

# Differential effects of oilseed supplements on methane production and milk fatty acid concentrations in dairy cows

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*It is known that supplementing dairy cow diets with full-fat oilseeds can be used as a strategy to mitigate methane emissions, through their action on rumen fermentation. However, direct comparisons of the effect of different oil sources are very few, as are studies implementing supplementation levels that reflect what is commonly fed on commercial farms. The objective was to investigate the effect of feeding different forms of supplemental plant oils on both methane emissions and milk fatty acid (FA) profile. Four multiparous, Holstein-Friesian cows in mid-lactation were randomly allocated to one of four treatment diets in a 4 × 4 Latin square design with 28-day periods. Diets were fed as a total mixed ration with a 50 : 50 forage : concentrate ratio (dry matter (DM) basis) with the forage consisting of 75 : 25 maize silage : grass silage (DM). Dietary treatments were a control diet containing no supplemental fat, and three treatment diets containing extruded linseed (EL), calcium salts of palm and linseed oil (CPLO) or milled rapeseed (MR) formulated to provide each cow with an estimated 500 g additional oil/day (22 g oil/kg diet DM). Dry matter intake (DMI), milk yield, milk composition and methane production were measured at the end of each experimental period when cows were housed in respiration chambers for 4 days. There was no effect of treatment diet on DMI or milk protein or lactose concentration, but oilseed-based supplements increased milk yield compared with the control diet and milk fat concentration relative to control was reduced by 4 g/kg by supplemental EL. Feeding CPLO reduced methane production, and both linseed-based supplements decreased methane yield (by 1.8 l/kg DMI) and intensity (by 2.7 l/kg milk yield) compared with the control diet, but feeding MR had no effect on methane emission. All the fat supplements decreased milk total saturated fatty acid (SFA) concentration compared with the control, and SFA were replaced with mainly cis-9 18:1 but also trans FA (and in the case of EL and CPLO there were increases in polyunsaturated FA concentration). Supplementing dairy cow diets with these oilseed-based preparations affected milk FA profile and increased milk yield. However, only the linseed-based supplements reduced methane production, yield or intensity, whereas feeding MR had no effect.*

**Keywords:** linseed, rapeseed, bovine, saturated fatty acids, trans fatty acids

## Implications

Feeding supplemental fat to ruminants decreases enteric methane emission, but there are relatively few direct comparisons of the effects of feeding different fat sources. In the present study not all oilseed sources decreased methane emissions when fed at the same level, despite effects on milk fatty acid (FA) profile for all the supplements fed. Therefore, higher feeding levels may be required to achieve both lower methane emissions and improved milk FA profile.

## Introduction

Currently there is considerable interest in developing nutritional strategies to reduce methane emissions by ruminant food-producing animals. It is well established that feeding supplemental fat (excluding calcium salts) to ruminants can reduce methane production, both on a daily and dry matter intake (DMI) basis (Beauchemin *et al.*, 2009; Martin *et al.*, 2010; Grainger and Beauchemin, 2011), the main reason being that supplemental lipids provide metabolizable energy (ME) to the diet which is not fermented, therefore reducing excess hydrogen available for methane synthesis. It is also suggested that lipid supplements rich in monounsaturated

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fatty acids (MUFA) or polyunsaturated fatty acids (PUFA) provide an alternative to methane synthesis for hydrogen disposal in the rumen (Clapperton, 1974; Fievez *et al.*, 2003). In addition, some FA can have direct toxic effects on cellulolytic microbes and fibre digestion, thereby reducing methanogenesis (Martin *et al.*, 2010). These microbial changes result in a shift in fermentation pattern towards propionate, which reduces hydrogen available for methane synthesis. It has been suggested that the effectiveness of lipid supplements to reduce methane production is inversely proportional to the degree of saturation of the component FA (Giger-Reverdin *et al.*, 2003). However previous studies have demonstrated little difference between MUFA- and PUFA-rich supplements in their ability to decrease methane emissions (Beauchemin *et al.*, 2009), and it is thought that the form of the lipid fed (and therefore rumen availability) is possibly more important (Martin *et al.*, 2008).

There has long been interest in feeding oilseed supplements to decrease milk saturated FA (SFA) by replacement with MUFA and/or PUFA (Kliem and Shingfield, 2016), as it has been shown that milk and dairy products contribute substantially to adult SFA consumption in European countries (Hulshof *et al.*, 1999). Current evidence is inconsistent for the effect of dairy SFA in particular on cardiovascular disease risk (Lovegrove and Givens, 2016). However the impact of dietary SFA on blood cholesterol is indisputable (Givens, 2008). Effectiveness of oilseed supplements for decreasing milk SFA concentration is dependent on source and form of oilseed (Glasser *et al.*, 2008; Kliem and Shingfield, 2016). Greater effects are observed if greater amounts are fed (e.g. ca. 1.2 kg oil/cow per day; Givens *et al.*, 2003), however negative effects on DMI, milk yield and milk composition mean that this strategy is less likely to be practical on commercial farms. Significant decreases in milk SFA concentration compared with control diets can be obtained by feeding oilseeds at more modest (e.g. 350 to 400 g oil/day) levels (Collomb *et al.*, 2004; Kliem *et al.*, 2016), and recent evidence demonstrates that this strategy can successfully be transferred to commercial practice (Kliem *et al.*, 2016). However a review of available literature suggests that these low levels of lipid supplementation (around 2 g/kg DM) may have little impact on methane production (Martin *et al.*, 2010), and feeding growing or lactating cattle either 260 or 280 g oil/day as extruded linseed (EL) had no significant effect on methane emissions (Hammond *et al.*, 2015; Livingstone *et al.*, 2015). The review of Martin *et al.* (2010) also highlighted a lack of direct comparisons between oilseed types on methane emissions, with most studies utilising different forms of the same oilseed.

The objective of our study was therefore to investigate whether different selected oilseed supplements, when fed to provide similar increases in diet oil concentration had any impact on both milk FA concentrations and methane emissions of lactating dairy cows.

## Material and methods

### *Experimental design, animals and management*

Four multiparous Holstein-Friesian cows of mean  $\pm$  standard error parity  $4.0 \pm 0.82$ , milk yield  $45.8 \pm 1.27$  kg/day and  $169 \pm 14.4$  days in lactation were used. Animals were randomly allocated to one of four treatments in a  $4 \times 4$  Latin square design experiment with 28-day periods. As only two cows could be housed in respiration chambers at any one-time cows started the experiment in pairs, staggered by 1 week. During weeks 1 to 3 of each period cows adapted to diet changes whilst kept in an open yard bedded on rubber mats and wood shavings, and individual feeding was achieved using an electronic identification system and pneumatic feed barrier (Insentec, Marknesse, The Netherlands). Fresh water was available *ad libitum*. During week 4 of each period cows were transferred to respiration chambers and held in individual tie stalls for four  $\times$  24 h measurements of methane emission and feed intake were obtained as described in detail previously (Reynolds *et al.*, 2014; Hammond *et al.*, 2016). The methane analysers were calibrated at the beginning and end of each daily measurement period. At the time of the present study measured CO<sub>2</sub> recoveries for the two chambers averaged 101.2% and 100.8%. Although in the chambers cows were restrained using head yokes, bedded using rubber mats and wood shavings, had continuous access to drinking water through drinking bowls, and were milked at 0530 and 1600 h.

### *Experimental diets*

Diets were offered *ad libitum* (fed for 10% refusals) as total mixed rations (TMR; forage : concentrate ratio 50 : 50 on a DM basis) with the forage consisting of maize silage and grass silage (750 and 250 g/kg of forage DM, respectively). Treatments consisted of a control diet (control) containing no added fat source, or similar diets with the addition of 22 g oil/kg DM as either EL (86 g/kg DM; Lintec, BOCM Pauls Ltd, Wherstead, Suffolk, UK), calcium salts of palm and linseed oil (CPLO) FA (44 g/kg DM; Flaxpro, Volac International Ltd, Royston, UK) or milled rapeseed (MR) (59 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. These were the same supplements as those used in the study of Kliem *et al.* (2016) and supplemented diets were formulated to achieve an increase in oil intake of 500 g/day at 22 kg predicted DMI.

Diets were formulated to be isonitrogenous and contain similar levels of NDF (Table 1), with supplemental oil primarily replacing starch from ground wheat and increasing diet ME concentration relative to the control diet. Cows were offered diets at 0900 h (2/3) and 1600 h (1/3). Refused feed was removed and weighed before the morning feeding.

### *Experimental sampling*

Individual forage components of experimental diets, the concentrate portion and TMR were sampled on days 22 to 27

**Table 1** *Ingredients and measured chemical composition of experimental diets fed to lactating dairy cows (g/kg dry matter (DM) or as stated)*

	Treatments <sup>1</sup>			
	Control	EL	CPO	MR
<b>Ingredients</b>				
Maize silage	370	370	370	370
Grass silage	120	120	120	120
Grass hay	10	10	10	10
Straw	10	10	10	10
Cracked wheat	110	65	60	72
DDGS wheat	43	43	43	43
Soyabean meal	76	67	74	69
Rapeseed meal	76	67	74	69
Palm kernel meal	32	32	32	32
Molassed sugar beet feed	32	32	32	32
Soyabean hulls	84	61	94	78
Molasses	17	17	17	17
Bicarbonate	4	4	4	4
Salt	4	4	4	4
Limestone	2	2	2	2
Minerals	9	9	9	9
Extruded linseed	0	86	0	0
Calcium salt of linseed and palm oil	0	0	44	0
Milled rapeseed	0	0	0	59
<b>Chemical composition</b>				
DM (g/kg fresh)	574	559	562	549
Organic matter	911	883	896	898
CP	177	174	180	170
NDF	359	336	348	391
ADF	215	202	214	246
Starch	199	174	166	160
Water soluble carbohydrates	29	11.6	15.5	26.8
Predicted ME (MJ/kg DM)	11.9	12.3	12.4	12.3
<b>Fatty acids</b>				
16:0	4.5	5.9	10.9	5.5
18:0	0.7	1.4	1.6	1.1
<i>cis</i> -9 18:1	5.7	8.9	11.8	18.3
18:2n-6	12.0	15.7	14.6	15.8
18:3n-3	2.7	15.0	8.7	5.2
Total fatty acids	32	53	54	54

EL = extruded linseed; CPO = calcium salts of palm and linseed oil; MR = milled rapeseed; DDGS = distillers' dried grains with solubles.

<sup>1</sup>Where EL, CPO and MR are diets containing ~500 g oil/day equivalent of extruded linseed, calcium salts of palm and linseed oil and milled rapeseed, respectively.

of each experimental period and added to a composite sample. Forage DM concentrations were determined daily by drying at 100°C for 23 h to ensure that the DM composition of experimental diets was maintained. Refused feed was removed before the morning feeding and weighed daily; fresh weights were recorded and during week 4 of each period a weekly composite of refused feed was dried at 60°C for 48 h to determine individual daily DM intake (DMI). Samples of dietary components, TMR and refusals (if appreciable) were retained at -20°C for chemical analysis.

Cows were milked twice daily, at 0530 and 1600 h. When in respiration chambers cows were milked using a pipeline system into buckets and milk yield determined gravimetrically and recorded. Samples of milk for the determination of fat, protein and lactose concentration were collected during the last 6 days of each experimental period, treated with potassium dichromate preservative (1 mg/ml; Lactabs, Thompson & Capper, Runcorn, UK), and held at 4°C until analyzed. Additional samples of milk were collected from two consecutive milkings during the last 24 h of each experimental period and stored at -20°C until composited using proportions based on milk yield immediately prior to FA analysis.

#### Chemical analysis

Chemical composition of oven dried (60°C), milled (1 mm screen) samples of forages and concentrates were determined using methods described and referenced by Kliem *et al.* (2008) for NDF, ADF, organic matter, CP, water soluble carbohydrates, starch and FA concentrations. Feed FA quantification was achieved using methyl heneicosanoate (H3265, Sigma-Aldrich Company Ltd, Dorset, UK) in toluene as an internal standard.

Milk fat, CP and lactose were determined by mid-IR spectroscopy (Foss Electric Ltd, York, UK). Lipid in 1 ml milk was extracted, transesterified and resulting FA methyl esters (FAME) separated using the methods of Kliem *et al.* (2013). Carbon deficiency in the flame ionization detector response for FAME containing 4- to 10-carbon atoms was accounted for using a combined correction factor which also converted FAME to FA (Ulberth *et al.*, 1999). All milk FA results were expressed as g/100 g total FA.

#### Statistical analysis

Intake, milk production, milk composition, methane production and milk FA composition data obtained during the 4 days of methane emission measurements were averaged for each cow and period ( $n = 16$ ) and analyzed using the mixed procedure of SAS (Statistical Analysis Systems software package version 8.2; SAS Institute, Cary, NC, USA) and models testing fixed effects of period and treatment and random effect of cow, with period as a repeated effect within cow, and the Kenward Rogers option used for denominator degrees of freedom. Compound symmetry, heterogeneous compound symmetry, first-order autoregressive or a heterogeneous first-order regressive covariance structures were used for repeated measures analysis, based on goodness of fit criteria (Bayesian Information Criteria, BIC) for each variable analyzed. Each treatment mean was compared with the control diet using Dunnett's comparisons. Least square means  $\pm$  SEM are reported and treatment effects are considered significant at  $P \leq 0.05$ .

#### Results

Analysis of the supplements confirmed the FA profile of each, with CPO containing the greatest amount of 16:0 (146 g/kg

DM compared with 18 and 21 g/kg DM for EL and MR, respectively) and 18:0 (18 g/kg DM compared with 8.0 and 5.0 g/kg DM for EL and MR, respectively). The MR supplement contained the most (208 g/kg DM and 72 g/kg DM) *cis*-9 18:1 and 18:2n-6, whereas EL contained the most (145 g/kg DM) 18:3n-3, closely followed by CPO (138 g/kg DM). Total FA contents of each supplement were 263, 386 and 501 g/kg DM for EL, MR and CPO, respectively.

Differences were observed in FA profile of the TMR diets (Table 1). The CPO diet contained approximately double the amount of 16:0 than the other diets (Table 1). The MR diet contained the most *cis*-9 18:1, whereas the EL diet contained the most 18:3n-3. As intended, including these supplements caused an increase in total FA content of the diet when compared with the control diet (Table 1).

There was no effect ( $P=0.441$ ) of treatment diets on DMI (Table 2). There were however effects on intakes of individual FA (Table 2). Intake of 16:0 was the highest ( $P<0.05$ ) for CPO followed by EL. Cows on all three supplement diets consumed more ( $P<0.001$ ) 18:0 than those on the control diet (Table 2). Supplementation increased ( $P<0.001$ ) the intake of *cis*-9 18:1, 18:2n-6, 18:3n-3 and total FAs when compared with the control (Table 2).

Including oilseed-based supplements increased ( $P=0.010$ ) daily milk yield. However there were no treatment effects ( $P>0.05$ ) on milk component yields (Table 2) apart from lactose yield which increased ( $P=0.009$ ) following EL supplementation when compared with the control. There were no effects of supplements on milk component concentration except for an 11% decrease in fat concentration when EL was fed, compared with control (Table 2).

Daily methane production (l/day) was reduced ( $P=0.012$ ) by 10% by the CPO diet compared with control (Table 3), and both linseed-based supplements reduced methane production per kg DMI (by on average 7%;  $P<0.03$ ) and per kg milk yield (by on average 15%;  $P<0.002$ ) compared with control. In contrast feeding MR had no effect ( $P=0.886$ ) on methane emissions.

Short- and medium-chain FA concentrations in milk fat were affected by treatment diet (Table 4). Concentrations of 8:0, 10:0, 14:0, 15:0 and 16:0 were all lower ( $P<0.05$ ) following supplementation when compared with the control diet, which contributed towards an overall lower ( $P=0.029$ ) concentration of short- and medium-chain ( $\leq 14:0$ ) SFA. Conversely 18:0 concentration was increased ( $P=0.001$ ) following supplementation (more so for rapeseed- than linseed-based diets). Despite this there was still an overall reduction in concentration of total SFA when compared with the control diet (average decrease of 8.3 g/100 g FAs).

Oilseed supplementation increased ( $P=0.008$ ) *trans*-9 16:1 but decreased ( $P<0.05$ ) *cis*-9 10:1, *cis*-9 12:1 and *cis*-9 16:1 concentrations (Table 4). There were changes in other MUFA concentrations, such that most *trans*-18:1 isomers and *cis*-13 18:1 and *cis*-16 18:1 increased (Table 5) following supplementation with EL, CPO and MR compared with the control diet. This resulted in an overall increase in both total *cis*- and *trans*-MUFA.

Concentrations of PUFA in milk fat were also affected by supplementation. There was an effect of diet ( $P=0.035$ ) on 18:3n-3, where EL increased the concentration three-fold (to 0.98 g/100 g FA) compared with the control diet (Table 4). This resulted in an increased ( $P<0.05$ ) concentration of total

**Table 2** Effect of oilseed supplementation of dairy cow diets on dry matter and fatty acid intake, and milk and constituent yield (least square mean results, units as specified)

	Treatments <sup>1</sup>				SEM	P diet <sup>2</sup>
	Control	EL	CPO	MR		
DM intake (kg/day)	21.4	21.5	20.9	21.9	1.18	0.441
Fatty acid intake (g/day)						
16:0	97.6	129.8*	222.1*	123.1	8.12	0.035
18:0	15.7	29.1*	32.1*	23.9*	1.42	<0.001
<i>cis</i> -9 18:1	119	201*	248*	390*	14.0	<0.001
18:2n-6	255	341*	299*	348*	14.2	<0.001
18:3n-3	63.2	320.7*	178.3*	113.2*	7.93	<0.001
Total fatty acids	688	1161*	1113*	1194*	54.2	<0.001
Yield						
Milk (kg/day)	31.5	34.5*	33.5*	33.6*	1.92	0.010
Fat (g/day)	1142	1140	1228	1204	195.0	0.158
Protein (g/day)	1027	1055	1032	1054	52.2	0.995
Lactose (g/day)	1369	1530*	1454	1427	161.0	0.043
Concentration (g/kg)						
Fat	36.4	32.4*	35.5	35.3	4.19	0.056
Protein	32.5	30.8	30.8	31.4	0.72	0.137
Lactose	43.2	43.5	42.8	42.6	2.21	0.252

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

<sup>1</sup>Where EL, CPO and MR are diets containing ~500 g oil/day equivalent of extruded linseed, calcium salts of palm and linseed oil and milled rapeseed, respectively.

<sup>2</sup>Overall effect of treatment diet.

\*Within rows treatments are different ( $P<0.05$ ) from the control based on Dunnett's pdiff test.

**Table 3** Effect of oilseed supplementation of dairy cows diets on methane production (least square mean results, units as specified)

	Treatments <sup>1</sup>				SEM	P diet <sup>2</sup>
	Control	EL	CPLO	MR		
CH <sub>4</sub> (l/day)	598	560	539*	601	42.4	0.025
CH <sub>4</sub> (l/kg of DMI)	28.0	25.7*	26.2*	27.8	2.01	0.035
CH <sub>4</sub> (l/kg of milk)	19.1	16.2*	16.4*	18.3	1.41	0.003

EL = extruded linseed; CPLO = calcium salts of palm and linseed oil; MR = milled rapeseed; DMI = dry matter intake.

<sup>1</sup>Where EL, CPLO and MR are diets containing ~500 g oil/day equivalent of extruded linseed, calcium salts of palm and linseed oil and milled rapeseed, respectively.

<sup>2</sup>Overall effect of treatment diet.

\*Within rows treatments are different ( $P < 0.05$ ) from the control based on Dunnett's pdiff test.

n-3 PUFA for EL (and CPLO) treatments (Table 4). A similar increase was observed in the concentration of total n-6 PUFA (Table 4), due to increased ( $P < 0.05$ ) concentrations of *trans*-9, *trans*-12 18:2, *cis*-9, *trans*-12 18:2 and *trans*-9, *cis*-12 18:2 isomers after cows were fed the EL diet compared with control (Table 6). Increases ( $P < 0.05$ ) were also observed in other 18:2 isomers such as *trans*-11, *cis*-15 18:2 and *cis*-9, *trans*-13 18:2 when EL was fed compared with the control diet (Table 6).

## Discussion

Dietary strategies to mitigate methane emissions by dairy cows need to be commercially practical, and not have any negative impact upon milk production and composition. Feeding linseed- and rapeseed-based supplements has been shown to be an effective strategy for decreasing methane emissions from ruminants (Martin *et al.*, 2010) as well as decreasing milk fat SFA/increasing unsaturated fatty acid concentrations (Glasser *et al.*, 2008). However, the effectiveness depends upon the oil concentration of the supplement, the supplement form and the amount of supplement fed, and must be balanced with any negative effects on cow production and health.

In the current study, supplementing cow diets with 22 g oil/kg DM in the form of EL, CPLO and MR had no effect on DMI and increased milk yield when compared with a control diet containing no supplemental oil. There are a plethora of older studies reporting positive effects of feeding supplemental fats on milk yield (Palmquist and Jenkins, 2017), but the milk yield response depends on DMI, which is in part dependent on the degree of saturation of the lipid fed (Palmquist and Jenkins, 2017). As reported by Firkins and Eastridge (1994), negative effects of fat supplements on DMI are typically greater as the iodine value (unsaturation) of the lipid fed increases. Inconsistent effects of supplemental plant oils on milk yield reported in the literature may also be due to the amounts fed. At lower supplementation levels ( $\leq 500$  g oil/day), unsaturated plant oils have been shown to increase milk yield (AlZahal *et al.*, 2008) or have no effect (Collomb

*et al.*, 2004; Kliem *et al.*, 2016) when compared with control diets containing no supplemental oil. At higher intake levels ( $> 500$  g oil/day) both DMI and milk yield can be decreased (Chilliard *et al.*, 2009), but not always (Kliem *et al.*, 2011). Feeding higher levels of oil supplements ( $\geq 50$  g oil/kg DM) can have a negative impact on ruminal and total tract organic matter and NDF digestion (Firkins and Eastridge, 1994). In addition, stage of lactation/production level can also affect the milk yield response to lipid supplementation, with cows in early lactation or of higher genetic merit being more likely to show positive milk yield responses to supplementation (Grainger and Beauchemin, 2011). In the present study the supplemented diets were formulated to have an increased ME concentration, so as there was no effect of treatments on DMI the increased milk yield following oilseed supplementation can be attributed to the increased provision of energy provided by the supplements.

The effect of oilseed supplementation on milk composition is also dependent on type, form and amount of oilseed fed. In general, feeding plant oils in their partially disrupted seed form has less impact on milk fat and protein concentration than feeding plant oils *per se* (Beauchemin *et al.*, 2009; Givens *et al.*, 2009; Kliem *et al.*, 2011), possibly due to a degree of rumen protection inferred by seed components. In a recent study at our location (Livingstone *et al.*, 2015), feeding EL at a lower level than in the present study had no effect on milk fat concentration in diets containing greater than 300 g NDF/kg DM. However, in the present study the EL supplement caused a decrease in milk fat concentration, similar to that observed by Chilliard *et al.* (2009), after feeding a similar amount of EL. However a later study reported no effect of EL supplementation (560 g oil/day) on milk fat concentration when fed in a diet with low NDF content (174 g/kg DM; Oeffner *et al.*, 2013). This suggests that in addition to the amount and form of the plant oil fed, basal diet composition (e.g. NDF concentration) can also influence the response of milk fat concentration to supplemental plant oils.

Only the CPLO supplement decreased methane production (l/day; Table 3). Both linseed-containing supplements also decreased methane yield and intensity, whereas there was no effect of MR on methane emissions. A previous study reported a decrease in methane yield (g/kg DMI) after feeding 750 g oil/cow per day as crushed linseed and canola when compared with a control diet, but no effect was observed with crushed sunflower seed (Beauchemin *et al.*, 2009). The differences in effects of oilseed supplements on methane production observed in the present study is unlikely to be due to the degree of unsaturation of supplemental oils; intake of PUFA (18:2n-6 + 18:3n-3) was highest for the EL group, but those of CPLO and MR were comparable. In addition, complete biohydrogenation of 1 mol 18:3n-3 will only spare 0.75 mol CH<sub>4</sub> (Martin *et al.*, 2010). It has been reported that 18:3n-3 has a greater toxicity to cellulolytic bacteria than 18:2n-6 (Maia *et al.*, 2007), which can result in a shift in rumen fermentation towards propionate, and thus an increase in hydrogen utilization. Cows consuming the EL

**Table 4** Effect of oilseed supplementation of dairy cow diets on milk fatty acid composition (least square mean results as g/100 g fatty acids)

Fatty acids	Treatments <sup>1</sup>				SEM	P diet <sup>2</sup>
	Control	EL	CPLO	MR		
4:0	2.54	2.78	2.65	2.75	0.184	0.497
6:0	1.64	1.61	1.48	1.75	0.150	0.128
8:0	1.07	0.87*	0.89*	1.06	0.087	0.033
10:0	2.78	2.06*	2.11*	2.49	0.193	0.052
<i>cis</i> -9 10:1	0.28	0.20*	0.22*	0.22*	0.028	0.025
12:0	3.77	2.80	2.87	3.26	0.208	0.154
<i>cis</i> -9 12:1	0.11	0.07*	0.07*	0.07*	0.009	0.024
13:0	0.11	0.06	0.05	0.06	0.020	0.506
13:0 iso	0.03	0.03	0.03	0.03	0.002	0.740
13:0 anteiso	0.10	0.07	0.06*	0.07	0.010	0.113
14:0	12.7	10.3*	10.4*	11.4	0.343	0.060
14:0 iso	0.08	0.07	0.07	0.08	0.006	0.509
<i>trans</i> -9 14:1	0.29	0.23	0.23	0.23	0.016	0.400
<i>cis</i> -9 14:1	1.13	0.95*	0.92*	0.92*	0.071	0.077
15:0	1.08	0.87*	0.87*	0.80*	0.070	0.020
15:0 anteiso	0.53	0.48	0.45*	0.47*	0.039	0.072
16:0	32.8	23.5*	30.6*	25.7*	1.37	<0.001
16:0 iso	0.21	0.17	0.19	0.20	0.022	0.403
<i>trans</i> -6 + 7 + 8 16:1	0.040	0.054	0.055	0.059	0.0096	0.523
<i>trans</i> -9 16:1	0.040	0.082*	0.055	0.068*	0.0115	0.013
<i>trans</i> -11 + 12 + 13 16:1	0.16	0.20*	0.18	0.19	0.020	0.154
<i>cis</i> -9 16:1 <sup>3</sup>	1.29	1.01*	1.21	0.97*	0.072	0.012
<i>cis</i> -11 16:1	0.51	0.50	0.48	0.48	0.043	0.59
<i>cis</i> -13 16:1	0.21	0.13	0.12	0.11*	0.006	0.079
17:0	0.53	0.49	0.40	0.43	0.034	0.350
17:0 iso	0.36	0.40*	0.33	0.35	0.035	0.024
18:0	9.7	13.3*	11.2	14.5*	0.48	0.002
18:0 iso	0.03	0.04	0.04	0.04	0.010	0.755
<i>trans</i> 18:1 total	3.0	5.7*	3.4*	4.8*	0.34	0.008
<i>cis</i> 18:1 total	17.7	24.8*	22.7*	22.5*	1.66	0.049
Non-CLA 18:2 total <sup>4</sup>	2.4	3.7*	2.9*	2.4	0.26	0.001
CLA total <sup>5</sup>	0.43	0.77*	0.60*	0.69*	0.077	0.006
18:3n-6	0.033	0.013*	0.020	0.025	0.0059	0.181
18:3n-3	0.31	0.98*	0.43	0.52	0.116	0.035
19:0 <sup>6</sup>	0.07	0.15	0.08	0.10	0.011	0.246
20:0	0.16	0.16	0.16	0.24*	0.006	<0.001
<i>cis</i> -8 20:1	0.12	0.05	0.04	0.05	0.029	0.456
<i>cis</i> -11 20:1	0.04	0.07	0.09	0.10*	0.016	0.166
20:2n-6	0.03	0.04	0.03	0.03	0.004	0.133
20:3n-6	0.11	0.07	0.13	0.11	0.018	0.327
20:3n-3	0.05	0.02	0.02	0.03	0.015	0.566
20:4n-6	0.12	0.11	0.16	0.12	0.026	0.685
20:5n-3	0.04	0.06	0.09	0.03	0.028	0.655
22:0	0.06	0.04	0.03	0.04	0.025	0.870
22:4n-6	0.04	0.02	0.03	0.02	0.005	0.090
22:5n-3	0.10	0.11	0.09	0.06	0.017	0.219
Σ SFA	72.2	60.0*	65.3*	66.5*	2.06	<0.001
Σ SFA<=14:0	24.9	23.0	20.4*	21.2	1.62	0.019
Σ <i>trans</i> total	4.1	8.4*	5.0*	6.1*	0.51	0.034
Σ <i>trans</i> -MUFA	3.6	6.3*	3.9*	5.5*	0.37	0.017
Σ <i>cis</i> -MUFA	20.8	27.5*	25.4	24.8	1.67	0.085
Σ n-6 PUFA	2.3	2.5*	2.5*	2.2	0.17	0.015
Σ n-3 PUFA	0.65	1.78*	0.87*	0.78	0.143	<0.001
n-6:n-3	3.7	1.3*	3.0	3.1	0.24	0.008

EL = extruded linseed; CPLO = calcium salts of palm and linseed oil; MR = milled rapeseed; CLA = conjugated linoleic acid; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

<sup>1</sup>Where EL, CPLO and MR are diets containing ~ 500 g oil/day equivalent of extruded linseed, calcium salts of palm and linseed oil and milled rapeseed, respectively.

<sup>2</sup>Overall effect of treatment diet.

<sup>3</sup>Co-elutes with 17:0 anteiso.

<sup>4</sup>All 18:2 isomers excluding CLA.

<sup>5</sup>Including *cis*-9, *trans*-11 CLA, *trans*-7, *cis*-9 CLA, *trans*-8, *cis*-10 CLA, *trans*-10, *cis*-12 CLA.

<sup>6</sup>Co-elutes with *cis*-15 18:1.

\*Within rows treatments are different ( $P < 0.05$ ) from the control based on Dunnett's pdiff test.

diet had a higher 18:3n-3 intake than cows consuming the other supplements, and the milk fat content was lower, consistent with a shift from acetate to propionate in the rumen as observed by Gonthier *et al.* (2004). The difference in response between oilseed supplement types could also be due to differences in the carbohydrate contents of the different diets, with the MR diet containing a greater amount of

NDF and ADF and less starch than that of the other diets, which can also effect methane yield (Hammond *et al.*, 2015).

A meta-analysis of methane production following different oilseed-supplemented diets suggested that each 1% addition in supplemental fat intake to the diet DM results in a mean decrease in methane yield (l/kg DMI) of 3.8% when compared with a control diet (Martin *et al.*, 2010). Both the EL

**Table 5** Effect of oilseed supplementation of dairy cow diets on milk fat 18:1 isomer composition (least square mean results as g/100 g fatty acids)

Fatty acids	Treatments <sup>1</sup>				SEM	P diet <sup>2</sup>
	Control	EL	CPLO	MR		
<i>trans</i> -4 18:1	0.00	0.02*	0.03*	0.05*	0.005	0.006
<i>trans</i> -5 18:1	0.00	0.00	0.01	0.03*	0.006	0.018
<i>trans</i> -6-8 18:1	0.25	0.55*	0.43	0.53*	0.056	0.066
<i>trans</i> -9 18:1	0.20	0.38*	0.28	0.37	0.046	0.056
<i>trans</i> -10 18:1	0.61	1.23*	0.79	0.75	0.272	0.058
<i>trans</i> -11 18:1	0.65	1.25*	0.86	1.19*	0.188	0.003
<i>trans</i> -12 18:1	0.39	0.66*	0.56	0.65*	0.068	0.043
<i>trans</i> -15 18:1	0.59	1.04	0.38	1.10	0.187	0.170
<i>trans</i> -16 18:1 <sup>3</sup>	0.32	0.85*	0.51*	0.52*	0.052	< 0.001
<i>cis</i> -9 18:1 <sup>4</sup>	16.5	22.9*	21.2*	21.2*	1.62	0.071
<i>cis</i> -11 18:1	0.59	0.60	0.59	0.63	0.074	0.899
<i>cis</i> -12 18:1	0.24	0.44*	0.33	0.35	0.046	0.076
<i>cis</i> -13 18:1	0.09	0.15*	0.11	0.10	0.020	0.030
<i>cis</i> -16 18:1	0.05	0.13*	0.08*	0.08*	0.010	< 0.001

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

<sup>1</sup>Where EL, CPLO and MR are diets containing ~ 500 g oil/day equivalent of extruded linseed, calcium salts of palm and linseed oil and milled rapeseed, respectively.

<sup>2</sup>Overall effect of treatment diet.

<sup>3</sup>Co-elutes with 18:1 *cis*-14.

<sup>4</sup>Co-elutes with 18:1 *trans*-13/14.

\*Within rows treatments are different ( $P < 0.05$ ) from the control based on Dunnett's pdiff test.

and CPLO methane responses in the present study (mean decreases of 3.4% and 2.9% per additional 1% supplemental fat, respectively) approach this value, but not the MR response as discussed above (mean decrease of 0.3% per additional 1% supplemental fat).

The most effective strategy for decreasing milk fat SFA concentration is by supplementing cow diets with oilseed preparations (Glasser *et al.*, 2008; Kliem and Shingfield, 2016). Increases in the supply of  $\geq 16$ -carbon FA from the diet inhibit acetyl CoA carboxylase transcription and activity in the mammary gland, decreasing *de novo* synthesis (Barber

*et al.*, 1997). In the present study, supplementing cow diets with ~500 g additional oil per day decreased milk SFA compared with the control diet, with the linseed-containing diets being more effective than MR. This was partially due to enhanced milk fat 18:0 concentration with the MR diet, which may have been partially derived from rumen biohydrogenation of dietary *cis*-9 18:1. This process for *cis*-9 18:1 is more complete than that for 18:2n-6 and 18:3n-3 (Doreau and Chilliard, 1997). Previous research involving the same EL supplements fed at lower oil inclusion levels (280 to 350 g/day) reported no significant effects on milk SFA concentration (Livingstone *et al.*, 2015; Kliem *et al.*, 2016), highlighting the variability of the response and suggesting that in order to achieve a consistent effect on milk SFA at least 500 g/day or more of additional oil should be fed.

Milk SFA were mainly replaced with *cis*-MUFA following supplementation, the most predominant being *cis*-9 18:1. Intake of *cis*-9 18:1 was highest for the MR diet, and the appearance of *cis*-9 18:1 in milk is associated with both increased intake and also increased rumen outflow of 18:0 following complete biohydrogenation of dietary MUFA and PUFA that is subsequently desaturated by mammary  $\Delta 9$  desaturase. A comprehensive meta-analysis of 106 experiments using lactating cows concluded that plant oils and oilseeds all increase milk fat *cis*-9 18:1 concentrations (Glasser *et al.*, 2008). The EL supplement also increased *cis*-12 18:1 and *cis*-16 18:1 concentrations, which tend to be higher following linseed supplementation (Lerch *et al.*, 2012) and are biohydrogenation intermediates of 18:3n-3 (Shingfield *et al.*, 2010). In the present study increases in the concentrations of *trans*-FA in milk fat were observed when oilseed supplements were fed, particularly for EL. These increases reflect the higher intake of PUFA for the EL diet. In particular, *trans*-10 18:1 and *trans*-11 18:1 and most of the *trans*-18:2 isomers (including *trans*-11, *cis*-15 18:2 and *cis*-9, *trans*-13 18:2) were higher in concentration in milk from cows supplemented with EL. *Trans*-10 18:1 is thought to arise as an intermediate of rumen 18:2n-6 biohydrogenation in response to certain rumen conditions, such as when rumen pH is decreased (Bauman *et al.*, 2011). Intake of 18:2n-6 was similar for both EL and MR diets,

**Table 6** Effect of oilseed supplementation of dairy cow diets on milk fat 18:2 isomer composition (least square mean results as mg/100 g fatty acids)

Fatty acids	Treatments <sup>1</sup>				SEM	P diet <sup>2</sup>
	Control	EL	CPLO	MR		
<i>trans</i> -9, <i>trans</i> -12 18:2	2.4	28.7*	4.9	9.0	6.69	0.060
<i>cis</i> -9, <i>trans</i> -12 18:2	30.0	52.5*	35.0	40.0	5.77	0.102
<i>cis</i> -9, <i>trans</i> -13 18:2	180	528*	314	263	58.9	0.005
<i>cis</i> -9, <i>trans</i> -14 18:2	67.6	222.3*	119.9	107.6	25.82	0.004
<i>cis</i> -10, <i>trans</i> -14 18:2	142.3	89.3*	110.5	115.4	12.86	0.127
<i>trans</i> -9, <i>cis</i> -12 18:2	12.5	42.5*	30.0*	15.0	5.68	0.006
<i>trans</i> -11, <i>cis</i> -15 18:2	50.6	535.3*	166.0*	98.2	46.14	< 0.001
<i>cis</i> -9, <i>cis</i> -12 18:2	1871	2212*	1991	1821	157.5	0.096

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

<sup>1</sup>Where EL, CPLO and MR are diets containing ~ 500 g oil/day equivalent of extruded linseed, calcium salts of palm and linseed oil and milled rapeseed, respectively.

<sup>2</sup>Overall effect of treatment diet.

\*Within rows treatments are different ( $P < 0.05$ ) from the control based on Dunnett's pdiff test.

and yet only EL increased milk *trans*-10 18:1. It may be that the EL diet resulted in a lower rumen pH resulting in this alternative biohydrogenation pathway, but unfortunately this was not measured. *Trans*-11 18:1 and *trans*-11, *cis*-15 18:2 are intermediates of rumen 18:3n-3 metabolism (Shingfield *et al.*, 2010), and *cis*-9, *trans*-13 18:2 is thought to arise in milk following increased availability of *trans*-13 18:1 for mammary  $\Delta 9$  desaturation (Rego *et al.*, 2009). There was a distinct lack of difference between the control and CPLO diets in terms of concentration of biohydrogenation intermediates, despite both CPLO and EL being sources of 18:3n-3. In fact, EL provided over twice the amount of 18:3n-3 than CPLO in terms of intake (274 v. 128 g/day). In addition, the calcium salt preparation would have afforded some degree of rumen inertness for CPLO PUFA.

The main objective of the present study was to demonstrate whether oilseed supplements fed at a commercially practical level have an impact on both methane emissions and milk FA profile. A previous study involving the same supplements fed at a slightly lower level reported modest but significant improvements in milk FA profile, in terms of replacing milk SFA with unsaturated FA (Kliem *et al.*, 2016). Results from the present study and our previous study with EL (Livingstone *et al.*, 2015) suggest that this milk FA response may be inconsistent at these low (but more practical in commercial situations) inclusion levels. Methane emissions were lower with the linseed-based supplements but there were no noticeable effects with the MR diet. In order to achieve both objectives consistently a higher dietary inclusion level of MR will be needed.

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### Declaration of interest

The authors declare no conflicts of interest.

### Ethics statement

All regulated experimental procedures used were licensed and inspected by the UK Home Office under the Animals (Scientific Procedures) Act, 1996.

### Software and data repository resources

Data are stored on a secure server at the University of Reading.

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