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K. E. Kliem[1], D. J. Humphries[1], P. Kirton[1], D. I. Givens[2] and C. K. Reynolds[1]

[1] *Animal, Dairy and Food Chain Sciences, School of Agriculture, Policy and Development, University of Reading, Reading, Berkshire, UK, RG6 6AR*

[2] *Institute for Food Nutrition and Health, University of Reading, Reading, RG6 6AR, United Kingdom.*

Corresponding author: Kirsty E. Kliem. E-mail: k.e.kliem@reading.ac.uk

Short title: Fat supplements, methane and milk fatty acids

Abstract

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 x 4 Latin square design with 28-day periods. Diets were fed as a TMR 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/d (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, whilst 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 profile for all the supplements fed. Therefore, higher feeding levels may be required to achieve both lower methane emissions and improved milk fatty acid 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 DM intake (**DMI**) basis (Beauchemin *et al.*, 2009; Martin *et al.*, 2010; Grainger and Beauchemin, 2011), the main reason being that supplemental lipids provide metabolisable energy 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 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 fatty acids (**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 & 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/d; 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 – 400 g oil/d) 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/d as extruded linseed 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.

Materials and methods

Experimental Design, Animals and Management

Four multiparous Holstein-Friesian cows of mean ± standard error parity 4.0 ± 0.82, milk yield 45.8 ± 1.27 kg/d and 169 ± 14.4 days in lactation were used. Animals were randomly allocated to one of four treatments in a 4 x 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 one week. During weeks 1-3 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 x 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_2 recoveries for the two chambers averaged 101.2 and 100.8%. Whilst 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 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 extruded linseed (86 g/kg DM; **EL**; Lintec, BOCM Pauls Ltd., Wherstead, Suffolk, UK), calcium salts of palm and linseed oil FA (44 g/kg DM; **CPLO**; Flaxpro, Volac International Ltd., Royston, UK), or milled rapeseed (59 g/kg DM; **MR**; provided for the study by BOCM Pauls Ltd., Wherstead, Suffolk, UK.). The milled rapeseed 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/d 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 09:00 h (2/3) and 16:00 h (1/3). Refused feed was removed and weighed prior to the morning feeding.

Experimental Sampling

Individual forage components of experimental diets, the concentrate portion and TMR were sampled on days 22-27 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 prior to 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 intakes. Samples of dietary components, TMR and refusals (if appreciable) were retained at -20°C for chemical analysis.

Cows were milked twice daily, at 0530 h 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 six days of each experimental period, treated with potassium dichromate preservative (1 mg/ml, Lactabs, Thomson

and Capper, Runcorn, UK), and held at 4° C until analyzed. Additional samples of milk were collected from 2 consecutive milkings during the last 24 h of each experimental period and stored at -20ºC until composited using proportions based on milk yield immediately prior to FA analysis.

Chemical Analysis

Chemical composition of oven dried (60ºC), 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, crude protein, and lactose were determined by mid-infrared 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 d of methane emission measurements were averaged for each cow and period (n = 16) and analysed 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 (BIC) for each variable analysed. Each treatment mean was compared with the control diet using Dunnett's comparisons. Least square means ± 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 CPLO 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 18:2 n-6, whereas EL contained the most (145 g/kg DM) 18:3 n-3, closely followed by CPLO (138 g/kg DM). Total FA contents of each supplement were 263, 386 and 501 g/kg DM for EL, MR and CPLO, respectively.

Differences were observed in FA profile of the TMR diets (Table 1). The CPLO 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 18:3 n-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 DM intake (**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 CPLO 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, 18:2 n-6, 18:3 n-3 and total fatty acids 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/d) was reduced (*P*=0.012) by 10% by the CPLO 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 (<=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 fatty acids).

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, CPLO 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 18:3 n-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 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 (≤ 500 g oil/d), 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/d) 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 (≥ 50 g oil/kg DM) can have a negative

299 impact on ruminal and total tract organic matter and NDF digestion (Firkins and
300 Eastridge, 1994). In addition, stage of lactation/production level can also affect the
301 milk yield response to lipid supplementation, with cows in early lactation or of higher
302 genetic merit being more likely to show positive milk yield responses to
303 supplementation (Grainger and Beauchemin, 2011). In the present study the
304 supplemented diets were formulated to have an increased ME concentration, so as
305 there was no effect of treatments on DMI the increased milk yield following oilseed
306 supplementation can be attributed to the increased provision of energy provided by
307 the supplements.
308
309 The effect of oilseed supplementation on milk composition is also dependent on type,
310 form and amount of oilseed fed. In general, feeding plant oils in their partially disrupted
311 seed form has less impact on milk fat and protein concentration than feeding plant oils
312 per se (Beauchemin *et al.*, 2009; Givens *et al.*, 2009; Kliem *et al.*, 2011), possibly due
313 to a degree of rumen protection inferred by seed components. In a recent study at our
314 location (Livingstone *et al.*, 2015), feeding EL at a lower level than in the present study
315 had no effect on milk fat concentration in diets containing greater than 300 g NDF/kg
316 DM. However, in the present study the EL supplement caused a decrease in milk fat
317 concentration, similar to that observed by Chilliard *et al.* (2009), after feeding a similar
318 amount of EL. However a later study reported no effect of EL supplementation (560 g
319 oil/d) on milk fat concentration when fed in a diet with low NDF content (174 g/kg DM;
320 Oeffner *et al.*, 2013). This suggests that in addition to the amount and form of the plant
321 oil fed, basal diet composition (e.g. NDF concentration) can also influence the
322 response of milk fat concentration to supplemental plant oils.
323

Only the CPLO supplement decreased methane production (L/d; 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/d as crushed linseed and canola when compared with a control diet, but no effect was observed with crushed sunflowerseed (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 (18:2 n-6 + 18:3 n-3) was highest for the EL group, but those of CPLO and MR were comparable. In addition, complete biohydrogenation of 1 mol 18:3 n-3 will only spare 0.75 mol CH_4 (Martin *et al*., 2010). It has been reported that 18:3 n-3 has a greater toxicity to cellulolytic bacteria than 18:2 n-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 diet had a higher 18:3 n-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 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 ≥ 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 18:2 n-6 and 18:3 n-3 (Doreau and Chilliard, 1997). Previous research involving the same EL supplements fed at lower oil inclusion levels (280 – 350 g/d) 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/d 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 Δ9 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 18:3 n-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 18:2 n-6 biohydrogenation in response to certain rumen conditions, such as when rumen pH is decreased (Bauman *et al.*, 2011). Intake of 18:2 n-6 was similar for both EL and MR diets, 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 18:3 n-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 Δ^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 18:3 n-3. In fact, EL provided over twice the amount of 18:3 n-3 than CPLO in terms of intake (274 vs 128 g/d). 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 committee

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 sources

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

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

	Treatments[1]			
	Control	EL	CPLO	MR
Ingredients				
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[1]	43	43	43	43
Soybean 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
Chemical composition				
DM (g/kg fresh)	574	559	562	549
Organic matter	911	883	896	898
Crude protein	177	174	180	170
Neutral detergent fibre	359	336	348	391
Acid detergent fibre	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
Fatty acids				

16:0	4.5	5.9	10.9	5.5
18:0	0.7	1.4	1.6	1.1
18:1 *cis*-9	5.7	8.9	11.8	18.3
18:2 n-6	12.0	15.7	14.6	15.8
18:3 n-3	2.7	15.0	8.7	5.2
Total fatty acids	32	53	54	54

[1]Where EL, CPLO and MR are diets containing ~500 g oil/d equivalent of extruded linseed, calcium salts of palm and linseed oil and milled rapeseed, respectively.

[2]DDGS – Distillers' dried grains with solubles

584 **Table 2**. Effect of oilseed supplementation of dairy cow diets on dry matter and fatty acid intake, and milk and constituent yield (least square

585 mean results, units as specified).

	Treatments[1]					*P* Diet[2]
	Control	EL	CPLO	MR	s.e.m.	
DM intake (kg/d)	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
18:1 *cis*-9	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/d)	31.5	34.5*	33.5*	33.6*	1.92	0.010
Fat (g/d)	1142	1140	1228	1204	195.0	0.158
Protein (g/d)	1027	1055	1032	1054	52.2	0.995
Lactose (g/d)	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

[1] Where EL, CPLO and MR are diets containing ~500 g oil/d equivalent of extruded linseed, calcium salts of palm and linseed oil and milled rapeseed, respectively.

[2] Overall effect of treatment diet. Within rows treatments with superscript asterisks 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[1]					*P* Diet[2]
	Control	EL	CPLO	MR	s.e.m.	
CH_4, L/d	598	560	539*	601	42.4	0.025
CH_4, L/kg of DMI	28.0	25.7*	26.2*	27.8	2.01	0.035
CH_4, L/kg of milk	19.1	16.2*	16.4*	18.3	1.41	0.003

[1] Where EL, CPLO and MR are diets containing ~500 g oil/d equivalent of extruded linseed, calcium salts of palm and linseed oil and milled rapeseed, respectively.

[2] Overall effect of treatment diet. Within rows treatments with superscript asterisks are different ($P<0.05$) from the control based on Dunnett's pdiff test.

613 **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 acid	Treatments[1]					*P* Diet[2]
	Control	EL	CPLO	MR	s.e.m.	
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
10:1 *cis*-9	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
12:1 *cis*-9	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
14:1 *trans*-9	0.29	0.23	0.23	0.23	0.016	0.400
14:1 *cis*-9	1.13	0.95*	0.92*	0.92*	0.071	0.077

Fatty acid	Treatments[1]					*P* Diet[2]
	Control	EL	CPLO	MR	s.e.m.	
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
16:1 *trans*-6+7+8	0.040	0.054	0.055	0.059	0.0096	0.523
16:1 *trans*-9	0.040	0.082*	0.055	0.068*	0.0115	0.013
16:1 *trans*-11+12+13	0.16	0.20*	0.18	0.19	0.020	0.154
16:1 *cis*-9[3]	1.29	1.01*	1.21	0.97*	0.072	0.012
16:1 *cis*-11	0.51	0.50	0.48	0.48	0.043	0.59
16:1 *cis*-13	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
18:1 *trans* total	3.0	5.7*	3.4*	4.8*	0.34	0.008

Fatty acid	Treatments[1]					*P* Diet[2]
	Control	EL	CPLO	MR	s.e.m.	
18:1 *cis* total	17.7	24.8*	22.7*	22.5*	1.66	0.049
Non CLA 18:2 total[4]	2.4	3.7*	2.9*	2.4	0.26	0.001
CLA total[5]	0.43	0.77*	0.60*	0.69*	0.077	0.006
18:3 n-6	0.033	0.013*	0.020	0.025	0.0059	0.181
18:3 n-3	0.31	0.98*	0.43	0.52	0.116	0.035
19:0[6]	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
20:1 *cis*-8	0.12	0.05	0.04	0.05	0.029	0.456
20:1 *cis*-11	0.04	0.07	0.09	0.10*	0.016	0.166
20:2 n-6	0.03	0.04	0.03	0.03	0.004	0.133
20:3 n-6	0.11	0.07	0.13	0.11	0.018	0.327
20:3 n-3	0.05	0.02	0.02	0.03	0.015	0.566
20:4 n-6	0.12	0.11	0.16	0.12	0.026	0.685
20:5 n-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

Fatty acid	Treatments[1]					*P* Diet[2]
	Control	EL	CPLO	MR	s.e.m.	
22:4 n-6	0.04	0.02	0.03	0.02	0.005	0.090
22:5 n-3	0.10	0.11	0.09	0.06	0.017	0.219
Σ SFA[7]	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 *trans* total	4.1	8.4*	5.0*	6.1*	0.51	0.034
Σ *trans* MUFA[8]	3.6	6.3*	3.9*	5.5*	0.37	0.017
Σ *cis* MUFA	20.8	27.5*	25.4	24.8	1.67	0.085
Σ n-6 PUFA[9]	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

[1] Where EL, CPLO and MR are diets containing ~500 g oil/d equivalent of extruded linseed, calcium salts of palm and linseed oil and milled rapeseed, respectively.

[2] Overall effect of treatment diet. Within rows treatments with superscript asterisks are different (P<0.05) from the control based on Dunnett's pdiff test.

[3] Co-elutes with 17:0 anteiso

[4] CLA – conjugated linoleic acid. All 18:2 isomers excluding CLA

[5] Including *cis*-9, *trans*-11 CLA, *trans*-7, *cis*-9 CLA, *trans*-8, *cis*-10 CLA, *trans*-10, *cis*-12 CLA

[6] Co-elutes with *cis*-15 18:1

[7] SFA – saturated fatty acids

[8] MUFA – monounsaturated fatty acids

[9] PUFA – polyunsaturated fatty acids

637 **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

638 acids)

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

cis-16 18:1	0.05	0.13*	0.08*	0.08*	0.010	<0.001

[1] Where EL, CPLO and MR are diets containing ~500 g oil/d equivalent of extruded linseed, calcium salts of palm and linseed oil and milled rapeseed, respectively.

[2] Overall effect of treatment diet. Within rows treatments with superscript asterisks are different (P<0.05) from the control based on Dunnett's pdiff test.

[3] Co-elutes with 18:1 *cis*-14

[4] Co-elutes with 18:1 *trans*-13/14

655 **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

656 acids)

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

657 [1] Where EL, CPLO and MR are diets containing ~500 g oil/d equivalent of extruded linseed, calcium salts of palm and linseed oil and milled

658 rapeseed, respectively.

659 [2] Overall effect of treatment diet. Within rows treatments with superscript asterisks are different (P<0.05) from the control based on Dunnett's pdiff

660 test.