Applied Biochemistry and Microbiology, Vol. 37, No. 3, 2001, pp. 314–317. Translated from Prikladnaya Biokhimiya i Mikrobiologiya, Vol. 37, No. 3, 2001, pp. 364–368.
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Adaptive Changes in Fatty Acid Compositions of Whitefish *Coregonus lavaretus* L. Tissue Lipids Caused by Anthropogenic Factors

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Received August 03, 2000

Abstract—Adaptive processes in the body of whitefish (*Coregonus lavaretus* L.) caused by anthropogenic effects on aquatic systems were studied. It was demonstrated that the content of fatty acid acyls correlated with the water pollution level. The role of a decrease in the content of arachidonic acid in fish reproduction under adverse conditions is discussed. It is underlined that the quantitative alterations in the gonad and liver fatty acid patterns are unidirectional. The compensatory character of the changes discovered is hypothesized.

The survival of hydrobionts under changing environmental conditions depends in many respects on their adaptive potential. Studies of biochemical reactions of the body are essential for assessing the vital activity of ichthyofauna in industrially polluted aquatic systems, as changes in different biochemical indices allow one to evaluate the effects of pollution components and the adaptive potential of hydrobionts. Various ecological factors cause rather quick and distinct changes in the structure of fish biological membranes. The diverse functions of the biological membrane and the activity of membrane-associated enzymes under both normal and extreme conditions depend on the state of its lipid component. It is known that fatty acids are the most labile component of lipids and that their biochemical rearrangements represent a universal adaptation mechanism [1]. Alterations in the quantitative ratios of fatty acid acyls upon changing of the water quality in a biotope demonstrate a sufficiently distinct dependence pattern of the fish biochemical status on the ecological conditions and physiological status of individuals [2].

As whitefishes are evolutionarily young and display adaptive plasticity [3] and a high sensitivity to water pollution [4, 5], they are a convenient object for physiological and biochemical studies.

The goal of this work was to study changes in the fatty acid patterns in whitefish gonad and liver tissues in response to the toxic effects of industrial discharges.

MATERIALS AND METHODS

Female whitefishes of the fourth growth stage were caught in the basin of Lake Imandra (Kola Peninsula) at sites with different pollution levels: Lake Syav and the Kislaya Bay, a site where wastewaters of copper–nickel, mining refinery, and apatite–nepheline plants were mixed; the Pirenga River, a distribution zone of the transit flow to the lake outlet; and the Kunchast Bay, a remote site without direct pollution.

Liver and gonad tissue samples (500 mg from each of five fishes) were homogenized in a chloroform–methanol (2 : 1 v/v) mixture containing 0.001% ionol (an antioxidant), the same mixture was added to a tenfold final volume, and the samples were stored at –4°C until analyzed. Lipids were extracted according to [6] and subjected to direct methanolysis [7]. The resulting mixtures of fatty acid methyl esters were analyzed by gas–liquid chromatography in a Khrom-4 (Russia) chromatograph using 15% polyethylene glycol adipate on Chromosorb W/AW (Merck, Germany). Fatty acids were identified through calculating their equivalent chain-length values and comparing them with standards [8]. Quantitative ratios of acids in the samples were assessed as described in [9].

RESULTS AND DISCUSSION

Up to 30 components, minor ones included, were detected in the fatty acid spectra of whitefish liver and gonad tissues. The fatty acid compositions displayed high contents of polyenoic acids (up to 55% of the total content) mainly due to the ω3 series acids. Palmitic (16 : 0), palmitoleic (16 : 1), oleic (18 : 1), arachidonic (20 : 4ω6), eicosapentaenoic (20 : 5ω3), and docosahexaenoic (22 : 6ω3) acid were relatively predominant both in the liver and roe. It is known that this fatty acid set is mediated by a low-temperature aquatic medium [1] and provides a necessary viscosity of biological membranes. A high content of polyunsaturated fatty acids in the membranes confers the low viscosity on these membranes and the resulting high activities of the membrane proteins [2, 10, 11].

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Table 1. Fatty acid composition of whitefish liver (% of Σ)

Fatty acids	Kunchast Bay	Pirenga River	Lake Syav	Kislaya Bay
10 : 1	–	–	–	0.1 ± 0.0**
13 : 0	–	–	–	0.3 ± 0.0
14 : 0	–	0.7 ± 0.0	–	0.6 ± 0.0
15 : 0	0.4 ± 0.0**	0.4 ± 0.0	0.2 ± 0.0	0.4 ± 0.0
15 : 1	0.1 ± 0.0	–	0.1 ± 0.0	–
16 : 0	19.1 ± 2.0	16.7 ± 2.0	14.6 ± 2.1	16.8 ± 1.8
16 : 1	3.9 ± 0.4	8.2 ± 1.1*	9.2 ± 1.2*	10.1 ± 1.1*
16 : 2ω4	–	–	0.3 ± 0.0	–
16 : 3ω4	–	–	0.4 ± 0.0	–
17 : 0	0.7 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
17 : 1	0.5 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
18 : 0	4.2 ± 0.5	3.4 ± 0.4	4.2 ± 0.3	4.6 ± 0.5
18 : 1	15.3 ± 1.9	18.4 ± 2.3	21.5 ± 3.2	19.0 ± 2.2
18 : 2ω6	2.1 ± 0.2	2.1 ± 0.3	1.5 ± 0.2	1.6 ± 0.2
18 : 3ω6	–	0.2 ± 0.0	–	–
18 : 3ω3	1.1 ± 0.1	1.4 ± 0.2	1.6 ± 0.3	0.9 ± 0.1
18 : 4ω3	0.7 ± 0.0	1.8 ± 0.3	1.6 ± 0.2	0.8 ± 0.1
20 : 2ω6	0.2 ± 0.0	0.1 ± 0.0	–	–
20 : 4ω3	–	0.2 ± 0.0	–	–
20 : 4ω6	7.9 ± 0.9	6.4 ± 0.5*	3.7 ± 0.4*	3.6 ± 0.4*
20 : 5ω3	9.3 ± 1.0	12.4 ± 1.5*	16.2 ± 2.1*	15.8 ± 1.8*
22 : 4ω6	0.3 ± 0.0	–	–	–
22 : 5ω6	1.1 ± 0.1	1.7 ± 0.2	–	–
22 : 5ω3	3.7 ± 0.4	2.4 ± 0.2	4.1 ± 0.5	3.6 ± 0.4
22 : 6ω3	29.4 ± 4.2	22.8 ± 3.6*	20.2 ± 3.8*	21.2 ± 2.2*

Notes to Tables 1 and 2:

* Significant difference with the control (Kunchast Bay) at $P \leq 0.05$.

** 0.0 indicates that the mean error is less than 0.05.

Minus (–) indicates that the acid was not detected.

Table 2. Fatty acid composition of whitefish roe (% of Σ)

Fatty acids	Kunchast Bay	Pirenga River	Lake Syav	Kislaya Bay
12 : 1	–	–	–	0.1 ± 0.0**
14 : 0	1.9 ± 0.2	2.2 ± 0.3	2.9 ± 0.3	3.3 ± 0.3
14 : 1	0.3 ± 0.0**	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
15 : 0	0.4 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
15 : 1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
16 : 0	15.8 ± 2.3	15.2 ± 2.1	13.0 ± 1.1	12.4 ± 1.5
16 : 1	9.8 ± 1.8	12.6 ± 1.3*	14.7 ± 1.5*	19.0 ± 1.8*
16 : 2ω4	0.1 ± 0.0	–	1.0 ± 0.1	0.8 ± 0.1
16 : 3ω4	0.2 ± 0.0	0.2 ± 0.0	1.5 ± 0.2	1.1 ± 0.2
17 : 0	0.4 ± 0.0	0.4 ± 0.0	0.1 ± 0.0	0.3 ± 0.0
17 : 1	0.5 ± 0.0	0.5 ± 0.0	–	–
18 : 0	1.9 ± 0.2	1.8 ± 0.3	1.8 ± 0.2	1.7 ± 0.2
18 : 1	18.7 ± 1.9	19.8 ± 2.0	20.6 ± 3.1	18.0 ± 2.1
18 : 2ω6	2.8 ± 0.3	2.5 ± 0.3	1.7 ± 0.7*	1.7 ± 0.2*
18 : 3ω6	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	–
18 : 3ω3	2.2 ± 0.2	1.9 ± 0.2	2.0 ± 0.2	1.7 ± 0.3
18 : 4ω3	1.6 ± 0.2	2.6 ± 0.3	2.2 ± 0.2	1.7 ± 0.2
20 : 0	–	–	0.3 ± 0.0	0.2 ± 0.0
20 : 2ω9	0.1 ± 0.0	–	–	–
20 : 2ω6	0.5 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	–
20 : 3ω6	0.3 ± 0.0	0.3 ± 0.0	–	–
20 : 4ω3	–	–	0.3 ± 0.0	–
20 : 4ω6	5.4 ± 0.6	3.0 ± 0.5*	1.8 ± 0.2*	2.1 ± 0.2*
20 : 5ω3	10.5 ± 2.5	11.5 ± 2.3	15.4 ± 2.3*	14.4 ± 1.5*
22 : 4ω6	0.5 ± 0.0	–	0.7 ± 0.0	–
22 : 5ω6	1.4 ± 0.0	1.2 ± 0.2	0.7 ± 0.0	0.4 ± 0.0
22 : 5ω3	3.1 ± 0.4	2.0 ± 0.3	2.5 ± 0.3	2.5 ± 0.3
22 : 6ω3	21.2 ± 3.5	21.3 ± 2.8	16.0 ± 2.0*	18.1 ± 2.0*

A comparison of aquatic sites with different pollution levels showed that the liver (Table 1) and gonad (Table 2) fatty acid patterns of the whitefish samples from the Pirenga River exhibit the values intermediate between those from the control clean site (Kunchast Bay) and polluted sites (Lake Syav and Kislaya Bay).

Thus, these data suggest a dependence between the quantitative content of fatty acid acyls in fish tissues and the pollution level. Comparison of the total contents of saturated, monoenoic, and polyenoic acid illustrates this effect best (Fig. 1). For example, the total content of saturated and polyenoic acids in the liver decreased with pollution, whereas the total monoenoic acid content increased. The quantitative changes in the gonad fatty acid contents followed the same pattern.

Note that monoenoic acids, especially, palmitoleic (16 : 1) acid, displayed the most pronounced changes. The palmitoleic content (% of the total) in the gonads increased 1.9-fold in the polluted area compared with the clean site; in the liver, 2.6-fold.

The pattern of polyenoic acids also underwent essential changes. For example, the contents of 18 : 2ω6, 20 : 4ω6, and 22 : 6ω3 in both the liver and gonads were decreased in the samples from polluted sites, whereas the content of 20 : 5ω3 acid was increased. Presumably, these acids play an important role in com-

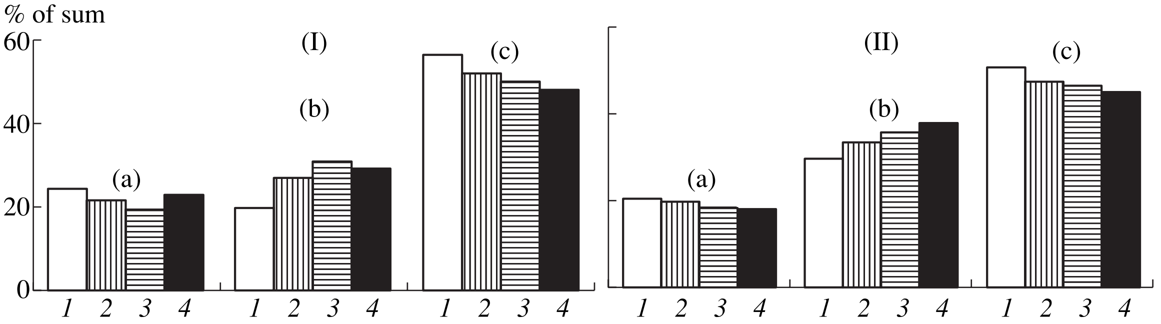

Fig. 1. Fatty acid composition in the (I) liver and (II) roe of whitefish from the basin of Lake Imandra, namely, (*1*) Kunchast Bay, (*2*) Pirenga River, (*3*) Lake Syav, and (*4*) Kislaya Bay: (a) saturated, (b) monoenoic, and (c) polyenoic fatty acids.

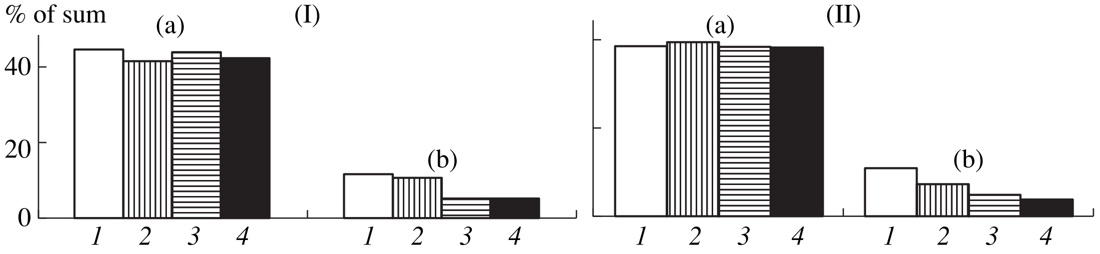

Fig. 2. Total (a) ω3-series and (b) ω6-series fatty acids in the (I) liver and (II) roe of whitefish from the basin of Lake Imandra: (*1*) Kunchast Bay, (*2*) Pirenga River, (*3*) Lake Syav, and (*4*) Kislaya Bay.

pensating adverse effects on the body. For example, it is known that the contents of the acids listed change under stressful conditions such as water temperature and salinity variations and pollution by toxicants [12, 13]. Most likely, changes in environmental parameters affect the systems of polyene desaturation and elongation and, possibly, increase the oxidation processes in the fish body. For example, the authors of [14] explained the deficiency of 22 : 6ω3 acid observed in marine fish with a low conversion rate of 20 : 5ω3 acid into 22 : 6ω3 acid. Both the impairment in Δ4-desaturation and the activation of oxidation or retroconversion of docosahexaenoate [15] may underlie the increase in the content of 20 : 5ω3 and the simultaneous decrease in the content of 22 : 6ω3 acid. However, it is possible that the changes observed resulted not from direct conversion of 22 : 5ω3 into 22 : 6ω3 through Δ4-desaturation but are mediated by a set of transformations through elongation of 22 : 5ω3 to 24 : 5ω3 acid, its subsequent Δ6-desaturation to 24 : 6ω3, and final peroxisomal β-oxidation to 22 : 6ω3 [16, 17]. Such metabolic reactions were found in rats [18] and rainbow trout [19].

Decreases in the ratios of 20 : 4ω6 to 18 : 2ω6 acids, reflecting the rate of essential linoleic acid conversion into arachidonic acid, with an increase in the pollution level in both the liver and gonads are of special importance. The biosynthesis of arachidonic (20 : 4ω6) acid from the alimentary precursor, linoleic (18 : 2ω6) acid, through elongation and desaturation reactions is the main pathway providing the body with this compound. The efficiency of this biosynthesis depends on both the amount of linoleic acid and the activities of the desaturation and elongation enzymes [20]. Remember that the content of 20 : 4ω6 acid in the polluted site decreased twofold in the liver and threefold in the gonads. Because this acid is a precursor of prostaglandins and thromboxanes [21–23], this finding suggests that the decrease observed, indirectly affecting the hormone levels, is involved in changing the strategy of the fish life cycle with a delayed maturation [24].

Note that the whitefish liver and gonad samples from the clean site contained 44.2 and 38.6% ω3 series acids, respectively, and 11.6 and 11.2% ω6 series acids. The ω3 to ω6 ratios equaled 3.8 and 3.5 for the liver and gonads, respectively. These values fall in the range typical of freshwater fish (1–4) [25].

The toxicological effects of industrial wastewater altered the characteristics described above. Upon all the quantitative changes in the fatty acid relative contents in both the liver and gonads, the total contents of ω3 fatty acids remained constant, whereas the total content of ω6 fatty acids decreased more than twofold (Fig. 2). Thus, the ω3 to ω6 ratio increased more than twofold in both the liver and gonads, suggesting that the functional status of biological membranes of the tissues in question altered within the range of the body compensatory potential.

These data suggest that alterations in the liver and gonad fatty acid compositions were unidirectional. It is likely that the overall lipid metabolism of the body had changed to support the optimal physicochemical status of the membrane lipids in order to provide for the adaptation of fishes to new environmental conditions.

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