

*Effects of forage type and extruded linseed supplementation on methane production and milk fatty acid composition of lactating dairy cows*

Article

Accepted Version

Livingstone, K. M., Humphries, D. J., Kirton, P., Kliem, K. E., Givens, D. I. and Reynolds, C. K. (2015) Effects of forage type and extruded linseed supplementation on methane production and milk fatty acid composition of lactating dairy cows. *Journal of Dairy Science*, 98 (6). pp. 4000-4011. ISSN 0022-0302 doi: <https://doi.org/10.3168/jds.2014-8987> Available at <https://centaur.reading.ac.uk/40227/>

It is advisable to refer to the publisher's version if you intend to cite from the work. See [Guidance on citing](#).

To link to this article DOI: <http://dx.doi.org/10.3168/jds.2014-8987>

Publisher: Elsevier

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the [End User Agreement](#).

[www.reading.ac.uk/centaur](http://www.reading.ac.uk/centaur)

**CentAUR**

Central Archive at the University of Reading

Reading's research outputs online

1 **Interpretive summary:**

2

3 **Effect of forage type and extruded linseed supplementation on methane**  
4 **production and milk fatty acid composition of lactating dairy cows**

5 Livingstone

6 In contrast to previous studies, replacing grass silage with maize silage in dairy cow  
7 diets did not affect methane production per unit of feed consumed, in part due to low  
8 NDF concentration of the grass silage fed. Similarly, feeding extruded linseed had no  
9 effect on methane production, but the amount of oil fed was relatively low. Feeding  
10 extruded linseed and feeding more maize silage both decreased saturated fatty acid  
11 concentration of milk fat, and therefore represent a potential strategy for removing  
12 saturated fatty acids from the food chain.

13

14

15 .

16

17 DIET EFFECTS ON MILK FATTY ACIDS AND METHANE<sup>2033</sup>

18

19

20 **Effects of forage type and extruded linseed supplementation on methane**  
21 **production and milk fatty acid composition of lactating dairy cows**

22

23 **K. M. Livingstone<sup>1</sup>, D. J. Humphries, P. Kirton, K. E. Kliem, D. I. Givens,**  
24 **and C. K. Reynolds<sup>2</sup>,**

25 Food Production and Quality Division, Centre for Dairy Research, School of  
26 Agriculture, Policy and Development, University of Reading, PO Box 237, Earley  
27 Gate, Reading, RG6 6AR, UK.

28

29

30 <sup>1</sup>Present address: Human Nutrition Research Centre, Institute of Cellular Medicine,  
31 Newcastle University, Newcastle upon Tyne, NE4 5PL, UK.

32 <sup>2</sup>Corresponding author: c.k.reynolds@reading.ac.uk

33

34

35

36

37

38

39

40

41

**ABSTRACT**

42  
43 Replacing dietary grass silage (GS) with maize silage (MS) and dietary fat  
44 supplements may reduce milk concentration of specific saturated fatty acids (SFA)  
45 and can reduce methane production by dairy cows. The present study investigated the  
46 effect of feeding an extruded linseed supplement on milk fatty acid (FA) composition  
47 and methane production of lactating dairy cows, and whether basal forage type, in  
48 diets formulated for similar NDF and starch, altered the response to the extruded  
49 linseed supplement. Four mid-lactation Holstein-Friesian cows were fed diets as total  
50 mixed rations, containing either high proportions of MS or GS, both with or without  
51 extruded linseed supplement, in a 4 x 4 Latin square design experiment with 28-day  
52 periods. Diets contained 500 g forage/kg DM containing MS and GS in proportions  
53 (DM basis) of either 75:25 or 25:75 for high MS or high GS diets, respectively.  
54 Extruded linseed supplement (275 g/kg ether extract, dry matter [DM] basis) was  
55 included in treatment diets at 50 g/kg DM. Milk yields, DM intake (DMI), milk  
56 composition, and methane production were measured at the end of each experimental  
57 period when cows were housed in respiration chambers. Whilst DMI was higher for  
58 the MS-based diet, forage type and extruded linseed had no significant effect on milk  
59 yield, milk fat, protein, or lactose concentration, methane production, or methane per  
60 kg DMI or milk yield. Total milk fat SFA concentrations were lower with MS  
61 compared with GS-based diets (65.4 vs. 68.4 g/100g FA, respectively) and with  
62 extruded linseed compared with no extruded linseed (65.2 vs. 68.6 g/100g FA,  
63 respectively) and these effects were additive. Concentrations of total *trans* FA were  
64 higher with MS compared with GS-based diets (7.0 vs. 5.4 g/100g FA, respectively)  
65 and when extruded linseed was fed (6.8 vs. 5.6 g/100g FA, respectively). Total n-3  
66 FA were higher when extruded linseed was fed compared with no extruded linseed

67 (1.2 vs. 0.8 g/100g FA, respectively), while total n-6 polyunsaturated FA were higher  
68 when feeding MS compared with GS (2.5 vs. 2.1 g/100g FA, respectively). Feeding  
69 extruded linseed and MS both provided potentially beneficial decreases in SFA  
70 concentration of milk, and there were no significant interactions between extruded  
71 linseed supplementation and forage type. However, both MS and extruded linseed  
72 increased *trans* FA concentration in milk fat. Neither MS nor extruded linseed had  
73 significant effects on methane production or yield, but the amounts of supplemental  
74 lipid provided by extruded linseed was relatively small.

75 **Key words:** Methane, forage type, linseed, milk fatty acids

76

77

## INTRODUCTION

78 There is currently considerable interest in developing management practices to reduce  
79 methane emissions attributable to ruminant meat and milk production and there are  
80 numerous dietary strategies that may be effective in reducing methane production or  
81 yield (methane per unit feed DMI). Previous studies have shown that replacing  
82 dietary ADF or NDF with starch (Mills et al., 2001), reducing NDF intake (Aguerre et  
83 al., 2011) and replacing grass silage (Reynolds et al., 2010) or alfalfa silage (Hassanat  
84 et al., 2013) with maize silage can reduce methane yield, but the effects are not  
85 consistent. In growing beef cattle effects of feeding maize silage as a replacement for  
86 GS on methane yield depending varied from positive to negative over the course of  
87 the experiment (Staerfl et al., 2012). In lactating dairy cows, incremental replacement  
88 of alfalfa silage with MS had quadratic effects on methane production and yield such  
89 that methane production was higher when the silages were fed as a 50:50 mixture  
90 (Hassanat et al., 2013). Somewhat similarly, incremental replacement of GS with MS

91 had a quadratic effect on methane production but linearly decreased methane yield in  
92 lactating dairy cows (van Gastelen et al., 2015).

93

94 In addition to effects of forage type and composition, the reducing effects of a variety  
95 of supplemental dietary lipids on methane production and(or) yield have been  
96 demonstrated in cattle and sheep (e.g. Beauchemin et al., 2008; Grainger and  
97 Beauchemin, 2011), with the longer chain PUFA shown to be particularly effective in  
98 some studies (Blaxter and Czerkawski, 1966; Clapperton, 1974) but not in all  
99 experiments (Grainger and Beauchemin, 2011).. Lipids in the diet provide  
100 metabolizable energy, whilst replacing fermentable substrates that contribute to  
101 methane synthesis in the rumen. In addition, rumen available MUFA and PUFA  
102 provide an alternative to methane synthesis for hydrogen disposal by rumen archaea,  
103 as well as having direct effects on rumen microflora that reduce methanogenesis  
104 (Beauchemin et al., 2008). It has previously been reported that feeding supplemental  
105 linseed oil as free oil or crushed or extruded linseed reduced methane production and  
106 yield of lactating dairy cows, but DMI and milk yield were also reduced (Martin et al.,  
107 2008).

108

109 There is also interest in developing dairy cow feeding strategies that reduce milk fat  
110 concentrations of SFA, as dairy fat is a substantial dietary source of SFA in European  
111 diets (Givens, 2008). The potential for these particular SFA to raise low density  
112 lipoprotein cholesterol in humans has been implicated as a risk factor for  
113 cardiovascular disease (**CVD**), which is the main cause of premature death in the UK  
114 (Givens, 2008). The cow's diet is a major determinant of milk FA composition  
115 (Chilliard and Verlay, 2004) and studies have shown that alteration of dietary forage

116 type (Ferlay et al., 2006) and inclusion of dietary fat supplements (Kliem et al., 2009)  
117 are both means of modifying milk FA composition.

118 In Northern Europe, maize silage (**MS**) and grass silage (**GS**) are conserved forages  
119 commonly fed to lactating dairy cows and have been examined in various studies to  
120 investigate their differing effect on milk FA composition (Nielsen et al., 2006, Kliem  
121 et al., 2008, Samková et al., 2009; van Gastelen et al., 2015). Evidence indicates that  
122 feeding cows MS compared with GS has little effect on total SFA but can alter  
123 individual SFA concentrations (Kliem et al., 2008; van Gastelen et al., 2015). In  
124 contrast, supplemental oilseeds and plant and marine oils lower total SFA  
125 significantly, whilst increasing unsaturated FA (Chilliard et al., 2001; Givens et al.,  
126 2009). Increasing MS in the diet can also increase trans FA (Kliem et al., 2008; van  
127 Gastelen et al., 2015) through incomplete ruminally biohydrogenation of dietary  
128 unsaturated FA, although changes are of lesser magnitude than those increases  
129 reported following supplementation with dietary oils (Chilliard et al., 2007). At  
130 current intake levels negative effects of ruminant derived *trans* on human health are  
131 equivocal (Bendsen et al., 2011), but any increases in milk fat should be minimized.

132 The production response to supplemental lipid is known to vary with forage type  
133 (Grainger and Beauchemin, 2011), and the objectives of the present study were to  
134 investigate the effects of dietary forage type (MS vs. GS) in diets formulated to  
135 contain similar amounts of NDF and starch and feeding ELS on methane production  
136 and milk FA composition in mid-lactation multiparous Holstein-Friesian dairy cows,  
137 and determine if the response to ELS was affected by forage type.

## 138 **MATERIAL AND METHODS**

### 139 **Animals and Diets**



140 All experimental procedures were licensed, regulated and monitored by the UK Home  
141 Office under the Animals (Scientific Procedures) Act, 1996. Four mid-lactation  
142 multiparous Holstein-Friesian dairy cows averaging ( $\pm$  SEM)  $643 \pm 40$  kg BW and  $60$   
143  $\pm 8$  DIM at the start of the study were randomly allocated to one of four experimental  
144 diets using a 4 x 4 Latin square design balanced for first order carry over effects with  
145 28 day periods. Cows were milked twice daily at approximately 0630 and 1630 h.  
146 When not restrained for measurements cows were housed in a cubicle yard with  
147 rubber chip-filled mattresses and wood shavings as additional bedding and were  
148 milked in a herringbone parlour. Whilst in the cubicle yard cows were fed  
149 individually using an electronic identification controlled pneumatic feed barrier  
150 (Insentec, Marknesse, The Netherlands) and drinking water was available ad libitum.

151

## 152 **Experimental Design and Treatments**

153 Throughout the study cows were fed one of 4 experimental diets as a TMR (Table 1)  
154 provided for ad libitum DMI (10 % refusals). Basal diets were high MS or high GS  
155 diets, with and without supplemental (50 g/kg diet DM) ELS (containing 275 g ether  
156 extract/kg DM; Lintec, BOCM Pauls Ltd, Wherstead, UK); providing four treatments  
157 in a 2 x 2 factorial design. Diets were based on diets used in a previous study  
158 (Reynolds et al., 2010) and were formulated to be isonitrogenous and have similar  
159 NDF and starch concentrations based on preliminary analyses of available silages and  
160 expected composition of concentrates. Animals were fed twice daily receiving 2/3 of  
161 their daily allocation in the morning and the remaining 1/3 in the afternoon. Refused  
162 TMR was removed and weighed daily before the morning feeding.

163

## 164 **Experimental measurements and sample collection**

165 Cows were weighed at the beginning of the study and the end of each period. Feed  
166 intake was recorded daily. Representative samples of the four TMR diets, individual  
167 forages (MS, GS) and concentrates (concentrates blends and Lintec) were taken on the  
168 last 5 days of each treatment period, bulked and stored in sealed bags at -20°C. At the  
169 end of the trial bulked samples were thawed, mixed, and split into sub-samples for  
170 further analyses. A representative sample of refused feed was taken during the last 5  
171 days of each experimental period and analysed for DM content (100°C for 24 h) to  
172 determine individual DM intakes. Sub-samples of forages and concentrates were  
173 stored frozen at -20°C until analysed for chemical composition.

174

175 Milk yields were recorded daily throughout the study. Milk samples were taken  
176 during the last 5 days of each period and preserved with potassium dichromate (1  
177 mg/ml; Lactabs, Thomson and Capper, Runcorn, UK) for the determination of milk  
178 composition. Additional untreated milk samples were taken on the last day of each  
179 period, composited according to yield, and stored at -20°C prior to FA analysis.

180

181 For the last 5 days of each period cows were housed individually in one of 2 open-  
182 circuit respiration chambers and four 24 h measurements of methane and carbon  
183 dioxide production, oxygen consumption, and heat production were obtained as  
184 described previously (Reynolds et al., 2014). Whilst in the chambers cows were  
185 restrained using head yokes, bedded using wood shavings on rubber mats, had  
186 continuous access to drinking water through drinking bowls, and were milked using a  
187 pipeline system.

188

189 ***Chemical analyses***

190 Diet components were analysed for NDF, ADF, organic matter, CP, water soluble  
191 carbohydrates, starch and estimated ME concentrations as described previously  
192 (Kliem et al., 2013; Reynolds et al., 2014). In addition, oven-dried (60°C) and milled  
193 (1 mm screen) samples of forages and concentrates were analysed for FA  
194 concentration using an adapted one-step extraction–transesterification method as  
195 described by Kliem et al. (2013). Based on this method, toluene was used as an  
196 extraction solvent, methanolic sulphuric acid (2%, v/v) as the methylating reagent and  
197 tritridecanoin (T3882, Sigma–Aldrich Company Ltd, Dorset, UK) in toluene as an  
198 internal standard.

199

200 Mid-infrared spectroscopy (Foss Electric Ltd, York, UK) was used to determine milk  
201 fat, protein, casein, lactose, and urea concentrations and 4% FCM yield calculated as  
202 described by Reynolds et al. (2014). Milk samples were analysed for FA composition  
203 as described by Kliem et al. (2008 and 2013). Briefly, samples were thawed in warm  
204 water (40°C), cooled to room temperature, and shaken to ensure homogeneity. Lipid  
205 in 1 ml milk was extracted using ethanol, diethyl ether and hexane. Using sodium  
206 methoxide in methanol, extracted FA were base-catalyzed transmethylated to fatty  
207 acid methyl esters (**FAME**) and calcium chloride was used to remove methanol  
208 residues. Subsequent FAME samples were separated using a flame ionization detector  
209 (**FID**) gas chromatograph (GC 3400 Varian Inc., Palo Alto, CA). Milk fat FAME  
210 were identified based on retention time comparisons with a mixture of authentic  
211 standards (GLC #463, Nu-Chek-Prep Inc., Elysian, MN; and O4754, O9881, E4762,  
212 V1381, Sigma-Aldrich Company Ltd., Dorset, UK) and cross referencing with  
213 published literature. Correction factors, to account for the carbon deficiency in the  
214 FID response for FAME containing 4- to 10- carbon atoms, were estimated using a

215 reference butter oil of known composition (CRM 164, Bureau of European  
216 Communities, Brussels, Belgium). After correcting FAME to FA, all results were  
217 expressed as g/100 g total FA.

218

### 219 **Statistical Analyses**

220 Results averaged for each cow and sampling period were analysed using mixed  
221 models procedures testing for fixed effects of period, forage, ELS, and forage by ELS  
222 interaction and random effects of cow (SAS Version 9.2, SAS Institute, Cary, NC,  
223 USA). Period by forage interaction was included in the statistical model but removed  
224 when declared non-significant ( $P > 0.10$ ). Period was treated as a repeated effect  
225 within individual cows using the compound symmetry covariance structure, which  
226 was found to have the best fit based on Akaike information criterion. Denominator  
227 degrees of freedom were calculated using the Kenward-Roger method. Least square  
228 means are reported and treatment effects were considered significant at  $P < 0.10$ .

229

## **RESULTS**

### 230 ***Dietary composition and intake and milk yield and composition***

231 In comparison with the GS diets, the MS diets contained higher OM, NDF, and starch  
232 concentrations ( $P < 0.02$ ), while CP, ADF, and ash concentrations were higher for the  
233 GS diets ( $P < 0.020$ ; Table 2). The MS diets were higher in 18:0, *cis*-9 18:1, and 18:2  
234 n-6 ( $P < 0.003$ ), and lower in 18:3 n-3 ( $P < 0.02$ ) than the GS diets. The dietary  
235 concentration of 16:0 was not affected by forage type ( $P = 0.575$ ). The addition of  
236 ELS to the diets increased the concentration of all FA measured ( $P < 0.003$ ), and the  
237 increase in *cis*-9 18:1 was greater for the MS diet. Total FA concentrations were  
238 similar in MS and GS diets without added ELS, and were increased by ELS addition

239 to a greater extent with the MS compared with the GS diet (forage by ELS interaction,  
240  $P < 0.03$ ).

241

242         Supplementation with ELS had no effect on DMI ( $P = 0.31$ ), but DMI was  
243 higher for MS compared with GS diets ( $P < 0.10$ , Table 3). Intakes of 18:0, *cis*-9  
244 18:1, 18:2 n-6, and total FA were lower on GS than MS diets ( $P < 0.001$ ; Table 3).  
245 Intake of 18:3 n-3 was higher for GS diets ( $P < 0.001$ ) and the increase in 18:3 n-3  
246 intake with ELS addition was greater for the MS than GS diets (forage by ELS  
247 interaction,  $P < 0.02$ ). Milk or 4 % FCM yield, milk composition, and milk  
248 component yield were not affected by diet forage type or ELS addition (Table 3).

249

#### 250 *Methane Emission and Respiratory Exchange*

251 Methane production (L/d) and yield (L/kg DMI) were not affected by diet (Table 4).  
252 Similarly, methane production per litre milk yield was not affected by diet forage type  
253 or ELS addition. Cows fed higher MS diets had higher oxygen consumption ( $P <$   
254  $0.03$ ), carbon dioxide production ( $P < 0.04$ ), and heat production ( $P < 0.03$ ) than  
255 when fed higher GS diets (Table 4).

256

#### 257 *Effect of Forage Type on Milk FA Composition*

258 Milk fat total SFA concentration was lower when higher MS diets were fed ( $P =$   
259  $0.076$ ), but there was no forage type effect for most individual milk SFA ( $P > 0.10$ ),  
260 with the exception of 13:0 iso ( $P = 0.034$ ), 13:0 anteiso ( $P < 0.058$ ), 14:0 ( $P = 0.082$ ),  
261 15:0 ( $P = 0.009$ ), and 24:0 ( $P = 0.010$ ), which were lower on MS-based diets  
262 compared with GS-based diets (Table 5).

263 .

264 Feeding higher MS diets increased all *trans* 18:1 isomers ( $P < 0.06$ ), leading to  
 265 overall higher total *trans* MUFA ( $P = 0.009$ ) concentrations relative to GS-based diets  
 266 (Tables 5 and 6). Forage type had no effect on total *cis*-MUFA (Table 5) and most  
 267 18:2 isomers (Table 7), although *cis*-11 18:1, *cis*-12 18:1, *cis*-13 18:1, *cis*-16 18:1,  
 268 *cis*-11 20:1, and *cis*-9, *cis*-12 18:2 were higher ( $P < 0.05$ ) on MS relative to GS  
 269 (Tables 6 and 7), and *cis*-9 10:1, *cis*-9 12:1 and *cis*-9 14:1 were lower ( $P < 0.05$ ;  
 270 Table 5). Concentrations of 20:3 n-3 ( $P < 0.024$ ), 20:5 n-3 ( $P < 0.020$ ) and 22:2 n-6  
 271 ( $P < 0.001$ ) were higher in milk fat from cows fed the GS-based diets than the MS-  
 272 based diets (Table 5). Total n-6 PUFA concentrations in milk fat were higher with  
 273 MS-based diets ( $P=0.001$ ).

274

#### 275 *Effect of Extruded Linseed Supplementation on Milk FA Composition*

276 Including ELS in the diets lowered total milk SFA ( $P = 0.055$ , Table 5). Milk fat  
 277 concentrations of 16:0 ( $P = 0.012$ ), 17:0 ( $P = 0.009$ ), 18:0 iso ( $P = 0.052$ ), and 24:0  
 278 ( $P = 0.022$ ) were lower and 18:0 ( $P = 0.039$ ) and 19:0 ( $P = 0.005$ ) were higher when  
 279 ELS was fed. Concentrations of *cis*-9 16:1 ( $P = 0.020$ ) were lower and *cis*-16 18:1 ( $P$   
 280  $= 0.014$ ) and *cis*-7 19:1 ( $P = 0.025$ ) were higher when ELS was fed.

281

282 Including ELS in the diet increased *trans* MUFA ( $P = 0.027$ ) and total *trans* ( $P =$   
 283  $0.030$ ) isomer concentrations compared with non-linseed diets (Table 5). This change  
 284 in *trans* profile was characterized by a greater ( $P = 0.024$ ) total *trans* 18:1 isomer  
 285 concentration (Tables 5 and 6) in milk fat when ELS was fed: *trans*-11 16:1 ( $P =$   
 286  $0.063$ ) and *trans*-13+14 18:1 ( $P = 0.002$ ), *trans*-15 18:1 ( $P = 0.0002$ ), and *trans*-16  
 287 18:1 ( $P < 0.001$ ). Similarly, ELS supplementation increased ( $P < 0.001$ ) total non-  
 288 conjugated linoleic acid (CLA) *trans* 18:2 isomers compared with non-linseed diets

289 (Table 7) by increasing *cis*-9, *trans*-12 18:2 ( $P = 0.02$ ), *cis*-9, *trans*-13 18:2 ( $P <$   
290  $0.001$ ), *trans*-9, *cis*-12 18:2 ( $P = 0.008$ ), *trans*-11, *cis*-15 18:2 ( $P < 0.001$ ) and *trans*-  
291 12, *cis*-15 18:2 ( $P = 0.028$ ). No effect of ELS was seen in total *cis*-MUFA  
292 concentrations ( $P > 0.05$ , Table 5), although *cis*-12 18:1 ( $P < 0.021$ ) and *cis*-16 18:1  
293 ( $P < 0.014$ ) concentrations were higher when ELS was fed. No interactions between  
294 forage type and ELS were shown in *trans* 18:1 or 18:2 isomers ( $P > 0.05$ ; Tables 5, 6  
295 and 7), with the exception of *trans*-5 18:1 ( $P = 0.016$ , Table 6) and *cis*-9, *trans*-12  
296 18:2 ( $P = 0.055$ ), *cis* 9, *trans*-13 18:2 ( $P = 0.082$ ), and *cis*-10, *trans*-14 18:2 ( $P =$   
297  $0.024$ , Table 7).

298

299 Milk fat concentrations of n-3 PUFA were higher ( $P < 0.001$ ) with ELS  
300 supplementation (Table 5), mainly due to increases in 18:3 n-3 ( $P < 0.001$ ) and 20:5  
301 n-3 ( $P = 0.025$ ). In contrast, 18:3 n-6 ( $P = 0.036$ ), 20:3 n-6 ( $P = 0.034$ ), 22:4 n-6 ( $P =$   
302  $0.028$ ), and 22:2 n-6 ( $P < 0.095$ ) concentrations were lower in milk fat when ELS was  
303 fed, although there was no effect on total n-6 PUFA concentrations ( $P > 0.10$ , Table  
304 5).

305

306

## DISCUSSION

### *Intake and Milk Yield and Composition*

308 Silage type significantly influences lactation performance, with increases in DMI and  
309 milk yield often observed as MS replaces GS in mixed forage diets (O'Mara et al.,  
310 1998; Kliem et al., 2008). In the present study, DMI was greater when higher MS  
311 diets were fed, which was associated with a numerical increase (1.2 kg/d) in milk  
312 yield and reduction (3.2 g/kg) in milk fat concentration. However, as reported

313 previously (O'Mara et al., 1998; Kliem et al., 2008) milk yield per kg DMI was  
314 numerically lower for higher MS diets.

315

316 No effect of ELS was observed on DMI or milk yield (Table 3). Supplemental dietary  
317 lipid has been shown to increase milk yield (Chilliard and Ferlay, 2004), but the  
318 responses are inconsistent across studies (Grainger and Beauchemin, 2011). This is in  
319 part due to differences in experimental design, diet composition, and the type of fat  
320 fed, as well as stage of lactation (Grainger and Beauchemin, 2011). For example,  
321 feeding extruded flax seed reduced milk yield in late lactation cows (Gonthier et al.,  
322 2005), whilst feeding supplemental lipid may be more likely to increase milk yield in  
323 early lactation, depending on the basal diet and type of lipid fed (Grainger and  
324 Beauchemin, 2011). Increased concentrations of readily available lipid in the rumen  
325 can be detrimental to normal rumen function and can impair fibre digestion and milk  
326 fat synthesis. In previous studies, supplemental ELS reduced milk yield and/or milk  
327 fat concentration (Martin et al., 2008; Kliem et al., 2009), yet in contrast, Hurtaud et  
328 al. (2010) reported an increase in milk yield following ELS supplementation. The lack  
329 of an effect of ELS in the present study may be due to the relatively low level of ELS  
330 inclusion in the diet and the stage of lactation of the cows at the start of the initiation  
331 of the trial.

332

### 333 ***Effects of Forage Type and Extruded Linseed on Methane Production***

334 We observed no effect of dietary forage type on methane production or yield. As  
335 noted previously, studies have found that greater concentrations of starch and lower  
336 concentrations of NDF in rations fed to cattle reduce methane production or yield, or  
337 both (Mills et al., 2001; Aguerre et al., 2011; Grainger and Beauchemin, 2011).



338 Similarly, replacing barley, alfalfa, or grass silage with MS (Hassanat et al., 2013;  
339 Benchaar et al., 2014; van Gastelen et al., 2015) has reduced methane yield for diets  
340 fed to lactating dairy cows, but the effects have been linear (van Gastelen et al., 2015),  
341 curvilinear (Hassanat et al., 2013), or variable over time/age in growing cattle (Staerfl  
342 et al., 202). Basal diets for the present study were based on previous studies, where  
343 feeding higher MS diets reduced methane yield compared with higher GS diets for  
344 lactating dairy cows (Reynolds et al., 2010). The lower methane yield for higher MS  
345 diets was observed despite TMR starch and NDF concentrations being similar for  
346 higher MS and higher GS diets. As in the present study (Table 1), this was achieved  
347 in the study of Reynolds et al. (2010) by adding maize meal to the GS diets and  
348 adding molassed sugar beet feed to the MS diets. This suggests that the source of the  
349 starch and NDF, and the resulting rates of fermentation in the rumen, may also  
350 determine methane yield. In this regard, Moe and Tyrrell (1979) reported that in  
351 addition to intakes of starch and NDF, their digestibility was also an important  
352 determinant of methane production by lactating and non-lactating dairy cattle.  
353 Although diets were formulated to have equal concentrations of starch and NDF in the  
354 present study, starch concentration was higher in MS compared to GS diets, but NDF  
355 concentration was also higher in the MS diets. This was due to differences in the NDF  
356 and starch concentrations of the GS and MS fed during the study compared to the  
357 concentration measured when treatment diets were formulated. Therefore, the higher  
358 concentration of NDF in the MS diets may have counteracted negative effects of  
359 higher starch concentration and MS composition per se on methane yield compared to  
360 GS diets. In addition, the difference in DMI between GS and MS diets was greater in  
361 the previous study (Reynolds et al., 2010), which may also explain differences in the  
362 response of methane yield to forage type between the present and previous study.

363

364 In the present study there was no significant effect of feeding ELS at 50 g/kg diet DM  
365 on methane production or yield. Feeding linseed oil to sheep has previously been  
366 shown to reduce methane production (Blaxter and Czerkawski, 1966; Clapperton,  
367 1974). Furthermore, feeding linseed oil as extruded or crushed linseed (or flax seed)  
368 decreased both methane production and methane yield of lactating dairy cows (Martin  
369 et al., 2008; Beauchemin et al., 2009). Indeed, supplemental dietary fat typically  
370 reduces methane yield of ruminants (Beauchemin et al., 2008; Grainger and  
371 Beauchemin, 2011). The effects of supplemental fat on methane yield are  
372 multifactorial, but are dominated by the provision of a source of digestible energy that  
373 is not fermented in the rumen (Grainger and Beauchemin, 2011). Based on results of  
374 a meta-analysis of published results, Grainger and Beauchemin (2011) concluded that  
375 increasing dietary inclusion of fat caused a linear reduction in methane yield and that  
376 within what were considered to be practical levels of dietary fat inclusion, there was  
377 no apparent difference in the magnitude of the effect of different types and forms of  
378 fat supplements on methane yield of cattle or sheep. Based on their analysis of data in  
379 cattle, methane yield was reduced by 1 g/kg diet DM for every 10 g/kg increase in  
380 dietary fat concentration on a DM basis. In the present study, the average increase in  
381 dietary FA concentration measured (8.1 g/kg DM) was associated with a numerical  
382 reduction in average methane yield (-2.15 g/kg DM), which is more than the decrease  
383 predicted based on the data summarized by Grainger and Beauchemin (2011). This  
384 suggests that the lack of a significant effect of supplemental ELS in the present study  
385 was in part due to the relatively low amount of fat inclusion in the diets. In this regard  
386 the amount fed was approximately twice the amount recommended in UK commercial

387 practice, which would be expected to have only a small effect on methane yield based  
388 on the numerical reduction observed in the present study.

389

390 A relationship between concentrations of a number of FA in milk fat and methane  
391 production or yield by lactating dairy cows has been reported (Chilliard et al., 2009;  
392 Dijkstra et al., 2011; Mohammed et al., 2011). Chilliard et al. (2009) reported that the  
393 large decrease in methane production of dairy cows when linseed oil was fed (Martin  
394 et al., 2008) was associated with a decrease in 8:0 and 16:0 and an increase in total 18  
395 carbon FA and *cis*-9, *trans*-13 18:2 concentrations in milk fat. We observed a  
396 significant increase in *cis*-9, *trans*-13 and decrease in 16:0 when ELS was fed that was  
397 not associated with a significant effect of ELS on methane production. In addition,  
398 there was no effect of ELS at the levels provided on 8:0 concentrations. As discussed  
399 previously, these discrepancies may reflect differences in the amounts of ELS fed  
400 compared with the study of Martin et al. (2008), where supplemental ELS increased  
401 diet ether extract concentration from 26 to 70 g/kg DM. Moreover, the relationships  
402 between milk fat concentrations of individual FA and methane production observed  
403 by Chilliard et al. (2009) may be specific to the dietary treatments used in their study  
404 (supplemental linseed oil). A recent meta-analysis of data from cows fed a variety of  
405 diets found there was no relationship between milk fat concentration of 8:0 or total 18  
406 carbon FA and methane production (Williams et al., 2014), although van Lingen et al.  
407 (2014) recently reported a significant positive relationship between 8:0 and methane  
408 yield in lactating dairy cows.

409

410 ***Effects of Forage Type and Extruded Linseed on Milk FA Concentration***

411 Previous studies have shown that forage type and oil supplements can influence  
412 milk FA composition (Kliem et al., 2008; Samková et al., 2009; Sterk et al., 2011,  
413 Hurtaud et al., 2010). Supplementation of diets with PUFA-rich oil sources such as  
414 ELS is thought to inhibit *de novo* milk FA synthesis of short (4:0-10:0) and medium  
415 (12:0-16:0) chain SFA in the mammary gland (Palmquist et al., 1993); thus reducing  
416 total SFA. Palmquist et al. (1993) suggested that this is due to an increased supply of  
417 dietary- and ruminally-derived unsaturated FA that compete for esterification with  
418 short-chain FA synthesized in the mammary gland. Another possible mechanism is  
419 the inhibitory effect of *trans* 18 isomers produced during biohydrogenation on the *de*  
420 *novo* synthesis of short and medium chain SFA (Chilliard et al., 2001). Previous  
421 studies have confirmed this relationship and corroborate the significantly lower 16:0  
422 concentrations seen in the present study (Glasser et al., 2008). However, we observed  
423 no significant differences in the amounts of short-chain FA following ELS  
424 supplementation, which contradicts previous findings (Glasser et al., 2008). Chilliard  
425 and Ferlay (2004) suggested that short-chain FA are not affected by lipid  
426 supplementation. Instead, it is argued that short-chain FA can be partially synthesised  
427 by pathways independent to medium-chain FA, where the former does not rely on  
428 acetyl-CoA carboxylase (Palmquist and Jenkins, 1980). This may explain why ELS  
429 and forage type had very little effect on the short-chain FA and only a small effect on  
430 medium-chain FA. An additional explanation for this may also be due to the low  
431 linseed oil inclusion level in comparison to other studies, which have fed up to 1 kg of  
432 linseed oil.

433

434 Chilliard et al. (2001) suggested that there was insufficient evidence to confirm the  
435 effect of forage type, as a total mixed ration, on milk FA composition but that MS

436 may increase *de novo* short-chain FA synthesis. To date, few studies have addressed  
437 this, although Kliem et al. (2008) proposed that MS may increase *de novo* short- and  
438 medium-chain FA production via an increased supply of acetate to the mammary  
439 gland. There was little effect of MS on these FA in the present study, in part reflecting  
440 the relatively small differences in forage type (250 g/kg diet DM). However, van  
441 Gastelen et al. (2015) also observed no effect of incremental replacement of GS with  
442 MS on milk fat concentrations of short and medium chain FA, apart from a linear  
443 reduction in 4:0.

444

445 Consistent with previous studies, increases in both 18:0 and total *trans* isomers  
446 concentrations in milk fat were observed when ELS was fed (Kliem et al., 2009;  
447 Hurtaud et al., 2010), as well as increased concentrations of *trans* FA isomers for the  
448 MS diets (Kliem et al., 2008). Inclusion of dietary oils (Collomb et al., 2004) and  
449 particularly unprotected oils (Lor et al., 2005), leads to a characteristic increase in  
450 *trans* and conjugated linoleic acid isomers due to exposure of unsaturated FA to  
451 rumen microflora (Chilliard et al., 2001; Shingfield et al., 2005). As observed in the  
452 present study, Chilliard et al. (2009) identified *trans*-13+14 18:1, *cis*-9, *trans*-13 18:2  
453 and *trans*-11, *cis*-15 18:2 as intermediates of biohydrogenation of the ELS diets.  
454 Although the MS diets had higher concentrations of *cis*-9 18:1 than GS, milk fat *cis*-9  
455 18:1 did not significantly increase. Similarly, despite a higher intake of 18:0 from MS  
456 compared with GS, milk fat 18:0 was not significantly higher following the MS diet.  
457 Our observed effect of forage type on milk fat *trans*-18:1 isomers has been confirmed  
458 in other studies (Shingfield et al., 2005) and has been attributed to differences in  
459 forage digestibility (O'Mara et al., 1998). Additionally, feeding a high MS diet, rich in

460 *n*-6 PUFA and starch, leads to characteristic increases in *trans*-10 18:1 (Kliem et al.,  
461 2008), which is consistent with our findings.

462

463 Linseed supplementation has been used in previous studies to not only reduce milk  
464 SFA, but also increase *n*-3-PUFA. Although, our results showed that this strategy did  
465 increase total *n*-3 PUFA, whether this increase would translate to an important health  
466 benefit to the consumer is questionable. The present study showed a significant  
467 increase in EPA (MS: 34 to 45 mg/100g total FA, GS: 45 to 53 mg/100g total FA)  
468 after ELS supplementation. Based on the enrichment of EPA seen in the present  
469 study, a 100 ml glass of this milk would only contribute up to 0.4% of the 450 mg  
470 daily intake for long-chain PUFA recommended for UK adults (Givens, 2008).  
471 Although not substantial, these calculations do not include other *n*-3 FA and dairy  
472 products. In addition, supplementation of the dairy cow's diet with ELS may  
473 represent a sustainable alternative to the use of marine oils, which have environmental  
474 and economic implications.

475

476

477 Growing public interest in lowering SFA consumption to improve human health  
478 means that any decrease in milk SFA concentrations following forage and lipid  
479 supplementation has public health incentives. Our study found only three minor  
480 interactions between forage type and ELS supplementation for the selected milk FA,  
481 which are in line with findings by Sterk et al. (2011). While lipid supplementation,  
482 and possibly MS, provided potentially beneficial decreases in SFA, the current  
483 concerns linking *trans* FA to increased risk of CVD mean that the significantly higher  
484 total *trans* concentrations following both MS and ELS supplementation may

485 counteract the beneficial decreases in SFA concentration. The question of whether  
486 ruminant *trans* are of similar risk to CVD as industrial *trans* remaining largely  
487 unanswered (Bendsen et al., 2011). Nonetheless, the implementation of *trans* labelling  
488 suggests that increases should be minimised, and development of lipid protection  
489 technologies is required to minimise their production. As current UK intakes of long  
490 chain PUFA are inadequate (Givens, 2008), enrichment of milk in this way may have  
491 long-term implications for human health. Nevertheless, it is questionable whether the  
492 magnitude of the changes in long chain PUFA concentrations seen in this study would  
493 produce a meaningful impact on health on a population level.

494

495

## CONCLUSIONS

496 The present study demonstrated that a relatively low inclusion level of oilseed (ELS)  
497 supplement can partially replace milk SFA with MUFA and PUFA, including long-  
498 chain PUFA, thereby offering a sustainable means of modifying milk FA  
499 composition, irrespective of whether MS or GS diets are fed. Methane production  
500 was not significantly affected, but numerical reductions observed were in line with  
501 predictions based on the relatively low amount of linseed oil fed. In contrast to other  
502 studies where replacing GS with MS increased starch and decreased NDF in the diets  
503 fed, replacing GS with MS in diets formulated for similar NDF and starch  
504 concentrations did not reduce methane production or yield, in part due to a lower NDF  
505 concentration in the GS than expected. Decreases in SFA and increases in  
506 unsaturated FA concentrations in milk fat were observed that if considered at a  
507 population level, including implications for other dairy products and dairy-containing  
508 foods, may contribute to a lower risk of CVD. However, there is a need to balance  
509 changes in beneficial PUFAs and detrimental SFA and *trans* FA, while avoiding any

510 effects on cow performance. These priorities remain a challenge to the agriculture and  
511 food sectors and require further exploration.

512

513

### ACKNOWLEDGMENTS

514 Funding by Marks and Spencer plc is gratefully acknowledged. The contributions of  
515 staff at the Centre for Dairy Research of the University of Reading for the care and  
516 management of animals used and for technical assistance during the study is also  
517 much appreciated.

518

### REFERENCES

519 Aguerre, M. J., M. A. Wattiaux, J. M. Powell, G. A. Broderick, and C. Arndt. 2011.  
520 Effect of forage-to-concentrate ratio in dairy cow diets on emission of methane,  
521 carbon dioxide, and ammonia, lactation performance, and manure excretion. *J. Dairy*  
522 *Sci.* 94:3081-3093. <http://dx.doi.org/10.3168/jds.2010-4011>.

523

524 Beauchemin, K. A., M. Kreuzer, F. O'Mara, and T. A. McAllister. 2008. Nutritional  
525 management for enteric methane abatement: a review. *Austr. J. Exper. Agric.* 48:21-  
526 27. <http://dx.doi.org/10.1071/EA07199>.

527

528 Benchaar, C., F. Hassanat, R. Gervais, P. Y. Chouinard, H. V. Petit, and D. I. Massé.  
529 2014. Methane production, digestion, ruminal fermentation, nitrogen balance, and  
530 milk production of cows fed corn silage- or barley silage-based diets. *J. Dairy Sci.*  
531 97:961-974. <http://dx.doi.org/10.3168/jds.2013-7122>.

532

533 Bendsen, N. T., R. Christensen, E.M. Bartels, and A. Astrup. 2011. Consumption of  
534 industrial and ruminant *trans* fatty acids and risk of coronary heart disease: a



535 systematic review and meta-analysis of cohort studies. *Eur. J. Clin. Nutr.* 65:773-83.  
536 <http://dx.doi:10.1038/ejcn.2011.34>

537

538 Blaxter, K. L., and J. Czerkawski. 1966. Modifications of methane production of the  
539 sheep by supplementation of its diet. *J. Sci. Food Agric.* 17:417-421.  
540 <http://dx.doi.org/10.1002/jsfa.2740170907>.

541

542 Brask, M., P. Lund, A. L. F. Hellwing, M. Poulsen, and M. R. Weisbjerg. 2013.  
543 Enteric methane production, digestibility and rumen fermentation in dairy cows fed  
544 different forages with and without rapeseed fat supplementation. *Anim. Feed Sci.*  
545 *Tech.* 184:67-79.

546 Chilliard, Y. and A. Ferlay. 2004. Dietary lipids and forages interactions on cow and  
547 goat milk fatty acid composition and sensory properties.  
548 *Reprod. Nutr. Dev.* 44: 467–492 467. <http://dx.doi: 10.1051/rnd:2004052>

549

550 Chilliard, Y., A. Ferlay, and M. Doreau. 2001 Effect of different types of forages,  
551 animal fat or marine oils in cow's diet on milk fat secretion and composition,  
552 especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. *Livest.*  
553 *Prod. Sci.* 70: 31-48.

554

555 Chilliard, Y., C. Martin, J. Rouel, and M. Doreau, M. 2009. Milk fatty acids in dairy  
556 cows fed whole crude linseed, extruded linseed, or linseed oil, and their relationship  
557 with methane output. *J. Dairy Sci.* 92:5199-5211. [http://dx.doi.org/10.3168/jds.2009-](http://dx.doi.org/10.3168/jds.2009-2375)  
558 2375.

559

- 560 Clapperton, J. L. 1974. The effect of trichloroacetamide, chloroform and linseed oil  
561 given into the rumen of sheep on some of the end-products of rumen digestion. *Br. J.*  
562 *Nutr.* 32:155-161. <http://dx.doi.org/10.1079/BJN19740065>.
- 563
- 564 Collomb, M., R. Sieber, and U. Bütikofer. 2004 CLA isomers in milk fat from cows  
565 fed diets with high levels of unsaturated fatty acids. *Lipids.* 39: 355-364.
- 566
- 567 Dijkstra, J., S. M. van Zijderveld, J. A. Apajalahti, A. Bannink, W. J. J. Gerrits, J. R.  
568 Newbold, H. B. Perdok, H. Berends. 2011. Relationships between methane  
569 production and milk fatty acid profiles in dairy cattle. *Anim. Feed Sci. Tech.*  
570 166:590–595. <http://dx.doi.org/10.1016/j.anifeedsci.2011.04.042>.
- 571
- 572 Ferlay, A., B. Martin, P. Pradel, J.B. Coulon, and Y. Chilliard. 2006. Influence of  
573 Grass-Based Diets on Milk Fatty Acid Composition and Milk Lipolytic System in  
574 Tarentaise and Montbéliarde Cow Breeds. *J. Dairy Sci.* 89: 4026-4041.
- 575
- 576 Givens, D. I. 2008. Session 4: Challenges facing the food industry in innovating for  
577 health Impact on CVD risk of modifying milk fat to decrease intake of SFA and  
578 increase intake of *cis*-MUFA. *Proc. Nutr. Soc.* 67:419-427.  
579 <http://dx.doi.org/10.1017/S0029665108008707>.
- 580
- 581 Givens, D. I., K. E. Kliem, D. J. Humphries, K. J. Shingfield, and R. Morgan. 2009.  
582 Effect of replacing calcium salts of palm oil distillate with rapeseed oil, milled or  
583 whole rapeseeds on milk fatty-acid composition in cows fed maize silage-based diets.  
584 *Animal.* 3:1067-1074. <http://dx.doi.org/10.1017/S175173110900442X>.

585

586 Glasser, F., A. Ferlay, and Y. Chilliard. 2008. Oilseed Lipid Supplements and Fatty  
587 Acid Composition of Cow Milk: A Meta-Analysis. *J. Dairy Sci.* 91: 4687-4703.  
588 <http://dx.doi.org/10.3168/jds.2008-0987>.

589

590 Gonthier, C., A. F. Mustafa, D. R. Ouellet, P. Y. Chouinard, R. Berthiaume, and H. V.  
591 Petit. 2005. Feeding Micronized and Extruded Flaxseed to Dairy Cows: Effects on  
592 Blood Parameters and Milk Fatty Acid Composition. *J. Dairy Sci.* 88:748-756.

593

594 Grainger, C., and K. A. Beauchemin. 2011. Can enteric methane emissions from  
595 ruminants be lowered without lowering their production? *Anim. Feed Sci. Tech.* 166–  
596 167:308-320. <http://dx.doi.org/10.1016/j.anifeedsci.2011.04.021>

597

598 Hassanat, F., R. Gervais, C. Julien, D. I. Massé, A. Lettat, P. Y. Chouinard, H. V.  
599 Petit, and C. Benchaar. 2014. Replacing alfalfa silage with corn silage in dairy cow  
600 diets: Effects on enteric methane production, ruminal fermentation, digestion, N  
601 balance, and milk production. *J. Dairy Sci.* 96:4553-4567.  
602 <http://dx.doi.org/10.3168/jds.2013-7122>.

603

604 Hurtaud, C., F. Faucon, S. Couvreur, and J. L. Peyraud. 2010. Linear relationship  
605 between increasing amounts of extruded linseed in dairy cow diet and milk fatty acid  
606 composition and butter properties. *J. Dairy Sci.* 93:1429-1443.  
607 <http://dx.doi.org/10.3168/jds.2009-2839>.

608

609 Kliem, K. E., P. C. Aikman, D. J. Humphries, R. Morgan, K. J. Shingfield, K. J. and  
610 D. I. Givens. 2009. Effect of replacing calcium salts of palm oil distillate with  
611 extruded linseeds on milk fatty acid composition in Jersey and Holstein cows.  
612 *Animal*. 3:1754-1762. <http://dx.doi.org/10.1017/S1751731109990723>.

613

614 Kliem, K. E., R. Morgan, D. J. Humphries, K. J. Shingfield, K. J. and D. I. Givens.  
615 2008. Effect of replacing grass silage with maize silage in the diet on bovine milk  
616 fatty acid composition. *Animal*. 2:1850-1858.  
617 <http://dx.doi.org/10.1017/S1751731108003078>.

618

619 Kliem, K. E., C. K. Reynolds , D. J. Humphries , R. M. Kirkland , C. E. S. Barratt, K.  
620 M. Livingstone, and D. I. Givens. 2013. Incremental effect of a calcium salt of *cis*-  
621 monounsaturated fatty acids supplement on milk fatty acid composition in cows fed  
622 maize silage-based diets. *J. Dairy Sci.* 96:3211-3221.  
623 <http://dx.doi.org/10.3168/jds.2012-6211>.

624

625 Loor, J. J., A. Ferlay, A. Ollier, M. Doreau, and Y. Chilliard. 2005. Relationship  
626 Among *Trans* and Conjugated Fatty Acids and Bovine Milk Fat Yield Due to Dietary  
627 Concentrate and Linseed Oil. *J. Dairy. Sci.* 88:726-740.

628

629 Martin, C., J. Rouel, J. P. Jouany, M. Doreau, and Y. Chilliard. 2008. Methane  
630 output and diet digestibility in response to feeding dairy cows crude linseed, extruded  
631 linseed, or linseed oil. *J. Anim. Sci.* 86: 2642-2650.  
632 <http://dx.doi.org/10.2527/jas.2007-0774>

633

634 Mills, J. A. N., J. Dijkstra, A. Bannink, S. B. Cammell, E. Kebreab, and J. France.  
635 2001. A mechanistic model of whole-tract digestion and methanogenesis in the  
636 lactating dairy cow: Model development, evaluation and application. *J. Anim. Sci.*  
637 79:1584-1597.

638

639 Moe, P. W. and H. F. Tyrrell. 1979. Methane production in dairy cows. *J. Dairy Sci.*  
640 62:1583-1586. [http://dx.doi.org/10.3168/jds.S0022-0302\(79\)83465-7](http://dx.doi.org/10.3168/jds.S0022-0302(79)83465-7).

641

642 Mohammed, R., S. M. McGinn, and K. A. Beauchemin. 2009. Prediction of enteric  
643 methane output from milk fatty acid concentrations and rumen fermentation  
644 parameters in dairy cows fed sunflower, flax, or canola seeds. *J. Dairy Sci.* 94:6057-  
645 6068. <http://dx.doi.org/10.3168/jds.2011-4369>.

646

647 Nielsen, T. S., K. Sejrsen, H. R. Andersen, P. Lund, and E. M. Straarup. 2004. Effect  
648 of silage type and energy concentration on conjugated linoleic acid (CLA) in milk fat  
649 from dairy cows. *Anim. Feed Sci. Technol.* 13:697-700.

650

651 O'Mara, F. P., J. J. Fitzgerald, J. J. Murphy, and M. Rath. 1998. The effect on milk  
652 production of replacing grass silage with maize silage in the diet of dairy cows.  
653 *Livest. Prod. Sci.* 55:79-87.

654

655 Palmquist, D. L., A. D. Beaulieu, and D. M. Barbano. 1993. Feed and animal factors  
656 influencing milk fat composition. *J. Dairy Sci.* 76:1753-1771.  
657 [http://dx.doi.org/10.3168/jds.S0022-0302\(93\)77508-6](http://dx.doi.org/10.3168/jds.S0022-0302(93)77508-6).

658

659 Palmquist, D. L. and T. C. Jenkins. 1980. Fat in Lactation Rations : Review. *J. Dairy*  
660 *Sci.* 63:1-14. [http://dx.doi.org/10.3168/jds.S0022-0302\(80\)82881-5](http://dx.doi.org/10.3168/jds.S0022-0302(80)82881-5)

661

662 Reynolds, C. K., L. A. Crompton, J. A. N. Mills, D. J. Humphries, P. Kirton, A. E.  
663 Relling, T. H. Misselbrook, D. R. Chadwick, and D. I. Givens. 2010. Effects of diet  
664 protein level and forage source on energy and nitrogen balance and methane and  
665 nitrogen excretion in lactating dairy cows. Pages 463-464 in *Proceedings of the 3rd*  
666 *International Symposium on Energy and Protein Metabolism.* G. M. Corvetto, ed.  
667 *EAAP Publ. No. 127, Wageningen Academic Publishers, The Netherlands.*

668

669 Reynolds, C. K., D. J. Humphries, P. Kirton, M. Kindermann, S. Duval, and W.  
670 Steinberg. 2014. Effects of 3-nitrooxypropanol on methane emission, digestion, and  
671 energy and nitrogen balance of lactating dairy cows. *J. Dairy Sci.* 97:3777-3789.  
672 <http://dx.doi.org/10.3168/jds.2013-7397>.

673

674 Samková, E., M. Pešek, J. Špička T. Pelikánová and O. Hanuš. 2009. The effect of  
675 feeding diets markedly differing in the proportion of grass and maize silages on  
676 bovine milk fat composition. *Czech J. Anim. Sci.* 54:93-100.

677

678 Shingfield, K. J., C. K. Reynolds, B. Lupoli, V. Toivonen, M. P. Yurawecz, P.  
679 Delmonte, J. M. Griinari, A. S. Grandison, and D. E. Beaver. 2005. Effect of forage  
680 type and proportion of concentrate in the diet on milk fatty acid composition in cows  
681 given sunflower oil and fish oil. *Anim. Sci.* 80:225-238.  
682 <http://dx.doi.org/10.1079/ASC41820225>.

683

- 684 Staerfl, S. M., J. O. Zeitz, M. Kreuzer, and C. R. Soliva. 2012. Methane conversion  
685 rate of bulls fattened on grass or maize silage as compared with IPCC default values,  
686 and the long-term methane mitigation efficiency of adding acacia tannin, garlic, maca  
687 and lupine. *Agric. Ecosyst. Environ.* 148:111-120.
- 688
- 689 Sterk, A., B. E. O. Johansson, H. Z. H. Taweel, M. Murphy, A. M. van Vuuren, W. H.  
690 Hendriks, and J. Dijkstra. 2011. Effects of forage type, forage to concentrate ratio, and  
691 crushed linseed supplementation on milk fatty acid profile in lactating dairy cows. *J.*  
692 *Dairy Sci.* 94:6078-6091. <http://dx.doi.org/10.3168/jds.2011-4617>.
- 693
- 694 van Gastelen, S., E. C. Antunes-Fernandes, K. A. Hettinga, G. Klop, S. J. J. Alferink,  
695 W. H. Hendriks, and J. Dijkstra. 2015. Enteric methane production, rumen volatile  
696 fatty acid concentrations, and milk fatty acid composition in lactating Holstein-  
697 Friesian cows fed grass silage- or corn silage-based diets. *J. Dairy Sci.* 98:1915–1927.
- 698
- 699 van Lingen, H. J., L. A. Crompton, W. H. Hendriks, C. K. Reynolds, and J. Dijkstra.  
700 2014. Meta-analysis of relationships between methane production and milk fatty acid  
701 profile in dairy cattle. *J. Dairy Sci.* 97:7115–7132.  
702 <http://dx.doi.org/10.3168/jds.2014-8268>.
- 703 Williams, S. R. O., P. J. Moate, M. H. Deighton, M. C. Hannah, and W. J. Wales.  
704 2014. Methane emissions of dairy cows cannot be predicted by the concentrations of  
705 C8:0 and total C18 fatty acids in milk. *Anim. Prod. Sci.* 54:1757-1761.  
706 <http://dx.doi.org/10.1071/AN14292>.
- 707
- 708

**Table 1.** Ingredients and chemical composition of the experimental diets (g/kg DM or as stated).

	Treatment <sup>1</sup>			
	MS	ML	GS	GL
Ingredients				
Grass silage <sup>2</sup>	125	125	375	375
Maize silage <sup>3</sup>	375	375	125	125
Cracked wheat	100	100	100	100
Maize meal	0	0	100	100
Molassed sugar beet feed	50	50	0	0
Soyabean hulls	92	79	98	86
Wheat feed	92	60	90	57
SoyPass <sup>®4</sup>	26	26	26	26
Soybean meal	62	57	51	46
Rapeseed meal	43	43	0	0
Molasses	15	15	15	15
Di-calcium phosphate	5	5	5	5
Salt <sup>5</sup>	5	5	5	5
Minerals and vitamins <sup>6</sup>	10	10	10	10
Extruded linseed <sup>7</sup>	0	50	0	50

<sup>1</sup>Maize silage-based TMR (MS), maize silage-based TMR with extruded linseed (ML), grass silage-based TMR (GS), grass silage-based TMR with extruded linseed (GL).

<sup>2</sup>Containing (g/kg DM): crude protein (159), NDF (339), sugars (18.4) and ash (92).

<sup>3</sup>Containing (g/kg DM): crude protein (70), NDF (346), starch (344), sugars (13.2), and ash (31).

<sup>4</sup>Rumen bypass soybean meal, Borregaard LignoTech, KW Alternative Feeds, Bury St. Edmunds, UK)

<sup>5</sup>Pioneer Rocksalt, Broste Ltd., Norfolk, UK.

<sup>6</sup>Dairy Direct, Bury St Edmunds, Suffolk, UK.

<sup>7</sup>Lintec, BOCM Pauls Ltd., Wherstead, UK. Declared composition (g/kg DM): crude protein (196), NDF (295), sugars (41.5), and ash (49.1).



709

710

711 **Table 2.** Composition of the total mixed rations fed (g/kg unless stated) on a dry  
 712 matter (DM) basis.

	Treatments <sup>1</sup>				SEM	P < <sup>2</sup>		
	MS	ML	GS	GL		F	L	F*L
Organic matter	932	937	924	925	2.5	0.014	0.304	0.581
Crude protein	157	157	166	163	2.3	0.010	0.507	0.373
NDF	320	334	303	308	4.9	0.006	0.115	0.383
ADF	220	218	240	227	4.8	0.016	0.129	0.210
Starch	223	211	194	186	4.1	0.001	0.078	0.632
Sugars	33.9	34.3	34.2	35.5	2.3	0.611	0.552	0.780
Ash	68.3	63.5	76.3	74.8	2.5	0.014	0.304	0.581
ME, MJ/kg DM	11.4	11.3	11.2	11.3	0.08	0.339	0.515	0.216
Fatty acids								
16:0	3.05	3.52	3.11	3.39	0.052	0.575	0.003	0.195
18:0	0.53	0.86	0.44	0.77	0.025	0.007	0.001	0.875
18:1 <i>cis</i> -9	4.17	5.86	3.08	4.20	0.085	0.001	0.001	0.009
18:2 n-6	9.80	10.56	8.36	9.31	0.162	0.001	0.003	0.601
18:3 n-3	2.72	8.32	4.73	9.07	0.279	0.015	0.001	0.106
Total fatty acids	21.88	31.29	21.84	28.63	0.400	0.026	0.001	0.028

<sup>1</sup>Maize silage-based TMR (MS), maize silage-based TMR with extruded linseed (ML), grass silage-based TMR (GS), grass silage-based TMR with extruded linseed (GL).

<sup>2</sup>Probability for the effect of forage (F), extruded linseed (L), or their interaction (F\*L).

713

**Table 3.** Effects of extruded linseed supplementation and dietary forage on dry matter (DM) and fatty acid intake and milk and constituent yield.

	Treatments <sup>1</sup>					P < <sup>2</sup>		
	MS	ML	GS	GL	SEM	F	L	F*L
DM intake, kg/d	20.3	21.2	19.2	19.7	1.1	0.094	0.310	0.712
Fatty acid intake, g/d								
16:0	60.1	70.7	57.5	66.6	3.24	0.125	0.002	0.691
18:0	11.2	18.8	9.86	16.4	0.66	0.002	0.001	0.186
18:1 <i>cis</i> -9	62.0	96.5	47.8	75.3	3.38	0.001	0.001	0.123
18:2 n-6	199	224	47.8	49.0	9.87	0.001	0.007	0.814
18:3 n-3	61.1	176	84.3	180	5.72	0.005	0.001	0.019
Total FA	461	660	403	568	25.40	0.002	0.001	0.296
Yield								
Milk, kg/d	36.1	37.4	35.7	35.4	1.1	0.358	0.710	0.519
4% FCM, kg/d	32.4	33.8	35.1	32.2	2.0	0.763	0.665	0.230
Fat, g/d	1200	1258	1387	1203	125.5	0.51	0.528	0.244
Protein, g/d	1143	1199	1149	1126	30.3	0.310	0.608	0.239
Lactose, g/d	1624	1670	1659	1598	92.7	0.851	0.941	0.589
Casein, g/d	850	895	870	841	32	0.642	0.816	0.329
Concentration								
Fat, g/kg	33.0	33.6	38.9	34.1	3.4	0.223	0.400	0.300
Protein, g/kg	31.6	32.1	32.3	31.8	0.5	0.609	0.955	0.200
Lactose, g/kg	45.0	44.6	46.3	45.3	1.4	0.453	0.587	0.808
Casein, g/d	23.5	24.0	24.4	23.8	0.51	0.276	0.805	0.134
Urea, mg/dL	23.2	22.1	23.1	21.1	1.6	0.651	0.264	0.708

<sup>1</sup>Maize silage-based TMR (MS), maize silage-based TMR with extruded linseed (ML), grass silage-based TMR (GS), grass silage-based TMR with extruded linseed (GL).

<sup>2</sup>Probability for the effect of forage (F), extruded linseed (L), or their interaction (F\*L).

715 **Table 4.** Effects of extruded linseed (Lintec) supplementation and dietary forage source on methane production and respiratory exchange of  
 716 lactating dairy cows.

	Treatment <sup>1</sup>				SEM	P < <sup>2</sup>		
	MS	ML	GS	GL		F	L	F*L
CH <sub>4</sub> , L/d	598	580	567	553	35.0	0.274	0.520	0.939
CH <sub>4</sub> , MJ/d	23.7	22.9	22.4	21.8	1.39	0.274	0.520	0.939
CH <sub>4</sub> , L/kg DMI	29.5	27.5	30.4	28.1	2.47	0.635	0.213	0.939
CH <sub>4</sub> , L/kg milk	16.5	15.5	16.1	15.7	1.09	0.878	0.391	0.719
O <sub>2</sub> consumed, L/d	7046	7081	6318	6626	294.2	0.026	0.427	0.523
CO <sub>2</sub> produced, L/d	7124	7212	6468	6659	329.8	0.037	0.559	0.828
Heat, MJ/d <sup>3</sup>	148.0	148.3	132.5	140.0	5.8	0.023	0.361	0.394

<sup>1</sup>Maize silage-based TMR (MS), maize silage-based TMR with extruded linseed (ML), grass silage-based TMR (GS), grass silage-based TMR with extruded linseed (GL).

<sup>2</sup>Probability for the effect of forage (F), extruded linseed (L), or their interaction (F\*L).

<sup>3</sup>Calculated based on respiratory exchange and methane production.

**Table 5.** Effects of extruded linseed supplementation and dietary forage on milk fatty acid composition (g/100g total fatty acids)

Fatty acid	Treatments <sup>1</sup>				SEM	P < <sup>2</sup>		718
	MS	ML	GS	GL		F	L	
4:0	3.1	3.3	3.4	3.1	0.23	0.657	0.754	0.112
6:0	2.3	2.3	2.5	2.45	0.21	0.137	0.756	0.762
8:0	1.3	1.3	1.5	1.4	0.14	0.147	0.667	0.939
10:0	3.0	2.8	3.2	3.2	0.27	0.179	0.554	0.584
10:1 <i>cis</i> -9	0.26	0.26	0.34	0.32	0.031	0.021	0.616	0.646
12:0	3.4	3.1	3.6	3.5	0.23	0.170	0.412	0.469
12:1 <i>cis</i> -9	0.07	0.06	0.09	0.08	0.006	0.042	0.292	0.565
13:0 iso	0.000	0.000	0.004	0.005	0.002	0.034	0.645	0.645
13:0 anteiso	0.02	0.02	0.03	0.04	0.021	0.058	0.833	0.768
13:0 <sup>3</sup>	0.09	0.09	0.10	0.10	0.014	0.170	0.589	0.639
14:0	11.3	10.8	11.7	11.6	0.36	0.082	0.349	0.554
14:1 <i>trans</i> -9	0.20	0.20	0.23	0.21	0.010	0.054	0.418	0.223
14:1 <i>cis</i> -9	0.91	0.91	1.03	0.96	0.111	0.049	0.334	0.337
15:0	0.93	0.86	1.04	1.02	0.084	0.009	0.259	0.442
15:1 <i>trans</i> -5	0.02	0.02	0.030	0.02	0.005	0.317	0.171	0.638
16:0 iso	0.21	0.22	0.23	0.20	0.018	0.948	0.318	0.106
16:0	29.8	25.7	30.8	28.1	1.66	0.126	0.012	0.503
16:1 <i>cis</i> -9 <sup>4</sup>	1.7	1.5	1.8	1.5	0.105	0.662	0.020	0.473

16:1 <i>cis</i> -11	0.03	0.03	0.04	0.03	0.008	0.484	0.812	0.812
16:1 <i>cis</i> -13	0.04	0.04	0.07	0.06	0.012	0.101	0.764	0.780
16:1 <i>trans</i> -6-7	0.02	0.02	0.02	0.02	0.005	0.229	0.878	0.721
16:1 <i>trans</i> -8	0.016	0.010	0.002	0.009	0.008	0.131	0.799	0.181
16:1 <i>trans</i> -9 <sup>5</sup>	0.37	0.40	0.38	0.36	0.026	0.478	0.726	0.233
16:1 <i>trans</i> -10	0.011	0.003	0.005	0.012	0.005	0.665	0.884	0.063
16:1 <i>trans</i> -11	0.03	0.05	0.03	0.04	0.011	0.435	0.063	0.263
16:1 <i>trans</i> -12	0.14	0.12	0.11	0.11	0.009	0.136	0.442	0.642
17:0	0.57	0.52	0.59	0.54	0.046	0.108	0.009	0.761
18:0 iso	0.19	0.16	0.20	0.17	0.026	0.313	0.052	0.663
18:0	9.35	10.5	8.7	9.7	0.60	0.138	0.039	0.857
18:1 <i>trans</i> total	5.2	6.3	3.6	4.9	0.63	0.008	0.024	0.801
18:1 <i>cis</i> total	19.1	21.4	18.4	19.4	1.58	0.227	0.143	0.528
Non-CLA <sup>6</sup> 18:2 total	0.73	1.1	0.75	1.09	0.14	0.974	<.0001	0.361
CLA total	0.57	0.66	0.46	0.57	0.09	0.146	0.128	0.875
18:3 <i>cis</i> -6,9,12	0.02	0.01	0.03	0.01	0.006	0.443	0.036	0.370
18:3 <i>cis</i> -9,12,15	0.44	0.8	0.50	0.78	0.039	0.438	<.0001	0.205
19:0 <sup>7</sup>	0.16	0.25	0.15	0.23	0.039	0.591	0.005	0.704
19:1 <i>cis</i> -7	0.007	0.011	0.004	0.015	0.003	0.881	0.025	0.239
20:0	0.12	0.13	0.12	0.12	0.007	0.604	0.980	0.570
20:1 <i>cis</i> -5	0.000	0.000	0.002	0.000	0.001	0.356	0.356	0.356
20:1 <i>cis</i> -9	0.09	0.10	0.10	0.10	0.008	0.551	0.660	0.283
20:1 <i>cis</i> -11	0.05	0.05	0.04	0.04	0.005	0.047	1.000	0.820

20:2 n-6	0.007	0.001	0.000	0.000	0.004	0.418	0.524	0.562 <sup>19</sup>
20:3 n-3	0.000	0.001	0.008	0.005	0.002	0.024	0.642	0.289 <sub>20</sub>
20:3 n-6	0.10	0.08	0.10	0.07	0.012	0.743	0.034	0.943 <sub>21</sub>
20:4 n-6	0.10	0.10	0.13	0.11	0.022	0.361	0.654	0.470 <sup>22</sup>
20:5 n-3	0.03	0.05	0.05	0.05	0.004	0.020	0.025	0.669
22:0	0.010	0.001	0.001	-0.001	0.006	0.418	0.524	0.562
22:1 <i>cis</i> -13	0.001	0.000	0.000	0.000	0.001	0.356	0.356	0.356
22:2 n-6	0.014	0.010	0.043	0.038	0.004	<.0001	0.095	0.775
22:3 n-3	0.001	0.003	0.012	0.006	0.004	0.196	0.670	0.378
22:4 n-6	0.02	0.01	0.02	0.01	0.004	0.647	0.028	0.926
22:5 n-3	0.09	0.08	0.09	0.08	0.015	0.886	0.362	0.977
22:6 n-3	0.003	0.000	0.000	0.000	0.002	0.356	0.356	0.356
24:0	0.02	0.01	0.03	0.02	0.007	0.010	0.022	0.584
$\sum \leq 14:0$	24.8	23.7	26.3	25.8	1.28	0.124	0.475	0.799
$\sum$ saturates	67.5	63.3	69.7	67.1	2.57	0.076	0.055	0.586
$\sum$ <i>cis</i> MUFA	21.4	23.6	21.1	21.8	1.63	0.306	0.185	0.479
$\sum$ <i>trans</i> MUFA	5.9	6.9	4.2	5.5	0.66	0.009	0.027	0.831
$\sum$ <i>trans</i> total	6.4	7.6	4.7	6.1	0.71	0.011	0.030	0.832
n-3 PUFA	0.73	1.2	0.83	1.2	0.08	0.268	<.0001	0.293
n-6 PUFA	2.6	2.5	2.2	2.1	0.14	0.001	0.187	0.766
Fatty acids (g/100g fat)	93.7	93.5	93.4	93.6	0.12	0.232	0.880	0.181

<sup>1</sup>Maize silage-based TMR (MS), maize silage-based TMR with extruded linseed (ML), grass silage-based TMR (GS), grass silage-based TMR with extruded linseed (GL).

<sup>2</sup>Probability for the effect of forage (F), extruded linseed (L), or their interaction (F\*L).

<sup>3</sup>Co-elutes with *cis*-9 12:1

<sup>4</sup>Co-elutes with 17:0 anteiso

<sup>5</sup>Co-elutes with 17:0 iso

<sup>6</sup>All 18:2 isomers excluding CLA

<sup>7</sup>Co-elutes with *cis*-15 18:1



724 **Table 6.** Effects of extruded linseed supplementation and dietary forage on milk 18:1 isomer composition (g/100g total fatty acids)

Fatty acid	Treatment <sup>1</sup>				SEM	P < <sup>2</sup>		
	MS	ML	GS	GL		F	L	F*L
<i>cis</i> -9 18:1 <sup>3</sup>	17.4	19.4	17.2	17.9	1.40	0.371	0.189	0.482
<i>cis</i> -11 18:1	0.75	0.73	0.54	0.58	0.123	0.016	0.922	0.598
<i>cis</i> -12 18:1	0.46	0.57	0.29	0.41	0.048	0.005	0.021	0.935
<i>cis</i> -13 18:1	0.12	0.12	0.09	0.11	0.019	0.046	0.180	0.422
<i>cis</i> -16 18:1	0.05	0.08	0.03	0.04	0.015	0.003	0.014	0.408
<i>trans</i> -5 18:1	0.030	0.015	0.004	0.018	0.005	0.044	0.849	0.016
<i>trans</i> -6,-7,-8 18:1	0.39	0.45	0.23	0.30	0.058	0.004	0.103	0.791
<i>trans</i> -9 18:1	0.33	0.38	0.21	0.27	0.063	0.045	0.268	0.888
<i>trans</i> -10 18:1	0.92	0.88	0.41	0.54	0.313	0.038	0.784	0.624
<i>trans</i> -11 18:1	1.3	1.6	0.86	1.18	0.194	0.056	0.114	0.947
<i>trans</i> -13-14 18:1	0.93	1.25	0.81	1.09	0.190	0.060	0.002	0.722
<i>trans</i> -15 18:1	0.54	0.72	0.50	0.66	0.063	0.058	0.002	0.746
<i>trans</i> -16 18:1 <sup>4</sup>	0.46	0.63	0.40	0.58	0.049	0.028	0.001	1.000

725 <sup>1</sup>Maize silage-based TMR (MS), maize silage-based TMR with extruded linseed (ML), grass silage-based TMR (GS), grass silage-based TMR  
726 with extruded linseed (GL).

727 <sup>2</sup>Probability for the effect of forage (F), extruded linseed (L), or their interaction (F\*L).

728 <sup>3</sup>Co-elutes with *cis*-10 18:1

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

730 **Table 7.** Effects of extruded linseed supplementation and dietary forage on milk 18:2 isomer composition (g/100g total fatty acids).

Fatty acid	Treatment <sup>1</sup>				SEM	P < <sup>2</sup>		
	MS	ML	GS	GL		F	L	F*L
<i>cis</i> -9, <i>cis</i> -12 18:2	2.30	2.20	1.80	1.70	0.14	0.002	0.377	0.759
<i>cis</i> -9 <i>cis</i> -15 18:2	0.05	0.05	0.06	0.05	0.010	0.424	0.475	0.279
<i>cis</i> -9, <i>trans</i> -12 18:2	0.06	0.06	0.04	0.06	0.009	0.140	0.020	0.055
<i>cis</i> -9, <i>trans</i> -13 18:2	0.21	0.38	0.23	0.34	0.074	0.324	0.001	0.082
<i>cis</i> -9, <i>trans</i> -14 18:2	0.11	0.16	0.11	0.15	0.029	0.597	0.001	0.417
<i>cis</i> -10, <i>trans</i> -14 18:2	0.15	0.11	0.13	0.14	0.009	0.441	0.145	0.024
<i>trans</i> -9, <i>cis</i> -12 18:2	0.02	0.03	0.01	0.02	0.004	0.125	0.008	0.452
<i>trans</i> -11, <i>cis</i> -15 18:2	0.06	0.19	0.09	0.20	0.026	0.320	0.0001	0.518
<i>trans</i> -12, <i>cis</i> -15 18:2	0.03	0.03	0.02	0.04	0.006	0.593	0.028	0.302
<i>trans</i> -11, <i>trans</i> -15 18:2	0.05	0.05	0.04	0.05	0.006	0.140	0.715	0.472

<sup>1</sup>Maize silage-based TMR (MS), maize silage-based TMR with extruded linseed (ML), grass silage-based TMR (GS), grass silage-based TMR with extruded linseed (GL).

<sup>2</sup>Probability for the effect of forage (F), extruded linseed (L), or their interaction (F\*L).