

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

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Kliem, K. E. ORCID: <https://orcid.org/0000-0002-0058-8225>,
Humphries, D. J., Kirton, P. ORCID: <https://orcid.org/0009-0001-1941-003X>, Givens, D. I. ORCID: <https://orcid.org/0000-0002-6754-6935> and Reynolds, C. K. ORCID:
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1 **Differential effects of oilseed supplements on methane production and milk**
2 **fatty acid concentrations in dairy cows**

3

4 K. E. Kliem¹, D. J. Humphries¹, P. Kirton¹, D. I. Givens² and C. K. Reynolds¹

5

6 ¹ *Animal, Dairy and Food Chain Sciences, School of Agriculture, Policy and*

7 *Development, University of Reading, Reading, Berkshire, UK, RG6 6AR*

8 ² *Institute for Food Nutrition and Health, University of Reading, Reading, RG6 6AR,*

9 *United Kingdom.*

10

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

12 **Short title:** Fat supplements, methane and milk fatty acids

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26 **Abstract**

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

51 CPLO there were increases in polyunsaturated FA concentration). Supplementing
52 dairy cow diets with these oilseed-based preparations affected milk FA profile and
53 increased milk yield. However, only the linseed-based supplements reduced methane
54 production, yield, or intensity, whilst feeding MR had no effect.

55

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

57

58 **Implications**

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

65

66

67 **Introduction**

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

76 polyunsaturated fatty acids (**PUFA**) provide an alternative to methane synthesis for
77 hydrogen disposal in the rumen (Clapperton, 1974; Fievez *et al.*, 2003). In addition,
78 some fatty acids (**FA**) can have direct toxic effects on cellulolytic microbes and fibre
79 digestion, thereby reducing methanogenesis (Martin *et al.*, 2010). These microbial
80 changes result in a shift in fermentation pattern towards propionate, which reduces
81 hydrogen available for methane synthesis. It has been suggested that the
82 effectiveness of lipid supplements to reduce methane production is inversely
83 proportional to the degree of saturation of the component FA (Giger-Reverdin *et al.*,
84 2003). However previous studies have demonstrated little difference between MUFA-
85 and PUFA-rich supplements in their ability to decrease methane emissions
86 (Beauchemin *et al.*, 2009), and it is thought that the form of the lipid fed (and therefore
87 rumen availability) is possibly more important (Martin *et al.*, 2008).

88

89 There has long been interest in feeding oilseed supplements to decrease milk
90 saturated FA (**SFA**) by replacement with MUFA and/or PUFA (Kliem and Shingfield,
91 2016), as it has been shown that milk and dairy products contribute substantially to
92 adult SFA consumption in European countries (Hulshof *et al.*, 1999). Current evidence
93 is inconsistent for the effect of dairy SFA in particular on cardiovascular disease risk
94 (Lovegrove & Givens, 2016). However the impact of dietary SFA on blood cholesterol
95 is indisputable (Givens, 2008). Effectiveness of oilseed supplements for decreasing
96 milk SFA concentration is dependent on source and form of oilseed (Glasser *et al.*,
97 2008; Kliem and Shingfield, 2016). Greater effects are observed if greater amounts
98 are fed (e.g. ca. 1.2 kg oil/cow/d; Givens *et al.*, 2003), however negative effects on
99 DMI, milk yield and milk composition mean that this strategy is less likely to be practical
100 on commercial farms. Significant decreases in milk SFA concentration compared with

101 control diets can be obtained by feeding oilseeds at more modest (e.g. 350 – 400 g
102 oil/d) levels (Collomb *et al.*, 2004; Kliem *et al.*, 2016), and recent evidence
103 demonstrates that this strategy can successfully be transferred to commercial practice
104 (Kliem *et al.*, 2016). However a review of available literature suggests that these low
105 levels of lipid supplementation (around 2 g/kg DM) may have little impact on methane
106 production (Martin *et al.*, 2010), and feeding growing or lactating cattle either 260 or
107 280 g oil/d as extruded linseed had no significant effect on methane emissions
108 (Hammond *et al.*, 2015; Livingstone *et al.*, 2015). The review of Martin *et al.* (2010)
109 also highlighted a lack of direct comparisons between oilseed types on methane
110 emissions, with most studies utilising different forms of the same oilseed.

111

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

116

117 **Materials and methods**

118 *Experimental Design, Animals and Management*

119 Four multiparous Holstein-Friesian cows of mean \pm standard error parity 4.0 ± 0.82 ,
120 milk yield 45.8 ± 1.27 kg/d and 169 ± 14.4 days in lactation were used. Animals were
121 randomly allocated to one of four treatments in a 4 x 4 Latin Square design experiment
122 with 28-day periods. As only two cows could be housed in respiration chambers at any
123 one time cows started the experiment in pairs, staggered by one week. During weeks
124 1-3 of each period cows adapted to diet changes whilst kept in an open yard bedded
125 on rubber mats and wood shavings, and individual feeding was achieved using an

126 electronic identification system and pneumatic feed barrier (Insentec, Marknesse, the
127 Netherlands). Fresh water was available *ad libitum*. During week 4 of each period
128 cows were transferred to respiration chambers and held in individual tie stalls for four
129 x 24 h measurements of methane emission and feed intake were obtained as
130 described in detail previously (Reynolds *et al.*, 2014; Hammond *et al.*, 2016). The
131 methane analysers were calibrated at the beginning and end of each daily
132 measurement period. At the time of the present study measured CO₂ recoveries for
133 the two chambers averaged 101.2 and 100.8%. Whilst in the chambers cows were
134 restrained using head yokes, bedded using rubber mats and wood shavings, had
135 continuous access to drinking water through drinking bowls, and were milked at 0530
136 and 1600 h.

137

138 *Experimental Diets*

139 Diets were offered *ad libitum* (fed for 10% refusals) as TMR (Forage:concentrate ratio
140 50:50 on a DM basis) with the forage consisting of maize silage and grass silage (750
141 and 250 g/kg of forage DM, respectively). Treatments consisted of a control diet
142 (control) containing no added fat source, or similar diets with the addition of 22 g oil/kg
143 DM as either extruded linseed (86 g/kg DM; **EL**; Lintec, BOCM Pauls Ltd., Wherstead,
144 Suffolk, UK), calcium salts of palm and linseed oil FA (44 g/kg DM; **CPLO**; Flaxpro,
145 Volac International Ltd., Royston, UK), or milled rapeseed (59 g/kg DM; **MR**; provided
146 for the study by BOCM Pauls Ltd., Wherstead, Suffolk, UK.). The milled rapeseed
147 supplement was manufactured by crushing rapeseed in a hammer mill using wheat
148 feed as a carrier in proportions of 75:25 on a fresh weight basis, respectively. These
149 were the same supplements as those used in the study of Kliem *et al.* (2016) and

150 supplemented diets were formulated to achieve an increase in oil intake of 500 g/d at
151 22 kg predicted DMI.

152

153 Diets were formulated to be isonitrogenous and contain similar levels of NDF (Table
154 1), with supplemental oil primarily replacing starch from ground wheat and increasing
155 diet ME concentration relative to the control diet. Cows were offered diets at 09:00 h
156 (2/3) and 16:00 h (1/3). Refused feed was removed and weighed prior to the morning
157 feeding.

158

159 *Experimental Sampling*

160 Individual forage components of experimental diets, the concentrate portion and TMR
161 were sampled on days 22-27 of each experimental period and added to a composite
162 sample. Forage DM concentrations were determined daily by drying at 100°C for 23 h
163 to ensure that the DM composition of experimental diets was maintained. Refused
164 feed was removed prior to the morning feeding and weighed daily; fresh weights were
165 recorded and during week 4 of each period a weekly composite of refused feed was
166 dried at 60°C for 48 h to determine individual daily DM intakes. Samples of dietary
167 components, TMR and refusals (if appreciable) were retained at -20°C for chemical
168 analysis.

169

170 Cows were milked twice daily, at 0530 h and 1600 h. When in respiration chambers
171 cows were milked using a pipeline system into buckets and milk yield determined
172 gravimetrically and recorded. Samples of milk for the determination of fat, protein and
173 lactose concentration were collected during the last six days of each experimental
174 period, treated with potassium dichromate preservative (1 mg/ml, Lactabs, Thomson

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

179

180 *Chemical Analysis*

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

187

188 Milk fat, crude protein, and lactose were determined by mid-infrared spectroscopy
189 (Foss Electric Ltd., York, UK). Lipid in 1 ml milk was extracted, transesterified and
190 resulting FA methyl esters (**FAME**) separated using the methods of Kliem *et al.* (2013).
191 Carbon deficiency in the flame ionization detector response for FAME containing 4- to
192 10-carbon atoms was accounted for using a combined correction factor which also
193 converted FAME to FA (Ulberth *et al.*, 1999). All milk FA results were expressed as g
194 /100 g total FA.

195

196 *Statistical Analysis*

197 Intake, milk production, milk composition, methane production and milk FA
198 composition data obtained during the 4 d of methane emission measurements were
199 averaged for each cow and period (n = 16) and analysed using the mixed procedure

200 of SAS (Statistical Analysis Systems software package version 8.2, SAS Institute,
201 Cary, NC, USA) and models testing fixed effects of period and treatment and random
202 effect of cow, with period as a repeated effect within cow, and the Kenward Rogers
203 option used for denominator degrees of freedom. Compound symmetry,
204 heterogeneous compound symmetry, first-order autoregressive or a heterogeneous
205 first-order regressive covariance structures were used for repeated measures
206 analysis, based on goodness of fit criteria (BIC) for each variable analysed. Each
207 treatment mean was compared with the control diet using Dunnett's comparisons.
208 Least square means \pm SEM are reported and treatment effects are considered
209 significant at $P \leq 0.05$.

210

211 **Results**

212 Analysis of the supplements confirmed the FA profile of each, with CPLO containing
213 the greatest amount of 16:0 (146 g/kg DM compared with 18 and 21 g/kg DM for EL
214 and MR, respectively) and 18:0 (18 g/kg DM compared with 8.0 and 5.0 g/kg DM for
215 EL and MR, respectively). The MR supplement contained the most (208 g/kg DM and
216 72 g/kg DM) *cis*-9 18:1 and 18:2 n-6, whereas EL contained the most (145 g/kg DM)
217 18:3 n-3, closely followed by CPLO (138 g/kg DM). Total FA contents of each
218 supplement were 263, 386 and 501 g/kg DM for EL, MR and CPLO, respectively.

219

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

225

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

232

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

239

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

245

246 Short and medium chain FA concentrations in milk fat were affected by treatment diet
247 (Table 4). Concentrations of 8:0, 10:0, 14:0, 15:0 and 16:0 were all lower ($P<0.05$)
248 following supplementation when compared with the control diet, which contributed
249 towards an overall lower ($P=0.029$) concentration of short and medium chain ($\leq 14:0$)

250 SFA. Conversely 18:0 concentration was increased ($P=0.001$) following
251 supplementation (more so for rapeseed- than linseed-based diets). Despite this there
252 was still an overall reduction in concentration of total SFA when compared with the
253 control diet (average decrease of 8.3 g/100 g fatty acids).

254

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

261

262 Concentrations of PUFA in milk fat were also affected by supplementation. There was
263 an effect of diet ($P=0.035$) on 18:3 n-3, where EL increased the concentration three-
264 fold (to 0.98 g/100 g FA) compared with the control diet (Table 4). This resulted in an
265 increased ($P<0.05$) concentration of total n-3 PUFA for EL (and CPLO) treatments
266 (Table 4). A similar increase was observed in the concentration of total n-6 PUFA
267 (Table 4), due to increased ($P<0.05$) concentrations of *trans*-9, *trans*-12 18:2, *cis*-9,
268 *trans*-12 18:2 and *trans*-9, *cis*-12 18:2 isomers after cows were fed the EL diet
269 compared with control (Table 6). Increases ($P<0.05$) were also observed in other 18:2
270 isomers such as *trans*-11, *cis*-15 18:2 and *cis*-9, *trans*-13 18:2 when EL was fed
271 compared with the control diet (Table 6).

272

273 **Discussion**

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

283

284 In the current study, supplementing cow diets with 22 g oil/kg DM in the form of EL,
285 CPLO and MR had no effect on DMI and increased milk yield when compared with a
286 control diet containing no supplemental oil. There are a plethora of older studies
287 reporting positive effects of feeding supplemental fats on milk yield (Palmquist and
288 Jenkins, 2017), but the milk yield response depends on DMI, which is in part
289 dependent on the degree of saturation of the lipid fed (Palmquist and Jenkins, 2017).
290 As reported by Firkins and Eastridge (1994), negative effects of fat supplements on
291 DMI are typically greater as the iodine value (unsaturation) of the lipid fed increases.
292 Inconsistent effects of supplemental plant oils on milk yield reported in the literature
293 may also be due to the amounts fed. At lower supplementation levels (\leq 500 g oil/d),
294 unsaturated plant oils have been shown to increase milk yield (AlZahal *et al.*, 2008) or
295 have no effect (Collomb *et al.*, 2004; Kliem *et al.*, 2016) when compared with control
296 diets containing no supplemental oil. At higher intake levels ($>$ 500 g oil/d) both DMI
297 and milk yield can be decreased (Chilliard *et al.*, 2009), but not always (Kliem *et al.*,
298 2011). Feeding higher levels of oil supplements (\geq 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

324 Only the CPLO supplement decreased methane production (L/d; Table 3). Both
325 linseed-containing supplements also decreased methane yield and intensity, whereas
326 there was no effect of MR on methane emissions. A previous study reported a
327 decrease in methane yield (g/kg DMI) after feeding 750 g oil/cow/d as crushed linseed
328 and canola when compared with a control diet, but no effect was observed with
329 crushed sunflowerseed (Beauchemin *et al.*, 2009). The differences in effects of oilseed
330 supplements on methane production observed in the present study is unlikely to be
331 due to the degree of unsaturation of supplemental oils; intake of PUFA (18:2 n-6 +
332 18:3 n-3) was highest for the EL group, but those of CPLO and MR were comparable.
333 In addition, complete biohydrogenation of 1 mol 18:3 n-3 will only spare 0.75 mol CH₄
334 (Martin *et al.*, 2010). It has been reported that 18:3 n-3 has a greater toxicity to
335 cellulolytic bacteria than 18:2 n-6 (Maia *et al.*, 2007), which can result in a shift in
336 rumen fermentation towards propionate, and thus an increase in hydrogen utilization.
337 Cows consuming the EL diet had a higher 18:3 n-3 intake than cows consuming the
338 other supplements, and the milk fat content was lower, consistent with a shift from
339 acetate to propionate in the rumen as observed by Gonthier *et al.* (2004). The
340 difference in response between oilseed supplement types could also be due to
341 differences in the carbohydrate contents of the different diets, with the MR diet
342 containing a greater amount of NDF and ADF and less starch than that of the other
343 diets, which can also effect methane yield (Hammond *et al.*, 2015).

344 A meta-analysis of methane production following different oilseed-supplemented diets
345 suggested that each 1% addition in supplemental fat intake to the diet DM results in a
346 mean decrease in methane yield (L/kg DMI) of 3.8 % when compared with a control
347 diet (Martin *et al.*, 2010). Both the EL and CPLO methane responses in the present
348 study (mean decreases of 3.4 and 2.9 %, per additional 1 % supplemental fat,

349 respectively) approach this value, but not the MR response as discussed above (mean
350 decrease of 0.3 % per additional 1 % supplemental fat).

351

352 The most effective strategy for decreasing milk fat SFA concentration is by
353 supplementing cow diets with oilseed preparations (Glasser *et al.*, 2008; Kliem and
354 Shingfield, 2016). Increases in the supply of ≥ 16 -carbon FA from the diet inhibit acetyl
355 CoA carboxylase transcription and activity in the mammary gland, decreasing de novo
356 synthesis (Barber *et al.*, 1997). In the present study, supplementing cow diets with
357 ~500 g additional oil per day decreased milk SFA compared with the control diet, with
358 the linseed-containing diets being more effective than MR. This was partially due to
359 enhanced milk fat 18:0 concentration with the MR diet, which may have been partially
360 derived from rumen biohydrogenation of dietary *cis*-9 18:1. This process for *cis*-9 18:1
361 is more complete than that for 18:2 n-6 and 18:3 n-3 (Doreau and Chilliard, 1997).
362 Previous research involving the same EL supplements fed at lower oil inclusion levels
363 (280 – 350 g/d) reported no significant effects on milk SFA concentration (Livingstone
364 *et al.*, 2015; Kliem *et al.*, 2016), highlighting the variability of the response and
365 suggesting that in order to achieve a consistent effect on milk SFA at least 500 g/d or
366 more of additional oil should be fed.

367

368 Milk SFA were mainly replaced with *cis*-MUFA following supplementation, the most
369 predominant being *cis*-9 18:1. Intake of *cis*-9 18:1 was highest for the MR diet, and the
370 appearance of *cis*-9 18:1 in milk is associated with both increased intake and also
371 increased rumen outflow of 18:0 following complete biohydrogenation of dietary MUFA
372 and PUFA that is subsequently desaturated by mammary $\Delta 9$ desaturase. A
373 comprehensive meta-analysis of 106 experiments using lactating cows concluded that

374 plant oils and oilseeds all increase milk fat *cis*-9 18:1 concentrations (Glasser *et al.*,
375 2008). The EL supplement also increased *cis*-12 18:1 and *cis*-16 18:1 concentrations,
376 which tend to be higher following linseed supplementation (Lerch *et al.*, 2012) and are
377 biohydrogenation intermediates of 18:3 n-3 (Shingfield *et al.*, 2010). In the present
378 study increases in the concentrations of *trans* FA in milk fat were observed when
379 oilseed supplements were fed, particularly for EL. These increases reflect the higher
380 intake of PUFA for the EL diet. In particular, *trans*-10 18:1 and *trans*-11 18:1 and most
381 of the *trans*-18:2 isomers (including *trans*-11, *cis*-15 18:2 and *cis*-9, *trans*-13 18:2)
382 were higher in concentration in milk from cows supplemented with EL. *Trans*-10 18:1
383 is thought to arise as an intermediate of rumen 18:2 n-6 biohydrogenation in response
384 to certain rumen conditions, such as when rumen pH is decreased (Bauman *et al.*,
385 2011). Intake of 18:2 n-6 was similar for both EL and MR diets, and yet only EL
386 increased milk *trans*-10 18:1. It may be that the EL diet resulted in a lower rumen pH
387 resulting in this alternative biohydrogenation pathway, but unfortunately this was not
388 measured. *Trans*-11 18:1 and *trans*-11, *cis*-15 18:2 are intermediates of rumen 18:3
389 n-3 metabolism (Shingfield *et al.*, 2010), and *cis*-9, *trans*-13 18:2 is thought to arise in
390 milk following increased availability of *trans*-13 18:1 for mammary Δ^9 desaturation
391 (Rego *et al.*, 2009). There was a distinct lack of difference between the control and
392 CPLO diets in terms of concentration of biohydrogenation intermediates, despite both
393 CPLO and EL being sources of 18:3 n-3. In fact, EL provided over twice the amount
394 of 18:3 n-3 than CPLO in terms of intake (274 vs 128 g/d). In addition, the calcium salt
395 preparation would have afforded some degree of rumen inertness for CPLO PUFA.
396
397 The main objective of the present study was to demonstrate whether oilseed
398 supplements fed at a commercially practical level have an impact on both methane

399 emissions and milk FA profile. A previous study involving the same supplements fed
400 at a slightly lower level reported modest but significant improvements in milk FA profile,
401 in terms of replacing milk SFA with unsaturated FA (Kliem *et al.*, 2016). Results from
402 the present study and our previous study with EL (Livingstone *et al.*, 2015) suggest
403 that this milk FA response may be inconsistent at these low (but more practical in
404 commercial situations) inclusion levels. Methane emissions were lower with the
405 linseed-based supplements but there were no noticeable effects with the MR diet. In
406 order to achieve both objectives consistently a higher dietary inclusion level of MR will
407 be needed.

408

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418 **Declaration of interest**

419 The authors declare no conflicts of interest.

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421 **Ethics committee**

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

424 **Software and data repository sources**

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

426

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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	29	11.6	15.5	26.8
carbohydrates				
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 <i>cis</i> -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

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

570 ²DDGS – Distillers’ dried grains with solubles

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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				s.e.m.	<i>P</i> Diet ²
	Control	EL	CPLO	MR		
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 <i>cis</i> -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

586 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
587 rapeseed, respectively.

588 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
589 test.

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

	Treatments ¹				s.e.m.	<i>P</i> Diet ²
	Control	EL	CPLO	MR		
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

601 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
 602 rapeseed, respectively.

603 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
 604 test.

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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				s.e.m.	<i>P</i> Diet ²
	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
10:1 <i>cis</i> -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 <i>cis</i> -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 <i>trans</i> -9	0.29	0.23	0.23	0.23	0.016	0.400
14:1 <i>cis</i> -9	1.13	0.95*	0.92*	0.92*	0.071	0.077

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

Fatty acid	Treatments ¹				s.e.m.	P Diet ²
	Control	EL	CPLO	MR		
18:1 <i>cis</i> 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 <i>cis</i> -8	0.12	0.05	0.04	0.05	0.029	0.456
20:1 <i>cis</i> -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				s.e.m.	P Diet ²
	Control	EL	CPLO	MR		
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 <i>trans</i> total	4.1	8.4*	5.0*	6.1*	0.51	0.034
Σ <i>trans</i> MUFA ⁸	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 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

614 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
615 rapeseed, respectively.

616 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
617 test.

618 ³ Co-elutes with 17:0 anteiso

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

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

621 ⁶ Co-elutes with *cis*-15 18:1

622 ⁷ SFA – saturated fatty acids

623 ⁸ MUFA – monounsaturated fatty acids

624 ⁹ PUFA – polyunsaturated fatty acids

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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					<i>P</i> Diet ²
	Control	EL	CPLO	MR	s.e.m.	
<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 ³	0.32	0.85*	0.51*	0.52*	0.052	<0.001
<i>cis</i> -9 18:1 ⁴	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
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639 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
640 rapeseed, respectively.

641 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
642 test.

643 ³ Co-elutes with 18:1 *cis*-14

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

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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				s.e.m.	P Diet ²
	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

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.