

## Effect of dietary fat supplements on levels of n-3 poly-unsaturated fatty acids, trans acids and conjugated linoleic acid in bovine milk

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### Abstract

The effects of three fat supplements on milk yield and composition were measured using 12 mid-lactation in-calf Holstein-Friesian cows in a balanced incomplete change-over design over three periods each of 3 weeks. All cows received a basal diet consisting of 36 kg/day grass silage (dry matter (DM) 270 g/kg, metabolizable energy (ME) 11.6 MJ/kg DM) and 7 kg/day of a concentrate mixture containing (g/kg) rolled barley (501), molassed sugar-beet pulp shreds (277), soya-bean meal (208) and a standard cow mineral supplement (14). Treatments were CON (control-no supplement); LIN and FISH (250 g/day of either linseed oil or marine oil, providing approximately 0.046 of ME intake) or TOA (95 g/day of tuna orbital oil, providing 0.018 of total ME intake).

There were no significant effects on silage DM intake or milk yield (means 9.25 and 17.2 kg/day respectively). The FISH and TOA treatments depressed ( $P < 0.05$ ) milk fat concentration (45.4, 44.6, 34.5 and 41.6 (s.e.d. 1.08) g/kg for CON, LIN, FISH and TOA respectively; note — the same treatment order is used for all results quoted). Compared with values for CON, yield of fat (g/day) was significantly ( $P < 0.05$ ) greater for LIN and significantly lower for FISH (739, 808, 572 and 732, s.e.d. 28.7). All three oil supplements reduced ( $P < 0.05$ ) milk protein content (33.6, 32.5, 30.6 and 32.4 (s.e.d. 0.43) g/kg) but, apart from a small increase for LIN, protein yield (g/day) was unaffected (545, 586, 510 and 574, s.e.d. 20.2).

The concentrations (g/100 g) of short-chain fatty acids ( $\leq C_{14}$ ) and  $C_{16}:0$  in milk fat were lower ( $P < 0.05$ ) for LIN than for the other treatments. All supplements increased the concentrations of  $C_{18:1}$  ( $P < 0.05$ ), the value for LIN being greater ( $P < 0.05$ ) than for the other treatments (21.0, 27.2, 25.3 and 23.7, s.e.d. 0.74). The FISH and TOA treatments increased ( $P < 0.05$ ) the concentrations of long chain ( $\geq C_{20}$ ) (n-3) poly-unsaturated fatty acids (PUFA), (0.19, 0.17, 0.49 and 0.27, s.e.d. 0.026) but less than proportionately 0.03 of dietary intake of these acids was transferred to milk, probably because they were found to be mostly in the phospholipid and cholesterol ester fractions of plasma. The FISH and TOA treatments increased ( $P < 0.05$ ) the percentages of total trans fatty acids in milk fat (1.13, 2.19, 10.26 and 3.62, s.e.d. 0.728) whilst a significant ( $P < 0.05$ ) increase in conjugated linoleic acid (CLA) was observed only for FISH (0.16, 0.28, 1.55, and 0.52, s.e.d. 0.154). Concentrations of CLA and total trans acids in milk were highly correlated ( $r = 0.91$ ,  $n = 36$ ,  $P < 0.001$ ) whilst trans acids in milk were inversely correlated with milk fat content ( $r = -0.63$ ,  $n = 36$ ,  $P < 0.001$ ) supporting the theory that milk fat depression may be caused by increased supply of trans fatty acids to the mammary gland. The health implications of these changes in milk fat composition are discussed.

**Keywords:** fatty acids (trans), linoleic acid (conjugated), milk fat, polyenoic fatty acids.

### Introduction

Increasingly, milk 'quality' is perceived by the consumer not just as the avoidance of harmful constituents in the product, but additionally that it should have positive attributes for health and well-being. Consequently, there is increased interest in various manipulations of milk composition such as

increasing the protein:fat ratio or increasing the proportion of total or specific unsaturated fatty acids in milk fat. Recently, much attention has been paid to the beneficial effects of n-3 poly-unsaturated fatty acids (n-3 PUFA) on human health particularly with respect to the vascular system and heart disease, immune and inflammatory responses and in early

development (Committee on the Medical Aspects of Food (COMA), 1994; Nettleton, 1994). Similarly, the powerful anti-carcinogenic properties of conjugated linoleic acids (CLA) has focused attention on this milk constituent (Parodi, 1997; Ip *et al.*, 1994).

However, it is essential that alterations in milk production systems aimed at increasing levels of beneficial components of milk do not also increase those that are potentially harmful to health. Concern is increasing about trans fatty acids in the human diet (Lichtenstein, 1993; British Nutrition Foundation (BNF), 1995). There is increasing evidence that trans acids promote cardiovascular disease and may interfere with the metabolism of essential fatty acids although there is debate about the effects of individual trans isomers (American Society for Clinical Nutrition (ASCN)/American Institute of Nutrition (AIN), 1996). A recent epidemiological study (Kohlmeier *et al.*, 1997) found an association between trans fatty acid levels in adipose tissue and incidence of breast cancer in women.

Recently, marine oils rich in (n-3) PUFAs, have been added to proprietary dairy diets with the primary aim of depressing milk fat levels to allow greater milk volumes to be produced without exceeding milk quota restrictions. The fat-depressing effect of fish oils has been known for many years (e.g. Storry *et al.*, 1974) but recent evidence suggests that increased output of trans acids from the rumen is the main mechanism for this effect (Gaynor *et al.*, 1994 and 1995).

The aim of the present experiment was to evaluate three dietary fat supplements in terms of their effects on milk output and composition with particular reference to effects on (n-3) PUFA, CLA and trans fatty acids. Linseed and fish oils were chosen because their high contents of (n-3) PUFA would be expected to have large effects on rumen function (especially biohydrogenation). Tuna orbital oil, with a high content of docosahexaenoic acid ( $C_{22:6}$  (n-3)), was given at a lower rate to provide the same daily amount of  $C_{22:6}$  (n-3) as the fish oil to investigate the possibility that such a supplement might lead to increased (n-3) PUFA levels in milk fat whilst minimizing trans fatty acid formation in the rumen.

## Material and methods

### *Cows and housing*

Twelve mid-lactation in-calf Holstein-Friesian cows were used. Mean (s.d.) pre-experiment live weight, condition score and milk yield were 636 (47.8) kg, 2.6 (0.47) and 20.9 (3.00) kg/day, respectively. All cows had calved during September/October 1997 and mean days since calving at the start of experiment

was 169 (s.d. 11.1). Cow health was recorded continuously. Cows were housed in a single group and given food individually by Calan Broadbent feeders. The protocol for this experiment was approved by the Animal Experiments Committee of the Scottish Agricultural College and the experiment was conducted in accordance with the Home Office Animals (Scientific Procedures) Act 1986.

### *Diets and design*

All cows received a basal diet consisting of 36 kg/day grass silage and 7 kg of a concentrate mixture containing (g/kg) rolled barley (501), molassed sugar-beet pulp shreds (277), soya-bean meal (Hipro 208) and a standard cow mineral supplement (14). Silage and concentrate were offered in two approximately equal meals at 07:00 and 17:00 h. Water was available continuously to the cows. The silage was made from a primary regrowth of a predominantly perennial ryegrass sward harvested in mid July and ensiled using 31/t of Add Safe (Trouw Nutrition, UK).

There were four dietary treatments: CON — no supplement; LIN — 250 g/day linseed oil (Unitrition Ltd, UK); FISH — 250 g/day fish oil (Isaac Spencer Ltd, UK) and TOA — 95 g/day tuna orbital oil (Scotia Pharmaceuticals, UK). The lower daily intake for TOA was chosen to provide a similar intake of docosahexaenoic acid ( $C_{22:6}$  n-3) to that provided by the FISH treatment (preliminary analysis of these oils gave values of 24.9 and 8.5 g/100 g oil for TOA and FISH respectively). The oil supplements were hand-mixed each day with the concentrate mixture and given as two equal meals. A balanced incomplete change-over experimental design was used, in which each cow received three of the four treatments, over three periods each of 3 weeks. Each treatment was evaluated by nine cows in a balanced, statistically valid manner. All sampling was carried out during week three of each period which, on the basis of previous experiments (N. W. Offer, unpublished), should have allowed sufficient time to minimize carry-over effects between treatments. The possibility of such effects was however investigated (see statistical methods). The design allowed the experiment to be completed in three periods thus avoiding the problems of using cows in very late lactation and also minimized the cost of the expensive tuna orbital oil. The cows were initially divided into three blocks of four cows according to yield, recorded 2 weeks before the start of the experiment. Each cow in a block was then randomly allocated to one of the four dietary treatments.

### *Milk yield and composition*

The cows were milked twice daily at 06:30 and 16:30 h. Yields were recorded daily. Milk samples

were retained for analysis on 3 days (both a.m. and p.m.) during the last week of each period. Samples were preserved with Bromopol (D&F Control Systems Inc., Knoll MicroCheck, Notts, UK) and bulked samples, representative of the milk produced by each cow over the three sampling days, were analysed for fat, protein and lactose (by infrared analyser, Milkoscan, Foss Instruments, UK), for fat by the Rose-Gotlieb method (British Standards Institution, 1987), and for citrate by high performance liquid chromatography (Marsili *et al.*, 1981). Individual fatty acids were measured on samples stored at  $-20^{\circ}\text{C}$  by gas chromatography (GC) and by GC-mass spectroscopy (GCMS for trans fatty acids and CLA).

#### Plasma

Samples (three per cow) of blood were taken by jugular venipuncture at approximately 11:00 h on 1 day during the last week of each period. Plasma was removed immediately following centrifugation and was stored at  $-20^{\circ}\text{C}$  prior to lipid analysis.

#### Food analysis

Samples of concentrate and silage were taken daily as meals were weighed out. Oven dry matter (DM) was measured on all samples. Detailed analyses were carried out on bulked samples representative of the foods given during the last week of each experimental period. Details of standard laboratory methods used are given by Dewhurst *et al.* (1996). The concentration of digestible organic matter in the silage DM (DOMD g/kg DM) was predicted from the near infrared reflectance spectra using the UK standard (ME-tick) model (Offer *et al.*, 1996).

#### Lipid analysis

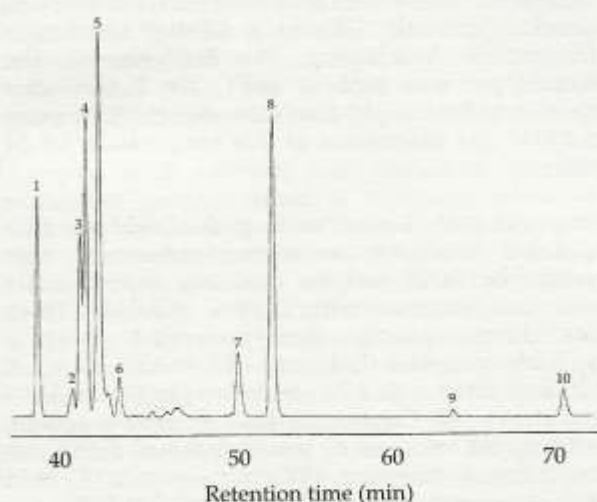
Total lipids were obtained from foods, plasma and milk by standard methods using chloroform:methanol extraction (Christie, 1984). The cholesteryl ester, phospholipid, triacylglycerol and free fatty acid fractions of total lipid from plasma were isolated by thin-layer chromatography on silica gel G using a solvent system of hexane:diethyl ether:formic acid (80:20:1, v/v). The separated bands were visualized under ultra-violet light after spraying the plates with a 0.1% (w/v in methanol) solution of 7-dichlorofluorescein.

The acyl components of total lipids extracted from plasma and food, and of the lipid classes isolated from plasma, were converted to their fatty acid methyl esters by refluxing with methanol:toluene:sulphuric acid (20:10:1 v/v) (Christie *et al.*, 1970). Starting quantities were adjusted so that between 2 and 10 mg of lipid was subject to the methylation procedure. The methyl esters were analysed by gas-liquid chromatography using a capillary column

(Carbowax, 30 m  $\times$  0.25 mm, film thickness 0.25  $\mu\text{m}$ , Alltech, Carnforth, UK) in a CP9001 instrument (Chrompack, Middleburg, The Netherlands). The temperature was held at  $185^{\circ}\text{C}$  for 2 min after injection of the sample, then increased at  $5^{\circ}\text{C}/\text{min}$  up to  $230^{\circ}\text{C}$  and maintained at this temperature for 24 min.

Fatty acid methyl esters were synthesized from milk lipid by incubation at room temperature with methanolic KOH and the resulting methyl esters were then extracted with heptane (Christie, 1984). Gas chromatography was performed using a capillary column (Econocap EC-WAX, 30 m  $\times$  0.25 mm, fitted with a 3 m retention gap) in an Ai 94 Instrument (Ai, Cambridge, UK). Following sample injection, the temperature was maintained at  $65^{\circ}\text{C}$  for 2 min, then increased at  $15^{\circ}\text{C}/\text{min}$  up to  $210^{\circ}\text{C}$ , held at this temperature for 10 min, increased at  $10^{\circ}\text{C}/\text{min}$  up to  $230^{\circ}\text{C}$ , and maintained at  $230^{\circ}\text{C}$  for a further 25 min.

The levels of the cis and trans isomers of  $\text{C}_{18:1}$  and  $\text{C}_{16:1}$  and the cis-9, trans-11 isomer of CLA in milk and plasma were measured on a Fisons MD800 GC mass spectrometer using a SGE 60 m column (BP  $\times$  70, 0.22 mm i.d., 0.25 mm film thickness). Samples were processed through the column isothermally at a temperature of  $155^{\circ}\text{C}$  for 85 min, the temperature was then ramped to  $240^{\circ}\text{C}$  for 10 min to remove any residual components from the column. Figure 1 shows part of a chromatograph obtained under these conditions showing typical separations of trans fatty acids and CLA. The trans-10, and trans-11 isomers of  $\text{C}_{18:1}$  were well resolved from the main cis-9 peak but the trans-6, -7, -8, and -9 isomers remained unresolved as one peak whilst the trans-12, -13, -14, -15 isomers were unresolved from the main cis-9 peak. Thus, values quoted for trans-9  $\text{C}_{18:1}$  included any trans-6, -7, -8, and -9 isomers. Similarly, values given for cis-9  $\text{C}_{18:1}$  included any trans-12, -13, -14, -15 isomers. The data of Molkentin and Precht (1995) suggests that levels of these potential contaminating isomers are very low. Hence, it is unlikely that this problem significantly affected the results. Individual standards of trans-6, -7, -9 and -11 were obtained from Sigma, Aldrich Co. Ltd (Fancy Road, Poole, Dorset BH12 4QH), the trans-10 isomer was identified by interpolation of retention times and mass spectra data from the NIST software reference library (National Institute of Standards and Technology, Department of Commerce, USA). The identification of the cis-9, trans-11 isomer of CLA was confirmed using a relatively pure reference standard (0.83 cis-9, trans-11; 0.03 cis-9, cis-11; 0.05 cis-9, trans-12; 0.02 cis-9, cis-12) obtained from Dr W. Christie (Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA). Further reference



**Figure 1** Total mass ion scan by gas chromatography - mass spectrometry for fatty acid methyl esters of lipid extracted from milk produced by a cow receiving FISH oil. Peak identification:— (1)  $C_{18:0}$ , (2) unresolved trans-6, trans-7 and trans-9,  $C_{18:1}$ , (3) trans-10,  $C_{18:1}$ , (4) trans-11,  $C_{18:1}$ , (5) cis-9,  $C_{18:1}$ , (6) cis-11,  $C_{18:1}$ , (7) cis-9, cis-12,  $C_{18:2}$ , (8)  $C_{19:0}$  [internal standard], (9) cis-9, cis-12, cis-15,  $C_{18:3}$ , (10) CLA, cis-9, trans-11,  $C_{18:2}$ .

to a mixed standard of four CLA isomers supplied by Sigma demonstrated that the above conditions gave well resolved peaks for the cis-9, trans-11; trans-10, cis-12; 8:10; 11:13 (exact geometry unknown) isomers. The cis-9, trans-11 isomer was the only one found in the milk samples and all values reported as CLA are for this isomer.

All gas-chromatography data were analysed by an EZ Chrom Data System (Scientific Software Inc., San Ramon, CA, USA) using  $C_{19:0}$  as an internal standard. The data processing system enabled expression of the fatty acid composition in terms of g/100 g lipid and also allowed calculation of the total amount of fatty acid derived from the lipids of each sample. The identification of the peaks was validated by comparison with the retention times of standard fatty acid methyl ester mixtures (Sigma, Poole, UK).

#### Statistical methods

Statistical analysis was carried out using GENSTAT V (Lawes Agricultural Trust, 1987). The residual maximum likelihood directive was used with the following model: fixed = (period + treatment + period  $\times$  treatment) random = cow/period. Least significant differences between treatment means were calculated from the resulting standard errors of difference for treatment of using a *t* value of 2.09

(error d.f. = 19,  $P = 0.05$ ). The possibility of carry-over effects was investigated by using the identity of the previous treatment as a covariate. This proved to be not significant ( $P > 0.05$ ) for any of the measurements and this covariance correction was not used. Correlations and preliminary exploration of the data were undertaken using the MINITAB statistical software (MINITAB Inc., 1980).

## Results

### Food composition

The silage had the following composition (g/kg DM unless specified): dry matter 268 g/kg, crude protein (CP) 146, neutral-detergent fibre (NDF) 513, acid hydrolysed ether extract (AHEE) 58, digestible organic matter 710, metabolizable energy (ME, MJ/kg DM) 11.6, pH 3.8 and ammonia nitrogen (g N per kg total N) 48. Values for the concentrate mix (without added fat) were: CP 205, NDF 236, AHEE 43, starch 259, neutral cellulase/gaminase digestibility (%) 86.7 and ME 13.2 MJ/kg DM.

The fatty acid compositions measured were typical of the foods. The lipid of silage and linseed showed high contents (g/100 g lipid) of  $C_{18:3}$  (n-3) (45.0 and 50.3 respectively), whilst  $C_{18:2}$  (n-6) was predominant in the basal concentrate (50.1). The FISH and TOA oils were characterized by their contents of long chain ( $>C_{20}$  acids) being particularly rich in  $C_{20:5}$  (n-3), (16.2 and 5.0 respectively) and  $C_{22:6}$  (n-3), (8.5 and 24.9 respectively). No trans fatty acids were detected in any of the feed ingredients.

### Nutrient intake

The cows remained healthy throughout the experiment. Table 1 gives the observed intakes for the four treatments. There were no refusals of silage or concentrate during the recording weeks of the experiment and at no time did refusals exceed 250 g/day. Forage:concentrate ratio (DM basis) was approximately 60:40 for all treatments. Dietary treatment had no significant ( $P > 0.05$ ) effect on silage intake.

Total fat intake averaged about 800 g/day for the control diet, approximately two-thirds of which was derived from the silage. The LIN and FISH supplements increased total fat intake to slightly more than 1000 g/day, representing a 0.29 proportional increase, whilst the TOA supplement, which was given at 95 g/day, increased lipid intake by approximately 0.11.

Compared with the CON treatment, the LIN supplement greatly increased intakes of  $C_{18:1}$ ,  $C_{18:2}$  and  $C_{18:3}$ . The main effects of the FISH and TOA supplements were to increase intakes of  $C_{16:0}$ ,  $C_{16:1}$

Table 1 Mean intakes of dry matter, oil and individual fatty acids

|                         | Treatment         |                   |                   |                   | s.e.d. |
|-------------------------|-------------------|-------------------|-------------------|-------------------|--------|
|                         | CON               | LIN               | FISH              | TOA               |        |
| Dry matter (kg/day)     |                   |                   |                   |                   |        |
| Silage                  | 9.39              | 9.34              | 8.99              | 9.28              | 0.165  |
| Concentrate             | 6.22              | 6.22              | 6.22              | 6.22              |        |
| Oil supplement          | 0                 | 0.250             | 0.250             | 0.095             |        |
| Total                   | 15.6              | 15.8              | 15.5              | 15.6              |        |
| Oil (g/day)             |                   |                   |                   |                   |        |
| Silage                  | 541               | 538               | 518               | 535               | 9.5    |
| Concentrate             | 269               | 269               | 269               | 269               |        |
| Oil supplement          | 0                 | 250               | 250               | 95                |        |
| Total                   | 810 <sup>a</sup>  | 1057 <sup>c</sup> | 1037 <sup>c</sup> | 899 <sup>b</sup>  | 9.5    |
| Fatty acids (g/day)     |                   |                   |                   |                   |        |
| C <sub>14:0</sub>       | 0                 | 0                 | 18.4              | 1.7               |        |
| C <sub>16:0</sub>       | 149 <sup>a</sup>  | 165 <sup>b</sup>  | 188 <sup>c</sup>  | 166 <sup>b</sup>  | 1.6    |
| C <sub>16:1</sub> (n-7) | 9.6 <sup>a</sup>  | 9.6 <sup>a</sup>  | 30.7 <sup>c</sup> | 14.0 <sup>b</sup> | 0.17   |
| C <sub>16:2</sub> (n-4) | 0                 | 0                 | 2.9               | 0                 |        |
| C <sub>16:3</sub>       | 0                 | 0                 | 4.1               | 0                 |        |
| C <sub>17:0</sub>       | 0                 | 0                 | 0                 | 1.2               |        |
| C <sub>17:1</sub> (n-7) | 0                 | 0                 | 0                 | 1.1               |        |
| C <sub>18:0</sub>       | 16.5 <sup>a</sup> | 28.5 <sup>d</sup> | 25.6 <sup>c</sup> | 22.9 <sup>b</sup> | 0.19   |
| C <sub>18:1</sub> (n-9) | 42.8 <sup>a</sup> | 88.3 <sup>d</sup> | 85.6 <sup>c</sup> | 56.2 <sup>b</sup> | 0.19   |
| C <sub>18:1</sub> (n-7) | 2.8               | 2.8               | 2.8               | 5.4               |        |
| Total C <sub>18:1</sub> | 45.8              | 91.3              | 88.6              | 61.9              | 0.19   |
| C <sub>18:2</sub> (n-6) | 202 <sup>b</sup>  | 239 <sup>a</sup>  | 202 <sup>b</sup>  | 204 <sup>b</sup>  | 1.2    |
| C <sub>18:3</sub> (n-3) | 259 <sup>b</sup>  | 384 <sup>a</sup>  | 249 <sup>b</sup>  | 257 <sup>b</sup>  | 4.3    |
| C <sub>18:4</sub> (n-3) | 0                 | 0                 | 5.4               | 0                 |        |
| C <sub>20:0</sub> (n-9) | 0                 | 0                 | 3.5               | 0                 |        |
| C <sub>20:4</sub> (n-6) | 0                 | 0                 | 2.7               | 1.6               |        |
| C <sub>20:5</sub> (n-3) | 0                 | 0                 | 40.4              | 4.8               |        |
| C <sub>22:0</sub>       | 7.8               | 7.8               | 7.5               | 7.7               | 0.14   |
| C <sub>22:1</sub> (n-9) | 0                 | 0                 | 4.1               | 0                 |        |
| C <sub>22:3</sub> (n-6) | 0                 | 0                 | 0                 | 1.8               |        |
| C <sub>22:5</sub> (n-3) | 0                 | 0                 | 4.6               | 1.0               |        |
| C <sub>22:6</sub> (n-3) | 0                 | 0                 | 21.1              | 23.7              |        |
| C <sub>24:0</sub>       | 5.8               | 5.8               | 5.5               | 5.7               | 0.10   |

<sup>a,b,c</sup> Means in a row not sharing common superscripts differ significantly ( $P < 0.05$ ). Zero values indicate that levels of fatty acids in the foods were below measurable limits ( $<0.5$  g/100 g fat). Values for s.e.d. were not calculated in these cases but

Table 2 Mean milk production data

|                                    | Treatment         |                   |                   |                   | s.e.d. |
|------------------------------------|-------------------|-------------------|-------------------|-------------------|--------|
|                                    | CON               | LIN               | FISH              | TOA               |        |
| Milk composition (g/kg)            |                   |                   |                   |                   |        |
| Fat (by IR)                        | 45.4 <sup>c</sup> | 44.6 <sup>c</sup> | 34.5 <sup>a</sup> | 41.6 <sup>b</sup> | 1.08   |
| Protein                            | 33.6 <sup>c</sup> | 32.5 <sup>b</sup> | 30.6 <sup>a</sup> | 32.4 <sup>b</sup> | 0.43   |
| Lactose                            | 44.9              | 45.9              | 45.9              | 45.7              | 0.38   |
| Fat (Rose-Gotlieb)                 | 47.4 <sup>c</sup> | 47.0 <sup>c</sup> | 38.3 <sup>a</sup> | 44.4 <sup>b</sup> | 1.21   |
| Citrate (mg/kg)                    | 914               | 947               | 935               | 954               | 22.9   |
| Urea N (mg/kg)                     | 192               | 187               | 201               | 201               | 5.6    |
| Yield of milk constituents (g/day) |                   |                   |                   |                   |        |
| Fat (by IR)                        | 739 <sup>b</sup>  | 808 <sup>c</sup>  | 572 <sup>a</sup>  | 732 <sup>b</sup>  | 28.7   |
| Protein                            | 545 <sup>ab</sup> | 586 <sup>c</sup>  | 510 <sup>a</sup>  | 574 <sup>bc</sup> | 20.2   |
| Lactose                            | 755               | 836               | 782               | 829               | 33.5   |

<sup>a,b,c</sup> Means in a row not sharing common superscripts differ significantly ( $P < 0.05$ ).

C<sub>18:1</sub> and the C<sub>20:5</sub> and C<sub>22:6</sub> (n-3). The increases in fatty acid intake (compared with values for CON) were less for TOA than for FISH except for C<sub>22:6</sub> (n-3), for which intakes were similar as intended (21.1 and 23.7 g/day for FISH and TOA respectively).

#### Milk yield and composition

Table 2 gives mean values for concentration and yield of major constituents. Treatment had no significant effect on yield ( $P > 0.05$ ) although there was a trend towards higher yields for LIN and TOA ( $P > 0.05$ ,  $P < 0.1$ ). The FISH and TOA supplements depressed milk fat concentration (measured by IR and Rose-Gotlieb,  $P < 0.05$ ) compared with values for CON but LIN had no effect. The FISH treatment

caused the greatest depression of milk fat concentration ( $P < 0.05$ ). All three oil supplements lowered milk protein concentration compared with the control value but this effect was greatest for the FISH treatment ( $P < 0.05$ ). Fat concentration measured by the Rose-Gotlieb method was higher than that from the Milkoscan IR instrument. The difference was proportionately greater for the FISH treatment suggesting that the changes in milk fatty acid composition caused by this supplement cause the IR method to under-estimate milk fat by a greater amount than for the other treatments. Mean daily fat yield was proportionately reduced by 0.23 by the FISH treatment whilst the LIN treatment increased fat yield by 0.09 compared with that for the control

Table 3 Milk fatty acid composition (g/100 g fat)

|                                     | Treatment           |                    |                    |                    | s.e.d. |
|-------------------------------------|---------------------|--------------------|--------------------|--------------------|--------|
|                                     | CON                 | LIN                | FISH               | TOA                |        |
| C <sub>4:0</sub>                    | 1.80                | 1.78               | 1.81               | 1.80               | 0.050  |
| C <sub>6:0</sub>                    | 1.00                | 0.98               | 0.93               | 1.00               | 0.046  |
| C <sub>8:0</sub>                    | 0.64                | 0.60               | 0.59               | 0.63               | 0.026  |
| C <sub>10:0</sub>                   | 1.76 <sup>b</sup>   | 1.51 <sup>a</sup>  | 1.58 <sup>a</sup>  | 1.68 <sup>b</sup>  | 0.055  |
| C <sub>12:0</sub>                   | 2.70 <sup>c</sup>   | 2.12 <sup>a</sup>  | 2.36 <sup>b</sup>  | 2.48 <sup>bc</sup> | 0.113  |
| C <sub>14:0</sub>                   | 9.88 <sup>b</sup>   | 8.77 <sup>a</sup>  | 10.26 <sup>b</sup> | 9.93 <sup>b</sup>  | 0.338  |
| C <sub>14:1</sub> (n-5)             | 0.97 <sup>b</sup>   | 0.80 <sup>a</sup>  | 1.07 <sup>b</sup>  | 1.05 <sup>b</sup>  | 0.060  |
| C <sub>15:0</sub>                   | 1.16 <sup>b</sup>   | 0.97 <sup>a</sup>  | 1.25 <sup>c</sup>  | 1.18 <sup>bc</sup> | 0.043  |
| C <sub>16:0</sub>                   | 40.2 <sup>b</sup>   | 34.0 <sup>a</sup>  | 39.6 <sup>b</sup>  | 39.5 <sup>b</sup>  | 0.75   |
| C <sub>16:1</sub> (n-7)             | 2.23 <sup>b</sup>   | 1.87 <sup>a</sup>  | 3.41 <sup>d</sup>  | 2.48 <sup>c</sup>  | 0.107  |
| C <sub>17:0</sub>                   | 0.88                | 0.61               | 0.73               | 0.81               | 0.106  |
| C <sub>18:0</sub>                   | 12.31 <sup>c</sup>  | 15.63 <sup>d</sup> | 6.74 <sup>a</sup>  | 10.51 <sup>b</sup> | 0.828  |
| C <sub>18:1</sub>                   | 21.0 <sup>a</sup>   | 27.2 <sup>d</sup>  | 25.3 <sup>c</sup>  | 23.7 <sup>b</sup>  | 0.74   |
| C <sub>18:2</sub> (n-6)             | 2.03 <sup>c</sup>   | 1.75 <sup>a</sup>  | 2.52 <sup>d</sup>  | 1.81 <sup>a</sup>  | 0.085  |
| C <sub>18:3</sub> (n-3)             | 0.72 <sup>a</sup>   | 0.84 <sup>b</sup>  | 0.74 <sup>a</sup>  | 0.71 <sup>a</sup>  | 0.029  |
| C <sub>18:4</sub>                   | 0.002               | 0.013              | 0.00               | 0.00               | 0.007  |
| C <sub>20:0</sub>                   | 0.20                | 0.13               | 0.07               | 0.17               | 0.055  |
| C <sub>20:1</sub> (n-9)             | 0.18                | 0.21               | 0.25               | 0.15               | 0.041  |
| C <sub>20:4</sub> (n-6)             | 0.09 <sup>a</sup>   | 0.07 <sup>a</sup>  | 0.17 <sup>b</sup>  | 0.09 <sup>a</sup>  | 0.010  |
| C <sub>20:5</sub> (n-3)             | 0.09                | 0.10               | 0.11               | 0.11               | 0.012  |
| C <sub>22:1</sub> (n-9)             | 0.032 <sup>a</sup>  | 0.003 <sup>a</sup> | 0.150 <sup>b</sup> | 0.015 <sup>a</sup> | 0.016  |
| C <sub>22:5</sub> (n-3)             | 0.055 <sup>ab</sup> | 0.047 <sup>a</sup> | 0.298 <sup>c</sup> | 0.095 <sup>b</sup> | 0.023  |
| C <sub>22:6</sub> (n-3)             | 0.037 <sup>a</sup>  | 0.024 <sup>a</sup> | 0.084 <sup>c</sup> | 0.066 <sup>b</sup> | 0.007  |
| Summary                             |                     |                    |                    |                    |        |
| Total saturated                     | 72.6 <sup>c</sup>   | 67.2 <sup>a</sup>  | 66.1 <sup>a</sup>  | 69.7 <sup>b</sup>  | 0.83   |
| Total ≤C <sub>14</sub>              | 18.8 <sup>b</sup>   | 16.6 <sup>a</sup>  | 18.6 <sup>b</sup>  | 18.6 <sup>b</sup>  | 0.52   |
| Total PUFA                          | 3.03 <sup>a</sup>   | 2.84 <sup>a</sup>  | 3.92 <sup>b</sup>  | 2.89 <sup>a</sup>  | 0.104  |
| Total (n-3) PUFA                    | 0.91 <sup>a</sup>   | 1.01 <sup>b</sup>  | 1.23 <sup>c</sup>  | 0.99 <sup>ab</sup> | 0.041  |
| Total ≥C <sub>20</sub> (n-3) PUFA   | 0.186 <sup>a</sup>  | 0.169 <sup>a</sup> | 0.489 <sup>c</sup> | 0.273 <sup>b</sup> | 0.026  |
| Total trans acids                   | 1.13 <sup>a</sup>   | 2.19 <sup>ab</sup> | 10.26 <sup>c</sup> | 3.62 <sup>b</sup>  | 0.728  |
| CLA                                 | 0.16 <sup>a</sup>   | 0.28 <sup>a</sup>  | 1.55 <sup>b</sup>  | 0.52 <sup>a</sup>  | 0.154  |
| Individual trans acids              |                     |                    |                    |                    |        |
| C <sub>16:1</sub> trans-9           | 0.04 <sup>a</sup>   | 0.07 <sup>a</sup>  | 0.45 <sup>c</sup>  | 0.14 <sup>b</sup>  | 0.034  |
| C <sub>18:1</sub> trans-9           | 0.11 <sup>a</sup>   | 0.29 <sup>b</sup>  | 0.71 <sup>c</sup>  | 0.46 <sup>b</sup>  | 0.044  |
| C <sub>18:1</sub> trans-10          | 0 <sup>a</sup>      | 0.26 <sup>a</sup>  | 1.61 <sup>b</sup>  | 0.38 <sup>a</sup>  | 0.327  |
| C <sub>18:1</sub> trans-11          | 1.03 <sup>a</sup>   | 1.57 <sup>ab</sup> | 7.50 <sup>c</sup>  | 2.65 <sup>b</sup>  | 0.599  |
| C <sub>18:1</sub> trans-9 + 10 + 11 | 1.09 <sup>a</sup>   | 2.12 <sup>ab</sup> | 9.81 <sup>c</sup>  | 3.48 <sup>b</sup>  | 0.697  |

abc,d Means in a row not sharing common superscripts differ significantly ( $P < 0.05$ ).

( $P < 0.05$ ). The LIN treatment also increased the yield of milk protein compared with the value for the control ( $P < 0.05$ ). Milk citrate and urea levels were unaffected by treatment ( $P > 0.05$ ).

#### Milk fatty acid composition

Table 3 gives the mean fatty acid content of milk fat. The proportion of short-chain fatty acids in milk fat ( $\leq C_{14}$ ) was reduced by the LIN treatment compared with values for CON ( $P < 0.05$ ). All three oil supplements lowered the proportion of saturated fatty acids, the LIN and FISH oils having the greatest effect. Proportions of  $C_{16:0}$  were reduced by LIN ( $P < 0.05$ ) but the other two supplements had no effect ( $P > 0.05$ ). The FISH and TOA supplements reduced  $C_{18:0}$  proportions compared with the value for the control, FISH having the greatest effect ( $P < 0.05$ ). The LIN treatment, by contrast, increased proportions of  $C_{18:0}$  ( $P < 0.05$ ). There were significantly different levels of  $C_{18:1}$  in milk fat among all treatments ( $P < 0.05$ ); the level being lowest for CON followed by TOA, FISH and LIN. The FISH treatment gave milk containing the highest levels of  $C_{16:1}$  and  $C_{18:2}$  ( $P < 0.05$ ).

The FISH and TOA treatments increased ( $P < 0.05$ )

the percentages of total trans fatty acids in milk fat, the value for FISH being significantly higher ( $P < 0.05$ ) than for all other treatments. The pattern of response for the individual trans isomers was broadly similar. The LIN treatment caused a significant ( $P < 0.05$ ) increase in trans fatty acid proportions only in the case of the  $C_{18:1}$  trans-9 isomer. The cis-9, trans-11 isomer of CLA was the only one found in the milk samples. The FISH treatment led to a significant ( $P < 0.05$ ) increase in CLA content in milk fat compared with the value for CON. Although mean CLA values were higher for LIN and TOA than for CON, the differences were not significant ( $P > 0.05$ ) because of large between-cow variation.

Only the FISH supplement raised the proportion of total PUFA in milk ( $P < 0.05$ ) but both LIN and FISH increased proportions of (n-3) PUFA with FISH having the greatest effect ( $P < 0.05$ ). Long-chain ( $\geq C_{20}$ ) (n-3) PUFA levels were raised by the FISH and TOA supplements compared with values for the other two treatments, with FISH again giving the greatest response ( $P < 0.05$ ). For fatty acids in this group, the greatest response was for  $C_{22:5}$  (n-3) with a smaller increase for  $C_{22:6}$  (n-3).

Table 4 Fatty acid composition of plasma lipid (g/100 g total plasma fatty acids)†

| Plasma lipid component | Treatment | Fatty acids       |                   |                   |                     |                    |                           |
|------------------------|-----------|-------------------|-------------------|-------------------|---------------------|--------------------|---------------------------|
|                        |           | Trans $C_{18:1}$  | $C_{20:5}$ (n-3)  | $C_{22:5}$ (n-3)  | $C_{22:6}$ (n-3)    | TPUFA (n-3)        | TPUFA $\geq C_{20}$ (n-3) |
| Cholesteryl esters     | CON       | nd                | 1.32 <sup>a</sup> | 0.02              | 0.09 <sup>ab</sup>  | 12.6 <sup>a</sup>  | 1.43 <sup>a</sup>         |
|                        | LIN       | nd                | 1.29 <sup>a</sup> | 0.02              | 0.16 <sup>abc</sup> | 14.5 <sup>c</sup>  | 1.46 <sup>a</sup>         |
|                        | FISH      | nd                | 2.77 <sup>b</sup> | 0.03              | 0.21 <sup>c</sup>   | 14.1 <sup>bc</sup> | 3.02 <sup>b</sup>         |
|                        | TOA       | nd                | 1.36 <sup>a</sup> | 0.02              | 0.06 <sup>a</sup>   | 13.0 <sup>ab</sup> | 1.44 <sup>a</sup>         |
|                        | s.e.d.    |                   | 0.147             | 0.011             | 0.046               | 0.54               | 0.161                     |
| Triglycerides          | CON       | nd                | nd                | 0.03              | 0.05                | 0.22               | 0.16                      |
|                        | LIN       | nd                | nd                | 0.02              | 0.06                | 0.15               | 0.08                      |
|                        | FISH      | nd                | 0.02              | 0.02              | 0.08                | 0.10               | 0.05                      |
|                        | TOA       | nd                | nd                | 0.02              | 0.07                | 0.33               | 0.28                      |
|                        | s.e.d.    |                   | 0.004             | 0.009             | 0.182               | 0.173              | 0.179                     |
| Free fatty acids       | CON       | nd                | nd                | 0.03              | 0.06                | 0.16               | 0.09                      |
|                        | LIN       | nd                | 0.01              | 0.04              | 0.06                | 0.19               | 0.11                      |
|                        | FISH      | nd                | 0.02              | 0.02              | 0.03                | 0.13               | 0.07                      |
|                        | TOA       | nd                | nd                | 0.04              | 0.05                | 0.17               | 0.09                      |
|                        | s.e.d.    |                   | 0.004             | 0.011             | 0.023               | 0.037              | 0.031                     |
| Phospholipids          | CON       | 0.27 <sup>a</sup> | 1.06 <sup>a</sup> | 0.74 <sup>a</sup> | 0.37 <sup>a</sup>   | 4.19 <sup>a</sup>  | 2.17 <sup>a</sup>         |
|                        | LIN       | 0.31 <sup>a</sup> | 1.12 <sup>a</sup> | 0.78 <sup>a</sup> | 0.35 <sup>a</sup>   | 4.82 <sup>b</sup>  | 2.25 <sup>a</sup>         |
|                        | FISH      | 2.20 <sup>b</sup> | 2.51 <sup>b</sup> | 0.96 <sup>b</sup> | 0.67 <sup>b</sup>   | 5.70 <sup>c</sup>  | 4.16 <sup>b</sup>         |
|                        | TOA       | 0.38 <sup>a</sup> | 1.16 <sup>a</sup> | 0.77 <sup>a</sup> | 0.54 <sup>b</sup>   | 4.59 <sup>ab</sup> | 2.47 <sup>a</sup>         |
|                        | s.e.d.    | 0.193             | 0.101             | 0.051             | 0.040               | 0.238              | 0.165                     |

<sup>abc</sup> Treatment means in a column, within each plasma lipid component, not sharing a common superscript differ significantly ( $P < 0.05$ ). The code 'nd' indicates none or trace quantities detected.

† Key to abbreviations: TPUFA (n-3) — total (n-3) polyunsaturated fatty acid; TPUFA  $\geq C_{20}$  (n-3) — total  $\geq C_{20}$  (n-3) polyunsaturated fatty acid; trans  $C_{18:1}$  — trans-10 + trans-11  $C_{18:1}$ .

*Plasma fatty acid composition*

The cholesterol ester (CE) fraction contained the highest proportions (g/100 g total plasma lipid) of total plasma fatty acids (mean 52) followed by the phospholipid fraction (PL, mean 33). Relatively small quantities of fatty acids were found in the triglyceride (TG, mean 4) and free fatty acid fractions (FFA, mean 3). These proportions were unaffected by oil supplementation ( $P > 0.05$ ).

The concentrations of selected fatty acids in the four plasma lipid components are shown in Table 4. The FFA and TG fractions contained only very low proportions of PUFA (both total and (n-3)). Trans fatty acids were found in measurable quantities only in the PL fraction where the levels were significantly higher for FISH than for the other treatments ( $P < 0.05$ ). The FISH supplement increased concentrations of plasma  $C_{20:5}$  (n-3),  $C_{22:6}$  (n-3) and total  $\geq C_{20}$  (n-3) PUFA in CE and PL fractions compared with values for the other treatments ( $P < 0.05$ ). The TOA treatment increased concentrations of plasma  $C_{22:6}$  (n-3) in the PL fraction compared with values for the CON and LIN treatments ( $P < 0.05$ ). No CLA was detected in the plasma lipid samples, the reason for which is unknown but may be due to isomerization during the acid methylation procedure used to process these samples.

*Correlations between trans acids, CLA and milk fat*

Table 5 shows correlations between trans fatty acids, CLA and total fat in milk and between these and total trans fatty acids in plasma. Milk fat concentration was negatively correlated with trans fatty acids in milk and plasma ( $P < 0.001$ ). CLA and trans fatty acid levels in milk were very highly correlated as were trans fatty acids in milk and plasma ( $P < 0.001$ ).

**Discussion***Effects on milk fat content and fatty acid composition*

The FISH treatment caused a 10.9 g/kg reduction in milk fat concentration, a smaller reduction (3.8 g/kg) was observed for TOA, whilst LIN had no effect. These responses would be of considerable commercial significance to the producer depending on the milk pricing and quota schemes in operation.

The route and mechanisms by which dietary fatty acids are incorporated into milk have been described in detail in several review articles (Bauman and Davis, 1974; Patton and Jensen, 1975; Dils *et al.*, 1977; Moore and Christie, 1979; Mepham, 1986; Barber *et al.*, 1997). Various hypotheses have been put forward to explain milk fat depression caused by high levels of dietary starch (low fibre) or unsaturated fat. The 'glucose-insulin' theories attribute milk fat depression to reduced supply of lipid precursors to the mammary gland as a result of altered rumen fermentation patterns (reduced acetate + butyrate/propionate ratio). This would reduce *de novo* synthesis directly (less acetate and butyrate) and would reduce the supply of long-chain fatty acids by altering the partition of plasma lipids toward adipose tissue (McClymont and Vallance, 1962). However, the glucogenic theory is discredited, at least as the sole mechanism, as generally there is no relationship between serum glucose and insulin levels and milk fat depression for individual cows (Gaynor *et al.*, 1995).

Alternative theories for milk fat depression assume a direct inhibition of milk fat synthesis within the mammary gland by compound(s) derived directly from the diet or following ruminal or animal metabolism of dietary components. Inhibition could be due to increased supply of fatty acids in general or by specific fatty acids with powerful individual

**Table 5** Correlation coefficients between milk fat content and proportions of trans acids and CLA in milk and plasma lipid (no. = 36)

| Milk              | Milk           |                |                 |                 |             | Plasma |             |
|-------------------|----------------|----------------|-----------------|-----------------|-------------|--------|-------------|
|                   | $C_{16:1} t-9$ | $C_{18:1} t-9$ | $C_{18:1} t-10$ | $C_{18:1} t-11$ | total trans | CLA    | total trans |
| Fat concentration | -0.60          | -0.66          | -0.51           | -0.60           | -0.63       | -0.63  | -0.56       |
| $C_{16:1} t-9$    |                | +0.90          | +0.83           | +0.94           | +0.98       | +0.85  | +0.97       |
| $C_{18:1} t-9$    |                |                | +0.72           | +0.89           | +0.93       | +0.83  | +0.85       |
| $C_{18:1} t-10$   |                |                |                 | +0.62           | +0.77       | +0.48  | +0.78       |
| $C_{18:1} t-11$   |                |                |                 |                 | +0.98       | +0.96  | +0.95       |
| total trans       |                |                |                 |                 |             | +0.91  | +0.98       |
| CLA               |                |                |                 |                 |             |        | +0.88       |

Correlations with absolute  $r$  values  $> 0.55$  are significant  $P < 0.001$ , others are significant  $P < 0.01$ .

effects. A number of studies have implicated trans fatty acids arising from incomplete rumen biohydrogenation of PUFAS as a major cause of milk fat depression (Pennington and Davis, 1975; Selner and Schultz, 1980; Wonsil *et al.*, 1994; Gaynor *et al.*, 1995; Griinari *et al.*, 1997b). Inhibition of milk fat synthesis in the mammary gland probably occurs at one or more of the following critical points in the pathways: (i) acetyl CoA carboxylase (ii) stearoyl-CoA desaturase and (iii) acyl transferase. The general inhibitory effect of cis fatty acids (from adipose tissue or directly from the diet) on mammary lipogenesis is probably mediated mainly by the first mechanism (Barber *et al.*, 1997) but the mode of action of trans fatty acids is less certain. Askew *et al.* (1971) showed inhibition of acyl transferases by trans acids *in vitro* but there is no clear evidence to rule out the other mechanisms.

Reduction of *de novo* synthesis of fatty acids in the mammary gland would be expected to reduce the output of short-chain fatty acids ( $\leq C_{14}$ ) as these arise mainly by this route. For the LIN treatment, there was a significant reduction in the concentration of these fatty acids in milk fat but no effect on daily output of these fatty acids. This suggests that the change in milk fatty acid composition was caused mainly by the dilution effect of increased incorporation of dietary fatty acids from the supplement. The lack of a significant effect on milk citrate concentration supports this conclusion as inhibition of *de novo* synthesis is associated with increased milk citrate levels (Faulkner and Pollock, 1989). The FISH supplement did not affect proportions of total short-chain fatty acids in milk fat, although daily output of these acids was proportionately reduced by 0.23. This suggests that there was substantial inhibition of *de novo* synthesis, although there was little effect on milk citrate concentration. However, the possible inhibition of the other critical points in milk fat synthesis cannot be ruled out. Neither concentration nor yield of short chain milk fatty acids were significantly affected by the TOA supplement.

There was a significant negative correlation between milk fat content and the concentration of trans acids in plasma or milk across all treatments (see Table 5). Plasma concentrations of trans fatty acids in the present experiment should be viewed with caution because of the possibility that the acid methylation procedure used in their measurement may have caused isomerizations of  $C_{18:1}$  to occur, although no referenced work could be found to support this theory. The significant negative correlation between concentrations of trans fatty acids in the lipids of plasma or milk with milk fat concentration is consistent with the theory that increased supply of

trans fatty acids to the mammary gland depresses milk fat concentration. The involvement of trans fatty acids would explain why Storry *et al.* (1974) found that 300 g/day of protected cod liver oil (very little trans acid formation in the rumen) had no effect on short and medium fatty acid secretion in milk whilst the unprotected oil caused similar reductions in daily output to those found in the present study.

There is uncertainty as to the relative efficacy of the different trans isomers as inhibitors of milk fat synthesis. Although direct evidence is lacking, the trans-10 isomer of  $C_{18:1}$  has been implicated as the most active isomer causing reduced milk fat synthesis (Griinari *et al.*, 1997b). Newbold *et al.* (1998) found a negative correlation between milk fat content and milk trans-10  $C_{18:1}$  but not with milk trans-11  $C_{18:1}$ . Thus, milk fat depression could arise as a result of a change in rumen metabolism leading to an increased proportion of trans-10  $C_{18:1}$  even if total trans output was unaltered. Griinari *et al.* (1997a) suggest that a shift towards formation of trans-10  $C_{18:1}$  at the expense of trans-11  $C_{18:1}$  as a result of altered rumen microbial population explains the milk fat depression observed when high concentrate diets are given. In the present experiment, significant negative correlations were found between concentration of fat in milk and proportions of all three trans isomers in milk fat (trans-9, -10 and -11). However, the values for all three trans isomers were highly correlated, which was not the case in the experiment of Newbold *et al.* (1998), and treatments had no significant effects on the relative proportions of the individual trans isomers. Thus, the relationship between milk fat concentration and levels of trans-11  $C_{18:1}$  in milk fat in the present experiment could be due to the correlation of the latter with the trans-10 isomer. It is not clear what controls the relative levels of the different trans isomers and their variable correlation. Griinari *et al.* (1997a) showed that isomers of CLA infused into the abomasum are active inhibitors of fat synthesis in the mammary gland and that the trans-10, cis-12 isomer of CLA is the most active inhibitor and not the more abundant cis-9 trans-11 isomer. This may explain the relationship between milk fat level and milk trans-10  $C_{18:1}$  observed by Newbold *et al.* (1998) since a significant proportion of the trans-10, cis-12 isomer of CLA may be formed from trans-10  $C_{18:1}$  in the animal's tissues (Griinari *et al.*, 1997a).

#### Transfer of (n-3) PUFAs into milk fat

The LIN treatment supplied an extra 125 g/day of dietary (n-3)  $C_{18:3}$  but only proportionately 0.12 of this was apparently transferred to milk fat. Proportional transfers of additional total (n-3)  $C_{20:22}$  PUFAs in the FISH and TOA oils were less than 0.03.

The exception to this was for (n-3)  $C_{22:5}$  which had an apparent proportional percentage transfer of approximately 0.30 for the FISH treatment. However, much of this probably arose from chain elongation of (n-3)  $C_{20:5}$  in the mammary gland since plasma levels of the latter were markedly increased by the FISH oil which provided 40 g/day of this PUFA. Levels of (n-3)  $C_{20:5}$  and  $C_{22:6}$  were comparable with those found by Mansbridge *et al.* (1998) for an equivalent level of added fish oil. However, the latter workers found a quadratic effect of fish oil level for (n-3)  $C_{20:5}$  which suggested that efficiency of transfer increased at higher levels of oil intake (>250 g/day). This observation is supported by a number of experiments (reviewed by Palmquist *et al.*, 1993) which show that high levels (up to 0.15 of milk fatty acids) of (n-3) PUFAs can be achieved by abomasal infusion of high levels of fish oil or by feeding high levels of rumen-protected fish oil.

The poor transfer of PUFAs to milk fat found in the present study may have arisen because of their biohydrogenation in the rumen and/or because they are partitioned towards other tissues within the body. Extensive biohydrogenation of (n-3)  $C_{18:3}$  is well documented (Harfoot and Hazelwood, 1988; Wachira *et al.*, 1998) and explains the low transfer of this PUFA. However, for the  $C_{20:22}$  (n-3) PUFAs there are conflicting reports on the degree of biohydrogenation to be expected. Ashes *et al.* (1992) and N. W. Offer (unpublished) found only low (less than proportionately 0.20) extents of biohydrogenation *in vitro*. However, Wachira *et al.* (1998), using cannulated wether lambs, found 0.60 of (n-3)  $C_{20:5}$  and  $C_{22:6}$  to be hydrogenated in the rumen. Spain *et al.* (1995) found that infusion of small amounts (48 ml/day) of fish oil into the duodenum increased plasma levels of (n-3) PUFAs but that this was not the case when infused into the rumen. It seems likely that *in vitro* systems underestimate the degree of biohydrogenation of these PUFAs. The extent of biohydrogenation was not measured in the present experiment but it seems likely that a substantial proportion of  $C_{20:22}$  (n-3) PUFAs must have been absorbed, since the FISH and TOA treatments caused significant increases in the levels of these PUFAs in the plasma, and there was no evidence of the biohydrogenation products of these acids in plasma or milk.

The mammary gland obtains fatty acids from the circulation largely by the action of the enzyme lipoprotein lipase (LPL) on the TG component of chylomicrons and very-low density lipoprotein (VLDL) (Speake *et al.*, 1985; Olivecrona and Bengtsson-Olivecrona, 1987; Scow and Chernick, 1987). By contrast, the PL component of plasma

lipoprotein is a very poor substrate for LPL (Olivecrona and Bengtsson-Olivecrona, 1987). In the present study, the  $C_{20:22}$  (n-3) PUFAs in the plasma were mainly present in the PL fraction with only extremely low levels of these fatty acids carried by the TG and FFA fractions. Moreover, the feeding of fish oils (FISH and TOA) produced significant increases in the levels of  $C_{22:6}$  (n-3) in plasma PL but not in TG. Thus, the site of the  $C_{20:22}$  (n-3) PUFAs in plasma lipid fractions is a likely explanation for the very low efficiency of transfer of these fatty acids from diet to milk.

The trans fatty acids, like the long-chain (n-3) PUFAs, were almost totally confined to the plasma PL fraction, yet substantial amounts of the former were transferred into the milk. The reason for this difference is not clear but possibly these two types of fatty acid may be located on different lipoproteins subject to different tissue uptake mechanisms. This aspect requires further study.

#### Formation of trans acids in the rumen

The FISH supplement led to much greater increases in the proportions of trans  $C_{18:1}$  in the lipids of both the plasma and the milk than were found for the other oil supplements. A comparison between the FISH and TOA treatments may provide an insight into the effects of individual fatty acids on trans fatty acid formation. Intakes (g/day) of  $C_{22:6}$  (n-3),  $C_{18:2}$  (n-6) and  $C_{18:3}$  (n-3) were similar for these treatments, but intakes of  $C_{20:5}$  (n-3),  $C_{16:1}$  and  $C_{18:1}$  were higher for FISH than for TOA. The increased trans acid output in milk for FISH may have been caused by the higher intake of  $C_{20:5}$  (n-3) for this treatment, since chain elongation of  $C_{16:1}$  to  $C_{18:1}$  is unlikely. The LIN treatment provided even more  $C_{18:1}$  with much less effect on trans  $C_{18:1}$  output. The  $C_{20:5}$  (n-3) PUFA may inhibit the enzyme which catalyses the final biohydrogenation step in the rumen ( $C_{18:1}$  trans-11 to  $C_{18:0}$ ) as free  $C_{18:2}$  (n-6) has been shown to do (Harfoot, 1978). Appropriate studies *in vitro* could help to resolve this question.

#### Formation of CLA

Levels of CLA and trans acids in milk were found to be highly correlated ( $r = +0.91$ ). Formation of the conjugated dienes is the first step in the biohydrogenation pathway of  $C_{18:2}$  and occurs rapidly (Harfoot *et al.*, 1973) but it is the penultimate step (the hydrogenation of the trans monoene) that is thought to be rate limiting and subject to modification as discussed above, which would not explain the observed association between milk CLA and trans monoenes. Griinari *et al.* (1997a) observed that trans-11  $C_{18:1}$ , but not CLA, accumulates in the rumen which may explain why no measurable

quantities of CLA were detected in plasma in the present experiment, although this could also have been caused by isomerization during the acid methylation procedure (Kramer *et al.*, 1997).

It is probable that CLA is formed from trans monoenes within the animal tissues such as the mammary gland. Mahfouz *et al.* (1980) showed that the delta-9 desaturase enzyme can convert trans-11 C<sub>18:1</sub> to cis-9 trans-11 C<sub>18:2</sub> in microsomal preparations from rat liver. Infusion of trans monoenes into the abomasum (J. M. Griinari, unpublished) increased milk CLA levels and supports this hypothesis as does the close correlation between CLA and trans C<sub>18:1</sub> acids observed in the present experiment. Similar positive correlations between CLA and trans C<sub>18:1</sub> acids have been reported by Jian *et al.* (1996) for diets of varied forage:concentrate ratio.

#### Implications for human health

Assuming an average UK daily adult consumption of dairy fat of 25 g/day (Ministry of Agriculture, Fisheries and Food, 1995), the observed changes in concentrations in milk fatty acid concentration (for LIN, FISH and TOA respectively to CON) equate to additional daily intakes (mg/day) of 25, 80 and 20 total (n-3) PUFA; 0, 76 and 22 long chain ( $\geq$ C<sub>20</sub>) (n-3) PUFA; 30, 350 and 90 of CLA and 27, 2280 and 620 of total trans acids for the LIN, FISH and TOA treatments respectively.

The additional intake of  $\geq$ C<sub>20</sub> (n-3) PUFA (76 mg/day) would be approximately equivalent to that from the consumption of 0.36 g of cod liver oil. Whether this would have real benefits for human health is debatable. Judged against the range of recommended adult intakes of total (n-3) PUFA of 250-800 mg/day (BNF, 1995), the additional intake appears small. However, it would provide between 0.38 and 0.76 of the estimated minimum human requirement for C<sub>20:5</sub> (n-3) and C<sub>22:6</sub> (n-3) (100 to 200 mg/day, Farrell, 1998).

The increase in intake of CLA which would result from the consumption of milk produced with the FISH supplement appears significant. Intakes of CLA have been estimated to be in the range 50 to 180 mg/day for adults in the USA (McGuire *et al.*, 1997) although Chin *et al.* (1992) suggested that it could be as high as 1 g/day. Thus the increase in intake of 350 mg/day for the FISH treatment would be substantial relative to the basal intake of CLA. Knekt *et al.* (1996) found a reduced incidence of breast cancer in women associated with increased intake of only two glasses of milk per day which would provide only about 55 mg of CLA. However, extrapolation of rat studies, which showed anticarcinogenic responses to CLA,

suggest that as much as 3.5 g/day of CLA would be needed to promote similar benefits in humans (Ha *et al.*, 1989).

The per capita consumption of trans fatty acids is estimated to be 8 to 13 g/day for the USA (ASCN/AIN, 1996) and 4 to 6 g/day in the UK (BNF, 1995). Thus an increased intake of 2.3 g/day (assuming all milk was produced using the FISH treatment) would raise concerns because of epidemiological evidence linking trans fatty acid intake and increased risk of heart disease and cancer (e.g. Hu *et al.*, 1997). However, evidence of this type does not necessarily implicate trans acids in milk fat as risk factors for heart disease and cancer. Most (approximately 65 percent) of the trans fatty acids in the western human diet arise from hydrogenated vegetable oils in manufactured foods (ASCN/AIN, 1996) which contain mainly the trans-9 and trans-10 isomers of C<sub>18:1</sub> rather than trans-11 which predominates in milk fat. The possibility that the former two isomers are more harmful than the latter is supported by the epidemiological study of Willett *et al.* (1993). It is also possible that the CLA in milk (or other factors) protect from the potentially harmful effects of the trans fatty acids especially as their concentrations appear always to be positively correlated.

In conclusion, the use of unsaturated oils in dairy cow diets has profound effects on the concentration of fat in milk and on its fatty acid profile which are important in terms of potential effects on human health. Whilst significant increases in beneficial components, such as the (n-3) PUFAs and CLA, may be achieved, it is important to consider effects on the concentrations of trans fatty acids. Widespread adoption of these methods of milk fat manipulation should not be undertaken until the precise effects on human health have been established.

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