Chemical and nutritional studies of flaxseed (variety Linott) in rats

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*The nutritional effects of flaxseed (*Linum usitatissimum, *variety Linott) were studied in the rat. In addition, thermal and storage stabilities of flaxseed were evaluated. Weanling rats were fed diets containing ground flaxseed at levels of 0, 10, 20, or 40% for 90 days. No differences were found in the food intake nor in body and organ weights. Serum triglyceride, total cholesterol, and the low-density lipoprotein (LDL) cholesterol concentrations were significantly lower in the rats fed the 20% and 40% flaxseed diets compared to the 0% flaxseed group. The high-density lipoprotein (HDL) cholesterol concentration and the LDL:HDL–cholesterol ratio were generally lower in the flaxseed-fed rats when compared to the 0% flaxseed group, but a significant lowering occurred only in the 40% flaxseed group. The incorporation of flaxseed in the diet caused significant elevations in the levels of the α-linolenic acid in adipose tissue and in organs. Higher amounts of eicosapentaenoic, docosapentaenoic, and docosahexaenoic acids were observed in the heart and liver of flaxseed-fed rats when compared to the 0% flaxseed group. A significant lowering of tissue vitamin E levels and an elevation of urinary thiobarbituric reacting substances occurred only in the 40% flaxseed group, which suggested that low to moderate intakes of flaxseed did not impart an oxidative stress on rat tissues. Dietary fiber in flaxseed appeared to be largely fermentable and was associated with a large increase in fecal moisture. The phytate in flaxseed had no effect on zinc status. The oil in both the intact and the ground flaxseeds was found to be thermally and oxidatively stable.*

Keywords: flax; flaxseed; flaxseed fiber; α-linolenic acid; n-3 fatty acids; phytic acid

Introduction

The flaxseed (*Linum usitatissimum*) is one of the richest sources of α-linolenic acid (18:3n-3). Flaxseed is mainly used for the production of industrial linseed oil and is not widely recognized as an edible grain. Nevertheless, it has a history of use in Europe. In North America, flaxseed at low levels has been accepted as a component in some brands of cereal, in specialty breads, and as a seed dressing on buns and various other bakery products. The promotion of flaxseed as a dietary source of 18:3n-3 could lead to increased incorporation of flaxseed in a variety of food products.[1]

Flaxseed may contain some anti-nutritional factors, particularly cyanogenic glycosides.[2] The well-known toxicity of cyanogenic glycosides is due to their hydrolysis by β-glycosidase to hydrogen cyanide (HCN), which is a potent respiratory inhibitor. Flaxseed is reported to contain a vitamin B_6 antagonist, namely 1-[(N-γ-L-glutamyl)-amino]-D-proline (linatine) and its presence has limited the use of flaxseed meal in poultry feeds.[3] Flaxseed also contains phytic acid which, with phytate:zinc molar ratios in excess of 12 when dietary calcium was 0.75%, or with ratios in excess of six when calcium was 1.75%, was shown to inhibit growth and decrease bone zinc levels in rats.[4]

Linseed oil, the oil of the flaxseed, has been studied in both animals and humans.[5-10] However, studies on the nutritional effects of flaxseed in humans or animals are scarce. A recent paper[11] reported that pigs fed a diet supplemented with 5% flaxseed for 8 weeks had increased total n-3 fatty acids in all organs. Another recent report indicated that flaxseed is hypocholesterolemic in rats.[12] The purpose of the present study was to examine in rats the nutritional effects of the intake of diets supplemented with high levels of flaxseed. In this context, the influence of the flaxseed diet on tissue

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Received April 10, 1991; accepted October 23, 1991.

Table 1 Fatty acid composition of flaxseed and the diets

Fatty acid	% Total fatty acids				
	Flaxseed	0% FS	10% FS	20% FS	40% FS
12:0	—	0.2	0.2	0.2	0.2
14:0	TR	0.7	0.7	0.7	0.7
16:0	7.5	18.5	16.7	15.5	13.5
17:0	—	0.2	0.2	0.2	0.2
18:0	2.5	5.4	5.7	5.9	6.3
20:0	—	0.3	0.2	0.1	0.1
ΣSaturates[a]	10.0	25.3	23.7	22.6	21.0
16:1	TR	1.1	0.9	0.9	0.9
18:1	20.6	30.6	29.7	28.5	27.3
20:1	—	0.3	0.3	0.2	0.2
ΣMonoenes[a]	20.6	32.0	31.0	29.7	28.6
18:2n-6	14.8	42.0	32.8	25.4	13.7
18:3n-3	54.4	0.7	12.4	22.2	36.5
ΣPolyunsaturates[a]	69.4	42.7	45.2	47.6	50.2
18:2n-6:18:3n-3	0.27	60.00	2.65	1.14	0.38

[a] Total includes minor fatty acids. Flaxseed contained 0.2% *trans* 18:3n-3.

lipid status, zinc status, fatty acid composition, lipid peroxidation, and vitamin E levels of some tissues was studied. Since flaxseed has been used to ameliorate constipation, dietary fiber was measured in diets and feces, and fecal characteristics were assessed. It is generally recognized that 18:3n-3, in an isolated form or as present as a component in an oil, is susceptible to heat and autoxidation. However, the stability of 18:3n-3 as present in seeds is not known. Therefore, since flaxseed is rich in 18:3n-3, the thermal and storage stabilities of the unextracted oil in flaxseed were also studied.

Materials and methods

Flaxseed

Flaxseed (*Linum usitatissimum*) was of the Linott variety grown in 1988 in Western Canada and was supplied to us by the Flax Growers, Western Canada. Proximate analysis of the seed showed, by wt., 29.4% fat, 25.3% protein, 26.5% fiber, 6.4% carbohydrates, and 9.4% water. The fatty acid composition is given in *Table 1*.

Flaxseed storage stability

Whole and coarsely ground flaxseeds were placed in glass jars and stored at room temperature or at 4° C. Samples were withdrawn after 0, 4, 8, 16, and 44 weeks and analyzed for fat content, fatty acid composition, and peroxide value (POV).

Flaxseed heat stability

Whole and coarsely ground seeds were heated in an oven at 100° C and 350°C for 20, 40, and 60 min. Samples were analyzed for fat content, fatty acid composition, and POV.

Animals and diets

Weanling male Sprague-Dawley rats from Charles River Canada Inc., St. Constant, Quebec (body weight 57.9 ± 2.3 g) were randomized according to body weight into four dietary groups. Rats were fed the purified diets (*Table 2*) for 90 days. The source of fat in the reference diet [0% flaxseed (FS)] was a blend of lard (29.8%) and corn oil (70.2%). The other diets contained 10%, 20%, and 40% ground flaxseed (abbreviated as 10% FS, 20% FS and 40% FS). The levels of the other ingredients were adjusted so as to make all the diets approxiamtely isocaloric and contain approximately the same amount of fat (14.8%–17.5%), protein (18.2%–19.2%) and fiber plus wood cellulose (12.8%–13.2%). The fatty acid composition of the diets is given in *Table 1*. The phytate, zinc, and calcium levels, as well as the

Table 2 Composition of the diets

Ingredient	% Weight			
	0% FS	10% FS	20% FS	40%FS
Flaxseed	—	10.00	20.00	40.00
Casein[a]	20.00	17.50	14.90	9.90
Lard	4.47	5.00	5.64	6.76
Corn oil	10.53	7.90	5.23	—
Alphacel[a]	13.20	10.50	7.80	2.20
Mineral mixture-AIN 76[a]	3.50	3.50	3.50	3.50
Vitamin mixture-AIN 76A[a]	1.00	1.00	1.00	1.00
Sucrose[a]	10.00	10.00	10.00	10.00
Cornstarch[a]	36.60	33.90	31.20	25.90
Choline bitartrate[b]	0.40	0.40	0.40	0.40
DL-methionine[b]	0.30	0.20	0.15	0.10
L-glutamic acid[b]	—	0.10	0.15	0.20
Fat (wt.%)[c]	14.8	15.8	16.7	17.5
Protein[d] (wt.%)	18.2	18.4	18.6	19.2
Carbohydrates[d] (wt%)	46.4	44.5	42.5	38.5
Energy (kJ/100 g)	1647	1655	1657	1630
% En from flaxseed	0	7.7	15.4	31.2

[a] Teklad (Madison, WI, USA).
[b] Sigma Chemicals (St. Louis, MO, USA).
[c] Fat was extracted three times during the experiment.
[d] Calculated from the food composition data.

Table 3 Phytate, zinc and calcium concentration of the flaxseed and diets

Diet or constituent	Phytate	Zn	Ca	[Phytate]:[Zn]	[Phytate] [Ca]:[Zn]
	(mmol/kg)			molar ratio	
Flaxseed	24.2	0.82	53.7	29.5	1585
0% FS	0.0	0.46	131.9	—	—
10% FS	2.4	0.54	133.0	4.5	595
20% FS	4.9	0.62	136.8	7.8	1065
40% FS	9.7	0.79	146.2	12.3	1802

[phytate]/[zinc] and the [phytate] [calcium]/[zinc] molar ratios are shown in *Table 3*.

The rats were kept in individual cages in an air-conditioned room (22° C) and maintained on a 12-hour light:dark cycle. Food and water were provided ad libitum. Diets were prepared weekly and stored at −4° C until used. The oxidative stability of the diets was monitored periodically using POV determinations. The POV were normally less than 0.05 milliequivalents per Kg diet. Diets were given each day and any unconsumed food was weighed and discarded.

On experiement days 27, 59, and 89, six animals from each dietary group were placed in individual metabolic cages and overnight fasting urine samples were collected. Each morning of the experimental days from 41–44, feces were collected and their individual weight and volume were determined immediately. Feces were pooled for each group for total fat, pH, and fiber fermentability measurements.

On day 90, after an overnight fast, animals were killed by exsanguination from the abdominal aorta while under pentobarbital anesthesia. Blood was collected for immediate analysis. Heart, liver, kidney, spleen, white and brown adipose (subscapular region) tissues were dissected, weighed, and immediately frozen on dry ice. They were stored at −80° C under nitrogen until analyzed.

Analytical methods

The fat in the various samples was extracted with chloroform-methanol and transmethylated.[7] The fatty acid methyl esters were analyzed by gas liquid chromatography (GLC) using a Supelcowax-10 flexible fused silica capillary column (30 m × 0.32 mm I.D.) in a Varian Vista 6000 Chromatograph. The POV was determined according to the American Oil Chemists' Society[13] official method Cd 8-53. Alpha- and gamma-tocopherols (vitamin E) in tissues were determined by the high performance liquid chromatography method of Thompson and Hatina.[14]

Urine peroxides (as thiobarbituric acid reacting substances, TBARS) were determined at pH 3.5 using 1,1,3,3-tetramethoxy-propane as the standard.[8] Urine TBARS were expressed as μmol/mmol creatinine.

For fecal determinations, a 0.5 g portion of the pooled feces was mixed with 5 mL distilled water and pH was measured at room temperature. Three samplings were used for triplicate analyses. Fat in the feces was extracted as described for other samples.

The total, soluble, and insoluble dietary fiber in the flaxseed and the feces were analyzed as described by Mongeau and Brassard.[15] The insoluble (neutral detergent) fiber fractions were analyzed as described by Mongeau and Brassard.[16] The total dietary fiber content was also measured using the methods of the Association of Official Analytical Chemists'[17] and Jeraci et al.[18] The wet volume of the fecal matter was measured as described by Mongeau et al.,[19] and fecal water content was estimated by freeze-drying. The fermentability or extent of digestion of flaxseed by intestinal microflora was assessed by the difference between intake and fecal excretion.

Phytate in the diet samples was analyzed by the method of Latta and Eskin.[20] Zinc and calcium in diet samples and zinc in liver and tibia were determined by flame atomic absorption spectroscopy following dry ashing and dissolution of the ash in 3.0 N HCl.

The blood was centrifuged at 1300 × g for 20 min at 4° C and the serum separated. Serum total- and high-density lipoprotein (HDL)-cholesterol, and triglycerides were determined enzymatically using the Boehringer Mannheim cholesterol c-system (Boehringer Mannheim GMBH Diagnostica, Mannheim, Germany). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedelwald equation.[21] Serum glucose was analyzed using A-Gent Clinical Chemistry Kits and an ABA-VP discrete auto analyzer (Abbott Laboratories, Diagnostic Division, Mississauga, Ontario, Canada).

Statistics

All data are reported as mean ± standard deviation. The data were examined using analysis of variance and significant differences between means were determined by the Duncan's New Multiple Range Test. All statistical procedures were done using the PCANOVA statistical software package (Human Systems Dynamics, Northridge, CA, USA).

Results

Stability studies

Oxidative and thermal stability of the intact oil in both whole and coarsely ground flaxseed were evaluated by monitoring the changes in the POV, oil content, and the fatty acid composition. The POV during the 44 weeks of storage at 4° C as well as at room temperature (22° C) did not change and were normally less than 0.5 milliequivalents per Kg flaxseed (data not shown). The heat treatment and 100° C and 350° C caused only an insignificant increase in the POV. For example, after 1 hr of heating at 350° C, the POV were only 1.83 and 1.95 milliequivalents per kg of whole and ground flaxseed, respectively. During the 44 weeks of storage and heat treatments, the oil content remained around 29%–30% and the fatty acid composition did not differ from that of fresh flaxseed (*Table 1*). Most importantly, heat treatment had no effect on the concentration of 18:3n-3 in the seed. Examination of the fatty acid methyl esters by GLC did not reveal the presence of any new *trans*-geometric isomers of 18:3n-3 acid nor cyclic fatty acids in heat treated flaxseed.

Table 4 Effect of flaxseed on serum lipids of rats

	mmol/L*			
	0% FS	10% FS	20% FS	40% FS
Total CH	3.83 ± 0.65[a]	3.54 ± 0.59[ab]	3.02 ± 0.52[bc]	2.56 ± 0.59[c]
HDL-CH	1.98 ± 0.36[a]	2.05 ± 0.47[a]	1.80 ± 0.39[ab]	1.61 ± 0.38[b]
LDL-CH	0.89 ± 0.49[a]	0.73 ± 0.27[ab]	0.59 ± 0.34[b]	0.33 ± 0.23[c]
LDL:HDL-CH	0.45 ± 0.27[a]	0.36 ± 0.13[a]	0.32 ± 0.17[ab]	0.19 ± 0.11[b]
TG	2.22 ± 0.95[a]	2.22 ± 0.48[a]	1.70 ± 0.44[b]	1.70 ± 0.32[b]
Glucose	8.29 ± 1.22	8.24 ± 1.24	8.54 ± 1.63	8.57 ±1.37

CH, cholesterol; TG, triacylglycerol; LDL, low-density lipoprotein; HDL, high-density lipoprotein.
* Values are the mean ± standard deviation of 15 rats per group.
Means across a horizontal row with different superscripts are significantly different ($P < 0.05$).

Table 5 Effect of flaxseed on urinary TBARS and tissue tocopherols

	Urinary TBARS (μmol/mmol creatinine*)			
Days on diet	0% FS	10% FS	20% FS	40% FS
30	1.05 ± 0.20	1.03 ± 0.19	0.80 ± 0.08	1.11 ± 0.10
60	0.67 ± 0.12[ab]	0.60 ± 0.07[a]	0.62 ± 0.03[a]	0.75 ± 0.08[b]
90	0.51 ± 0.09[a]	0.52 ± 0.06[a]	0.56 ± 0.08[ab]	0.62 ± 0.07[b]
	α-Tocopherol (μg/g tissue*)			
Tissue	0% FS	10% FS	20% FS	40% FS
Liver	60.12 ± 12.42[a]	56.34 ± 6.44[ab]	46.62 ± 13.55[bc]	40.70 ±9.27[c]
Heart	51.67 ± 14.84[a]	50.31 ± 5.25[ab]	42.70 ± 11.27[ab]	42.15 ± 7.86[b]
Spleen	45.55 ± 5.39[ab]	47.18 ± 8.99[a]	41.73 ± 4.23[b]	36.34 ± 4.74[c]
Adipose (white)	23.59 ± 5.87[ab]	23.38 ± 3.52[ab]	28.86 ± 5.43[a]	21.28 ± 6.51[b]
	γ-Tocopherol (μg/g tissue*)			
Liver	7.53 ± 2.27[a]	5.82 ± 1.25[b]	5.19 ± 1.60[bc]	4.33 ± 1.01[c]
Heart	7.77 ± 2.96	5.93 ± 1.50	5.89 ± 2.35	5.56 ± 2.43
Spleen	9.63 ± 2.85[a]	8.40 ± 2.89[ab]	7.84 ± 1.89[ab]	6.63 ± 1.44[b]
Adipose (white)	6.53 ± 1.40[ab]	5.29 ± 1.30[a]	7.48 ± 0.79[b]	6.54 ± 1.67[ab]

* Values are the mean ± standard deviation of six rats per group.
Means across a horizontal row with different superscripts are significantly different ($P < 0.05$).

Body weights, organ weights and food consumption

Rats fed the different diets consumed similar amounts of food (21.5, 22.9, 20.9, and 21.1 g/rat/day for 0, 10, 20, and 40% FS groups respectively) and appeared healthy. At necropsy, the body, liver, heart, kidney, and spleen weights for the 0% FS group (n = 15) were 511.9 ± 54.1 g, 17.4 ± 3.1 g, 1.6 ± 0.6 g, 3.4 ± 0.4 g, and 0.8 ± 0.2 g, respectively. The body and organ weights for the other groups were not significantly different from the 0% FS group.

Serum glucose, triglyceride, total-, HDL- and LDL-cholesterol

Fasting serum glucose level was not affected by the incorporation of flaxseed in the diet (*Table 4*). Serum lipids were not changed by 10% FS, but higher levels, as compared to 0% FS, lowered the plasma triglyceride, total-, and LDL-cholesterol concentrations. The HDL-cholesterol and the ratio of LDL:HDL-cholesterol were generally lowered with increasing flaxseed in the diet, but significant lowering, compared to the 0% FS group occurred only when the diet contained 40% FS.

TBARS and vitamin E in tissues and diets

Urinary excretion of TBARS was higher only in rats fed the 40% FS for 60 and 90 days (*Table 5*). In all the dietary groups, urinary TBARS expressed on a creatinine basis decreased with time during the 90 days of feeding. Because creatinine excretion increased from 2.1 ± 1.0, 5.7 ± 3.0 to 11.9 ± 4.3 mmol/L at 30, 60, and 90 days respectively, reflecting the changing body weight, total excretion of TBARS/24 hr actually increased, although we did not measure it directly.

Both α- and γ-tocopherols tended to decrease with increasing dietary flaxseed (*Table 5*). Alpha-tocopherol was significantly lower in the liver, heart, and spleen of rats fed the 40% FS diet than those fed the 0% FS diet. Gamma-tocopherol was significantly lower only in the liver and spleen of the 40% FS group.

Table 6 (n-6) and (n-3) Fatty acids of brown and white adipose tissues, and organs of male rats

Fatty acid	% Total fatty acids*			
	0%	10% FS	20% FS	40% FS
Brown adipose tissue				
18:2n-6	33.7 ± 1.8[a]	26.6 ± 1.6[b]	18.1 ± 4.9[c]	18.5 ± 4.7[c]
20:4n-6	0.8 ± 0.1[a]	0.7 ± 0.0[b]	0.4 ± 0.1[c]	0.5 ± 0.1[c]
18:3n-3	0.4 ± 0.2[a]	3.8 ± 0.2[b]	12.5 ± 4.2[c]	13.1 ± 3.7[c]
20:5n-3	0	0	0	0.1 ± 0.1
22:5n-3	0	0	0.1 ± 0.1	0.2 ± 0.1
22:6n-3	0	0	0.1 ± 0.1	0.2 ± 0.1
White adipose tissue				
18:2n-6	36.2 ± 2.1[a]	30.1 ± 0.7[b]	23.1 ± 3.8[c]	14.7 ± 0.4[d]
20:4n-6	0.5 ± 0.1[a]	0.2 ± 0.1[b]	0.2 ± 0.2[b]	0.1 ± 0[b]
18:3n-3	0.4 ± 0.0[a]	5.9 ± 0.5[b]	14.2 ± 1.1[c]	17.0 ± 1.3[d]
20:5n-3	0	0	0.1 ± 0.1	0.1 ± 0.1
22:5n-3	0	0	0	0.1 ± 0.1
22:6n-3	0	0	0	0.1 ± 0.1
Liver fatty acids				
18:2n-6	22.9 ± 5.1[a]	19.7 ± 1.2[a]	18.2 ± 1.7[ab]	14.1 ± 0.3[b]
20:4n-6	10.8 ± 3.0[a]	11.6 ± 2.0[a]	10.8 ± 2.1[a]	7.4 ± 0.7[b]
18:3n-3	0.3 ± 0.1[a]	2.4 ± 0.3[b]	4.3 ± 1.0[c]	7.9 ± 0.7[d]
20:5n-3	0.1 ± 0.0[a]	0.6 ± 0.1[b]	1.1 ± 0.1[c]	3.4 ± 0.3[e]
22:5n-3	0.2 ± 0.1[a]	0.7 ± 0.2[a]	0.8 ± 0.1[a]	1.7 ± 0.9[b]
22:6n-3	1.1 ± 0.4[a]	3.2 ± 0.5[b]	3.5 ± 0.8[b]	3.0 ± 0.2[b]
Heart fatty acids				
18:2n-6	18.4 ± 2.8	20.7 ± 1.3	21.2 ± 1.6	17.9 ± 1.3
20:4n-6	16.2 ± 2.7[a]	15.0 ± 2.0[ab]	13.3 ± 2.6[bc]	10.8 ± 3.2[c]
18:3n-3	0.8 ± 0.2[a]	2.1 ± 2.0[ab]	5.1 ± 4.3[bc]	6.8 ± 3.7[c]
20:5n-3	0.3 ± 0.1[a]	0.2 ± 0.1[a]	0.3 ± 0.0[a]	0.6 ± 0.1[b]
22:5n-3	0.7 ± 0.3[a]	2.0 ± 0.7[b]	2.4 ± 1.0[b]	2.9 ± 0.9[b]
22:6n-3	3.1 ± 0.7[a]	5.9 ± 2.4[b]	7.4 ± 3.4[b]	5.4 ± 2.0[b]
Kidney fatty acids				
18:2n-6	29.5 ± 0.8[a]	27.5 ± 3.0[a]	22.0 ± 0.6[b]	14.9 ± 0.7[c]
20:4n-6	6.9 ± 1.6[a]	6.6 ± 0.9[a]	5.0 ± 0.9[b]	5.8 ± 1.2[b]
18:3n-3	0.4 ± 0.0[a]	8.0 ± 0.5[b]	8.1 ± 0.8[b]	16.0 ± 1.5[c]
20:5n-3	0[a]	0.1 ± 0.1[a]	0.3 ± 0.1[a]	1.0 ± 0.2[b]
22:5n-3	0	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.1
22:6n-3	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.5 ± 0.1

* Values are the mean ± standard deviation of six rats per group.
Means across a horizontal row with different superscripts are significantly different ($P < 0.05$).

Analysis of the ground flaxseed showed only traces of α-tocopherol. The corn oil used in the diets had approximately 10 IU/100 g oil. The AIN vitamin mixture at the 1% level in the diet has been formulated to provide 5 IU/100 g diet. We did not measure the α-tocopherol content in the diets per se, but calculations using the values above show 6.1, 5.8, 5.5, and 5.0 IU/100 g diet in 0%, 10%, 20%, and 40% FS diets, respectively.

Fatty acids in tissues

Changes in the fatty acid composition (detailed data not shown) of brown and white adipose tissues generally reflected the composition of the dietary fats. Although the levels of 18:3n-3 in brown and white adipose tissues (*Table 6*) increased significantly with the increasing amounts of flaxseed in the diet, the increase in adipose tissue was less than the increase in 18:3n-3 in the respective diets. As compared to the 0% FS group, liver, heart, and kidney tissues of rats fed the flaxseed diets had higher levels of 18:3n-3 and its desaturation/elongation products, particularly 20:5n-3, 22:5n-3, and 22:6n-3 (*Table 6*). The long-chain n-3 fatty acids were more concentrated in liver and heart than in kidney. In liver, the concentration of 22:6n-3 increased three-fold when flaxseed in the diet was increased from 0% to 10%, but further increases in flaxseed did not proportionately elevate the 22:6n-3 concentration. However, 20:5n-3, and to a certain extent 22:5n-3, increased with increasing flaxseed in the diet. In heart, as opposed to liver, flaxseed did not increase the level of 20:5n-3, except in the 40% dietary group, where a slight, but significant increase occurred.

Although the flaxseed diets contained much lower levels of 18:2n-6 than the 0% FS diet (*Table 1*), feeding flaxseed did not proportionately decrease the levels of 18:2n-6 or its desaturation and elongation product, 20:4n-6, in organ lipids (*Table 6*). In heart, the 18:2n-6 level was unaffected, but 20:4n-6 gradually decreased with the increase of flaxseed in the diet. In liver, significantly lower levels of both 18:2n-6 and 20:4n-6 occurred only in the 40% FS group, whereas in kidney, in addition to the 40% FS group, the 20%

Table 7 The liver and tibia zinc concentration of rats fed the various diets

Diet	Liver Zn μg/g dry wt.*	Tibia Zn μg/g dry wt.*
0% FS	80.6 ± 5.15[a]	212 ± 9.5
10% FS	89.2 ± 1.48[ab]	235 ± 9.8
20% FS	90.5 ± 6.02[b]	227 ± 22.2
40% FS	94.7 ± 5.29[b]	224 ± 13.8

* Values are the mean ± standard deviation of six rats per group. Means in the same column with different superscripts are significantly different ($P < 0.05$).

FS group also had lower levels compared to the 0% FS group.

Phytate, zinc, and calcium

The phytate content of flaxseed was 24.24 mmol/kg, while in the diets it ranged from 2.42 to 9.70 mmol/kg, resulting in [phytate]:[zinc] molar ratios ranging from 4.47 in the 10% FS diet to 12.33 in the 40% FS diet (*Table 3*). The calcium content of the diets was approximately 0.5%, resulting in [phytate][calcium]:[zinc] molar ratios of 595–1802. These phytate levels, however, had little effect on zinc status as shown by the bone zinc levels (*Table 7*). The liver levels of zinc increased slightly. The concentration of zinc in the livers of rats fed the 0% FS diet was significantly lower than the levels in the livers of animals fed 20% and 40% FS diets.

Dietary fiber and fecal characteristics

By one method,[15] the total dietary fiber content of flaxseed was 26.5% ± 0.3% (mean of triplicate analyses) with 39% of it being water-soluble. Other methods gave values of 26.2%[17] and 27.7%.[18] In all the diets the sum of purified wood cellulose (PWC) and flaxseed fiber was around 13% (*Table 8*). The flaxseed total dietary fiber content varied from 0% (0% FS diet) to 10.6% (40% diet).

About 6% of insoluble fiber was fermented in the 0% FS group and increased to 32% in the 40% FS group (*Table 8*). The material measured as insoluble fiber was from multiple sources and the contribution of each source varied with the diet. The lignin intake in all groups was below 0.2 g/rat/day and its fermentability appeared to be negligible. Most hemicelluloses in the 40% FS diet were from flaxseed and out of 0.76 g/rat/day ingested, 0.27 g was recovered in feces, showing a fermentability of 64%.

The food intake during the 4-day feces collection period (days 41–44) was within 20.8 ± 1.3 g (10% FS) and 21.8 ± 2.0 g (40% FS). Fecal wet weight increased by 84% ($P < 0.01$) from 5.5 (0% FS diet) to 10.1 g/rat/day (40% FS diet) (*Table 9*). When expressed as g per 100 g food ingested, wet weight increased by 80% ($P < 0.01$) from 25.7 ± 1.5 to 46.3 ± 7.2, but dry weight increased only by 20% from 15.8 ± 0.8 to 19.0 ± 1.9. Fecal volume increased by 52% ($P < 0.01$) from 6.7 (0% FS diet) to 10.2 mL/rat/day (40% FS diet). Fecal moisture was increased ($P < 0.01$) from 38.9 (0% FS diet) to 58.4% (40% FS diet) (*Table 9*).

The mean daily fat intake increased from 3.14 g (21.2 g of diet containing 14.8% fat) in the 0% FS group to 3.82 g (21.8 g of diet containing 17.5% fat) in the 40% FS group. The fat content of feces pooled for each group (triplicate analyses) increased threefold from 3.19 ± 0.14 (0% FS diet) to 9.72% ± 0.15 (40% FS diet). Taking into account the fecal wet weight excretion, the mean daily fat excretion ranged from 0.17 to 0.98 g. This was associated with a gradual decrease in pH, which was 1.2 units lower in the 40% FS group (6.13 ± 0.008) than in the 0% FS group (7.35 ± 0.04) (*Table 9*).

Automated consistometry[19] measurements showed a 62% increase in fecal softness between the 10% and 40% FS groups; the 41% difference between the 20% and 40% FS groups was also significant ($P < 0.05$). A comparison with the 0% FS group was not possible

Table 8 Flaxseed fiber and wood cellulose in diets and in vivo cellulose fermentability

	Wt.%			
	0% FS	10% FS	20% FS	40% FS
Flaxseed fiber and wood cellulose in diets[a]				
Wood cellulose	13.2	10.5	7.8	2.2
Flaxseed insoluble fiber	0.0	1.6	3.2	6.4
Flaxseed soluble fiber	0.0	1.0	2.1	4.2
Total	13.2	13.1	13.1	12.8
In vivo insoluble fiber fermentability[b]				
Intake (g/rat/day)[c]	2.89 ± 0.22	2.85 ± 0.19	2.39 ± 0.23	1.90 ± 0.18
Excretion (g/rat/day)[d]	2.70 ± 0.01	2.31 ± 0.03	1.99 ± 0.02	1.29 ± 0.01
Disappearance (%)[e]	6.5	18.8	16.5	32.1

[a] Calculated according to amounts added to diets.
[b] Insoluble fiber includes hemicellulose, cellulose, and lignin.
[c] Mean ± standard deviation for 10 animals.
[d] Mean ± standard deviation of a triplicate analyses in pooled feces of each group.
[e] Disappearance (or fermentability) = {[(intake) − (excretion)]/[intake]} × 100.

Table 9 Fecal characteristics during the 4-day (experimental days 41–44) collection period

	0% FS	10% FS	20% FS	40% FS
Wet weight g/rat/day	5.5 ± 0.6[a]	6.1 ± 0.7[a]	6.9 ± 1.1[a]	10.1 ± 1.8[b]
Wet volume ml/rat/day	6.7 ± 0.6[a]	7.3 ± 0.8[a]	8.1 ± 1.2[a]	10.2 ± 1.8[b]
Water content g/100 g wet wt.	38.9 ± 1.6[a]	42.9 ± 2.7[b]	46.9 ± 5.3[bc]	58.4 ± 4.6[c]
Fat content g/100 g wet wt.	3.19	5.62	7.79	9.72
pH	7.35	6.88	6.48	6.13

Values are the mean ± standard deviation of 15 rats per group, except for fat content and pH, where the values are for a pooled sample of all the 15 rats in the group.
Means across a horizontal row with different superscripts are significantly different ($P < 0.05$).

because fecal pellets (collected immediately after defecation) were dry and friable, and their rupture invalidated the measurement.

Discussion

The high level of 18:3n-3 has caused concern regarding the oxidative and thermal stability of the oil in flaxseed. The results reported here, however, demonstrated the stability of oil in flaxseed. The thermal stability of 18:3n-3 in both intact and ground flaxseed contrasted with that in extracted oils.[22-24] Heat treatment of linseed oil, the oil of flaxseed, resulted in the formation of polymers, *trans*-geometrical isomers of 18:3n-3 (*t*18:3n-3) and cyclic fatty acids.[22,23] The virtual absence of these thermal products in heated flaxseed suggests that the structure of the seed protected 18:3n-3 from geometrical isomerization and cyclization.

The measurement of routine indicators of nutritional adequacy such as body and organ weights demonstrated that incorporation of flaxseed into the diet had no detrimental effect on growth rate of the rat. Although flaxseed is known to contain potential antinutritional factors, particularly a vitamin B_6 antagonist[3] and cyanogenic glycosides,[2] there were no obvious deleterious effects in the present study.

The n-3 fatty acid data presented here showed that 18:3n-3 in flaxseed was bioavailable and incorporated into tissues and organs of the rat. The presence of C_{20} and C_{22} n-3 fatty acids in organs, particularly liver, which is the major site of fatty acid conversion, confirmed that rat can convert dietary 18:3n-3 to its longer and more unsaturated fatty acid derivatives.[6] This conversion was also noted in humans.[9,10] Pigs fed flaxseed also readily absorbed and metabolized 18:3n-3.[11]

The concentration of 22:6n-3 in the liver increased when flaxseed in the diet increased from 0% to 10%, but a further increase of flaxseed had no effect on the 22:6n-3 concentration. The concentration of 20:5n-3, however, significantly increased with all increases of flaxseed. This observation may indicate a close regulation of the conversion of 20:5n-3 to 22:6n-3 in the liver. It is interesting to note that maximum concentration of 22:6n-3 in the liver occurred when the ratio of 18:2n-6:18:3n-3 in the diet was about 2.7.

The incorporation of dietary 18:2n-6 in the organ lipids and the conversion of 18:2n-6 to 20:4n-6 were related to the level of flaxseed in the diets. It is noteworthy that only the 40% FS diet had any appreciable significant impact on the lowering of 20:4n-6 in the organ lipids examined. The 20% FS had only a marginal influence and the 10% FS had no effect. The relative amounts of dietary 18:2n-6 and 18:3n-3 are important, because these two fatty acids compete for incorporation into tissues, and for Δ6 desaturation and elongation.[25] In rats a higher dietary ratio of saturates to 18:2n-6 favors the conversion of 18:3n-3 to long-chain n-3 fatty acids more than the conversion of 18:2n-6 to long-chain n-6 fatty acids.[6] In the present study the diets were designed to provide equal amounts of saturated fatty acids, but actual chemical analysis showed a gradual, but an insignificant, increase in saturates with increasing flaxseed in the diets. This small variation in saturated fatty acids would not be expected to affect the competition between 18:2n-6 and 18:3n-3. Therefore, the lower levels of 20:4n-6 in rats fed high flaxseed diets (20% and 40%) were likely due to the inhibition of the conversion of 18:2n-6 to 20:4n-6 by the lower ratio of 18:2n-6:18:3n-3 in these diets. In the 20% FS diet the ratio of 18:2n-6:18:3n-3 was 1.14, which may be close to the ratio required to inhibit the conversion of 18:2n-6 to 20:4n-6 in rats.

The formation of urinary TBARS indicated an elevation of lipid peroxidation with a high intake of flaxseed. This was possibly a consequence of the increased accumulation of n-3 fatty acids in the tissues. As the long chain n-3 fatty acids are oxidatively unstable, a higher sensitivity to membrane peroxidation could be expected in animals fed the n-3 fatty acids.[26,27] Rats fed a high-fat diet (20%) with 28% of the fat from linseed oil, have also been shown to have elevated urinary and tissue TBARS.[8] Although the excretion of TBARS appears to decrease with time, the six-fold increase in creatinine excretion from 30–90 days was greater than the halfing of TBARS when expressed on a creatinine basis. Thus, total TBARS excretion/24 hr would be expected to triple from 30–90 days and increase the requirement for vitamin E.[26,27] Also, the flaxseed diets with lower levels of corn oil had lower levels of α-tocopherol. These factors could contribute to the lower levels of tissue α-tocopherols

in flaxseed fed rats. Another possibility is that α-tocopherol could have been destroyed at a stage before absorption, for example during the preparation of the diets, while in the diet cups, or in the digestive tract of the rat. Consequently, the tissues of the rats fed flaxseed, because of the high 18:3n-3 content, were low in α-tocopherol. It should be emphasized that although a lowering of vitamin E was observed generally, a significant drop occurred only in the 40% flaxseed group. A similar situation occurred with the TBARS values. Thus, in the rat, a low to moderate intake of flaxseed did not impart a general oxidative stress on the tissues, but a high intake of flaxseed may have done so.

The serum lipid data suggested that flaxseed resulted in hypolipidemia. Most importantly in the rat, flaxseed lowered total- and LDL-cholesterol as well as the LDL:HDL-cholesterol ratio. The hypocholesterolemic effect of flaxseed in rats has also been observed by Kritchvesky et al.[12] Generally marine n-3 fatty acids are not considered as hypocholesterolemic,[28] but the effect of 18:3n-3 is not well understood. In the present study incorporation of flaxseed resulted in a large increase in the ratio of 18:3n-3:18:2n-6 in the diets, and a moderate increase of total polyunsaturated fatty acids with a parallel decrease in the saturated fatty acids. These overall changes in the fatty acid profiles may have contributed to the observed lowering of serum cholesterol. The fiber content of flaxseed also may have played a role in its hypocholesterolemic effect.

The Linott variety contained lower levels of phytate, calcium, and zinc than those reported in other varieties.[29] These differences have been related to differences in soil mineral content.[28]

The long-recognized negative effect of phytate on zinc bioavailability is exacerbated by high levels of calcium.[4,30,31] Thus, this inhibitory effect is best defined by the [phytate] [calcium]:[zinc] molar ratio.[31,32] For instance, in rats, reduced zinc status has been reported with [phytate]:[zinc] molar ratios of 12 with a calcium concentration of 0.75% and 6 with a calcium concentration of 1.75%.[4] This represents a [phytate] [calcium]:[zinc] molar ratio of approximately 2200. In man, [phytate] [calcium]:[zinc] molar ratios as low as 200–400 have been reported to impair zinc absorption.[32] In the present study, no effect of phytate was seen on zinc status, as assessed by the tibia zinc concentration, probably because of the lower calcium concentration. The [phytate] [calcium]:[zinc] molar ratio was only 1600.

Unlike the bone levels, the zinc concentration in liver increased with increasing amounts of flax in the diet. This may have been associated with increasing amounts of zinc introduced into the diet by the addition of flaxseed.

Flaxseed fiber is about 40% water-soluble and 60% insoluble (*Table 8*). Soluble fiber is often associated with viscous properties in the small intestine, and with rapid and extensive bacterial fermentation in the large intestine. Insoluble fiber such as that in wheat bran is slowly and less extensively (30%–50%) fermented in humans and rats,[33-35] and provides bulking effects by virtue of its physical presence, water-holding capacity, and bacterial growth. In the present experiment, flaxseed fiber showed a good fecal bulking effect and its fecal moistening effect (58%, *Table 9*) compared favorably with that of wheat bran in rats (around 50%).[34]

The insoluble fiber intake decreased when flaxseed fiber increased because of the different proportions of cellulose and flaxseed fiber in the diets. The fermentability was the percentage of ingested insoluble fiber not recovered in pooled feces for each group. Insoluble fiber in 40% FS diets (*Table 8*) was 32% fermented but 25% of it was added wood cellulose. Since the latter is practically unfermentable in humans and in rats, the 32% fermentability observed for insoluble fiber suggests that a substantial portion of the insoluble fiber from flaxseed was fermented. Because wood cellulose can negatively affect bacterial growth in the large intestine,[36] the fermentability of the flaxseed fiber fractions may have been underestimated. The 39% water-soluble fiber of flaxseed was presumed to be fermentable. It thus appears that the fermentability of total dietary fiber in flaxseed would be more than 50% and most likely in the 60%–70% range. Fermentation products, notably short-chain fatty acids, and their absorption from the large intestine were associated with beneficial effects, including a decreased pH in the large intestine lumina and the possible regulation of lipid metabolism in liver by propionate.[37]

The low standard deviation values of triplicate analysis of pH and total fat from pooled feces suggest significant differences between 0% and 40% FS groups. The 305% increase in total fat excretion is relevant because in a similar experiment in which fecal fat was measured for each rat, a 2% increase in dietary fiber from wheat bran significantly increased total fat excretion by 35% ($P < 0.05$).[38]

Bach Knudsen et al.[39] speculated that the higher fat excretion associated with intake of certain fiber sources is due to a higher fecal bile acid excretion indirectly caused by fiber fermentation. The lower pH in the lumen of cecum and colon influences the growth and metabolism of colonic epithelial cells and would modify bile acid degradation by inhibiting the 7-alpha-dehydroxylation of primary bile acids. The present results do not provide information to verify this hypothesis but indicate that the lipid metabolism with dietary flaxseed was different from that with dietary cellulose. Comparison of fat intake and excretion during the four-day collection period showed that 40% FS rats excreted more (0.81 g) and ingested more (0.68 g) fat than 0% FS rats. It is not known if the hypocholesterolemic effect of flaxseed was due to both its oil and its fiber.

Based on the criteria examined, no adverse effects were observed in the rats fed Linott flaxseed, even at high intakes. The α-linolenic acid in flaxseed was bioavailable and is converted by rats to long chain n-3 fatty acids. The flaxseed fiber may also be of benefit in the lowering of serum lipids.

Acknowledgments

The authors thank Laurent Samure, Kevin Kittle, Dominique Patry, and Donna Beauchamp of the Animal Resources Division, Health Protection Branch for the care of the animals. The technical assistance of Bart Belonge, Rene Brassard, Alex Giroux, Elaine O'Grady, Rudy Hollywood, Gerard Pelletier, and Keith Trick is greatly appreciated.

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