Composition of Muscle Tissue Lipids of Silver Carp and Bighead Carp

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ABSTRACT: Lipids have a complex role in the nutritional value of food. Some polyunsaturated fatty acids, characterized as essential, are extremely important for human health. This is primarily related to α-linolenic acid (18:3n-3), eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3). Content of polyunsaturated n-3 fatty acids is usually much higher in lipids of marine fish than in freshwater fish. Previous investigations have shown that muscle tissue of silver carp and bighead carp from fish farms may be a rich source of essential fatty acids. Because of that, the objective of this work was to examine contents and composition of fatty acids and total lipids in the muscle tissue of silver and bighead carp, with the aim to find out whether there are significant differences in this respect between the two species and to what extent the harvest season can influence the composition of lipids in these freshwater fish. This study showed that there is no significant difference either in the content of polyunsaturated n-3 and n-6 fatty acids, or in the n-6/n-3 fatty acid ratio in these two fish species. The lipids of both the silver and bighead carp from the spring harvest have significantly higher contents of the n-3 acids and a significantly lower n-6/n-3 ratio than fish from the autumn harvest.

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Lipids have a very complex role in human nutrition. Along with proteins and carbohydrates they represent the source of energy, and because of the presence of essential and polyunsaturated fatty acids they have an important biological function.

Some polyunsaturated fatty acids, characterized as essential, are of extreme biological importance for human health. Hence, lipids are valued not only from the aspect of energy supply but also as a source of essential acids. Of special importance are fatty acids of the n-3 series, primarily found in algae, but also present in fish lipids.

The essential and long-chain polyunsaturated fatty acids have an indispensable role in the synthesis of prostaglandins, tromboxaines and leukotrienes, and eicosanoids. Some recent studies have demonstrated the great importance of the n-3 fatty acids, so that the quality of lipids is presently evaluated on the basis of the n-6/n-3 ratio of essential fatty acids, as well as the ratio of α-linolenic acid (18:3n-3) vs. eicosapentaenoic acid (EPA) (20:5n-3) and docosahexaenoic acid (DHA) (22:6n-3). According to the recommendations of the World Health Organization (1–3), about 30% of human energy requirements should be satisfied from fats, whereby saturated acids should make about 10%, and polyunsaturated ones up to 7% (1–3). As for the intake of α-linolenic acid, the recommended value is from 0.8 to 1.1 g/d, whereas the recommended total intake of EPA and DHA is in the range of 0.3–0.4 g/d (4). It is also thought that daily intake of 2 g/d of n-3 polyunsaturated fatty acids may completely satisfy the daily needs of the human organism (4). The n-3 polyunsaturated fatty acids are found only in fish lipids and flax seed oil (1). A low n-6/n-3 ratio is desirable, although the WHO/FAO (2,3) recommendation is that in total daily diet the n-6/n-3 ratio should be 5:1.

Total annual yields of freshwater fish are much lower than of marine fish, and this is probably the reason why the amount of data on the composition and quality of the former is relatively small. There are significant differences between freshwater fish and marine fish with respect to the content and composition of fatty acids. On average, freshwater fish contain half as much EPA and DHA as marine fish (5). The mentioned average composition of fatty acids should be taken conditionally because the differences between different species may be much greater than between the average contents in the two fish groups. Gruger (6) published data concerning the composition of fatty acids of 95 marine animals, mainly fish species. If only some of the data are compared it can be seen, for example, that the lowest EPA content is found in whale (up to 0.9%), whereas in fish species the EPA content may vary in the range from 2.4 to 22%. A similar situation is observed with DHA content: The lowest content is again in whale (up to 1.1%), and in fish species it ranges from 1.3 to 37.5%. Similar differences have also been found for other fatty acids.

Hearn (7) presented data on total lipid content and composition of fatty acids in 41 freshwater and marine fish species of interest to human health. According to these data the highest

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oil contents are in eel (21.5%) and herring (11.2%), carp having only 4.5%. Cod fish and herring have most favorable (lowest) n-6/n-3 ratio (1:15 and 1:13, respectively). In contrast, this ratio in carp is 1.7:1, n-3 fatty acid content is lower, and n-6 series content is much higher than in the former species.

Even on the basis of these scarce research results it can be concluded that the importance of freshwater fish can keep pace with marine fish (7). Like the marine ones, freshwater fish contain different amounts of lipids depending on species (6), though differences can appear caused by other factors, mainly related to diet (8). A similar situation is also observed in regard to lipid composition, i.e., the content of polyunsaturated fatty acids and the n-6/n-3 ratio (6–8).

Contents of the n-3 polyunsaturated fatty acids are usually higher in lipids of marine than freshwater fish. Of the freshwater fish, a higher content of n-3 fatty acids is found in herbivorous species using phytoplankton for nutrition. Previous investigations have shown that muscle tissue of silver carp and bighead carp from fish farms can be a rich source of essential fatty acids, as they contain up to 6.6% of EPA and up to 6.0% of DHA (9–11). For this reason, the tissue of these fish, though containing little fat, can be judged from a nutritional point of view as the most favorable among the freshwater fish (9–10). Positive clinical effects of essential fatty acids have been found in cases of cardiovascular disease (9), and a procedure has been developed for separating and concentrating polyunsaturated fatty acids from this fish oil (10).

This paper describes a study of the content and composition of fatty acids of total muscle tissue lipids of silver carp and bighead carp, with the aim of establishing whether there is a significant difference between the two species, and to what extent the harvest season can influence their lipid composition.

MATERIALS AND METHODS

Material and measuring techniques. Investigations were carried out on the samples of two-year-old silver carp (*Hypophthalmichtys molitrix* Val.) and bighead carp (*Aristichthys nobilis* Val.) of both spring and autumn harvest from the Fishfarm Despotovo in Vojvodina, Yugoslavia. Spring samples were taken in the first half of March of 1993, and autumn ones in the second half of November of the same year.

Muscle tissue samples of each fish were taken from the middle part of the trunk, from the dorsal fin to the lateral line, in a length of 5 cm, from both sides of the body. The samples without skin were homogenized on a Moulinex mill (Paris, France) for 1 min. Total lipid content in such samples was 1.3 and 2.4% in silver carp and bighead carp, respectively. The homogenized samples, tightly packed in bags impermeable to gases (polyester film/aluminum foil/polyethylene film), were sealed in the nitrogen atmosphere. They were then frozen and kept until the chromatographic experiment at −18°C for a short time (up to 1 wk).

Qualitative and quantitative determination of fatty acids. The above samples were thawed at 4°C over 1–2 h and then thoroughly mixed. Total lipids were extracted with *n*-heptane at room temperature (12). Saponification of lipids and esterification of fatty acids were carried out by the method of Wijngaarden (13), modified by Vörösbaranyi (12). The dried fatty acid methyl esters were transferred to vials (Hewlett-Packard 4330.0525; Germany), closed in the nitrogen atmosphere, and kept until chromatographing at −18°C.

Qualitative and quantitative analyses of fatty acid methyl esters were carried out on a Hewlett-Packard 5640B gas chromatograph, using two stainless columns. The 3.0 m long, 2.5 mm i.d. columns were filled with Chromosorb WAW (Supelco, Bellefonte, PA) (100/120 mesh), to which Supelco 10% liquid phase SP 2330 (cyanopropyl-silicon oil, 68%) was added. The column was stabilized at 200°C during 36 h at a helium flow rate of 20 mL/min. A volume of 1.2 mL of *n*-heptane solution of fatty acid methyl esters was injected for separation. Separation conditions were: column temperature 192°C; injector temperature 250°C; flame-ionizing detector temperature 300°C; helium flow rate 23.1 mL/min; hydrogen flow rate to the detector 23.2 mL/min; air flow rate to the detector 300 mL/min.

Identification of fatty acids was carried out on the basis of retention times of the standard mixtures of fatty acid methyl esters RM-3, polyunsaturated fatty acid (PUFA)-1, and PUFA-2, purchased from Supelco.

Experimental results (five examinations) were treated statistically by the procedure of two-factor variance analysis (first factor fish species, second factor harvesting season) (14). The calculated variances were used in the Least Significant Difference (LSD) test. The level of statistical significance was set at $P \leq 0.05$ (5%).

RESULTS AND DISCUSSION

Composition of muscle tissue lipids. Results obtained by measuring the content and composition of fatty acids in muscle tissue lipids of silver carp and bighead carp (mean value of spring and autumn samples) are presented in Table 1. In the same table are also given the data obtained by Mieth *et al.* (11) on fatty acid contents in the muscle tissue of silver carp.

Contents of saturated fatty acids are higher in the muscle tissue lipids of silver carp (30.5%) than in those of bighead carp (29.4%), and the difference is statistically significant. However, the contents of total monounsaturated fatty acids were highest: 46.3 and 48.7% for silver carp and bighead carp, respectively; the difference between them is statistically significant.

Oleic acid (18:1) is the most abundant fatty acid in both fish species (32.2 and 35.0% in silver carp and bighead carp, respectively), the difference between them being statistically significant, which is also reflected in the significance of difference in the contents of total monounsaturated fatty acids.

These results showed that the content of palmitic acid in the investigated fish is at the level of the mean value for freshwater fish (5). On the other hand, the oleic acid (18:1) content is significantly higher than the average value.

The muscle tissue lipids of silver carp contain 23.2%, and those of bighead carp 21.7% of PUFA (Table 1). PUFA are

TABLE 1
Mean Contents of Fatty Acids in the Muscle Tissue Lipids of Silver Carp and Bighead Carp[a]

Fatty acid	Silver carp (%) (Mieth *et al.*, 11)	Our study Silver carp (%)	Our study Bighead carp (%)	LSD	Significance of difference
10:0	0.1				
12:0	0.1	0.1	0.2		
13:0	0.1				
14:0	4.5	3.0	2.7	0.141	NS
15:0	0.3				
16:0	15.4	22.2	21.0	0.42	*
17:0	0.5				
18:0	3.2	5.2	5.5	0.405	NS
19:0	0.2				
Total saturated	24.4	30.5	29.4	0.42	*
12:1	0.1				
14:1	0.4	0.6	0.7		
16:1	10.5	13.3	12.9	0.409	NS
18:1	24.8	32.2	35.0	0.534	*
20:1	2.5	0.2	0.1		
22:1	2.9				
Total monounsaturated	41.2	46.3	48.7	0.524	*
14:2	0.6	1.0	1.1		
16:2	1.0				
18:2n-6	4.3	3.8	4.0	0.529	NS
128:3n-3	7.0	6.7	6.0	0.35	*
18:3n-6	0.4				
18:4n-3		0.4	0.9	0.124	*
20:2	0.5				
20:3	0.5				
20:4n-6		2.1	1.8	0.112	*
20:4n-3	3.3	0.9	0.6	0.085	*
20:5n-3	6.6	3.5	3.1	0.167	*
22:3	0.1				
22:4n-6	0.5	0.04	0.04		
22:5n-6	1.4	0.6	0.4		
22:5n-3	2.0	0.7	0.5	0.081	*
22:6n-3	6.0	3.5	3.3	0.164	NS
Total polyunsaturated	34.2	23.2	21.7	1.937	NS
n-6	11.0	6.6	6.2	0.53	NS
n-3	21.6	15.7	14.4	0.532	NS
Ratio n-6/n-3	0.5	0.42	0.43	0.019	NS

[a]NS, not significant; *significant; LSD, least significant difference.

composed of true essential acids (linoleic, linolenic, and arachidonic), EPA and DHA (being the precursors in the synthesis of eicosanoids, are considered essential), and all others. In view of such a character of PUFA, the lipids of both fish species are of high biological importance.

The muscle tissue lipids of silver carp and bighead carp contain approximately the same amounts of EPA (3.5 and 3.1%, respectively) and DHA (3.5 and 3.3%, respectively). The EPA content is significantly higher with silver carp, whereas the difference in DHA contents is not statistically significant. Compared to the literature data (11), we found approximately half of the EPA and DHA content.

One of the criteria for estimating the biological value of lipids is the n-6/n-3 polyunsaturated fatty acid ratio. The lipids with lower ratios are considered biologically more important, and it is desirable for this ratio to be less than 0.5. With silver carp the n-6/n-3 ratio is 0.42 and with bighead carp 0.43, and the difference is not significant.

On the basis of the fatty acid composition and the lipid contents in fish muscle tissue it is possible to calculate that bighead carp contains more linolenic acid (0.141 g/100 g) than silver carp (0.086 g/100 g). Total contents of EPA and DHA are also higher in bighead carp (0.150 g/100 g) than in silver carp (0.089 g/100 g).

If we compare our results with those from the literature (11), it is evident that there are some differences in the fatty acid contents. These differences, however, are not significant (see Table 1) and they can be primarily ascribed to the difference in fish diets. Besides, our results were obtained for the muscle tissue samples taken from the dorsal part, whereas the literature data (11) are related to the whole fish trunk.

Effect of harvesting season on fatty acid composition. Results of the determination of content and composition of fatty acids in lipids of silver carp muscle tissue of the spring and autumn harvest are presented in Table 2.

Content of saturated fatty acid in silver carp lipids is higher

TABLE 2
Contents of Fatty Acids in Muscle Tissue Lipids of Silver Carp[a]

Fatty acid	Spring (%)	Autumn (%)	LSD	Significance of difference
12:0	0.2	0.1		
14:0	2.8	2.8	0.199	NS
16:0	21.3	23.1	0.594	*
18:0	5.5	5.0	0.573	NS
Total saturated	29.8	31.0	0.593	*
14:1	0.8	0.4		
16:1	13.9	12.6	0.578	*
18:1	29.9	34.6	0.755	*
20:1	0.1	0.2		
Total monounsaturated	44.7	47.8	0.742	*
14:2	1.0	0.9		
18:2n-6	3.5	4.1	0.748	NS
18:3n-3	6.4	7.1	0.495	NS
18:4n-3	0.6	0.2	0.175	*
20:4n-6	2.5	1.8	0.159	*
20:4n-3	1.0	0.8	0.120	NS
20:5n-3	4.3	2.6	0.236	*
22:4n-6	0.02	0.5		
22:5n-6	0.7	0.5		
22:5n-3	0.7	0.6	0.115	NS
22:6n-3	4.8	2.3	0.231	*
Total polyunsaturated	25.4	21.0	2.739	NS
n-6	6.7	6.5	0.794	NS
n-3	17.8	13.6	0.752	*
Ratio n-6/n-3	0.37	0.48	0.023	*

[a]See Table 1 for abbreviations.

TABLE 3
Contents of Fatty Acids in Muscle Tissue Lipids of Bighead Carp[a]

Fatty acid	Spring (%)	Autumn (%)	LSD	Significance of difference
12:0	0.2	0.2		
14:0	2.5	2.8	0.199	NS
16:0	20.1	21.9	0.594	*
18:0	5.1	5.9	0.573	NS
Total saturated	27.9	30.8	0.593	*
14:1	0.7	0.6		
16:1	12.8	13.0	0.578	NS
18:1	34.7	35.3	0.755	NS
20:1	0.1	0.1		
Total monounsaturated	48.4	49.0	0.742	*
14:2	1.0	1.1		
18:2n-6	4.1	3.8	0.748	NS
18:3n-3	5.8	6.1	0.495	NS
18:4n-3	1.2	0.6	0.175	*
20:4n-6	1.8	1.9	0.159	NS
20:4n-3	0.8	0.5	0.120	*
20:5n-3	3.7	2.5	0.236	*
22:4n-6	0.01	0.06		
22:5n-6	0.5	0.2		
22:5n-3	0.6	0.5	0.115	NS
22:6n-3	4.0	2.5	0.231	*
Total polyunsaturated	23.4	19.9	2.739	NS
n-6	6.4	6.0	0.794	NS
n-3	16.1	12.7	0.752	*
Ratio n-6/n-3	0.39	0.47	0.023	*

[a]See Table 1 for abbreviations.

in autumn (Table 2). The content of monounsaturated fatty acids is 44.72% in spring and 47.79% in autumn, the difference being statistically significant. PUFA in fish lipids make 25.42 and 21.03% in spring and autumn, respectively, and the difference is not statistically significant.

Content of n-6 fatty acids is approximately independent of the season (6.7 and 6.5%), whereas n-3 acids are more abundant in spring (17.8%) than in autumn (13.6%), the difference being statistically significant. The n-6/n-3 ratio in spring (0.37) is significantly lower than in autumn (0.48). These results, and especially the contents of PUFA and n-3 fatty acids and the n-6/n-3 ratio, qualify the investigated fish meat as a biologically very important food, similar to that of marine fish (6,7).

Similar results were obtained for the muscle tissue lipids of bighead carp (Table 3). As with silver carp, the muscle tissue lipids of bighead carp have a significantly higher content of saturated fatty acids (Table 3) in autumn (30.8%) than in spring (27.9%). Monounsaturated fatty acids are also more abundant in autumn (49.0 vs. 27.9%), and the difference is statistically significant. As for other fatty acids and the n-6/n-3 ratio, the results are the same as those obtained for silver carp. In spring, the contents of PUFA (23.4%) and n-6 fatty acids (6.3%) are higher. At the same time, n-3 fatty acid content (16.1%) is significantly higher, whereas the ratio of n-6/n-3 fatty acids is significantly lower (0.39).

In regard to the results for significance of difference in dependence on the harvest season, the results are similar to those described above. So it can be concluded that the meat of the two fish species is of similar biological value.

On surveying the literature at our disposal we concluded that of the freshwater fish species, PUFA content is higher in whitefish (15,16), *Acantholingua ohridana* (17), trout (18–20), and some other commercially less important fish species. With carp, the content of PUFA can amount even to 30% (8) with special feeding. Other freshwater fish species contain less than 20% of these fatty acids.

The investigated fish lipids have increased contents of linoleic and linolenic acid. Linoleic acid (18:2n-6) in both species is present at a level of about 4%. The content (in the range of 6–7%) of linolenic acid (18:3n-3) is somewhat enhanced, whereas the contents of octadecatetraenoic (18:4n-3) and eicosatetraenoic (20:4n-3) acid in muscle tissue lipids of the investigated fish is below 1%. The EPA (20:5n-3) content in the muscle tissue lipids of both fish species is in the range from 2.5 to 3.7%. Arachidonic acid (20:4n-6), a true essential acid, is present in both species at about 2%. The content of docosapentaenoic acid (22:5n-3) is low (both fish species contain about 0.5 to 0.7%), whereas the DHA (22:6n-3) content is from about 2.5% to more than 4.5%.

Saturated fatty acids are not characteristic and predominant in fish lipids as in other animal lipids. Their content in lipids of the investigated fish is in the range from about 28 to about 31%, and they are higher in autumn. However, the difference for individual acids depending on the season is less than 2%.

Contents of individual saturated fatty acids in muscle tissue lipids of the investigated fish are similar; palmitinic acid is most abundant, being in the range of 20–23%.

The investigated fish lipids contain predominantly monounsaturated fatty acids. They are less abundant in silver carp (45–48%) than in bighead carp (48–49%). Most abundant is oleic acid, being in the range of 30–35%. Seasonal variations of monounsaturated acids are more pronounced than with the saturated ones, the content in both fish species being higher in autumn. A high total content of monounsaturated fatty acids makes the lipids of these fish liquid at room temperature.

Contents of PUFA in the muscle tissue lipids of the investigated fish is in the range from 19.5 to above 25%, and with both species is higher in spring.

In addition to total PUFA content, n-3 fatty acid contents and n-6/n-3 ratios are also essential in the evaluation of biological value of lipids. It is desirable that the n-3 fatty acid content be enhanced relative to n-6 fatty acids, which should result in a lower n-6/n-3 ratio. As the n-3 fatty acids, especially those with 20 and 22 carbon atoms, shorten the process of biosynthesis of eicosanoids, their high lipid content can effectively prevent the appearance and development of cardiovascular and other diseases, as confirmed by clinical investigations (21–31). Hence, concentrates of the n-3 polyunsaturated fatty acids are already commercially available in capsule form (32).

Lipids of both fish species contain more n-3 PUFA (up to almost 18%), and much less n-6 fatty acid (about 6%), so that the n-6/n-3 ratio is in the range from about 0.37 to about 0.48.

In view of such results for the content of n-3 fatty acids and the n-6/n-3 ratio in lipids, both fish species can be considered as biologically very valuable. If the obtained values are compared to those for marine fish (5–7), it can be seen that there is a relatively small number of species having an n-6/n-3 ratio which is significantly lower than 0.5, and there are those with which this ratio exceeds 2.0. As both investigated fish species contain more n-3 fatty acids in spring, having at the same time a more favorable n-6/n-3 ratio, spring harvest fish have a higher biological value.

The results obtained for the content of PUFA, and the n-6/n-3 ratio indicate that both fish species are biologically very valuable. With people consuming such fish, the risk of cardiovascular and other diseases should be lowered. Clinical investigations on the effect of the intake of some other fish species have been of concern of many researchers. Studies on curing cardiovascular diseases by diets rich in the presently investigated fish, carried out in a Hungarian hospital, gave very good results (9,10). Therefore, we can accept the statement by Sučić (33) "that at least one third of people with hyperlipoproteinemia, especially those with increased triglycerides, should not be treated with drugs, provided strict dietary instructions are observed."

For the nutritive evaluation of fish, in addition to composition of the lipids involved, the content of essential fatty acids is as important as the lipid content. On the basis of these data it is possible to estimate whether the kind and amount of the consumed fish can satisfy daily requirements of the human organism for these fatty acids.

On the basis of lipid content in the investigated muscle tissue of the two fish species and composition of fatty acids involved, it is evident that linolenic acid content is somewhat higher in bighead carp (0.141 g/100 g vs. 0.086 g/100 g). The same conclusion holds for the total EPA and DHA content (0.150 g/100 g vs. 0.089 g/100 g).

In order to satisfy the respective daily needs in EPA and DHA of 0.3 g/d and 0.4 g/d, respectively (4,38,39), it can be calculated that daily consumption of bighead carp should be in the range of 200–267 g and that of silver carp in the range of 337–449 g.

As for the daily intake of linolenic acid, on the basis of the recommended value of 0.8–1.1 g/d (4,38,39) it would be necessary to take daily between 567 g and 780 g of bighead carp, or from 930 to 1.279 g of silver carp. However, in contrast to EPA and DHA, this fatty acid is also present in some other food products.

Therefore, fish intake is of essential importance for the evaluation of nutritional value to satisfy daily EPA and DHA requirements. On the basis of this requirement, bighead carp should be preferred to silver carp. As the consumption of both investigated fish species in normal amounts can satisfy the daily needs, it is evident that their nutritional values are very high.

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