Protection of conjugated linoleic acids from ruminal hydrogenation and their incorporation into milk fat


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Abstract

In vitro incubations were used to assess the hydrogenation of conjugated linoleic acid (CLA) isomers 9-cis 11-trans and 10-trans 12-cis present in synthetically produced CLA-60. About 80–90% of the unprotected CLA was hydrogenated when incubated at 38°C for 24 h anaerobically with sheep rumen fluid, the main end product of hydrogenation being trans-octadecenoic acid (C_{18:1}). Encapsulation of the CLA in a matrix of protein provided a protection of about 70% with a 30% hydrogenation of the CLA isomers, resulting in no significant change in the trans-C_{18:1} but an increase in the level of stearic acid (C_{18:0}).

Feeding sheep with unprotected CLA or protected CLA increased the proportion of isomers 9-cis 11-trans and 10-trans 12-cis in abomasal digesta. The concentration of the CLA isomers leaving the abomasum and available for absorption at the small intestine was about 3.5–4% higher for the protected CLA, confirming protection imparted by encapsulation.

Feeding lactating goats with protected CLA increased the proportion of isomers 9-cis 11-trans and 10-trans 12-cis in milk fat. The total CLA levels were enhanced by about 10-fold above the control levels present in milk fat with an efficiency of transfer into milk fat of 36–41% and 21–30%, respectively, for the two isomers.

Keywords: Rumen hydrogenation; Conjugated linoleic acid; Protected fat supplements; Milk fat

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1. Introduction

Conjugated linoleic acids (CLA) have recently been recognised as a nutrient that exerts important physiological effects in animal studies, including anti-carcinogenic, anti-atherogenic, immunomodulating growth promotant, lean body mass promotant and anti-diabetic (Pariza, 1997; Houseknecht et al., 1998).

In the diet of many consumers meat and dairy products would be a significant source of CLA (Dhiman et al., 1999a); these foods contain a mixture of positional and geometric isomers (about seventeen isomers have been identified) but the 9-cis 11-trans-octadecaenoic acid is the principal form present (Sehat et al., 1998). This CLA is an intermediary product in the ruminal bio-hydrogenation of dietary C_{18:3} (linolenic) and C_{18:2} (linoleic) acids present in pasture lipids (Kepler et al., 1966), but there is also a suggestion that the 9-cis 11-trans can be synthesised de novo from 11-trans, which is also an intermediary in the sequence of the bio-hydrogenation of these fatty acids (Chouinard et al., 1999).

The potential health benefits of CLA to consumers have intensified the research effort to enhance the levels of these fatty acids in meat and dairy products. Various attempts have been made to increase the CLA levels in ruminant products by grazing pasture, feeding with large quantities of unsaturated fats (Kelly et al., 1998) and the latter together with manipulating the ratio of forage to concentrate in the diet (Griinari et al., 1998). There was a large variability in the response with cows at the same stage of lactation, fed on similar diets and under similar management conditions (Kelly et al., 1998). In a study by Dhiman et al. (1999b) the level of CLA in cow’s milk was enhanced from 0.3% to 0.6% by feeding with extruded soybean at a level of 12% of dry matter in the diet. While, Chouinard et al. (1999) infused increasing doses of a commercially available mixture of CLA into the abomasum of dairy cows which significantly increased the CLA content of milk fat to maximal levels of the two major isomers 9-cis 11-trans (1.9%) and 10-trans 12-cis (1.1%) at the highest infusion rate of 150 g/day.

In this paper we examine a more practical approach of feeding ruminants on protected CLA supplements to deliver CLA post abomasum at the small intestine. In addition the bio-availability and transfer of CLA isomers into milk fat has been assessed.

2. Materials and methods

2.1. Fat supplements

CLA-60 was provided by Bioriginal Food & Science Corporation; 102 Melville Street Saskatoon, Saskatchewan, CANADA S7J 0R. The fatty acid composition of unprotected CLA and protected CLA supplements used in these studies are presented in Table 1. The CLA supplements were prepared by homogenisation of a mixture containing equal parts of CLA and casein and the subsequent treatment of the homogenate with formaldehyde using procedures described by Ashes et al. (1979) and Gulati et al. (1999). (1) Unprotected (UP) and protected (PR) CLA-casein (1:1, w/w) freeze dried preparations were used in the initial in vitro studies; (2) Unprotected (UP) and protected (PR) CLA-
casein (1:1, w/w) fluid bed dried preparations were used in in vitro assessment and animal studies where larger quantities of material were required.

2.2. In vitro rumen incubations

2.2.1. Animals and diets

Three Merino sheep with rumen cannulae were used as donors and were fed once daily with 800 g of a basal ration of pelleted mature lucerne hay:oat grain (60:40; w/w).

2.2.2. Measuring the hydrogenation of CLA supplements

Rumen fluid (100 ml) was collected at 0800 h (prior to feeding) from three sheep and pooled in a pre-warmed thermos flask, strained through a muslin cloth, gassed with nitrogen and kept at 39°C. Incubations were initiated within 15 min of collection of rumen fluid. Fat supplements (100 mg) equivalent to 50 mg of oil were incubated with 10 ml of rumen fluid anaerobically at 39°C for 24 h. Six replicate incubations were set up. All the fatty acids were measured by gas liquid chromatography procedures described by Gulati et al. (1999). The methyl esters of CLA isomers and the C\textsubscript{18:1}-\textit{cis} and -\textit{trans} isomers were separated by gas-chromatography (Perkin Elmer, auto sampler) on a BPX70 column (50 m, bonded fused silica phase) with a spit ratio of 20:1, using helium as a carrier gas. The Column was temperature programmed from 150–210°C at 2°C/min. Hydrogenation of the unsaturated lipids was calculated using the formula: protection (%) = 18 : 2 after incubation/18:2 before incubation × 100.

2.3. To study the fate of fatty acids post-rumen

2.3.1. Animals and diets

Three Merino sheep (body weight of 40 kg) with cannulae in the abomasum were fed daily at 8:00 a.m. with 800 g of the pelleted basal ration (see Section 2.2 for details). During the first treatment period, the sheep were given the same quantity of the basal ration sprayed with 40 g of unprotected CLA (CLA-60) for 3 days. This feeding period

<table>
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<th></th>
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<th>C\textsubscript{18:0}</th>
<th>C\textsubscript{18:1}</th>
<th>C\textsubscript{18:2}</th>
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<tr>
<td></td>
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<td>11-trans</td>
<td>10-trans</td>
<td>12-cis</td>
<td>Other isomers</td>
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<td>4.49</td>
<td>18.33</td>
<td>6.13</td>
<td>21.31</td>
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</tbody>
</table>

\textsuperscript{a} See Section 2 for details of diets.
\textsuperscript{b} Freeze dried.
\textsuperscript{c} Fluid bed dried.
was sufficient to induce major changes in the proportion of fatty acids that are present in low concentrations Gulati et al. (1999). This was followed by a seven day control period to prevent any carry-over (lag) effects, during which time the sheep were fed on the basal diet. The seven day period was sufficient to bring the values to the control level and these were monitored prior to a second treatment period being imposed in which sheep were given 800 g of the basal ration with 80 g of a protected CLA-casein (1:1, w/w) to provide 40 g CLA-60/day for 3 days.

2.4. Measuring the fatty acid composition of abomasal digesta

Abomasal digesta (20 ml) were collected before CLA supplementation and at various times after feeding at (0, 50, 54, 61, 85, 116, 164, and 260 h after CLA supplementation commenced). Fresh abomasal digesta samples were analysed for fatty acid composition by the procedures of Gulati et al. (1999). Differences between means were calculated using a standard t-test procedure with a pooled estimate of variance.

2.5. For milk trials

2.5.1. Animals and diets

Two Saanen goats (approximately 60 kg body weight) in the first 3 months of lactation and producing about 1.5–3 l of milk per day were given a total of 2400 g/day of a basal ration of pelleted lucerne hay:oat grain (60:40, w/w) supplemented with minerals, divided evenly between AM and PM feeds. During the treatment period, the goats were given the basal ration + 80 g of a protected CLA-casein (1:1, w/w) per day for 4 days which allowed sufficient time to induce a change in the fatty acid profiles of ruminant milk (Gulati et al., 1997).

2.6. Collection of milk samples and fatty acid analysis

Goats were machine milked once daily in the morning before feeding; milk volumes were recorded and 50 ml milk samples were collected and kept at 4°C for analysis. The fatty acid composition of milk fat was determined by gas–liquid chromatography and the differences between means were calculated using procedures set out in Section 2.3. Fatty acid composition of neutral lipid and phospholipid fractions of milk fat were determined by the procedures of Scott et al. (1993)

3. Results and discussion

The unprotected and protected CLA contained about 21% of the 9-cis 11-trans isomer and 30% of the 10-trans 12-cis isomer of the fatty acids and there was no significant change in composition during the preparation of the unprotected or protected supplements (Table 1).

When unprotected CLA or the protected CLA supplements were incubated with rumen fluid, the C_{18:2} and the CLA isomers 9-cis 11-trans, 10-trans 12-cis present in
unprotected CLA were extensively hydrogenated to the C18:1-trans fatty acid (Fig. 1). The accumulation of the trans-intermediates is probably due to an excess of free fatty acids which inhibit the final hydrogenation of C18:1-trans isomers to stearic acid. In contrast the protected CLA supplement was hydrogenated to a lesser extent with only a small increase in the C18:1-trans fatty acid but an increase in stearic acid C18:0 (Fig. 2). This demonstrates protection of the CLA isomers and the C18:2, the results being similar to that reported for the protection of C18:1, C18:2 and C18:3 in canola/soybean supplements by Gulati et al. (1997). Fig. 3 shows a comparison of the in vitro protection of the CLA supplements. In the unprotected CLA, C18:2 and the CLA isomers 9-cis 11-trans and 10-trans 12-cis were 70–90% hydrogenated (i.e. 10–30% protected) while in the protected CLA supplement the hydrogenation is much lower at about 30%, thus imparting an in vitro ruminal protection of 70%.

Fig. 4a and b shows the levels of CLA isomers 9-cis 11-trans and 10-trans 12-cis fatty acids appearing at the time intervals post-feeding in abomasal digesta from three sheep fed on a pelleted basal diet either sprayed with 40 g CLA-60 (unprotected) or mixed with 80 g protected CLA-casein (1:1, w/w), to supply an equivalent level of 40 g CLA in the (protected) supplement. Following 3 days of supplementation, the concentrations of CLA isomers 9-cis 11-trans and 10-trans 12-cis at the abomasum reached a peak at 61 h (approximately 7 h after the final feeding period) and eventually declined over time when the CLA diet was replaced with the basal diet (Fig. 4a and b). The level of CLA isomers appearing at the abomasum in sheep on the protected CLA supplement was 3.5–4%
Fig. 2. In vitro hydrogenation of protected CLA isomers and C₁₈ unsaturated fatty acids by rumen microorganisms. Supplements (100 mg) of protected CLA-casein (1:1, w/w) were anaerobically incubated with 10 ml of strained sheep rumen fluid at 38°C for 24 h. Mean and error bars for six replicates. (■) 0 h unincubated (□) 24 h incubated.

Fig. 3. A comparison of the in vitro ruminal protection of C₁₈:2 and CLA isomers 9-cis 11-trans and 10-trans 12-cis. (■) Protected CLA supplement (□) unprotected CLA supplements.
The fatty acid composition of milk from goats fed on rumen protected CLA supplements (40 g/day of protected CLA-60) is shown in Fig. 5a and b. The levels of the two major CLA isomers were 2.8 and 2.4% for the 9-cis 11-trans and 10-trans 12-cis fatty acids ($n = 2$). This was significantly higher ($P < 0.001$) than values reported by Dhiman et al. (1999b) and Chouinard et al. (1999), who gave extruded oilseeds to cows and increased the CLA concentrations from 0.3 to 0.6%, while infusing (50 g/day) of CLA-60 into the abomasum increased the 10-trans 12-cis and 9-cis 11-trans isomer to 0.5 and 1%. In addition the fatty acids derived by mammary synthesis $C_{10–C_{14}}$...
(lauric-myristic) were depressed by CLA supplementation (Fig. 5a; Table 2) and this is consistent with the decrease in milk fat levels observed previously by Chouinard et al. (1999). The fatty acid composition of neutral lipids (NL) and phospholipid (PL) fractions prepared from milk fat showed that the percent distribution of the two CLA isomers 10-trans 12-cis and 9-cis 11-trans was similar for the two fractions (Table 3). These data also show that the ratio of C18:0:C18:1 changed from 0.58 to 1.18% in NL and 0.51 to 1.02% in the PL and this is an indication of inhibition of the \( \Delta 9 \) desaturase enzyme by CLA and confirms the previous observations of Chouinard et al. (1999). The protected CLA isomers 9-cis 11-trans and 10-trans 12-cis consumed were transferred into milk fat with an efficiency of 36–41% and 21–30% in goats (Table 3). This was higher than the levels achieved by Chouinard et al. (1999), for these two isomers (22 and 10%) by infusing CLA-60 into the abomasum of dairy cows. The effect of feeding protected CLA on milk fat, protein and production need further long term feeding trials to be undertaken.

These studies demonstrate that the bio-hydrogenation of CLA fatty acids by rumen micro-organisms can be prevented by encapsulating the CLA in a matrix of protected protein. Further studies are required to improve protection procedures and to set the optimal threshold levels of CLA isomers required in ruminant products, as it has been demonstrated that the potency of CLA fatty acids is 100-fold higher than fish oil fatty acids to elicit responses in disease-prevention in animal models (Ip et al., 1994).
### Table 2
Fatty acid composition (% w/w) of neutral lipid and phospholipid fraction of milk from goats fed on diets of protected CLA

<table>
<thead>
<tr>
<th></th>
<th>C₁₀:₀</th>
<th>C₁₂:₀</th>
<th>C₁₄:₀</th>
<th>C₁₆:₀</th>
<th>C₁₈:₀</th>
<th>C₁₈:₁&lt;sup&gt;t&lt;/sup&gt;</th>
<th>C₁₈:₁&lt;sup&gt;c&lt;/sup&gt;</th>
<th>C₁₈:₂</th>
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<td></td>
<td></td>
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<td>9-cis 11-trans</td>
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<td>7.55</td>
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<td>23.59</td>
<td>14.71</td>
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<td>2.16</td>
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<td>0.237</td>
<td>0.418</td>
<td>0.171</td>
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<td>0.84</td>
<td>0.017</td>
<td>0.017</td>
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<tr>
<td>CLA Mean</td>
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<td>2.42</td>
<td>6.74</td>
<td>22.11</td>
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<td>2.58</td>
<td>19.99</td>
<td>2.73</td>
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<td>0.201</td>
<td>0.114</td>
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<td>0.922</td>
<td>0.141</td>
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<tr>
<td>CONTROL Mean</td>
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<td>2.67</td>
<td>6.74</td>
<td>21.93</td>
<td>15.10</td>
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<td>0.041</td>
<td>0.131</td>
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</tr>
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<td>1.74</td>
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<td>21.91</td>
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<sup>a</sup> Neutral lipid and phospholipid fraction were prepared using procedures of Scott et al. (1993).

<sup>b</sup> CLA isomers: 9-cis 11-trans and 10-trans 12-cis.
Acknowledgements

We wish to thank Mr. G. Paget for skilled technical assistance and Bioriginal Food & Science Corporation, Canada for providing the CLA-60 used in these studies.

References


Table 3

Efficiency of transfer of ruminal protected CLA fatty acids into milk a

<table>
<thead>
<tr>
<th>CLA isomers consumed (g/day)</th>
<th>Milk fat (g/day)</th>
<th>CLA fatty acid isomers in milk fat (% w/w)</th>
<th>Secretion into milk (g/day)</th>
<th>Transfer into milk of CLA isomers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat</td>
<td>1</td>
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<td>10.91</td>
<td>126</td>
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<tr>
<td></td>
<td>2</td>
<td>7.39</td>
<td>10.91</td>
<td>93.84</td>
</tr>
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</table>

a For details of diets see Section 2 materials and methods 80 g CLA supplement is a mixture of CLA-casein (1:1, w/w) providing 40 g of CLA-60 in the diet.