Improving the fatty acid profile of winter milk from housed cows with contrasting feeding regimes by oilseed supplementation

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ABSTRACT

Many studies show concentrations of nutritionally desirable fatty acids in bovine milk are lower when cows have no access to grazing, leading to seasonal fluctuations in milk quality if cows are housed for part of the year. This study investigated the potential to improve the fatty acid profiles of bovine milk by oil-seed supplementation (rolled linseed and rapeseed) during a period of indoor feeding in both organic and conventional production systems. Both linseed and rapeseed increased the concentrations of total mono-unsaturated fatty acids, vaccenic acid, oleic acid and rumenic acid in milk, but decreased the concentra-tion of the total and certain individual saturated fatty acids. Linseed resulted in greater changes than rapeseed, and also significantly increased the concentrations of α-linolenic acid, total polyunsaturated fatty acids and total omega-3 fatty acids. The response to oilseed supplementation, with respect to increasing concentrations of vaccenic acid and omega-3 fatty acids, appeared more efficient for organic compared with conventional diets.

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1. Introduction

Milk and dairy products are important sources of fatty acids (FA) in the human diet (Haug, Hostmark, & Harstad, 2007; Mills, Ross, Hill, Fitzgerald, & Stanton, 2011), with up to 36% of infant fat intake being from dairy products in some countries (Food Standards Agency, 2009). However, there are health concerns about the high concentrations of saturated fatty acids (SFA) in milk fat. Most importantly, lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0) have all been linked to negative effects on human health, especially an increased risk of cardiovascular dis-ease, although more recent reviews recommend the main target of improving milk quality should be a decrease in C16:0, due to its relatively high concentrations in milk fat (Haug et al., 2007).

A number of recent studies show that the concentrations of total and specific monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) increase when cows consume high fresh grass or grass/clover (and to a lesser extent conserved) forage and low concentrate diets (Butler et al., 2008; Stergiadis et al., 2012). This included increases in PUFA, such as omega-3 fatty acids (n-3)

and rumenic acid (RA, c9t11 C18:2), and the MUFA oleic acid (OA, c9 C18:1), which have been linked to health benefits (Haug et al., 2007; Mills et al., 2011). High fresh forage intake also improved the ratio of omega-3:omega-6 fatty acids (n-3/n-6) in milk (Butler et al., 2008; Stergiadis et al., 2012) in line with dietary recommendations (European Food Safety Authority, 2010). How-ever, when cows are housed, the milk concentrations of desirable MUFA and PUFA are known to decrease, due to lack of fresh forage in the diet. Seasonal changes in dairy diets on many farms have been shown to result in variable milk fat composition throughout the year with differences being more marked in organic systems where high intakes of grazed forage in summer are replaced with conserved forage based diets in winter (Butler, Stergiadis, Seal, Eyre, & Leifert, 2011; Stergiadis et al., 2012). Therefore, there is a need to develop strategies to improve winter milk quality in both conventional and organic production systems.

One approach to increase the MUFA and PUFA content in milk and reduce concentrations of the main undesirable SFA is to sup-plement winter dairy diets with vegetable oils or oilseeds (Chilliard et al., 2007; Glasser, Ferlay, & Chilliard, 2008). However, the efficiency of this approach to raise the MUFA and PUFA concen-trations in milk is relatively poor. For example, Chilliard et al. (2007) reported only 7% of α-linolenic acid (ALA, c9c12c15 C18:3) and 15% of linoleic acid (LA, c9c12 C18:2) consumed by cows was transferred into milk with the balance lost through

hydrolysis, isomerisation and biohydrogenation (RBH) in the rumen. This is even more marked for longer chain n-3, such as eicosapentaenoic acid (EPA, c6c9c12c15c18 C20:5), docosapentaenoic acid (DPA, c7c10c13c16c19 C22:5) and docosahexaenoic acid (DHA, c4c7c10c13c16c19 C22:6) (Chilliard et al., 2007). Transfer rates are also reported to depend on the type of oilseed supplement used, the proportion and composition of FA in the diet, productivity of the cows and the proportion of concentrate in the diet (Chilliard et al., 2007; Zachut et al., 2010). The metabolism of dietary MUFA and PUFA in the rumen involves hydrogenation to SFA; in addition, SFA leaving the rumen can be further transformed in the mammary gland before being secreted into milk. For example, Δ9-desaturase enzymes convert SFA (e.g. C14:0 C16:0 and C18:0) and MUFA (e.g. vaccenic acid; t11 C18:1) into MUFA and PUFA, respectively, although the latter will be dominated by the conversion of VA to RA (Chilliard et al., 2007; Destaillats, Trottier, Galvez, & Angers, 2005). Supplementation of dairy diets with oilseeds has shown variant results on milk yield mainly due to contrasting basal diets between the studies (Glasser et al., 2008).

To our knowledge there are no studies reporting both the impact of oilseed supplementation on milk fat profiles and the relative efficiency of this practice under contrasting feeding regimes and management practices (organic, conventional) for housed dairy cows. Provided the main nutritional differences between commonly used organic and conventional dairy regimes (higher forage:concentrate ratio and clover inclusion in the organic silages) influence rumen kinetics and lipid metabolism (Dewhurst et al., 2003), responses in milk FA profiles after oilseed supplementation may differ between the two systems. This study therefore aimed to (a) quantify the effect of dietary linseed and rapeseed supplementation of 'winter indoor diets' and (b) identify the impact of this oilseed supplementation in organic and conventional dairy systems under identical environmental conditions and stockmanship. The overall goal was to provide protocols for dairy producers to improve the nutritional quality of winter milk.

2. Materials and methods

2.1. Experimental design

This study was based on two experiments carried out in two separate winter feeding seasons (2007 and 2010). Each experiment was carried out over a six week period using animals in two parallel herds of Holstein–Friesian cows at Newcastle University's Nafferton farm. The herds, established in 2006, are treated as independent units although under common supervision; one herd managed to organic standards (Soil Association, 2010), which allowed a system comparison without the bias of differing stockmanship and environmental conditions. Nafferton farm dairy herds are run along the lines of typical commercial production systems; management, including feeding, reflect practice on many comparable conventional and organic units. Each experiment consisted of two separate but simultaneous trials, one performed in the conventional and one in the organic herd, resulting in four different trials: (a) year 1, conventional herd (trial C1), (b) year 1, organic herd (trial O1), (c) year 2, conventional herd (trial C2) and (d) year 2, organic herd (trial O2). Both experiments were of a nested design with cows in each herd randomly allocated to treatment groups, blocked for lactation number, days in milk, milk yield, gross milk composition (fat, protein and lactose) and somatic cell count (SCC) based on the last recording prior to selection. In both experiments, milk samples proportionate to yield were taken from individual cows twice in 24 h (morning and afternoon milking) during weeks 1, 3 and 6, with samples mixed before being stored at −20 °C until analysis. Cows from both herds were loose housed with fresh straw bedding added daily and feed offered once a day as a mixed ration, with additional concentrate feed provided in the milking parlour twice per day. The organic herds received a mixed ration based on silage made from organically managed ryegrass/white clover and red clover swards, and conventional cows were fed a diet based on silage made from pure ryegrass swards. Table 1 lists the quantities of silage and other ingredients included in the

Table 1
Ingredients and composition of concentrate diets and conserved forage intake in both experiments (kg DM/cow/day). Diets were planned to be iso-nitrogenous within management systems (conventional, organic) for each experiment.

	Experiment 1						Experiment 2			
	Conventional (C1)			Organic (O1)			Conventional (C2)		Organic (O2)	
	Control	Linseed	Rapeseed	Control	Linseed	Rapeseed	Control	Linseed	Control	Linseed
Silage[a]	12.8	13.0	11.9	13.3	14.1	12.4	10.7	9.9	14.0	15.4
Straw[b]	–	–	–	–	–	–	0.3	0.4	0.3	0.6
Concentrates										
Wheat	3.5	3.0	2.9	2.9	2.9	2.6	2.7	2.3	2.3	2.2
Extracted rapeseed meal: extracted soyabean meal	1.8	1.4	1.4	–	–	–	0.8	1.0	–	–
Beans	–	–	–	1.2	–	0.2	1.6	–	1.6	0.3
Molasses	0.5	–	–	–	–	–	0.7	0.7	–	–
Rolled rapeseed	–	–	1.2	–	–	1.0	–	–	–	–
Rolled linseed	–	1.5	–	–	1.4	–	–	2.0	–	2.0
Minerals/vitamins[c]	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	–	–
Compound feed	2.5	2.5	2.5	2.5	2.5	2.5	3.3	3.3	3.7	3.7
Estimated intakes[d]										
Dry matter (kg)	21.2	22.0	20.4	20.2	21.1	18.8	20.1	19.7	21.9	24.1
Metabolisable energy (MJ/kg DM)	10.3	10.8	10.7	9.9	10.2	10.1	9.7	10.2	9.7	10.3
Neutral detergent fibre (% of DMI)	30.4	29.8	29.4	29.9	29.9	29.9	26.7	25.4	28.1	28.2
Crude protein (% of DMI)	15.0	15.0	14.9	14.7	14.3	14.4	13.7	13.7	12.9	12.9
Lipid intake (kg/cow/day)	0.8	1.3	1.2	0.6	1.1	1.0	0.8	1.4	0.7	1.4
Concentrates (% of DMI)	39.7	41.3	42.1	34.2	33.3	34.5	45.5	47.6	34.7	33.9

[a] Conventional silage was made of grass while organic silage was a mixture of organically grown grass and clover.
[b] Straw was not included in the diet in trial 1 but cows bedded daily on fresh straw.
[c] Organic supplements excluded vitamins.
[d] Based on weighed feed dispensed in each group.

mixed ration, further details on silage composition are given in Table S1 (supplementary data). Standards for organic dairy production related to housing, grazing, health and fertility treatments but also to feeding – at least 60% of dry matter intake (DMI) has to be forage (Soil Association, 2010), which means that, in practice, concentrate intakes on organic farms are often lower than in conventional herds. However, in an attempt to narrow the differences between herds, a relatively high level of concentrate feeding (within the standards) was adopted for organic cows in this study to reduce the impact of differing the forage:concentrate ratio on FA profiles. Forage proportion of DMI averaged 66% across trials and treatments for the organic herd, compared with 57% for the conventional cows. Although forage type is not defined by organic regulations, in the absence of nitrogen fertilizer, farms rely on legumes for nitrogen fixation, resulting in a high proportion of clover in organic forages. This study aimed to assess the impact of the same batch of oilseeds against contrasting production systems. In Experiment 1, conventionally produced linseed and rapeseed were fed to both herds (under a derogation in existing EU regulation in 2007 for organic cows) whereas in Experiment 2, organic linseed was used. The work described in this study was carried out in accordance with EU Directive 2010/63/EU for animal experiments.

2.1.1. Experiment 1

This experiment served as a pilot study to investigate differences in the impact of linseed and rapeseed on milk FA composition. Results were used to select the appropriate oilseed for further investigation to optimise the desirable impact in Experiment 2 (main study). Forty-two cows from the conventional herd (trial C1) and forty-six from the organic herd (trial O1) were used over a six week period. Cows from each herd were divided into three dietary subgroups (control, rapeseed and linseed), of which thirteen to seventeen cows were used for milk sampling. The basal diets and oilseed supplements used for the organic and conventional subgroups are described in Table 1. Experimental diets were designed to be iso-nitrogenous within each trial with rolled oilseed replacing a combination of protein feeds and rolled wheat in the control diet. Rapeseed was fed at 1.25 kg/cow per day and linseed at 1.5 kg/cow per day – delivering a target of 600 g of oil/cow per day. Concentrations of (a) diet components, (b) FA composition of total mixed rations (c) chemical composition of silages and (d) FA and chemical composition of oilseeds are shown in Tables 1 and 2, S1 (supplementary data) and S2 (supplementary data), respectively. The quantities of the dominant fatty acids in rapeseed and linseed provided through dietary supplementation are shown in Fig. S1 (supplementary data).

2.1.2. Experiment 2

This was the main study in the sequence of experiments. It assessed the impact of linseed supplementation, at a higher level than in Experiment 1, on the milk FA profile, to potentially maximise the desirable impact without compromising milk yield and solids contents. Forty cows from each of the conventional (trial

C2) and the organic (trial O2) herds were divided into two equal subgroups (control and linseed), and were used for milk sampling over a six week period. Feeding was similar to Experiment 1 except rolled beans were included in the conventional basal diets and chopped straw in both the conventional and organic basal diets (due to limited silage stocks caused by poor weather conditions in the 2009 growing season). Rolled linseed supplementation was 40% higher than in Experiment 1 (2.1 kg), delivering 710 g oil/cow per day. Details of (a) diet components, (b) FA composition of total mixed rations, (c) chemical composition of silages, (d) FA composition of individual feed components and (e) FA and chemical composition of oilseeds are shown in Tables 1 and 2, S1 (supplementary data), S2 (supplementary data) and S3 (supplementary data), respectively. In addition, (a) the total OA, LA and ALA provided through the different experimental diets in Experiment 2 and (b) the quantities of the dominant FA in oilseeds provided through dietary supplementation in both trials are shown in Figs. 1 and S1 (supplementary data), respectively.

2.2. Chemical analysis of feed and milk

2.2.1. Feed chemical composition analysis

Feeds provided in Experiment 2, DM was determined by oven drying feeds at 105 °C for 16 h and the organic matter content of feeds was determined by ashing at 550 °C for 6 h (AOAC, 1990). Acid detergent fibre, neutral detergent fibre, starch and sugar contents were determined as described by Khan and Chaudhry (2010). The feed oil content was determined by extraction with petroleum spirit under controlled conditions (Ministry of Agriculture, 1973). The feed protein content was measured in dry feed using a LECO FP-428 protein analyser (Daun, Buhr, Mills, Diosady, & Mag, 1993). Prediction of silage chemical composition was performed by Promar Labs using Near Infrared Reflectance Spectroscopy in both experiments.

2.2.2. Milk yield and basic composition analysis

Milk yield was automatically measured in the parlour during milking and the energy corrected milk yield (ECM) was calculated as shown by Peterson et al. (2012); ECM = [0.327 × yield (kg/d)] + [12.86 × fat (kg/d)] + [7.65 × protein (kg/d)]. Aliquots of samples were submitted to the National Milk Record laboratory (Harrogate, UK) for standard analyses of fat, protein and lactose using a Milkoscan FT 6000 (Foss Electric, Hillerod, Denmark) and for SCC using a Fossomatic instrument (Foss Electric).

2.3. Fatty acid determination in feed and milk

2.3.1. Chemicals and analytical standards for FA analysis of lipids

For the FA profile analyses of milk and feed samples, hexane (⩾99.9%), toluene (⩾99.5%), and 0.5 M sodium methoxide in

Table 2
Fatty acid (FA) composition (% of total FA; only FA with concentrations over 1% of total FA are presented) of total mixed rations used as basal diets in the conventional and organic cows, with or without linseed supplementation, in trial 2.

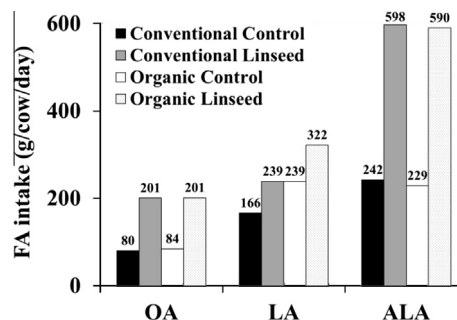|	Conventional		Organic	
	Control	Linseed	Control	Linseed
C16:0 (Palmitic)	16.8	9.51	17.2	9.54
C18:0 (Stearic)	2.11	4.16	3.58	4.26
c9 C18:1 (OA)	6.49	14.6	5.26	15.0
c9c12 C18:2 (LA)	20.6	16.3	20.9	17.1
c9c12c15 C18:3 (ALN)	43.8	52.0	44.1	51.2

Fig. 1. Dietary supply of α-linolenic acid (ALA), linoleic acid (LA) and oleic acid (OA) in trials C2 and O2.

methanol were purchased from Sigma–Aldrich (Gillingham, UK). Methanol (⩾99.8%) and acetyl chloride (⩾98.0%) were purchased from Fischer Scientific Ltd. (Loughborough, UK) and 12 N hydrochloric acid was purchased from VWR (Lutterworth, UK). Analytical standards used for the peak identification on the chromatograms were; (i) 52 FA methyl esters standard (GLC463, Nu-Chek Prep Inc., Elysian, MN, USA), (ii) 37 FA methyl esters standard (18919, Supelco, Bellefonte, PA, USA) and (iii) c9t11 C18:2 conjugated standard (1245, Matreya, Pleasant Gap, PA, USA).

2.3.2. Animal feed fatty acid analysis

Lipids were extracted with petroleum spirit under controlled conditions (Ministry of Agriculture & F. a. F, 1973). Fifty mg of the extracted lipid was transferred in a glass tube and the same methylation and esterification procedure was followed as described in Butler et al. (2011).

2.3.3. Milk fatty acid analysis

The method used for the FA analysis of milk samples in Experiment 1 was previously described by Butler et al. (2011). An improved analytical method was used in Experiment 2, which was based on the methylation and esterification protocols for milk FA described by Chilliard, Martin, Rouel, and Doreau (2009). Analysis of FAME was carried out with a gas chromatograph (Shimadzu, GC-2014, Kyoto, Japan) using a Varian CP-SIL 88 fused silica capillary column (100 m × 0.25 mm ID × 0.2 μm film thickness). Modifications in the chromatographic conditions and gradient in the original method of Chilliard et al. (2009) were applied in our equipment to ensure optimum peak separation. Purified helium was used as a carrier gas with a head pressure of 109.9 kPa and a column flow of 0.39 ml/min. A split injection system was used with an auto injector (Shimadzu, AOC-20i) with a split ratio of 50.0 and an injector temperature of 255 °C. FAME peaks were detected by flame ionisation detection at 260 °C. 1 μl of sample was injected at an initial column temperature of 70 °C, which was held for 1 min. The temperature was then raised at 5 °C/min to 100 °C, where it was held for 2 min, and then increased at 10 °C/min to 160 °C, where it was held for 90 min. Finally, the temperature was increased to 240 °C at a rate of 5 °C/min, thus giving a final gradient of 155 min total runtime. Peaks were identified using the commercial FAME standards described above, and were confirmed by using GC–MS (Shimadzu; GC–MS-QP2010; Kyoto, Japan), operating under the same analytical conditions. Literature resources which present peak separation in chromatograms in detail, were used for the identification of peaks for which a standard was not available, such as isomers of C18:1 (Griinari et al., 1998; Loor, Ueda, Ferlay, Chilliard, & Doreau, 2004; Shingfield et al., 2006), non-conjugated isomers of C18:2 (Loor et al., 2004; Shingfield et al., 2006) and conjugated linoleic acid isomers (Shingfield et al., 2006). Quantification of FA was based on peak areas of individual FA, expressed as a percentage of the total peak areas for quantified FA. Correction factors for the peak areas were used for the short chain SFA (C4:0-C10:0) by using the 52 FAME standard and the following formula:

$$\text{Corrected area in sample} = (\% \text{ of FA in the standard, based}$$
$$\text{on chemical composition}$$
$$/\% \text{ FA in the standard found in GC})$$
$$\times \text{area in sample.}$$

2.3.4. Calculated dietary intakes

Dietary intakes (g) of individual FA and FA groups in milk from different experimental groups were calculated as follows.

Reported dairy fat intakes (g) (Wollff & Precht, 2002) × milk fat content (g/100 g milk) × % of individual FA or FA group in total FA × 0.933 (correction factor representing% of FA in total milk fat (Glasser, Doreau, Ferlay, & Chilliard, 2007)).

2.3.5. ALA recovery from feed to milk

Recovery of ALA from provided feed to milk was calculated (in Experiment 2) as ALA in milk (g)/ALA intake (g), where:

ALA in milk (g) = yield (g) × [milk fat content (g/100 g milk)/100)] × [ALA (g/100 g total FA) × 0.933 (correction factor representing% of FA in total milk fat (Glasser et al., 2007))/100)].
ALA intake = feed provided (g DM) × [feed lipid content (g/100 g DM)/100] × % of [ALA (g/100 g total FA in feed)/100].

2.4. Statistical analysis

Prior to analyses, variables expressed as proportions (individual FA and SFA, MUFA, PUFA) were arcsine transformed, milk SCC was log10 transformed in both trials and EPA, DPA and n-3 were cube root transformed in trial C1. Analyses of variance (ANOVA) were derived separately for each trial from linear mixed-effects models (Pinheiro & Bates, 2000). Dietary treatment (Experiment 1; control, linseed, rapeseed, Experiment 2; control, linseed) and sampling date (Experiment 1 and 2; 1st week, 3rd week, 6th week) were fixed factors and individual cows were the random factor. Significant dietary treatment, sampling date and interaction means were compared using Tukey's honest significant difference test (P < 0.05) where appropriate, based on a mixed-effects model. Analyses were performed in the R statistical environment (R Development Core team, 2009) and residual normality was assessed using the qqnorm function (Crawley, 2007), with no data showing deviation from normality.

3. Results

Oilseed supplementation did not affect the milk yield, fat and protein concentrations or SCC in any trial (Tables 3; trials C1, O1 and 4; trials O1, O2). However, oilseed supplementation caused significant changes in FA profiles in milk from both organic and conventional cows, with similar trends observed in both experiments, but due to the design differences between the trials these are described separately below. All differences discussed in the results section were statistically significant (P < 0.05) unless otherwise stated.

3.1. Experiment 1

3.1.1. Trial C1

In the conventional herd, there was no effect of oilseed supplementation on milk yield, ECM and milk fat and protein concentrations, although an effect of date was detected (supplementary information; Table S4). The fat and protein content of milk decreased from the 1st to 6th week of the experiment, while the SCC increased. However, the main effect of oilseed supplementation (Table 3), sampling date (supplementary information; Table S4) or both were detected for a range of nutritionally relevant FA and FA groups. Milk from the linseed diets showed higher concentrations of MUFA, PUFA, n-3, C18:0, VA, ALA and RA and a higher n-3/n-6, but lower concentrations of SFA, C12:0, C14:0 and C16:0 (Table 3). Milk from rapeseed-fed cows also had higher concentrations of MUFA, C18:0, OA, VA and RA but lower concentrations of SFA, C12:0, C14:0 and C16:0 than milk from cows on non-supplemented diets, while ALA was also depressed (Table 3). When milk from linseed-fed and rapeseed-fed cows was compared, the former had higher concentrations of PUFA, n-3, n-6, VA and ALA (Table 3).

Table 3
Main effect means ± SE and ANOVA P-values for the effect of oilseed supplementation (control, linseed, rapeseed) on milk yield, and basic and fatty acid composition (g/kg total fatty acids) of milk from the conventional (trial C1) and organic (trial O1) cows in experiment 1.

	Conventional (C1)			ANOVA P-values[a]	Organic (O1)			ANOVA P-values[a]
	Control	Linseed	Rapeseed		Control	Linseed	Rapeseed	
	(n = 38)	(n = 35)	(n = 40)		(n = 44)	(n = 37)	(n = 41)	
Yield (kg/cow/day)	33.4 ± 1.5	31.4 ± 1.6	30.1 ± 1.3	NS	28.9 ± 1.5	31.4 ± 1.7	29.9 ± 1.3	NS
ECM[b]	35.8 ± 1.6	33.6 ± 1.7	32.9 ± 1.3	NS	31.3 ± 1.6	33.5 ± 1.6	32.2 ± 1.2	NS
Fat (g/kg milk)	40.2 ± 1.0	40.9 ± 1.1	40.9 ± 1.1	NS	39.6 ± 1.2	39.1 ± 1.4	40.3 ± 1.3	NS
Protein (g/kg milk)	34.1 ± 0.6	33.2 ± 0.7	33.1 ± 0.5	NS	33.3 ± 0.5	33.2 ± 0.6	32.5 ± 0.5	NS
SCC ($\times 10^3$)[c]	304 ± 68	238 ± 83	266 ± 87	NS	364 ± 120	294 ± 73	137 ± 20	NS
SFA[d]	(n = 42)	(n = 39)	(n = 45)		(n = 51)	(n = 42)	(n = 44)	
C12:0	45.4 ± 1.1[a]	34.7 ± 1.3[b]	35.8 ± 0.9[b]	***	43.9 ± 1.1[a]	32.1 ± 0.8[b]	35.4 ± 1.0[b]	***
C14:0	133 ± 2[a]	115 ± 2[b]	115 ± 2[b]	***	130.2 ± 1.6[a]	111.3 ± 1.7[b]	117.9 ± 1.9[b]	***
C16:0	355 ± 5[a]	291 ± 5[b]	291 ± 6[b]	***	347 ± 5[a]	293 ± 5[b]	299 ± 5[b]	***
C18:0	106 ± 3[b]	156 ± 5[a]	151 ± 5[a]	***	113 ± 3[c]	156 ± 4[a]	140 ± 3[b]	***
MUFA[e]								
OA	191 ± 4[b]	233 ± 3[a]	244 ± 6[a]	***	184 ± 4[b]	221 ± 4[a]	230 ± 5[a]	***
VA	8.3 ± 0.4[c]	13.6 ± 0.5[a]	10.9 ± 0.4[b]	***	8.9 ± 0.3[c]	18.4 ± 0.6[a]	13.0 ± 0.5[b]	***
PUFA[f]								
LA	12.9 ± 0.3	13.2 ± 0.3	11.9 ± 0.3	††	19.3 ± 0.5	20.0 ± 0.7	19.0 ± 0.7	NS
ALA	5.79 ± 0.19[b]	8.26 ± 0.19[a]	5.17 ± 0.14[c]	***	11.99 ± 0.26[b]	15.83 ± 0.27[a]	10.27 ± 0.36[c]	***
RA	4.40 ± 0.16[c]	6.50 ± 0.28[a]	5.51 ± 0.21[b]	***	4.26 ± 0.15[b]	7.30 ± 0.25[a]	6.63 ± 0.26[a]	***
EPA	0.72 ± 0.08	1.13 ± 0.36	0.78 ± 0.06	NS	0.98 ± 0.07	1.08 ± 0.10	0.88 ± 0.09	NS
DPA	1.18 ± 0.48	0.94 ± 0.21	0.78 ± 0.21	NS	0.98 ± 0.08	0.95 ± 0.10	0.99 ± 0.12	NS
FA groups								
SFA	742 ± 5[a]	695 ± 4[b]	692 ± 6[b]	***	740 ± 4[a]	689 ± 4[b]	691 ± 5[b]	***
MUFA	230 ± 4[b]	272 ± 3[a]	282 ± 6[a]	***	220 ± 4[b]	263 ± 3[a]	268 ± 5[a]	***
PUFA	28.1 ± 0.8[b]	33.3 ± 0.7[a]	26.3 ± 0.4[b]	***	40.3 ± 0.7[b]	48.4 ± 1.0[a]	40.8 ± 1.1[b]	***
n-3[g]	7.7 ± 0.5[b]	10.8 ± 0.5[a]	6.7 ± 0.3[b]	***	13.9 ± 0.3[b]	17.9 ± 0.3[a]	12.2 ± 0.4[c]	***
n-6[h]	16.0 ± 0.4[a]	16.1 ± 0.4[a]	14.1 ± 0.3[b]	*	22.1 ± 0.5	23.2 ± 0.8	22.0 ± 0.8	NS
n-3/n-6	0.49 ± 0.03[b]	0.68 ± 0.04[a]	0.48 ± 0.02[b]	***	0.65 ± 0.02[b]	0.80 ± 0.03[a]	0.57 ± 0.02[b]	***

[a] Significances were declared at $P < 0.001$, $P < 0.01$, $P < 0.05$, $0.05 < P < 0.10$ (trend), $P > 0.10$ (nonsignificant; NS). Means within the same treatment with different letters are significantly different ($P < 0.05$) according to Tukey's honestly significant difference test.
[b] Energy corrected milk yield = [0.327 × yield (kg/d)] + [12.86 × fat (kg/d)] + [7.65 × protein (kg/d)], as proposed by Peterson et al. (2012).
[c] Somatic cell count.
[d] SFA: C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, C20:0, C22:0, C24:0.
[e] MUFA: c9 C14:1, c9 C16:1, oleic acid (c9 C18:1; OA), vaccenic acid (t11 C18:1; VA), c8 C20:1.
[f] PUFA: linoleic acid (c9c12 c18:2; LA), α-linolenic acid (c9c12c15 C18:3; ALA), rumenic acid (c9t11 C18:2; RA), t10c12 C18:2, c8,c11,c14 C20:3, c5c8c11c14 C20:4, eicosapentaenoic acid (c5c8c11c14c17 C20:5; EPA), docosapentaenoic acid (c7c10c13c16c19 C22:5; DPA).
[g] n-3 FA: ALA, EPA, DPA.
[h] n-6 FA: LA, c8,c11,c14 C20:3, c5c8c11c14 C20:4, c13c16 C22:2, t10c12 C18:2.

3.1.2. Trial O1

In the organic herd, there was also no effect of oilseed supplementation on milk yield, ECM and for the milk fat and protein concentrations (Table 3) but an effect of date was detected for the milk fat content (supplementary information; Table S4); that was higher in 6th than in 1st week of the experiment. However, the main effects of oilseed supplementation (Table 3), sampling date (supplementary information; Table S4) or both were detected for a range of nutritionally relevant FA and FA groups. As with the conventional herd, milk from linseed diets showed higher concentrations of MUFA, PUFA, n-3, C18:0, VA, ALA and RA and higher n-3/n-6, but lower concentrations of SFA, C12:0, C14:0 and C16:0 (Table 3). Milk from rapeseed-fed cows also had higher concentrations of MUFA, C18:0, OA, VA, ALA and RA and lower concentrations SFA, C12:0, C14:0, C16:0, LA and ALA than milk from non-supplemented cows (Table 3). When milk from linseed-fed and rapeseed-fed cows was compared, the former had higher concentrations of PUFA, n-3, n-6, VA and ALA, similarly to the conventional herd, while RA/VA was also lower (Table 3).

3.2. Experiment 2

3.2.1. Trial C2

In the conventional herd, oilseed supplementation did not affect milk yield, ECM or milk fat and protein concentrations (Table 4) although an effect of date was detected; the fat content of milk was increased from the 1st to the 6th week of the experiment (supplementary information; Table S5). However, the main effects of oilseed supplementation (Table 4), sampling date (supplementary information; Table S5) or both, were detected for a range of nutritionally relevant FA and FA groups. Linseed supplementation increased the MUFA, PUFA, n-3, n-6, C18:0, OA, VA, ALA, RA and n-3/n-6, but decreased concentrations of SFA, C12:0, C14:0 and C16:0 (Table 4).

3.2.2. Trial O2

In the organic herd, the milk yield, ECM, milk fat and protein concentrations and SCC were not influenced by oilseed supplementation or date (Tables 4 and supplementary information Table S5). However, the main effects of oilseed supplementation or sampling date or both were detected for a range of nutritionally relevant FA and FA groups (Tables 4 and supplementary information Table S5). Linseed supplementation increased MUFA, PUFA, n-3, n-6, C18:0, OA, VA, ALA, RA and n-3/n-6, but decreased concentrations of SFA, n-6, C12:0, C14:0, C16:0 and LA (Table 4).

4. Discussion

4.1. Impact of oilseed supplementation

As previously reported, milk yield, fat and protein content and SCC were not affected by oilseed supplementation (Collomb

Table 4
Main effect means ± SE and ANOVA *P*-values for the effect of oilseed supplementation (control, linseed) on milk yield, and basic and fatty acid (g/kg total fatty acids) composition of milk from the conventional (trial C2) and organic (trial O2) cows in experiment 2.

	Conventional (C2)		ANOVA	Organic (O2)		ANOVA *P*-values[a]
	Control	Linseed	*P*-values[a]	Control	Linseed	
	(*n* = 57)	(*n* = 57)		(*n* = 60)	(*n* = 60)	
Yield (kg/cow/day)	25.8 ± 0.8	25.4 ± 0.8	NS	28.3 ± 1.1	30.3 ± 1.1	NS
ECM[b]	28.7 ± 0.8	28.8 ± 0.7	NS	29.9 ± 0.8	32.3 ± 0.7	NS
Fat (g/kg milk)	42.4 ± 0.6	44.5 ± 0.8	NS	38.9 ± 0.5	40.0 ± 0.5	NS
Protein (g/kg milk)	32.6 ± 0.4	32.2 ± 0.4	NS	30.7 ± 0.3	30.0 ± 0.3	NS
SCC (×10³)[c]	288 ± 61	349 ± 134	NS	214 ± 48	187 ± 46	NS
SFA[d]	(*n* = 60)	(*n* = 60)		(*n* = 60)	(*n* = 60)	
C12:0	40.4 ± 0.6	30.2 ± 0.5	***	29.8 ± 0.6	21.6 ± 0.5	***
C14:0	127 ± 1	104 ± 1	***	110 ± 1	86 ± 1	***
C16:0	351 ± 3	247 ± 4	***	306 ± 3	220 ± 4	***
C18:0	91.5 ± 1. 3	147.8 ± 3.3	***	104.2 ± 1.3	160.4 ± 3.3	***
MUFA[e]						
OA	167 ± 2	228 ± 3	***	215 ± 2	251 ± 3	**
VA	7.0 ± 0.2	13.4 ± 0.7	***	10.7 ± 0.2	19.3 ± 0.7	***
PUFA[f]						
LA	11.7 ± 0.2	11.3 ± 0.2	NS	21.7 ± 0.2	17.7 ± 0.2	***
ALA	5.55 ± 0.09	10.52 ± 0.18	***	10.82 ± 0.09	16.86 ± 0.18	***
RA	3.65 ± 0.09	6.06 ± 0.23	***	5.44 ± 0.09	8.12 ± 0.23	***
EPA	0.34 ± 0.01	0.22 ± 0.01	***	0.43 ± 0.01	0.27 ± 0.01	***
DPA	0.74 ± 0.02	0.63 ± 0.02	***	0.88 ± 0.02	0.68 ± 0.02	***
FA groups						
SFA	742 ± 3	646 ± 5	***	673 ± 3	596 ± 5	***
MUFA	224 ± 2	304 ± 4	***	277 ± 2	336 ± 4	***
PUFA	33.2 ± 0.4	50.5 ± 0.9	***	50.6 ± 0.4	67.8 ± 0.9	***
n-3[g]	8.8 ± 0.1	17.1 ± 0.3	***	14.2 ± 0.1	25.4 ± 0.3	***
n-6[h]	15.4 ± 0.2	16.6 ± 0.3	*	25.7 ± 0.2	23.4 ± 0.3	**
n-3/n-6	0.57 ± 0.01	1.03 ± 0.01	***	0.55 ± 0.01	1.10 ± 0.01	***

[a] Significances were declared at *P* < 0.001 = ***, *P* < 0.01 = **, *P* < 0.05 = *, 0.05 < *P* < 0.10 = † (trend), *P* > 0.10 = NS (non-significant). Means within the same treatment with different letters are significantly different (*P* < 0.05) according to Tukey's honestly significant difference test.
[b] Energy corrected milk yield = [0.327 × yield (kg/d)] + [12.86 × fat (kg/d)] + [7.65 × protein (kg/d)], as proposed by Peterson et al. (2012).
[c] Somatic cell count.
[d] SFA: C4:0, C6:0, C7:0, C8:0, C9:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C23:0, C24:0.
[e] MUFA: c9 C14:1, t9 C16:1, c9 C16:1, c9 C17:1, t6+t7+t8 C18:1, t9 C18:1, t10 C18:1, VA, t12+t13+t14 C18:1, OA, c11 C18:1, c12 C18:1, c13 C18:1, c14+t16 C18:1, c15 C18:1, c8 C20:1, c13 C22:1.
[f] PUFA: c9t13 C18:2, t9t12 C18:2, t8c13 C18:2, c9t12 C18:2, t9c12 C18:2, t11c15 C18:2, LA, c9c15 C18:2, c12c15 C18:2, c6c9c12 C18:3, ALA, RA, unknown conjugated C18:2 isomers, c11c14 C20:2, c8c11c14 C20:3, c11c14c17 C20:3, c5c8c11c14 C20:4, c13c16 C22:2, c13c16c19 C22:3, EPA, DPA.
[g] n-3 FA: t11c15 C18:2, c9c15 C18:2, ALA, c11c14c17 C20:3, EPA, c13c16c19 C22:3, DPA.
[h] n-6 FA: t9t12 C18:2, c9t12 C18:2, t9c12 C18:2, LA, c6c9c12 C18:3, c11c14 C20:2, c8c11c14 C20:3, c5c8c11c14 C20:4, c13c16 C22:2.

et al., 2004). This suggested that the addition of between 1.6 and 2.1 kg of rolled oilseeds/cow per day to winter diets for conventional and organic cows, as in this study, would not affect milk production or total solids; the two parameters dairy producers are currently paid for.

Across the experiments and production systems, linseed supplementation of silage diets during winter housing: (a) decreased the milk concentrations of SFA, C12:0, C14:0 and C16:0 and (b) increased milk concentrations of a range of nutritionally desirable FA (including MUFA, OA, VA, PUFA, n-3, ALA and RA) and the n-3/n-6 ratio, to levels similar to or higher than those found in milk from grazing (Butler et al., 2008, 2011), being in line with previous studies (Akraim, Nicot, Juaneda, & Enjalbert, 2007; Collomb et al., 2004). On the other hand, linseed supplementation reduced concentrations of long chain n-3 (EPA and DPA) in Experiment 2, conforming to a recent meta-analysis citing a range of studies in which linseed and other oil supplements decreased concentrations of long chain (>18 C) unsaturated FA (Glasser et al., 2008). Currently, our estimated dietary intakes of n-6 are higher than necessary, of medium chain n-3 (especially ALA) are adequate, whereas consumption of long chain n-3 fatty acid is inadequate (European Food Safety Authority, 2010). Although EPA plus DHA intake from dairy products is relatively low, any reduction is undesirable and would exacerbate the shortfall on the 250 mg/day

recommended intake for adults (European Food Safety Authority, 2010). In this respect, FA profiles in milk from feeding fresh forage (Stergiadis et al., 2012) are more desirable than those arising from linseed supplements.

In contrast to linseed, rapeseed supplementation only increased concentrations of MUFA, OA (the dominant FA found in rapeseed oil), VA and RA, but did not affect the total and individual n-3, thus being in line with previously reported results (Glasser et al., 2008), although some studies report no effect of rapeseed feeding on milk VA and RA concentrations (Collomb et al., 2004; Egger et al., 2007). Differences in the impact of linseed and rapeseed supplementation on milk FA profiles were expected, since: (a) the dominant FA differ – ALA in linseed as opposed to OA in rapeseed (Glasser et al., 2008) and (b) higher ALA intakes result in more ALA escaping RBH and being secreted into milk (Collomb et al., 2004; Egger et al., 2007). However, as previously reported (Akraim et al., 2007), increasing the ALA intake by supplementation in this study depressed the relative proportion transferred into milk for both conventional (1.9% vs. 2.3% for control; trial C2) and organic (3.2% vs. 4.9% for control; trial O2) linseed diets (individual results not shown). In contrast to ALA, both oilseeds were expected to raise concentrations of VA and RA (Glasser et al., 2008). Higher intakes of both ALA (linseed) and OA (rapeseed) are likely to increase RBH intermediates such as VA, leaving the rumen and transported to the mammary gland

for secretion, or as the precursor for RA synthesis (Destaillats et al., 2005; Loor et al., 2004), thus explaining their increased concentrations in milk.

Also in agreement with previous studies (Akraim et al., 2007; Collomb et al., 2004; Glasser et al., 2008), both oilseed supplements improved milk composition by reducing concentrations of the main nutritionally undesirable SFA (C12:0, C14:0, and C16:0) and total SFA. This may be explained by greater intake of unsaturated fats, reported to inhibit the *de novo* synthesis of short and medium chain saturated FA, including palmitic acid (Akraim et al., 2007; Chilliard et al., 2007; Zachut et al., 2010).

4.2. Differing responses between production systems

Here we report for the first time the impact of oilseed supplementation in two different production systems under the same husbandry and environmental background. Changes in response to supplementation did differ between the organic and conventional herds. This was more apparent in Experiment 2 when the rise in milk n-3 and ALA concentrations from linseed supplementation was greater for cows under organic (+8.4 and +6.3 g/kg total FA, respectively) compared with conventional (+5.4 and +5.3 g/kg total FA, respectively) management. Since unsupplemented cows on control diets showed organic milk to be higher in ALA and n-3 than conventional milk, this indicated that the response in this study to linseed was additive to the benefits offered by the basal diets, with respect to ALA and n-3 FA transfer into milk. In addition, the depression in milk LA and n-6 concentrations by linseed supplementation of organic diets was not detected in conventionally managed cows, whereas for milk OA, linseed gave a greater increase for cows under conventional management (+61 vs. +36 g/kg total FA). Together these differences suggested that the impact of dietary practices to improve winter milk quality will vary between conventional and organic production.

The greater impact of linseed on ALA concentrations in milk from organic cows (in both experiments) may be explained by more dietary ALA escaping hydrogenation. Including clover in organic forage is likely to reduce RBH of unsaturated FA due to a combination of lower rates of lipolysis and reduced rumen retention compared with soley grass based forages used in conventional systems (Dewhurst et al., 2003). Reducing the extent of RBH would lead to less C18:0 produced, and hence availability for mammary desaturation, which could also explain the greater increase in OA seen in conventional milk from feeding linseed. However, overall concentrations of C18:0 were higher in organic milk indicating either greater production as a result of hydrogenation of elevated LA intakes by organic diets or lower utilisation of stearic acid for OA synthesis in the udders of organic cows. This latter theory may also be supported by the lower C14:1/C14:0 ratio (the most reliable indicator for Δ9-desaturase activity; results not shown; (Griinari et al., 2000)) in organic milk.

Reduced LA concentrations in milk from linseed supplementation has been reported by Rego et al. (2009) for basal diets rich in ALA, possibly suggesting competition between ALA and LA, with respect to RBH and/or uptake by the mammary gland. In this study, it appeared that adding extra ALA against a background diet, which appears to allow more ALA to reach the mammary gland (organic-linseed), would decrease milk LA compared with a diet supplying less LA and ALA (organic-control). The greater depression in milk LA concentrations from linseed supplementation under organic management, despite an apparently larger boost in LA intake, also suggested a greater hydrogenation of LA in preference to ALA – a finding also supported by a greater increase VA concentrations (one of the main LA RBH products) (Destaillats et al., 2005), in milk from organically managed cows.

These differing responses to oilseed supplementation appear to be explained by differences in basal diets used in each system, particularly a higher proportion of forage and the inclusion of clover in silage, which are obligatory under organic regulations (Soil Association, 2010) and likely to influence rumen dynamics.

4.3. Potential impact on consumers' nutrition

Linseed increased the content of the most nutritionally desirable FA in milk to levels similar to or even higher than those achieved by grazing cows (Butler et al., 2008, 2011), which could be important in human nutrition. Many FA elevated by linseed feeding are associated with reduced risk of hypertension and coronary heart disease, certain cancers, obesity and type 2 diabetes, and improve neurological, anti-inflammatory and immune system function (Haug et al., 2007; Mills et al., 2011). Also, although there are no published daily recommended intakes for VA and RA, increasing their concentrations in milk is considered desirable since conjugated linoleic isomers have anticarcinogenic, antiobese, immunomodulating and antidiabetic properties (Haug et al., 2007; Mills et al., 2011) and up to 30% of VA consumed can be converted to RA by humans (Turpeinen et al., 2002).

The perceived harmful effect of SFA on increasing CVD risk in humans has led to a substantial decline in whole milk and butter consumption over the last 30 years in many countries, although intake of other high-fat dairy products, such as cheese, has increased (Kliem & Givens, 2011). Kliem, Shingfield, Livingstone, and Givens (2013), suggest a decline of up to 15 g SFA/100 g FA in winter milk is required to impact on public health and savings in health care costs. In Experiment 2 of this study, organic milk from linseed supplementation contained 14.6 g less SFA per 100 g of FA than conventional milk from control cows. Combining the optimum feeding strategy (organic; higher forage:concentrate ratio and use of grass/clover silage) with oilseed supplementation (2.1 kg linseed/cow/day) may produce milk with improved health properties, although this theory needs to be investigated in larger farm-based surveys to test if these changes in milk SFA content apply at a national scale.

Milk consumption in Europe varies greatly with reported intakes ranging from 519 g (Spain) to 1360 g (Finland) per person per day (Wollf & Precht, 2002), which has a bearing on intakes of potentially harmful and beneficial FA. Recommended intakes for SFA and n-3 are set at <10% energy intake and 1–2% energy intake, respectively (European Food Safety Authority, 2010). Assuming an average daily adult energy intake of 8368 kJ (Anderson, 1994), and an energy content of 37.7 kJ/g fat (Akoh, 1995), these recommended intakes corresponds to <22.2 g SFA and 2.2–4.4 g of n-3. Based on the results from this study, organic dairy products from linseed-supplemented cows would provide 4 g less SFA at low dairy intakes, rising to 12 g less per day at high dairy intakes, compared with products from conventional unsupplemented cows (representing common winter feeding practice in UK). However, in both cases high milk consumption would exceed the SFA recommended intakes; by 8 g in organic-linseed or by 20 g in conventional-unsupplemented milk. In addition, over the range of European reported dairy consumption (Wollf & Precht, 2002), organic milk from linseed-supplemented diets would supply 11–59% of the recommended intakes for n-3 but the correspondent contributions for conventional milk from unsupplemented cows would only reach 4–23%. This indicates that, although oilseed supplements are a reliable way to improve milk FA profiles (Glasser et al., 2008), its combination with an appropriate basal diet is important to maximise their impact on milk quality.

5. Conclusion

In conclusion, although oilseed supplementation reduced concentrations of SFA and increased nutritionally desirable FA (e.g. RA or ALA or both), it did reduce concentrations of longer chain n-3 and can therefore not fully compensate for the lack of fresh forage in the diet of housed cows. Rapeseed was inferior to linseed supplementation, with respect to improving milk fat composition, because it did not increase ALA or n-3 concentrations. The basal diets (e.g. conserved forage type, forage:concentrate ratios or their combination) were also relevant for milk fat profiles and interacted with the response to oilseed supplementation. A proposed combination of dietary factors (high forage:concentrate ratio and grass/clover silage in basal diet with 2.1 kg of supplemented rolled linseed) appeared to maximise the positive impact of decreasing SFA and increasing n-3 in milk without compromising milk production and solids content.

Acknowledgements

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.05.021.

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