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Sex-linked differences in fatty acid composition of rainbow trout (*Oncorhynchus mykiss*) liver oil

M. T. Satué & M. C. López

Nutrición y Bromatología, Dpto Ciencias Fisiológicas, Humanas y de la Nutrición, Facultad de Farmacia, Universidad de Barcelona, Barcelona, Spain

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Fatty acid analysis of the liver oil of male and female farmed rainbow trout (*Oncorhynchus mykiss*) was carried out by gas–liquid chromatography with flame ionization detection (FID). The polyunsaturated fatty acid fraction was much higher in males than in females, while the opposite was true for the monounsaturated fatty acid fraction.

The $n-3$ series fatty acid content is greater than the $n-6$ series fatty acid content. Docosahexaenoic acid (DHA) appears, in both types of sample, in greater amounts than eicosapentaenoic acid (EPA). In general, values found for female samples are in line with those reported in the literature. Male samples, although they followed the same pattern, showed significant differences ($P < 0.05$) from females.

The high DHA/EPA ratios found in rainbow trout liver oil make it suitable for industrial uses, such as infant formula supplementation. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Many studies have been reported on the beneficial effects of $n-3$ series long-chain polyunsaturated fatty acids (LC-PUFA) (Kinsella, 1988; Bjerve, 1990; Kelly, 1991). Of these, a decrease in cardiovascular disease (Kinsella *et al.*, 1990*a*), improvement of autoimmune and inflammatory disorders (Kelley *et al.*, 1991), tumour cell killing ability (Dianzani, 1989) and retina and brain development (Crawford, 1993) are the most cited. Fatty fish is the main source of $n-3$ LC-PUFA; trout, classified as a fatty fish and amenable to farming, is a useful source of these fatty acids.

Farming fish for human consumption leads to the production of large quantities of guts which are rejected. Fish liver is well known for its high content of fat, and is a good source of fish oil. We present a study of the fatty acid composition of trout liver oil in an attempt to promote the exploitation of these guts.

MATERIALS AND METHODS

Samples

Liver oil was analysed from six female (1.61–2.63 kg) and ten male (1.12–2.40 kg) adult trout (*Oncorhynchus mykiss*), provided by a fish farm. The trout were all fed on the same diet and were captured in December 1991 at the same pond to avoid seasonal and habitat differences. The fish were put into polyethylene bags, immediately frozen and stored at −20°C for future analysis.

Reagents and standards

The petroleum ether (40–65°C) used for Soxhlet extraction was from SDS (Peypin, France). The methanol (pro-analysis grade), chloroform (pro-analysis grade) and anhydrous sodium sulphate (pro-analysis grade) used for oil extraction in the Folch–Lees method were purchased from Probus (Badalona, Spain). The methylation reagent, sodium methoxide (0.5% CH_3ONa), was prepared in our laboratory with sodium (pro-synthesis grade) and methanol (pro-analysis grade), both from Merck (Schuchardt, Germany), as well as boron trifluoride–methanol (BF_3 CH_3OH; pro-synthesis grade) and n-hexane (pro-analysis grade). All the fatty acid standards were from Supelco (Bellefonte, PA, USA); the internal standard ($C_{12:0}$) was from Sigma (St. Louis, MO, USA).

Methods

Oil extraction

The trout were gutted and the livers were dissected to obtain the oil. Each liver was thoroughly mixed with anhydrous sodium sulphate to avoid the formation of emulsions during fat extraction. A fraction of the mix was introduced into a Soxhlet device and extracted with

Table 1. Response factors and recovery percentages calculated for the major fatty acids

Fatty acid	Response factor (mean (SD), $n=3$)	Recovery (%, mean (SD), $n=10$)
$C_{14:0}$	1.09(0.03)	95.8(3.80)
$C_{16:0}$	1.11(0.02)	93.3(2.19)
$C_{16:1}$ $n-7$	1.12(0.03)	98.1(3.99)
$C_{18:0}$	1.14(0.03)	94.6(2.78)
$C_{18:1}$ $n-9$	1.14(0.02)	96.0(2.14)
$C_{18:2}$ $n-6$	1.16(0.03)	97.9(2.81)
$C_{20:1}$ $n-9$	1.18(0.03)	95.3(3.96)
$C_{22:1}$ $n-11$	1.19(0.04)	96.1(2.41)
$C_{20:5}$ $n-3$	1.24(0.03)	94.2(3.92)
$C_{22:6}$ $n-3$	1.27(0.04)	96.3(3.97)

SD, standard deviation.

petroleum ether. When extraction was complete, the solvent was removed under reduced pressure at 30°C using a rotary evaporator, and the total fat content was calculated. The rest of the liver was extracted following the Folch–Lees method (Folch *et al.*, 1957), consisting of solvent extraction (chloroform–methanol, 2:1, v/v) at ambient temperature. The oil obtained was kept at −20°C in a dark glass vial until fatty acid analyses were performed.

Methyl ester formation

We used the technique described by Morrison & Smith (1964). In a 20-ml glass, screw-capped tube with 20 mg of the internal standard ($C_{12:0}$), 200 mg of oil was weighed. Then 5 ml of 0.5% sodium methoxide was added and the tube was placed in a boiling water bath for approximately 15 min (until only one phase could be seen). After cooling, 6 ml of boron trifluoride–methanol was added and the tube was again placed in the boiling bath for another 15 min. The tube was then immediately cooled and the methyl esters formed were extracted with 2 ml of hexane. For satisfactory separation of the organic phase 2.5–3 ml of a saturated solution of NaCl was added, the tube was vortex-mixed and the hexane phase was then transferred to a screw-capped tube containing a small amount of anhydrous sodium sulphate.

Fatty acid analysis

A 5-µl aliquot of the methyl esters formed was injected into a Perkin Elmer Sigma 300 gas–liquid chromatograph, equipped with a flame ionization detector (FID) and connected to an HP3379A integrator. The carrier gas used was helium at a flow rate measured at the end of the column of 0.83 ml min^{-1} and a split ratio of 1:108. The detector gas flow (nitrogen and hydrogen) was optimized. Twenty-two fatty acids were separated on a cyanosilicone column SP-2330 (30 m×0.25 mm i.d.×0.20 µm film thickness; Supelco) in a relatively short time (29 min) starting at 160°C (hold 1 min) and temperature programming at a rate of 4°C min^{-1} to

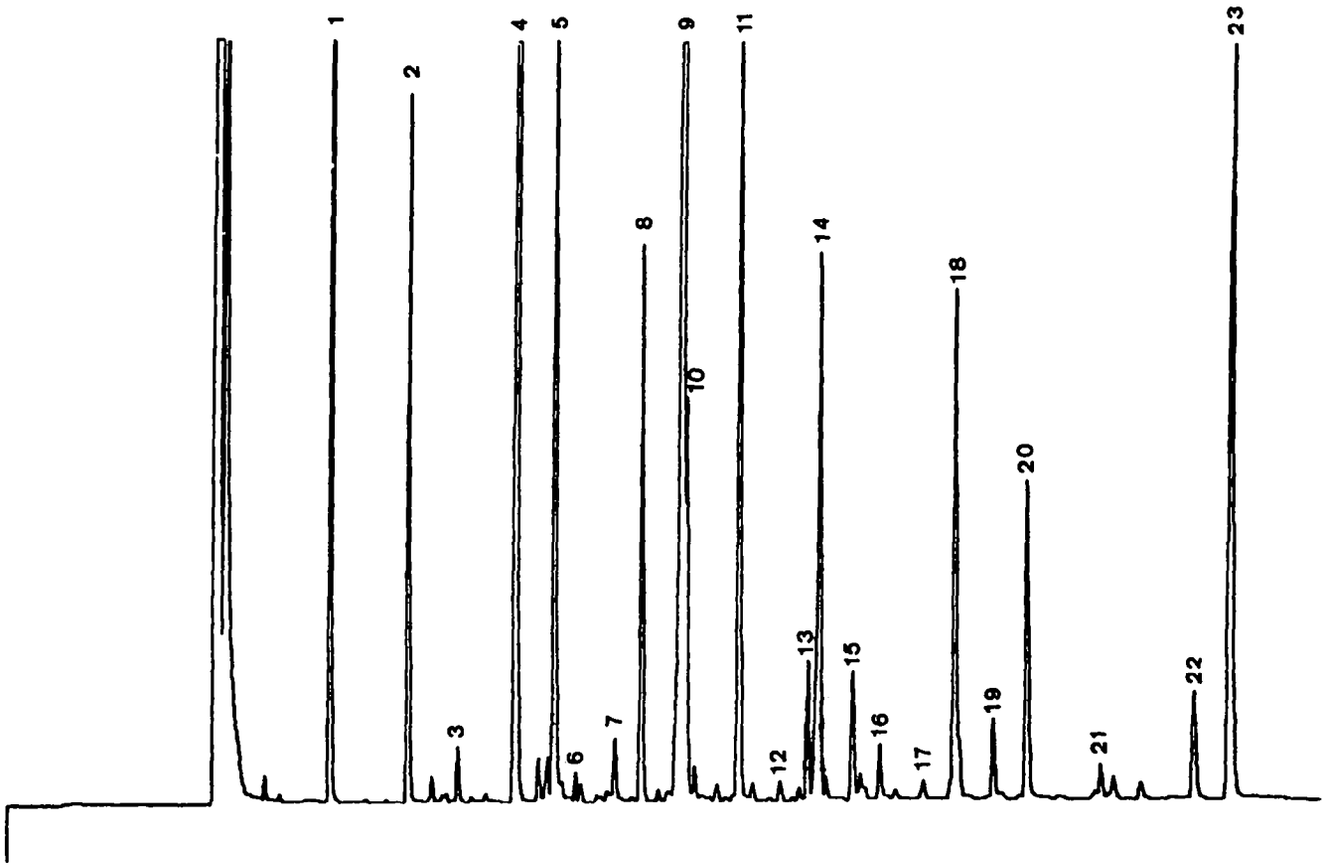

Fig. 1. Chromatogram of the fatty acid profile of a trout liver oil. Peaks: 1, $C_{12:0}$ (internal standard); 2, $C_{14:0}$; 3, $C_{15:1}$; 4, $C_{16:0}$; 5, $C_{16:1}$ $n-7$; 6, $C_{17:0}$; 7, $C_{17:1}$ $n-8$; 8, $C_{18:0}$; 9, $C_{18:1}$ $n-9$; 10, $C_{18:1}$ $n-7$; 11, $C_{18:2}$ $n-6$; 12, $C_{20:0}$; 13, $C_{18:3}$ $n-3$; 14, $C_{20:1}$ $n-9$; 15, $C_{18:4}$ $n-3$; 16, $C_{20:2}$ $n-6$; 17, $C_{22:0}$; 18, $C_{22:1}$ $n-11$; 19, $C_{20:4}$ $n-6$; 20, $C_{20:5}$ $n-3$; 21, $C_{22:4}$ $n-6$; 22, $C_{22:5}$ $n-3$; 23, $C_{22:6}$ $n-3$.

260°C (hold 3 min). The injector and detector temperature were both set at 260°C. Identification was achieved by comparison with appropriate standards and by means of the linear regression between logarithms of retention times and number of carbon atoms. Quantification was based on the internal standard calibration, using methyl laurate, which was not present in the trout liver oil, as internal standard. Empirical response factors were determined with a quantitative mixture of all the fatty acids identified including the internal standard. Response factor (RF) for each fatty acid was calculated as:

$$RF = (W_x/W_{st}) \times (A_{st}/A_x)$$

where W_x is the amount of fatty acid weighed in the mixture, W_{st} is the weight of internal standard used, and A_{st} and A_x are the areas obtained, respectively, for the internal standard and the fatty acid in the analysis of the fatty acid mixture.

Statistics

The Stratgraphics package (Version 4.0, STSC Inc. and Statistical Graphics Corporation) was used for the analysis of variance (ANOVA) of results.

RESULTS AND DISCUSSION

For method validation, we assessed repeatability by derivatizing and injecting ten aliquots of a given sample and found variation coefficients ranging from 1.07% to 1.50%, which are lower than the values calculated by Horwitz (1982) for intralaboratory analysis. Response factor values calculated for the fatty acids identified, and related to the internal standard, ranged between 1.1 ± 0.03 and 1.3 ± 0.04. The standard addition method was used to test the accuracy: a constant amount of fatty acid standard was added to ten aliquots of a sample to obtain the recovery. Mean recoveries were all greater than 93% (Table 1). Detection and quantification limits (15–23 ng and 17–25 ng, respectively) were acceptable according to the Kateman & Pijpers (1981) criterion.

Figure 1 shows a typical chromatogram of trout liver oil. The fatty acid composition, the percentages of saturated, monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids and the ratios $n-3/n-6$ and DHA/EPA are shown in Table 2.

Marked differences were found in liver composition of female and male trout. Satué *et al.* (1994), Ota & Takagi (1990) and Jhaveri & Constantinides (1982) had

Table 2. Mean values for the fatty acids of male and female samples

Fatty acid	Female ($n=6$)			Male ($n=10$)		
	% (mean, SD)	% of SFA	mg kg^{-1} (mean, SD)	% (mean, SD)	% of SFA	mg kg^{-1} (mean, SD)
$C_{14:0}$	2(0.3)	6.55	14(3.8)	2(0.4)	5.52	9(3.3)
$C_{16:0}$	16(1.5)	63.39	135(29.9)	22(1.5)	74.52	114(33.5)
$C_{17:0}$	0.15(0.03)	0.58	1.3(0.44)	0.3(0.08)	0.90	2.1(1.44)
$C_{18:0}$	7(1.2)	26.28	58(18.1)	5(0.6)	16.57	27(10.0)
$C_{20:0}$	0.2(0.04)	0.62	1.6(0.36)	—	—	—
$C_{22:0}$	0.7(0.27)	2.58	6(2.9)	0.8(0.399)	2.49	6(2.6)
Total saturates	26(2.5)		213(51.1)	30(1.7)		158(49.6)
$C_{15:1}$	0.2(0.05)	0.42	1.5(0.3)	0.4(0.14)	1.63	3(1.9)
$C_{16:1}$ $n-7$	5(1.9)	11.37	43(11.4)	2.2(0.66)	9.34	12(6.5)
$C_{17:1}$ $n-8$	0.4(0.06)	0.85	3(0.9)	0.3(0.07)	1.13	2(1.1)
$C_{18:1}$ $n-9$	28(8.6)	62.51	241(54.1)	12(2.0)	48.1	63(21.4)
$C_{18:1}$ $n-7$	4(0.8)	7.84	28(6.1)	2.1(0.27)	8.84	12(5.2)
$C_{20:1}$ $n-9$	5(0.6)	10.16	40(11.1)	2(0.8)	9.43	13(7.5)
$C_{22:1}$ $n-11$	3.1(0.68	6.84	27(8.2)	5.2(0.5)	21.53	29(11.9)
Total monounsaturates	45(9.3)		379(129.0)	24(3.5)		133(65.2)
$C_{18:2}$ $n-6$	4(0.6)	13.03	32(6.2)	6(0.7)	13.96	34(12.3)
$C_{18:3}$ $n-3$	0.5(0.19)	1.59	4(1.0)	0.6(0.10)	1.39	5(2.8)
$C_{18:4}$ $n-3$	0.2(0.03)	0.64	1.7(0.32)	0.4(0.16)	0.89	3(2.7)
$C_{20:2}$ $n-6$	1(0.2)	3.26	8(3.1)	1(0.4)	1.61	7(2.8)
$C_{20:4}$ $n-6$	0.6(0.26)	1.87	5(0.8)	0.7(0.28)	1.46	5(3.0)
$C_{20:5}$ $n-3$	3(1.0)	10.96	28(7.7)	7(1.7)	16.09	41(12.4)
$C_{22:4}$ $n-6$	0.6(0.29)	2.03	6(2.4)	0.5(0.13)	1.07	4(1.4)
$C_{22:5}$ $n-3$	1.4(0.44)	4.78	13(3.7)	2(0.2)	3.96	15(6.3)
$C_{22:6}$ $n-3$	18(5.2)	61.84	169(58.8)	27(4.2)	59.57	162(52.8)
Total polyunsaturates	29(7.3)		267(79.2)	46(3.8)		274(83.1)
Total $n-3$	24(6.5)		216(68.5)	38(4.5)		224(67.6)
Total $n-6$	6(1.0)		51(12.5)	8(1.1)		50(18.7)
$n-3/n-6$	4(0.8)		4(0.8)	5(1.0)		5(1.0)
DHA/EPA	6(1.1)		6(1.1)	4(1.1)		4(1.9)

Values are expressed as percentages of total fatty acids and as mg kg^{-1}; SD, standard deviation.
SFA, saturated fatty acids; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

previously reported differences related to sex in fatty acid composition of trout (*Oncorhynchus mykiss*) flesh oil, chum salmon (*Oncorhynchus keta*) liver and dogfish (*Squalus acanthias*) muscle oil, respectively. The main fatty acids in trout liver oil were, in order: in males, $C_{22:6}$ $n-3$, $C_{16:0}$, $C_{18:1}$ $n-9$, $C_{22:5}$ $n-3$, $C_{18:2}$ $n-6$; in females, $C_{18:1}$ $n-9$, $C_{22:6}$ $n-3$ $C_{16:0}$, $C_{18:0}$, $C_{16:1}$.

Saturated fatty acid (SFA) percentages were only slightly different (26%, 30%) but significant ($P = 0.0012$); they were in the middle of the range reported for various freshwater fish (Gibson, 1983; Vlieg & Body, 1988; Wang *et al.*, 1990) and were higher than in other fish liver oils of marine importance (McGill & Moffat, 1992). Palmitic acid ($C_{16:0}$), reported by Ackman & Eaton (1966) as a key metabolite not influenced by diet in fish, was the saturated fatty acid found in highest concentration (63.4% of saturates in females and 74.5% in males). The value for males was much higher than those found in the literature (59.6–66.5%; McGill & Moffat, 1992) and is statistically different ($P = 0.0001$) from the $C_{16:0}$ content found in females.

In the monoene fraction, $C_{18:1}$ $n-9$ (oleic acid) was the major fatty acid in both sexes, with higher concentrations in females (62.5% of total MUFA vs. 48.1%; $P < 0.0001$). These values are similar to those reported in marine fish liver oil (McGill & Moffat, 1992) and in muscle oil composition either in marine fish (Vlieg *et al.*, 1993) or in freshwater fish (Aggelousis & Lazos, 1991). We found a considerable proportion (2–5%) of LC-MUFA ($C_{20:1}$ and $C_{22:1}$) in both kinds of sample, other authors having reported much lower ($< 0.1\%$) values (Ackman *et al.*, 1980; Aggelousis & Lazos, 1991) for freshwater fish. Nevertheless, the values found for these fatty acids are in the range reported in other studies (McGill & Moffat, 1992), where values in liver oil up to 17.5% of total fatty acids could be found. When studying trout feed composition these fatty acids appeared in even greater proportions ($C_{20:1}$ $n-9$, 7–9%; $C_{22:1}$ $n-11$, 14%) and, although trout have the capacity to metabolize these fatty acids through a peroxisomal oxidative path, up to 8% can be incorporated without transformation into adipose tissue (Henderson & Sargent, 1985). Long-chain monoenes are oxidized slowly in mammals and are suspected of causing cardiac lipidosis (Vles, 1975). It may thus be necessary to monitor fish feed in order to obtain a quality product. MUFA constituted the largest proportion of total unsaturated fatty acids in the liver oil of female trout (60.3%), which is within the range usually reported. However, liver oil from male trout showed a high proportion of polyunsaturates (65.7%) due to the high concentration of DHA found. Moreover, significant differences between sexes were found in the percentages of both MUFA and PUFA ($P < 0.001$); in females, the MUFA fraction was much higher than in males (44.7% vs. 24.0% of total fatty acids) while the opposite was true in the PUFA fraction (29.5% vs. 46.0%). In the $n-6$ group, significant differences ($P < 0.001$) were found between sexes in linoleic acid ($C_{18:2}$ $n-6$), the predominant fatty acid with 3.8% in females and 6.4% in males. Arachidonic acid ($C_{20:4}$ $n-6$), another important fatty acid involved in eicosanoid formation, showed, for both types of sample, similar values (no significant differences for $P = 0.3862$) which are lower than those found in the literature for freshwater fish muscle oil (Karahadian & Lindsay, 1989; Aggelousis & Lazos, 1991) and for marine liver oil (0.4–2; McGill & Moffat, 1992).

In the $n-3$ group, significant differences were found between males and females for DHA content ($P = 0.0018$) and for EPA ($P = 0.0001$). The lower content of $n-3$ PUFA in females may be related to egg formation. Do *et al.* (1980) reported a high percentage of $C_{22:6}$ in *Tilapia* egg lipid as compared to body and intestinal lipids. Some authors point out that the main essential fatty acid requirement of rainbow trout is for the $n-3$ series, and there are indications that longer chain forms may promote egg quality (Bromage *et al.*, 1992; Cowey, 1992).

There was a significant difference in the $n-3/n-6$ ratio in females (3.9) and males (4.6) ($P = 0.0002$). These values are lower than ratios found by McGill & Moffat (1992) for some marine liver oils (4.3–18.9) and at the same level as those reported for some marine oils (Vlieg *et al.*, 1993), but are clearly higher than data reported for muscle oil of some freshwater fish (Karahadian & Lindsay, 1989; Aggelousis & Lazos, 1991).

Despite the differences found in fatty acid composition in liver oil of male and female trout, in the LC-PUFA group we noted a greater content of DHA than of EPA in all samples analysed. Connor *et al.* (1990), Anderson *et al.* (1990) and Foote *et al.* (1990) pointed out the role of DHA in brain and retina development; in human milk DHA has been identified in appreciable amounts (Koletzko *et al.*, 1988) and the Nutrition Committee of ESPGAN (1991) recommends the adaptation of infant formula to human milk. Fish oil is considered a good source of $n-3$ series LC-PUFA, but other studies (Karahadian & Lindsay, 1989; Aggelousis & Lazos, 1991; McGill & Moffat, 1992) have reported low DHA/EPA ratios in freshwater fish species (0.53–1.6) and in marine fish liver oil (0.7–1.8). Due to its influence in eicosanoid formation (Goodnight *et al.*, 1982), EPA can alter prostaglandin metabolism and coagulation in newborns (Kinsella *et al.*, 1990*b*; Innis, 1991). In contrast, as shown by our results (DHA/EPA 3.9–5.8), trout liver oil, especially that from males, which is significantly higher in DHA than that from females ($P = 0.0052$), may be a suitable source of DHA for infant formula supplementation.

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