Animal (2007), **1:6**, pp 835–843 © The Animal Consortium 2007
doi: 10.1017/S175173110700002X

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Conjugated linolenic acid (CLnA), conjugated linoleic acid (CLA) and other biohydrogenation intermediates in plasma and milk fat of cows fed raw or extruded linseed

F. Akraim[1†], M. C. Nicot[1], P. Juaneda[2] and F. Enjalbert[1‡]

[1]*Ecole Nationale Vétérinaire de Toulouse, Laboratoire de Nutrition, 23 Chemin des Capelles, BP 87614, F-31076 Toulouse Cedex 3, France;* [2]*Institut National de la Recherche Agronomique, UMR Flavic, 17 Rue Sully, BP 86510, F-21065 Dijon Cedex, France*

(Received 6 November 2006; Accepted 15 March 2007)

Thirty lactating dairy cows were used in a 3 × 3 Latin-square design to investigate the effects of a raw or extruded blend of linseed and wheat bran (70:30) on plasma and milk fatty-acids (FA). Linseed diets, containing 16.6% linseed blend on a dry-matter basis, decreased milk yield and protein percentage. They decreased the proportions of FA with less than 18 carbons in plasma and milk and resulted in cis*-9,* cis*-12,* cis*-15 18:3 proportions that were more than three and four times higher in plasma and milk, respectively, whereas* cis*-9,* cis*-12 18:2 proportions were decreased by 10–15%. The* cis*-9,* trans*-11,* cis*-15 18:3 isomer of conjugated linolenic acid was not detected in the milk of control cows, but was over 0.15% of total FA in the milk fat of linseed-supplemented cows. Similarly, linseed increased plasma and milk proportions of all biohydrogenation (BH) intermediates in plasma and milk, including the main isomer of conjugated linoleic acid* cis*-9,* trans*-11 18:2, except* trans*-4 18:1 and* cis*-11,* trans*-15 18:2 in plasma lipids. In milk fat, compared with raw linseed, extruded linseed further reduced 6:0–16:0 even-chain FA, did not significantly affect the proportions of 18:0,* cis*-9 18:1 and* cis*-9,* cis*-12 18:2, tended to increase* cis*-9,* cis*-12,* cis*-15 18:3, and resulted in an additional increase in the proportions of most BH intermediates. It was concluded that linseed addition can improve the proportion of conjugated linoleic and linolenic acids, and that extrusion further increases the proportions of intermediates of ruminal BH in milk fat.*

Keywords: conjugated linoleic acid, conjugated linolenic acid, extrusion, linseed, milk fatty acid

Introduction

Because of ruminal biohydrogenation (BH) of fatty-acids (FA), milk has a low concentration of polyunsaturated FA (PUFA), particularly omega-3 FA, which are thought to have beneficial effects on human health (Simopoulos, 2002). Milk can contain FA originating in ruminal PUFA BH, which can also affect human health. The main isomer of conjugated linoleic acid (CLA), *cis*-9, *trans*-11 18:2, is the first intermediate of *cis*-9, *cis*-12 18:2 BH, and has been shown to inhibit carcinogenesis (Parodi, 1999). In the rumen, CLA is reduced to *trans*-11 18:1, further hydrogenated to 18:0 (Harfoot and Hazlewood, 1988). The first step of *cis*-9, *cis*-12, *cis*-15 18:3 ruminal BH is an isomerisation of the *cis*-12 double bond to a *trans*-11 double bond, resulting in an isomer of conjugated linolenic acid (CLnA), *cis*-9, *trans*-11, *cis*-15 18:3 (Harfoot and Hazlewood, 1988); chemically prepared isomers of this natural CLnA exhibit stronger cytotoxic effects than CLA on human tumour cells (Igarashi and Miyazawa, 2000). In the rumen, CLnA is reduced to *trans*-11, *cis*-15 18:2, which can be reduced to either *trans*-11 C18:1, *cis*-15 18:1 or *trans*-15 18:1; the latter two are not further hydrogenated in the rumen, but *trans*-11 18:1 can be reduced to 18:0 (Harfoot and Hazlewood, 1988). Besides these well-known pathways, which mainly result in *trans*-11 isomers, rumen BH can result in many other *cis* or *trans* positional isomers of 18:2 or 18:1 FA, specially *trans*-13 and/or *trans*-14 isomers when linseed is added to the diet (Loor *et al.*, 2004).

Among oilseeds, linseed has the highest proportion of *cis*-9, *cis*-12, *cis*-15 18:3, and also contains *cis*-9, *cis*-12 18:2, so that the addition of linseed to the diet of dairy cows could improve PUFA and conjugated FA contents in milk fat; higher proportions of *cis*-9, *cis*-12, *cis*-15 18:3 and

† Present address: Animal Production Department, Faculty of Agriculture, Omar Almukhtar University, PO Box 919, Al-Baida, Libya.
‡ E-mail: f.enjalbert@envt.fr

CLA have been observed in plasma and milk fat after the dietary addition of linseeds or linseed oil (Gonthier *et al*., 2005; Loor *et al*., 2005). Higher proportions of other BH intermediates, particularly *trans*-11, *cis*-15 18:2 and *trans*-6–*trans*-11 18:1, were found in plasma and milk after linseed oil supplementation (Loor *et al*., 2005). On the contrary, little is known about the concentration of CLnA in milk (Destaillats *et al*., 2005), and the effects of diet on this FA have not been studied. Extrusion of oilseeds has been shown to increase the proportions of main BH intermediates in milk fat, with canola (Bayourthe *et al*., 2000), soya beans (Chouinard *et al*., 2001) and linseed (Gonthier *et al*., 2005), and increased (Chapoutot and Sauvant, 1997) or decreased (Chouinard *et al*., 2001; Gonthier *et al*., 2005) milk PUFA have been reported. The effects of linseed extrusion on CLnA or minor *trans*-18:1 intermediates have not been studied.

The objectives of this study were to investigate the effects of raw or extruded linseed on plasma and milk FA profiles in lactating dairy cows, focusing on PUFA and BH intermediates.

Material and methods

Experimental design and diets

All procedures for this study complied with the Guide for the Care and Use of Agriculture Animals in Agricultural Research and Teaching (Federation of Animal Sciences Societies, 1999). Thirty lactating Holstein cows, 116 ± 64 days in milk, were assigned to three groups of 10 cows according to parity, milk production, milk fat and protein contents, and days in milk. One cow was excluded during the second period because of an acute mastitis. Groups of cows were housed in three adjacent pens. In a 3 × 3 Latin-square design, the groups of cows were assigned to one of the following three diets: (1) control diet (C), (2) diet with raw linseed (RL) and (3) diet with extruded linseed (EL). Diets were based on maize silage, and contained similar amounts of crude protein (CP) and fibre (Table 1). Supplemented linseed was a blend of 70% linseed and 30% wheat bran to avoid oil losses during extrusion, and the word linseed will designate this blend throughout the text. Linseed was crushed through a 3-mm screen. For EL, the mixture was preconditioned at 50°C before extrusion at 120°C. Cows were fed by pen, and had free access to the diet all day long. Diet ingredients were mixed and distributed once per day in the morning, at the rate of 23 kg dry matter (DM) per cow, corresponding to the actual *ad libitum* DM intake measured before experiment. Water was available *ad libitum*. Each period lasted 21 days: the cows were adapted to diets for 19 days and sampled during the last 2 days.

Table 1 *Ingredients and chemical composition of diets*

	Diets		
	Control	Raw linseed	Extruded linseed
Ingredients (% dry matter)†			
Maize silage	60.31	61.39	61.12
Dehydrated alfalfa	8.24	6.26	6.29
Wheat	5.58	0.00	0.00
Concentrate 1	13.48	14.13	14.06
Fat supplement	1.31	0.00	0.00
Concentrate 2	9.34	0.00	0.00
Raw linseed	0.00	16.42	0.00
Extruded linseed	0.00	0.00	16.79
Salt	0.44	0.44	0.44
Mineral–vitamin mix	1.31	1.34	1.33
Chemical composition (% dry matter)			
Crude protein	15.43	15.84	15.79
NDF	42.08	43.10	43.07
ADF	24.36	24.90	24.40
Total fatty acids	3.26	5.60	5.74
Fatty acids profile (g/100 g FA)			
16:0	26.71	10.14	10.38
18:0	3.37	3.30	3.42
cis-9 18:1	22.28	18.72	18.87
cis-9, *cis*-12 18:2	32.66	25.45	25.24
cis-9, *cis*-12, *cis*-15 18:3	7.86	39.72	39.01

†Details of ingredients are as follows. Concentrate 1: composed of protected soya-bean meal, protected sunflower meal, maize germ meal, urea, palm kernel meal and cane vinasse; 44.7% CP (dry-matter basis). Fat supplement: calcium salts of palm and soya-bean oil. Concentrate 2: composed of maize, barley, wheat, sorghum, maize cob, wheat bran and cane molasses; 24.6% CP (dry-matter basis). Raw and extruded linseed: blend linseed/wheat bran (70:30). Vitamin–mineral mix: contained on a per kg basis: 70 g P, 210 g Ca, 60 g Mg, 10 g Na, 300 000 IU vitamin A, 60 000 IU vitamin D3, 700 mg vitamin E, 4500 mg Zn, 3500 mg Mg, 800 mg Cu, 70 mg I, 20 mg Co, 15 mg Se.

Samples and chemical analysis

Samples of diet ingredients were taken and frozen until analysis for DM and CP (Association of Official Analytical Chemists, 1996), NDF and ADF according to the method of Van Soest *et al*. (1991) and FA content and profile as explained here-under. Blood samples were taken at 1400 h on day 20 and at 0800 h on day 21 from the coccygeal vessels using heparinised tubes, and immediately centrifuged. Plasma was kept at –20°C until analysis. At the evening milking of day 20 and the morning milking of day 21, a 50 ml milk sample was immediately frozen and kept for FA analysis, and a 10 ml sample was used for determination of fat and protein content. The samples from the two milkings were mixed before analysis. Milk fat and true protein contents were determined by IR analysis (Milkoscan 605, Foss Electric, F-75001 Paris).

Milk samples for FA analysis were freeze-dried (Vitris Freezemobile 25; Vitris Gardiner, NY). Plasma total lipids were extracted as described by Folch *et al*. (1957) and 19:0 was used as an internal standard. FAs in plasma lipids extracts and in unextracted diet ingredients and milk samples were methylated using sodium methoxide followed by boron trifluoride as described by Park and Goins (1994). This method, which successively uses basic and acid transmethylations, allows methylation of all lipid classes,

including non-esterified fatty acids, and does not alter the stereochemistry of CLA double-bonds (Duckett *et al*., 2002). One part of FA methyl esters from each sample was fractionated by argentation thin-layer chromatography (Ag-TLC) (plates 20 × 20 cm, Silica gel 60, Merk KGaA, Germany) to separate the *trans*-18:1 FA, as described by LeDoux *et al*. (2002). Total and *trans*-18:1 FA profiles were analysed by GLC (Agilent 6890N, equipped with a model 7683 auto injector, Network GC System, Palo Alto, CA, USA). The column was a fused silica capillary (CPSil88, 100 m × 0.25 mm ID, 0.2 μm film thickness, Chrompack-Varian, Middleburg, The Netherlands).

For plasma analysis, flame ionisation detector temperature was maintained at 260°C and the injector at 255°C, and a splitless injection with an automatic injector was used. Helium was the carrier gas with a constant pressure (24.6 p.s.i.). The samples were injected in 0.5 μl of hexane. Initial temperature of the oven was 70°C, held for 1 min, increased by 5°C/min to 100°C, held at 100°C for 2 min, increased by 10°C/min to 175°C, held at 175°C for 40 min, increased by 5°C/min to a final temperature of 225°C and maintained at 225°C for 15 min, as described by Loor *et al*. (2002). *Trans*-10 18:1 and *trans*-11 18:1 were not completely separated with this method, and were considered together and designated as *trans*-10 + 11 18:1. Plasma analysis was performed before the publication of Loor *et al*. (2004), indicating coelution of *cis*-15 18:1 and 19:0. Because we utilised 19:0 as an internal standard, we used a different method for determination of milk FA, with the same initial temperatures of the detector and injector, and with a split ratio of 1:50. Hydrogen was the carrier gas with a constant pressure (23.2 p.s.i.). The samples were injected with an automatic injector, in 0.1 μl of hexane. Initial temperature of the oven was 60°C, held for 1 min, increased by 20°C/min to 150°C, held at 150°C for 10 min, increased by 2°C/min to 175°C, held at 175°C for 20 min, increased by 10°C/min to a final temperature of 225°C and maintained at 225°C for 10 min. In addition to the separation of *cis*-15 18:1 from 19:0, this method allows a better separation of *trans*-10 and *trans*-11 18:1 when their proportions are very different.

Identification and quantification of peaks were made by comparison with commercial standards when available (Sigma, St. Louis, USA). Identification of *trans*-4 to *trans*-8 18:1, *trans*-12 to *trans*-16 18:1, and *trans*-11, *cis*-15 18:2 was made by comparison with published chromatograms (Precht and Molkentin, 1999). *Trans*-10 18:1 and *trans*-11 18:1, measured together in plasma or their sum in milk samples, were used as an internal standard to quantify *trans*-18:1 FA determined by Ag-TLC. After quantification of the amounts of *trans*-18:1 isomers, *cis*-9 18:1 was corrected by subtracting the overlapping *trans* isomers (*trans*-15 18:1 in plasma samples and *trans*-13 and *trans*-14 18:1 in milk samples) as suggested by Precht and Molkentin (1999).

CLnA was identified by gas chromatography–mass spectroscopy (GC–MS). The FA methyl esters were converted into 2-alkenyl-4,4-dimethyloxazoline (DMOX) derivatives according to Yurawecz *et al*. (1994). Briefly, 100 μl of 2-amino-2-methyl-1-propanol were added to the fatty-acid methyl ester (FAME). The reaction mixture was maintained at 170°C for 8 h under nitrogen atmosphere. The analysis of 4,4-DMOX derivatives was achieved using a GC-2010 coupled with a QP-2010 mass spectrometer (Shimadzu, Champs-sur-Marne, France). The column described above was utilised. Helium was used as the carrier gas at a constant velocity of 24.3 cm/s. The oven was programmed from 60 to 210°C at 20°C/min and the final temperature was maintained for 50 min. The injector in splitless mode was maintained at 250°C. The electron impact mass spectra were recorded at 70 eV between 100 and 450 amu. The identified CLnA was 9,11,15 18:3, the most probable configuration being *cis*-9, *trans*-11, *cis*-15 18:3 (Harfoot and Hazlewood, 1988; Destaillats *et al*., 2005).

Low area peaks were rejected from quantification, the rejection threshold corresponding to around 0.02% of injected FA. *Trans*-10, *cis*-12 18:2 was under area rejection threshold in nearly all plasma and milk samples, so that the only measured CLA isomer was *cis*-9, *trans*-11 18:2, and the term CLA will refer to this isomer in this paper. Figure 1 presents chromatograms of a whole milk sample and from an Ag-TLC extract of milk.

Calculations and statistical analysis

Desaturase ratios between *cis*-9 unsaturated FA and their precursors, which can serve as a proxy for Δ-9 desaturase activity (Bauman *et al*., 2001), were calculated as described by Kelsey *et al*. (2003).

Milk production, protein and fat content, proportions of each FA and desaturase ratios were compared with SYSTAT (Statistical Packages for the Social Sciences, 1998), using the model

$$\text{Variable} = \text{mean} + \text{cow effect} + \text{period effect} + \text{diet effect} + \text{experimental error}.$$

Differences among treatments were assessed using contrasts between control and mean values of linseed diets, and between raw and extruded linseeds. Significance was declared at $P < 0.05$, and tendencies at $0.05 < P < 0.10$.

Results

Milk yield and composition

Milk production was lower for cows fed linseed diets than for those fed diet C (Table 2). Linseed had no significant ($P = 0.109$) effect on milk fat percentage but decreased protein percentage. Yields of milk protein and milk fat were lower for cows fed linseed. Compared with diet RL, diet EL tended to decrease yields of milk, milk fat and milk protein, without affecting milk fat and protein percentages.

Plasma fatty acids profile

Plasma FA profiles are presented in Tables 3 and 4. The proportions of 12:0, 14:0, 15:0, 16:0, *cis*-9 16:1, 17:0, *cis*-9

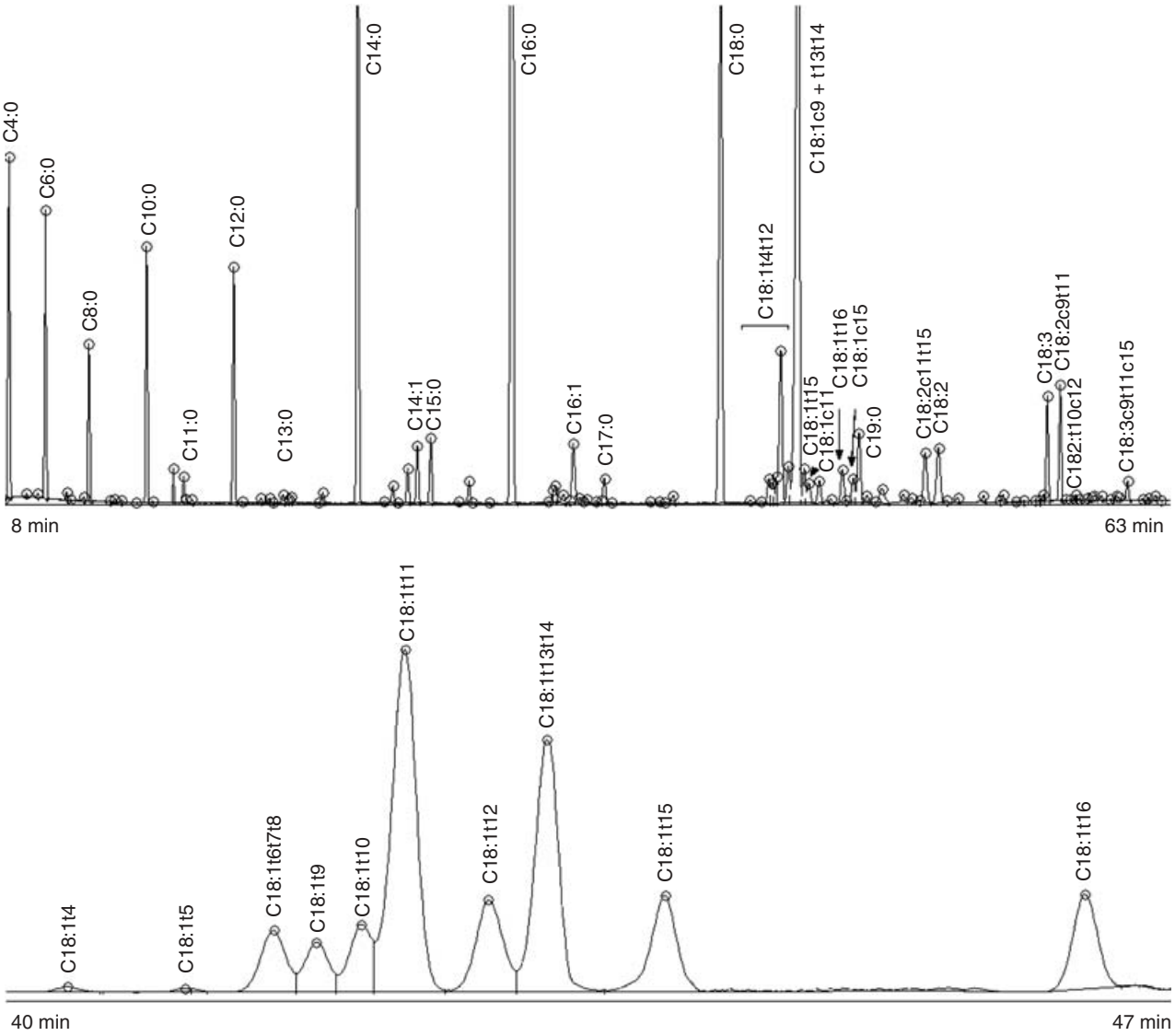

Figure 1 GLC chromatograms of fatty-acid methyl esters (FAMEs) obtained from milk fat of a cow receiving extruded linseed: total FAMEs (upper graph) and *trans*-18:1 fraction obtained by argentation thin-layer chromatography (on lower graph).

Table 2 *Least-square means of milk yield and composition of lactating cows fed control diet or diets supplemented with raw or extruded linseed*

	Diet				Contrast	
	Control	Raw linseed	Extruded linseed	s.e.	Control *v.* linseed	Raw *v.* extruded
Milk (kg/day)	34.86	33.34	32.30	0.38	<0.001	0.052
Composition (%)						
Fat	4.61	4.49	4.36	0.09	0.109	0.329
Protein	2.95	2.90	2.89	0.02	0.048	0.682
Production (kg/day)						
Fat	1.59	1.50	1.41	0.04	0.003	0.059
Protein	1.03	0.97	0.93	0.01	<0.001	0.075

18:1, *cis*-9, *cis*-12 18:2, and *cis*-11 18:1 were decreased, but the proportions of 18:0, *cis*-9, *cis*-12, *cis*-15 18:3, *trans*-5 to *trans*-16 18:1, CLA, and CLnA were increased in the plasma of lactating cows fed linseed diets compared with those fed C diet.

Feeding diet EL decreased the proportions of 12:0, 14:0, 16:0, 17:0 and 18:0, and increased the proportions of *cis*-9, *cis*-12, *cis*-15 18:3, *trans*-10 + 11 to *trans*-16 18:1 FA, CLA, and CLnA, compared with diet RL. The proportion of *trans*-11, *cis*-15 18:2 was not affected by treatments.

Table 3 *Least-square means of proportions of fatty acids (other than biohydrogenation intermediates) in the plasma of lactating cows fed control diet or diets supplemented with raw or extruded linseed*

	Diet				Contrast	
Fatty acid (%)	Control	Raw linseed	Extruded linseed	s.e.	Control *v.* linseed	Raw *v.* extruded
12:0	0.20	0.16	0.10	0.01	<0.001	<0.001
14:0	0.99	0.85	0.69	0.03	<0.001	<0.001
15:0	0.47	0.43	0.43	0.01	<0.001	0.401
16:0	10.23	8.17	7.72	0.10	<0.001	0.001
cis-9 16:1	1.11	0.97	0.98	0.02	<0.001	0.695
17:0	0.47	0.43	0.42	0.01	<0.001	0.013
18:0	13.81	14.49	14.09	0.13	0.005	0.039
cis-9 18:1	8.20	7.74	7.93	0.13	0.021	0.309
cis-9, *cis*-12 18:2	44.13	37.88	37.07	0.29	<0.001	0.052
cis-9, *cis*-12, *cis*-15 18:3	4.52	13.53	14.91	0.20	<0.001	<0.001

Table 4 *Least-square means of proportions of biohydrogenation intermediates in the plasma of lactating cows fed control diet or diets supplemented with raw or extruded linseed*

	Diet				Contrast	
Fatty acid (%)	Control	Raw linseed	Extruded linseed	s.e.	Control *v.* linseed	Raw *v.* extruded
Total *trans*-18:1	1.10	2.23	3.04	0.10	<0.001	<0.001
trans-4 18:1	0.023	0.021	0.024	0.003	0.887	0.487
trans-5 C18:1	0.017	0.020	0.024	0.001	0.003	0.097
trans-6 + 7 + 8 18:1	0.024	0.062	0.041	0.009	0.031	0.2315
trans-9 18:1	0.047	0.079	0.067	0.010	0.042	0.406
trans-10 + 11 18:1	0.41	0.64	0.95	0.03	<0.001	<0.001
trans-12 18:1	0.13	0.28	0.35	0.01	<0.001	<0.001
trans-13 + 14 18:1	0.28	0.70	0.99	0.04	<0.001	<0.001
trans-15 18:1	0.11	0.26	0.35	0.02	<0.001	<0.001
trans-16 18:1	0.09	0.22	0.27	0.01	<0.001	0.003
cis-11 18:1	0.48	0.38	0.36	0.02	<0.001	0.413
cis-9, *trans*-11 18:2	0.14	0.20	0.26	0.01	<0.001	<0.001
trans-11, *cis*-15 18:2	0.52	0.49	0.40	0.04	0.123	0.141
9,11,15 18:3	0.14	0.22	0.27	0.01	<0.001	0.008

Milk fatty acids profile

Milk FA profiles are presented in Tables 5 and 6. Feeding linseed did not affect the milk fat proportions of 4:0 but decreased the proportions of 18:2, *cis*-11 18:1 and FA from 6:0 to 17:0 except 13:0. The proportions of 18:0, *cis*-9 18:1, *cis*-9, *cis*-12, *cis*-15 18:3, all individual *trans*-18:1, *cis*-15 18:1, CLA and *trans*-11, *cis*-15 18:2 were increased with linseed-supplemented diets. CLnA was under the rejection threshold in the milk from cows fed diet C. Linseed diets resulted in a lower desaturase ratio (Table 7) of 18:0, but did not affect desaturase ratios of 14:0, 16:0 and *trans*-11 18:1.

Compared with diet RL, diet EL increased the proportions of *trans*-6 + 7 + 8 to *trans*-15 18:1, CLA, *trans*-11, *cis*-15 18:2 and CLnA, tended to increase the proportion of *cis*-9, *cis*-12, *cis*-15 18:3, reduced the proportions of all even-chain FA from 6:0 to 16:0, but did not affect the proportions of 18:0, *cis*-9 18:1 and *cis*-9, *cis*-12 18:2. Extrusion did not affect the desaturase ratios of milk fat.

Discussion

Milk production and composition, plasma and milk fatty acids other than biohydrogenation intermediates

Feeding linseed reduced milk production in this experiment. The Literature on the response of milk production to diets supplemented with 10–15% of linseed (DM basis) report either reduction (Kennelly (1996) with 10% linseed; Petit *et al.* (2005)) or little effect (Kennelly (1996) with 15% linseed; Gonthier *et al*. (2005)). Petit *et al*. (2005) explained their observed decreased production by a lower DM intake, but this parameter was not measured in our experiment.

Linseed feeding decreased milk protein percentage in our experiment, which is consistent with the results of Kennelly (1996) but contrasts with the observations of Gonthier *et al*. (2005) on late lactation dairy cows. This lowered milk protein percentage with high fat diets has been related to a decreased mammary blood flow (Cant *et al*., 1993).

Table 5 *Least-square means of proportions of fatty-acids (other than biohydrogenation intermediates) in the milk of lactating cows fed control diet or diets supplemented with raw or extruded linseed*

Fatty acid (%)	Diet				Contrast	
4:0	2.69	2.69	2.57	0.07	0.475	0.225
6:0	2.16	2.01	1.82	0.05	<0.001	0.010
8:0	1.33	1.23	1.06	0.02	<0.001	<0.001
10:0	2.74	2.37	2.06	0.06	<0.001	<0.001
11:0	0.25	0.21	0.19	0.02	0.047	0.533
12:0	3.95	3.15	2.54	0.08	<0.001	<0.001
13:0	0.19	0.15	0.16	0.02	0.111	0.847
14:0	11.41	9.95	9.15	0.11	<0.001	<0.001
cis-9 14:1	0.99	0.76	0.72	0.04	<0.001	0.438
15:0	0.98	0.84	0.81	0.02	<0.001	0.266
16:0	33.59	24.35	23.27	0.29	<0.001	0.011
cis-9 16:1	1.73	1.23	1.23	0.04	<0.001	0.995
17:0	0.63	0.57	0.57	0.01	<0.001	0.987
18:0	9.40	14.20	14.39	0.28	<0.001	0.632
cis-9 18:1	18.65	21.84	22.03	0.28	<0.001	0.633
cis-9, *cis*-12 18:2	1.92	1.70	1.62	0.04	<0.001	0.152
cis-9, *cis*-12, *cis*-15 18:3	0.27	0.95	1.20	0.10	<0.001	0.072

Table 6 *Least-square means of proportions of biohydrogenation intermediates in the milk of lactating cows fed control diet or diets supplemented with raw or extruded linseed*

Fatty acid (%)	Diet				Contrast	
	Control	Raw linseed	Extruded linseed	s.e.	Control *v.* linseed	Raw *v.* extruded
Total *trans*-18:1	3.18	6.61	8.91	0.27	<0.001	<0.001
trans-4 18:1	0.020	0.034	0.038	0.002	<0.001	0.270
trans-5 18:1	0.016	0.025	0.031	0.003	0.003	0.175
trans-6 + 7 + 8 18:1	0.10	0.16	0.26	0.01	<0.001	<0.001
trans-9 18:1	0.17	0.24	0.36	0.01	<0.001	<0.001
trans-10 18:1	0.44	0.61	1.09	0.06	<0.001	<0.001
trans-11 18:1	0.87	1.51	2.07	0.09	<0.001	<0.001
trans-12 18:1	0.31	0.67	0.74	0.02	<0.001	0.022
trans-13 + 14 18:1	0.72	1.91	2.64	0.16	<0.001	0.003
trans-15 18:1	0.28	0.79	0.93	0.04	<0.001	0.008
trans-16 18:1	0.28	0.73	0.80	0.03	<0.001	0.101
cis-11 18:1	0.53	0.45	0.45	0.02	<0.001	0.992
cis-15 18:1	0.07	0.54	0.79	0.04	<0.001	<0.001
cis-9, *trans*-11 18:2	0.59	0.84	1.12	0.04	<0.001	<0.001
trans-11, *cis*-15 18:2	0.11	0.60	0.92	0.03	<0.001	<0.001
9,11,15 18:3		0.15	0.18	0.01		0.002

Table 7 *Least-square means of desaturase ratios in the milk of lactating cows fed control diet or diets supplemented with raw or extruded linseed*

	Diet				Contrast	
	Control	Raw linseed	Extruded linseed	s.e.	Control *v.* linseed	Raw *v.* extruded
cis-9 14:1/(14:0 + *cis*-9 14:1)	0.077	0.069	0.075	0.004	0.341	0.294
cis-9 16:1/(16:0 + *cis*-9 16:1)	0.048	0.050	0.051	0.002	0.285	0.675
cis-9 18:1/(18:0 + *cis*-9 18:1)	0.66	0.61	0.61	0.01	<0.001	0.810
cis-9, *trans*-11 18:2/(*trans*-11 18:1 + *cis*-9, *trans*-11 18:2)	0.39	0.39	0.39	0.02	0.967	0.865

Both linseed forms reduced proportions of all FA with less than 18 carbons in plasma and milk, except for the proportions of 4:0 and 13:0 in milk. Because of their lower proportion in milk fat and because of the lower milk fat production, the daily amount of short-chain FA was decreased with linseed diets: the total output of 8:0–14:0-

saturated even-chain FA was 315, 258 and 211 g/day with diets C, RL and EL, respectively ($P < 0.001$ for both contrasts C *v.* linseed and RL *v.* EL), which represented a 18% and 33% decrease, respectively, and explained the reduction of milk fat yield. With raw linseed, Gonthier *et al.* (2005) reported a 38% decrease of 8:0–14:0 daily output, and a 34% decrease was observed due to linseed oil (Loor *et al.*, 2005). Similar effect was observed after soybean (Chouinard *et al.*, 1997) or sunflower (Schingoethe *et al.*, 1996) dietary addition.

Milk FA from 6:0 to 14:0 and part of 16:0 are synthesised by the mammary gland, and increasing amounts of unsaturated fat supplement are known to inhibit the synthesis of these FA (Clapperton and Banks, 1985). Among possible reasons for this inhibition, Grummer (1991) cited a direct inhibition of mammary acetyl-CoA carboxylase, which is mediated by *trans*-10, *cis*-12 18:2 (Baumgard *et al.*, 2000), but in our experiment, milk proportion of *trans*-10, *cis*-12 18:2 was very low with all diets. Loor *et al.* (2005) suggested that milk fat depression could also be explained by other FA, including *trans*-10 18:1, which exhibited high proportions in milk with low proportions of 6:0–14:0 in our experiment. However, Lock *et al.* (2007) recently demonstrated that this FA does not reduce milk fat synthesis in dairy cows.

Compared with diet C, linseed feeding increased 18:0 and decreased *cis*-9 18:1 proportions in plasma lipids by 3.5% and 4.5%, respectively. Consequently, the lowered 18:0 desaturase ratio with linseed diets could have been due to this lowered *cis*-9 18:1/18:0 ratio in plasma rather than to a decreased activity of mammary Δ9-desaturase. Moreover, 14:0, 16:0 and *trans*-11 18:1 desaturase ratios were not affected by linseed addition.

In spite of a 35% increase in *cis*-9, *cis*-12 18:2 dietary concentration, the linseed diets decreased *cis*-9, *cis*-12 18:2 proportion in both plasma lipids and milk fat: the *cis*-9, *cis*-12 18:2 milk excretion decreased from 30.3 g/day with diet C to 25.8 and 22.4 g/day with diets RL and EL, respectively, and the lower milk yield with linseed diets could only account for a little part of these differences. This demonstrates a lower transfer from diet to milk, due to a higher ruminal disappearance of *cis*-9, *cis*-12 18:2, a lower mammary uptake, a competition between *cis*-9, *cis*-12 18:2 and *cis*-9, *cis*-12, *cis*-15 18:3 in the mammary gland, or both.

Linseed feeding increased the plasma proportion of *cis*-9, *cis*-12, *cis*-15 18:3 by only three times in our experiment, whereas the dietary amount was nine times higher than in diet C. A similar three-fold increase in plasma lipids has been observed after linseed addition by Gonthier *et al.* (2005). This low transfer from diet to plasma is due to the extensive BH of *cis*-9, *cis*-12, *cis*-15 18:3 in the rumen, and suggests that ruminal *cis*-9, *cis*-12, *cis*-15 18:3 BH extent is increased when dietary supply is high. The mammary gland can efficiently uptake blood *cis*-9, *cis*-12, *cis*-15 18:3 because its proportion in milk FA reaches 13.9% after a duodenal daily infusion of 500 g of linseed oil (Petit *et al.*, 2002). In our experiment, the transfer rate of *cis*-9, *cis*-12, *cis*-15 18:3 from diet to milk decreased from 7.45% with diet C to 3.10% with linseed diets.

The proportion of *cis*-9, *cis*-12, *cis*-15 18:3 was increased in the plasma, and tended to be increased in the milk with diet EL compared with diet RL, suggesting a lower ruminal BH after extrusion. On the contrary, Gonthier *et al.* (2005) reported that the extrusion of linseed decreased milk *cis*-9, *cis*-12, *cis*-15 18:3 proportion. The discrepancy between our results and theirs could be attributed to the difference of the extrusion temperature of linseed, which was 155°C in their experiment compared with 120°C in our experiment. However, Chouinard *et al.* (1997) demonstrated using soya beans that increasing extrusion temperature from 120 to 140°C has only minor effects on milk PUFA.

Plasma and milk biohydrogenation intermediates

Linseed supplement was the only source of CLnA in the milk because there was no detectable CLnA in the milk of cows fed diet C. Destaillats *et al.* (2005) found 0.03% of CLnA in the fat of Canadian summer milk. Our rejection threshold was under this value, but lack of CLnA with C diet in our experiment could be related to the low amount of dietary *cis*-9, *cis*-12, *cis*-15 18:3, because dietary *cis*-9, *cis*-12, *cis*-15 18:3 is mainly provided by grass or linseed supplements, which were not ingredients of our diet C.

Proportions of CLnA and CLA were increased in both plasma lipids and milk fat by linseed supplementation. The same effect was observed for *trans*-11, *cis*-15 18:2 in milk fat, but not in plasma lipids where a high variability was noticed. Plasma and milk CLnA and *trans*-11, *cis*-15 18:2 originate from the ruminal BH of *cis*-9, *cis*-12, *cis*-15 18:3 (Harfoot and Hazlewood, 1988), so that increased values could be expected with linseed addition. Milk CLnA possibly could also originate from mammary Δ9-desaturation of *trans*-11, *cis*-15 18:2, but the ratio of CLnA to *trans*-11, *cis*-15 18:2 was higher in plasma lipids than in milk fat, which does not support this hypothesis. However, plasma non-esterified FA and triacylglycerols are the primary FA sources for the mammary gland, so that the interpretation of this difference of ratio can be biased if CLnA and *trans*-11, *cis*-15 18:2 have different distributions among plasma lipid classes.

Milk CLA can originate from ruminal CLA, the first intermediate of *cis*-9, *cis*-12 18:2 BH, but most milk CLA originates in mammary Δ9-desaturation of *trans*-11 18:1 (Griinari *et al.*, 2000; Lock and Garnsworthy, 2002). Because *trans*-11 18:1 is only a minor isomerisation product of *cis*-9 18:1 in the rumen (Mosley *et al.*, 2002), most ruminal *trans*-11 18:1 originates in *cis*-9, *cis*-12 18:2 and *cis*-9, *cis*-12, *cis*-15 18:3 BH (Harfoot and Hazlewood, 1988), which can explain the higher proportion of this FA after linseed addition. In plasma lipids, linseed feeding decreased *cis*-9, *cis*-12 18:2, but increased CLA. This higher ratio of CLA to *cis*-9, *cis*-12 18:2 could be due to an inhibition by *cis*-9, *cis*-12, *cis*-15 18:3 of the ruminal reductase, which converts CLA to *trans*-11 18:2 (Troegeler-Meynadier *et al.*, 2003).

Total *trans*-18:1 percentage in the milk fat of cows fed diet C in our experiment was similar to the percentage reported in French and German milks from cows under various nutritional and management conditions: 3.3–3.8% (Wolff *et al*., 1998). Linseed feeding in our experiment increased the proportions of *trans*-18:1 FA, in agreement with the results reported by Loor *et al*. (2005) with a linseed oil supplement.

From the BH pathways of *cis*-9, *cis*-12 18:2 and *cis*-9, *cis*-12, *cis*-15 18:3 described by Harfoot and Hazlewood (1988), we could have expected very different patterns of positional distribution of *trans*-18:1 isomers between C and linseed diets, because *cis*-9, *cis*-12 18:2 was the major PUFA in our diet C, and *cis*-9, *cis*-12, *cis*-15 18:3 was the major PUFA with linseed diets. *Trans*-11 18:1 should have largely been the most important isomer with diet C, whereas *cis*-15 18:1, *trans*-11 18:1 and *trans*-15 18:1 should have been the major isomers with linseed diets, and important proportions of *trans*-13 + 14 18:1 could also be expected (Ward *et al*., 1964; Loor *et al*., 2004). In our experiment, with diet C, the *trans*-13 + 14 18:1 proportion was 66% and 55% of that of *trans*-10 + 11 18:1 in plasma and milk, respectively, and proportions of *trans*-12 18:1, *trans*-15 18:1, and *trans*-16 18:1 were all around 10% of the total *trans*-18:1 in plasma and milk. *Trans*-12 18:1, *trans*-15 18:1, and *trans*-16 18:1 have already been suggested to be produced during *cis*-9, *cis*-12 18:2 BH (Loor *et al*., 2002), in addition to their production during *cis*-9 18:1 (Mosley *et al*., 2002) and *cis*-9, *cis*-12, *cis*-15 18:3 BH, and Piperova *et al*. (2002) published a distribution of *trans*-18:1 isomers close to ours in the milk from cows fed a diet without added fat, and where *cis*-9, *cis*-12, *cis*-15 18:3 represented 10.8% of the total dietary FA. On the contrary, Loor *et al*. (2005) found, with a diet without added fat but where *cis*-9, *cis*-12, *cis*-15 18:3 represented 25.8% of the total dietary FA, that *trans*-13 + 14 18:1 proportion was only 24% of that of *trans*-10 + 11 18:1 in milk, but 61% in plasma. After linseed oil supplementation, these authors found a distribution of major *trans*-18:1 isomers that was closer to our values with both added linseed forms, where *trans*-10 + 11 18:1 averaged 29.2% and 33.5% of plasma and milk total *trans*-18:1, respectively, and *trans*-13 + 14 18:1 averaged 33.5% and 29.3% of plasma and milk *trans*-18:1, respectively. In our experiment, relative to total *trans*-18:1, *trans*-12 18:1 and *trans*-16 18:1 were not changed by linseed addition. *Trans*-15 18:1 and *cis*-15 18:1, which are considered to be important final products of *cis*-9, *cis*-12, *cis*-15 18:3 BH (Harfoot and Hazlewood, 1988), increased by 244% and 677% in milk fat after linseed addition, but in spite of these large increases, these isomers were minor compared with *trans*-10 18:1, *trans*-11 18:1, or *trans*-13 + 14 18:1. The relative increase was higher for *cis*-15 than for *trans*-15 18:1, which was consistent with the large increase of *trans*-11, *cis*-15 18:2, the direct precursor of *cis*-15 18:1 in the rumen.

Most studies on the relationship between *trans*-18:1 and cardiovascular risk in human have compared *cis*-9 18:1 to *trans*-9 18:1, and the biological properties of this isomer have often been extrapolated to all *trans*-18:1 isomers. However, the position of the double bond can strongly affect the biological effect of *trans*-18:1 isomers, making this extrapolation suspect (Bauman *et al*., 2004). *Trans*-9 18:1 represents about 6.9% of *trans*-18:1 FA in the fat of German milks produced under various feeding conditions (Wolff *et al*., 1998). In our experiment, this proportion was 5.3% with diet C and was decreased by linseed feeding. However, linseed increased the proportion of this isomer relative to total FA by 35% and 106% with diets RL and EL, respectively.

Compared with diet RL, diet EL resulted in higher proportions of CLnA and CLA in plasma and milk fat, and increased *trans*-11, *cis*-15 18:2 proportion in milk fat. Higher CLA proportion with extruded than raw oilseeds has already been reported (Chouinard *et al*., 2001; Gonthier *et al*., 2005). In agreement with Gonthier *et al*. (2005), feeding EL in our experiment also increased proportions of total and most individual 18:1 BH intermediates percentages in plasma and milk, compared with RL. The relative increase in milk was over 40% for *trans*-6 to *trans*-11 18:1 and *cis*-15 18:1, but was only 18% for *trans*-15 18:1, and around 10% for *trans*-12 and *trans*-16 18:1. Extrusion of oilseeds is known to result in increased proportions of *trans* intermediates in milk fat (Chouinard *et al*., 1997; Chouinard *et al*., 2001) but our results show that the effects differ according to the *trans*-18:1 isomer, suggesting that extrusion affects the pathways of ruminal BH.

Conclusion

Including linseed in the diet of dairy cows resulted in a lower milk production and protein percentage. Milk proportions of FA from 6:0 to 16:0 decreased, but the proportions of *cis*-9 18:1, *cis*-15 18:1, all *trans*-18:1 isomers, CLA, *trans*-11, *cis*-15 18:2, CLnA and *cis*-9, *cis*-12, *cis*-15 18:3 increased when the diet contained raw or extruded linseed. With these diets, plasma and milk proportions of *trans*-13 + 14 18:1 were in the same range than that of *trans*-10 + 11 18:1, and were more than twice that of *trans*-15 18:1. EL, when compared with RL, tended to lower milk, milk fat and milk protein daily productions, further decreased the milk fat proportions of FA from 6:0 to 16:0, and increased the milk fat proportions of *trans*-9 18:1, *trans*-10 18:1, *trans*-11 18:1, *trans*-13 + 14 18:1, *trans*-15 18:1, *cis*-15 18:1, CLA, *trans*-11, *cis*-15 18:2 and CLnA. The present experiment provides the first evidence that milk CLnA can be manipulated via dietary linseed.

Acknowledgement

The authors thank Valorex (7 La Messayais, 35210 Combourtillé, France) for supply of linseed sources.

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