLA isomers from other *trans* C18:1 isomers, by action of the delta-9 desaturase on *trans* C18:1. In particular, it was shown in sheep that delta-9 desaturase expression (mRNA) decreases in adipose tissue and increases in mammary tissue with the onset of lactation [142]. A high mammary desaturase activity could explain why the CLA (product) to *trans*-11 C18:1 (precursor) ratio was fairly constant in several trials, although this ratio varied between experimental conditions and

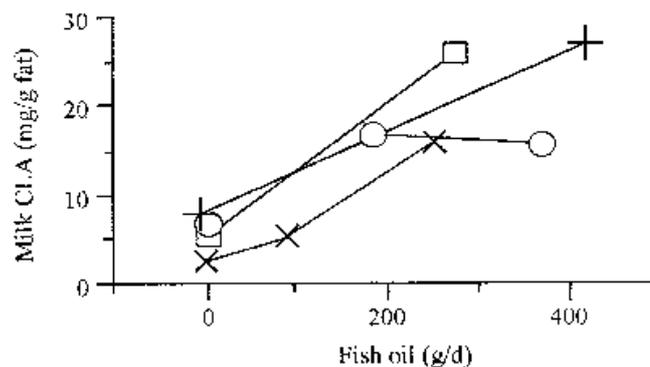


Figure 5. Relationship between fish oil intake and cow milk CLA (from [35] (□), [36] (○), [84] (+), and [117] (×)).

laboratories [69]. These authors suggest that ca. 33% of *trans*-11 C18:1 taken up by the mammary gland is desaturated to *cis*-9, *trans*-11 CLA, i.e. the major (more than 90%) CLA isomer found in milk fat. The presence of *trans*-11 C18:1 in ruminant milk fat could enhance its value for human consumers, since rodent tissues can also convert *trans*-11 C18:1 into CLA [126]. However, the desaturation of C18:0 to C18:1 has not been detected in the human mammary gland [81] despite the fact that *delta*-9 desaturase genes have been identified in human tissues [138].

Besides this, some CLA is probably absorbed from the digestive tract and taken up by the mammary gland of ruminant species. CLA isomers infused into the abomasum of cows were transferred into milk with an efficiency of between 10 and 26% [39] or 21 to 33% [40].

3.3.2. The isomers of *trans*-octadecenoic acid and the regulation of milk fat secretion

Milk fat content is greatly decreased by low fibre, high grain diets and by feeding unprotected, unsaturated lipid supplements, especially fish oils (review by Doreau et al. [54]). A role for *trans* C18:1 in these responses has been hypothesised for some time ([42, 131], reviews by Chilliard et al. [28, 31] and Palmquist [119]). The effect of high grain diets on milk FA composition differs between corn and barley diets. The former increases more C18:2 and probably *trans* C18:1, and decreases more C4 to C16 saturated FA (review by Palmquist et al. [121]), probably in relation with the higher lipid content of corn compared to barley. It was recently shown, by direct experimental approaches, that the *trans* isomers of C18:1 [64, 87, 125] and CLA [39, 103] are very efficient at inhibiting milk fat synthesis and secretion. Furthermore, *trans*-11 C18:1 is very efficiently taken up by the mammary gland [137] and could inhibit the desaturation of C18:0 by the mammary

gland [57]. However, there was no decrease in milk fat content in some trials where *trans* C18:1 was greatly increased [6, 88, 145].

A new insight arose recently when Griinari et al. [70] showed that low fibre diets greatly increased the proportion of *trans*-10 C18:1 (and not *trans*-11 isomer) and *trans*-10, *cis*-12 CLA isomers in milk fat. Furthermore, this team demonstrated that this CLA isomer depresses milk fat content, whereas the more frequent *cis*-9, *trans*-11 CLA does not [14, 69]. However, fish oil supplementation sharply decreased milk fat content without significantly increasing *trans*-10, *cis*-12 CLA, whereas 96% of the CLA increase was due to the *cis*-9, *trans*-11 isomer [35].

The different effects of the different *trans* C18:1 isomers, and of the different CLA isomers on the successive steps of milk (and body) fat synthesis (FA synthesis, uptake, desaturation and esterification, fat globule synthesis and secretion) remain to be unravelled, in ruminant as well as in monogastric species. Inhibition of FA synthesis and desaturation have been suggested in the ruminant mammary gland [14, 40]. It was shown in rodents that the *trans*-10, *cis*-12 CLA, but not the *cis*-9, *trans*-11 isomer, decreases the hepatic microsomal *delta*-9 desaturation activity [17]. In addition, the various effects of these isomers on human health also need to be elucidated.

Since the melting point of *trans*-FA is very different from that of the corresponding *cis* isomers, and because lipid fluidity in cell structures could limit fat globule packaging and/or secretion, it is likely that the percentage of the different FA, as well as their position on the glycerol molecule, contribute together via the fluidity of milk fat to an overall regulatory mechanism, thus integrating the complex effects of the numerous physiological and environmental factors that can modify milk fat composition [74]. Other dietary factors could interact further, since Focant et al. [61] observed that vitamin E restores milk fat content in

oil supplemented diets, despite a high level of *trans* C18:1.

3.3.3. *Dietary factors that affect trans fatty acids and CLA content in milk fat*

Griinari and Bauman [69] have proposed that dietary factors which affect milk CLA content could be grouped into one of two categories. The first would be factors that provide lipid substrates for formation of CLA or *trans* C18:1 in the rumen. The second would be factors that change the microbial activity associated with ruminal biohydrogenation. Literature data are presented in Table V according to the ranges of observed milk CLA content. However, no definitive conclusions can be drawn from these data, because a large proportion of the data arose from indirect comparisons of experiments in different laboratories or experimental conditions, and also because a lot of the potential interactions in practical farm conditions have not yet been studied. It nevertheless appears that plant oils high in linoleic acid (e.g. sunflower, soya and rapeseed) are very efficient at increasing milk CLA content (Fig. 6). Besides directly increasing the yield of CLA and *trans* C18:1, it is likely that linoleic acid inhibits the final reduction of *trans* C18:1, thus increasing its accumulation in the rumen [69]. Calcium salts of rapeseed also increase milk CLA content [38], in agreement with the concept that calcium salts are not resistant to ruminal biohydrogenation. Furthermore, vegetable oils are more efficient than extruded seeds (which are themselves more efficient than raw seeds) at increasing milk CLA content (Tab. V and Fig. 6). This potency could be inversely related to the protection of PUFA against biohydrogenation. On the other hand, supplementation with animal fats is not very efficient at increasing CLA content (Fig. 6) because of their low PUFA content.

When soya oil was offered 24-times daily, instead of twice, the milk fat content

increased, and the percentage of *trans* C18:1 decreased whereas that of C18:0 increased [6]. This suggests that ruminal hydrogenation was more complete and that milk CLA synthesis was probably decreased.

Feeding 4% rapeseed oil to dairy goats [110] greatly increased milk fat CLA content (by 204%), and more efficiently than similar doses of rapeseed in dairy cow diets (Fig. 6). It should be stressed that the milk yield and composition responses to dietary fat differ notably between goats and cows. Feeding vegetable oils or seeds increases milk fat content in goats (review by Chilliard and Bocquier [24]), whereas it generally decreases it in cows [21]. This peculiarity of goats could be related to some differences in the metabolism of *trans* FA in the rumen or in the mammary gland.

Feeding linseed oil (a C18:3-rich oil) greatly increases milk fat CLA content [36, 48] and is at least as efficient as C18:2-rich vegetable oils (Fig. 6). Since C18:3 is not a precursor of CLA in the rumen, this suggests that feeding linseed oil results in a large increase in the production of ruminal *trans*-11 C18:1, which can be used by the mammary gland for CLA synthesis.

Dietary fish oil is more efficient at increasing milk CLA content (Fig. 5) than an equal amount of plant oils. Biohydrogenation of long-chain PUFA (EPA and DHA) is unlikely to yield CLA or *trans*-11 C18:1 directly. However, fish oil increases ruminal [145], and milk [27, 34, 35, 99] *trans*-11 C18:1, possibly through inhibition of the reduction of this FA in the rumen. There is a linear relationship between milk fat CLA and *trans* C18:1 content across a variety of feeding conditions (Fig. 7). However, the CLA: *trans* C18:1 ratio is much lower with fish oil. It could therefore be hypothesised that either the very high level of *trans* C18:1 exceed the desaturating capacity of the mammary gland, or that some specific FA from fish oil (EPA, DHA or intermediate of biohydrogenation) inhibits the delta-9 desaturase activity. The latter hypothesis,

Table V. Effects of dietary factors on milk fat CLA content in dairy cows (literature review)^a.

low values (0.2–0.8%)	medium values (0.8–1.6%)	high values (> 1.6%)
corn silage	fresh pasture/young grass	rapeseed oil ^c
grass silage/hay/pasture	low fiber diets	soybean oil ^c
animal or vegetable saturated fats	restricted feeding	sunflower oil ^c
raw soybeans	extruded soybeans	linseed oil ^c
micronized soybeans	peanut oil	calcium salts of soybean and linseed oils
soybeans treated by heat processing	rapeseed oil ^b	fish oils
extruded soybeans or cottonseeds	soybean oil ^b	
dietary buffers	linseed oil ^b	
	calcium salts of rapeseed oil	
	ionophores (transient ?)	

^a Several interactions between dietary factors are likely to change the response to each factor alone, implying that dose-responses suggested by this table are only indicative (from [2, 35, 36, 37, 47, 48, 49, 70, 77, 83, 84, 90, 91, 101, 102, 117, 123, 127, 133, 136]).

^b At low doses.

^c At medium doses.

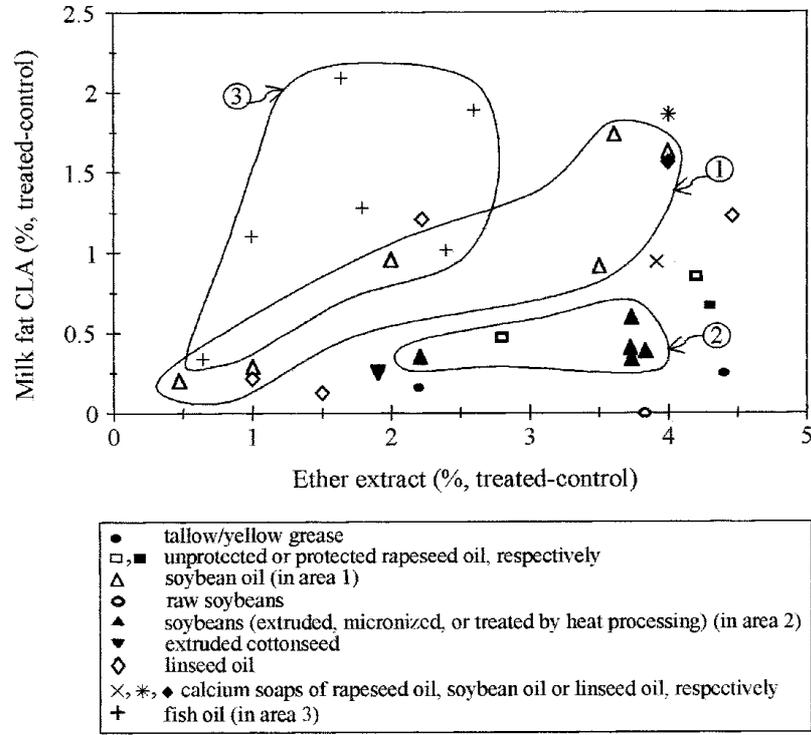


Figure 6. Effect of the nature and the amount of fat supplements on CLA content in cow milk fat (from [34, 35, 36, 48, 49, 51, 84, 117, 127, 136]).

however, does not explain the low CLA: *trans* C18:1 ratio observed with monensin supplementation ([127]; Fig. 7), and this therefore favours the first hypothesis. However, the effects of ionophores (probably a transient effect) and of forage: concentrate ratio were variable for different studies, as discussed by Griinari and Bauman [69].

Pasture feeding increases milk CLA (see above), especially with grass at an early growth stage [37]. The high C18:3 content of young grass (see above) and its low fibre content probably interact to increase the production of CLA or its *trans* C18:1 precursors. The influence of cow breed on milk CLA is either not significant or of limited extent, with milk from Montbeliardes showing slightly higher values [102].

Only limited data are available relative to dietary influences on the distribution of individual CLA isomers. The effect of the diet on milk fat CLA is largely accounted for by a change in the concentration of *cis*-9, *trans*-11 CLA isomer. This is consistent with the relative importance of the endogenous synthesis of *cis*-9, *trans*-11 CLA from *trans*-11 C18:1 by Δ -9 desaturase. Similarly, *trans*-7, *cis*-9 CLA can be produced from *trans*-7 C18:1 by Δ -9 desaturase. Therefore, it is no surprise that *trans*-7, *cis*-9 is typically found as the second largest CLA isomer (3 to 16% of total CLA isomers) in milk fat [149]. Recently, milk fat was studied from cows fed diets that result in the formation of high levels of biohydrogenation intermediates in the rumen i.e. pasture feeding and supplementation with

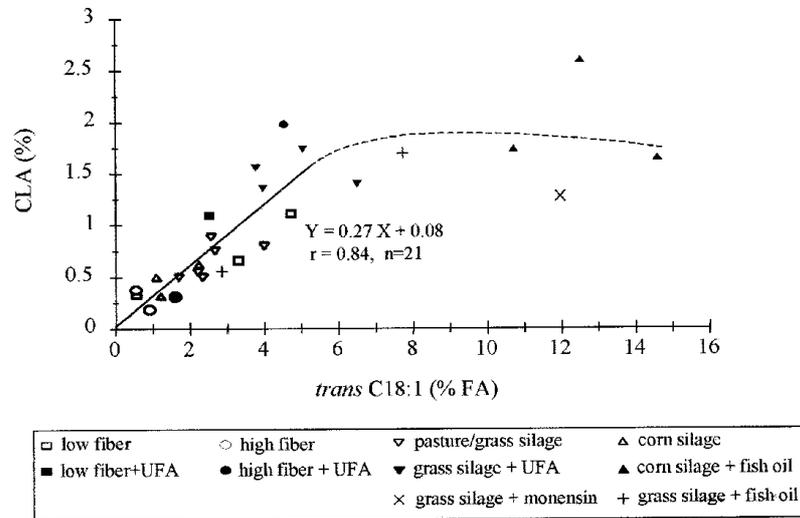


Figure 7. Relationship between CLA and *trans* C 18:1 in milk fat of cows fed various diets (from [34, 35, 36, 70, 77, 83, 117, 127, 136]). The linear regression ($n = 21$) was calculated without fish oil (+, ▲) and monensin (×) data.

dietary oils (peanut, sunflower, linseed and fish oil). These dietary treatments produced a wide range of *cis/trans*-CLA isomer concentrations. The following positive associations between dietary treatment and specific *cis/trans*-CLA isomers in milk fat were observed: pasture feeding and *cis/trans* 11,13; peanut oil and *trans*-7, *cis*-9 CLA; sunflower oil and *trans*-10, *cis*-12; linseed oil and *cis*-12, *trans*-14; fish oil and *cis*-9, *trans*-11 CLA ([35], Griinari M., Bauman D.E., Chilliard Y., Nurmela K., and Perajoki P., quoted in [68]).

4. CONCLUSION

As a means of modifying milk FA composition, dietary factors are very effective, easy to use and rapidly achieve their effect. The recent progress in the knowledge on the mechanisms of lipid digestion and metabolism in ruminants, as well as on the potential beneficial health effects of specific FA for humans, will undoubtedly

stimulate the development of new feeding strategies for lactating ruminants. This would include pasture management, concentrate formulation and techniques of protection for plant lipids.

Bearing in mind that medical evaluation of the value of the different milk FA for human health will probably continue to evolve in the future, the first goal of animal nutritionists is to freely explore the range of possible changes in milk FA composition, taking into account as many FA as possible. The second goal is to predict the laws of response of these FA to dietary factors, using modelling techniques. The third, but by no means least important goal, is to evaluate the direct or indirect consequences of changes in feeding management and in milk FA composition on the other aspects of the quality of dairy products. This includes milk triglyceride and fat globule structure, as well as manufacturing characteristics, oxidative stability, organoleptic quality, consumer choices and human health. This last goal could only be achieved through the

development of integrated multidisciplinary fundamental and applied research.

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