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

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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.

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16

DIET EFFECTS ON MILK FATTY ACIDS AND METHANE2033

Effects of forage type and extruded linseed supplementation on methane

production and milk fatty acid composition of lactating dairy cows

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42 **ABSTRACT**

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 ruminaly 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.

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 (± SEM) 643 ± 40 kg BW and 60

143   ± 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**

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

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

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

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 <div align="center">**RESULTS**</div>

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**

307   *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 (Loor 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**

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708

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

| | Treatment[1] | | | |
	MS	ML	GS	GL
Ingredients				
Grass silage[2]	125	125	375	375
Maize silage[3]	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®[4]	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[5]	5	5	5	5
Minerals and vitamins[6]	10	10	10	10
Extruded linseed[7]	0	50	0	50

[1]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).

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

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

[4]Rumen bypass soybean meal, Borregaard LignoTech, KW Alternative Feeds, Bury St. Edmunds, UK)

[5]Pioneer Rocksalt, Broste Ltd., Norfolk, UK.

[6]Dairy Direct, Bury St Edmunds, Suffolk, UK.

[7]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[1]					P <[2]		
	MS	ML	GS	GL	SEM	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 *cis*-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

[1]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).
[2]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[1]					P <[2]		
	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 *cis*-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

[1]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).

[2]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[1]					P <[2]		
	MS	ML	GS	GL	SEM	F	L	F*L
CH_4, L/d	598	580	567	553	35.0	0.274	0.520	0.939
CH_4, MJ/d	23.7	22.9	22.4	21.8	1.39	0.274	0.520	0.939
CH_4, L/kg DMI	29.5	27.5	30.4	28.1	2.47	0.635	0.213	0.939
CH_4, L/kg milk	16.5	15.5	16.1	15.7	1.09	0.878	0.391	0.719
O_2 consumed, L/d	7046	7081	6318	6626	294.2	0.026	0.427	0.523
CO_2 produced, L/d	7124	7212	6468	6659	329.8	0.037	0.559	0.828
Heat, MJ/d[3]	148.0	148.3	132.5	140.0	5.8	0.023	0.361	0.394

[1]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).

[2]Probability for the effect of forage (F), extruded linseed (L), or their interaction (F*L).

[3]Calculated based on respiratory exchange and methane production.

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

| Fatty acid | Treatments[1] | | | | | P <[2] | | 718 |
	MS	ML	GS	GL	SEM	F	L	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 *cis*-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 *cis*-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 [3]	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 *trans*-9	0.20	0.20	0.23	0.21	0.010	0.054	0.418	0.223
14:1 *cis*-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 *trans*-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 *cis*-9[4]	1.7	1.5	1.8	1.5	0.105	0.662	0.020	0.473

16:1 *cis*-11	0.03	0.03	0.04	0.03	0.008	0.484	0.812	0.812
16:1 *cis*-13	0.04	0.04	0.07	0.06	0.012	0.101	0.764	0.780
16:1 *trans*-6-7	0.02	0.02	0.02	0.02	0.005	0.229	0.878	0.721
16:1 *trans*-8	0.016	0.010	0.002	0.009	0.008	0.131	0.799	0.181
16:1 *trans*-9[5]	0.37	0.40	0.38	0.36	0.026	0.478	0.726	0.233
16:1 *trans*-10	0.011	0.003	0.005	0.012	0.005	0.665	0.884	0.063
16:1 *trans*-11	0.03	0.05	0.03	0.04	0.011	0.435	0.063	0.263
16:1 *trans*-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 *trans* total	5.2	6.3	3.6	4.9	0.63	0.008	0.024	0.801
18:1 *cis* total	19.1	21.4	18.4	19.4	1.58	0.227	0.143	0.528
Non-CLA[6] 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 *cis*-6,9,12	0.02	0.01	0.03	0.01	0.006	0.443	0.036	0.370
18:3 *cis*-9,12,15	0.44	0.8	0.50	0.78	0.039	0.438	<.0001	0.205
19:0[7]	0.16	0.25	0.15	0.23	0.039	0.591	0.005	0.704
19:1 *cis*-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 *cis*-5	0.000	0.000	0.002	0.000	0.001	0.356	0.356	0.356
20:1 *cis*-9	0.09	0.10	0.10	0.10	0.008	0.551	0.660	0.283
20:1 *cis*-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 [719]
20:3 n-3	0.000	0.001	0.008	0.005	0.002	0.024	0.642	0.280 [720]
20:3 n-6	0.10	0.08	0.10	0.07	0.012	0.743	0.034	0.943 [721]
20:4 n-6	0.10	0.10	0.13	0.11	0.022	0.361	0.654	0.470 [722]
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 *cis*-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
$\sum$ *cis* MUFA	21.4	23.6	21.1	21.8	1.63	0.306	0.185	0.479
$\sum$ *trans* MUFA	5.9	6.9	4.2	5.5	0.66	0.009	0.027	0.831
$\sum$ *trans* 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

[1]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).

[2]Probability for the effect of forage (F), extruded linseed (L), or their interaction (F*L).

[3]Co-elutes with *cis*-9 12:1

[4]Co-elutes with 17:0 anteiso

[5]Co-elutes with 17:0 iso

[6]All 18:2 isomers excluding CLA

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

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

725   [1]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   [2]Probability for the effect of forage (F), extruded linseed (L), or their interaction (F*L).

728   [3]Co-elutes with *cis*-10 18:1

729   [4]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).

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

[1]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).

[2]Probability for the effect of forage (F), extruded linseed (L), or their interaction (F*L).

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