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

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Interpretive summary:

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Livingstone

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

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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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ABSTRACT

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

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

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

INTRODUCTION

There is currently considerable interest in developing management practices to reduce methane emissions attributable to ruminant meat and milk production and there are numerous dietary strategies that may be effective in reducing methane production or yield (methane per unit feed DMI). Previous studies have shown that replacing dietary ADF or NDF with starch (Mills et al., 2001), reducing NDF intake (Aguerre et al., 2011) and replacing grass silage (Reynolds et al., 2010) or alfalfa silage (Hassanat et al., 2013) with maize silage can reduce methane yield, but the effects are not consistent. In growing beef cattle effects of feeding maize silage as a replacement for GS on methane yield depending varied from positive to negative over the course of the experiment (Staerfl et al., 2012). In lactating dairy cows, incremental replacement of alfalfa silage with MS had quadratic effects on methane production and yield such that methane production was higher when the silages were fed as a 50:50 mixture (Hassanat et al., 2013). Somewhat similarly, incremental replacement of GS with MS had a quadratic effect on methane production but linearly decreased methane yield in lactating dairy cows (van Gastelen et al., 2015).

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

There is also interest in developing dairy cow feeding strategies that reduce milk fat concentrations of SFA, as dairy fat is a substantial dietary source of SFA in European diets (Givens, 2008). The potential for these particular SFA to raise low density lipoprotein cholesterol in humans has been implicated as a risk factor for cardiovascular disease (**CVD**), which is the main cause of premature death in the UK (Givens, 2008). The cow’s diet is a major determinant of milk FA composition (Chilliard and Verlay, 2004) and studies have shown that alteration of dietary forage type (Ferlay et al., 2006) and inclusion of dietary fat supplements (Kliem et al., 2009) are both means of modifying milk FA composition.

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

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

MATERIAL AND METHODS

Animals and Diets

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

Experimental Design and Treatments

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

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

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

Mid-infrared spectroscopy (Foss Electric Ltd, York, UK) was used to determine milk fat, protein, casein, lactose, and urea concentrations and 4% FCM yield calculated as described by Reynolds et al. (2014). Milk samples were analysed for FA composition as described by Kliem et al. (2008 and 2013). Briefly, samples were thawed in warm water (40°C), cooled to room temperature, and shaken to ensure homogeneity. Lipid in 1 ml milk was extracted using ethanol, diethyl ether and hexane. Using sodium methoxide in methanol, extracted FA were base-catalyzed transmethylated to fatty acid methyl esters (**FAME**) and calcium chloride was used to remove methanol residues. Subsequent FAME samples were separated using a flame ionization detector (**FID**) gas chromatograph (GC 3400 Varian Inc., Palo Alto, CA). Milk fat FAME were identified based on retention time comparisons with a mixture of authentic standards (GLC #463, Nu-Chek-Prep Inc., Elysian, MN; and O4754, O9881, E4762, V1381, Sigma-Aldrich Company Ltd., Dorset, UK) and cross referencing with published literature. Correction factors, to account for the carbon deficiency in the FID response for FAME containing 4- to 10- carbon atoms, were estimated using a reference butter oil of known composition (CRM 164, Bureau of European Communities, Brussels, Belgium). After correcting FAME to FA, all results were expressed as g/100 g total FA.

Statistical Analyses

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

RESULTS

Dietary composition and intake and milk yield and composition

In comparison with the GS diets, the MS diets contained higher OM, NDF, and starch concentrations ($P < 0.02$), while CP, ADF, and ash concentrations were higher for the GS diets ($P < 0.020$; Table 2). The MS diets were higher in 18:0, *cis*-9 18:1, and 18:2 n-6 ($P < 0.003$), and lower in 18:3 n-3 ($P < 0.02$) than the GS diets. The dietary concentration of 16:0 was not affected by forage type ($P = 0.575$). The addition of ELS to the diets increased the concentration of all FA measured (P < 0.003), and the increase in *cis*-9 18:1 was greater for the MS diet. Total FA concentrations were similar in MS and GS diets without added ELS, and were increased by ELS addition to a greater extent with the MS compared with the GS diet (forage by ELS interaction, $P < 0.03$).

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

Methane Emission and Respiratory Exchange

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

Effect of Forage Type on Milk FA Composition

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

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

Effect of Extruded Linseed Supplementation on Milk FA Composition

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

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

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

Milk fat concentrations of n-3 PUFA were higher (P < 0.001) with ELS supplementation (Table 5), mainly due to increases in 18:3 n-3 (P < 0.001) and 20:5 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 = 0.028), and 22:2 n-6 (P < 0.095) concentrations were lower in milk fat when ELS was fed, although there was no effect on total n-6 PUFA concentrations (P > 0.10, Table 5).

DISCUSSION

Intake and Milk Yield and Composition

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

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

Effects of Forage Type and Extruded Linseed on Methane Production

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

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

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

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

Effects of Forage Type and Extruded Linseed on Milk FA Concentration

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

Chilliard et al. (2001) suggested that there was insufficient evidence to confirm the effect of forage type, as a total mixed ration, on milk FA composition but that MS may increase *de novo* short-chain FA synthesis. To date, few studies have addressed this, although Kliem et al. (2008) proposed that MS may increase *de novo* short- and medium-chain FA production via an increased supply of acetate to the mammary gland. There was little effect of MS on these FA in the present study, in part reflecting the relatively small differences in forage type (250 g/kg diet DM). However, van Gastelen et al. (2015) also observed no effect of incremental replacement of GS with MS on milk fat concentrations of short and medium chain FA, apart from a linear reduction in 4:0.

Consistent with previous studies, increases in both 18:0 and total *trans* isomers concentrations in milk fat were observed when ELS was fed (Kliem et al., 2009; Hurtaud et al., 2010), as well as increased concentrations of *trans* FA isomers for the MS diets (Kliem et al., 2008). Inclusion of dietary oils (Collomb et al., 2004) and particularly unprotected oils (Loor et al., 2005), leads to a characteristic increase in *trans* and conjugated linoleic acid isomers due to exposure of unsaturated FA to rumen microflora (Chilliard et al., 2001; Shingfield et al., 2005). As observed in the present study, Chilliard et al. (2009) identified *trans*-13+14 18:1, *cis*-9, *trans*-13 18:2 and *trans*-11, *cis*-15 18:2 as intermediates of biohydrogenation of the ELS diets. Although the MS diets had higher concentrations of *cis*-9 18:1 than GS, milk fat *cis*-9 18:1 did not significantly increase. Similarly, despite a higher intake of 18:0 from MS compared with GS, milk fat 18:0 was not significantly higher following the MS diet. Our observed effect of forage type on milk fat *trans*-18:1 isomers has been confirmed in other studies (Shingfield et al., 2005) and has been attributed to differences in forage digestibility (O'Mara et al., 1998). Additionally, feeding a high MS diet, rich in *n*-6 PUFA and starch, leads to characteristic increases in *trans*-10 18:1 (Kliem et al., 2008), which is consistent with our findings.

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

Growing public interest in lowering SFA consumption to improve human health means that any decrease in milk SFA concentrations following forage and lipid supplementation has public health incentives. Our study found only three minor interactions between forage type and ELS supplementation for the selected milk FA, which are in line with findings by Sterk et al. (2011). While lipid supplementation, and possibly MS, provided potentially beneficial decreases in SFA, the current concerns linking *trans* FA to increased risk of CVD mean that the significantly higher total *trans* concentrations following both MS and ELS supplementation may counteract the beneficial decreases in SFA concentration. The question of whether ruminant *trans* are of similar risk to CVD as industrial *trans* remaining largely unanswered (Bendsen et al., 2011). Nonetheless, the implementation of *trans* labelling suggests that increases should be minimised, and development of lipid protection technologies is required to minimise their production. As current UK intakes of long chain PUFA are inadequate (Givens, 2008), enrichment of milk in this way may have long-term implications for human health. Nevertheless, it is questionable whether the magnitude of the changes in long chain PUFA concentrations seen in this study would produce a meaningful impact on health on a population level.

CONCLUSIONS

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

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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).

Table 2. Composition of the total mixed rations fed (g/kg unless stated) on a dry 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).

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).

Table 4. Effects of extruded linseed (Lintec) supplementation and dietary forage source on methane production and respiratory exchange of 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.

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

Fatty acid	Treatments[1]				SEM	P <[2]		
	MS	ML	GS	GL		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
20:3 n-3	0.000	0.001	0.008	0.005	0.002	0.024	0.642	0.280
20:3 n-6	0.10	0.08	0.10	0.07	0.012	0.743	0.034	0.943
20:4 n-6	0.10	0.10	0.13	0.11	0.022	0.361	0.654	0.470
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
∑ ≤ 14:0	24.8	23.7	26.3	25.8	1.28	0.124	0.475	0.799
∑ saturates	67.5	63.3	69.7	67.1	2.57	0.076	0.055	0.586
∑ *cis* MUFA	21.4	23.6	21.1	21.8	1.63	0.306	0.185	0.479
∑ *trans* MUFA	5.9	6.9	4.2	5.5	0.66	0.009	0.027	0.831
∑ *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

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

	Treatment[1]					P <[2]		
Fatty acid	MS	ML	GS	GL	SEM	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

[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*-10 18:1

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

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).