

# The role of dairy products in supplying conjugated linoleic acid to man's diet: a review

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Health benefits for man have been associated with conjugated linoleic acid (CLA) and dairy products are highlighted as offering the best opportunity to increase CLA consumption. CLA is synthesised in the rumen as an intermediate in the biohydrogenation of linoleic acid to stearic acid. The supplies of both intermediates and endproducts of biohydrogenation are affected by the substrate supply and extent of biohydrogenation, thus influencing the CLA content of milk from ruminants. The majority of CLA is present in the rumen in the form of the *cis*-9,*trans*-11 isomer. The transfer efficiency of CLA to milk fat is affected by the presence of different isomers of CLA. Ruminant mammary and adipose cells are able to synthesise *cis*-9,*trans*-11-CLA from *trans*-11-18:1 (vaccenic acid) by the action of the  $\Delta^9$ -desaturase enzyme. Plant oils are high in both linoleic and linolenic acids, which results in increased CLA production in the rumen and in the mammary gland. The CLA content of milk increases when cows are offered grazed grass. Many published studies examining the CLA concentration of processed milk were confounded by variations in the concentration of CLA in the raw milk.

## Conjugated linoleic acid: Dairy products: Ruminants

### Introduction

Conjugated linoleic acid (CLA) consists of fatty acids with eighteen C and two double bonds separated by one single bond (MA McGuire, MK McGuire, MS McGuire and JM Griinari, unpublished results). CLA is a collective term describing a mixture of positional and geometric isomers of linoleic acid with conjugated double bonds. The two unsaturated double bonds in CLA are usually either the C atoms in positions 9 and 11 or at positions 10 and 12 (from the

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**Abbreviation:** CLA, conjugated linoleic acid.

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carboxylic end); however there are other possibilities. At each double bond position, it is possible for the H atoms to be in either the *cis* or *trans* configuration. The *cis*-9,*trans*-11-CLA isomer is believed to be the active form because only this isomer is incorporated into the phospholipid fraction of tissues of animals fed a mixture of CLA isomers (Ha *et al.* 1990).

Recently, there has been a surge of interest in CLA in man's diet because of increasing evidence, based largely on animal studies, suggesting potential benefits of CLA for man's health (Ip *et al.* 1994). Although CLA occurs naturally in many foodstuffs, the principal dietary sources are dairy products and other foods derived from ruminant animals (Chin *et al.* 1992). Since current dietary recommendations include eating less animal fat, dietary CLA concentration is believed to have declined during the past 20 years (Stanton *et al.* 1997). It is likely that the health benefits derived from CLA have therefore declined in relation to the decrease in consumption of animal fat.

The aim of the present literature review is to summarise and critically assess the literature and assess current knowledge on CLA in the ruminant. The metabolic pathways involved in the production of CLA in the rumen will be summarised. In addition, it will confirm which raw materials contribute to CLA concentration in the milk, discuss feeding strategies to increase the CLA in milk and establish the nature of the processing effect on CLA in milk products.

### **Clarification of metabolic pathways involved in the conversion of dietary polyunsaturated fatty acids to conjugated linoleic acid by the rumen bacteria**

Since CLA content in products from ruminants is higher than those from non-ruminants, the present review will concentrate on the conversion in rumen microflora as opposed to other microflora.

#### *Synthesis by rumen bacteria*

The rumen is the site of intense microbial lipid metabolism. Lipolysis of dietary glycolipids, phospholipids and triacylglycerol releases free fatty acids, which are hydrogenated to a large extent (Harfoot & Hazlewood, 1997). The amount of conjugated dienoic acids in cows' milk (Bartlett & Chapman, 1961) and butter (Parodi, 1977) has been correlated positively with dietary intake of linoleic acid, indicating that CLA formed in the rumen is incorporated into milk fat (Bartlett & Chapman, 1961; Parodi, 1977). Kepler *et al.* (1966) identified the *cis*-9,*trans*-11-CLA isomer as an intermediate in the biohydrogenation of linoleic acid by the rumen micro-organism *Butyrivibrio fibrisolvens*.

In a review, Vivani (1970) proposed that CLA was also formed as an intermediate in the biohydrogenation pathway of linoleic acid. However, in the biohydrogenation studies with rumen micro-organisms,  $\alpha$ -linolenic acid (*cis*-9,*cis*-12,*cis*-15-octadecatrienoic acid) has been shown to be converted to *cis*-9,*trans*-11,*cis*-15-conjugated triene, then to *trans*-11,*cis*-15-18:2, and finally to an octadecenoic acid which is either *trans*-11, *trans*-15, or *cis*-15 (Harfoot & Hazlewood, 1988). Therefore, the pathways from  $\alpha$ -linolenic acid do not involve CLA as an intermediate. Harfoot & Hazlewood (1988) and Vivani (1970) investigated the biohydrogenation pathway of linoleic acid. Although linoleate isomerase and CLA reductase have been purified from the bacteria *Butyrivibrio fibrisolvens* (Kepler & Tove, 1967; Hughes *et al.* 1982), in general, no one species of micro-organism carries out the full sequence of biohydrogenation (Harfoot & Hazlewood, 1988).

The extent of rumen biohydrogenation mainly depends on the type of diet. This has been shown to be due to a drop in pH, limiting at first lipolysis, and thus hydrogenation, which occurs only on free fatty acids (Van Nevel & Demeyer, 1996). A large amount of dietary linoleic acid and a decrease in the rate of hydrogenation are the two main factors that contribute to an increase in the concentration of the intermediate compounds CLA and *trans* monounsaturated fatty acids.

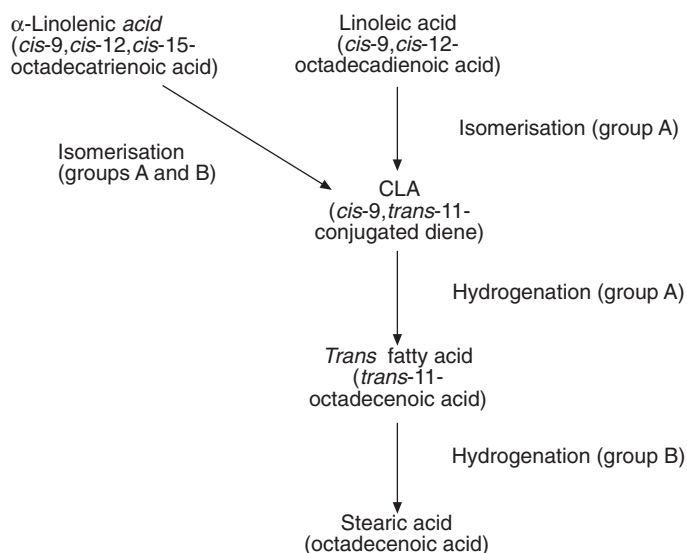
### Pathways

The pathway of biohydrogenation of linoleic acid to stearic acid by rumen micro-organisms is shown in Fig. 1.

### Control of rumen biohydrogenation

Little information is currently available regarding the biochemical mechanism that regulates the metabolism of the different CLA isomers in the ruminant animal. Changes in substrate supply and extent of biohydrogenation will affect the supply of intermediate and endproducts of biohydrogenation, thus influencing the CLA content of milk from ruminants (Kelly *et al.* 1998a, Dhiman *et al.* 1999b). However, it is the penultimate step (the hydrogenation of the *trans* monoene) that is thought to be rate limiting and subject to modification.

Free fatty acids liberated by lipolysis are adsorbed onto particles, where they are both hydrogenated and/or incorporated into the lipid fraction of the solid-associated bacteria



**Fig. 1.** Pathway of biohydrogenation of linoleic and α-linolenic acids to stearic acid by the rumen. CLA, conjugated linoleic acid. Adapted from Harfoot & Hazlewood (1988). Note that group A bacteria mostly hydrogenate linoleic and α-linolenic acids to *trans*-11-octadecenoic acid. Group B bacteria are capable of hydrogenating octadecenoic acid to stearic acid.

(Demeyer & Doreau, 1999). According to Harfoot & Hazlewood (1997), the balance between these processes is one of the factors determining the extent of fatty acid biohydrogenation in the rumen. A range of hydrogenating bacteria has been isolated and can be divided into groups A and B. Group B bacteria are capable of hydrogenating a wide range of octadecenoic acids to stearic acid. Group A hydrogenate linoleic and  $\alpha$ -linolenic acids mainly to the *trans*-11-octadecenoic acid or vaccenic acid, with smaller amounts of other positional and stereo-isomers of the same acid. The initial step in the biohydrogenation of linoleic acid involves the isomerisation of the *cis*-9,*cis*-12 isomer to the *cis*-9,*trans*-11 isomer, which is followed by a preferential reduction of the *cis*-9 double bond to form the *trans*-11:18:1. The *trans*-18:1 isomer normally comprises about 70 g/100 g rumen 18:1 acids, and it was shown in a continuous culture of rumen micro-organisms that free fatty acid may contain up to seven *trans*- and six *cis*-18:1 acids. The *trans*-11 isomer represented at least 80 g/100 g total *trans*-18:1 acids (Fellner *et al.* 1995). *Trans*-18:1 and CLA constituted 13.0 and 0.5 g/100 g total fatty acid respectively and the conjugated diene isomers were shown to contain 33.0 g *cis*-9,*trans*-11 isomer/100 g (Fellner *et al.* 1997). Biohydrogenation only occurs with free fatty acid, but the system is easily overloaded, with inhibition of the process by the free acids and accumulation of *trans*-18:1 and CLA (Fellner *et al.* 1997). The lipase enzyme responsible for lipid breakdown is inhibited by low pH. This may explain the decreased degree of saturation in rumen and duodenal lipids (Kobayashi *et al.* 1992) and/or body fat in animals fed on concentrate diets. Gerson *et al.* (1985) showed that other factors in addition to pH probably relate to changes in microbial populations.

In addition to vaccenic acid, *trans*-10-octadecenoic acid is also found in cows' milk (Griinari *et al.* 1998). Verhulst *et al.* (1985) isolated a micro-organism that converts linoleic acid to *trans*-10,*cis*-12-CLA, so it is likely, by analogy to vaccenic acid, that *trans*-10-octadecenoic acid may form in the rumen via microbial metabolism of linoleic acid to *trans*-10,*cis*-12-CLA, which is then biohydrogenated at the *cis*-12 bond. Since mammals do not possess  $\Delta^{12}$ -desaturase, it follows that the *trans*-10,*cis*-12-CLA reported in ruminant tissues would originate from *trans*-10,*cis*-12-CLA that was absorbed from the gastrointestinal tract.

Although the pathway of hydrogenation of linoleic acid in the rumen is well established, the effects of polyunsaturated fatty acid concentration on the individual enzymes are unclear. The isomerase that catalyses the first step in which CLA is produced has been purified from rumen bacteria and found to be inhibited by high concentrations of linoleic and  $\alpha$ -linolenic acid (Keppler & Tove, 1967). However, Choi *et al.* (1997) fed four dietary fat supplements, differing in polyunsaturated fatty acid concentration, and found that there was no effect on loss of linoleic acid in the rumen, which averaged 91.0 g/100 g fatty acids across treatments (Choi *et al.* 1997), suggesting that isomerase was not inhibited at the concentrations fed. However, increased concentrations of CLA and *trans*-18:1 fatty acids indicated inhibition of the reductase enzymes which convert CLA to *trans*-vaccenic acid and the latter to stearic acid.

#### *Effects of fat protection on conjugated linoleic acid production in the rumen*

Since the 1970s different attempts have been made to protect lipids against biohydrogenation. Although the degree of protection is sometimes uncertain, this technique is to date the only one which results in large amounts of polyunsaturated fatty acids escaping rumen degradation. When protected fat is offered to dairy cows the CLA content of their milk is reduced. This effect is due to protection of the lipid molecule, resulting in the group A isomerisation bacteria being unable to convert linoleic acid into CLA. Among the techniques that have been investigated, the use of Ca salts is very popular. The ability of Ca salts to prevent interactions

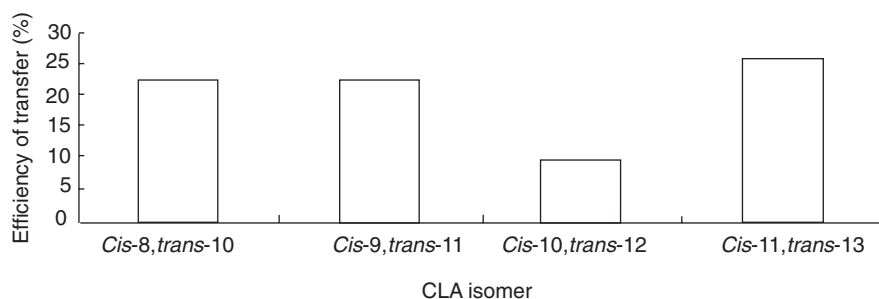
between fatty acids and microbes has been demonstrated for palm oil fatty acids (Chilliard *et al.* 2000). Further investigations are required to produce a method of fat protection that allows fatty acid isomerisation but protects from excessive biohydrogenation.

### *Absorption and transport*

The epithelial cells are the site for esterification of glycerol into triacylglycerols and phospholipids, which are transported into the lymph as chylomicrons and VLDL, which is the main route in ruminants, and further into the blood where these lipoproteins are found together with LDL and HDL. LDL and VLDL deliver most preformed fatty acids to the mammary gland. Although HDL account for approximately 900 g/kg blood lipids they consist largely of phospholipids, cholesterol and cholesteryl esters, containing the major proportion of polyunsaturated fatty acids (Mansbridge & Blake, 1997). Lipoproteins transport fatty acid mainly to the mammary gland in dairy cattle and mainly to adipose and muscle tissue in fattening animals. When fatty acids are needed for energy production, that is, synthesis of milk fat, VLDL lose most of their triacylglycerol and are converted into LDL and HDL. Chouinard *et al.* (1999) reported that there appears to be some selectivity in the uptake or incorporation of the *cis*-9,*trans*-11 isomer over the *trans*-10 isomer of CLA in dairy cows. There were differences in the efficiency of transfer of CLA to milk fat among the isomers. Only about 10 g/100 g dietary supplement of *cis*-10,*trans*-12-CLA isomer was transferred to milk fat, whereas the *cis*-8,*trans*-10, *cis*-9,*trans*-11- and *cis*-11,*trans*-13-CLA isomers were transferred to milk fat with over twice the efficiency (22.0–26.0 g/100 g; Fig. 2) (Chouinard *et al.* 1999). Studies in lactating dairy cows have also found that the transfer efficiency of a dietary supplement of the *cis*-10,*trans*-12-CLA isomer was only about half of that observed for the *cis*-9,*trans*-11-CLA isomer (Chouinard *et al.* 1999).

### *Adipose tissue and the mammary gland as conjugated linoleic acid depots*

Baumgard *et al.* (2000) reported that the *trans*-10,*cis*-12-CLA isomer would decrease milk fat synthesis. CLA may be inhibiting the activity or synthesis of key enzymes involved in *de novo* fatty acid synthesis. The specific mechanisms whereby CLA alters lipid metabolism are not clear. One mechanism may involve increases in rates of lipolysis and fatty acid oxidation in



**Fig. 2.** Apparent efficiency of abomasally-infused conjugated linoleic acid (CLA) isomers into milk fat of dairy cows. Adapted from Chilliard *et al.* (2000).

adipose tissue. In lactating cows, circulating concentrations of plasma non-esterified fatty acids are highly correlated with rates of lipolysis and the relatively minor changes observed with CLA supplementation suggested that CLA had little or no effect on lipolysis (Bauman *et al.* 1988). When CLA supplement was infused abomasally to by-pass rumen biohydrogenation, Chouinard *et al.* (1999) observed a dramatic reduction in milk fat of lactating cows, whereas milk yield and protein were unaffected. The addition of the CLA supplement increased the milk fat content of CLA in a dose-dependent manner from approximately 7 mg/g fat at the zero dose to 64 mg/g fat at the high dose of 150 g CLA supplement/d (90 g actual CLA isomers/d). The milk content of CLA had typically reached a constant value by 4–5 d after initiation of the daily CLA supplement infusion. Only about 10 g/100 g dietary supplement of *cis*-10,*trans*-12-CLA isomer was transferred to milk fat, whereas the *cis*-8,*trans*-10-, *cis*-9,*trans*-11- and *cis*-11,*trans*-13-CLA isomers were transferred to milk fat with over twice the efficiency (22.0–26.0 g/100 g).

Another possible mechanism by which CLA might alter lipid metabolism would be to reduce tissue uptake of fatty acids. This involves lipoprotein lipase, and the activity of this enzyme was decreased when 3T3-L1 adipocyte cultures were incubated with CLA (Park *et al.* 1997, 1999). If lipoprotein lipase in the mammary gland was affected in this study then a reduction in the use of preformed fatty acids for milk fat synthesis would be expected. A reduction was observed, but effects on *de novo*-synthesised fatty acids were more extensive.

#### De novo synthesis

In certain circumstances, fatty acids can be synthesised *de novo* from acetate. Adipose tissue is the major site of fatty acid synthesis in ruminants, except during lactation when the mammary gland becomes the predominant site. Synthesis of fatty acids up to palmitic acid takes place in the cytoplasm from acetyl-CoA and  $\beta$ -hydroxybutyrate derived from mitochondrial oxidation. Mitochondria elongate palmitic acid to longer-chain fatty acids up to C<sub>22</sub>, whereas microsomes are capable of elongation as well as desaturation of fatty acids  $\geq$ C<sub>18</sub>. In the mammary gland, *de novo* synthesis is generally limited to short-chain fatty acids and medium-chain fatty acids (up to C<sub>16:0</sub>).

However, Corl *et al.* (1998) suggested a possible synthesis of CLA in the mammary gland from *trans*-11-18:1 by the  $\Delta^9$ -desaturase. Offer *et al.* (1999) also suggested that CLA is formed from *trans* monoenes within the animal tissues such as the mammary gland. Griinari & Bauman (1999) suggested that the ruminant mammary glands and the adipose cells are able to synthesise *cis*-9,*trans*-11-CLA from *trans*-11-18:1 and other CLA isomers from other *trans*-18:1 isomers by action of the  $\Delta^9$ -desaturase on *trans*-18:1. Ward *et al.* (1998) showed in sheep that the  $\Delta^9$ -desaturase expression decreased in adipose tissue and increased in mammary tissue with the onset of lactation. Griinari & Bauman (1999) suggested that about 33 g/100 g *trans*-11-18:1 taken up by the mammary gland is desaturated to *cis*-9,*trans*-11-CLA. The presence of *trans*-18:1 in ruminant milk could enhance its value for consumption by man, since rodent tissues can also convert *trans*-18:1 into CLA (Santora *et al.* 2000). The  $\Delta^9$ -desaturase genes have been identified in tissues from human subjects (Tocher *et al.* 1998) but the desaturation of 18:0 to 18:1 has not been detected in the mammary gland of human subjects (Jensen, 1999).

### Identification of feeding strategies which promote conjugated linoleic acid production in the rumen and secretion in milk

Griinari & Bauman (1999) 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*-18:1 in the rumen. The second would be factors that change the microbial activity associated with rumen biohydrogenation. Plant oils high in linoleic acid (e.g. sunflower, soyabean and rapeseed) are very efficient at increasing milk CLA content. Besides increasing the yield of CLA and *trans*-18:1 directly, it is likely that linoleic acid inhibits the final reduction of *trans*-18:1, thus increasing its accumulation in the rumen (Griinari & Bauman, 1999). Large variations in CLA content of milk have been reported. Typical concentrations of CLA in milk fat are 3–6 g/kg fat, but CLA concentration in milk can vary widely among herds (Riel, 1963), which may be a consequence of dietary differences. However, the specific factors that cause these variances have not been extensively investigated.

#### *Dietary*

The CLA content of milk and milk products can be altered by affecting rumen production of CLA or *trans*-11-18:1, or by dietary supplementation with these fatty acids (Chouinard *et al.* 1999). Indeed, Jiang *et al.* (1996) reported a variation of 0.25–1.77 g CLA/100 g milk fatty acids and suggested that there is scope for increasing the CLA content of milk through changes to the cows' diet. Furthermore, in ruminant tissue and milk, concentration, as well as isomer distribution, of CLA are likely to be modulated by the microbial population in the rumen, which in turn is influenced by the quantity and composition of animal feed and its dietary oils content (Vivani, 1970).

#### *Oils*

Dhiman *et al.* (1997) showed that free oils (rich in linoleic or linolenic acid) in the diets of dairy cows increased the CLA content of milk. Free oil is not normally included in the diet as it produces inhibitory effects on microbial (particularly cellulolytic) activity in the rumen (Jenkins, 1993). In comparison, dietary oil in the form of intact seeds does not change milk CLA (Dhiman *et al.* 1997). Milk fat depression commonly occurs when diets high in plant oils are fed (Davis & Brown, 1970) and high intakes of dietary fat may also cause milk protein concentration and yield to decrease. In this case the dietary fat adversely affects microbial fermentation and microbial protein yield thereby decreasing the supply of amino acids available for absorption by the cow (Palmquist & Jenkins, 1980).

Dhiman *et al.* (1999a) showed that feeding fish meal increased CLA content of milk by a small margin and Franklin *et al.* (1999) reported that cows fed marine algae had a greater concentration of CLA in their milk. In accordance with Davis & Brown (1970) with regard to plant oils, fish oil was toxic to rumen micro-organisms as it caused a decrease in concentration of milk fat. Furthermore, supplementation of fish oil at 200–400 g/d to dairy cows resulted in decreased DM intake (Doreau & Chilliard, 1997); this is likely to be a result of rumen micro-organism toxicity.

*Plant oils*

MA McGuire, MK McGuire, MS McGuire and JM Griinari (unpublished results) fed cows four different concentrations of maize oil (500 g linoleic acid/kg) and found that CLA increased in a dose-dependent manner from 2.3–6.9 mg/g milk fat. However, there was large variation among cows in the concentration of CLA even though DM intake and milk yields were similar among the eight cows. Griinari *et al.* (1996) also showed that the addition of dietary unsaturated fatty acids such as maize oil and changing forage:concentrate ratios enhanced the CLA content of milk fat. In contrast, Dhiman *et al.* (1999a) found that supplying an additional 10 g CLA/kg fat in the diet through high-oil maize and high-oil maize silage did not influence the CLA content of milk.

Stanton *et al.* (1997) reported that a supplement of full-fat rapeseed (high in oleic acid) caused a greater increase in CLA content of milk than did soyabean oil (high in linoleic acid). Stanton *et al.* (1997) found that rapeseed supplementation resulted in an increase of 650 g CLA/kg milk over non-supplemented control, but the total fat concentration was not reported. It is not known whether the effect of rapeseed oil was due to its relatively low linoleic acid content or an effect of large amounts of oleic acid. Twofold increases in fat and CLA content were reported in milk from goats when 40 g rapeseed oil/kg diet was fed (Mir *et al.* 1999). It should be noted that the milk yield and composition responses to dietary fat differ notably between goats and cows. Feeding vegetable oils or seeds increased milk fat content in goats (for review, see Chilliard & Bocquier, 1993) whereas it generally decreased it in cows (Chilliard, 1993). These differences observed with goats could be related to differences in the metabolism of *trans* fatty acids in the rumen or in the mammary gland. Jahreis *et al.* (1996) reported that feeding dairy cows increasing amounts of rapeseed oil (control 470 g fat, treatment one 200 g oil, treatment two 400 g oil/cow per d) correlated with elevated *trans*-fatty acid contents in milk fat from 2.2 g/100 g total fatty acid in the control group to 3.4 and 4.4 g/100 g respectively.

Kelly *et al.* (1998a) reported that feeding sunflower oil (high in linoleic acid) increased CLA concentrations to 24.4 g/kg milk fat compared with values of 13.3 and 16.7 g/kg fat for high-oleic (peanut oil) and high-linolenic acid oils (linseed oil), respectively. These studies suggested that, given an adequate dietary intake of linoleic acid, dietary constituents that provide rumen substrates for the optimal growth of bacteria producing linoleic acid isomerase would maximise CLA output. Feeding linseed oil (linolenic-rich) greatly increased CLA content in milk fat (Dhiman *et al.* 1997; Chouinard *et al.* 1998) and was shown to be as efficient as sources of linoleic acid (Chilliard *et al.* 2000). Linolenic acid is not a precursor of CLA in the rumen and it has been suggested (Chilliard *et al.* 2000) that feeding linseed oil results in a large increase in the production of rumen *trans*-11-18:1, which can be used by the mammary gland for CLA synthesis (Fig. 2).

When soyabean oil was offered twenty-four times daily instead of twice, the milk fat content increased and the percentage of *trans*-18:1 decreased, whereas that of 18:0 increased (Banks *et al.* 1980). This suggests that rumen hydrogenation was more complete and that the milk CLA synthesis was probably decreased.

The CLA content of milk and cheese may also be increased by the addition of extracted soyabeans and cottonseed to the diets of dairy cows. Dhiman *et al.* (1999a) suggested that to make oil more readily available for digestion, the soyabeans and cottonseeds can be processed through an extruder to rupture the seeds. Dhiman *et al.* (1999b) reported that contents of CLA in milk and cheese were doubled from 0.34 g/100 g fatty acid to 0.69 g/100 g fatty acid by the inclusion of full-fat extruded soyabeans.

Full-fat rapeseed supplementation of diets fed to lambs or dairy cows have been shown to increase CLA content of meat (Mir *et al.* 1997) and milk (Stanton *et al.* 1997) respectively.

### *Other fats*

Doyle (1998) reported that supplements of restaurant grease (consisting of both vegetable oil and animal fat) and tallow both increased fat content of milk, but only the grease increased CLA. However, Dhiman *et al.* (1999b) warned that an increase in CLA may be accompanied by an increase in oxidised fats, which can produce 'off' flavours in the milk and in addition milk yield may decrease on some of these diets (Doyle, 1998).

### *Grazing*

Seasonal variations in the CLA content of milk are very marked, with values during the summer often up to two or three times higher than during the winter (Jahreis *et al.* 1997; Parodi, 1999). From studies of other *trans* fatty acids a seasonal effect for example on *trans* vaccenic acid content in milk fat is known (Precht, 1995). Moreover, Jahreis *et al.* (1997) reported that grazing of fresh polyunsaturated fatty acid-rich grass increased the formation of both CLA and *trans*-vaccenic acid in milk.

The high content of CLA in milk from cows offered pasture has also been attributed to the linoleic acid content of the forage although the proportion of linoleic acid is low compared with  $\alpha$ -linolenic acid (Garton, 1960). Feeding sheep diets high in  $\alpha$ -linolenic acid increases the rumen content of *trans*-18:1 fatty acid as a result of incomplete hydrogenation (Czerkawski *et al.* 1975).

Recently it was shown that high-concentrate diets and low rumen pH result in increased rumen *trans*-fatty acid generation (Kalscheur *et al.* 1997). Jahreis *et al.* (1997) reported that milk from cows grazing pasture had higher CLA content compared with cows offered maize silage and high-cereal-based concentrates. Increasing the proportion of grazed grass from pasture in the diet of dairy cows linearly increased the CLA content of milk (Dhiman *et al.* 1999a). Cows grazing pasture permanently had five times more CLA compared with cows fed total mixed ration containing conserved forage–grain (50:50, w/w). Conversely, feeding hay did not influence milk CLA content.

Boylston *et al.* (1996) did not observe any seasonal CLA variations in dairy cows fed the same total mixed ration throughout the year. Therefore, it seems seasonal variation in CLA content of milk can be attributed to the proportion of grazed grass in the diet. Indeed, Precht & Molquentin (1997) found that milk fat from cows consuming pasture contained a mean of 12.0 g CLA/kg milk compared with 4.5 g CLA/kg in milk from cows fed hay, silage and concentrates.

Jahreis *et al.* (1997) suggested that the reason that the CLA content in the milk from cows living indoors all year was low compared with pasture fed cows, was that the diet fed to these cows was low in polyunsaturated fatty acids, and therefore a deficiency of substrate for biohydrogenation by rumen bacteria existed. Although not the only determinant, the amount of dietary polyunsaturated fatty acid determines the generation of *trans* fatty acids by rumen bacteria.

### *Forage:concentrate ratio*

Lower forage:concentrate ratios in dairy cattle diets have also been shown to increase CLA concentration in milk (Jiang *et al.* 1996). Jiang *et al.* (1996) were able to double the CLA content from 5.04 to 11.28 g/kg fat by feeding a higher concentrate:roughage ratio to dairy cows, without appreciably increasing the percentage milk fat.

*Animal factors*

Depending on feeding and milk performance, genetic constitution and stage of lactation, the composition of bovine milk fat is subject to strong variations. Indeed, Doyle (1998) reported that survey results demonstrate that CLA concentration in milk from New York, USA, herds ranges from 2.2 to 20.1 g/kg fat. Moreover, Lawless *et al.* (1999) proposed that the influence of cow breed on milk CLA is either not significant or limited, with milk from Montbeliardes showing slightly higher values than Holstein-Friesians and Normandes offered grazed grass. Recently, Kelly *et al.* (1998b) examined this variation for cows fed either a pasture diet or a total mixed ration. Although they observed that individuals maintained relatively constant milk fat concentrations of CLA across time, there was a threefold variation in milk CLA content among individuals (2.4–7.0 g/kg milk fat) even though all cows were at a similar stage of lactation, consumed the same diet through a total mixed ration at similar intakes and produced similar amounts and composition of milk. The CLA content in milk fat from cows in the grazing group (Kelly *et al.* 1998b) showed significantly ( $P < 0.05$ ) higher individual cow variation as the proportion of pasture in the ration increased (3.0–9.0 and 6.3–18.1 g/kg milk fat for total mixed ration and grazing respectively). Kelly *et al.* (1998a) reported substantial individual variation (9.9–51.7 g CLA/kg fat) in cows at the same stage of lactation that consumed a total mixed ration supplemented with 53 g sunflower oil/kg DM. The variation was significantly lower for supplements of peanut oil and linseed oil. The results suggest additional factors such as individual genetic regulation of rumen microflora may operate (Moore *et al.* 1993).

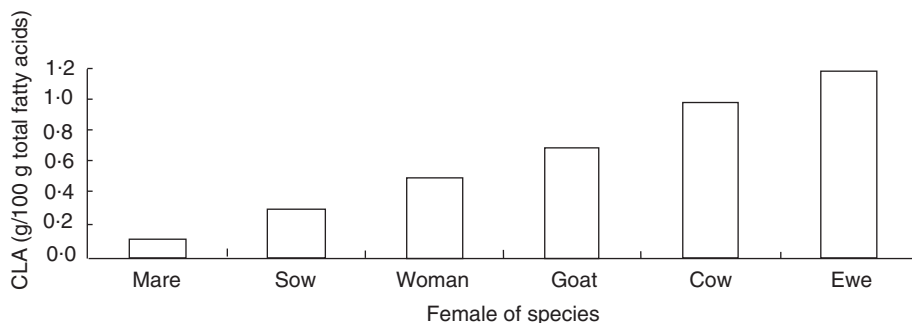
Stanton *et al.* (1997) reported that certain breeds of cattle and some individual cows appear to be more efficient at incorporating CLA into milk, with a range of 3–25 g/kg milk fat, when offered grazed grass. Older cows (>4 years old) tended to produce milk with more CLA, as did those fed a higher grass allowance.

*Relationship with trans fatty acids*

Rumen biohydrogenation results in a characteristic pattern of positional isomers of *trans*-18:1 where *trans*-11 is the major isomer comprising at least 80.0 g/100 g *trans*-18:1 (Kemp *et al.* 1984). This pattern is reflected in the tissue lipids and milk fat of ruminants (Wolff, 1995). Chemical hydrogenation of polyunsaturated fatty acids produces a distinctly different pattern of positional *trans* isomers, where the proportions of isomers with *trans* double bonds in positions 9, 10 and 11 are almost equal (Fig. 3).

The increased concentration of both *trans*-18:1 and CLA in milk fat compared with hydrogenated vegetable oil could result from similar proportional inhibition of both enzymes required for production of *trans*-18:1 and CLA by the dietary *n*-3 polyunsaturated fatty acids or feedback inhibition of CLA reductase by increased concentrations of *trans*-18:1 in the rumen (Griinari *et al.* 1997). Enser *et al.* (1999) reported a strong linear correlation between CLA and *trans*-18:1 concentrations in beef cows. A similar relationship between these two fatty acids has been reported in milk indicating that the effect stems from the rumen rather than specific metabolism in the mammary gland. Furthermore, this correlation could reflect the desaturation of vaccenic acid by  $\Delta^9$ -desaturase.

In summary, there is scope for increasing CLA concentrations in milk by feeding to affect rumen production of CLA. This may include pasture management, concentrate formulation and techniques for protection of plant oils.



**Fig. 3.** Conjugated linoleic acid (CLA) content in milk fat of different species. Adapted from Jahreis *et al.* (1999).

### Dietary sources of conjugated linoleic acid for man

#### *Dairy products*

Table 1 shows the CLA concentration in different foods and Table 2 indicates the major sources of CLA. Milk fat has the greatest potential for high CLA and certainly elevated concentrations of *cis*-9,*trans*-11 isomer. MA McGuire, MK McGuire, MS McGuire and JM Griinari (unpublished results) showed that human milk is devoid of CLA when ruminant products are removed from their diet. Fig. 4 shows that the CLA content in milk fat is greatest for ruminant animals, particularly cows and sheep. The primary isomer of CLA, *cis*-9,*trans*-11-octadecadienoic acid accounts for more than 82.0 g/100 g total CLA isomers in dairy products (Chin *et al.* 1992). However, the CLA content in milk and cheeses varies considerably, ranging from approximately 3 to 9 g/kg fat (Chin *et al.* 1992). Dhiman *et al.* (1999b) reported CLA contents in samples of milk of 3.4, 6.9 and 6.0 g/kg milk fat from cows offered diets containing 135 g soyabean meal/kg, 120 g full-fat soyabeans/kg or 120 g full-fat cotton seed/kg respectively and reported no change in CLA content in mozzarella cheese processed from the same milk.

#### *Meat*

Interestingly, pork, chicken and eggs contain CLA and it is likely that rumen bacteria are not the only source for the biosynthesis of CLA (Ip *et al.* 1994). Nonetheless, Chin *et al.* (1992) confirms that meat from ruminants generally contains more CLA than meat from non-ruminants. Interestingly, turkey meat contains higher concentration of CLA than chicken, but reasons for this difference are unclear.

#### *Fish and other marine foods*

The concentration of CLA in fish and seafood is very low; however, this is of little relevance in the UK since intakes of fish and seafood are declining, from 27 g/d in 1948, to approximately 20 g/d in 1990 (Ministry of Agriculture, Fisheries and Food, 1995).

**Table 1.** Total conjugated linoleic acid and *cis*-9,*trans*-11-conjugated linoleic acid in different food products\*

Food	Total CLA (g/kg fat)	<i>cis</i> -9, <i>trans</i> -11 CLA (g/100 kg total CLA)
Butter	9.4–11.9	91.0
Processed cheese	3.2–8.9	17.0–90.0
Natural cheese	0.6–7.1	17.0–90.0
Yoghurt	5.1–9.0	82.0
T-bone steak (cooked)	4.7–9.9	65.0
T-bone steak (raw)	4.4–6.6	59.0
Vegetable oils	0.2	45.0
Milk fat	2.0–30.0	90.0

CLA, conjugated linoleic acid.

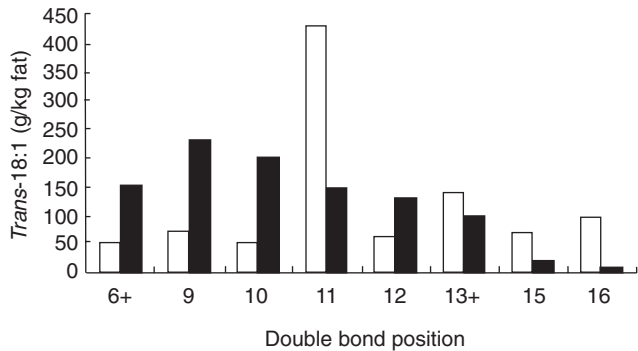
\*Adapted from O'Shea *et al.* (1998) and Chin *et al.* (1992).

**Table 2.** The contribution of various components of the UK diet to conjugated linoleic acid intake\*

Food source	Assumed average fat intake from the food source (g/d)	CLA intake by man (mg CLA/d)
Meat	17.5	49.00
Fish	1.3	0.55
Cheese	4.6	25.30
Butter	4.5	27.50
Milk	8.5	53.00
Vegetable, salad oils and margarine	9.3	1.90

CLA, conjugated linoleic acid.

\*Adapted from Ministry of Agriculture, Fisheries and Food (1995) and Chin *et al.* (1992).



**Fig. 4.** Distribution of *trans*-18:1 isomers in milk fat (□), and hydrogenated vegetable oil (■) where cows were offered various different feeds. Adapted from Precht & Molkentin (1995). The hydrogenated vegetable oil was margarine and cooking oil.

*Synthetic sources*

In a study by Ip *et al.* (1999), dairy cows were fed in such a way that resulted in the production of high-CLA butter. Subsequently, a group of rats were fed either high-CLA butter or a synthetic CLA source. The rats consuming high-CLA butter consistently accumulated more total CLA in their tissues compared with those consuming the artificial CLA. The high-CLA butter fat also provided a source of vaccenic acid (*trans*-11-18:1) and this may be converted to *cis*-

9,*trans*-11-CLA by the  $\Delta^9$ -desaturase enzyme (Griinari & Bauman, 1999), hence providing higher concentrations of CLA than the synthetic CLA treatment.

One potential method of supplying CLA in man's diet would be to provide it in a synthetic form. However, it is likely that providing CLA in the form of dairy products may supply the precursor for the endogenous synthesis of CLA as well as containing more of the biologically active isomer of CLA than synthetic sources.

### *Plants*

The CLA content of plant oils is very low, ranging from 0.1 to 0.7 g CLA/kg fat (Chin *et al.* 1992). Chin *et al.* (1992) observed that the distribution of individual CLA isomers in plant oils is also different from that in animal fat. Indeed, beef tallow contained four times more CLA than plant oil and whereas more than 800 g CLA/kg beef tallow is the *cis*-9,*trans*-11-CLA isomer, only half of the CLA was found to be in the form of the *cis*-9,*trans*-11 isomer in commercial plant oils. Plant oils are an inferior source of CLA in the human diet; safflower contains the most CLA, but only half is present in the form of the *cis*-9,*trans*-11 isomer and higher concentrations of the *cis*-10,*trans*-12 isomer. Consequently, plant oils are unlikely to be a major source of CLA in man's diet.

### *Dietary intakes*

The CLA contribution from various components of the UK diet is shown in Table 2. Intakes of the various food sources were obtained from Ministry of Agriculture, Fisheries and Food (1995) and CLA concentrations of the foods were adapted from Chin *et al.* (1992) for meat, fish, cheese, butter, milk and vegetable, salad oils and margarine.

## **Non-dietary sources of conjugated linoleic acid**

### *Conjugated linoleic acid content of human tissues*

Chin *et al.* (1994) showed that intestinal bacteria of rodents are capable of converting unsaturated fatty acids to CLA, although it is not known whether this capacity is present in human gut microflora. It is well known that human milk, serum and adipose tissue can contain CLA (mainly the *cis*-9,*trans*-11 isomer) (Fritsche & Steinhart, 1998). Jiang *et al.* (1999) conducted a study on 123 men using weighed dietary records, blood sampling and adipose tissue biopsy. A correlation was shown between *cis*-9,*trans*-11-18:2 concentration of CLA in adipose tissue and milk fat intake. The CLA concentration in adipose tissue was twice as high as that in serum, but there was no significant correlation between the amount of CLA in adipose tissue and in serum (Jiang *et al.* 1999). It is likely that vaccenic acid is used for CLA production by  $\Delta^9$ -desaturase enzyme in the tissues of man, as clearly occurs in rodents.

Chin *et al.* (1992) proposed that CLA found in the tissues of non-ruminants may either be a consequence of dietary intake, or perhaps due to the conversion of linoleic acid to the *cis*-9,*trans*-11-CLA isomer by bacterial flora. Indeed, Chin *et al.* (1992) indicated that micro-organisms in the rat gastrointestinal tract are capable of synthesising CLA from linoleic acid, although there would be no absorption of CLA produced by micro-organisms in the hindgut.

### Clarification of the effects of various milk processing techniques on conjugated linoleic acid concentrations in milk products

Garcia-Lopez *et al.* (1994) reported large differences in CLA content among dairy products, which led the investigators to speculate that processing conditions may influence CLA content substantially. Indeed, Ha *et al.* (1989) reported that the CLA content of dairy products ranged from 0.0283 g/kg for raw whole milk to 18.2 g/kg for processed cheese. In contrast, Shantha *et al.* (1995) found that CLA concentration did not sharply increase during processing of dairy products, nor did CLA content decrease during storage.

The effect of processing was evaluated by comparing, on a fat basis, the amount of CLA contributed by the raw ingredients with that found in processed cheese at different points in the processing line (Garcia-Lopez *et al.* 1994). There was an average increase in CLA content of 144 g/kg during processing. The increase in CLA resulted mainly from heating the raw ingredients and not from any other step in the processing line. These results are consistent with those obtained by Shantha *et al.* (1992) and Aneja & Murthi (1991).

Care must be exercised when comparing the CLA content and isomer distribution profiles for the various dairy products. First, it must be remembered, as reviewed by Reil (1963), that the CLA content of milk fat can be expected to vary between 2 g/kg and 30 g/kg. This variation is seasonal, highest values occurring when pastures are lush and rich in polyunsaturated fatty acids. Thus the CLA values of the various dairy products and different varieties of natural cheeses reported by Ha *et al.* (1989) and Chin *et al.* (1992) probably do not represent different types of products or processing conditions, but rather reflect the fluctuating CLA concentration of the raw materials. Second, an outstanding characteristic of the conjugated fatty acids is their instability even at ambient temperatures.

#### *Processing mechanisms*

CLA was found at higher concentrations in all cheddar-based processed cheeses than in unprocessed cheddar, except for American cheese (Dhiman *et al.* 1999b). Indeed, Dhiman *et al.* (1999b) reported that the processing of milk into mozzarella cheese did not alter the fatty acid composition of CLA and among all cheese sampled, blue, Edam and Swiss cheese had the highest CLA concentrations.

#### *Temperature*

There is debate as to the effects of processing on CLA content of dairy products. Dairy products such as cheeses (both natural and processed), milk and yoghurts that have undergone a variety of heat-processing treatments generally contain increased concentrations of CLA (Shantha *et al.* 1992). In contrast, Chin *et al.* (1992) reported that CLA in natural cheeses was comparable with that in unprocessed milk. Moreover, total CLA content and the *cis*-9,*trans*-11 isomer were comparable in processed and natural cheeses, indicating that heat treatment during processing does not alter total CLA content or *cis*-9,*trans*-11 isomer concentration.

High processing temperature (80°C) increased the formation of CLA in processed cheese, but no increase in CLA formation was observed with increasing temperature when the cheese was processed under N<sub>2</sub> instead of atmospheric conditions (Kanner *et al.* 1987). Thus, both pro-

cessing temperature and the presence of air play a role in the formation of CLA. Indeed, oxygen radicals, such as the hydroxyl radical, are known to initiate lipid oxidation by causing the formation of lipid free radicals (Kanner *et al.* 1987). In contrast, Steinhart (1996) proposed that oxidative reactions have no important effect on CLA during food processing and storage, nor does conventional processing alter the total CLA content of foods although concentrations of *cis*-9,*trans*-11-CLA may be lower after processing.

### *Environmental*

Ha *et al.* (1989) speculated that synthetic CLA could originate during the auto-oxidation of linoleic acid under anaerobic conditions. Auto-oxidation of linoleic acid involves the formation of an alkyl radical (chemically or enzymically), stabilised by its resonance structures, followed by the addition of O to produce the corresponding hydroperoxides. Under anaerobic conditions, however, the conjugated diene system could be established by the addition of H to the conjugated alkyl radical. Proteins may act as H donors and anaerobic conditions could be generated in isolated pockets during the heating step (Ha *et al.* 1989). This hypothesis seems to be supported by the findings of Shantha *et al.* (1992) who found that increasing the whey-protein concentration from 0 to 60 g/kg results in an increase in CLA content of processed cheese of 1.73 mg/g fat. Indeed, Shantha *et al.* (1992) reported that lipid peroxides were not detected when the cheese was processed under either atmospheric conditions or N<sub>2</sub> indicating that any linoleic acid radicals formed during processing were primarily being converted to CLA instead of lipid peroxides.

Caric & Kalab (1987) reported an increase in CLA concentrations with addition of whey protein concentrate at concentrations normally found in processed cheeses. However, the CLA content of the whey-protein concentrate was less than 0.075 g/100 g total fatty acid methyl esters. Therefore, the maximum amount of CLA contributed by the whey-protein concentrate was 0.016 g/kg fat, whereas the total increase in CLA content of processed cheese containing 60 g whey-protein concentrate/kg was approximately 10.7 g/kg fat. This indicates that the observed increase in CLA was not solely due to fat contributed by whey-protein concentrate. The ability of the whey-protein concentrate low-molecular-mass fraction to increase the formation of CLA in processed cheese could be due to interactions between the low-molecular-mass components and the linoleic acid radicals resulting in CLA.

### *Additives*

As previously mentioned, CLA may be formed by initial formation of the linoleic acid radical, the dienyl radical can then abstract a proton from an H donor to give rise to CLA (Ha *et al.* 1989). It is possible that certain additives may act as H donors, and Shantha & Decker (1993) reported that the H donor propyl gallate was more effective than butylated hydroxytoluene for CLA-forming activity (Table 3).

The observed differences in the CLA forming ability of these could be in part related to their differing solubility characteristics. Cysteine and ascorbic acid, well-documented proton donors, gave similar increases in CLA concentration (Shantha & Decker, 1993).

**Table 3.** The effects of antioxidants and reducing agents on conjugated linoleic acid concentration in processed cheese\*

Treatment	CLA concentration (g/kg fat)	
	<i>cis</i> -9, <i>trans</i> -11 isomer	Total CLA
Control (natural cheddar cheese)	3.30	4.14
Butylated hydroxytoluene	3.77	4.80
Ascorbate	4.28	5.88
Cysteine	4.09	5.71
Propyl gallate	4.69	6.60

CLA, conjugated linoleic acid.

\*Adapted from Shantha &amp; Decker (1993).

### Ageing

Werner *et al.* (1992) analysed three 13-month-aged cheeses and one unaged cheese for CLA concentration and isomer distribution. The CLA concentration in the fat of the aged and unaged cheeses ranged from 5.05 to 5.39 g CLA/kg fat, with 80.0 g *cis*-9,*trans*-11 isomer/100 g CLA. Werner *et al.* (1992) concluded that ageing-time had negligible effects on the total CLA concentration of cheese; however, CLA concentration in raw milk was not measured. Indeed, Lin *et al.* (1995) reported that mean CLA contents of raw milk and cheeses aged 0 and 180 d were 3.44, 3.51 and 3.32 mg/100 g fat respectively. With the exception of cheeses aged 180 d, CLA content was not significantly affected by ageing.

### Conclusions

CLA is present in products from ruminant animals including milk, dairy products and meat. Although it is also found in both plants and fish, it is present in much lower quantities than in ruminant products and it is unclear what the source of CLA is. Currently, there is scarce information in the published literature examining the mechanism that regulates the formation of the different CLA isomers within the dairy cow. The supplies of intermediate and endproducts of biohydrogenation are affected by the substrate supply and extent of biohydrogenation, thus influencing the CLA content of milk from ruminants. The majority of CLA is present in the rumen in the form of the *cis*-9,*trans*-11 isomer. The *cis*-9,*trans*-11-CLA isomer is produced as an intermediate in the biohydrogenation of linoleic acid by the rumen micro-organism *Butyrivibrio fibrisolvens*.

It is not known whether pH *per se* or the change in bacterial population is responsible for CLA manipulation. pH in the rumen may simply reflect other changes responsible for the shift in hydrogenation, rather than causing changes. The linoleic acid concentration in the rumen will affect biohydrogenation and consequently CLA production in the rumen. CLA can also be synthesised in the mammary gland and adipose tissue from vaccenic acid. However, the conditions required in the rumen that promote increases of *trans*-11:18:1 remain unclear. The efficiency of transfer of CLA to milk fat can vary between CLA isomers. The *cis*-10,*trans*-12-CLA isomer was transferred to milk fat less efficiently than the *cis*-8,*trans*-10-, *cis*-9,*trans*-11- and *cis*-11,-*trans*-13-CLA isomers.

Several dietary strategies may be adopted to increase the CLA content of milk fat. It appears that plant oils high in linoleic acid, for example sunflower and soyabean, are very efficient at increasing milk CLA content. When cows are offered grazed grass the CLA content of

their milk increases, especially when the grass is at an early growth stage. The high linolenic acid content of the grass and its low fibre content probably interacts to increase the production of CLA or its *trans*-18:1 precursors. It may be possible to increase the CLA content of milk further by combining grazing with addition of oils to the diet. Animal variation in the CLA content of milk was high, affecting the CLA content of milk almost as much as the diet offered. The reasons for this variation between individuals are unknown, but may be found in the rumen micro-flora and rumen pH, in the feeding behaviour of the cow, in the efficiency of incorporation of CLA into the milk or in the conversion of vaccenic acid to CLA in the mammary gland.

The processing conditions for milk products that are important for CLA formation are: temperature, presence of O<sub>2</sub>, additives and ageing. However, increases in CLA result mainly from heating the raw ingredients and not from any other step in the processing line. When investigating the effects of processing on CLA concentration care must be taken to remove any variation caused by the CLA content of the raw milk. Although there is little evidence to show that increases in CLA concentration due to processing result in CLA in the form of the *cis*-9,*trans*-11 isomer, there is also little evidence to the contrary.

Increasing the concentration of CLA in food is a possible way for man to increase his CLA intake and obtain the potential benefits of CLA consumption (Chamruspollert & Sell, 1999). Investigation is required both to quantify rumen conditions that promote CLA synthesis and to determine the scope for increasing CLA consumption through dairy products. There is tremendous scope for increasing CLA concentration in milk by feeding to affect rumen production of CLA; however, this is complicated by large between-animal variations. In addition, further investigation is required to enhance CLA supply through increased intake of vaccenic acid.

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