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ORIGINAL ARTICLE

Effect of Linseed Fed as Rolled Seeds, Extruded Seeds or Oil on Fatty Acid Rumen Metabolism and Intestinal Digestibility in Cows

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Abstract Linseed, a source of linolenic acid, is used in ruminant diets to increase polyunsaturated fatty acids (FA) in animal products. Seed processing is known to have an impact on FA rumen metabolism, but few data are available for linseed. We studied the effect of linseed lipid on ruminal metabolism and intestinal digestibility in cows. Three modes of linseed processing: rolled linseed (RL), extruded linseed (EL) and linseed oil plus linseed meal (LO), supplemented at 7.5% of DM intake, were compared to a control diet (C). Duodenal flows, intestinal digestibility and plasma composition were determined. The duodenal flow of linolenic acid was similar among diets. The sum of t10 and t11-18:1, which were coeluted, was increased with lipid-supplemented diets and represented more than 60% of *trans* 18:1 for EL and LO diets. The main 18:2 isomers were c9, c12 and t11, c15 among the non-conjugated isomers, and t11, t13 among CLA. Linseed supplementation increased the duodenal flow of unsaturated intermediates of biohydrogenation, and this effect was more pronounced for extruded seeds and oil than for rolled seeds. For most 18-carbon FA, intestinal digestibility was slightly higher for C and LO diets than for RL and EL. Plasma concentrations of non-conjugated 18:2 and linolenic acid were similar among the lipid-supplemented diets. Within diet, profiles of 18:1 isomers (except c9) remained very similar between duodenal and plasma FA.

Keywords Linseed · Extrusion · Fatty acids · Rumen metabolism · Digestion · Cow

Abbreviations

BH	Biohydrogenation
C	Control diet
CLA	Conjugated linoleic acid
DM	Dry matter
EL	Extruded linseed
FA	Fatty acid
LO	Linseed oil
RL	Rolled linseed

Introduction

Linolenic acid, along with other fatty acids (FA) of the n-3 series, is known to have positive effects on human health, particularly by decreasing the incidence of cardiovascular diseases [1]. It has been suggested that the nutritional value of milk and beef could be improved by increasing linolenic acid content in ruminant diets. The two main sources of linolenic acid are grass, both fresh and ensiled [2], and linseed. Linseed is easy to incorporate in production rations and, despite extensive biohydrogenation (BH) in the rumen; it has been demonstrated to increase linolenic acid content significantly in both milk [3, 4] and meat [5–8]. Furthermore, these authors showed that animal products following linseed intake are enriched in c9, t11-18:2, which is an isomer of conjugated linoleic acid (CLA) that may have a positive effect on human health by decreasing the

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incidence of cancer [9, 10]. Linseed incorporation also increases proportions of other FA such as t11-18:1 or c11, t15-18:2. These modifications in product composition arise from modifications in ruminal FA metabolism [11]. At the same time, it has been shown that moderate amounts of linseed added to rations do not impair organic matter or fiber digestion [12], whereas important amounts of linseed oil strongly reduced organic matter and fiber digestion [13].

Oilseeds can be either given as whole seeds or processed by different techniques, the most common being extrusion. In the case of linseeds, since digestion may not be optimized following the intake of whole seeds that have not been mechanically treated, it is recommended to supply them as rolled or ground seeds [7]. Theoretically, since the extrusion process breaks down plant cells, it is expected that cytosolic triglycerides are more accessible to rumen bacteria than when whole seed is given, meaning they are more rapidly available for lipolysis and BH. In two experiments recently carried out with extruded linseed, Akraim et al. [14] did not find any effect of linseed extrusion on duodenal FA composition, whereas Gonthier et al. [15] found that extrusion increased the disappearance of linolenic acid but also increased the proportion of intermediate compounds such as *trans* 18:1 FA in duodenal flow. These inconsistent data need to be verified, especially because Akraim et al. [14] did not measure duodenal flows while Gonthier et al. [15] did not determine *cis* and *trans* 18:1 composition in detail. Moreover, oil within the seed needs to be compared with free oil, firstly to evaluate the effects of oil accessibility according to its form of presentation, and secondly because published experiments have focused exclusively on either linseed oil or linseed but never the two in the same experiment and in the same amount. The aim of this study was thus (1) to investigate the consequences of a linseed supply on the changes in organic matter and fiber digestion (evaluated in a complementary paper [16]) and in the composition of absorbable FA; (2) to compare three modes of processing, i.e. rolled seeds, extruded seeds, and oil of this FA composition.

Materials and Methods

Experimental Design, Animals and Diets

All details on experimental design, animals, diets and samplings were described by Doreau et al. [16]. Briefly, four dry cows fitted with ruminal and duodenal cannulas were used in a 4 × 4 Latin square design with 5-week periods, with sampling during the last week of each period. They received four treatments: (1) control diet (C); (2) diet C supplemented with 7.5% rolled linseeds on dry matter (DM) basis (RL); (3) diet C supplemented with 7.5% extruded linseeds on DM basis (EL); (4) diet C supplemented with 4.9% linseed oil and 2.6% linseed meal on DM basis (LO). Diets were given in limited amounts (i.e. 90% of individual voluntary intake determined in a pre-experimental period) to avoid refusals and differences in intake among diets. All diets comprised 60% corn silage, 10% hay, 14% dairy concentrate, 15% experimental concentrates, 0.5% urea and 0.5% of a concentrate mineral–vitamin premix (DM basis). Extruded linseeds were supplied as Croquelin® (Valorex, Combourtillé, France) which is an extruded mixture of 50% linseeds, 30% wheat bran and 20% sunflower meal (DM basis). Extrusion was performed with a one-screw extruder at 120°C after a low-temperature (72°C) preconditioning with steam for 157 min. In the RL diet, the linseeds of the same batch as that used to make Croquelin® were rolled with a roll crusher. Particle size, determined by dry sieving, was 22.6% > 2.0 mm, 62.0% between 0.8 and 2.0 MM; 9.4% between 0.4 and 0.8 mm, and 6.0 > 0.4 mm; the proportion of intact seeds was very low. Linseed oil was obtained by cold pressing and provided by Vandeputte (Mouscron, Belgium). Corn silage was offered in two equal portions at 9.30 a.m. and 4.30 p.m. Hay and concentrates, including oil, were offered at 9.00 a.m.

Fatty Acid Analyzes, Duodenal Flow and Intestinal Digestibility Calculations

Fatty acids in feedstuffs, duodenal digesta and feces were directly transmethylated, essentially as described by Sukhija and Palmquist [17] with modifications according to Loor et al. [11] in order to decrease isomerization of *cis–trans* conjugated double bonds due to too high temperature and too acidic pH. Total plasma lipids were extracted according to the method described in Folch et al. [18] using a chloroform/methanol mixture (2:1, by vol) (3 mL plasma + 20 mL mixture) and then rinsed with 6 mL distilled water and recovered in 1 mL hexane. Extracted FA were methylated with 2 mL of 0.5 N $NaOCH_3$ at 50°C for 45 min followed by 2 mL 5% HCl in methanol at 50°C for 45 min. After neutralization with 6% K_2CO_3 and additional hexane, a hexanoic phase containing FA methyl esters was recovered. Methyl tricosanoate was used as internal standard. Samples were injected by auto-sampler into a Varian CP-3800 gas chromatograph equipped with a flame ionization detector. Methyl esters from all samples were separated on a 100 m × 0.25 mm i.d. CP-Sil 88 fused-silica capillary column. Hydrogen was the carrier gas. Injector pressure was held constant at 23 psi. Pure methyl ester standards purchased from Supelco (Bellefont, USA), Sigma (St Louis, USA), Nu-Chek Prep (Elysian, USA), and Biovalley (Conches, France) were used to identify peaks, except for

certain *trans* and *cis* 18:1 isomers that were identified in order of elution and according to Kramer et al. [19], and t11, c15-18:2 that was identified according to Ulberth and Henninger [20].

Satisfactory separations of most *cis* and *trans* 18:1 and CLA isomers were obtained with a single chromatographic run. There was a partial coelution of different monounsaturated *cis* and *trans* 18-carbon isomers as well as c9, c11-18:2, and 21:0, as previously reported with the CP-Sil 88 100 m capillary column. In particular, due to the imperfect separation between t10 and t11-18:1 in some samples, the results give the sum of these two isomers.

Duodenal flows and intestinal digestibility of fatty acids were determined from duodenal flow of DM and fecal DM as described by Doreau et al. [16], and from FA determination in duodenal contents and feces. Rumen BH was computed as (intake − duodenal flow)/intake × 100.

Statistical Analyses

Statistical analyzes of fatty acid duodenal flows and digestibilities were performed as a 4 × 4 Latin square using the PROC MIXED procedure of the SAS statistical analysis software suite [21]. The statistical model included the effect of cow as random effect and the effects of period and treatment as fixed effects. Differences among means were determined using the Student–Newman–Keuls test. Differences at $P < 0.10$ were considered to be significant.

Results

Fatty acid intake was increased by 235 g/day on average between the C diet and the lipid-supplemented diets, with similar dry matter intakes (Table 1). This increase was mainly due to linolenic acid (+140 g/day) and, to a lesser extent, linoleic acid (+30 g/day) and oleic acid (+40 g/day).

Table 1 Dry matter and fatty acid intake (g/day, except dry matter in kg/day)

	Diet			
	C	RL	EL	LO
Dry matter (kg/day)	10.5	10.0	9.9	9.9
Total FA	269.4	514.8	501.2	497.7
16:0	41.6	54.0	54.0	51.7
18:0	6.6	16.6	16.0	14.9
c9 18:1	52.7	92.9	93.2	93.1
c9, c12 18:2	112.6	145.0	142.0	142.9
c9, c12, c15 18:3	40.8	188.5	179.0	175.5

C control diet, *RL* control diet supplemented with 7.5% rolled linseed, *EL* control diet supplemented with 7.5% extruded linseed, *LO* control diet supplemented with 4.9% of linseed oil and 2.6% linseed meal

Duodenal Flow of Fatty Acids

Duodenal flows of total FA were similar among the three lipid-supplemented diets and higher than for the control diet (Table 2). The ratio of duodenal flow to intake of total FA was 1.18, 0.87, 0.96 and 0.98 for diets C, RL, EL and LO, respectively (SEM = 0.03), and was significantly lower for lipid-supplemented diets than for the C diet ($P < 0.01$). The difference among diets in duodenal flow of total FA was due to differences in 18-carbon FA. Duodenal flows of 18:0 and total *cis* 18:1 FA was lower for diet C than for the other 3 diets. Duodenal flows of total *trans* 18:1 and total CLA were highest with the EL and LO diets, lowest with the C diet, and intermediate with the RL diet (not significantly different from the other three diets). Duodenal flow of total non-conjugated 18:2 was higher with diets EL and LO than with diets C and RL. Duodenal flow of linolenic acid was numerically maximal with the EL diet and minimal with the C diet, but the differences were not significant due to high inter-animal variability. No significant differences were found for the other FA. Except for t5 and c9-18:1, there were between-diet differences for all the 18:1 isomers (Table 3). For t6 + t7 + t8, t9, t12, t13 + t14 + c6, c10 + t15, c12, c13, c14 + t16 and c15-18:1, the duodenal flows were similar among linseed-supplemented diets and significantly higher than with control diet. For the coelution t10 + t11 and c11-18:1, the duodenal flows were higher with EL and LO diets compared with C and RL diets. For t4-18:1, the duodenal flow was higher with EL and LO diets than with the RL diet, all three surpassing the C diet.

Among non-conjugated 18:2, the flow of linoleic acid did not differ among diets; the flow of t11, c15-18:2 was higher with the EL and LO diets than with the C and RL diets, the flow of c9, t12-18:2 was higher with the RL, EL and LO diets than with the C diet, and the flows of t9, t12 and t9, c12-18:2 were higher with the LO diet than with the C diet and intermediate with RL and EL diets (Table 3). Among CLA, t10, c12-18:2 flow did not differ among diets, the duodenal flow of the coelution of c9, t11 and t8, c10-18:2 was higher with the EL and LO diets than with the C and RL diets, the flow of the coelution of t11, c13 and c9, c11-18:2 was higher with the LO diet than with the C and RL diets, EL diet being intermediate, and the flow of t11, t13 and of other t, t-CLA was higher with the RL, EL and LO diets than with the C diet.

The BH of oleic and linoleic acids was higher with the RL diet than with the C diet, and intermediate with EL and LO diets (Table 4). The biohydrogenation of linolenic acid did not significantly differ among diets (Table 4).

Table 2 Duodenal flow of main fatty acids (g/day)

	Diet				SEM	Statistical level
	C	RL	EL	LO		
Total FA	317.4 a	447.6 b	472.9 b	486.6 b	14.58	$P < 0.01$
12:0	1.0	0.8	0.8	0.9	0.08	NS
14:0	2.6	2.3	2.4	2.6	0.17	NS
Iso 15:0	1.7	1.4	1.5	1.6	0.10	NS
Anteiso 15:0	3.8	3.4	3.5	3.9	0.14	NS
15:0	3.4	3.1	3.2	3.5	0.18	NS
Iso 16:0	3.1	2.6	2.5	2.9	0.22	NS
16:0	49.0	53.5	55.0	56.3	2.35	NS
17:0	2.0	1.9	1.8	2.0	0.11	NS
18:0	168.8 a	252.3 b	228.9 ab	227.9 ab	10.52	$P < 0.01$
Sum of *cis* 18:1	22.2 a	38.0 b	39.0 b	40.1 b	2.94	$P < 0.05$
Sum of *trans* 18:1	32.1 a	56.1 ab	82.4 b	93.3 b	6.16	$P < 0.01$
Sum of non-conjugated 18:2	15.2 a	17.5 a	31.6 b	33.3 b	2.69	$P < 0.01$
Sum of CLA	1.0 a	1.7 ab	2.7 b	3.0 b	0.21	$P < 0.01$
18:3n-3	2.1	5.9	7.8	4.7	1.69	NS
20:0	1.8	1.8	2.0	2.0	0.08	NS
22:0	1.2	1.2	1.4	1.4	0.056	NS
24:0	1.4	1.4	1.6	1.7	0.078	NS

C control diet, *RL* control diet supplemented with 7.5% rolled linseed, *EL* control diet supplemented with 7.5% extruded linseed, *LO* control diet supplemented with 4.9% linseed oil and 2.6% linseed meal

Means with different letters within a row are significantly different ($P < 0.05$)

Fatty Acid Digestibility

Intestinal digestibility of total FA was higher with the C and LO diets than with the RL diet, the EL diet being intermediate and not significantly different from the three other diets (Table 5). Digestibility of 12- to 17-carbon FA, and digestibility of linolenic acid did not differ among diets. Digestibility of *cis* and *trans* 18:1 isomers and of 18:2 isomers was highest with the LO diet and lowest with the RL diet. Considering 18:1 isomers, digestibility was the lowest with the RL diet for all 18:1 isomers, the difference being significant for most of them (Table 6). Digestibility was the highest with the LO diet for most isomers, especially those for which duodenal flow was high, i.e. t10, t11, c9 and the coelution of c10 and t15-18:1. Digestibility of t11, c15-18:2 did not differ among diets, whereas digestibility of linoleic acid was lower with the RL diet than with the other three diets. Digestibility of individual CLA isomers and minor non-conjugated 18:2 isomers was not calculated because the very low duodenal flows resulted in inaccurate measurements.

Plasma Fatty Acids

Among the saturated FA, only 14- to 16-carbon FA and 17:0 differed among diets in their plasma content, with a higher proportion for C diet compared to the others (Table 7). Among monounsaturated FA, the proportion of oleic acid ranged between 9.8 and 11.5% according to diet and did not differ significantly among diets (Table 8). The percentage of t11-18:1, which was the main *trans* 18:1 isomer, was higher with the EL and LO diets than with the C and RL diets. There were no between-diet differences in t4, t5, t9, t10, c9, c10 + t15, c11 and c13-18:1 (Table 8). There were higher proportions of the coelution of t13, t14 and c6-18:1 with the RL and LO diets than with the C diet, the EL diet being intermediate. There was a higher proportion of c12-18:1 with the RL diet than with the C and EL diets, the LO diet being intermediate.

Conjugated linoleic acids were present in very small amounts. There was a lower proportion of c9, t11-isomer (coeluted with t8, c10) in total FA with the RL diet (0.19%) than with the LO diet (0.36%), the C (0.27%) and EL (0.34%) diets being intermediate ($P < 0.05$). Other isomers were found in some animals, but only in trace amounts.

Discussion

Duodenal Flow and Biohydrogenation

The rumen balance of total FA is consistent with the equation published in Schmidely et al. [22], with a positive balance (a net synthesis of both C12–C17 and C18 FA) in the C diet, and a slightly negative balance for the lipid-supplemented diets.

The main monounsaturated C18 isomers at duodenum for lipid-supplemented diets were found in peaks corresponding to the coelution of t10 and t11-18:1, the coelution of t13, t14 and c6-18:1, c9-18:1 and the coelution of c10

Table 3 Duodenal flow of unsaturated 18-carbon fatty acids (g/day)

	Diet				SEM	Statistical level
	C	RL	EL	LO		
18:1 isomers						
t4	0.37 a	0.57 b	0.69 c	0.72 c	0.021	$P < 0.01$
t5	0.27	0.42	0.38	0.39	0.095	NS
t6 + t7 + t8	1.48 a	2.62 b	3.25 b	3.72 b	0.208	$P < 0.01$
t9	1.13 a	1.80 b	2.07 b	2.18 b	0.174	$P < 0.05$
t10 + t11	18.22 a	23.98 a	51.58 b	59.29 b	4.720	$P < 0.01$
t12	2.87 a	5.70 b	6.66 b	7.12 b	0.405	$P < 0.01$
t13 + t14 + c6	5.80 a	17.83 b	14.77 b	17.32 b	1.344	$P < 0.01$
c9	10.74	11.44	15.47	14.70	1.643	NS
c10 + t15	5.22 a	11.79 b	10.85 b	11.46 b	0.670	$P < 0.01$
c11	2.61 a	2.79 a	3.61 b	3.70 b	0.231	$P < 0.05$
c12	2.14 a	4.42 b	3.76 b	3.73 b	0.335	$P < 0.05$
c13	0.25 a	0.59 b	0.58 b	0.64 b	0.048	$P < 0.01$
is-14 + t16	2.47 a	6.39 b	4.81 b	5.14 b	0.306	$P < 0.01$
c15	0.70 a	3.76 b	2.87 b	3.37 b	0.328	$P < 0.01$
Non-conjugated 18:2						
t9, t12	0.06 a	0.16 ab	0.20 ab	0.33 b	0.043	$P < 0.05$
c9, t12	0.46 a	0.89 b	0.78 b	0.77 b	0.033	$P < 0.01$
t9, c12	0.06 a	0.13 ab	0.11 ab	0.16 b	0.019	$P < 0.05$
t11, c15	1.49 a	4.72 a	12.50 b	14.01 b	1.374	$P < 0.01$
c9, c12	10.59	7.89	12.13	12.02	1.225	NS
Conjugated 18:2 (CLA)						
c9, t11 + t8, c10	0.22 a	0.16 a	0.57 b	0.64 b	0.084	$P < 0.05$
t10, c12 + 21:0	0.09	0.08	0.10	0.12	0.011	NS
t11, c13 + c9, c11	0.12 a	0.07 a	0.46 ab	0.59 b	0.070	$P < 0.01$
t11, t13	0.13 a	0.66 b	0.64 b	0.80 b	0.064	$P < 0.01$
Sum of other t, t-CLA	0.46 a	0.77 b	0.89 b	0.89 b	0.054	$P < 0.01$

C control diet, *RL* control diet supplemented with 7.5% rolled linseed, *EL* control diet supplemented with 7.5% extruded linseed, *LO* control diet supplemented with 4.9% linseed oil and 2.6% linseed meal

Means with different letters within a row are significantly different ($P < 0.05$)

Table 4 Ruminal biohydrogenation of unsaturated FA (%)

	Diet				SEM	Statistical effect
	C	RL	EL	LO		
c9-18:1	79.2 a	87.3 b	83.4 ab	84.1 ab	1.88	$P < 0.10$
c9, c12-18:2	90.7 a	94.5 b	91.5 ab	91.6 ab	0.90	$P < 0.10$
c9, c12, c15-18:3	94.9	97.0	95.8	97.3	0.91	NS

C control diet, *RL* control diet supplemented with 7.5% rolled linseed, *EL* control diet supplemented with 7.5% extruded linseed, *LO* control diet supplemented with 4.9% linseed oil and 2.6% linseed meal

Means with different letters within a row are significantly different ($P < 0.05$)

and t15-18:1. These data are similar to those found with linseed oil-supplemented diets [11, 23] and in vitro data [24], and these isomers most probably arise from partial BH of linolenic acid and dietary c9-18:1. The high amount of t11, c15 among non-conjugated 18:2 isomers and t11, t13 among CLA confirms the importance of carbon 13 and carbon 15 desaturated C18 as BH intermediates of linolenic acid [24]. In all diets, the duodenal flows of total CLA represented less than 10% of the non-conjugated 18:2.

Among the different lipid-supplemented diets, RL exhibited the highest BH for oleic and linoleic acid, whereas linolenic acid BH did not seem to be affected by the supplement. The duodenal flows are consistent, since the RL diet induced a higher flow of 18:0 and lower flows of BH intermediates (*trans* 18:1 and 18:2) compared to EL and LO diets.

The most surprising result of this experiment was the numerically higher amount of linolenic acid at the duodenum with the EL diet than with the RL diet. Other data showed either a lower amount of linolenic acid with extruded linseed [15] or no variation [14]. A part of these differences among trials may be explained by differences in technological processes. In our trial, the small particle size of rolled linseeds probably results in a rapid release of oil. The extrusion process apparently shows limited

Table 5 Intestinal digestibility of main fatty acids (%)

	Diet				SEM	Statistical level
	C	RL	EL	LO		
Total FA	75.7 b	66.9 a	72.4 ab	76.3 b	2.05	$P < 0.10$
12:0	47.2	37.7	42.0	43.5	5.70	NS
14:0	45.0	38.6	47.3	43.6	5.22	NS
Iso 15:0	54.0	49.2	50.7	52.2	6.06	NS
Anteiso 15:0	63.1	56.6	59.2	60.3	4.13	NS
15:0	41.6	36.9	39.2	41.7	6.15	NS
Iso 16:0	37.0	35.4	31.2	32.8	6.67	NS
16:0	69.6	65.2	66.0	70.0	2.67	NS
17:0	31.3	17.1	17.8	18.3	8.11	NS
18:0	79.5 b	65.4 a	69.9 ab	73.8 ab	3.08	$P < 0.1$
Sum of *cis* 18:1	77.80 ab	74.5 a	76.9 ab	82.1 b	1.00	$P < 0.01$
Sum of *trans* 18:1	85.4 bc	78.6 a	81.6 ab	87.6 c	0.89	$P < 0.10$
Sum of non-conjugated 18:2	73.6 a	68.1 a	79.9 ab	85.7 b	2.74	$P < 0.05$
Sum of CLA	60.6 ab	48.2 a	60.9 ab	80.6 b	5.26	$P < 0.05$
18:3n-3	66.6	61.9	72.2	83.8	7.77	NS
20:0	69.7 b	59.7 a	66.2 b	69.1 b	2.12	$P < 0.10$
22:0	57.7 ab	52.8 a	58.6 ab	62.8 b	2.16	$P < 0.10$
24:0	58.9 ab	52.3 a	60.3 ab	63.7 b	2.17	$P < 0.05$

C control diet, *RL* control diet supplemented with 7.5% of rolled linseed, *EL* control diet supplemented with 7.5% extruded linseed, *LO* control diet supplemented with 4.9% linseed oil and 2.6% linseed meal

Means with different letters within a row are significantly different ($P < 0.05$)

Table 6 Intestinal digestibility of main 18:1 and 18:2 isomers (%)

	Diet				SEM	Statistical level
	C	RL	EL	LO		
18:1 isomers						
t4	80.2	77.6	82.3	83.6	2.02	NS
t5	90.8 b	85.0 a	89.5 b	91.6 b	1.20	$P < 0.05$
t6 + t7 + t8	89.3 b	83.6 a	85.5 a	89.1 b	0.85	$P < 0.01$
t9	89.1 b	84.2 a	84.1 a	88.2 b	1.08	$P < 0.05$
t10 + t11	84.2 bc	75.6 a	80.0 ab	87.9 c	1.76	$P < 0.01$
t12	86.4 ab	82.2 a	85.2 ab	88.4 b	0.81	$P < 0.01$
t13 + t14 + c6	88.5 b	82.5 a	84.6 ab	87.3 ab	1.14	$P < 0.05$
c9	70.8 ab	59.3 a	64.6 ab	74.4 b	3.03	$P < 0.05$
c10 + t15	83.3 b	75.7 a	79.7 ab	84.1b	0.92	$P < 0.01$
c11	75.7 ab	71.9 a	77.7 ab	80.6 b	1.00	$P < 0.01$
c12	88.3 a	90.1 ab	91.6 b	91.1 ab	0.45	$P < 0.01$
c13	96.3	91.9	95.2	94.4	1.73	NS
c14 + t16	86.2 b	73.8 a	77.8 ab	81.1 ab	2.28	$P < 0.05$
c15	86.5	85.4	87.6	89.1	1.16	NS
18:2 isomers						
t11, c15	74.0	65.3	78.4	92.1	6.65	NS
c9, cis12	73.4*b*	61.4a	78.1b	78.0b	3.53	$P < 0.05$

C control diet, *RL* control diet supplemented with 7.5% rolled linseed, *EL* control diet supplemented with 7.5% extruded linseed, *LO* control diet supplemented with 4.9% linseed oil and 2.6% linseed meal

Means with different letters within a row are significantly different ($P < 0.05$)

differences in temperature with processes used in other publications, but preconditioning before extrusion modifies biohydrogenation [14], due to changes in oil availability (Enjalbert, Chesneau, Troegeler-Meynadier, Nicot, unpublished data). Our finding is confirmed by a series of 6 feeding trials on bulls and steers receiving linseeds either rolled or extruded with the same process [5]: muscles contained more linolenic acid with EL than with RL. Similarly, Petit et al. [25] observed that extrusion of soybean and canola led to higher linoleic acid deposits in lamb

Table 7 Plasma content of main fatty acids (% total FA, except total FA in g/L)

	Diet				SEM	Statistical level
	C	RL	EL	LO		
Total FA (g/L)	1.114 a	1.640 b	1.614 b	1.599 b	0.127	$P < 0.10$
12:0	0.094	0.063	0.056	0.066	0.014	NS
Iso 14:0	0.086 b	0.038 a	0.036 a	0.070 ab	0.009	$P < 0.05$
14:0	0.570 b	0.364 a	0.373 a	0.373 a	0.011	$P < 0.01$
Iso 15:0	0.272 c	0.156 a	0.181 ab	0.206 b	0.011	$P < 0.05$
Anteiso 15:0	0.508 b	0.345 a	0.305 a	0.391 a	0.028	$P < 0.05$
15:0	0.639 b	0.454 a	0.441 a	0.478 a	0.022	$P < 0.01$
Iso 16:0	0.406 b	0.273 a	0.257 a	0.305 a	0.022	$P < 0.05$
16:0	13.962 b	11.951 a	11.585 a	11.648 a	0.182	$P < 0.01$
Iso 17:0	0.426	0.305	0.298	0.367	0.028	NS
17:0	0.972 b	0.718 a	0.732 a	0.762 a	0.029	$P < 0.01$
18:0	23.969	24.623	22.525	23.834	0.835	NS
Sum of *cis* 18:1	13.241	13.120	11.613	13.232	0.818	NS
Sum of *trans* 18:1	3.103 a	4.130 a	5.214 b	6.185 b	0.358	$P < 0.10$
Sum of non-conjugated 18:2	27.831	27.378	28.927	26.945	1.236	NS
Sum of CLA	0.300 ab	0.212 a	0.397 b	0.421 b	0.044	$P < 0.10$
18:3 n-3	0.364 b	0.212 ab	0.153 a	0.234 ab	0.047	$P < 0.10$
20:0	0.045	0.044	0.070	0.084	0.026	NS
22:0	0.026	0.022	0.000	0.054	0.020	NS
24:0	0.064	0.007	0.015	0.000	0.018	NS

C control diet, *RL* control diet supplemented with 7.5% rolled linseed, *EL* control diet supplemented with 7.5% extruded linseed, *LO* control diet supplemented with 4.9% linseed oil and 2.6% linseed meal

Means with different letters within a row are significantly different ($P < 0.05$)

Table 8 Plasma content of 18:1 isomers (% total FA)

	Diet				SEM	Statistical level
	C	RL	EL	LO		
t4	0.020	0.007	0.034	0.032	0.010	NS
t5	0.010	0.049	0.018	0.054	0.015	NS
t6 + t7 + t8	0.151 a	0.187 ab	0.206 ab	0.251 b	0.012	$P < 0.01$
t9	0.207	0.217	0.210	0.270	0.022	NS
t10	0.248	0.212	0.238	0.357	0.053	NS
t11	1.500 a	1.561 a	3.142 b	3.620 b	0.336	$P < 0.01$
t12	0.301 a	0.549 b	0.426 ab	0.460 ab	0.032	$P < 0.01$
t13 + t14 + c6	0.655 a	1.346 b	0.939 ab	1.140 b	0.018	$P < 0.05$
c9	11.487	10.737	9.798	11.184	0.764	NS
c10 + t15	0.122	0.155	0.187	0.173	0.036	NS
c11	0.672	0.502	0.500	0.501	0.047	NS
c12	0.538 a	1.006 b	0.637 a	0.762 ab	0.073	$P < 0.05$
c13	0.098	0.071	0.044	0.080	0.017	NS
c14 + t16	0.241 a	0.409 b	0.285 a	0.337 ab	0.027	$P < 0.05$
c15	0.084 a	0.249 b	0.161 ab	0.196 ab	0.027	$P < 0.05$

CL control diet, *RL* control diet supplemented with 7.5% rolled linseed, *EL* control diet supplemented with 7.5% extruded linseed, *LO* control diet supplemented with 4.9% linseed oil and 2.6% linseed meal

Means with different letters within a row are significantly different ($P < 0.05$)

muscle. These authors explained the protection of poly-unsaturated FA in extruded oilseeds by the decrease in protein degradability following extrusion, which would protect lipid droplets against hydrogenation. However, in the present experiment, proteins were not protected by extrusion [16]. In the present experiment, measurements of the FA composition of rumen fluid [26] confirmed the fact that lipids are rapidly released in rumen from extruded seeds, leading to a higher passage rate towards the duodenum. Under this hypothesis, a slower release of FA from rolled seeds may lead to a more complete BH, whereas a more rapid release of FA may result in a higher bypass if the rates of isomerization and hydrogenation is lower than the rate of lipolysis. This latter hypothesis is consistent

with in vitro and in vivo data [27, 28]. Isomerization and hydrogenation rates may be lowered by the toxic effect of the free polyunsaturated FA released in the rumen. The duodenal flows of most FA obtained with the EL and LO diets were very similar and not significantly different. The majority of comparisons between extruded and raw oilseeds have been made on dairy cows through comparison of milk FA profiles, giving highly inconsistent data, with some authors observing lower levels of unsaturated FA with extruded seeds compared to raw seeds (Chouinard et al. [29] on soybean) while other authors reported the opposite trend (McNamee et al. [30] on rapeseed, McGuffey and Schingoethe [31] on sunflower seed, Chouinard et al. [32] on soybeans). The effects of extrusion on 18:0 and 18:1 isomers are also very different among studies.

Intestinal Digestibility

Intestinal digestibility of total FA and differences between the main FA are consistent with the means of published data [22, 33, 34]. For 18:1 FA, *trans* isomers proved to be more digestible than *cis* isomers, which is consistent with recent data (e.g. Doreau et al. [35, 36], Loor et al. [11], review by Glasser et al. [34]). This is probably due to the stereochemical configuration of these FA. In the present experiment, oleic acid had a lower digestibility than other *cis* isomers. This has been observed only by Doreau et al. [35] with forage diets, but not by other authors with forage or mixed diets containing linseeds. A reason could be that duodenal oleic acid is still mainly dietary oleic acid and hence in the esterified form whereas the other *cis* isomers are mainly BH intermediates and hence in the unesterified form. This lower intestinal digestibility corresponds, both in Doreau et al. [35] and in the present experiment, to a high level of BH.

In the present experiment, digestibilities of oleic, linoleic and linolenic FA were always lower for the RL diet compared to the other diets. In contrast, Gonthier et al. [15] observed a lower digestibility of the control diet compared to linseed-supplemented diets, and no difference among linseed-supplemented diets. The digestibility of the control diet may differ according to the basal ration. The differences observed among the lipid-supplemented diets in the present study may be partly due to between-diet differences in the availability of intracellular oil. However, the low duodenal flow of linolenic acid ($\leq$5% of intake) means that at least 95% of dietary lipids were hydrolyzed and hydrogenated, and virtually all the FA were released from their original matrix. The absence of variation of digestibility with diet for palmitic acid is explained by its origin: palmitic acid is derived mainly from dietary compounds other than linseeds, or from microbial synthesis. We observed the same differences in digestibility between the 3 forms of linseeds (i.e. RL $\leq$ EL $\leq$ LO) for 18:1 and 18:2 isomers resulting from BH. These differences among diets can be explained by differences in FA metabolism in the large intestine and/or by differences in mechanisms of FA absorption in the small intestine. These hypotheses remain to be evaluated.

As numerical differences in digestibility were low compared to differences in duodenal flows between FA, the apparently absorbed amounts were highly correlated to duodenal flows. However, between-diet differences in duodenal flow and digestibility may be compensated for or increased when apparently absorbed amounts are considered. Absorbed amounts of linolenic acid were similar between RL, EL and LO diets (3.7, 5.6 and 4.9 g/day, respectively) whereas they were lower with RL than EL and LO for linoleic acid (4.8, 9.5 and 9.4 g/day) and oleic acid (6.8, 10.0 and 10.9 g/day), as well as for BH intermediates such as the coelution of t10 and t11-18:1 (19.1, 41.3 and 52.1 g/day) and t11, c15-18:2 (2.9, 9.8 and 13.0 g/day). These differences may result in differences in plasma and milk composition. In particular, the milk and meat contents of CLA derived from absorbed vaccenic acid may be higher when linseed is extruded or as oil than it is rolled.

Use of Plasma FA Composition as a Predictor of BH Pathways

Total plasma FA composition does not reflect FA absorption because it contains all lipoproteic fractions and is a criterion of global lipid metabolism, including lipid mobilization, organ uptake, hepatic lipid metabolism [37]. For example, plasma FA composition always shows high levels of linoleic acid whereas the absorbed amounts of this FA are very low. However, studies performed by Sinclair et al. [38] with sheep, Scislowski et al. [6] with steers and Loor et al. [23] with dairy cows have shown that diet supplementation with linseed oil results in an increased proportion of linolenic acid in plasma lipids. This was not observed in the present experiment, perhaps due to the very low increase in absorbed linolenic acid in lipid-supplemented diets compared to the control diet. We observed a global increase in total plasma FA content, mainly due to 18-carbon FA, whose proportions in total FA increased at the expense of 12- to 17-carbon FA. Non-conjugated 18:2 isomers (mainly linoleic acid) and linolenic acids were present in much higher proportions in plasma and were not linked to the absorbed amounts, reflecting an endogenous regulation of their plasma levels, independently of their dietary supply.

Similarly, there was a higher proportion of c9-18:1 in plasma FA than in duodenal flow, probably because of endogenous delta-9 desaturation of 18:0 in enterocytes and

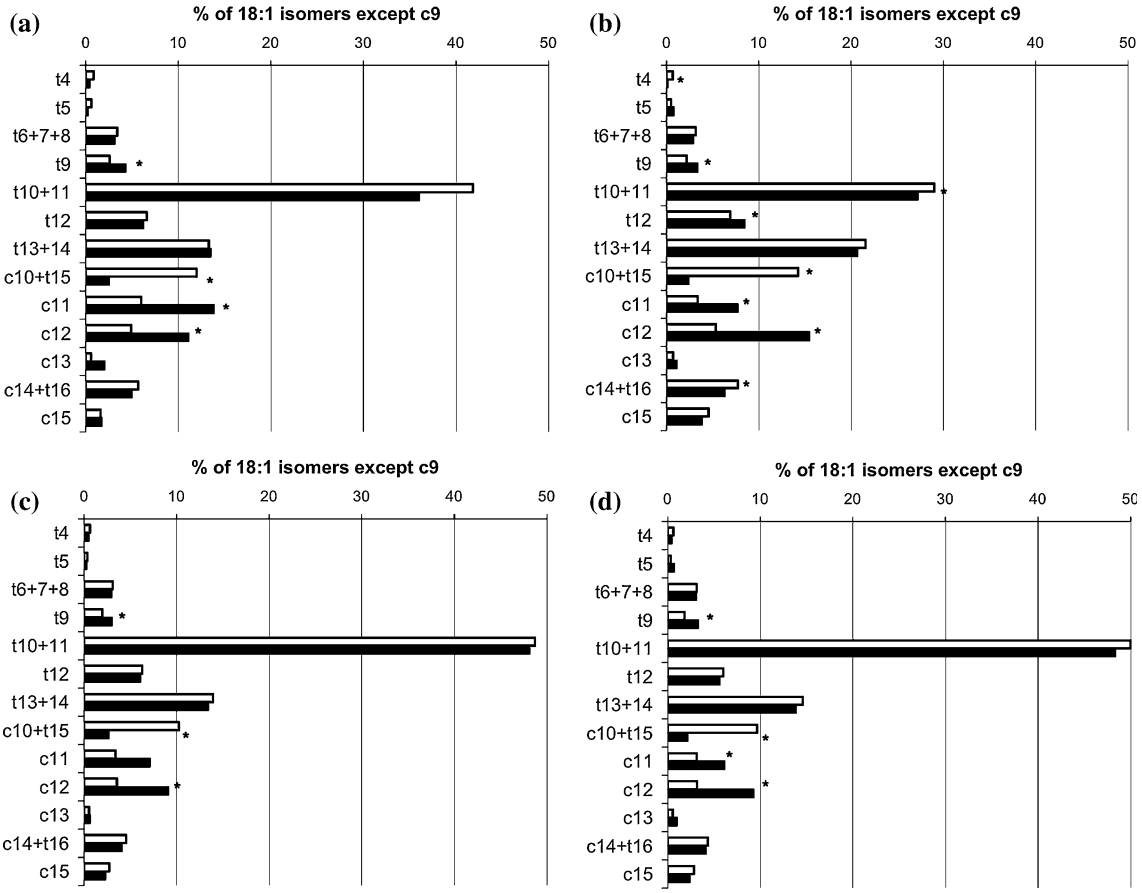

Fig. 1 Comparison of 18:1 isomers (expressed in % of total 18:1 isomers except c9-18:1) between duodenal fluid (*white bars*) and plasma (*black bars*) from cows fed a control diet (C, **a**, or a control diet supplemented with 7.5% rolled linseed (RL, **b**), 7.5% extruded linseed (EL, **c**) or 4.9% of linseed oil and 2.6% linseed meal (LO, **d**). *Asterisk* a significant difference between duodenum and plasma (Paired *T* test, $P < 0.05$)

other tissues. When 18:1 isomer proportions were compared between duodenum fluid and plasma (not taking into account c9-18:1 for the above-cited reason), *trans* 18:1 profiles in plasma closely reflected the respective profiles at the duodenum, which was also observed for some but not all *cis* 18:1 isomers, as shown by Fig. 1. Unfortunately, we did not determine the t10:t11 ratio due to coelutions in duodenal samples. This profile similarity has recently been observed in experiments on dairy cows receiving either two different diets supplied with linseed oil [3, 11] or receiving three different oils [4, 23]. However, in the latter experiments, the t10:t11 ratio in duodenal contents was imperfectly predicted by plasma composition. Additional experiments are required in order to propose prediction equations. The similarity of *trans* 18:1 profiles between duodenal and plasma FA means that these isomers have similar metabolisms (no difference in organ uptake or oxidation according to the position of the double bound).

In conclusion, linseed supplementation increased the duodenal flow of unsaturated intermediates of biohydrogenation, and this effect was more pronounced for extruded seeds and oil than for rolled seeds. Duodenal flow of linolenic acid was however similar among diets, due to its very high ruminal hydrogenation, which was not affected by linseed supplementation.

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