Conjugated linoleic acid—snake oil or wonder fat?

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Summary

Conjugated linoleic acid is a mixture of positional and geometric isomers of linoleic acid with conjugated double bonds located at positions 7,9–, 8,10–, 9,11–, 10,12– or 11,13– on the carbon chain. Substantial amounts of CLA are found in products of ruminant origin due to the presence of anaerobic bacteria in the rumen that are responsible for the synthesis of CLA or precursors. Over the past decade there has been increasing interest in this group of fatty acids, particularly because of the potent antioxidative and anticarcinogenic properties attributed to one or more of these isomers. In addition, under some circumstances, dietary conjugated linoleic acid has been shown to decrease fat accretion in rodents. In this review we have compiled some very recent data on the effects of CLA on lipid metabolism in large domestic animals, specifically pigs and dairy cows. There is growing evidence that at least one isomer of CLA is a potent inhibitor of fat accretion and milk fat secretion. While the broad ranging effects of CLA that are discussed may appear reminiscent of some previous much touted ‘snake oils’ it is hoped that this review may persuade the reader to rank CLA more as a ‘wonder fat’.

Introduction

Conjugated linoleic acid (CLA) represents a mixture of positional and geometric isomers of linoleic acid (cis–9, cis–12–octadecadienoic acid) with conjugated double bonds located at positions 7,9–, 8,10, 9,11–, 10,12– or 11,13– on the carbon chain. The double bonds can be in the cis– (c) or trans– (t) configuration depending upon the special arrangement of the hydrogen atoms attached to the carbon atoms of the double bond (Parodi 1997).

Presently, we know of seventeen positional and geometric isomers of CLA (Ha et al. 1989; Sehat et al. 1998; Yurawecz et al. 1998; Werner et al. 1992):

• c/t, c–8,10–, –9,11–, –10,12–; and –11,13–octadecadienoic acid accounts for the major isomers and t/c–7,9–, c,c–8,10–, –10,12–, –9,11–, –11,13–; t/t–8,10–, –10,12–, –9,11–, –11,13–octadecadienoic acid accounts for the minor isomers.

One of the most biologically active and most abundant CLA isomers is c–9, t–11–octadecadienoic acid (Figure 1). For many years the c–9,t–11 was thought to be the only isomer that could be incorporated into the phospholipids of cell membranes and non–phospholipid fractions of muscle and liver (Ha et al. 1990; Ip et al. 1991; Park et al. 1995). Today, with the ability to better separate the isomers of CLA, there are other isomers that are thought to be biologically active.

Larger quantities of CLA are found in animal sources rather than in vegetable sources, particularly in products of ruminant origin such as dairy products and red meats. This is due to the presence of anaerobic bacteria in the rumen that are responsible for the synthesis of CLA or precursors. Kepler et al. (1966) has demonstrated that CLA was the first intermediate in the biohydrogenation of linoleic acid to stearic acid by the rumen bacterium Butyrivibrio fbrisolvens and it was later identified in milk fat (Parodi 1977).

Figure 1 The chemical structure of linoleic acid (c–9, c–12–octadecadienoic acid) and the major conjugated linoleic acid (CLA) isomer (c–9, t–11–octadecadienoic acid) found in animal tissues (Chin et al. 1994a).
The beneficial effects of CLA were first recognised in the mid–1980s by scientists at the University of Wisconsin. Over the past decade extensive research has produced evidence that CLA has potent antioxidative and anticarcinogenic properties (see Belury 1995 for a review). CLA was found to inhibit mouse skin carcinogenesis induced by 7,12–dimethylbenz[a]anthracene (DMBA) (Ha et al. 1987; Pariza and Hargaves 1985), mouse forestomach tumours caused by benzo[a]pyrene (BP), and rat mammary carcinogenesis induced by DMBA (Ha et al. 1990; Ip et al. 1991; 1994). In the latter study, tumour incidence, tumour multiplicity and tumour weight in groups of rats supplemented with 0.5, 1, and 1.5% CLA were reduced by 32, 56 and 60%, respectively (Ip et al. 1991). In addition to its antioxidant and anticarcinogenic properties, CLA has been shown to reduce the catabolic effects of immune stimulation in mice, rats and chickens (Cook et al. 1993; Miller et al. 1994). Furthermore, CLA has been shown to be anti–atherogenic in rabbit and guinea pig models, and anti–diabetic in the Zucker diabetic fatty acid fa/fa rat model (Banni and Martin 1998).

Another biological effect of CLA relates to fat accretion and nutrient partitioning. CLA was found to significantly reduce body fat and increase lean body mass in laboratory animals (Albright et al. 1996; Chin et al. 1994b; Park et al. 1995). In one of the studies undertaken by Park et al. (1997), mice fed a CLA supplemented diet (5% corn oil plus 0.5% CLA) had 57–60% lower body fat and 5–14% increased lean body mass relative to controls. Furthermore, feed efficiency and growth rate were also improved when rats, mice, chicks and rabbits were fed diets supplemented with CLA (Chin et al. 1994b; Cook et al. 1993). For instance the feed:gain ratios of male and female rats were improved by 5 and 7% respectively (Chin et al. 1994b).

Although the effects of CLA on rodent performance and body composition have been known for a few years, the effect of CLA on body composition in larger animals such as pigs has not been studied extensively. This was mainly due to the high cost involved in CLA synthesis. Recently, a proprietary alkali isomerization method has been modified from an established technique (Official Methods of Analysis 1990) to allow the production of commercial quantities of CLA from vegetable oils containing substantial amounts of linoleic acid, such as sunflower oil. This has allowed the assessment of the effects of CLA in large farm animals. The purpose of this paper is to review current knowledge on the effects of CLA in large domestic animals, with particular focus on effects on fat metabolism.

**Effects of CLA in pigs**

Over the last 2 years there has been a number of short communications on the efficacy of CLA in reducing carcass fat content of pigs. In general, these have suggested that backfat depth is reduced (Dugan et al. 1997; Dunshea et al. 1998; Thiel et al. 1998) although some have reported no change (O’Quinn et al. 1998). Recently, scientists at the VIAS have conducted a comprehensive slaughter–balance study to determine the dose response to CLA. Briefly, sixty–six female cross bred (Large White x Landrace) pigs (initial weight 56.6 kg and initial P2 backfat 11.4 mm) were used. To obtain initial body composition, six pigs were slaughtered at 57 kg liveweight. The remaining 60 pigs were randomly allocated to one of 6 dietary treatments: 0, 1.25, 2.5, 5.0, 7.5 and 10.0 g of CLA–55 per kg diet (Natural Lipids Ltd., Hovdebygda, Norway). This commercial source of CLA contains 55% of CLA isomers and was prepared from sunflower oil. Thus, the final CLA concentrations for the six treatments were 0, 0.7, 1.4, 2.75, 4.1 and 5.5 g/kg of diet. At the completion of the study, pigs were slaughtered and the chemical composition of the carcass was determined.

Effects of dietary CLA on growth performance are given in Table 1. Dietary CLA had no significant effect on average daily gain or feed intake throughout the study. However, feed:gain was improved by dietary CLA with the responses being most pronounced over the first 4 weeks of the treatment. Over the entire 8 week period, CLA improved feed:gain by 0.2 kg/kg (–6.5%). The improvements in feed:gain were a combination of the small but non–significant changes in both growth rate and feed intake. Other workers have observed similar small improvements in feed:gain in pigs supplemented with dietary CLA (Dugan et al. 1997; Cook et al. 1998a; Thiel et al. 1998), although some have observed no change (O’Quinn et al. 1998).

Earlier studies had observed that dietary CLA supplementation of growing pigs generally resulted in less fat at slaughter as estimated by dissection of

<table>
<thead>
<tr>
<th>Table 1 Effect of dietary CLA on growth performance of finisher pigs (Ostrowska et al. 1998).</th>
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<tbody>
<tr>
<td><strong>Dose of CLA (g/kg)</strong></td>
</tr>
<tr>
<td>Daily gain (kg/d)</td>
</tr>
<tr>
<td>Feed intake (kg/d)</td>
</tr>
<tr>
<td>Feed:Gain</td>
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</table>

1L = linear effect of CLA; Q = quadratic effect of CLA; CC = CLA vs. control
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wholesale loin cuts (Dugan et al. 1997) or back fat thickness (Cook et al. 1998a; Thiel et al. 1998). However, O’Quinn et al. (1998) found that there was little effect of CLA (5g/kg CLA–60) on backfat thickness. In the present investigation we also obtained serial measures of back fat thickness by ultrasound throughout the study. Increases in back fat occurred during the 8 week treatment period in all groups, but the magnitude of the increase declined in a linear manner with increasing dietary level of CLA supplement (Figure 2). Although the numerical differences progressively increased during the 8 week treatment period, they were not significant until week 3 of treatment. At the highest level of CLA supplementation, P<sub>2</sub> back fat depth was reduced by 25% (6 mm) at week 8.

While backfat measures are indicative of whole body fat content, anecdotal evidence from the commercial sector is that this is not always the case. One of the decided advantages of the slaughter–balance studies is that it allows the determination of actual body composition and rates of tissue accretion. These data are presented in Table 2. Carcass water accretion increased with increasing dietary CLA reaching a maximum at a dietary CLA supplement of 5.0 g/kg of diet before decreasing, as indicated by the quadratic response. The carcass protein accretion response to dietary CLA was also quadratic in nature with protein accretion maximised at a dietary CLA supplement of 5.0 g/kg. Carcass fat accretion decreased linearly with increasing CLA supplement rates. At the highest level of CLA there was a 30% reduction (~86 g/d) in carcass fat accretion.

The improvements in body composition observed with CLA feeding were not accompanied by any detectable effects on meat quality (Table 3). Dietary CLA had no effect upon colour (L value), ultimate pH, drip and cooking loss and tenderness (shear force). O’Quinn et al. (1998) also reported that there were no effects of CLA on colour, marbling, drip loss or firmness.

At present there are very few data available on the effect of CLA on immune function in the pig. In one abstract, Cook et al. (1998b) reported that pigs fed dietary CLA (9.5 g/kg CLA–60) had elevated total white cell numbers, principally due to increased lymphocyte numbers. In a study conducted with Aleeta Knowles from the University of Sydney we fed 30 pigs various levels of CLA (0, 1.25, 2.5, 5.0, 7.5 and 10.0 g/CLA–55 per kg diet) for 8 weeks and obtained blood samples at slaughter for assessment of haematological and immune status. Unlike in the study of Cook et al. (1998b), dietary CLA had no effect upon total white cells, neutrophils, lymphocytes or monocytes (Table 4). However there was a significant decrease in eosinophils and IgG with increasing level of CLA in the diet, although at the highest level IgG did increase again. While not remarkable, the effects of CLA on immune function do open up some interesting avenues for future research. Just what the very marked reductions in eosinophil cell count and serum IgG mean in light of relatively little effect on other measures is unclear. For example, does a low level of IgG mean that the CLA treated pigs cannot produce IgG, or are they less challenged by the environment? It is well established that stimulation of the immune system can result in a decrease in growth (Klasing and Korver 1997), and it is intriguing to note that growth performance was maximised and IgG minimised at intermediate doses of CLA in our studies. These are obvious areas for further investigation.

**Mechanism of action of CLA on fat accretion**

The mechanism whereby CLA causes a reduced body fat accretion is not known. Effects could involve de novo lipogenesis, use of preformed fatty acids for lipid synthesis, rates of lipolysis, or some combination of these. In the studies outlined earlier the pigs were fed a high carbohydrate diet so de novo lipogenesis would

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Table 2  Effect of dietary CLA on carcass tissue accretion rates of finisher pigs (Ostrowska et al. 1999a).

<table>
<thead>
<tr>
<th>Dose of CLA (g/kg)</th>
<th>Significance&lt;sup&gt;1&lt;/sup&gt;</th>
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<tbody>
<tr>
<td></td>
<td>L</td>
</tr>
<tr>
<td>Water (g/d)</td>
<td>312</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>106</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>278</td>
</tr>
</tbody>
</table>

<sup>1</sup>L = linear effect of CLA; Q = quadratic effect of CLA; CC = CLA vs. control
represent the major mechanism of lipid synthesis, and it would appear that CLA was acting to reduce these rates. West et al. (1998) reported that the carcass fat content at the end of their study was less in CLA–treated mice fed either a high carbohydrate or a high fat diet. Thus, rates of de novo synthesis and use of preformed fatty acids might both be reduced by CLA. However, it is also possible that CLA could have effects on lipolysis where increased rates of fat mobilisation may be sufficiently increased to result in a net reduction in lipid accretion.

In order to investigate the interactions between dietary energy source and CLA we conducted a study with catheterised pigs. Twenty gilts (initial weight 65 kg) were fed either a low fat diet (25 g/kg) or a high fat (100 g/kg) diet with either 0 or 10 g/kg of CLA–55 for eight days; blood samples were taken frequently. The added fat in the high fat diet was provided as palm oil, thereby ensuring that saturated fatty acids were the predominant preformed fatty acids. While the diets had different energy contents the protein and lysine to DE ratios were similar. Pigs were offered approximately 90% of ad libitum DE intake and feed was delivered every 3 h to ensure a relatively steady state for measuring plasma metabolites. Prior to the study the pigs were fed an intermediate diet. In addition, plasma metabolite responses to insulin and epinephrine were measured on day 8.

While growth performance data are notoriously unreliable over short periods there were no significant effects of CLA on average daily gain (P = 0.77) or feed:gain ratio (P = 0.87). However, feed intake tended to be reduced by dietary CLA (P = 0.11). Others have seen a reduction in feed intake with CLA feeding, in particular in the early stages of feeding relatively high levels of CLA (Cook et al. 1998a). The major source of circulating NEFA is from hydrolysis of either circulating or adipose tissue triglycerides (Pethick and Dunshea 1995). The higher level of NEFA in the pigs fed the high fat diet (Table 5) probably reflects a higher rate of hydrolysis of circulating triglycerides of dietary origin in these pigs. The small but significant increase in plasma NEFA in the CLA fed pigs is possibly due to increased adipose tissue lipolysis. If increased fat mobilization is the source of increased plasma NEFA in CLA fed pigs it would only be a very small component (ca. 6 g/d, Dunshea et al. 1992a,b) of the reduction in fat accretion in pigs fed this level of CLA (86 g/d; Table 2). Evidence of an increase in adipose tissue fat mobilization during CLA feeding is provided by an increase in lipolysis in plasma NEFA after an intravenous epinephrine injection on day 8 of the study (Ostrowska et al. 1999b). Since the plasma NEFA response to epinephrine is an indicator of β-adrenergic stimulated lipolysis (Sechen et al. 1991; Dunshea and King 1995) these data suggest that this response is heightened during CLA feeding.

Table 3 Effect of dietary CLA on loin meat quality of finisher pigs (Ostrowska et al. unpublished data).

<table>
<thead>
<tr>
<th>Dose of CLA (g/kg)</th>
<th>Significance(^1)</th>
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<tr>
<td></td>
<td>L</td>
</tr>
<tr>
<td>L Value</td>
<td>50.4</td>
</tr>
<tr>
<td>Ultimate pH</td>
<td>5.45</td>
</tr>
<tr>
<td>48 h drip loss (%)</td>
<td>7.14</td>
</tr>
<tr>
<td>Cooking loss (%)</td>
<td>35.2</td>
</tr>
<tr>
<td>Shear force (kg)</td>
<td>5.38</td>
</tr>
</tbody>
</table>

\(^1\)L = linear effect of CLA; Q = quadratic effect of CLA; CC = CLA vs. control

Table 4 Effect of dietary CLA on blood haematology and IgG of finisher pigs (Ostrowska et al. unpublished data).

<table>
<thead>
<tr>
<th>Dose of CLA (g/kg)</th>
<th>Significance(^1)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>L</td>
</tr>
<tr>
<td>White cells (x10^6/mL)</td>
<td>19.50</td>
</tr>
<tr>
<td>Neutrophils(x10^5/mL)</td>
<td>4.44</td>
</tr>
<tr>
<td>Lymphocytes (x10^6/mL)</td>
<td>12.90</td>
</tr>
<tr>
<td>Monocytes (x10^3/mL)</td>
<td>1.10</td>
</tr>
<tr>
<td>Basophils (x10^6/mL)</td>
<td>0.017</td>
</tr>
<tr>
<td>Eosinophils (x10^5/mL)</td>
<td>1.09</td>
</tr>
<tr>
<td>IgG (ng/mL)</td>
<td>189.00</td>
</tr>
</tbody>
</table>

\(^1\)L = linear effect of CLA; Q = quadratic effect of CLA; CC = CLA vs. control
Alternatively, the increase in NEFA in CLA–fed pigs could be due to a reduced uptake of NEFA resulting from hydrolysis of circulating triglycerides. A reduced uptake of pre–formed fatty acids is clearly indicated by the significant increase in circulating triglycerides (23.0 vs. 25.2 mg/dL, P = 0.008) in pigs fed diets containing CLA, despite the reduced feed intake. Also, there was a substantial but non–significant increase (+24%) in serum triglycerides in pigs fed CLA (O’Quinn et al. 1998). Therefore it appears likely that the effect of CLA on fat accretion is via decreased adipose tissue triglyceride synthesis either from preformed fatty acids or from de novo synthesis. The primary substrate for de novo lipid synthesis is glucose, the uptake of which is dependent upon insulin. However, dietary CLA had little effect upon either plasma glucose or insulin. The ratio of insulin:glucose tended to be increased (P = 0.13) with CLA, particularly in pigs fed the low fat diet, and this may be indicative of slight insulin resistance. However, the glucose response to an insulin injection was not affected by dietary CLA level (Ostrowska et al. 1999b) suggesting little change in whole body insulin sensitivity or responsiveness.

Effects of CLA in the dairy cow

As mentioned previously, CLA is found in ruminant adipose tissue and milk fat and is the result of incomplete biohydrogenation of linoleic acid in the rumen. Given the intense interest in the anticarcinogenic and other putative health benefits of CLA there has been considerable effort to manipulate the level of CLA in milk fat. In particular, researchers at Cornell University have identified a number of dietary factors, such as the level of forage and oil in the diet, that can greatly influence the level of CLA in milk fat (see Kelly and Bauman 1996; Bauman et al. 1998 for reviews). For instance, the higher the level of pasture or fodder compared to concentrates in the diet, the higher the level of CLA. Also, the addition of plant or fish oils high in unsaturated fatty acids, particularly linoleic acid, increases the level of CLA in the milk. For example, Griinari et al. (1998) found that increasing the amount of forage from 20 to 50% of the diet combined with substituting saturated fat with unsaturated fat in the diet greatly increased the level of CLA in milk fat (Figure 3). Also, Kelly et al. (1998) reported that while replacing a total mixed ration with pasture resulted in a 33% reduction in milk yield of lactating dairy cows, the level of CLA in milk was increased by 115%. An important observation was that although for a particular diet the CLA level in the milk fat of a given cow was consistent there was considerable variation between animals. While some, if not most, of the CLA appearing in milk is the result of direct transfer of CLA absorbed from the gut there is now evidence that the cow herself can synthesize CLA from t–11 linoleic acid through delta–9 desaturase (Corl et al. 1998). It is possible that some of the variation in CLA content of milk seen between animals fed a similar diet may be due to differences in the cows ability to produce CLA from t–11 linoleic acid. It is likely that there will be further interest in these areas since any management option that results in an increase in the CLA content of milk may improve the nutritional profile of dairy products. Given the positive effect of forage and pasture on the CLA content of milk, the predominantly pasture based Australian dairy industry may be in a very good position to manipulate the CLA content of dairy products.

![Figure 3](image-url) Effect of dietary fat (saturated vs unsaturated) and dietary fodder content (20 or 50%) on the amount of c/t– 9,11 CLA in milk fat of dairy cows (Griinari et al. 1998).

Table 5  Effect of dietary fat and CLA on feed and DE intake and plasma constituents in finisher pigs. Data are mean of 8 days of feeding (Ostrowska et al. unpublished).

<table>
<thead>
<tr>
<th>Fat (g/kg)</th>
<th>25</th>
<th>100</th>
<th>Significance¹</th>
</tr>
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<tbody>
<tr>
<td>CLA–55 (g/kg)</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>DE intake (MJ/d)</td>
<td>37.5</td>
<td>35.4</td>
<td>35.4</td>
</tr>
<tr>
<td>Plasma NEFA (µmol/L)</td>
<td>78.8</td>
<td>88.0</td>
<td>123.0</td>
</tr>
<tr>
<td>Plasma glucose (mmol/L)</td>
<td>5.45</td>
<td>5.41</td>
<td>5.61</td>
</tr>
<tr>
<td>Plasma triglycerides (mg/dL)</td>
<td>21.1</td>
<td>23.7</td>
<td>24.8</td>
</tr>
<tr>
<td>Plasma insulin (mU/L)</td>
<td>15.9</td>
<td>18.0</td>
<td>12.0</td>
</tr>
</tbody>
</table>

¹ F = effect dietary fat; C = effect of dietary CLA
In a study to investigate the effects of CLA on mammary gland lipid metabolism in the dairy cow Chouinard et al. (1999) infused CLA into the abomasum of lactating dairy cows for 4 d. CLA was infused into the abomasum to ensure that CLA was not subject to biohydrogenation within the rumen. Infusion of CLA maximally inhibited milk fat content by 50% without affecting milk yield at the lowest dose investigated (50 g CLA–60/d). The CLA c/t– 8,10, c/t– 9,11 and c/t–11,13 CLA isomers were transferred to milk fat with an efficiency of about 24% whereas the transfer of the c/t– 10,12 isomer was only 10%. The differential in transfer efficiencies led these authors to hypothesise that the c/t– 10,12 CLA isomer was responsible for the reduction in milk fat secretion. Similarly, Loor and Herbein (1998) infused 200 g of linoleic acid, or a mixture of CLA and linoleic acid, into the abomasum of lactating dairy cows for 24 h. Compared to the infusion of linoleic acid alone the infusion of the CLA and linoleic acid mixture decreased milk fat content by approximately 34%. Based on the observed large reductions in medium chain fatty acids, both these groups of authors concluded that de novo fatty acid synthesis was inhibited by CLA. However, the increase in the proportion of long chain saturated fatty acids relative to their respective monounsaturates (e.g. stearic vs. oleic acid) suggests that CLA may also inhibit desaturase activity (Chouinard et al. 1999). While it is difficult from these data to determine whether there is an effect of CLA on the uptake of preformed fatty acids, the relative low transfer efficiencies for the CLA isomers would suggest that this process is altered by CLA. Alternatively, since these cows were in late lactation the low transfer efficiencies may be because absorbed fatty acids were being preferentially deposited in adipose tissue.

Conclusion

Conjugated linoleic acid represents a complex of various isomers of linoleic acid that may have various effects on many aspects of metabolism. Some of the anticarcinogenic and antioxidative properties of CLA appear to be due to the c/t– 9,11 isomer. In addition to these effects, there is now growing evidence that some CLA isomer(s), quite likely the c/t– 10,12 isomer, can decrease fat deposition and milk fat secretion. Dietary CLA manufactured from vegetable oil may offer the means to manipulate body composition in the pig and possibly other species. Manipulation of the diet of dairy cattle may be a means of increasing the CLA content of the milk of dairy cows to further increase the nutritive value of dairy products.

Acknowledgements

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References


