Abstract

Conjugated linoleic acid (CLA) is presently under extensive research because of its potent anticarcinogenic effects and also because of its effects on the immune system and on lipid metabolism. The biological effects of these acids are briefly reviewed. Ruminant edible products are the richest natural sources of CLA because they are supposed to be mainly derived from rumen biohydrogenation of linoleic acid. The end product of the main and more effective linoleic acid biohydrogenation pathway is the trans-11 C18:1. The contents of CLA and trans C18:1 acids are positively correlated in rumen contents, fat depots and milk, although their ratio (trans C18:1 / CLA) varies. Nutritional strategies for the enrichment of ruminant products with CLA will currently be achieved by increasing the supply of linoleic acid in reticulo-rumen metabolism, although this will also result in an increase in trans-11 C18:1. The inclusion in the diet of soybean oil up to 12% does not greatly affect the trans C18:1 / CLA ratio. The potential deleterious effects of trans octadecenoic acids on human health have been considered even if no consensus is reached. Therefore it will be important to manipulate rumen biohydrogenation in order to increase CLA output with a low trans C18:1 / CLA ratio. Although further investigation is needed, scarce data already suggest this could be achieved by increasing the supply of linoleic rich fat and modifying the basal diet or include ionophores. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: CLA; Biohydrogenation; Trans fatty acids; Lipid supplementation; Lamb
provoke alarmist reactions (as for trans fatty acids) or promote consumer interest in specific foods (such as eggs enriched in docosahexaenoic acid). We can, therefore expect increasing interest in foods “naturally” enriched in these conjugated linoleic acid isomers. The objective of this work is to briefly review some of the biological activities attributed to these fatty acids, their production in ruminants and possible strategies to increase them in ruminant milk and meat.

2. Conjugated octadecadienoic acids

Conjugated octadecadienoic acids are a group of geometric and positional isomers of linoleic acid (C18:2 n-6). They are collectively known as conjugated linoleic acid (CLA) and have double bonds which are separated by a single carbon-carbon bond (conjugated unsaturation) instead of the usual methylene interrupted double bond system. CLA isomers include both cis–cis, cis–trans and trans–trans geometry with double bonds at 9 and 11, 10 and 12 or 11 and 13 positions (Fig. 1). The predominant natural isomer in humans and animals is the cis-9, trans-11 octadecadienoic acid (Parodi, 1977; Britton et al., 1992; Chin et al., 1992; Parodi, 1994). We will use the designation “rumenic acid”, recently proposed by Kramer et al. (1998a), as the common name for this acid. Rumenic acid is considered to be the most biologically active CLA isomer because it is the predominant isomer incorporated in membrane phospholipids (Ha et al., 1990). However, extensive incorporation of cis-11, trans-13 isomer in swine hepatic and myocardic phospholipids was observed when a commercial CLA mixture was fed (Kramer et al., 1998b). Other results indicate that the biological activity of CLA is not restricted to

Fig. 1. Graphic representation of linoleic acid and its main conjugated isomers in the reticulo-rumen.
rumenic acid. Contrary to what is observed with mixed conjugated octadienoic acids, Lee et al. (1998) demonstrated that rumenic acid alone does not inhibit the expression of stearoyl-CoA desaturase mRNA.

Like all conjugated dienes, CLA absorbs UV light at 234nm. This allows it to be detected by UV spectrophotometry (Riel, 1963). This property has been extensively used to study the peroxidation of unsaturated fatty acids in biological systems because of the characteristic formation of conjugated diene fatty acid hydroperoxides (Logani and Davies, 1980). Conjugated unsaturation in both types of compounds causes difficulties in interpreting peroxidation data measured by this method (Banni et al., 1998), particularly when CLA content is high.

3. Biological activities of conjugated octadecadienoic acids

The role of CLA in animal metabolism is currently under strong investigation. The already known effects can be very diverse, and are not fully integrated into a comprehensive framework of knowledge. The interest of the scientific community was triggered by the accidental discovery of anticarcinogenic activity in fried beef extracts (Pariza and Hargraves, 1985) in which Ha et al. (1987) later isolated CLA isomers as being responsible for the anticarcinogenic action. Since then, the inhibitory action of CLA on several chemical induced carcinogenesis models was demonstrated, including epidermal (Ha et al., 1987; Belury et al., 1996), mammary (Ip et al., 1991, 1994b; Thompson et al., 1997) and gastrointestinal carcinomas (Ha et al., 1990; Liew et al., 1995). Physiological concentrations of CLA inhibits proliferation of several human cancer cell lines in vitro (Shultz et al., 1992a,b; Schonberg and Krokan, 1995). Anticarcinogenic effects of CLA appear to be dose dependent, from 0.1 to 1% in the diet (Ip et al., 1991, 1994b). When extrapolated to humans, this range of concentrations is only slightly higher than the estimated consumption of CLA in the USA population (Ip et al., 1994a).

Dietary CLA supplementation reduces the plasma concentration of low density lipoproteins and inhibits atherosclerosis onset in rabbits (Lee et al., 1994) and hamsters (Nicolosi et al., 1997) fed atherogenic diets.

Ha et al. (1990) claimed that CLA was effective preventing peroxide formation from unsaturated fatty acids, although nothing in the structure of CLA suggest that it can possess such antioxidant activity. These authors hypothesised that an oxidised derivative of CLA (formed by the introduction of a β-hydroxy acrolein moiety across the conjugated double-bound system) is the active antioxidant species rather than CLA itself. In later revaluations, van den Berg et al. (1995) and Banni et al. (1998) concluded that CLA has only a minor effect preventing linoleic acid oxidation, that was attributed to a competition effect, due to the higher susceptibility of CLA to oxidation. Some works even suggest that CLA can be a pro-oxidant agent (Schonberg and Krokan, 1995; Chen et al., 1997).

CLA participates in the modulation of the immune system, enhancing the mitogen-induced lymphocyte blastogenesis, lymphocyte cytotoxic activity and macrophage killing ability (Michal et al., 1992; Chew et al., 1997; Wong et al., 1997). CLA reduces catabolic effects in skeletal muscles in birds, rats and mice after immune stimulation, without compromising the efficacy of the immune response (Cook et al., 1993; Miller et al., 1994). The proposed mechanism to explain this action involves the reduction of prostaglandin E₂ (PGE₂) synthesis (Cook et al., 1993). The inhibitory effects of CLA on PGE₂ were confirmed in several tissues and experimental conditions (Liu and Belury, 1997; Sugano et al., 1997; Li and Watkins, 1998; Liu and Belury, 1998). The modulation of the eicosanoid synthesis by CLA probably includes the elongation and desaturation of CLA resulting in the synthesis of conjugated eicosatrienoic and eicosatetraenoic metabolites (Banni et al., 1996). These metabolites were found in phospholipids of the ovine hepatocyte (Banni et al., 1996) and in the liver (Sebedio et al., 1997) and mammary gland (Thompson et al., 1997) of rats fed CLA supplemented diets.

Experiments with pigs (Dugan et al., 1997) and mice (Park et al., 1997) fed a CLA supplemented diet, suggest that CLA can act as a fat to lean repartitioning agent. A similar effect was observed by Chin et al. (1994a) in rats. This effect on fat to lean repartition apparently results from the reduced
lipogenesis and increased lipolysis in adipose tissue (Park et al., 1997). Furthermore, West et al. (1998) concluded that in mice fed high-fat and low-fat CLA supplemented diets, CLA reduces body fat by decreasing feed intake, by increasing metabolic rate and by decreasing the night-time respiratory quotient. This lipid catabolic response is in agreement with observations by Belury et al. (1997), suggesting that CLA acts as a typical hepatic peroxisome proliferator. Besides the effects on lipid metabolism, the proposed reduction of PGE synthesis in skeletal muscles induced by CLA (Cook et al., 1993) may result in reduced proteolysis (Rodemann and Goldberg, 1982).

4. The dietary sources of CLA

CLA is present in a great variety of feeds, although usually in residual quantities (Forgerty et al., 1988; Chin et al., 1992; Lin et al., 1995). CLA concentration is higher in ruminant products, particularly rumenic acid (Parodi, 1977 and 1994). The presence of the conjugated double bond system in milk was first described by Booth et al. (1935). Riel (1963) studied and reviewed the presence of total conjugated dienes in milk fat and observed that they can vary between 2 and 28 mg/g, showing a marked seasonal fluctuation related to the feed cycle. However, common milk CLA concentrations are between 3 and 6 mg/g (Kelly et al., 1998a). Consequently, cheese and other dairy products are also excellent sources of CLA (Ha et al., 1989; Shantha et al., 1992; Werner et al., 1992; Lin et al., 1995). In some cheeses, an enrichment of CLA levels is possible during the technological process (Shantha et al., 1992; Werner et al., 1992). Ruminant meat is also an important source of CLA. In the case of mutton, 14.9 mg/g concentrations were found in intramuscular lipids in Australia (Forgerty et al., 1988), 5.6 mg/g in the USA (Chin et al., 1992) and 12.0 mg/g in Germany (Fritsche and Steinhart, 1998). In one of our studies we determined a 5.1 mg/g CLA concentration in the total fatty acids of the Longissimus dorsi muscle in lambs fed with dehydrated lucerne (Bessa et al., 1998).

Several methods such as alkaline isomerisation of vegetable oils (Kramer et al., 1998b) have been developed for the large scale synthesis of CLA which may allow the production of CLA rich meat and eggs through feeding regimes containing these synthesised CLA’s. In monogastrics, the inclusion of CLA in diets is particularly promising because its products have low CLA content and hydrogenation is minimal in the gastrointestinal tract. Kramer et al. (1998b) introduced 1% of a CLA commercial mix into pig diets which resulted in the enrichment of meat lipids with the isomers present in the mix.

5. Rumen biohydrogenation and CLA synthesis

Rumenic acid was initially identified by Kepler et al. (1966) as being an intermediate agent in the biohydrogenation of linoleic acid by the Butyrivibrio fibrisolvens rumen bacteria. The cis-12, trans-11 octadecenoate isomerase which catalyses the transformation of linoleic acid into rumenic acid needs the free COOH radical (Kepler et al., 1970). This implies a prior lipolysis of galactolipids, phospholipids and triglycerides of the diet before isomerisation takes place. The initial isomerisation is followed by the saturation of cis-9 double bond through the reductase characterised by Hughes et al. (1982) resulting in trans-vaccenic acid (trans-11 C18: 1), the major trans isomer of ruminant tissues.

This metabolic pathway (Fig. 2) is the best known and it is thought to be quantitatively the most expressive (Harfoot and Hazlewood, 1988). The diversity of octadecenoic (Table 1) and octadecadienoic isomers in the reticulo-rumen and fat produced by ruminants reflects the high biological diversity of the reticulo-rumen ecosystem. Scarce information is available concerning metabolic pathways and implicated microorganisms for the most part of these isomers, and especially for methylene interrupted octadecadienoic isomers. Butyrivibrio fibrisolvens and most bacteria capable of biohydrogenation are unable to hydrogenate monoenoic acids. In the reticulo-rumen only three bacterial strains which were able to hydrogenate trans-11 C18:1 and cis-9 C18:1, thus producing stearic acid (Harfoot and Hazlewood, 1988). The concentration of trans C18:1 isomers in the rumen is affected by several factors such as the concentration of unsaturated fatty acids (Bessa, unpublished.; Bateman and
that the accumulation of trans octadecenoic acids in the reticulo-rumen ecosystem may also be useful as a response to environmental stress such as low pH, presence of ionophores and high concentrations of fatty acids.

In monogastrics, gut microorganisms also produce CLA (Chin et al., 1994b), although to a lesser extent because of the reduced size and the anatomical position of fermentative compartments.

As shown by Pollard et al. (1980) and Holman and Mahfouz (1981) in mice, rumenic acid may also be synthesised by the desaturation of trans-vaccenic acid (trans-11 C18:1) resulting from the action of hepatic microsomal \( \Delta 9 \) desaturases. This mechanism of CLA formation may also be observed in ruminants, where trans-11 C18:1 availability is greater than in monogastric animals. The abomasal infusion of trans-11 C18:1 and trans-12 C18:1 in dairy cows induced an increase in rumenic acid concentration in the milk as well as that of cis-9, trans-12 C18:2 which was previously non existent (Corl et al., 1998). Bauman’s team at Cornell, Ithaca, is presently studying this hypothesis (Bauman et al., 1998). It is not likely that trans octadecenoic acid availability limits this pathway in ruminants, because the concentration of trans octadecenoic acids in the milk and fat depots is always much higher (3 to 12 times) than CLA. The possibility of humans synthesizing CLA from trans octadecenoic acids in the same way as ruminants (Parodi, 1994; Bauman et al., 1998) may bring new meaning to the role of trans octadecenoic acids, especially that of trans-11 C18:1, which is present mainly in ruminant fat.

Importance was given to trans octadecenoic

Table 1
Proportion of cis and trans octadecenoic isomers in cow milk fat (butter)\(^*\) (adapted from Wolff et al., 1995)

<table>
<thead>
<tr>
<th>trans isomers</th>
<th>% of total trans isomers</th>
<th>cis isomers</th>
<th>% of total cis isomers</th>
</tr>
</thead>
<tbody>
<tr>
<td>trans-8</td>
<td>1.5</td>
<td>cis-8</td>
<td>0.5</td>
</tr>
<tr>
<td>trans-9</td>
<td>13.6</td>
<td>cis-9</td>
<td>94.0</td>
</tr>
<tr>
<td>trans-10</td>
<td>6.4</td>
<td>cis-10</td>
<td>0.2</td>
</tr>
<tr>
<td>trans-11</td>
<td>64.4</td>
<td>cis-11</td>
<td>4.2</td>
</tr>
<tr>
<td>trans-12</td>
<td>2.4</td>
<td>cis-12</td>
<td>0.1</td>
</tr>
<tr>
<td>trans-13</td>
<td>2.3</td>
<td>cis-13</td>
<td>0.5</td>
</tr>
<tr>
<td>trans-14</td>
<td>3.6</td>
<td>cis-14</td>
<td>0.1</td>
</tr>
<tr>
<td>trans-15</td>
<td>2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>trans-16</td>
<td>3.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^*\) There are seasonal variations.
isomers in this paper because they are found in large quantities in ruminant tissues and because positive correlations between CLA and \textit{trans} C18:1 isomer concentrations were observed in milk (Jiang et al., 1996; Newbold et al., 1998), lamb muscle and adipose tissue (Fig. 3) (Bessa et al., 1998). Therefore, the formation of CLA rich products implies a parallel enrichment in \textit{trans} C18:1 isomers.

The \textit{trans} C18:1/CLA ratio may vary widely. In reticulo-ruminal contents and bacteria, Bessa (unpublished findings) found values that ranged from 25 to 32. In growing lambs, we found values of 8.7 in perirenal fat, 7.8 in subcutaneous fat and 3.4 in \textit{Longissimus dorsi} muscle lipids (Bessa et al., 1998). In cow milk, Jiang et al. (1996) determined a value of 4.0 for \textit{trans}-11 C18:1/CLA ratio. These differences may be partially explained by the possible preferential incorporation of CLA into phospholipids, preferential secretion of CLA in milk or perhaps by differences in \textit{Δ9} desaturase activity in several tissues.

The association between CLA and \textit{trans}-oc-tadecenoic acids may limit the interest of increasing their concentration in ruminant products. \textit{Trans}-oc-tadecenoic isomers have been blamed for the decrease of fat in cow milk (Gaynor et al., 1994; Wonsil et al., 1994; Gaynor et al., 1995) which may compromise the interest in increasing CLA concentration in milk. However, Griinari et al. (1998a) separated and identified cow milk \textit{trans}-oc-tadecenoic isomers with precision and observed that the milk fat content is negatively correlated with only one minor isomer (\textit{trans}-10 C18:1). These results were confirmed by Newbold et al. (1998) who showed that \textit{trans}-10 C18:1 concentration is not correlated with that of CLA or \textit{trans}-11 C18:1. Bauman et al. (1998) go even further in suggesting that the negative effects of CLA in milk (and body) fat synthesis could be due to isomers which have a \textit{trans}-10 bond.

\textit{Trans} acids may have negative effects not only on animal productivity but also on human health (Wahle and James, 1993). There is considerable concern and controversy regarding the latter, particularly in plasma lipoprotein concentrations and composition which are related to an increase in cardiovascular diseases (Judd et al., 1994; Precht and Molkentin, 1995; Ackman, 1997; ASCN/AIN Task Force on \textit{trans} Fatty Acids, 1996). Further studies need to be carried out regarding the specific biological effects of each isomer and the confirmation that \textit{trans}-11 C18:1 may be a quantitatively relevant precursor for rumenic acid.

### 6. Manipulation of rumen biohydrogenation as a way to enrich products in CLA

The level of CLA in milk reflects the quantity which is available for intestinal absorption (Loor and Herbein, 1997; Chouinard et al., 1998b). Therefore, feed manipulation which allows high CLA output in the reticulo-rumen is likely to increase CLA availability for deposition in tissues and secretion in milk.

The increase of linoleic acid intake (C18:2 n-6) is one of the feeding strategies for CLA enrichment in ruminant fat since linoleic acid is the main precursor of CLA. The main available sources of linoleic acid in animal feeds are cereal and oilseed grains or oils obtained from these. Kelly et al. (1998a) supplemented dairy cows with peanut oil (rich in \textit{cis}-9 C18:1), sunflower (rich in C18:2 n-6) and linseed (rich in C18:3 n-3), thus obtaining the following CLA concentrations in milk fat: 13.3 mg/g, 24.4 mg/g and 16.7 mg/g, respectively. We also obtained a significant increase for CLA concentration in lamb fat, after having included soybean oil up to 7% of dry matter (DM) in the diets (Fig. 4).
However, the concentration of CLA in milk fat increased clearly when dairy cows were fed with low linoleic acid diets, such as linseed oil calcium soaps (Chouinard et al., 1998a). The studied linolenic acid biohydrogenation pathways do not consider CLA as an intermediary agent but they produce trans-11 C18:1 as one of the final products (Harfoot and Hazlewood, 1988). Due to the extreme microbial diversity in the reticulo-rumen, alternative pathways may exist to linolenic acid biohydrogenation, involving CLA production.

CLA concentration in milk fat also seems to have increased when dairy cows are fed fish oil supplemented diets (Chouinard et al., 1998a). It is not clear if this results of an increase in CLA rumen production or of the action of Δ9 desaturase on trans-11 C18:1. In fact, the supplementation of ruminant diets with fish oils or fish meals leads to accumulation of trans octadecenoic acids in the rumen (Borsting et al., 1992; Scollan et al., 1997) which are reflected in milk fat composition (Wonsil et al., 1994; Mansbridge and Blake, 1997). Fish oils are usually poor in linoleic and linolenic acids and rich in oleic, palmitic and in very long n-3 polyunsaturated fatty acids (PUFA). These PUFA in fish oil and fishmeal have been considered resistant to biohydrogenation (Ashes et al., 1992). However, recently published in vivo studies, refer to the extensive biohydrogenation of these acids (Carro et al., 1997; Choi et al., 1997). In order to explain the great production of trans octadecenoic acid observed, it is necessary to admit to the possible oxidative shortening of carbon chains from these very long acids on a scale never demonstrated in the reticulo-rumen ecosystem or the direct cis–trans isomerization of oleic acid.

CLA levels in the milk of grazing animals are higher than those of animals in confinement (Kelly et al., 1998b; Griinari et al., 1998b). Pasture availability may explain the seasonal variation in CLA levels in milk observed by Riel (1963) in Canada and the high levels in sheep tissues in Australia (Forgerty et al., 1988). Green pasture may contain up to 3% fatty acids on a Dry Matter basis, of which about 90% will be unsaturated C18 acids (Murphy et al., 1995).

The effects of presentation and technological processing of lipid supplements on CLA production in the reticulo-rumen still need to be studied. For example, milk fat from dairy cows fed extruded soybean contains 11 to 12% trans-11 C18:1 versus only 2.5% in cows fed natural soybean (Chouinard et al., 1997). Although these authors did not determine the CLA level, we may suppose that its concentration had the same variation because of the high positive correlation between trans-11 C18:1 and CLA. In another study, Chouinard et al. (1998a) observed increases in CLA concentration in the milk of cows fed diets containing extruded, toasted and micronised soybeans when compared to raw soybeans.

Therefore, work on biohydrogenation seems important in order to increase CLA production and at the same time reduce the trans C18:1/CLA ratio. This may be obtained by controlling the enzymatic process which regulates CLA conversion into trans-11 C18:1. Hughes et al. (1982) isolated and characterized Butirivibrio fibrisolvens cis-9 trans-11 octadecadienoate reductase and concluded it was a cytoplasmatic enzyme requiring iron and had a maximum activity for pH values between 7.2 and 8.2.

The use of ionophore antibiotics in association with lipid supplementation may be one of the ways to decrease the trans C18:1/CLA ratio (Fellner et al., 1997). In a study in vitro, these authors observed that the simultaneous use of ionophores and linoleic acid infusion resulted in a decrease of the ratio of
Fig. 5. The trans C18:1/CLA ratio (A) and CLA concentration in % of total fatty acids (B) in mixed rumen bacteria continuous culture contents. Effect of ionophores with or without linoleic acid infusion (LA) – (Adapted from Fellner et al., 1997).

Table 2
Relation between trans fatty acids and CLA in milk – Interaction between the basal diet and lipid supplement for dairy cows (according to data published by Griinari et al., 1996)

<table>
<thead>
<tr>
<th>Type of diet</th>
<th>Lipid supplement</th>
<th>trans FA (mg/g)</th>
<th>CLA (mg/g)</th>
<th>trans FA/CLA ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>High forage</td>
<td>Saturated</td>
<td>1.4</td>
<td>0.2</td>
<td>7</td>
</tr>
<tr>
<td>High forage</td>
<td>Unsaturated</td>
<td>44.4</td>
<td>9.1</td>
<td>4.9</td>
</tr>
<tr>
<td>Low forage</td>
<td>Saturated</td>
<td>6.0</td>
<td>0.2</td>
<td>30</td>
</tr>
<tr>
<td>Low forage</td>
<td>Unsaturated</td>
<td>52.0</td>
<td>4.9</td>
<td>10</td>
</tr>
</tbody>
</table>

* Corn oil – rich in C18:2 n-6.

trans C18:1/CLA and in an increase in CLA proportion (Fig. 5).

The trans C18:1/CLA ratio may also be decreased by studying interactions between the basal diet and the type of lipid supplement. Although the use of increasing levels of lipids as supplements of the same diet did not change that ratio (Bessa et al., 1998), Griinari et al. (1996) showed that results depend on the type of basal diet being fed (Table 2).

Therefore, besides the traditional objective (i.e. total inhibition of biohydrogenation), new objectives are defined for the manipulation of biohydrogenation in the rumen. Attention is now centred on CLA, although little information is available on the effects of a series of linoleic acid, non-conjugated isomers on metabolism and human and animal health. These isomers positively correlate with CLA in the rumen content (Fellner et al., 1997), in milk fat (Sauer et al., 1998) and in sheep fat (Bessa et al., 1998).

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