Supplementing dairy cow diets with oilseed preparations has been shown to replace milk saturated fatty acids (SFA) with mono- and/or polyunsaturated fatty acids (MUFA, PUFA), which may reduce risk factors associated with cardio-metabolic diseases in humans consuming milk and dairy products. Previous studies demonstrating this are largely detailed, highly controlled experiments involving small numbers of animals, but in order to transfer this feeding strategy to commercial situations further studies are required involving whole herds varying in management practices. In experiment 1, three oilseed supplements (extruded linseed (EL), calcium salts of palm and linseed oil (CPLO) and milled rapeseed (MR)) were included in grass silage-based diets formulated to provide cows with ~350 g oil/day, and compared with a negative control (Control) diet containing no supplemental fat, and a positive control diet containing 350 g/cow per day oil as calcium salt of palm oil distillate (CPO). Diets were fed for 28-day periods in a 5 × 4 Latin Square design, and milk production, composition and fatty acid (FA) profile were analysed at the end of each period. Compared with Control, all lipid supplemented diets decreased milk fat SFA concentration by an average of 3.5 g/100 g FA, by replacement with both cis- and trans-MUFA/PUFA. Compared with CPO, only CPLO and MR resulted in lower milk SFA concentrations. In experiment 2, 24 commercial dairy farms (average herd size ± SEM 191 ± 19.3) from the south west of the United Kingdom were recruited and for a 1 month period asked to supplement their herd diets with either CPO, EL, CPLO or MR at the same inclusion level as the first study. Bulk tank milk was analysed weekly to determine FA concentration by Fourier Transform mid-IR spectroscopy prediction. After 4 weeks, EL, CPLO and MR all decreased herd milk SFA and increased MUFA to a similar extent (average −3.4 and +2.4 g/100 g FA, respectively) when compared with CPO. Differing responses observed between experiments 1 and 2 may be due in part to variations in farm management conditions (including basal diet) in experiment 2. This study demonstrates the importance of applying experimental research into commercial practice where variations in background conditions can augment different effects to those obtained under controlled conditions.

Keywords: milk fatty acids, saturated fatty acids, linseed, rapeseed

Implications

Saturated fatty acids (SFA) are thought to increase the risk of cardiovascular disease and in many Western diets, milk/dairy products are a major source of SFA. Supplementing dairy cow diets with oilseeds decreases milk SFA content, but to date this has not been demonstrated under both experimental and commercial conditions. This study showed that at low levels, oilseed supplements can have meaningful effects on milk fatty acid (FA) profiles (without affecting cow performance) both experimentally and commercially. Therefore, these oilseed preparations could all be used in dairy cow feeding as a part of an overall strategy for replacing SFA in the human diet.

Introduction

There is evidence that cardiovascular disease risk can be reduced by the isoenergetic replacement of SFA with cis-monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA) in the human diet (Vafeiadou et al., 2015). In the United Kingdom, milk and dairy products contribute about 25% and 28% of total SFA consumed by men and women, respectively (Bates et al., 2014), with higher contributions in other countries (Hulshof et al., 1999). However, instead of advocating population-wide decreases in milk and dairy product consumption, altering the FA composition of milk and dairy products by replacing SFA with MUFA and/or PUFA offers an opportunity to lower SFA intake while maintaining the contribution of these foods to the balanced human diet.
Supplementing dairy cow diets with oilseed preparations is an effective means of replacing milk fat SFA with unsaturated FA (Glasser et al., 2008). Kliem et al. (2011) reported a reduction in SFA from 67 to 57 g/100 g FA, whilst increasing cis-MUFA from 25 to 33 g/100 g FA after cows consumed around 1.2 kg oil as milled high oleic acid rapeseed/day. A study involving extruded linseed (EL) reported a change in SFA from 75 to 57 g/100 g FA, with SFA being replaced by MUFA (20 to 34 g/100 g FA) and PUFA (2.5 to 5.7 g/100 g FA) after feeding 960 g oil from the supplement (Ferlay et al., 2010). However, these studies involved feeding high levels of oilseeds, which may decrease milk fat and protein concentration, especially over longer periods (Lerch et al., 2012a). In addition, costs may restrict feeding at these levels on a herd basis.

To our knowledge there are few published studies investigating the effects of supplemental oilseeds on milk FA composition in commercial herds. Stergiadis et al. (2014) investigated the effects of full-fat rolled linseed or rapeseed supplementation in two groups of cows over 6-week periods, but fed 1.25 kg/cow per day of rapeseed or 1.5 kg/cow per day linseed, which may not be practical.

The objective of this study was to investigate whether selected oilseed supplements, when fed at similar oil intake, resulted in decreased SFA and increased unsaturated FA concentrations in milk fat, in both a controlled, experimental study and on commercial farms with the normal variation associated with commercial practice.

Material and methods

Experiment 1: individual cow study

Experimental design, animals and management. All experimental procedures used were licensed, regulated and inspected by the UK Home Office under the Animals (Scientific Procedures) Act, 1996. Five multiparous Holstein-Friesian cows of mean ± standard error parity 3.6 ± 0.93, milk yield 33.4 ± 1.18 l/day and 216 ± 8.8 days in lactation were used. Animals were randomly allocated to one of five treatments in a 5 × 4 Latin Square design experiment with 28-day periods. Cows were housed in a cubicle yard with rubber chip bedding. In the cubicle yard individual feeding was achieved using an electronic identification system and pneumatic feed barrier (Insetec, Marknesse, the Netherlands). Clean water was constantly available via a trough system. Cows were provided for the study by BOCM Pauls Ltd. The MR supplement was manufactured by crushing rapeseed in a hammer mill using wheat feed as a carrier in proportions of 75 : 25 on a fresh weight basis, respectively. Diets were formulated to be isonitrogenous (Table 1). Cows were offered diets as equal meals at 0830 and 1600 h.

Experimental diets. Diets were offered as total mixed rations (TMR; forage : concentrate ratio 50 : 50 on a dry matter (DM) basis) with the forage consisting of maize silage and grass silage (250 and 750 g/kg of forage DM, respectively; Table 1). Treatments consisted of a basal diet (Control), with the concentrate portion containing a minimal amount (6.8 g/kg DM) of a commercial fat supplement (calium salts of palm oil FA (CPO); Megalac®, Volac International Ltd, Royston, UK). Treatment diets were the basal diet with the addition of 350 g oil/cow per day supplied as CPO (17 g/kg DM), EL (55 g/kg DM; Lintec, BOCM Pauls Ltd, Wherstead, Suffolk, UK), CPO (28 g/kg DM; CPLO; Flaxpro, Volac International Ltd), or milled rapeseed (MR, 39 g/kg DM; provided for the study by BOCM Pauls Ltd). The MR supplement was manufactured by crushing rapeseed in a hammer mill using wheat feed as a carrier in proportions of 75 : 25 on a fresh weight basis, respectively. Diets were formulated to be isonitrogenous (Table 1). Cows were offered diets as equal meals at 0830 and 1600 h.

Experimental sampling. Individual forage components of experimental diets and the TMR were sampled daily and added to a weekly composite sample. Oven DM contents

| Ingredients | CPO | EL | CPLO | MR |
|-------------|-----|----|------|----|
| Maize silage | 120 | 120 | 120 | 120 |
| Grass silage | 360 | 360 | 360 | 360 |
| Grass hay | 15 | 15 | 15 | 15 |
| Straw | 15 | 15 | 15 | 15 |
| Concentrate mix | 416 | 416 | 416 | 416 |
| Soya bean meal | 9 | 16 | 0 | 8 |
| Soy hulls | 55 | 31 | 9 | 29 |
| Sodium bicarbonate | 4 | 4 | 4 | 4 |
| Salt | 4 | 4 | 4 | 4 |
| Limestone | 2 | 2 | 2 | 2 |
| CPO | 0 | 17 | 0 | 0 |
| EL | 0 | 0 | 55 | 0 |
| CPLO | 0 | 0 | 28 | 0 |
| MR | 0 | 0 | 0 | 39 |
| Chemical composition | | | | |
| DM (g/kg fresh) | 597 | 599 | 598 | 598 |
| Organic matter | 884 | 881 | 884 | 884 |
| CP | 185 | 186 | 185 | 189 |
| NDF | 348 | 333 | 328 | 339 |
| ADL | 207 | 195 | 191 | 199 |
| Starch | 133 | 133 | 136 | 137 |
| Water soluble carbohydrates | 25.4 | 26.9 | 26.1 | 25.8 |
| ME (MJ/kg DM) | 11.8 | 12.0 | 12.1 | 11.7 |
| Fatty acids | | | | |
| 18:0 | 6.6 | 13.7 | 7.1 | 9.7 |
| 18:1 cis-9 | 0.78 | 1.37 | 1.15 | 1.32 |
| 18:2 n-6 | 6.1 | 11.5 | 7.9 | 12.3 |
| 18:3 n-3 | 8.6 | 10.2 | 9.3 | 10.6 |
| Total fatty acids | 25.4 | 26.9 | 26.1 | 25.8 |

CPO = calcium salts of palm oil distillate; EL = extruded linseed; CPLO = calcium salts of palm and linseed oil distillate; MR = milled rapeseed; DM = dry matter; ME = metabolisable energy; DDGS = dried distillers grains with solubles.

1 Containing (g/kg DM): cracked wheat, 102; DDGS wheat, 43; soya bean meal 67; rapeseed meal, 73; palm kernel meal, 22; molasses sugar beet feed, 32; soya hulls, 30; Megalac®, 7; molasses, 17; urea, 5; minerals (KW Alternative Feeds Ltd, Barrow Hill Barns, Andover, UK), 9.

2 Dairy Direct, Church Farm, Bury St Edmunds, UK.
Table 2 Summary of commercial farms enrolled onto experiment 2

| Diet group | CPO | EL | CPLO | MR | SEM |
|------------|-----|----|------|----|-----|
| No. of farms | 5 | 6 | 6 | 5 | – |
| Average herd size | 119 | 213 | 180 | 141 | 19.3 |
| Average number of cows in milk | 252 | 111 | 804 | 8313 | 267.7 |
| Average grass silage : maize silage in forage portion of diet (fresh weight basis) | 57:43 | 75:25 | 55:45 | 59:41 | – |
| Ratio of all-year-round: seasonal calving herds | 2:3 | 5:1 | 2:4 | 4:1 | – |

CPO = calcium salts of palm oil distillate; EL = extruded linseed; CPLO = calcium salts of palm and linseed oil distillate; MR = milled rapeseed.

1SEM for n = 22 values.

2Information obtained from National Milk Records or from farms directly before the study.
Experiment 1: individual cow study

Overall diets were readily consumed with cows maintaining satisfactory DMI (24.0 kg DM/day) and milk yields (30.6 kg/day) throughout the study. Chemical analysis of the five treatment diets showed little difference in most components (Table 1). FA analysis of TMR subsamples demonstrated that the CPO diet was highest in 16:0 and 18:0, MR diet highest in cis-9 18:1 and 18:2 n-6 and CPLO diet highest in 18:3 n-3 (Table 1). Overall, there was little difference between the total FA content of CPO, CPLO and MR, whereas that of EL (and Control) was lower (Table 1).

There was no effect of treatment on DMI, milk yield or milk composition (Table 3), but an effect of diet ($P < 0.001$) was observed for FA intake. Cows fed CPO consumed the highest ($P < 0.05$) amounts of 16:0 and 18:0, and those fed MR consumed greater ($P < 0.05$) quantities of cis-9 18:1. The greatest ($P < 0.05$) amount of 18:2 n-6 and 18:3 n-3 was consumed by cows fed CPLO (Table 3).

Diet affected milk fat short and medium chain SFA concentrations (Table 4), with CPLO decreasing ($P < 0.05$) 8:0 and 10:0 concentrations. The lipid supplemented diets all decreased ($P < 0.05$) 14:0 and 15:0 concentrations when compared with Control, and there were variations in concentrations of 13:0 iso, 14:0 iso and 15:0 anteiso depending on treatment diet. There was no difference in 16:0 concentration between Control and CPO, but the other treatments all lowered ($P < 0.05$) 16:0 concentration compared with both Control and CPO. In contrast 18:0 was increased ($P < 0.05$) when EL, CPLO and MR were fed

### Table 3 Effect of lipid supplement on dry matter and fatty acid intake, and milk and constituent yield in experiment 1 (least square mean results)

|                        | Control | CPO      | EL       | CPLO     | MR       | SEMa   | Period | Diet     |
|------------------------|---------|----------|----------|----------|----------|--------|--------|----------|
| DM intake (kg/day)     | 24.1    | 23.6     | 24.1     | 24.1     | 24.2     | 0.79   | 0.005  | 0.870    |
| Fatty acid intake (g/day) | 16:0    | 157d     | 320a     | 172d     | 234b     | 194c   | 8.6    | 0.027    | <0.001   |
|                        | 18:0    | 18.7d    | 32.0a    | 27.8b    | 31.9a    | 25.3c  | 0.95   | 0.012    | <0.001   |
|                        | 18:1 cis-9 | 145a   | 270c     | 191d     | 297b     | 330a   | 9.0    | 0.011    | <0.001   |
|                        | 18:2n-6 | 208d     | 239c     | 224c     | 355a     | 271b   | 8.0    | 0.005    | <0.001   |
|                        | 18:3n-3 | 106d     | 104d     | 160b     | 192a     | 130c   | 4.5    | 0.008    | <0.001   |
| Total fatty acids      | 781c    | 1112a    | 917b     | 1159b    | 1111a    | 35.0   | 0.007  | 0.001    |
| Yield                  |         |          |          |          |          |        |        |          |
| Milk (kg/day)          | 30.1    | 30.2     | 30.5     | 31.5     | 30.6     | 1.20   | 0.470  | 0.770    |
| Fat (g/day)            | 1104    | 1161     | 1130     | 1161     | 1133     | 71.9   | 0.257  | 0.569    |
| Protein (g/day)        | 962     | 949      | 970      | 1004     | 968      | 37.6   | 0.050  | 0.454    |
| Lactose (g/day)        | 1354    | 1347     | 1377     | 1425     | 1382     | 58.5   | 0.364  | 0.734    |
| Concentration (g/kg)   |         |          |          |          |          |        |        |          |
| Fat                    | 37.1    | 38.4     | 37.4     | 36.8     | 36.8     | 2.17   | 0.287  | 0.064    |
| Protein                | 32.0    | 31.6     | 31.6     | 31.8     | 31.9     | 0.71   | 0.136  | 0.902    |
| Lactose                | 44.8    | 44.3     | 45.2     | 45.3     | 45.3     | 0.95   | 0.448  | 0.276    |

DM = dry matter.

a,b,c,dWhere there is an overall diet effect, values within rows with differing superscripts are significantly ($P < 0.05$) different.

1CPO, EL, CPLO and MR are diets containing 350 g oil/day equivalent of calcium salts of palm oil, extruded linseed, calcium salts of palm and linseed oil and milled rapeseed, respectively.

2Refers to the significance of overall effect of period and diet.

SEM for $n = 20$ measurements.
| Fatty acid                | Control | CPO | EL | CPLO | MR | SEM² | Period | Diet |
|--------------------------|---------|-----|----|------|----|------|--------|------|
| 4.0                      | 2.63    | 2.55| 2.68| 2.71 | 2.64| 0.054| 0.021  | 0.194|
| 6.0                      | 1.76    | 1.65| 1.76| 1.64 | 1.72| 0.056| 0.109  | 0.185|
| 8.0                      | 1.14    | 1.04| 1.10| 0.97 | 1.13| 0.063| 0.026  | 0.035|
| 10.0                     | 2.86    | 2.50| 2.75| 2.22 | 2.63| 0.186| 0.019  | 0.029|
| 10:1 cis-9               | 0.28    | 0.25| 0.27| 0.26 | 0.25| 0.020| <0.001 | 0.122|
| 12.0                     | 3.78    | 3.29| 3.57| 2.97 | 3.42| 0.231| 0.035  | 0.059|
| 12:1 cis-9               | 0.10    | 0.09| 0.09| 0.09 | 0.09| 0.008| 0.017  | 0.264|
| 13.0                     | 0.11    | 0.07| 0.10| 0.08 | 0.09| 0.008| 0.120  | 0.080|
| 13:0 iso                 | 0.032   | 0.030| 0.035| 0.027| 0.029| 0.002| 0.036  | <0.001|
| 14.0                     | 11.4    | 10.3| 10.9| 10.3| 10.6| 0.40 | 0.003  | 0.015|
| 14:0                     | 3.14    | 3.25| 2.87| 2.79 | 2.72| 0.65 | 0.594  | 0.022|
| 15:0                     | 0.56    | 0.48| 0.51| 0.45 | 0.46| 0.019| 0.045  | <0.001|
| 16.0                     | 11.4    | 10.3| 10.9| 10.3| 10.6| 0.40 | 0.033  | 0.017|
| 16:0 iso                 | 1.14    | 0.90| 1.00| 0.91 | 0.93| 0.054| 0.075  | 0.003|
| 17:0 iso                 | 0.56    | 0.48| 0.49| 0.46 | 0.48| 0.018| 0.009  | 0.009|
| 17:1 iso                 | 0.36    | 0.33| 0.34| 0.34 | 0.34| 0.018| 0.002  | 0.032|
| 17:1 cis-9               | 0.16    | 0.16| 0.16| 0.14 | 0.15| 0.012| 0.779  | 0.481|
| 18.0                     | 9.76    | 8.91| 11.19| 11.69| 13.20| 0.42 | 0.008  | 0.007|
| 18:0 iso                 | 0.048   | 0.078| 0.057| 0.126| 0.086| 0.0234| 0.178  | 0.151|
| 18:1 trans total         | 2.84    | 3.11| 3.30| 4.07 | 3.30| 0.153| 0.221  | 0.006|
| 18:2total                | 2.94    | 2.94| 3.26| 3.17| 2.85| 0.155| 0.107  | 0.006|
| 20.0                     | 0.15    | 0.14| 0.16| 0.14 | 0.15| 0.012| 0.779  | 0.481|
| 20:1 cis-11              | 0.55    | 0.49| 0.49| 0.47 | 0.48| 0.029| 0.25   | 0.024|
| 20:1 cis-13              | 0.21    | 0.17| 0.18| 0.16 | 0.19| 0.018| 0.206  | 0.009|
| 20:2 n-6                 | 0.54    | 0.43| 0.49| 0.46 | 0.48| 0.018| 0.002  | <0.001|
| 20:3 n-3                 | 0.36    | 0.33| 0.34| 0.34 | 0.34| 0.018| 0.002  | 0.032|
| 20:4 n-6                 | 0.16    | 0.16| 0.16| 0.14 | 0.15| 0.012| 0.779  | 0.481|
| 20:5 n-3                 | 0.59    | 0.52| 0.52| 0.52 | 0.59| 0.044| 0.116  | <0.001|
| 30:5 total               | 2.94    | 2.94| 3.26| 3.17| 2.85| 0.155| 0.107  | 0.006|
| CLA total                | 0.032   | 0.029| 0.029| 0.023| 0.023| 0.0057| 0.398  | 0.012|
| 30:5 n-3                 | 0.59    | 0.52| 0.52| 0.52 | 0.59| 0.044| 0.116  | <0.001|
| ΣSFA                     | 68.0    | 66.3| 65.6| 62.8 | 65.1| 0.90 | 0.073  | 0.021|
| ΣMUFA                    | 23.7    | 21.8| 24.0| 20.8 | 22.4| 0.86 | 0.060  | 0.215|
| ΣPUFA                    | 4.11    | 4.32| 4.84| 5.68 | 4.66| 0.172| 0.080  | <0.001|
| Σtrans MUFA              | 3.40    | 3.62| 3.87| 4.72 | 3.91| 0.154| 0.098  | 0.003|
| Σcis MUFA                | 23.8    | 25.1| 25.0| 27.2 | 26.3| 0.73 | 0.137  | 0.046|
| Σn-6 PUFA                | 2.53    | 2.57| 2.60| 2.49 | 2.37| 0.16 | 0.113  | 0.012|
| Σn-3 PUFA                | 0.91    | 0.81| 1.37| 1.22 | 0.90| 0.048| 0.141  | <0.001|
| n-6:3                     | 2.71    | 3.12| 3.02| 2.96 | 2.93| 0.075| 0.609  | <0.001|

**SFA** = saturated fatty acids; **MUFA** = monounsaturated fatty acids; **PUFA** = polyunsaturated fatty acids.

ab,c Where there is an overall diet effect, values within rows with differing superscripts are significantly (P < 0.05) different.

1CPO, EL, CPLO and MR are diets containing 350 g oil/day equivalent of calcium salts of palm oil, extruded linseed, calcium salts of palm and linseed oil and milled rapeseed, respectively.

2Refers to the significance of overall effect of period and diet.

3SEM for n = 20 measurements.

4Co-elutes with cis-15 18:1.

5All 18:2 isomers excluding CLA.

6Co-elutes with cis-15 18:1.

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compared with Control and CPO. This led to an overall effect of treatment diet ($P = 0.02$) on total SFA, but only CPLO and MR were lower ($P < 0.05$) than Control.

CPLO and MR increased ($P < 0.05$) total cis-MUFA compared with Control (Table 4), mainly due to numerical increases in cis-9 18:1 (Table 5). In addition, total trans MUFA concentrations were greater ($P < 0.05$) with EL, CPLO and MR compared with Control. This was mainly due to enhanced concentrations of some of the trans-18:1 isomers, notably trans-4 18:1, trans-6-8 18:1, trans-9 18:1, trans-12 18:1 and trans-16 18:1 (Table 5).

Treatment diet also affected milk fat PUFA concentration. Total n-6 PUFA concentration was lower ($P < 0.05$) with MR compared with the other diets, mainly due to a lower concentration of cis-9, cis-12 18:2 (Table 6). There were also differences in concentrations of minor n-6 PUFA, with EL enhancing ($P < 0.05$) cis-9, trans-12 and trans-9, trans-12 18:2 (Table 6) compared with the other diets. Concentrations of total n-3 PUFA were greater ($P < 0.05$) with EL and CPLO compared with Control, CPLO and MR (Table 4), largely due to 18.3 n-3 concentration, but also 20.5 n-3 (Table 4) and trans-11, cis-15 18:2 concentrations (Table 6). The greatest

### Table 5 Effect of lipid supplement on milk fat 18:1 isomer composition in experiment 1 (least square mean results as g/100 g fatty acids)

| Fatty acid | Control | CPO | EL | CPLO | MR | SEM | Period | Diet |
|------------|---------|-----|----|------|----|-----|--------|------|
| trans-4 18:1 | 0.003<sup>d</sup> | 0.026<sup>b</sup> | 0.018<sup>c</sup> | 0.041<sup>a</sup> | 0.034<sup>ab</sup> | 0.0038 | 0.052 | <0.001 |
| trans-5 18:1 | 0.050 | 0.055 | 0.068 | 0.018 | 0.098 | 0.0505 | 0.572 | 0.584 |
| trans-6-8 18:1 | 0.28<sup>d</sup> | 0.33<sup>c</sup> | 0.32<sup>c</sup> | 0.44<sup>a</sup> | 0.40<sup>b</sup> | 0.015 | 0.020 | <0.001 |
| trans-9 18:1 | 0.21<sup>d</sup> | 0.26<sup>c</sup> | 0.30<sup>b</sup> | 0.34<sup>a</sup> | 0.30<sup>c</sup> | 0.017 | 0.235 | <0.001 |
| trans-10 18:1 | 0.48 | 0.47 | 0.45 | 0.58 | 0.54 | 0.074 | 0.211 | 0.082 |
| trans-11 18:1 | 0.99 | 1.00 | 0.97 | 1.27 | 1.09 | 0.09 | 0.049 | 0.186 |
| trans-12 18:1 | 0.40<sup>c</sup> | 0.44<sup>bc</sup> | 0.52<sup>a</sup> | 0.56<sup>a</sup> | 0.50<sup>ab</sup> | 0.046 | 0.599 | 0.006 |
| trans-15 18:1 | 0.37 | 0.34 | 0.52 | 0.36 | 0.34 | 0.038 | 0.386 | 0.265 |
| cis-9 18:1<sup>1</sup> | 18.8 | 20.9 | 20.4 | 21.7 | 21.5 | 0.73 | 0.084 | 0.070 |
| cis-11 18:1 | 0.61 | 0.60 | 0.59 | 0.60 | 0.71 | 0.034 | 0.597 | 0.067 |
| cis-12 18:1 | 0.25<sup>b</sup> | 0.26<sup>b</sup> | 0.40<sup>b</sup> | 0.37<sup>a</sup> | 0.30<sup>b</sup> | 0.031 | 0.606 | 0.005 |
| cis-13 18:1 | 0.09 | 0.07 | 0.12 | 0.11 | 0.09 | 0.016 | 0.425 | 0.154 |
| cis-16 18:1<sup>1,4</sup> | 0.057<sup>c</sup> | 0.069<sup>b</sup> | 0.089<sup>a</sup> | 0.089<sup>a</sup> | 0.072<sup>b</sup> | 0.0053 | 0.174 | <0.003 |

<sup>a,b,c,d</sup>Where there is an overall diet effect, values within rows with differing superscripts are significantly ($P < 0.05$) different.

<sup>1</sup>CPO, EL, CPLO and MR are diets containing 350 g oil/day equivalent of calcium salts of palm oil, extruded linseed, calcium salts of palm and linseed oil and milled rapeseed, respectively.

<sup>2</sup>Refers to the significance of overall effect of period and diet.

<sup>3</sup>SEM for $n = 20$ measurements.

<sup>4</sup>Co-elutes with 18:1 cis-14.

<sup>1</sup>CPO, EL, CPLO and MR are diets containing 350 g oil/day equivalent of calcium salts of palm oil, extruded linseed, calcium salts of palm and linseed oil and milled rapeseed, respectively.

<sup>2</sup>Refers to the significance of overall effect of period and diet.

<sup>3</sup>SEM for $n = 20$ measurements.

### Table 6 Effect of lipid supplement on milk fat 18:2 isomer composition in experiment 1 (least square mean results as mg/100 g fatty acids)

| Fatty acid | Control | CPO | EL | CPLO | MR | SEM<sup>3</sup> | Period | Diet |
|------------|---------|-----|----|------|----|--------|--------|------|
| trans-11, trans-15 18:2 | 74.4<sup>a</sup> | 66.8<sup>c</sup> | 94.0<sup>a</sup> | 81.4<sup>b</sup> | 78.6<sup>bc</sup> | 6.41 | 0.007 | 0.010 |
| trans-9, trans-12 18:2 | 2.36<sup>a</sup> | 0.00<sup>b</sup> | 12.55<sup>a</sup> | 2.36<sup>b</sup> | 2.73<sup>b</sup> | 1.96 | 0.095 | 0.010 |
| cis-9, trans-12 18:2 | 20.7<sup>a</sup> | 26.7<sup>bc</sup> | 45.0<sup>a</sup> | 34.1<sup>b</sup> | 28.5<sup>bc</sup> | 3.49 | 0.013 | 0.004 |
| cis-9, trans-13 18:2 | 201<sup>b</sup> | 194<sup>d</sup> | 301<sup>a</sup> | 296<sup>a</sup> | 230<sup>b</sup> | 33.1 | 0.199 | 0.009 |
| cis-9, trans-14 18:2 | 84.0<sup>b</sup> | 84.9<sup>b</sup> | 136.3<sup>a</sup> | 112.3<sup>a</sup> | 65.0<sup>b</sup> | 13.6 | 0.675 | 0.008 |
| cis-10, trans-14 18:2 | 143 | 130 | 129 | 130 | 131 | 17.1 | 0.877 | 0.975 |
| trans-9, cis-12 18:2 | 29.8 | 29.9 | 40.9 | 40.2 | 29.2 | 2.11 | 0.578 | 0.212 |
| trans-11, cis-15 18:2 | 101<sup>b</sup> | 106<sup>b</sup> | 191<sup>a</sup> | 203<sup>a</sup> | 116<sup>b</sup> | 13.3 | 0.612 | <0.001 |
| cis-9, cis-12 18:2 | 2231<sup>ab</sup> | 2344<sup>a</sup> | 2240<sup>ab</sup> | 2164<sup>bc</sup> | 2079<sup>c</sup> | 164.2 | 0.010 | 0.026 |

<sup>a,b</sup>Where there is an overall diet effect, values within rows with differing superscripts are significantly ($P < 0.05$) different.

<sup>1</sup>CPO, EL, CPLO and MR are diets containing 350 g oil/day equivalent of calcium salts of palm oil, extruded linseed, calcium salts of palm and linseed oil and milled rapeseed, respectively.

<sup>2</sup>Refers to the significance of overall effect of period and diet.

<sup>3</sup>SEM for $n = 20$ measurements.
(P < 0.05) total CLA concentration was observed after CPLO was fed (Table 4) when compared with the other treatment diets.

Experiment 2: commercial farm study
The supplements used in the commercial farm study varied in terms of their FA profile. The CPO supplement contained the most 16:0 (48 g/100 g FA) compared with CPLO, EL and MR at 19, 7 and 5 g/100 g FA, respectively. MR was highest in cis-9 18:1 (57 g/100 g FA), followed by CPLO, CPO and EL at 40, 36 and 17 g/100 g FA, respectively. The greatest proportion of 18:2 n-6 was in MR (20 g/100 g FA), and EL was richest in 18:3 n-3 (51 g/100 g FA) compared with CPLO, MR and CPO with 22, 10 and <0.5 g/100 g FA, respectively.

There was no effect (P > 0.05) of treatment group or time on average yield sold from the farms per cow, with yields (±SEM) for the beginning and end of the experimental periods of: CPO 30.8 v. 31.4 (±3.45) l/cow, EL 28.5 v. 29.9 (±2.44) l/cow, CPLO 26.5 v. 27.1 (±2.67) l/cow and MR 23.5 v. 24.4 (±3.00) l/cow. Over the 4-week experimental period there was no overall diet effect on milk fat (P = 0.071) and protein (P = 0.122) concentrations (Figure 1a and b). There was, however, an effect of week for fat (P = 0.033) and protein (P < 0.001). There was an overall effect (P = 0.010)

![Figure 1](image-url)  
**Figure 1** Effect of lipid supplement on (a) bulk tank milk fat (g/100 g milk), (b) bulk tank milk protein (g/100 g milk), (c) bulk tank milk saturated fatty acid concentration (g/100 g fatty acids), (d) bulk tank monounsaturated fatty acid concentration (g/100 g fatty acids) and (e) bulk tank milk polyunsaturated fatty acid concentration (g/100 g fatty acids) of commercial dairy herds over a 4-week period (experiment 2). CPO, EL, CPLO and MR are diets containing 350 g oil/day equivalent of calcium salts of palm oil, extruded linseed, calcium salts of palm and linseed oil and milled rapeseed, respectively.
of diet on total SFA (Figure 1c). There was no difference \( (P = 0.149) \) between weeks 4 and 0 for CPO, but EL, CPLO and MR all decreased \( (P < 0.001) \) total SFA over the 4-week period (average decrease of 3.40 g/100 g FA, or 4.9\%). When comparing the mean of treatments within week 4, there was no difference \( (P > 0.05) \) between EL, CPLO and MR. For total MUFA there was an overall diet effect \( (P = 0.022; \text{Figure 1d}) \). Again, the CPO diet did not change MUFA between weeks 0 and 4 \( (P = 0.986) \) but EL, CPLO and MR all increased \( (P < 0.001) \) total MUFA (average increase at week 4 of 2.38 g/100 g FA, or 8.8\%), with no difference \( (P > 0.05) \) between total MUFA means for EL, CPLO and MR at week 4. Diet affected \( (P < 0.001) \) total PUFA concentration (Figure 1e), with EL, CPLO and MR all increasing \( (P < 0.05) \) PUFA over the 4-week period. At week 4 total PUFA concentration was highest \( (P < 0.01) \) in farms feeding EL, with the concentration for CPLO and MR farms being similar \( (P = 0.899) \).

### Discussion

Feeding linseed and rapeseed oil supplements is an effective strategy for decreasing milk fat SFA and increasing unsaturated FA concentrations (Glasser et al., 2008). However, effectiveness depends on the oil content of the oilseed, and the amount consumed by the cow. Studies have shown that consuming large amounts of crushed rapeseed results in a substantial decrease in SFA concentration, with a concomitant increase in unsaturated FA (e.g. Kliem et al., 2011). Even at modest levels \( (i.e. <500 \text{ g oil/cow per day}) \), supplementation with ground rapeseed and ground linseed decreased milk total SFA concentration (Collomb et al., 2004; Egger et al., 2007; Chen et al., 2008). However, it is still unknown whether lower levels, despite being more sustainable at a commercial farm level, would influence bulk milk FA profile for an entire herd. This knowledge is critical for the development of feeding strategies that will affect the entire milk pool, and therefore the milk and dairy product-consuming public.

In the present studies, the target oil intake was 350 g/cow per day. This was the maximum amount of supplement practical to feed taking into account the oil content of each supplement. Most studies investigating effects of oilseeds on milk FA profile have utilised a higher intake \( (e.g. >500 \text{ g oil/cow per day}) \) of oil (Glasser et al., 2008), but caution must be exerted at high inclusion levels due to the negative effect on the rumen function and cow performance (Lock and Shingleton, 2004). Increasing the amount fed in the present studies to for example 1 kg oil/cow per day would have meant feeding a total of 1.2 kg CPO, 4 kg EL, 2 kg CPLO and 3.3 kg MR supplements. Feeding incremental levels of MR up to 1250 g oil/cow per day \( (5.5 \text{ kg MR/day}) \) had no effect on DMI and milk yield and composition over a 28-day period (Kliem et al., 2011), but an earlier study involving feeding 1214 g oil/cow per day as whole cracked rapeseed decreased DMI, milk yield and milk fat yield (Givens et al., 2003). As the present study objective was to investigate levels of supplementation that are practical to feed taking into account the oil content of each supplement, it was not feasible to supplement at higher levels.

Feeding 350 g oil equivalent of all lipid supplements had no effect on DMI, milk yield or milk composition when compared with a control diet containing no lipid supplement, in agreement with previous studies whereby cows were supplemented with oilseeds supplying similar amounts of oil (Collomb et al., 2004; Oeffner et al., 2013). This is particularly important when developing a sustainable strategy for use on commercial dairy farms. Increasing the energy density of the diet \( (e.g. \text{by feeding supplemental oilseeds}) \) can sometimes decrease DMI, which can decrease milk yield (Chilliard et al., 2009), but this response varies with oilseed form and amount fed. Supplemental oilseeds can also decrease milk fat and protein concentration. ELs can depress milk fat synthesis when included in a diet higher in NDF content \( (420 \text{ to } 430 \text{ g/kg DM}, \text{Gonthier et al., 2005}; 308 \text{ g/kg DM}, \text{Chilliard et al., 2009}; 397 \text{ g/kg DM}, \text{Ferlay et al., 2010}) \) but have little effect when included in low NDF-containing diets \( (174 \text{ g/kg DM}, \text{Oeffner et al., 2013}) \), which may reflect the effect of linseed oil on fibre fermentation in the rumen. Experiment 1 in the current study involved TMR diets containing \( \sim330 \text{ g/kg DM NDF} \). However, at the level of oil fed a negative impact on milk fat secretion was unlikely. Experiment 2 resulted in a decreased fat concentration in bulk milk from EL farms after 4 weeks. This may reflect the basal diets fed, as this group fed the highest average ratio of grass silage : maize silage, but diets were not analysed for chemical composition, and detailed dietary information was unfortunately unavailable for some farms in this group. Earlier studies including MR in dairy cow diets reported no effect on milk fat and protein concentrations, at intake levels of up to 1345 g oil/cow per day (Givens et al., 2009; Kliem et al., 2011).

The lack of difference between EL and Control/CPO in terms of total SFA concentration in experiment 1 appeared to be due to enhanced \( 18:0 \) concentration despite decreased \( 16:0 \). The other linseed-containing supplement (CPLO) exerted a greater effect on total SFA, mainly due to the numerical decrease in SFA \( \leq 14:0 \), which is in agreement with an earlier study involving supplementation of calcium salts of linseed oil (Brzózska, 2006). A similar effect in SFA \( \leq 14:0 \) was observed in the present study when comparing Control with CPO. Intake of total FA from CPLO was greater than that of EL, which suggests that a greater quantity of longer chain PUFA escaped rumen metabolism and inhibited mammary synthesis \( (\text{via inhibition of acetyl CoA carboxylase activity}) \) of shorter chain SFA (Barber et al., 1997). The MR treatment appeared to only affect \( 14:0 \) and \( 16:0 \) concentration when compared with both Control and CPO, which is in agreement with earlier studies (Givens et al., 2009; Kliem et al., 2011). In contrast to experiment 1, experiment 2 saw all three oilseed supplements decrease total SFA over a 4-week period, when compared with CPO. The disparity between studies may be due to differences in the basal diets of farms enrolled.
in experiment 2, which were not under experimental control, or indeed average lactation stage of cows consuming supplements, which varied depending on farm due to differences in calving pattern. The interaction between oilseed supplementation and basal diet has been reported previously (Sterk et al., 2011), and a lower rumen pH (e.g. for high starch diets) affects a shift in rumen biohydrogenation pathways so that biohydrogenation of dietary MUFA/PUFA is less complete (Palmquist et al., 2005). The average forage: concentrate ratio across all farms (excluding three who were unable to provide this information) was 64:36, so overall a greater proportion of forage than in experiment 1. In addition, although the aim during experiment 2 was that each cow consumed 350 g/day additional oil, in reality this may not have occurred. Stage of lactation will affect DMI, and farms involved in experiment 2 were a mixture of all-year-round- and autumn-calving herds, meaning that, perhaps for some cows, all of the supplement was not consumed. Conversely, depending on feeding system, some cows may have had the opportunity to consume more than 350 g/day oil. This highlights the importance of conducting trials on commercial farms to verify experimental results.

The greatest milk fat concentration of cis-MUFA was observed for the CPL0 diet, which reflected milk fat cis-9 18:1 concentration. This was probably derived from both cis-9 18:1 intake, and via increased rumen outflow of 18:0 that was subsequently desaturated by mammary Δ9 desaturase, following biohydrogenation of dietary PUFA (for CPLO the daily intake of 18:2 n-6 + 18:3 n-3: was the greatest PUFA intake across all diets). Supplementation with the linseed-based supplements also increased cis-12 18:1 and cis-16 18:1 concentrations, which tend to be elevated following linseed supplementation (Lerch et al., 2012b) and are biohydrogenation intermediates of 18:3 n-3 (Shingfield et al., 2010). Increases in the concentration of individual trans-18:1 isomers following CPLO supplementation reflect the higher intake of PUFA. Trans-6-8 and trans-9 18:1 concentrations were greater in milk from CPLO and MR-fed cows than those supplemented with EL. This may be due to the increased intake of cis-9 18:1 on the CPLO and MR diets, as trans-7 and trans-9 18:1 largely originate from isomerisation of cis-9 18:1 in the rumen (Mosley et al., 2002). All three oilseed diets increased trans-12 18:1, which is an intermediate of cis-9 18:1, 18:2 n-6 and 18:3 n-3 biohydrogenation (Shingfield et al., 2010). There was no difference (P > 0.1) in milk fat total MUFA concentration between the CPO diet and those containing oilseed supplements. However, in experiment 2 the same supplements increased MUFA concentration after 4 weeks compared with CPO, when fed at the same rate. Again, this could be related to basal diet and/or consumption rate. Increasing the proportion of concentrates in the diet (from 60:40 to 40:60 forage:concentrate) resulted in a greater increase in total MUFA following EL supplementation (Neveu et al., 2013).

EL appeared to increase the concentration of most trans-18:2 isomers to a greater extent than the other supplements. A proportion of cis-9, trans-13 18:2 is synthesised endogenously by the action of mammary Δ9 desaturase on trans 18:1 (Shingfield et al., 2008), and trans-11, cis-15 18:2 is an intermediate of rumen 18:3 n-3 metabolism (Shingfield et al., 2010). Supplementing cow diets with 350 g oil as CPL0 resulted in the greatest increase in total trans FA compared with both the Control and the CPO diet. Supplementing with similar quantities of rapeseed oil (Halmemies-Beauchet-Filleau et al., 2011; Jacobs et al., 2011) and linseed oil (Jacobs et al., 2011) resulted in much greater concentrations of total trans FA.

The effectiveness of an oilseed supplement for increasing concentrations of milk PUFA depends on the PUFA content of the supplement, as well as its physical form. Transfer efficiency of 18:3 n-3 (daily yield of 18:3 n-3 from milk as a percentage of 18:3 n-3 intake) following EL supplementation is generally greater compared with supplementing as linseed oil (Chilliard et al., 2009), perhaps due to a degree of rumen protection by the EL matrix. In experiment 1, 18:3 n-3 transfer efficiency due to EL was greater (P < 0.001) than that of CPL0 (6.3% v. 4.6%, respectively). This, coupled with the greater concentration in milk fat trans FA following the CPL0 diet, suggests that the rumen inertness of PUFA in CPL0 was lower. However, there was no difference (P = 0.721) between EL and CPL0 in terms of transfer efficiency of 18:2 n-6. Rumen stability of calcium soaps of FA is assumed to be inversely related to the degree of FA unsaturation (Chouinard et al., 1998), so it is possible that 18:2 n-6 within the CPL0 diet may have experienced partial rumen protection.

The objective of the present experiments was to demonstrate whether inclusion of oilseed supplements in dairy cow diets at a commercially viable level could have meaningful impact on the FA profile of milk, in terms of decreasing SFA and increasing cis-MUFA and cis-PUFA. Results from experiment 1 suggest SFA can be decreased by an average of between 1.8 (compared with CPO) and 3.5 (compared with control diet) g/100 g FA. However, compared with CPO, oilseed supplements decreased bulk milk SFA concentration by on average 3.4 g/100 g in the commercial study. A decrease of this magnitude if applied to average UK winter (October to February) milk with a SFA concentration of 71.3 g/100 g FA (Kliem et al., 2013) would have small impact on UK adult SFA consumption if applied to all dairy products consumed (change from 13.4% to 13.2% and 12.9% to 12.7% food energy intake for men and women, respectively; intake data derived from Bates et al., 2014). Nonetheless it would be a move in the right direction, and would remove SFA from the food chain whilst replacing them with MUFA and PUFA, albeit with the inclusion of small proportions of trans FA. At current intake levels ruminant-derived trans FA are thought not to have negative effects on human health (Mozaffarian, 2006) although further research is required to inform on the isomer-specific effects of ruminant-derived trans FA (Gebauer et al., 2011).

In conclusion, results from these two experiments were in broad agreement, in that inclusion of modest amounts of different oilseed-based supplements in dairy cow diets
decreased milk SFA concentration by replacement with unsaturated FA. This effect can be observed in a change-over study involving five cows, and also a continuous study involving 3081 cows, and thus demonstrates successful transfer of scientific principle to commercial practice.

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