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Effects of Introducing Linseed in Livestock Diet on Blood Fatty Acid Composition of Consumers of Animal Products

Pierre Weill[a] Bernard Schmitt[b] Guillaume Chesneau[a] Norohanta Daniel[b] Faouzi Safraou[b] Philippe Legrand[c]

[a]Valorex, Combourtillé, [b]Centre d'Enseignement en Nutrition, Centre hospitalier de Bretagne sud, Lorient, et [c]Laboratoire de Biochimie ENSA/INRA, Rennes, France

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Linseed · α-Linolenic acid · Conjugated linoleic acid · Polyunsaturated fatty acids · Extrusion · Animal nutrition · Human nutrition · Animal lipids

Abstract
Reducing the ratio between essential fatty acids: C18:2 n–6/C18:3 n–3 down to 5 is recommended by Nutritional Guidelines. We studied the fatty acid (FA) changes in consumers' plasma following changes in livestock diet. First, a zootechnical study introduced 5% of extruded linseed into the diet of livestock to replace other oleaginous ingredients, and on an iso-nutritional values basis. The products from linseed-fed animals contained more n–3 fatty acids (precursor α-linolenic and derivatives obtained by elongations and desaturations) than control animal products (issued from animals fed without linseed), and more conjugated linoleic acids (CLA). The n–6/n–3 ratio was reduced by 54% in butter, 60% in meat and 86% in eggs. Following this, a double-blind, randomised, cross-over clinical study involving 75 healthy volunteers compared plasma and erythrocyte FA profiles in consumers of animal products (from livestock fed the linseed diet or from livestock fed standard diet). It showed modifications in the FA composition of the experimental human regimen with more C18:3 n–3 (1.65 vs. 0.75 g/day), and more n–3 derivatives. The C18:2 n–6/C18:3 n–3 ratio decreased (7 vs. 15). In volunteers' plasma, C18:3 n–3 increased in the essay group (0.93 vs. 0.44% of the FA), so did n–3 derivatives and CLA. The n–6/n–3 ratio decreased from 14.3 to 10.2. In erythrocytes, C20:5 n–3 increased in the essay group (0.59 vs. 0.45%) and so did C22:6 n–3. The n–6/n–3 ratio decreased in parallel from 4.2 to 3.8. Without any changes in consumers' eating habits, foodstuffs from animals fed linseed diets induced significant modifications of human plasma and erythrocyte fatty acid composition (comparable to that noted under the 'Cretan' diet) and a sharp increase in CLA.

Introduction

In terms of prevention, it is a well-known fact that nutrition in western countries is generally deficient in n–3 fatty acids, α-linolenic acid (ALA) C18:3 n–3 in particular, and proficient in n–6 fatty acids, C18:2 n–6 in particular. Indeed, results from epidemiological [1, 2], clinical [3] and interventional [4] studies have confirmed the increased mortality risk linked to cardiovascular disease (CVD) and cancer, related to such unbalanced dietary

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Dr. Philippe Legrand
ENSA/INRA. Laboratoire de Biochimie, 65, rue de Saint Brieuc
F–35042 Rennes Cedex (France)
Tel. +33 2 23 48 55 47, Fax +33 2 23 48 55 50
E-Mail Philippe.Legrand@agrorennes.educagri.fr

behaviour. Nutritional guidelines on lipid composition advocate reducing the C18:2 n–6/C18:3 n–3 ratio and increasing n–3 polyunsaturated fatty acid (PUFAs) intake [5]. In addition, recent studies have suggested a beneficial effect of dietary supplementation of certain conjugated linoleic acids (CLA) of dairy origin (rumenic acid C18:2 cis 9 trans 11 in particular) towards preventing breast cancer [6] and certain types of diabetes [7]. Various solutions have been proposed to increase dietary n–3 PUFA intake: consumption of fish and fish oil providing very-long-chain n–3 PUFA, consumption of C18:3 n–3 rich rapeseed oil or C18:3 n–3 rich vegetables. Among those plant sources, linseed holds a specific place. Extremely rich in C18:3 n–3, it was commonly used in Europe in the form of boiled ground seed added to livestock diet until the beginning of the 20th century [8, 9].

A number of studies have explored linseed or linseed oil supplementation to human regimens. But this involves a difficult technique due to the linseed oil peroxidation potential. Also, uncooked linseed contains cyanogenic compounds, which can be toxic to man at high concentration [10, 11]. Thus, a possible solution would be to introduce extruded (hence detoxified) linseed to the diet of animals destined for human consumption. Animal fats constitute about half of all human nutritional lipid intake in developed countries [12] and lipid profiles vary according to the diets given to the livestock [13]. Modern husbandry methods have profoundly modified livestock diet composition. ALA is a major component of growing plant membranes [14], especially grass and algae during the chlorophyll synthesis period (as in Cretan purslane). Replacing green forage by maize and soybean induced an increase in C18:2 n–6 (linoleic acid, LA) [15] in animal diets. So, by irreversibly withdrawing grass from livestock nutrition, modern farming has considerably reduced ALA availability in consumers' menus.

To restore sufficient ALA intake as a measure of preventing cardiovascular diseases and cancer, drastic changes in eating behaviour is sometimes needed. But major cultural resistance often confronts implementing these methods on a large scale and for sustained periods. Although it has been proved that the Cretan diet constitutes the best nutritional model for primary and secondary cardiovascular disease (CVD) prevention, it is nonetheless unlikely that such a model may be reproduced on a large scale in North European countries and in Anglo-Saxon countries, even if these have the highest prevalence of CVD.

This study tested the following dual hypotheses: (1) that an exclusive plant modification of livestock feeding by using linseed induces changes in the fatty acid contents of foodstuffs of animal origin by increasing n–3 (precursor and very-long-chain derivatives) fatty acids, and (2) that human consumption of those foodstuffs improves fatty acid composition in man and thus could constitute an effective measure of CVD prevention without the need to radically change previous nutritional habits.

Material and Methods

Livestock and Diets

The 'Linseed' (fed linseed) and 'Control' animals were of the same breed, same sex, same production levels and husbandry conditions. The animal diets presented in table 1 were identical in energy, nitrogen, minerals and vitamin contents for the 'Linseed' and 'Control' livestock. No anti-oxidant agent was added to the 'Linseed' diet. Also, all 'Linseed' or 'Control' diets were of plant origin, and any fat and protein of animal origin were excluded. The animal diet fatty acid composition and fat intakes are noted in table 2.

The linseed used in animal feed came from five C18:3 n–3 rich varieties (>230 g C18:3 n–3/kg dry matter – DM). It underwent a cooking-extrusion process meant to promote oil utilisation (free fat/total fat >75%), to inactivate lipases (peroxide index >20 mEq peroxide/kg DM after processing) and cyanogenic antinutritional factors (Linustatine, Neolinustatine, Linamarine) of the seeds (HCN <20 ppm/kg linseed). The oil content of extruded linseed was 413 g/kg DM. The FA composition of this oil was 57% C18:3 n–3, 16% C18:2 n–6, 18% C18:1 n–9 and 9% saturated fatty acids (SFA).

Volunteers

These were a population of healthy volunteers with normal lipid parameters, matched with regard to sex ratio (32 men and 43 women), age (34.6 ± 2 years, range 25–45), bodyweight and height (BMI = 25.4 ± 13), and plasma lipid values 21 days prior to the beginning of the experimentation (D-21) as follows: total cholesterol (5.1 ± 0.8 mmol/l), HDL-C (m = 1.75 ± 0.38 mmol/l), LDL-C (3.2 ± 0.77 mmol/l), triglycerides (0.88 ± 0.34 mmol/l, C18:3 n–3 (0.51% of total plasma FA) and CLA (0.32% of total plasma FA).

Protocol

A double-blind, randomized, cross-over trial involved 50 healthy volunteers, comparing two 35-day periods, with an 18-day 'wash-out' interval. During each period, volunteers were given food allocations, either from animals fed linseed (volunteers 'Essay' regimen), or from animals fed linseed-free diets (volunteers 'Control' regimen). These allocations constitute the total animal products intake.

A second group (so-called 'half-dose' group) of 25 volunteers was also conducted with a double-blind randomized cross-over protocol. These volunteers were also given food allocations either from animals fed linseed or linseed-free diets, but only for the morning and evening meals, the lunch meal being 'free' (out of experimentation) to represent the nutritional habits of people who work and cannot control the composition of their lunch. The aim of experimenting this 'half dose' group was to investigate any possibly dose-effect of the 'essay' regimens (red blood cell analysis was not conducted in this group).

Table 1. Composition of animal diets

Species	Diet	Amount of linseed g/animal/day	Extruded linseed % in ingested dry matter	Estimated daily intake g/animal/day	% Fat	% Protein
Dairy cow[1]	control	0		20,000	2.6	15.2
	linseed	1,000	5	20,000	4.5	15.2
Laying hens[2]	control	0		130	4.1	16.7
	linseed	13	10	130	6.5	16.9
Pig[3]	control	0	0	2,500	1.7	16.6
	linseed	62	2.5	2,500	2.2	16.7
Broiler chicken[4]	control	0	0	115	3.5	16.5
	linseed	4	3.5	115	4.2	16.5

[1] Composition of milking cows' diet is 60% corn silage, 10% grass silage, 3% hay, 17% soybean meal, and 10% ground wheat. 1 kg of extruded linseed was substituted for 0.8 kg of ground wheat and 0.2 kg of soybean meal.
[2] Composition of laying hen feed is 20% corn, 33.5% wheat, 7.5% wheat bran, 22% soybean meal, 5% sunflower meal, 1.5% rapeseed oil, 10.5% minerals and vitamins. 10% linseed was substituted for 6% corn, 1.5% soybean meal, 1.5% sunflower meal and 1% rapeseed oil.
[3] Composition of pig feed is 50% wheat, 10% barley, 9% wheat bran, 13.5% soybean meal, 12% pea, 0.5% rapeseed oil and 5% minerals, amino acids and vitamins. 2.5% linseed was substituted for 0.5% rapeseed oil and 2% pea.
[4] Composition of broiler feed is 35% corn, 34% wheat, 3% wheat bran, 21% soybean meal, 1.2% sunflower oil and 5.8% minerals, amino acids and vitamins. 3.5% linseed was substituted for 0.8% sunflower oil, 2% corn 0.7% soybean meal.

Table 2. Fat intake and fatty acid (FA) composition of animal diets

Species	Diet	Total fat intake g/animal/day	Total FA daily intake g/animal/day	C18:3 n–3 % of total FA	C18:2 n–6 % of total FA	C18:1 n–9 % of total FA	Saturated fatty acids % of total FA	C18:2 n–6/ C18:3 n–3
Dairy cow	control	520	364	5	65	14	15	13
	linseed	908	758	35	42	11	11	1.2
Laying hens	control	5.3	4.8	5	55	25	15	11
	linseed	8.5	7.6	45	45	5	5	1
Pig	control	42.5	38	7	50	26	17	7.1
	linseed	55	50	30	50	12	8	1.7
Chicken	control	4	3.6	10	60	16	14	5
	linseed	4.8	4.4	27	45	16	12	1.7

Strict recommendations were given regarding, in particular, the need for identical eating habits over both periods with a prohibition to consume any animal fat other than that provided for the experiment. Olive oil was the exclusive oil used either for seasoning or cooking, regardless of group ('Essay' and 'Control'). An overall dietary balance was observed equal to 35% of total energy supply as lipids. Volunteers were asked to accurately record their consumption for 2 weeks during each period. Five energy level groups were formed, corresponding to daily consumption levels from 1,400 to 2,600 kcal (5,859–10,881 kJ). Guidelines were given on the weekly consumption of eggs, butter and meat by each energy level group. The mean energy intake level was 2,020 kcal (8,454 kJ) per person per day, including 702 kcal (2,938 kJ) as lipids. Animal fat represented a mean daily energy supply of 485 kcal (2,030 kJ). Table 3 illustrates the mean daily consumption by volunteers.

Measurements and Titrations

Analytical Methods. Free fat in linseed was measured as follows: A 10-gram homogeneous sample of feed ground on a 3-mm grid was mixed with paraffin ether in three steps, with interspersed 5-min agitation and followed by filtration through an unpleated, ash-free and grease-free paper filter approximately 110 mm in diameter. The sol-

Table 3. Daily food intake by volunteers (g/volunteer/day)

	'Full-dose' group food intake	'Full-dose' group fat intake	'Half-dose' group food intake	'Half-dose' group fat intake
Butter	38	32	24	20
Milk	100	3.8	100	3.8
Total dairy products	138	35.8	124	23.8
Eggs	99	10	51	5.1
Meat and ham	43	1.1	22	22
Processed pork	28	5.6	14	14
Total pork	71	6.7	36	3.3
Chicken	33	1.3	16	0.6
Beef	6	0.1	3	0.1
Lamb	4	0.2	2	0.1
Total animal products	351	54	232	33
Olive oil	19	18	nd	nd
Other plant oils	3	3	nd	nd
Vegetables	300	2	nd	nd
Fruit	150		nd	nd
Starchy foods	400	2	nd	nd

For the 'full-dose' groups, animal products were exclusively provided by the experimental protocol, the average daily nutrient intake was 79 g fat, 93 g protein, 1 g mineral, and 0.7 g cholesterol and the average daily energy intake was 2,020 kcal.

For the 'half-dose' groups, the values represent only the controlled experimental part of the daily intake and do not represent the total daily intake, since the lunch was 'free', i.e. out of experimentation (see 'Materials and Methods').

vent was eliminated by distillation (BBS extractor). The residue was heated in an oven (103 ± 2°C) for at least 1 h. Fatty acid composition was performed as follows: hexane-extracted fat, following chlorhydric acid (3 mol/l) hydrolysis, according to European directive 98/64/CEE, was then derived with 0.5 mol/l NaOH, 12–15% bore trifluoride, according to French standard NF T-60 233. Gazeous phase chromatography was performed according to the above-described technique (except for the injection of 1 µl in 1/5th leakage mode). For identification of trans isomer of C18:1 and C18:2, we used a special column (100m-CP Sil88- and 120m -BPx70-). Heteroside hydrolysis with 10 ml sodium acetate (20 g/l solution) distillation was carried out in the presence of potassium iodide (50 g/l solution) by steam driving the cyanhydric acid, liberated by hydrolysis. The amount of acid thus obtained was directly titrated using a silver nitrate solution (0.02 *N* solution) in ammonia medium and in the presence of potassium iodide (Normes NF V03-772). The peroxide index was obtained by processing a test solution specimen in iso-octane acetic acid solution (3:2) with a potassium iodide solution. Liberated iodine titration was achieved through titrated 0.01 mol/l sodium thiosulfate solution (ISO 3960).

Blood Tests. Blood lipids were assayed on D –21, D +15, D +35, D +53, D +88, and D +102, including cholesterol (total, HDL and LDL) and triglycerides (Biotrol enzyme assay, Chennevières, France). Red blood cells (RBC) and plasma were separated by centrifugation after each blood sampling. Plasma and cell fatty acids were extracted by a method derived from French standard NF T60-233, 1977. It uses a 2-ml mixture of chlorhydric acid (3 mol/l) solution with an 8-ml isopropanol/hexane mixture (2:3). After evaporation, the fat extracted was methylated in the presence of 4 ml of a 0.5-mol/l methanolic solution of sodium hydroxide brought to the boil for 10 min, then by 5 ml of a 15% methanolic solution of bore trifluoride boiled for 2 min. Final extraction was performed with 2 ml heptane. The methylated solution was analysed on a Hewlett Packard 5890 gazeous phase chromatograph on a HP-FFAP column (30 m × 0.53 mm × 1 µm). A 2-µl volume was injected. Injector and detector temperature was 230°C. Oven temperature was programmed at 180–230°C in 5°C/min increments.

Animal Product Acceptability Assessment. Hedonic tests involving a jury of 50 independent consumers (Laboratoire Earning, Alfortville, France) for each product pair ('Essay' and 'Control') were carried out on the 11 products provided to the volunteers (3 batches of butter, 1 batch of eggs, 7 batches of meat). To appreciate the softness of butter, we measured the quantity of liquid content at 10°C in a so-called 'differential thermic analysis' (DTA).

Statistical Analysis

Statistical analysis of paired samples was carried out using Student's t test for normal data and Wilcoxon's test for nonparametric data (STATA 6.0 software).

Table 4. Animal product evaluation

Product	Diet	Preference[1]	Color[2]	Taste[2]	Flavor[2]	Softness[2]	Juiciness[2]
Lamb	control	24	6.4	6.6	6.9	7.1	6.5
	linseed	30	6.6	7.0	7.2	7.4	6.7
Beef A[3]	control	29	6.7	6.9	6.8	6.2	6.1
	linseed	22	7.0	7.2	6.6	5.7	6.5
Beef B[3]	control	19	7.0	7.2	6.9	6.5	7.0
	linseed	34	7.1	7.4	7.4	7.3	7.4
Pork	control	34*	6.3	6.6	6.3	5.9	5.0
	linseed	20	6.8	6.9	6.2	6.3	5.5
Broiler A[3]	control	21	6.1	5.8	6.1		6.0
	linseed	30	6.3	6.3	6.3		6.0
Broiler B[3]	control	25	5.8	5.9	5.9		5.6
	linseed	27	6.0	5.9	5.9		6.1
Ham	control	14	5.3	5.8		5.9	
	linseed	37*	7.3*	7.0*		7.9*	
Egg	control	16					
	linseed	38*					
Butter A[3]	control	20	7.2	6.2	6.8	7.4	
	linseed	31	7.1	6.1	7.1	7.5	
Butter B[3]	control	28	6.3	5.9	7.1	7.1	
	linseed	26	6.3	6.1	6.9	8.4*	
Butter C[3]	control	26	6.9	6.8	6.7		
	linseed	24	7.0	6.9	7.1		
All products	control	256					
	linseed	319*					

* $p < 0.05$.
1 Number of preference answers.
2 Notation on a 0 to 10 scale.
3 A, B, C are repetitions for different periods of production and consumption.

Results

Effect of the 'Extruded Linseed' Diet on Quality of Animal Products (Fat Content, Rheological and Sensitive Parameters)

The animal diets were iso-nitrogenous and iso-energetic, but not iso-fat: The 'Linseed' diets contained more fat, as shown in table 1. This difference did not appear in animal products (data not shown).

In our experiment, the use of extruded linseed (5% of DM composition of cows' diets) decreased the fat percentage in milk (the daily milk production was higher) but not the daily fat production (data not shown). For the other species, there was a little, nonsignificant decrease in fat content: Eggs exhibited a nonsignificant decrease in fat content balanced by a nonsignificant increase in egg weight, and there were no differences in meat, except a decrease in nonconsumed abdominal fat of broiler chicken (data not shown).

The softness of butter originated from linseed-fed animals increased by 36% (from 17% of liquid phase at 10°C in control butter to 23% in butter from linseed-fed animals). The hedonic test (preference questionnaire) was in favor of the linseed-fed animal products ($p < 0.05$) and the sensitive analysis elicited a repeated but nonsignificant preference for the linseed-fed animal products as shown in table 4.

Effect of the 'Extruded Linseed' Diet on the Fatty Acid Composition of Animal Products

Table 5 represents the fatty acid composition of food products originating either from animals fed linseed diets or from those fed control diets. These results show that linseed introduction very markedly modified the fatty acid profile of animal products. Beginning with saturated fatty acids, proportion of C16:0 decreased whereas C18:0 increased. Among the monoenes, C18:1 decreased in all products, except milk. Regarding PUFAs, these results

Table 5. Fatty acid composition of animal products (% of total FA)

	C16:0	C18:0	C18:1	C18:2 n–6	CLA	C18:3 n–3	C20:4 n–6	C20:5 n–3 + C22:6 n–3	C18:2 n–6/ C18:3 n–3	n–6/ n–3
Milk										
Control	34	9	20	2	0.5	0.3	0.2	0.1	6	7
Linseed diet	26	11	26	3	1.1	0.9	0.2	0.1	3	3
Egg										
Control	23	8	42	19		0.6	1.8	0.8	30	15
Linseed diet	21	9	39	17		6.6	1.1	1.6	3	2
Pork										
Control	23	13	44	11		1.1	1.3	0.2	10	7
Linseed diet	22	14	42	11		2.9	1	0.4	4	3
Chicken										
Control	22	7	41	17		1.4	1.9	0.2	12	10
Linseed diet	18	7	39	15		6	1.6	0.4	3	3

The composition of animal diets are presented in table 1.
CLA = Conjugated linoleic acids (96% of CLA is rumenic acid C18:2 cis9 trans11).

Table 6. Fatty acid composition (g/volunteer/day) of volunteers' regimen ('full-dose' groups)

Fatty acid	Control	Essay	
C16:0	15.8 ± 3.6	12.9 ± 3.1	**
C18:0	5.3 ± 1.1	6.1 ± 1.4	*
C18:1 n–9	26.3 ± 2.9	26.9 ± 3.2	
C18:1 trans11	0.39 ± 0.08	1.3 ± 0.3	**
C18:2 n–6	11.2 ± 0.7	11.2 ± 0.8	
C18:2 cis9 trans11	0.17 ± 0.05	0.37 ± 0.1	**
C18:3 n–3	0.75 ± 0.05	1.65 ± 0.29	**
C20:4 n–6	0.28 ± 0.07	0.20 ± 0.05	**
Long-chain (≥C20) n–3 PUFA	0.11 ± 0.03	0.25 ± 0.07	**
Σ n–3	0.86 ± 0.08	1.91 ± 0.36	**
Σ n–6	11.6 ± 0.8	11.3 ± 0.9	
C18:2 n–6/C18:3 n–3	14.9 ± 3.3	6.8 ± 1.2	**
Σ n–6/Σ n–3	13.5 ± 2.8	5.9 ± 1.1	**

Values are means ± SD.
Significant differences: * $p < 0.05$; ** $p < 0.01$.
The compositions of animal diets are presented in table 1.

revealed several effects: the C18:2 n–6/C18:3 n–3 ratio as well as the n–6 FA/n–3 FA ratio were significantly reduced; C18:3 n–3 proportion increased in all products, n–3 FA derivatives increased in all animal products whereas arachidonic acid C20:4 n–6 decreased. Lastly, ruminant lipids (milk and meats) from linseed-fed animals contained more CLA (essentially in the form of rumenic acid: C18:2 cis9 trans11).

Effects of Using Extruded Linseed in Livestock on the Fatty Acid Composition of the 'Essay' and 'Control' Regimens Eaten by the Volunteers

The difference between 'Essay' and 'Control' regimens was the fatty acid composition of animal products, inducing marked differences in the fatty acid composition of the regimens ingested by the volunteers. Results are presented in table 6. The proposed regimens, representative of eating traditions of Northern France, are rich in saturated fatty acids (SFAs). Regarding PUFAs, it appears that the amount of C18:3 n–3 increased from 750 mg/day in the 'control' regimen (0.33% of energy supply) to 1,650 mg/day in the 'full-dose essay' regimen (0.73% of energy supply). Furthermore, the C18:2 n–6/C18:3 n–3 ratio was reduced from 14.9 to 6.8, close to the commonly accepted recommendations. n–3 FA proportion increased (+119% for ALA and +141% for n–3 derivatives) in the 'full-dose essay' group as compared to the control group.

Among the SFAs, palmitic acid (C16:0) consumption decreased from 15.8 g/day in the 'control' regimen to 12.9 g/day in the 'full-dose essay' regimen whereas stearic acid C18:0 increased from 5.3 to 6.1 g/day. Lastly, rumenic acid (C18:2 cis9 trans11) and its parent transvaccenic acid (C18:1 trans11) increased by +114 and +233%, respectively, in the 'full-dose essay' group.

Effects of Consuming Animal Foodstuffs, Originating from Livestock Fed Extruded Linseed, on Plasma Fatty Acid Composition in Volunteers of 'Full-Dose Group'

The composition of plasma FA in volunteers after 'full-dose essay' and 'control' regimens is presented in table 7. These results show that introducing C18:3 n–3-rich extruded linseed in livestock feeding markedly modified the plasma FA composition of human consumers. C18:3 n–3 significantly increased from 0.44 to 0.93% (+111%) ($p < 0.001$) in the plasma. In the 'full dose essay' group, C16:0 and C20:4 n–6 decreased significantly while C18:2 cis9 trans11, C20:5 n–3, C22:5 n–3 and C22:6 n–3 increased also significantly. In contrast, there was no difference in C18:1 trans11 level, despite its high increase in the 'essay' regimen content.

Furthermore, there was a significant reduction of the C18:2 n–6/C18:3 n–3 ratio (–52%) and in the n–6/n–3 ratio (–28%). These changes in serum FA composition appeared very early following the initiation of the regimen (after 15 days of regimen, data not shown).

Table 7. Fatty acid composition (% total FA) of volunteers' serum lipids ('full-dose' groups)

Fatty acid	Control regimen	Essay regimen	
C16:0	19.5 ± 1.8	18.5 ± 1.7	*
C18:0	5.9 ± 0.9	5.7 ± 0.8	
C18:1 n–9	22.6 ± 2	23.3 ± 2.4	
C18:1 trans11	1.71 ± 0.2	1.78 ± 0.2	
C18:2 n–6	31.3 ± 3.6	31.6 ± 4.2	
C18:2 cis9 trans11	0.28 ± 0.07	0.42 ± 0.13	**
C18:3 n–3	0.44 ± 0.11	0.93 ± 0.30	**
C20:4 n–6	7.7 ± 1.1	6.6 ± 1.1	
C20:5 n–3	0.52 ± 0.22	0.80 ± 0.17	**
C22:5 n–3	0.35 ± 0.08	0.45 ± 0.10	**
C22:6 n–3	1.51 ± 0.24	1.67 ± 0.29	*
Σ n–6	41.3 ± 3.9	40.2 ± 4.5	
Σ n–3	2.88 ± 0.43	3.93 ± 0.41	**
C18:2 n–6/C18:3 n–3	71 ± 23	34 ± 12	**
Σ n–6/Σ n–3	14.3 ± 2.50	10.2 ± 1.8	**

Values are means ± SD.
Significant differences: * $p < 0.05$; ** $p < 0.01$.
The compositions of animal diets are presented in table 1.

Effects of Consuming Animal Foodstuffs, Originating from Livestock Fed Extruded Linseed, on Plasma Fatty Acid Composition in Volunteers of 'Half-Dose Group'

Even in the 'half-dose essay group' (where the lunch meal was 'free', i.e. out of experimentation), the same trends were observed: the composition of plasma FA in 'half-dose group' volunteers after essay and control regimens is presented in table 8. The C18:3 n–3 and C20:5 n–3 levels in serum of 'half-dose essay' group significantly increased (+84 and +38% respectively, $p < 0.01$). As compared to the control groups, the increase of CLA C18:2 cis9 trans11 and C18:1 n–9 levels are higher in 'half-dose essay' (table 8) than in 'full-dose essay' (table 7) groups; respectively +57% ($p < 0.01$) and +50% ($p < 0.01$) for CLA, +7% ($p < 0.01$) and +3% (NS) for C18:1 n–9. We also observed a decrease in C18:0 ($p < 0.01$) in the 'half-dose essay' group only. Finally, the n–6/n–3 ratios significantly decreased (–45% and –22% for precursor and precursors plus derivatives, respectively, in this 'half-dose essay' group as compared to the control.

Effects of Consuming Animal Foodstuffs, Originating from Livestock Fed Extruded Linseed, on Red Blood Cell Fatty Acid Composition in Volunteers

Red blood cell fatty acid composition is interesting to study because those fatty acids are mainly contained in membrane phospholipids. The evolution of that FA composition therefore reflects the structural changes induced in the cells by the diet and not the modifications of dietary plasma fatty acid fluxes. These results presented in table 9 demonstrate that after 35 days of 'essay' regimen, red blood cell fatty acid profiles were significantly modified, as follows: there was an increase in C20:5 n–3 (+32%, $p < 0.001$), in C22:5 n–3 (+10%, $p < 0.05$) and an increasing trend in C22:6 n–3 (+7%, $p = 0.06$). The n–6/n–3 FA ratio was reduced by 12% ($p < 0.001$) in relation with the increase in n–3 FA. In contrast, arachidonic acid (C20:4 n–6) proportion remained unchanged, conversely to what was noted in plasma. Concerning CLA (C18:2 cis9 trans11), the observed increase was not significant. Lastly, C16:0 decreased (–3%, $p < 0.01$) but not as much as described in plasma (–5%).

Effects of Consuming Animal Foodstuffs, Originating from Livestock Fed Extruded Linseed, on Circulating Cholesterol and Triglycerides

This study revealed that circulating cholesterol (data not shown) was not modified by the diet. As to circulating triglycerides (data not shown), no effect was observed despite the increase in n–3 fatty acids in the essay group regimen.

Table 8. Fatty acid composition (% total FA) of volunteers' serum lipids ('half-dose groups')

Fatty acid	Control regimen	Essay regimen	
C16:0	19.1 ± 2.1	18.3 ± 1.7	*
C18:0	6.0 ± 0.7	5.4 ± 0.8	**
C18:1 n–9	21.9 ± 2.2	23.4 ± 2.7	*
C18:1 trans11	1.6 ± 0.1	1.6 ± 0.4	
C18:2 n–6	31.7 ± 1.4	31.7 ± 3.8	
C18:2 cis9 trans11	0.26 ± 0.07	0.41 ± 0.1	**
C18:3 n–3	0.49 ± 0.14	0.90 ± 0.23	**
C20:4 n–6	7.1 ± 1.0	6.6 ± 1.2	
C20:5 n–3	0.7 ± 0.26	0.97 ± 0.28	**
C22:5 n–3	0.41 ± 0.10	0.43 ± 0.09	
C22:6 n–3	1.68 ± 0.36	1.77 ± 0.33	
Σ n–6	41.9 ± 4.3	41.0 ± 4.2	
Σ n–3	3.35 ± 0.63	4.14 ± 0.48	**
C18:2 n–6/C18:3 n–3	72 ± 20	39 ± 13	**
Σ n–6/Σ n–3	12.9 ± 2.7	10.0 ± 1.4	**

Values are means ± SD.
Significant differences: * $p < 0.05$; ** $p < 0.01$.
The compositions of animal diets are presented in table 1.

Table 9. Fatty acid composition (% total FA) of volunteers' red blood cells ('full-dose' groups)

Fatty acid	Control regimen	Essay regimen	
C16:0	18.6 ± 1.2	17.9 ± 1.0	**
C18:0	21.8 ± 2.0	21.9 ± 1.8	
C18:1 n–9	15.9 ± 1.5	15.7 ± 1.0	
C18:2 n–6	10.2 ± 2.5	10.2 ± 2.5	
C18:2 cis9 trans11	0.40 ± 0.11	0.50 ± 0.12	
C18:3 n–3	0.15 ± 0.05	0.24 ± 0.10	**
C20:4 n–6	15.8 ± 1.8	15.5 ± 1.5	
C20:5 n–3	0.45 ± 0.12	0.59 ± 0.11	**
C22:5 n–3	2.04 ± 0.39	2.24 ± 0.28	*
C22:6 n–3	4.77 ± 0.89	5.12 ± 0.72	
Σ n–6	31.4 ± 2.1	30.9 ± 1.5	
Σ n–3	7.4 ± 1.2	8.2 ± 0.9	**
C18:2 n–6/C18:3 n–3	68.0 ± 12.1	42.5 ± 9.9	**
Σ (n–6)/Σ (n–3)	4.2 ± 0.69	3.8 ± 0.49	**

Values are means ± SD.
Significant differences: * $p < 0.05$; ** $p < 0.01$.
The compositions of animal diets are presented in table 1.

Discussion

The α-linolenic intake (ALA) intake in western countries should be at least doubled to reach an acceptable level for C18:2 n–6/C18:3 n–3 ratio [5]. Recent reports indicate that the major part of the human α-linolenic acid intake comes from animal sources [16]. Even in a population of women in southern France, animal sources of ALA (mainly from dairy products) represent 75% of the total ALA intake [17]. So, it appeared interesting to check the hypothesis that a higher ALA intake, and a better n–6/n–3 ratio, could be obtained by increasing the ALA part in animal lipids. So we have described the effects of linseed use as a source of ALA, not directly in human regimens, but in animal diets.

A lot of experiments relate the effects of polyunsaturated fatty acids (PUFAs) introduced in animal diets on quality of animal products. Regarding dairy production, use of PUFAs is often reported to improve softness of butter and cheese [18, 19] but also to decrease the fat production (so called 'low fat syndrome') [20] and to increase the trans FA content in milk [21]. The most important cause of this decrease in milk fat production is probably the ruminal synthesis of C18:1 trans10 (C18:1 trans10 production is important in rumen acidosis conditions [21]) which affects the de novo lipid synthesis in the mammary gland. However, ALA supplementation is reported to increase the C18:1 trans11 production [21] (this fatty acid has no effect on de novo lipid synthesis in the mammary gland), as checked in our experiment, where the C18:1 trans11 represented more than 90% of the total C18:1 trans isomers (data not shown).

Concerning now monogastric animals, we observed nonsignificant but repeated decreases in fat content of animal products, probably because high amounts of ALA are reported to inhibit lipogenesis [22, 23].

Use of PUFAs is also reported to decrease sensitive and rheological quality of meats and eggs with development of off-flavors that often affect taste [24, 25] (so-called 'fish taint' due to fat peroxidation). These negative effects are also observed with linseed introduction, in the form of crude or ground seed, or in the form of linseed oil [26–28]. With a small amount of extruded linseed (extrusion inhibits lipase activity and leads to a slow oil liberation as presented in Materials and Methods) in animals' diets, we did not observe any deterioration in animal product quality as could be expected from above mentioned experiments using other forms of linseed.

The lipids originating from linseed-fed animals are interesting sources of ALA but also of n–3 derivatives (especially in meat and in eggs) due to the elongation desaturation occurring mainly in liver of animals. Some differ-

ences appeared also for saturated fatty acids (SFAs) content of animal products: less palmitic acid and more stearic acid in all the linsed-fed animal products. The decrease in palmitic acid content is probably due to a reduction of FA synthesis in the presence of higher ALA intake [29]. The increase in stearic acid is probably due to a reduction of its Δ9 desaturation in the same conditions [30]. Regarding the monoenes, we observed less C18:1 n–9 (this confirms the Δ9 desaturation reduction hypothesis) in all the linseed-fed animal products, except in milk (in dairy cows, Δ9 desaturation occurs in the mammary gland after an intensive PUFA hydrogenation in the rumen that produces stearic acid [31]). Lastly, the linseed-fed ruminant products were richer in C18:1 trans11 (vaccenic acid) and in CLA cis9 trans11 (rumenic acid), two FA produced from ALA after the isomerisation-hydrogenation occurring in the rumen [32].

Thus, introducing extruded linseed at indicated levels in animal diets increased the ALA content of all animal products. Consequently, the ALA intake in man is approximately doubled in 'essay' regimens without any change in consumers' habits. This enrichment is obtained without any measured negative side effect on product quality, and the FA balance of animal lipids seems better.

However, the major interest of our study was to reveal the high physiological relevance of this enrichment in volunteers' blood FA composition. The sera levels of ALA (respectively, 0.93 and 0.90% of the total FA in 'full-dose essay' and 'half-dose essay' groups) are very high in comparison with other studies at same ALA ingestion level but from plant oil origin (rapeseed [4], or linseed oil [33]). Similarly, the increases in blood red cell levels in C20:5 n–3, C22:5 n–3, C22:6 n–3 (mainly in phospholipids) are markedly higher than those observed after introduction of ALA from plant oils even at high doses in experimental human regimens [34, 35].

There are no definite well-known reasons to explain this high availability of animal sources of n–3 FA. Different and probably additive reasons can be proposed: (i) the origin and composition of n–3 FA (coming from various products) provide all the n–3 FA together and at the different meals; (ii) the relatively low amount of n–3 FA avoid their use as energetic substrate, whereas SFAs are a preferential source for β-oxydation; (iii) the phospholipids constitute an important part of animal lipids (mostly in eggs and meat) and when present in the regimen, they ensure an efficient supply form of very-long-chain FA (n–3 series in the present study).

It is worth underlining the significant increase in long-chain n–3 PUFAs in consumers' sera. This strongly suggests that a significant α-linolenic acid desaturation and elongation process occurs in animals, and probably in humans, promoting human supply of the whole n–3 group of FA, precursor or derivatives alike. Thus very-long-chain n–3 derivative synthesis was induced by the action of Δ6 and Δ5 desaturases, to the detriment of arachidonic acid synthesis, which was significantly reduced in serum by the essay regimen whereas linoleic acid proportion remained unchanged. This observation is consistent with the competition between linoleic and α-linolenic acids for the Δ6 desaturase [5].

In human sera from the 'essay' groups, it is interesting to notice that the increase in n–3 FA (including n–3 long-chain FA) is accompanied by a decrease in n–6 long-chain FA (especially C20:4 n–6). This decrease (observed here also in animals) contributes to the n–6-/n–3 ratio improvement. It is also interesting to observe that the sera modifications are not limited to the PUFAs. In 'essay' groups, we can observe a decrease in C16:0 ($p < 0.05$), and an increase in CLA cis9 trans11 ($p < 0.01$). In addition, we observed that the C18:1 trans11 sera level is not different in 'essay' and 'control' groups despite its higher intake in 'essay' groups.

It is not surprising that no changes were observed for the circulating cholesterol, probably because the amount of linoleic acid (known to reduce cholesterolemia) [36] was identical in both regimens (essay and control). In addition, no change was observed concerning triglyceride levels, which could be explained by (i) the short duration of the study; (ii) the moderate n–3 FA intake, including mainly ALA, and only few long-chain n–3 FA (known to reduce circulating triglycerides) [37], and (iii) the fact that volunteers were not hypertriglyceridemic.

The positive relationship between the ALA level in serum and the CVD prevention is strongly suggested in the literature [4, 5–38]. From these reports, the nutritional guidelines recommend to increase the ALA intake and to decrease the n–6/n–3 ratio in our regimens, as advice for CVD prevention. The higher ALA intake recommendation is mainly based on a better choice of oil, and on changes in food habits. But, in fact, ALA-rich oils (like rapeseed) cannot be used for cooking and changes in food habits occur very slowly [39]. In addition to these recommendations, the introduction of extruded linseed in animal diets can provide a new and complementary way of ALA enrichment in human regimens. This n–3 FA enrichment procedure appears to be highly relevant, even at low doses, and seems compatible with nutritional recommendations, especially for consumers who usually eat animal fats.

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