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Lipid Status and Fatty Acid Spectrum of the Black Baikalian Grayling *Thymallus arcticus baicalensis* Dybowski, 1874

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Abstract

For the first time the content of various classes of lipids and fatty acids in muscles and liver of the black Baikalian grayling *Thymallus arcticus baicalensis* Dybowski, 1874 was determined. It has been revealed that in all tissues under investigation there are neutral lipids (triglycerides) prevailing, whereas the content of phospholipids in red muscles is twice lower as compared to their content in white muscles. Among fatty acids of the total lipids in all the organs there are polyunsaturated acids (44.58–53.7 %) and saturated (19.06–30.94 %) acids prevailing. Polyunsaturated acids are mainly presented by acids 22:6n-3 and 20:5n-3 whose total fraction amounts to 19.5–39.1 %. The n-3/n-6 ratio between fatty acids of the mentioned series depends on tissue kind and varies within the range of 2.19–4.02. For the lipids of white muscles, the fraction of polyunsaturated fatty acids is higher at the expense of docosahexaenoic acid. Due to the total fat content and the balanced n-3/n-6 ratio between polyunsaturated fatty acids of corresponding series in the muscular tissue, the black Baikalian grayling could be use as a high-quality raw material for developing the dietary and specialized kinds of foodstuff as well as biologically active supplements.

Key words: fatty acids, black Baikalian grayling *Thymallus arcticus baicalensis*, muscles, liver, lipids

INTRODUCTION

The role of polyunsaturated fatty acids (PUFA) in the nutrition of a human and in the prophylaxis of many systemic diseases is well-known [1]. A special place among them is occupied by fatty acids of n-3 and n-6 series (ω3 and ω6 fatty acids), since they represent precursors of eucosanoids participating in the major physiological and biochemical processes in a human organism [2]. The diet of euhominids is basically characterized by the prevalence of fats from land mammals wherein n-6 PUFA are inherent.

Lipids of aquatic organisms differ from those of land animals and plants, particularly, in fatty-acid composition. For normal functioning of a human organism the acids belonging to n-3 series such as eucosapentaenic acid (20:5 n-3, EPA) and docosahexaenic acid (22:6 n-3, DHA) are required those are synthesized in aquatic organisms, mainly in micro-algae [3]. These acids enter through food chains into higher trophic levels whose terminal unit is presented by a human being. Until recent time the lipids of fresh-water fish species were of no interest as an object of biochemical research, since sea fishery satisfied population's requirements

for essential PUFA of series n-3 [4, 5]. However, for midland areas the problem of PUFA food sources is of urgent importance, which determines an increased interest with respect to biochemical studies concerning fresh-water fishes. They differ from sea fishes [6] in the content of lipids (in particular, in the n-3/n-6 PUFA ratio) which depends on fishes' feeding sources.

The contribution of fresh-water fish used to the dietary intake of the population of Eastern Siberia is great enough, however the materials concerning biochemical studies on the Baikal fishes are not numerous. Detailed characteristics of lipids and fatty acids (FA) is presented only for Baikal pelagic bullhead fishes [7–9] those do not represent commercial species, whereas the features of fat accumulation in black Baikalian grayling and Baikalian omul are described by the authors of [10–12]. In this connection the studies concerning nutritional value and fatty-acid composition of the Baikal Lake food fishes has of currently central importance.

The representatives of family Thymallidae live in almost all fresh-water reservoirs (rivers and lakes) of the northern hemisphere; in some countries they serve as objects of aquiculture [13]. The results of biochemical studies concerning graylings living in water reservoirs of Siberia have demonstrated that the composition of lipids includes essential PUFA, in particular 20:5n-3 and 22:6n-3 species [14]. The black Baikalian grayling dominates within the littoral zone of the Baikal Lake being of local fishing importance [15, 16]. Detailed characteristics of the lipids and FA of this species is significant both for the estimation of the food value the black Baikalian grayling, and for establishing its role in the lake ecosystem. The purpose of the present investigation consisted in the determination of the lipid status [17] and FA spectrum for the black Baikalian grayling.

EXPERIMENTAL

Gathering the material was performed within the water area of the Southern Baikal in the region of the Bolshiye Koty settlement and Berezovy cape in May–June, 2005. Graylings were caught using an entangling net with 14–24 mm mesh. The total length of fishes selected for the analysis ranged within 145–276 mm. Females and males were analyzed separately. As the material for the studies we used liver as well as red and white muscles of immature individuals. Fishes were prepared at immediately after catching, organs and tissues were fixed in the chloroform/methanol (1 : 2) mixture. The quantitative determination of total lipids was carried out according to Bligh and Dyer method [18]. The separation of lipids into classes was performed with the use of the method of column chromatography on silica gel [19].

Methyl esters of fatty acids (MEFA) from the total lipid extracts were obtained using the technique by Carreau and Dubacq [20] purifying them from impurities by means of thin layer chromatography (benzene as an eluent). The determination of MEFA was performed using the method of capillary column gas-liquid chromatography, with Supelcowax-10 polar phase with the help of Shimadzu 17A chromatograph (Japan), helium being a carrier gas. The identification of MEFA peaks in chromatographic profiles was carried out via the comparison of retention time values for methyl esters obtained from FA standards (Sigma, the USA) and via the calculation of equivalent chain length indices [21–23].

RESULTS AND DISCUSSION

Analysis of lipids

According to the content of lipids in muscles and liver (Table 1), the black Baikalian grayling belongs to moderately fat fishes. The fraction of lipids in white muscles is much lower as compared to their content in red muscles. The amount of the total lipids in all the organs of males is lower as compared to those

TABLE 1

Content of lipids in muscles and liver of the black Baikalian grayling, % of the wet mass

Sample	Males (n = 5)	Females (n = 7)
White muscles	0.59±0.12	0.84±0.20
Red muscles	4.52±0.10	6.93±0.75
Liver	4.84±0.15	8.81±0.02

TABLE 2

Structure lipid fractions in liver and muscles black Baikalian graylinga, % from a total sum of lipids

Sample	Class of lipids		
	Neutral lipids	Glycolipids	Phospholipids
White muscles	74.37±2.05/77.36±1.50	9.13±0.75/8.45±1.12	15.70±3.61/12.47±2.06
Red muscles	87.55±1.26/89.17±2.64	5.65±1.08/4.49±0.62	6.52±1.59/5.13±0.54
Liver	59.28±1.25/56.10±1.88	8.36±0.91/10.32±1.54	29.52±0.68/32.18±2.73

Note. The first value is data for males (n = 5), the second value is that for females (n = 7).

for females. For liver of all fishes, the content of the total lipids is higher than their content in muscles ranging from 4.84 to 8.81 %.

Table 2 demonstrates the distribution of lipids throughout the classes. The results of the analysis of the composition of lipid classes demonstrate neutral lipids (NL) to prevail in all the organs. It is known, that the lipid metabolism in fishes within the feeding period is characterized by the accumulation of a significant amount of storage lipids in the form of triglycerides (TG) those serve as an energy source required for fish growth and for maturing genital glands [24]. The two major factors such as nutrition and development of sexual products promote the accumulation of TG in fishes during the springtime period, which is quite reasonable for immature fresh-water fishes [9]. The analysis of the fish lipids has been performed within the period from May to June when the metabolism of lipids shifts toward TG biosynthesis *de novo* (especially for female individuals). Indeed, a number of papers concerning boreal fresh-water fish species demonstrate that the content of TG increases, first of all, during this period [25].

There are various mechanisms for the regulation of the metabolism of membrane lipids (phospholipids) inherent in fishes both under the adaptation with respect to the fluctuations of environmental factors, and under various physiological conditions of an organism. From data presented in Table 2 one can see that the content of phospholipids (PL) within the feeding period is higher as compared to the content of glycolipids (GL), however their distribution throughout the organs and tissues is uneven. The content of PL in the samples of liver is more than three times higher as compared to that of GL whereas the content of PL and GL in red muscles is almost the same. The distribution of GL and PL for males and females is of similar character. Phospholipids to a considerable extent accelerate the lipometabolic processes in liver, and the lipids either are used for obtaining energy, or pass into blood and then are delivered by blood to different tissues. Here fats could be included into structural elements of tissues, however their basic part is deposited in fatty "depots" where they are stored until there would appear energy requirement; then the fats are carried to liver again to be exposed to oxidation. Liver in fishes performs mainly metabolic function, whereas in the Baikal cisco fish species (Coregonidae) and grayling fish species (Thymallidae), liver also serves as a depository for carotenoids [8]. Such situation is not inherent in other fish species. A higher content of PL in grayling liver as compared to the content of GL could be connected, to all appearance, just with this fact, irrespective of the sexual identity of an individual.

Interesting regularities have been revealed concerning red muscles of the black Baikalian grayling (see Table 2). On the basis of the data obtained one could conclude that the intensity of metabolic processes is higher in red muscles. One of the most important features of red muscles in fishes consists in the ability to involve β-oxidation of FA [26, 27]. In white muscles the basic energy source is presented by pyruvate and amino acids (within the feeding period). Switching from one kind of the energy source to another is distinctly determined by time being rigorously regulated [28]. Thus, the increased content of the total lipids in red muscles of graylings could be connected with the feeding state of immature fishes. During the feeding period, the longest in the annual cycle of fishes (7–8 months), fishes actively eat, which results in establishing a positive lipid balance in fish body.

TABLE 3

Content of the basic fatty acids of the total lipids in muscles and liver of the black Baikalian grayling (percentage of the sum total for fatty acids)

Acid	Males (n = 5)			Females (n = 7)		
	Liver	Muscles		Liver	Muscles	
		red	white		red	white
14:0	1.96	6.07	3.38	1.57	2.99	1.91
15:0-i	0.20	0.83	0.39	0.19	0.57	0.49
15:0-ai	–	0.44	0.27	–	0.32	0.10
15:0	0.52	0.44	0.38	0.36	0.39	0.37
16:0	2.33	10.56	6.93	20.79	14.38	19.16
16:1n-9	0.39	0.48	0.38	0.56	0.47	0.39
16:1n-7	2.44	7.58	3.2	2.24	5.58	2.75
17:0-i	0.30	0.18	–	0.24	0.24	0.16
17:0-a	0.48	0.44	0.17	0.28	0.44	0.2
17:0	0.59	1.29	0.64	0.41	1.02	0.38
16:3n-3	0.28	0.26	0.20	0.27	0.52	0.23
18:0	4.56	2.43	3.97	3.00	2.99	3.92
18:1n-9	8.93	14.68	9.08	10.34	15.96	10.27
18:1n-7	2.86	3.26	2.29	2.99	4.28	2.72
18:2n-6	2.53	7.58	3.47	1.44	4.14	2.24
18:3n-3	1.72	5.50	2.56	0.81	3.16	1.62
18:4n-3	0.70	1.78	0.42	0.28	1.65	0.15
20:1n-9	0.23	0.49	0.25	0.25	0.50	0.24
20:2n-6	0.49	0.60	0.35	0.30	0.53	0.26
20:3n-6	0.43	0.29	0.32	0.10	–	0.59
20:4n-6	5.92	2.92	4.26	7.12	3.00	5.16
20:4n-3	0.50	1.38	0.75	0.74	0.65	0.54
20:5n-3 (EPA)	5.14	4.15	7.17	4.82	2.61	5.85
22:4n-6	0.58	0.36	0.27	2.25	0.30	0.12
22:5n-6	1.62	2.13	2.31	2.18	2.35	2.32
22:5n-3	0.98	1.72	1.55	0.73	1.28	1.17
22:6n-3 (DHA)	31.55	15.34	30.23	33.46	27.03	33.25
n-3	40.87	30.34	43.02	41.24	37.09	42.94
n-6	11.57	13.88	10.98	13.43	10.32	10.69
n-3/n-6	3.53	2.19	3.92	3.07	3.59	4.02
DHA/EPA	6.14	3.70	4.22	6.92	10.36	5.69
SFA	30.94	22.68	26.13	26.84	23.34	26.69
MUFA	14.85	26.49	15.20	16.38	26.79	16.37
PUFA	57.82	50.83	60.09	58.38	51.41	58.38

Note. SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids.

Analysis of fatty acids

The results of FA composition analysis for total lipids contained in muscles and liver of the black Baikalian grayling are presented in Table 3. It is seen that their composition is typical for freshwater organisms (from the lower invertebrates up to fishes).

It follows from the data concerning the content of FA in muscles that there are PUFA prevailing totally in all the samples. The content of docosahexaenoic acids (DHA) ranges within 15.34–33.46 %, and its content, as well as the fraction of arachidonic acid (20:4n-6, AA), in all the tissues of females is higher comparing to that for similar tissues of males. However, the distribution EPA exhibits an inverse character. For the tissues of all the fishes, the total content ω-3 PUFA was almost identical. The DHA/EPA ratio that characterizes nutritional properties of aquatic organisms [23], in all the tissues under investigation is higher for females as compared to this parameter for males. Besides PUFA, in total lipids the fraction of saturated FA is high, which, first of all, is inherent in palmitic acid (16 : 0), whose content varies within the range of 10.56–22.33 %.

The analysis of FA in liver, where the basic processes occur such as utilizing the acids supplied with food and the biosynthesis of short-chain saturated and monoene FA has demonstrated that there is a considerable difference between males and females. However, the greatest influence upon total FA balance in white muscles is exerted by such acids as 16:0, 20:5n3 and 22:6n3, whereas for red muscles the change in the composition are determined by such acids as 16:0, 18:1n9, 22:5n3 and 22:6n3. These data confirm the occurrence of oxidative FA metabolism in red muscles; they are in a good agreement with the results published earlier [29].

The n-3/n-6 PUFA ratio and the total content of EPA and DHA represent one of the paramount characteristics of the biological activity of lipids of aquatic organisms. The minimal PUFA content in the dietary intake of a human being should range from 8 to 23 %, whereas the optimum n-3/n-6 ratio ranges from 1 : 4 to 1 : 10 [24]. A diet characterized by a rather high content of n-6 and a low content n-3 PUFA, does not result in achieving required PUFA balance necessary for normal physiological condition of a human being [1]. In order to overcome n-3/n-6 FA imbalance, fish fats and the special medical preparations enriched with n-3 PUFA are used.

CONCLUSION

The content of PUFA in tissues of the black Baikalian grayling is two times higher than the minimum suggested by dieticians, whereas the optimum n-3/n-6 ratio amounts on the whole to 4 : 1. The lipids of the black Baikalian grayling represent a source of not only essential total n-3 PUFA, but also, first of all, DHA. Among the acids of n-3 family there are α-linolenic acid, eucosapentaenic and docosahexaenic acids those are contained in the Baikalian grayling characterizing the latter as a fish with high dietary parameters. Low EPA and DHA level in nutrition could cause serious problems with health, since these acids are required for realizing many functions of an organism, including the development and normal functioning of eyes and brain. These substances also help to cope with inflammatory processes, for example with arthritis; they promote the reduction of triglyceride content in blood whose content is directly connected with the diseases of cardiovascular system as well as metabolic disorder.

Thus, the data obtained concerning the lipid status and fatty acid spectrum of the black Baikalian grayling could be of interest for the studies on biological efficiency of water reservoirs, on the problems of fish adaptation to environmental conditions, for pharmaceutical industry and dietology, as well as for fish industry and nature-conservation organizations.

REFERENCES

1 A. P. Simopoulos, *Can. J. Physiol. Pharm.*, 75 (1997) 234.
2 R. T. Holman, Handbook of Essential Fatty Acid Biology: Biochemistry, Physiology and Behavioral Neurobiology, in S. Yehuda, D. I. Mostofsky (Eds.), Human Press, New York, 1997, pp. 139–182.
3 T. Kondo, K. Ogawa, T. Satake, *Clin. Cardiol.*, 9 (1986) 413.
4 C. C. Akoh, D. B. Min, Food Lipids: Chemistry, Nutrition, and Biotechnology, 2nd Ed., CRC Press, New York, 2002, pp. 866–872.
5 D. V. Jump, *J. Biol. Chem.*, 277 (2002) 8755.

6 R. J. Henderson, *Progr. Lipid Res.*, 26 (1987) 281.
7 S. J. Ju, J. R. Kucklick, T. Kozlova, H. R. Harvey, *J. Great Lakes Res.*, 23 (1997) 241.
8 T. A. Kozlova, S. V. Khotimchenko, *Comp. Biochem. Physiol.*, B, 105 (1993) 97.
9 T. A. Kozlova, S. V. Khotimchenko, *Ibid.*, 126 (2000) 477.
10 P. Ya. Tugarina, Khariusy Baikala, Nauka, Novosibirsk, 1981, pp. 5–49.
11 E. F. Kornakova, V. V. Asherstova, L. I. Egorova, IV Vsesoyuz. Soveshch. Limnologov "Krugovorot Veshchestva i Energii v Vodoemakh. Ryby i Rybnye Resursy" (Thesises), Irkutsk, 1977, pp. 258–261.
12 P. Ya. Tugarina, B. P. Tarlinskaya, L. I. Tyutrina, in: Produktivnost' Baikala i Antropogennye Izmeneniya Yego Prirody, Irkutsk, 1974, pp. 261–271.
13 N. E. Jones, W. M. Tonn, G. J. Scrimgeour, *Env. Biol. Fishes*, 67 (2003) 169.
14 N. N. Sushchik, *Comp. Biochem. Physiol.*, B, 134 (2003) 111.
15 N. G. Borisov, N. S. Ovsyannikov, Opredelitel' Promyslovykh Ryb SSSR, Pishch. Prom-st', Moscow, p. 181.
16 I. B. Knizhin, S. J. Weis, S. Sushnik, *Vopr. Ikhtiol.*, 46 (2006) 442.
17 V. N. Akulin, I. P. Levanidov, Razvitiye Issledovaniy v Oblasti Tekhnologii Ispolzovaniya Ryb i Nerybnykh Produktov. Problemy Dalnevostochnoy Rybokhozyaystvennoy Nauki, Agropromizdat, Moscow, 1985, pp. 101–115.
18 E. G. Bligh, W. J. Dyer, *Can. J. Biochem. Physiol.*, 37 (1959) 911.
19 M. Kates, Techniques of Lipidology: Isolation, Analysis, and Identification of Lipids, Elsevier, NY, 1973.
20 J. P. Carreau, J. P. Dubacq, *J. Chromatogr.*, 151 (1978) 384.
21 J. Forch, M. Lee, C. Sranleg, *J. Biol. Chem.*, 226, 2 (1987) 487.
22 J. Flanzy, M. Boudon, C. Leger *et al.*, *J. Chromatogr. Sci.*, 14 (1976) 17.
23 G. R. Jamieson and E. H. Ri, *Phytochem.*, 14 (1975) 2229.
24 G. Mourente, A. Rodriguez, D. R. Tocher, J. R. Sargent, *Aquaculture*, 112 (1993) 79.
25 I. Gill, R. Valivety, *Trends Biotech.*, 15 (1997) 401.
26 T. Ingemansson, A. Peltersson, P. Kaufmann, *J. Food Sci.*, 58, 3 (1993) 513.
27 M. Guppy, W. Hulbert, P. W. Hochachka, *J. Exp. Biol.*, 82 (1979) 303.
28 T. P. Mommsen, C. J. French, P. W. Hochachka, *Can. J. Zool.*, 58 (1980) 1785.
29 N. N. Sushchik, Zhirnye Kisloty Kak Markery Trofometabolicheskikh Vzaimodeystviy v Presnovodnykh Ekosistemakh (Author's Abstract of Doctoral Dissertation in Biology), Krasnoyarsk, 2006.