Elevation of Conjugated cis-9, trans-11-Octadecadienoic Acid in Bovine Milk Because of Dietary Supplementation

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ABSTRACT

Cows on pasture were fed full fat soybeans (toasted, flaked, and pelleted) or ground full fat rapeseeds to investigate effects on cis-9, trans-11-octadecadienoic acid in milk. Three herds of 16 cows each that were on pasture were fed 3.1 kg/d of unmolassed beet pulp (control), 3.0 kg/d of rapeseed concentrate, or 3.1 kg/d of a soybean supplement. The concentration of cis-9, trans-11-octadecadienoic acid in the milk of cows fed the rapeseed and soybean supplements was significantly higher than in the milk of cows fed the control diet during the feeding trial. Over the trial, the cis-9, trans-11-octadecadienoic acid concentration in the milk of individual cows varied from 6.8 to 25.7 mg/g of fat in the control herd, from 10.6 to 33.5 mg/g of fat in the herd fed the rapeseed concentrate, and from 8.8 to 30.5 mg/g of fat in the herd fed the soybean supplement. The concentration of cis-9, cis-12-octadecadienoic acid, the substrate for cis-9, trans-11-octadecadienoic acid synthesis in the rumen, was 4.9 g/100 g of fatty acid methyl esters in the milk fat of cows fed the soybean supplement, 2.5 g/100 g of fatty acid methyl esters in the milk fat of cows fed the rapeseed concentrate, and 2.3 g/100 g of fatty acid methyl esters in the milk fat of the control cows. Milk yield and milk constituent yields were not affected by supplementation of either full fat soybeans or rapeseeds compared with controls, but milk protein concentration was significantly reduced by both oilseed supplements.

(Key words: conjugated linoleic acid, milk, rapeseed, soybean)

Abbreviation key: CLA = conjugated linoleic acid, FAME = fatty acid methyl esters, FFR = full fat rapeseed, FFS = full fat soybean.

INTRODUCTION

Conjugated linoleic acid (CLA) has gained considerable attention in recent years because of its many beneficial effects, including anticarcinogenic activity (1, 10, 11, 12, 28, 31), antiatherogenic activity (18, 22, 23), the ability to reduce the catabolic effects of immune stimulation (5, 19), the ability to enhance growth promotion (3), and the ability to reduce body fat (26). Of the individual isomers of CLA, cis-9, trans-11 octadecadienoic acid has been implicated as the most biologically active because it is the predominant isomer incorporated into the phospholipids of cell membranes (10, 11). The cis-9, trans-11 CLA isomer is also the predominant isomer found in the diet; CLA arises from microbial biohydrogenation of dietary linoleic acid in the rumen, which is converted to CLA (15). Foods that contain animal fat, such as beef, lamb, milk, and dairy foods are, therefore, rich sources of CLA (2, 8). From animal studies, it has been predicted (12) that daily intakes of approximately 3 g of CLA are required to prevent cancer. The average estimated CLA intake has been reported to range from 0.35 to approximately 1 g/d (2, 8).

A number of factors have been shown to influence CLA concentrations in bovine milk fat, including lactation number (16, 32), dietary restriction (13), feed allowance (17, 32), seasonal variation (27, 29, 33), and dietary oils (6, 7, 14, 17, 32). A number of recent studies have demonstrated substantial increases in the CLA content of milk fat because of animal dietary and management practices. Full fat rapeseed (FFR) supplements have resulted in substantial increases in CLA in milk over unsupplemented controls (17, 32). In addition, supplements of soybeans (7, 17) and oils of soybean and linseed (7) have increased milk fat CLA concentrations. The method of processing oilseed supplements has been shown to influence the degree and extent of biohydrogenation (7, 21), and, therefore, milk fat CLA content (7). Low grass intake caused a reduction in the concentration of CLA over a 19-wk period (32). Dhiman et al. (6) have shown that cows on only pasture produced milk fat with a
higher CLA content than did cows receiving only one-third or two-thirds of their daily feed from pasture. Cows fed diets supplemented with fish meal also had an increased CLA content in milk fat (6), and the forage to concentrate ratio and the type and amount of fatty acids in the cattle feed were shown to affect CLA concentrations in bovine milk fat (14).

Recently, Stanton et al. (32) showed that CLA concentrations in milk fat are significantly increased for cows fed a diet supplemented with a high amount (1650 g/d per cow) of FFR compared with the CLA concentrations in the milk fat of cows fed pasture only or a diet supplemented with a low amount (825 g/d per cow) of FFR. For cows on pasture, dietary supplementation with FFR or full fat soybeans (FFS), which contain, respectively, approximately 25 and 55% cis-9, cis-12-C18:2 (linoleic acid), the substrate for CLA synthesis (15), has previously been shown to yield lower proportions of saturated fatty acids and higher amounts of C18-unsaturated fatty acids in milk fat (20). When supplements of FFS, which were toasted, flaked, and pelleted, were fed to cows on pasture, linoleic acid increased from 1.8 to 4.8 g/100 g of total milk fatty acids. Conversely, supplements of crushed FFR under similar conditions yielded only 2.3 g of linoleic acid/100 g of fatty acids (20). The aim of this study was to investigate whether FFS, which contains a higher proportion of substrate for CLA synthesis than does FFR and was previously shown to be a useful supplement for increasing milk fat CLA content of cows on pasture (32), was also a suitable supplement for increasing CLA content in milk fat.

MATERIALS AND METHODS

Reagents and Standards

Fatty acid methyl esters (FAME) C4:0 to C20:2 (all of >99% purity) and anhydrous Na2SO4 (99.7% purity) were obtained from Sigma Chemical Co. (St. Louis, MO). The CLA standard was a gift provided by M. Pariza (Food Research Institute, University of Wisconsin, Madison). Hexane, methanol, and chloroform were HPLC grade and were obtained from LabScan Analytical Sciences (Stillorgan, County Dublin, Ireland). Methanolic-HCl was obtained from Supelco Inc. (Bellefonte, PA), and KOH was obtained from Prolabo (Manchester, United Kingdom).

Dietary Treatments

Forty-eight cows were allotted into groups of first lactation and second lactation or greater; within these groups, cows were blocked into groups of 3, according to stage of lactation and milk yield. Within each block, cows were randomly assigned to one of three dietary treatments. These treatments were 1) pasture for ad libitum intake supplemented with 3.1 kg/d per cow of unmolassed beet pulp, 2) pasture for ad libitum intake supplemented with 3.0 kg/d per cow of a FFR concentrate (550 g/kg of FFR, 400 g/kg of unmolassed beet pulp, and 50 g/kg of molasses), and 3) pasture for ad libitum intake supplemented with 3.1 kg/d per cow of FFS supplement. The FFR, unmolassed beet pulp, and molasses were mixed in the correct proportions and were then ground through a 3-mm screen. This treatment has been shown previously (21) to make the oil available for rumen biohydrogenation. The FFS supplement consisted of FFS (toasted, flaked, and pelleted) that were not mixed with any other ingredient. The composition of these fat supplements was similar to that described previously (20). Cows were fed the diets for 32 d, and milk samples from individual cows were taken on d 11, 18, and 32 at the evening milking. Samples were obtained from the recording jars in the milking parlor after agitation and then analyzed for CLA and trans-vaccenic acid (trans-11-C18:1). Composite milk samples for each herd, consisting of the morning and evening milk of each individual cow (based on the morning and evening milk yields), were taken on d 18 of the feeding trial and analyzed for FAME profile. Individual cow yields were measured 5 d/wk, and milk composition (fat, protein, and lactose) was determined weekly on one successive morning and evening milk sample. Blood samples were collected once into heparinized tubes from the coccygeal vein of each cow during the 4th wk of the trial.

Fatty Acid Analysis

Quantification of the CLA and trans-11-C18:1 content in milk fat. Milk fat samples were obtained from whole milk by centrifugation, as described previously (21). The triglyceride and free fatty acid forms of CLA (cis-9, trans-11 isomer) in milk fat from individual cows sampled on d 11, 18, and 32 and the trans-11-C18:1 isomer in milk fat from individual cows sampled on d 32 of the feeding trial were quantified by GLC using a gas-liquid chromatograph (Varian 3500; Varian, Harbor City, CA) fitted with a flame ionization detector. The GLC was performed according to the method of Chin et al. (2) using acid-catalyzed methanolysis as previously described (32) with reference to the internal standard C13:0. Separation of the FAME was performed on a Supelcowax-10 capillary column (Supelco Inc.) (60 m × 0.32 mm i.d., 0.25-μm film thickness), using He as carrier gas at a
pressure of 1.86 bar. The injector temperature was programmed from 80°C to a final temperature of 200°C at a rate of 100°C/min without an initial delay and held for 20 min. The detector temperature was 250°C. The column temperature was programmed from an initial temperature of 50°C to a final temperature of 220°C, without an initial delay, at a rate of 20°C/min during each analysis. The column was held at the final temperature of 220°C for 60 min. Collected data were recorded and analyzed on a Minichrom PC system (VG Data System, Manchester, United Kingdom). The cis-9, trans-11 CLA isomer in milk fat samples was identified by retention time with reference to a CLA mix that was generously provided by the Food Research Institute. The trans-11-C18:1 isomer was identified by retention time with reference to standard trans-11-C18:1 FAME.

To calculate correction factors for the CLA isomer peaks, the internal standard C13:0 was used according to the following formula: $C_f = \frac{A_{IS} \times W_t}{(A_i \times W_{tIS})}$, where $C_f$ = correction factor for the actual CLA isomer, $A_{IS}$ = area of the internal standard (C13:0), $A_i$ = area of the CLA peak, $W_t$ = weight of the CLA isomer, and $W_{tIS}$ = weight of the internal standard. The quantity of CLA was expressed as milligrams per gram of fat, and the minimum detection limit was <0.05 mg CLA/g of fat. To quantify trans-11-C18:1 in milk fat, the response factors of the individual fatty acids were calculated relative to the area of C18:0, which was assigned a response factor of 1.00. trans-11-C18:1 was expressed as grams per 100 g of FAME.

**Quantification of FAME in milk fat and dietary oils.** Oil was extracted from ground rapeseeds and soybeans by the Folch wash method as described by Christie (4). The fatty acids from rapeseed and soybean oils and from composite milk samples taken on d 18 were analyzed according to their FAME by GLC using a gas-liquid chromatograph (Pye Unicam 204; Unicam, Cambridge, United Kingdom) fitted with a dual flame ionization detector. The FAME were prepared as described by Stanton et al. (32) but without the addition of the internal standard. Separation was performed on a 2.13-m glass column with an internal diameter of 2 mm, which was packed with 10% ethylene glycol adipate on a chromosorb WHP 100/120 mesh (Analabs, North Haven, CT). The N2 carrier gas and H2 flow rates were 20 ml/min and the air flow rate was 300 ml/min. The injector temperature was 200°C, and the detector temperature was 250°C. The column temperature was programmed from 80°C, with an initial delay of 2 min, at a rate of 16°C/min during each analysis and held at a final temperature of 200°C until all the fatty acids were eluted. Fatty acids were identified by their retention times with reference to standard FAME. Peak areas were computed using a microcomputer (Trilab 2000; Trivector Scientific Ltd., Turvey, Bedford, United Kingdom). The response factors of the individual fatty acids were calculated relative to the area of C16:0 that was assigned a response factor of 1.00. Fatty acids were expressed as grams per 100 g of FAME.

**Milk Composition**

Milk composition (fat, protein, and lactose) was determined by automated infrared analysis using a MilkoScan 605 (Foss Electric, Hillerød, Denmark).

**Blood Analysis**

Plasma was prepared from heparinized blood samples by centrifugation at 800 x g for 10 min at 4°C and analyzed for the following: BHBA, glucose, protein, albumin, globulin, and urea using a Cobas Mira Biochemical Analyzer (Roche Diagnostics, Basel, Switzerland).

**Experimental Design and Analysis**

Forty-eight cows were allocated into groups of first lactation and second lactation or greater and within these groups were blocked into groups of 3, according to stage of lactation and milk yield, thus giving a randomized block design (34). Within each block, cows were randomly assigned to one of three dietary treatments for 32 d. These treatments were FFR or FFS supplement or control diets. An ANOVA was carried out using Genstat (9) on CLA at each date; the effects in the model were blocks and diet. If a significant effect of treatment was found, then an orthogonal contrast was made between the control and FFR plus FFS and between FFR and FFS. Correlation coefficients between the concentrations of CLA and trans-11-C18:1 in milk fat obtained at d 32 of the trial were estimated using PROC CORR of SAS (30). Milk yield, constituent yield, and composition averaged over the 4 wk of the trial were analyzed using the GLM procedure of SAS (30); lactation number, calving date, and preexperimental data were used as covariates. The blood data were also analyzed using the GLM procedure of SAS, and lactation number was used as a covariate.

**RESULTS AND DISCUSSION**

**Milk Constituent Yield and Composition**

Supplementation with FFR and FFS compared with unmolassed beet pulp (control) did not signifi-

cantly influence milk yield or milk constituent yields (Table 1). In a previous study (20), supplementation with these feeds increased milk yields compared with those of a control treatment (without supplementation). This increase was likely due to increased energy intake than to any effect of FFR or FFS per se. Milk protein concentration was reduced \((P < 0.01)\) by supplementation with FFR and FFS, and milk fat concentration was reduced \((P < 0.05)\) by supplementation with FFR only (Table 1). The effect of the supplementation of FFR on milk fat was observed previously (20), but, in that study, there was no effect on protein.

### Blood Composition

Plasma glucose concentrations were lower for cows fed the FFR \((P < 0.05)\) and FFS \((P < 0.01)\) supplements than for cows on the control treatment (Table 2). This result might have been due to a negative effect of the unsaturated fat from the supplements on rumen fermentation, thereby reducing the quantity of propionate produced. The higher plasma urea \((P < 0.001)\) of cows fed the FFS supplement than of cows on the control treatment and those fed the FFR supplement (Table 2) is a reflection of the higher protein content of the FFS supplement. Full fat soybeans have a protein content of approximately 360 g/kg compared with 200 and 100 g/kg for FFR and unmolassed beet pulp, respectively.

### Fatty Acid Composition of FFR and FFS

The fatty acid composition of the rapeseed and soybean oils is shown in Table 3. Both oils contained negligible amounts of CLA but substantial amounts of C\(_{18}\) unsaturated fatty acids. Oleic acid (\(cis-9-C_{18:1}\)) was approximately three times higher in the rapeseed oil (61 g/100 g of FAME) than in the soybean oil (21 g/100 g of FAME), and the linoleic acid content was approximately three times lower in the rapeseed oil (19.5 g/100 g of FAME) than in the soybean oil (54.5 g/100 g of FAME). Rapeseed oil was more abundant in linolenic acid (\(C_{18:3}\); 10.0 g/100 g of FAME) than in soybean oil (7.6 g/100 g of FAME). The \(C_{18}\) polyunsaturated fatty acids are the substrates for CLA synthesis in the rumen (15). In addition to the \(C_{18}\) unsaturated fatty acids, palmitic acid (\(C_{16:0}\)) represented 11.4 g/100 g of FAME in the soybean oil (Table 3).

### Blood Metabolites

Table 2. Blood metabolites of cows on pasture and fed full fat soybean (FFS) or full fat rapeseed (FFR) supplements or fed unmolassed sugar beet pulp (control).

<table>
<thead>
<tr>
<th>Blood metabolite</th>
<th>Control</th>
<th>FFS</th>
<th>FFR</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHBA, mmol/L</td>
<td>0.42</td>
<td>0.47</td>
<td>0.37</td>
<td>0.05</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>3.55(^a)</td>
<td>2.99(^b)</td>
<td>3.12(^b)</td>
<td>0.095</td>
</tr>
<tr>
<td>Protein, g/L</td>
<td>77.4</td>
<td>78.7</td>
<td>79.9</td>
<td>1.22</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>35.9</td>
<td>35.8</td>
<td>37.5</td>
<td>0.49</td>
</tr>
<tr>
<td>Globulin, g/L</td>
<td>41.4</td>
<td>42.9</td>
<td>42.5</td>
<td>1.21</td>
</tr>
<tr>
<td>Urea, mmol/L</td>
<td>5.49(^b)</td>
<td>7.28(^a)</td>
<td>5.43(^b)</td>
<td>0.151</td>
</tr>
</tbody>
</table>

\(^{a,b}\)Means within rows without a common superscript letter differ \((P < 0.05)\).
Milk Fat CLA Content After Supplementation with FFR and FFS

In a previous study (32), the CLA content in the milk fat increased ($P < 0.001$) when cows on pasture were fed 3.0 kg/d per cow of a FFR concentrate, which was similar to the concentrate fed in this trial. In the present study, the effects of FFR and FFS dietary supplements on the concentration of CLA in bovine milk were compared by means of a one-sided statistical test because the control treatment was not expected to yield more CLA in the milk fat than FFR or FFS dietary supplements. Chromatograms of milk fatty acids from cows on pasture and fed the FFR supplement (Figure 1a), the FFS supplement (Figure 1b), unmolassed beet pulp (control) (Figure 1c), or the standard CLA mixture (Figure 1d) showed that the cis-9, trans-11 CLA isomer was the major form in milk fat and was increased by dietary supplementation; the trans-10, cis-12 CLA isomer remained a minor component of milk fat. Chin et al. (2) reported that the cis-9, trans-11 CLA isomer accounted for 92% of total CLA in bovine milk fat, and trans-10, cis-12 was a minor component. Throughout the feeding trial, on d 11, 18, and 32, the supplementation of pasture with FFR concentrate resulted in

Figure 1. The GLC chromatogram of fatty acid methyl esters, following separation by capillary GLC on a Supelcowax-10 column (Supelco Inc., Bellefonte, PA) of milk fat sample following supplementation with full fat rapeseed (a), full fat soybean (b), unmolassed sugar beet pulp (control) (c), or standard mixture of conjugated linoleic acid (CLA) isomers (d). The peaks indicated are 1, butyric acid, C$_4$:0; 2, caproic acid, C$_6$:0; 3, caprylic acid, C$_8$:0; 4, capric acid, C$_{10}$:0; 5, lauric acid, C$_{12}$:0; 6, tridecanoic acid, C$_{13}$:0 (internal standard); 7, myristic acid, C$_{14}$:0; 8, palmitic acid, C$_{16}$:0; 9, stearic acid, C$_{18}$:0; 10, oleic acid, cis-9-C$_{18}$:1; 11, trans-vaccenic acid, trans-11-C$_{18}$:1; 12, cis-9, trans-11 isomer of CLA; and 13, trans-10, cis-12 isomer of CLA.
TABLE 4. Conjugated linoleic acid (cis-9, trans-11-C_{18:2}; CLA) content in milk fat of cows on pasture and fed full fat soybean (FFS) or full fat rapeseed (FFR) supplement or unmolassed sugar beet pulp (control).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>FFS</th>
<th>FFR</th>
<th>SED(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d 11</td>
<td>10.7(^a)</td>
<td>14.9(^b)</td>
<td>19.7(^a)</td>
<td>1.35</td>
</tr>
<tr>
<td>d 18</td>
<td>13.4(^a)</td>
<td>17.2(^b)</td>
<td>21.9(^a)</td>
<td>1.62</td>
</tr>
<tr>
<td>d 32</td>
<td>16.6(^b)</td>
<td>19.6(^b)</td>
<td>24.0(^a)</td>
<td>1.78</td>
</tr>
</tbody>
</table>

\(^{a,b,c}\)Means within rows without a common superscript letter differ (\(P < 0.05\)). Residual df = 30.

\(^{1}\)Standard error of the difference.

higher \((P < 0.001)\) CLA concentrations in milk fat than with the control treatment (Table 4). The CLA content in the milk fat of cows fed the FFS supplement was higher \((P < 0.05)\) than in the milk fat of cows fed the control diet at d 11 and 18 of the feeding trial (Table 4). From d 11 through 32 of the trial, the CLA concentration in milk fat increased \((P < 0.001)\) for all treatments. Conjugated linoleic acid is not a constituent of rapeseed or soybean oils (Table 3), and so the increased concentrations of CLA obtained in the milk fat were not due to direct transfer from dietary sources but to microbial biohydrogenation by rumen bacteria.

The variation in CLA concentrations in milk fat between individual cows in this study was 6.5 to 33.1 mg/g of fat. Among individual cows, CLA concentrations in the milk fat in the control herd varied from 6.8 to 25.7 mg/g of fat; in the milk of cows fed the FFR concentrate, CLA concentrations ranged from 10.6 to 33.5 mg/g of fat; and, in the milk of cows fed the FFS supplement, the variation was from 8.8 to 30.5 mg/g of fat. Large variation in CLA concentrations in milk fat of individual cows has been observed previously; for example, Stanton et al. (32) reported values from 1.6 to 16.0 mg/g of fat, and Jiang et al. (13) reported 2.5 to 17.7 mg/g of fat. Factors known to influence the CLA concentrations in milk fat include feed allowance (17, 32), dietary oil supplements (6, 7, 14, 17, 32), dietary restriction (13), lactation number (16, 32), and seasonal factors (27, 29, 33).

### Milk Fatty Acid Profiles After Supplementation with FFR and FFS

The fatty acid profiles of the composite milk samples on d 18 of the trial are shown in Table 5; no statistical analysis was possible on these data. However, the following observations were made. The linoleic acid concentrations in milk fat obtained from herds fed either FFR or FFS were higher than those from the control herd, but the amount obtained following FFS supplementation was approximately twice \((4.9 \text{ g/100 g of FAME})\) that following FFR supplementation \((2.5 \text{ g/100 g of FAME})\). This approximately twofold increase in linoleic acid content in milk fat following FFS supplementation might be expected if complete hydrogenation did not occur because soybean oil contains over twice the amount of linoleic acid as does rapeseed oil. A similar increase in the linoleic acid content in milk fat was obtained previously because of FFS supplementation (20).

The linolenic acid content in composite milk samples from the herd fed the FFR supplement \((0.6 \text{ g/100 g of FAME})\) was lower than that of the herd fed the FFS supplement \((1.0 \text{ g/100 g of FAME})\) and that of the control herd \((0.7 \text{ g/100 g of FAME}; \text{ Table 5})\), although the linolenic acid content of the FFR supplement was substantially higher than that of the FFS supplement (Table 3). This result is indicative of extensive microbial biohydrogenation in the rumen following ingestion of the FFR supplement.

The concentrations of oleic acid were increased in the milk fat of herds fed either FFR or FFS supplements compared with concentrations in milk fat of control herds. Supplementation of FFR was more ef-

### TABLE 5. Milk fatty acid composition of cows on pasture and fed full fat soybean (FFS) or full fat rapeseed (FFR) supplement for 18 d or fed unmolassed sugar beet pulp (control).

<table>
<thead>
<tr>
<th>Fatty acid(^1)</th>
<th>Control</th>
<th>Full fat soybean</th>
<th>Full fat rapeseed</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(_{10:0})</td>
<td>2.47</td>
<td>2.00</td>
<td>1.59</td>
</tr>
<tr>
<td>C(_{10:1})</td>
<td>0.22</td>
<td>0.20</td>
<td>0.14</td>
</tr>
<tr>
<td>C(_{12:0})</td>
<td>3.25</td>
<td>2.47</td>
<td>2.17</td>
</tr>
<tr>
<td>C(_{12:1})</td>
<td>0.14</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>C(_{14:0})</td>
<td>11.30</td>
<td>9.15</td>
<td>8.96</td>
</tr>
<tr>
<td>C(_{14:1})</td>
<td>1.33</td>
<td>1.16</td>
<td>1.13</td>
</tr>
<tr>
<td>C(_{15:0})</td>
<td>1.66</td>
<td>1.21</td>
<td>1.26</td>
</tr>
<tr>
<td>C(_{16:0})</td>
<td>29.05</td>
<td>23.13</td>
<td>22.32</td>
</tr>
<tr>
<td>C(_{16:1})</td>
<td>2.06</td>
<td>1.86</td>
<td>2.13</td>
</tr>
<tr>
<td>C(_{17:0})</td>
<td>0.94</td>
<td>0.74</td>
<td>0.71</td>
</tr>
<tr>
<td>C(_{17:1})</td>
<td>0.46</td>
<td>0.35</td>
<td>0.36</td>
</tr>
<tr>
<td>C(_{18:0})</td>
<td>10.83</td>
<td>12.80</td>
<td>12.03</td>
</tr>
<tr>
<td>C(_{18:1})</td>
<td>24.90</td>
<td>31.32</td>
<td>35.34</td>
</tr>
<tr>
<td>C(_{18:2})</td>
<td>2.33</td>
<td>4.94</td>
<td>2.45</td>
</tr>
<tr>
<td>C(_{18:3})</td>
<td>0.71</td>
<td>1.03</td>
<td>0.57</td>
</tr>
<tr>
<td>CLA(^2)</td>
<td>1.74</td>
<td>2.23</td>
<td>2.49</td>
</tr>
<tr>
<td>C(_{20:0})</td>
<td>0.21</td>
<td>0.21</td>
<td>0.22</td>
</tr>
<tr>
<td>C(_{20:1})</td>
<td>0.20</td>
<td>0.29</td>
<td>0.44</td>
</tr>
<tr>
<td>C(_{20:2})</td>
<td>0.24</td>
<td>0.21</td>
<td>0.22</td>
</tr>
</tbody>
</table>

\(^{1}\)Means of duplicate sample analyses.

\(^{2}\)Total conjugated linoleic acid (CLA) isomers.
fective, resulting in an increase in oleic acid of approximately 40% compared with that of FFS supplementation, which yielded an increase of approximately 26% over the control treatment (Table 5). The amount of oleic acid in rapeseed oil was almost three times the amount in soybean oil (Table 3). This greater increase in oleic acid content in milk fat by FFR than with FFS was also observed by Murphy et al. (20). When fed to a group of hypercholesterolaemic patients, butter that was rich in monounsaturated fat made from the milk of cows fed FFR yielded beneficial changes in the plasma lipid fraction (24).

There was a strong correlation between CLA (cis-9, trans-11-C_{18:1}; CLA) and trans-11-C_{18:1} in milk fat (Figure 3). Trans-11-C_{18:1} is formed as an intermediate during the biohydrogenation of dietary linoleic acid to stearic acid (13, 15). The mean concentrations of trans-11-C_{18:1} in the milk fat of control cows was 4.1 ± 1.07 g/100 g of FAME and of cows fed the FFR and FFS supplements were 5.6 ± 0.81 and 4.7 ± 0.96 g/100 g of FAME, respectively. Separate regression lines relating CLA to trans-11-C_{18:1} were fitted for the three diets. These lines were compared for slopes and intercepts and were not significantly different. Therefore, the following single line was fitted (Figure 3): CLA = -0.05 + 0.46 trans-11-C_{18:1} (r = 0.83). A strong positive correlation between CLA and trans-11-C_{18:1} in milk fat has been observed previously (13), which suggests that the first two steps in the biohydrogenation of linoleic to stearic acid were not rate limiting.

CONCLUSIONS

The data indicate that supplementation of cows on pasture with similar amounts of oil derived from the FFR supplement, which was prepared using ground

Figure 3. Correlation between concentrations of conjugated linoleic acid (cis-9, trans-11-C_{18:2}; CLA) and trans-11-C_{18:1} in the milk fat of cows on pasture and fed unmolassed sugar beet pulp (control), full fat rapeseed (FFR) supplement, or full fat soybean (FFS) supplement for 32 d. Regression equation: CLA = -0.05 + 0.46 trans-11-C_{18:1}; r = 0.83. FAME = Fatty acid methyl esters.
FFR, and FFS, which consisted of toasted FFS, yielded significantly more CLA in the milk fat than did the control diet. The FFR supplement was more effective than the FFS supplement at increasing the CLA content in milk fat. However, the concentrations of linoleic and linolenic acids, which are the potential substrates for rumen biohydrogenation, thereby leading to CLA production (15), were higher in the FFS supplement (62.1 g/100 g of FAME) than in the FFR supplement (29.4 g/100 g of FAME). The linoleic acid concentrations in the milk of control cows and cows fed the FFR supplement were similar, but the cows fed the FFR supplement received approximately 42 g of linoleic acid/d per cow more than did the control cows, suggesting that extensive biohydrogenation of linoleic acid occurred in the rumen following ingestion of the FFR supplement. However, the linoleic acid content in the milk of cows fed the FFS supplement was approximately twice the linoleic acid content in the milk fat of the control cows or the cows fed the FFR supplement. These data indicate that linoleic acid ingested in the FFS supplement was not biohydrogenated extensively in the rumen.

Rapeseed oil is a relatively rich source of linolenic acid compared with soybean oil, and the amount of linoleic acid in rapeseeds is relatively low compared with its content in soybeans. Following supplementation of FFR, the linolenic acid concentration in milk fat was lower than in milk fat of cows fed the FFS supplement, although cows fed the FFR supplement received the most linolenic acid. This result is indicative of rumen biohydrogenation of the linolenic acid in the FFR supplement and might have contributed to the increased concentrations of CLA observed in milk following this dietary treatment.

It appears that conversion in the rumen of dietary C18 polyunsaturated fatty acids from the FFS supplement to CLA did not proceed at the optimal level, as is borne out by the lower trans-11-C18:1 concentrations in the milk of cows fed the FFS supplement than in those fed the FFR supplement. This result points toward the partial unavailability of the dietary oil in the FFS supplement, as opposed to inhibition of microbial activity by the supplemental fat, which is known to increase as the degree of unsaturation increases (25). A concentration of supplemental fat that was similar to that fed in this study, but in the form of pure soybean oil (4%), resulted in an approximately fourfold increase in the concentration of CLA in milk (20.8 mg/g of fatty acids) over the control (7). In this study, the concentration of CLA obtained in milk was 17.2 mg/g of fat following FFS supplementation, which represented a 27% increase in the CLA concentration in milk over the control on d 18 of the trial. The concentration of CLA obtained following FFR supplementation was 21.8 mg/g of fat, which represented approximately a 60% increase over the control on d 18 of the trial and is similar to the relative increase in the CLA concentration in milk obtained previously following FFR supplementation (32). The lower relative increase in the CLA concentration following supplementation with FFS, which was rich in potential substrates for rumen biohydrogenation, suggests that these fatty acids were not readily accessible to the rumen microorganisms.

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REFERENCES


