

Title	Fatty Acids in Lipids of Mature Chum Salmon, Oncorhynchus keta, with Special Reference to Phytanic Acid
Author(s)	OTA, Toru; TAKAGI, Toru
Citation	北海道大學水産學部研究彙報, 40(4), 313-322
Issue Date	1989-11
Doc URL	http://hdl.handle.net/2115/24042
Туре	bulletin (article)
File Information	40(4)_P313-322.pdf



Fatty Acids in Lipids of Mature Chum Salmon, Oncorhynchus keta, with Special Reference to Phytanic Acid

Toru OTA* and Toru TAKAGI*

Abstract

The component fatty acids of the lipid classes of serum, liver, and dorsal muscle from mature chum salmon, *Oncorhynchus keta*, were examined with special reference to phytanic acid.

The free fatty acids (FFA) in the serum contained more phytanic acid than sterol esters (SE), triacylglycerols (TG) and phospholipids (PL). Whereas, in the liver and dorsal muscle, this acid tended to be accumulated in the TG and SE.

A distinct difference in the phytanic acid content in the serum and liver was determined in salmon of different sex. The phytanic acid content in male serum and liver was 12 mg/100 ml and 22 mg/100 g, respectively. These levels were about twice as high as those of females.

It was assumed that the sex difference in the distribution of component fatty acids including phytanic acid in the lipid classes of mature chum salmon tissues was closely related to fish physiology in the spawning season, and also in the male the inactivation of lipid catabolism caused by completion of maturation took place in preference to that in the female.

Introduction

Three isoprenoid fatty acids [4, 8, 12-trimethyltridecanoic (4, 8, 12-TMTD), 2, 6, 10, 14-tetramethylpentadecanoic (pristanic) and 3, 7, 11, 15-tetramethylhexadecanoic (phytanic) acids] have been widely found in the marine organisms^{1~3,6,7}. Their occurrence and biochemistry have been summarized by some authors in datail^{1,4,5}.

The isoprenoid fatty acids have been found to constitute between 0.42-0.65% of the total fatty acids in marine fish, 0.40-1.57% in whale and 0.04-1.49% in zooplankton⁵⁾.

In humans, especially, a close relationship between phytanic acid and Refsum's syndrome has been revealed^{5,8,9)}. It has been suggested that the high concentration of this acid in the tissues is due to a decline in the catabolic process (in particular, α -oxidation).

Chum salmon, Oncorhynchus keta, migrate into rivers from the ocean for spawning and after spawning they die. During this time, numerous physiological and biochemical changes occur : maturation, starvation and utilization of stored chemical components¹⁰⁻¹⁴.

In order to clarify the relationship between the maturation and the lipid metabolism of chum salmon, it is necessary to investigate the lipid classes and fatty acid composition of the tissues in detail.

In the present study, the lipid class and fatty acid compositions of the dorsal

^{*} Laboratory of Marine Lipid Chemistry, Faculty of Fisheries, Hokkaido University (北海道大学水産学部魚油化学講座)

muscle, liver and serum of mature female and male chum salmon were investigated with special reference to phytanic acid.

Materials and Methods

Materials

Mature chum salmon, Oncorhynchus keta (3 females and 5 males), with an average body length of 69 cm were captured from Shiriuchi River in south Hokkaido, in December, 1982. Blood was collected from the caudal vessels with a hypodermic syringe. The serum was separated after centrifugation at 3,000 rpm for 15 min.

Young chum salmon having an average total length of 36 cm were caught from the North Pacific Ocean in July, 1981 at 55°59'N, 179°59'W as materials for comparison with mature chum salmon.

Extraction and separation of lipids

The lipids in the dorsal muscle, liver and serum were extracted by the method of Bligh and Dyer¹⁵⁾. Fractionation of the total lipids into neutral lipids (NL) and phospholipids (PL) was carried out by column chromatography on silicic acid (Silica gel 60, Merck) using chloroform and methanol as solvents. The NL was subsequently fractionated into the lipid classes by thin-layer chromatography (TLC) on silica gel plates (0.5 mm, Silica gel G) using hexane/diethyl ether/acetic acid (85:15:1, v/v) as the development solvent.

Fatty acid analysis

Fatty acid methyl esters (FAME) were prepared from the separated lipid classes by heating with 7% BF₃-methanol and/or 0.5N sodium methoxide-methanol followed by purification by TLC on silica gel plates (0.5 mm, Silica gel G, Merck) using hexane/diethyl ether (85:15, v/v) as the development solvent.

Separation of the isopreonoid fatty acid fraction from the total fatty acids for identification by gas chromatography-mass spectrometry (GC-MS) was carried out by argentation-TLC and urea fractionation.

The total FAME from chum salmon tissues was warmed with urea (9 parts) and methanol (50 parts) and this solution was stored at -20° C for 3 hrs and filtered. The non-urea complexing FAME was further fractionated by argentation-TLC (Silica gel G plates impregnated with 10% AgNO₃) using hexane/benzene (7:3 v/ v) as the development solvent.

GC analysis of FAME was carried out on a Yanagimoto G80 gas chromatograph equipped with a flame ionization detector. Two glass columns $(1.5 \times 3 \text{ mm i.d.})$ with 15% BDS on Chromosorb WAW (80-100 mesh) at 215°C and 5% DEGS on chromosorb WAW (100-120 mesh) at 190°C, respectively, were used. Individual fatty acids were identified by comparison with known standards and equivalent chain length (ECL), and quantified using a Shimadzu Chromatopac E1A.

GC-MS analysis of isoprenoid fatty acids was performed with a Hitachi 60 M operated at 20 eV and equipped with a glass column $(2 \text{ m} \times 3 \text{ mm i.d.})$ packed with 3% Silar 10 C on Gas Chrom Q (100-120 mesh) at 150°C.

Results

Identification of isoprenoid fatty acids

The gas chromatogram of the methyl esters of the isoprenoid acid fraction concentrated from chum salmon tissue showed three peaks with ECL values of 14.05 (A), 15.52 (B) and 16.86 (C), respectively, on the BDS column (Fig. 1). As shown in Fig. 2, the mass spectrum of component C showed a molecular ion peak (M^+) at m/e 326 and the fragment peaks at m/e 101 (basepeak) characteristic of the 3-methyl substituent, m/e 143 and 171 characteristic of the 7-methyl substituent, m/e 213 and 241 characteristic of the 11-methyl substituent and m/e 283 and 311 (M-15) characteristic of the 15-methyl substituent. From these results component C was confirmed to be methyl phytanate¹⁶). Similarly, the mass spectra of components A and B showed characteristic fragment peaks for methyl esters of 4, 8, 12-TMTD and pristanic acids (data not shown).

Lipid content and lipid class composition

Table 1 shows the lipid content and lipid class composition of chum salmon tissues. Lipids from each tissue were rich in PL which constituted from 42.4 to 75.0% of the total lipids except for that of the female dorsal muscle. It was clear that the serum contained more SE and less FFA; the liver contained more FFA and free sterols (ST) and less TG; and the dorsal muscle contained more TG and less SE than the other tissues. The contents of TG and FFA as mg per 100 ml in the serum were 60-89 mg and 14-19 mg, respectively. These levels were similar to the values reported by Patton et al.¹⁷⁾ for pink salmon collected from their spawning ground.

In young chum salmon liver, the FFA content was unexpectedly high. This may be partly due to hydrolysis of the lipids during the longer storage of the sample on board ship.

In the serum, the SE content was higher in the male than the female. On the other hand, in the dorsal muscle, the TG content was higher in the female than the male. Such sex differences were also observed in the fatty acid composition of the individual lipid classes as described below.



Fig. 1. Gas chromatogram on BDS column of methyl esters of isoprenoid fatty acid fraction. ECL: A-14.05, B-15.52, C-16.86

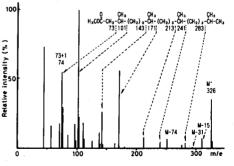


Fig. 2. Mass spectrum of methyl ester of isoprenoid fatty acid fraction C having ECL 16.86 on BDS column.

- 315 -

	Ser			Li	Dorsal muscle				
	Mature					ing	Mature		
	Female	Male	Female	Male	Female	Male	Female	Male	
Lipid content	mg/100 ml			C	%				
Lipid content	1030	983	2.8	3.8	3.9	3.8	2.8	1.8	
Lipid class (% of total lipids)		-							
Sterol esters ^{*1}	13.9	19.5	1.0	1.1	4.9	6.0	0.4	0.4	
Triacylglycerols	8.6	6.1	0.9	1.8	1.6	1.6	62.6	43.2	
Free fatty acids	1.4	1.9	14.1	12.5	39 .8	41.0	5.2	7.2	
Sterols	8.1	8.0	10.9	8.7	10.3	9.0	2.5	3.0	
Phospholipids	67.5	64.1	72.4	75.0	43.3	42.4	27.0	44.0	
Others*2	0.4	0.4	0.7	0.9	—	—	2.3	2.1	

Table 1. Lipid content and lipid class composition of chum salmon tissues.

*1 Includes a small amount of hydrocarbons.

*2 Mainly mono-and di-acylglycerols.

Fatty acid composition

The fatty acid composition of the lipid classes of chum salmon tissues are shown in Tables 2, 3 and 4. In general, the predominant saturated fatty acid was 16:0 except for serum FFA. The main components in the mono- and poly-unsaturated fatty acids were 16:1, 18:1, 20:1, 22:1, 20:5 (n-3) and 22:6 (n-3). Furan fatty acids^{18~20)} (mainly F_6 and F_4 acids) which are fatty acids characteristically found in fish lipids, were found only in the serum SE of mature chum salmon with ca. 10% of the total fatty acids.

Isoprenoid acids tended to be concentrated mainly in the lipid classes of the serum and liver rather than those of the dorsal muscle.

The occurrence and distribution of isoprenoid fatty acids in marine organisms have been summarized by several authors^{1,5)}. In this study, the isoprenoid acids as the sum of pristanic and phytanic acids were found in the lipid classes of mature chum salmon tissues in the range 0.3-11.9% of the total fatty acids. These levels were relatively high as compared to those from marine fish lipids²⁰⁾. As shown in Table 3, relatively high amounts (ca. 5%) of these acids were found in the liver TG of young chum salmon that live on plankton and also, phytanic acid in the TG of *E. pacifica* constituted 6.0% of the total fatty acids (data not shown). Hence, these acids were assumed to be concentrated in chum salmon tissues from the diets rather than endogenous *de novo* synthesis as stated by Ackman et al.¹⁾.

The distribution of phytanic acid in the lipid classes of serum was markedly different from those of the liver and dorsal muscle. This acid in the serum was found predominantly in the FFA, but accumulated in the TG or SE in the liver and dorsal muscle. Further, the comparison of the fatty acid composition of lipids in the male with that of the female definitely showed that the former TG and FFA contained more long-chain monounsaturated acids (20:1 and 22:1) as well as phytanic acid.

— 316 —

OTA & TAKAGI: Phytanic acid in lipids of chum salmon

		Fei	male	Male					
Fatty acid	SE*1	TG*1	FFA*1	PL*1	SE	TG	FFA	PL	
12:0	-		_				_		
Iso 14:0	0.1	0.2	0.2	_	0.2	0.1	0.1	_	
14:0*2	0.4	2.9	2.6	3.4	0.6	4.4	2.0	4.3	
15:0	0.2	0.2	0.3	0.4	0.3	0.1	0.2	0.5	
Pristanic	Tr*3	0.1	1.6	Tr	0.1	0.7	1.6	0.1	
16:0	23.7	7.9	10.1	23.4	32.8	5.8	6.9	26.9	
Phytanic	0.7	1.6	5.8	0.7	1.0	4.4	10.3	1.7	
18:0	5.3	1.0	4.9	8.9	1.8	1.6	3.3	2.7	
14:1	-	Tr	Tr	Tr	_	_	Tr	T	
16:1	1.4	10.6	4.5	3.6	1.4	9.1	2.9	4.(
17:1	0.4	0.8	0.3	0.4	0.3	0.2	Tr	0.2	
18:1	15.0	41.9	17.9	18.8	11.3	33.2	13.2	12.'	
20:1	0.6	2.1	8.2	1.5	1.1	7.1	14.4	1.1	
22:1	<u> </u>	0.9	13.3	0.6	0.2	6.2	26.2	1.5	
24:1		_	3.2		—	—	5.1		
18:2 (n-6)	0.2	0.9	0.3	0.2	0.4	1.1	0.3	0.8	
18:3 (n-6)	Tr		0.1	Tr	0.2	0.2	0.1	_	
18:3 (n-3)	0.1	1.2	0.3	0.2	0.3	1.2	0.2	0.6	
18:4 (n-3)	0.2	1.1	0.4	0.2	0.5	1.5	0.2	0.6	
20:2 (n-6)	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	
20:3 (n-6)	-	0.1	0.2	0.1		0.1	0.2	0.1	
20:4 (n-6)	0.8	0.7	1.0	1.4	1.5	0.2	0.2	1.	
20:4 (n-3)	0.4	1.8	0.7	0.6	1.0	2.2	0.5	0.9	
20:5 (n-3)	23.1	13.5	10.9	11.8	16.8	12.5	4.2	15.8	
22:5 (n-6)	Tr	Tr	—	0.1	0.1	0.2	_	0.5	
22:5 (n-3)	2.1	4.6	2.8	5.3	1.9	3.4	1.7	5.5	
22:6 (n-3)	14.7	4.8	10.1	17.2	14.8	2.6	5.3	16.'	
Total saturates	30.4	13.9	25.5	36.8	36.8	17.1	24.4	36.5	
Total monoenes	17.4	56.3	47.4	24.9	14.3	55.8	61.8	19.8	
Total polyenes	41.7	28.8	26.9	37.2	37.7	25.3	13.0	42.2	
Furan fatty acids	9.6		_	_	10.7		—		
Unknowns	0.9	1.1	0.2	1.1	0.5	1.8	0.7	1.9	

Table 2. Fatty acid composition of lipid classes of chum salmon serum (% of total fatty acids).

*1 SE-Sterol esters, TG-Triacylglycerols, FFA-Free fatty acids, PL-Phospholipids.

*2 Includes a small amount of 4, 8, 12-trimethyltridecanoic acid.

*3 Trace (less than 0.05%).

	Mature									_	You	ing			1	
Fatty acid		Fe	male			Ma			_		ıale		Male			
•	SE*1	TG* ¹	FFA*1	PL*1	SE	TG	FFA	PL	SE	TG	FFA	PL	SE	TG	FFA	PL
12:0									Tr*2	0.2	Tr	Tr	Tr	0.2	Tr	Tr
Iso 14:0	0.2	0.2	Tr	0.1	0.3	0.1	Tr	0.1	0.1	0.1	\mathbf{Tr}	Tr	0.1	0.1	\mathbf{Tr}	Tr
14:0*3	4.0	1.5	2.9	3.0	1.8	5.6	4.6	3.1	1.5	3.1	2.9	1.5	1.6	2.9	2.9	1.6
15:0	0.3	0.3	0.2	0.3	0.2	0.2	0.2	0.5	0.4	0.3	0.4	0.3	0.4	0.2	0.4	0.3
Pristanic	0.3	0.1	0.1	0.1	0.9	2.4	0.4	0.1	0.2	1.4	0.1	0.1	0.2	1.6	0.1	0.1
16:0	10.7	20.5	10.7	19.0	22.8	7.1	9.5	27.0	12.9	9.0	15.9	17.0	11.8	9.3	16.8	18.8
Phytanic	2.1	1.2	0.6	0.7	1.9	7.0	1.9	0.5	2.5	3.3	2.5	1.4	2.2	2.9	2.4	1.5
18:0	2.2	5.3	2.2	7.1	2.2	4.0	1.5	3.0	1.4	9.1	6.1	8.4	1.5	12.0	6.5	9.3
14:1	_		Tr	Tr	Tr	0.1	0.1	Tr	\mathbf{Tr}	0.1	0.1	Tr	Tr	0.1	0.1	Tr
16:1	8.8	5.9	7.3	3.3	7.1	6.2	7.1	2.1	3.9	4.3	4.4	0.6	3.6	3.6	4.4	0.5
17:1	0.7	0.6	0.5	0.4	0.6	0.3	0.5	0.4	0.8	0.5	1.0	0.5	0.8	0.4	0.9	0.5
18:1	38.2	19.2	28.5	22.0	20.7	31.5	25.7	16.2	14.4	31.0	23.8	16.7	14.2	28.8	23.2	16.6
20:1	4.0	1.8	3.0	1.9	5.2	12.5	9.0	2.6	10.0	8.7	3.8	3.6	13.7	8.2	3.8	3.8
22:1	3.0	0.8	1.4	0.2	5.2	13.0	8.5	0.7	7.2	5.5	1.6	0.9	9.9	6.1	1.8	1.0
24:1	- 1	_	_	_	_	0.6			0.6	0.6	\mathbf{Tr}	0.5	0.2	0.8	0.2	0.4
18:2 (n-6)	0.7	0.7	0.9	0.6	1.5	0.8	1.6	1.0	0.8	0.8	0.9	0.6	0.7	0.8	0.9	0.6
18:3 (n-6)	-	_	_	_	—	0.2	—	—	0.3	0.2	0.2	0.1	0.2	0.1	0.1	0.1
18:3 (n-3)	0.7	0.3	0.9	0.3	1.3	0.7	1.4	.0.6	0.3	0.2	0.7	0.3	0.3	0.2	0.7	0.3
18:4 (n-3)	0.9	0.8	1.1	0.3	1.5	0.4	1.1	0.4	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.1
20:2(n-6)	0.1	0.1	0.2	0.2	0.2	0.1	0.3	0.2	0.4	0.4	0.4	0.5	0.5	0.3	0.4	0.5
20:3 (n-6)	0.1	0.1	0.2	0.1	0.1	0.2	0.3	0.1	0.3	0.3	0.1	0.1	0.2	0.5	0.1	0.1
20:4 (n-6)	0.7	2.2	3.1	3.4	0.8	0.2	1.0	2.1	1.6	1.3	2.4	2.6	1.1	1.2	2.2	2.3
20:4 (n-3)	0.6	1.1	1.7	0.6	2.0	0.8	2.3	0.7	1.6	0.7	1.2	0.1	1.5	0.6	1.2	0.7
20:5 (n-3)	11.7	18.3	16.4	15.9	12.1	1.6	9.6	18.3	14.3	5.7	12.2	12.2	10.7	6.4	12.6	12.1
22:5 (n-6)	Tr	0.1	0.1	0.1	0.1	0.1	—	0.2	0.3	0.2	0.2	0.3	0.4	0.1	0.1	0.3
22:5(n-3)	4.6	3.6	5.3	4.9	2.7	2.2	5.8	5.0	2.8	2.3	2.9	3.6	4.3	0.8	2.2	3.5
22:6 (n-3)	4.1	13.1	12.7	13.7	7.0	1.7	7.3	13.2	14.2	9.1	14.1	26.3	15.1	7.8	13.9	23.1
Total saturates	19.8	29.1	16.7	30.3	30.1	26.4	18.1	34.3	19.0	26.5	27.9	28.7	17.8	29.2	29.1	31.6
Total monoenes	54.7	28.3	40.7	27.8	38.8	64.2	50.9	22.0	36.9	50.7	34.7	22.8	42.4	48.0	34.4	22.8
Total polyenes	24.2	40.4	42.6	40.1	29.3	9.0	30.7	41.8	37.1	21.4	35.5	47.0	35.2	19.0	34.6	43.7
Furan fatty acids	_	_	_	_		_		—	2.7	_	. —		0.3	_	_	_
Unknowns	1.3	2.2	0.1	1.9	1.8	0.5	0.2	2.0	4.2	1.4	1.8	1.4	4.4	3.8	1.9	1.9

Table 3. Fatty acid composition of lipid classes of livers of mature and young chum salmon (% of total fatty acids).

*1 SE-Sterol esters, TG-Triacylglycerols, FFA-Free fatty acids, PL-Phospholipids.

*² Trace (less than 0.05%).

*3 Includes a small amount of 4, 8, 12-trimethyltridecanoic acid.

Bull. Fac. Fish. Hokkaido Univ. 40(4), 1989.

OTA & TAKAGI: Phytanic acid in lipids of chum salmon

		Fei	nale	Male					
Fatty acid	SE*1	TG*1	FFA*1	PL*1	SE	TG	FFA	PL	
12:0	0.7	0.2	0.1		0.3	0.2	0.1	_	
Iso 14:0	1.3	Tr*2	0.1	Tr	2.1	Tr	0.1	Tr	
14:0*3	7.3	5.6	3.9	4.2	3.2	5.2	3.5	3.6	
15:0	1.1	0.3	0.4	0.5	0.5	0.2	0.3	0.3	
Pristanic	1.1	0.1	Tr	0.1	0.5	0.1	Tr	0.1	
16:0	22.1	9.1	17.4	29.8	29.7	6.3	18.0	29.4	
Phytanic	1.2	0.6	0.3	0.9	2.2	0.8	0.4	0.7	
18:0	5.0	2.8	2.7	4.4	2.8	2.5	2.1	3.5	
14:1	Tr	0.1	Tr	_	—	0.1	Tr		
16:1	15.6	10.0	8.1	5.4	11.0	8.5	6.1	4.1	
17:1	1.3	1.0	1.3	0.9	1.0	0.3	0.4	0.3	
18:1	16.5	31.1	24.9	16.3	15.8	24.9	18.0	12.2	
20:1	1.1	11.5	7.3	1.9	3.6	17.5	10.6	2.2	
22:1	0.5	9.6	5.0	0.6	2.6	17.1	8.5	0.7	
24 :1	Tr	0.4	0.2	0.2	Tr	1.3	0.6	0.1	
18:2 (n-6)	1.1	0.6	1.1	0.5	0.8	0.9	1.2	0.5	
18:3 (n-6)	0.2	0.2	0.1	Tr	·	0.2	_	-	
18:3 (n-3)	0.5	0.5	1.0	0.4	0.8	0.7	1.1	0.4	
18:4 (n-3)	0.3	0.6	1.0	0.3	0.4	0.6	0.6	0.4	
20:2 (n-6)	0.1	0.2	0.2	0.1	0.1	0.2	0.2	0.1	
20:3 (n-6)	-	0.1	0.2	0.1	—	0.1	0.2	0.1	
20:4 (n-6)	0.6	0.2	0.4	0.6	0.4	0.1	0.4	0.7	
20:4 (n-3)	0.5	0.7	1.3	0.6	1.3	0.5	1.1	0.4	
20:5 (n-3)	13.9	4.0	9.8	12.2	9.4	2.4	7.8	11.1	
22:5 (n-6)	_	0.1	0.1	0.1	Tr	0.1	0.1	0.2	
22:5 (n-3)	1.1	3.5	3.4	2.6	2.0	3.7	3.7	2.5	
22:6 (n-3)	3.7	6.5	9.6	16.6	6.5	5.0	14.5	26.1	
Total saturates	39.8	18.7	24.9	39.9	41.3	15.3	24.5	37.6	
Total monoenes	35.0	63.7	46.8	25.3	34.0	69.7	44.2	19.6	
Total polyenes	22.0	17.2	28.2	34.1	21.7	14.5	30.9	42.5	
Furan fatty acids	-	_	_	_	-	_	-	_	
Unknowns	3.2	0.4	0.2	0.7	3.0	0.5	0.4	0.4	

Table 4. Fatty acid composition of lipid classes of chum salmon dorsal muscle (% of total fatty acids).

*1 SE-Sterol esters, TG-Triacylglycerols, FFA-Free fatty acids, PL-Phospholipids.

*² Trace (less than 0.05%).
*³ Includes a small amount of 4, 8, 12-trimethyltridecanoic acid.

Discussion

In humans, a close relationship between the high accumulation of phytanic acid and Refsum's disease has been documented^{5,8,21,22)}. The high accumulation of this acid in the tissues of patients is considered to be due to the inactivation of lipid catabolism (particularly α -oxidation). In this study, the distribution of this acid among the lipid classes of mature chum salmon serum was different from that of the serum of patients with Refsum's disease in that this acid comprised 40% of the total fatty acids in the TG and 1% in the FFA²³.

In salmon, several physiological changes are known to occur during spawning. However, it is not clear whether or not the symptom (cerebellar ataxia and visual stenosis) similar to Refsum's disease occurs in mature chum salmon. The contents of the main fatty acids in the tissues of mature chum salmon are summarized in Table 5. The contents of phytanic acid in the liver and serum of the male were about twice as large as those of the female. These findings suggest that sex differences in the lipid metabolism of chum salmon occur during the spawning season.

As to the gonad development in salmonids, the gonadosomatic index of the testes of rainbow trout (Salmo gairdneri) reaches a maximum prior to spawning,

Fatter and	Sex	Serus	m	Live	r	Dorsal Muscle		
Fatty acid	Sex	mg/100 ml*1	Ratio*2	$mg/100 g^{*1}$	Ratio	mg/100 g	Ratio	
Phytanic	Female	5	0.42	12		14	1.40	
	Male	12	0.42	22	0.55	10		
16.0	Female	119	0.91	279	0.54	305	1 47	
	Male	131	0.91	515	0.34	208	1.47	
16.1	Female	25	1.14	71	0.95	195	2.22	
16:1	Male	22	1.14	75	0.90	88	4.42	
10.1	Female	123	1 60	389	0.92	602	2.35	
18:1	Male	77	1.60	422	0.92	256	2.00	
00.1	Female	9	0.00	36	0.38	201	1.37	
20:1	Male	13	0.69	96	0.30	147	1.37	
00 - 1	Female	6	0.43	8	0.13	162	1.21	
22:1	Male	14	0.45	61	0.15	134		
90 · F (- 9)	Female	75	0.02	263	0.79	133	1.66	
20:5 (n-3)	Male	81	0.93	362	0.73	80		
99.6(-9)	Female	86	1 10	221	0.94	192	1.00	
22:6 (n-3)	Male	77	1.12	263	0.84	178	1.08	

Table 5. Contents of major fatty acids in mature chum salmon tissues.

*1 The values were calculated from the contents of SE, TG, FFA and PL in the tissues and the ratios of fatty acids/fatty acid esters (0.4 for SE, 0.9 for TG and 0.6 for PL).

*² Female/Male.

followed by a decrease. Whereas that of the ovary increases gradually and reaches a maximum during the late spawning period²⁴). Such a difference in the procedure of gonad development between the female and male may affect the catabolism of the stored lipids. Hence, it can be suggested that the sex difference of the distribution of the lipid components as stated above is closely related to fish physiology during the spawning season. That is to say, the decline of lipid catabolism caused by completion of maturation in the male chum salmon occurs in preference to the female.

It is suggested that the isoprenoid acids have no important role as biomembrane constituents because of their low levels in the PL of the tissues examined (Tables 2, 3 and 4). It can be assumed that they are accumulated in the tissues when the normal oxidation process is blocked by inactivation of lipid catabolism in the late spawning season.

Selective utilization of lipids in fish under starvation, migration and maturation has been discussed by some authors. Ando et al.²⁵⁾ reported that no selective consumption of the fatty acids of chum salmon muscle lipids occured during spawning migration. However, although the changes in the lipids of chum salmon during the spawning season are well investigated, sex difference in the fatty acid composition among lipid classes has not been appreciably studied. In spite of no significant differences in the FFA content of the serum between the female and male as shown in Table 1, a distinct sex difference in the fatty acid composition was observed in the FFA, that is, the metabolically active component of blood and a major source of energy. These findings suggest that lipid metabolism (transfer and oxidation of fatty acids) between the female and male is affected by their maturation and proceeds with different activity during the spawning season.

Isoprenoid fatty acids have been used as an indication on food web research in marine biochemistry¹). In addition, phytanic acid may be available as an indication for physiological study in salmonids at spawning season.

The sex differences in the content of fatty acids in chum salmon tissues were also observed in the long-chain monounsaturated fatty acids. As shown in Table 5, the ratios of female to male of 20:1 and 22:1 in the serum were 0.69 and 0.43 respectively, and these levels were slightly low compared to those (0.91-1.60) of other fatty acids except phytanic acid. These results also suggest that the longchain monounsaturated fatty acids as well as phytanic acid are not subject to oxidation compared to other fatty acids as described by Beare-Rogers²⁶⁾.

References

- Ackman, R.G. and Hooper, S.N. (1968). Examination of isoprenoid fatty acids as distinguishing characteristics of specific marine oils with particular reference to whale oils. Comp. Biochem. Physiol. 24, 549-565.
- Hansen, R.P. and Meiklen, S. (1970). Isoprenoid fatty acids in Antarctic krill (Euphausia superba). J. Sci. Fd Agric. 21, 203-206.
- Ackman, R.G., Hooper, S.N. and Ke, P.J. (1971). The distribution of saturated and isoprenoid fatty acids in the lipids of three species of molluscs, *Littorina littorea*, *Crassostrea virginica* and Venus mercenaria. Comp. Biochem. Physiol. 39B, 579-587.
- Sen Gupta, A.K. (1972). Recent advances in the chemistry and biochemistry of methyl branched fatty acids. *Fette Seifen Anstrichm.* 74, 693-705.

Bull. Fac. Fish. Hokkaido Univ. 40(4), 1989.

- Lough, A.K. (1973). The chemistry and biochemistry of phytanic, pristanic and related acids. In "Progress in the Chemistry of Fats and Other Lipids" ed. by Holman, R.T., Pergamon Press, London, 14, 1-48.
- Carballeira, N.M., Maldonado, L. and Parras, B. (1987). Isoprenoid fatty acids from marine sponges. Are sponges selective? *Lipids* 22, 767-769.
- Ackman, R.G., Eaton, C.A., Sipos, J.C., Hooper, S.N. and Castell, J.D. (1970). Lipids and fatty acids of two species of North Atlantic krill (Meganyctiphanes norvegica and Thysanoëssa inermis) and their role in aquatic food web. J. Fish. Res. Bd. Canada 27, 513-533.
- Hansen, R.P. (1965). 3, 7, 11, 15-Tetramethylhexadecanoic acid: Its occurrence in the tissues of humans afflicted with Refsum's syndrome. *Biochim. Biophys. Acta* 106, 304-310.
- Eldjarn, L., Try, K., Ackman, R.G. and Hooper, S.N. (1968). Different ratios of the LDD and DDD diastereoisomers of phytanic acid in patients with Refsum's disease. *Biochim. Biophys.* Acta 164, 94-100.
- Zama, K. and Igarashi, H. (1954). Biochemical studies of the salmon, Oncorhynchus keta-II. The changes in the components of depot fats during spawning migration. Bull. Jap. Soc. Sci. Fish. 19, 1087-1091.
- Takahashi, H., Kaneko, H. and Ichisugi, T. (1976). Biochemical studies of the salmon and trout 1. Chemical component of the salmon (Oncorhynchus keta) during the spawning migration. J. Hokkaido Fish. Exp. Station 33, 1-6.
- Takahashi, H., Kaneko, H. and Ichisugi, T. (1978). Biochemical studies of the salmon and trout 2. Lipid composition of salmon (*Oncorhynchus keta*) during the spawning migration. J. Hokkaido Fish. Exp. Station 35, 8-13.
- 13) Hatano, M., Takama, K., Kojima, H. and Zama, K. (1983). Proximate composition of fall chum salmon. Bull. Jap. Soc. Sci. Fish. 49, 213-218.
- 14) Ando, S., Yamazaki, F. and Hatano, M. (1986). Influence of 17α-methyltestosterone on the level and composition of lipid in adult chum salmon muscle. Bull. Fac. Fish. Hokkaido Univ. 37, 246-251.
- Bligh, E.G. and Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911-917.
- Sen Gupta, A.K. and Peters, H. (1966). Isolierung und Strukturaufklärung polyverzweiter Fettsäuren aus Fischöl. Fette Seifen Anstrichm. 68, 349-360.
- Patton, S., Crozier, G.F. and Benson, A.A. (1970). Serum lipids and the death of spawning pacific salmon. Nature 225, 754-755.
- Glass, R.L., Krick, T.P., Sand, D.M., Rahn, C.H. and Schlenk, H. (1975). Furanoid fatty acids from fish lipids. *Lipids* 10, 695-702.
- Glass, R.L., Krick, T.P., Olson, D.L. and Thorson, R.L. (1977). The occurrence and distribution of furan fatty acids in spawning male freshwater fish. Lipids 12, 828-836.
- 20) Gunstone, F.D., Wijesundera, R.C. and Scrimgeour, C.M. (1978). The component acids of lipids from marine and freshwater species with special reference to furan-containing acids. J. Sci. Fd Agric. 29, 539-550.
- Avigan, J. (1966). The presence of phytanic acid in normal human and animal plasma. Biochim. Biophys. Acta 116, 391-394.
- 22) Skjeldal, O.H. and Stokke, O. (1987). The subcellular localization of phytanic acid oxidase in rat liver. Biochim. Biophys. Acta 921, 38-42.
- 23) Karlsson, K.-A, Norrby, A. and Samuelsson, B. (1967). Use of thin-layer chromatography for the preliminary diagnosis of Refsum's disease (Heredopathia atactica polyneuritiformis). *Biochim. Biophys. Acta* 144, 162-164.
- 24) Nomura, M. (1963). Studies on reproduction af rainbow trout, Salmo gairdneri, with special reference to egg taking-V. Development of gonads and size of fish spawned firstly. Bull. Jap. Soc. Sci. Fish. 29, 976-984.
- 25) Ando, S., Hatano, M. and Zama, K. (1985). A consumption of muscle lipid during spawning migration of chum salmon Oncorhynchus keta. Bull. Jap. Soc. Sci. Fish. 51, 1817-1824.
- 26) Beare-Rogers, J.L. (1977). Docosenoic acids in dietary fats. In "Progress in the Chemistry of Fats and Other Lipids" ed. by Holman, H.T., Pergamon Press, London, 15, 29-56.