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FATTY ACIDS COMPOSITION AND FAT SOLUBLE VITAMINS CONTENT OF BIGHEAD CARP *(ARISTICHTHYS NOBILIS)*

A. Merdzhanova, D. Dobreva, M. Stancheva

Medical University of Varna, 55 Marin Drinov St., 9000 Varna
a.merdzhanova@gmail.com

ABSTRACT

In the present study fatty acid composition and fat soluble vitamins content were analyzed in two season's samples (spring and autumn) freshwater bighead carp *(Aristichthys nobilis)*.

Analysis of fatty acid methyl esters was performed by gas chromatography system with MS detection. Vitamins A, D_3 and E were analyzed simultaneously using RP-HPLC system. The sample preparation procedure includes saponification and liquid-liquid extraction of the unsaponifiable matter.

The fatty acid and vitamins contents of the investigated fish species showed significant seasonal changes. The spring bighead carp characterized with saturated fatty acid (SFA) (37.5%) and mono unsaturated fatty acids (MUFA) (22.1%), and poly unsaturated fatty acids (PUFA) (40.4%), including essential omega 3 fatty acids (23.0%). The autumn samples showed higher SFA (40.5%) and MUFA (34.8%), and lower PUFA (24.6%), due to reduced omega 3 fatty acids (9.7%).

Similar amounts of alpha-tocopherol were found in two season's fish samples – 1097.0 μg.100g^{-1}ww for spring and 1051.8 μg.100 g^{-1}ww for autumn bighead carp. The higher amounts of all-trans retinol (15.7 μg.100 g^{-1}ww) and cholecalciferol (8.0 μg.100 g^{-1}ww) were found in spring fish samples, while in autumn bighead carp were found – 9.0 μg.100 g^{-1}ww and 5.4 μg.100g^{-1}ww, respectively.

Key words: *Aristichthys nobilis, fatty acids, retinol, alpha-tocopherol, cholecalciferol*

INTRODUCTION

Fish tissue is considered as a valuable source of proteins, fats, vitamins and minerals, which are very important for human health and might be obtained from the diet. Many studies suggest that fish is one of the most important dietary sources of vitamin A (all-trans-retinol), vitamin D_3 (cholecalciferol) and vitamin E (alpha-tocopherol) and also essential fatty acids (FA), but their contents depend on the fish species and season of catch. Therefore we need to determine their contents in the edible parts of fishes [1, 2].

Aquaculture in Bulgaria is mainly fresh water. One of the most farming warm-water fishes in our country are carps family (about 50% of total aquaculture production). After common carp, the bighead carp *(Aristichthys nobilis)* is another highly breaded member of this family – in freshwater farms and dam lakes [3].

There is limited information in the scientific literature about the nutritive composition, especially about vitamins, on bighead carp edible tissue.

The aim of this study was to determine and compare fat soluble vitamins content and fatty acids composition in bighead carp raw fillets in two seasons – spring and autumn.

MATERIALS AND METHODS

Collection of samples

Samples of Bighead carp fish species were caught from Pyasachnik Dam Lake (Plovdiv region), Spring and Autumn 2010. Three specimens of

each fish sample were used as a material for FA and vitamin analysis. The fishes were filleted with the skin and the samples were homogenized using kitchen homogenizer. The raw material was used for the determination of fat soluble vitamins content and FA composition.

Lipid extraction and fatty acid analysis

The samples of freshly prepared homogenate (5.000 ± 0.001g) were extracted in triplicate with chloroform:methanol (1:2 v/v) according to Bligh and Dyer procedure [4]. The chloroform layers were evaporated until dryness and quantified gravimetrically. Total lipid content of edible tissue was determined for each group (n = 3) and the results were presented as g per 100 g wet weight ($g.100g^{-1}ww$).

The dry residue of the chloroform fraction was methylated by base-catalyzed transmethylation using 2M KOH in methanol and n-hexane [5]. The hexane layer was separated and analyzed by GC-MS. All chemicals used in the experiments were analytical grade (Merck, Germany; Sigma-Aldrich, Germany).

Gas chromatography was performed by a model FOCUS Gas Chromatograph with auto sampler A3000, equipped with Polaris Q MS detector (Thermo Scientific, USA). The capillary column used was a TR-5 MS, 30 m length, 0.25 mm i.d. Helium was used as a carrier gas at a flow rate 1 ml/min. Peaks were identified according to two parameters: Retention Time (RT) based on available FAME mix standard (SUPELCO F.A.M.E. Mix C4 – C24) and mass spectra (ratio m/z) – compared to internal Data Base (Thermo Sciences Mass Library, USA). The recovery rates were calculated utilizing the external standard method. The results were expressed as FA % of total FA.

Extraction of fat soluble vitamins and HPLC analysis

The sample preparation includes several steps: an aliquot of the homogenized sample (1,000 ± 0.005 g) was weighed into a glass tube with a screw cap and 1% of methanolic L-ascorbic acid and 1M methanolic potassium hydroxide were added. Six parallel samples of fish edible tissue were prepared and saponified at 80°C for 20 min. The interested components were extracted with n-hexane and the extract was evaporated under nitrogen

[6]. The dry residue was dissolved in methanol and injected (20µl) into the liquid chromatography system.

Three fat soluble vitamins were analyzed simultaneously using reversed phase high performance liquid chromatography (RP-HPLC) system (Thermo Scientific Spectra SYSTEM) equipped with analytical column ODS2 Hypersil™ 250x4, 6 mm, 5u. All-trans retinol and cholecalciferol were detected by UV, alpha-tocopherol by fluorescence detection. The mobile phase was composed 97:3 = MeOH:H_2O, flow rate 1ml.min^{-1}. The qualitative analysis was performed by comparing the retention times of pure substances: at λ_{max} = 325 nm for retinol; λ_{max} = 265 nm for cholecalciferol and alpha-tocopherol fluorescence at λ_{ex} = 288 nm and λ_{em} = 332 nm. The quantitation was done by the method of external calibration comparing the chromatographic peak areas of the corresponding standards (Retinol, Supelco; DL-alpha Tocopherol, Supelco; Cholecalciferol, Supelco). The results were expressed as µg per 100g wet weight (µg.100g^{-1}ww). All chemicals used in the experiments were for liquid chromatography (Sigma-Aldrich, Germany).

Statistical analysis

The data were analyzed using Graph Pad Prism 5 software. Two-way ANOVA (nonparametric test) and student t-test statistical analysis was employed for the calculations. The differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Total lipid content and FA composition

The lowest total lipid amount was found in spring bighead carp (3.80±0.09g.100^{-1}g ww), whereas the autumn's samples presented the highest content (7.33±0.25g.100^{-1}g ww). Our results are higher for both season than presented by Vujkovic *et al.* (1999) and Hadjinikolova et al., 2008 [7, 8].

The typical FA composition of freshwater fish species is a result from the FA composition of their food – phytoplankton and other aquatics plants [2, 7, 9]. Vujkovic et al. were found a relative pattern MUFA>SFA>PUFA in bighead carp from local fish farm for both seasons – spring and autumn [2]. A deflection of this pattern was observed for spring bighead carp in our

investigation, in which PUFA content was significantly higher than SFA ($p<0.001$) and MUFA ($p<0.001$) (PUFA > SFA> MUFA). Significantly higher quantities in SFA compared to MUFA ($p<0.001$) and PUFA ($p<0.001$) were found for the autumn bighead carp ($p<0.05$) (SFA> MUFA> PUFA) (Fig. 1).

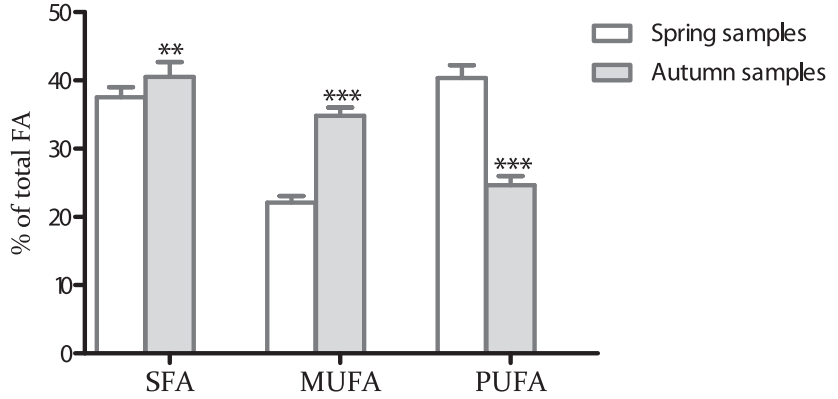

Figure 1. *Fatty acid composition in both season's bighead carp raw fillets *** $p<0.001$ and ** $p<0.05$*

Many authors found out a great variation in FA content with predomination of palmitic (C16:0) and stearic (C18:0) SFA among the fish species [2, 7]. Our results also revealed that in all studied fish species the dominating SFAs were palmityc, stearic and myristic acid (Table 1). The highest level of palmitic acid was measured for autumn sample (28.10%), which is in agreement with the results of Vujkovic et al. who found highest levels of palmitic and stearic acid in autumn bighead carp and silver carp species. Similar results were obtained by other authors [7, 10, 11].

Among MUFAs the highest levels were found for oleic acid followed by palmitoleic acid in two seasons studied fish species. The highest levels of erucic acid were measured in the spring carp. It may be supposed that the low MUFA levels in spring bighead carp are related to the lower concentrations of palmitoleic and oleic acids (Table 1). Few studies reported that the oleic acid is the main MUFA in freshwater fish species. According to Mieth and Vujkovic the highest levels of oleic acid were measured in autumn bighead carp fillets (up to 34.6%) compared to spring samples [7, 11]. Our results showed the same trends for this FA (Table 1).

Table 1 *Fatty Acid profile in bighead carp species (spring and autumn (mean ± SD)* [+]

Fatty acid % of total FA	Bighead carp Spring 2010	Bighead carp Autumn 2010
Saturated Fatty Acid		
C 12:0	2.10±0.50	1.00±0.05
C 14:0	2.96±0.70	2.30±0.45
C 16:0	14.70±1.20	28.10±1.65***
C 17:0	0.79±0.05	0.60±0.08
C 18:0	9.11±1.05	5.50±0.80***
C 20:0	2.09±0.72	0.55±0.05
C 21:0	0.00	0.09±0.01
C 22:0	2.56±0.70	0.55±0.04
C 23:0	0.46±0.02	0.15±0.01
C 24:0	2.04±0.90	1.50±0.30
Monounsaturated Fatty Acid		
C 14:1	1.65±0.50	0.75±0.10
C 16:1	5.25±0.90	10.50±1.05***
C 17:1	0.76±0.06	0.15±0.01
C 18:1 omega9	10.21±1.18	22.35±1.60***
C 20:1	1.30±0.35	0.50±0.03
C 22:1 omega9	1.91±0.60	0.30±0.02
C 24:1	1.04±0.25	0.25±0.02
Polyunsaturated Fatty Acid		
C 18:3 omega6	0.00	0.20±0.01
C 18:2 omega6	4.55±1.05	9.58±1.15***
C 18:3 omega3	2.21±0.54	0.80±0.10

C 20:5 omega3	5.81±0.80	2.55±0.75***
C 20:4 omega6	7.99±1.00	3.75±0.85***
C 20:3 omega6	1.55±0.45	0.65±0.10
C 20:2	1.60±0.40	0.60±0.05
C 20:3 omega3	1.85±0.50	0.58±0.02
C 22:6 omega3	13.15±1.05	5.71±0.70***
C 22:2	2.14±0.81	0.35±0.05

**** $p<0.001$; $^{+}$ all samples analyzed in triplicate*

Four major PUFAs were identified as dominant: C22:6 omega3, linoleic (LA, C18:2 omega 6), C20:5 omega3 and arachidonic acid (ARA, C20:4 omega 6) (Table 1). Significant amounts of the biologically important PUFAs such as C22:6 omega3, C18:2 omega6, C20:5 omega3 and C20:4 omega6 were found in all studied fish samples. The highest levels of C22:6 omega3 and EPA were measured in the spring fish fillets (33.00% and 22.80% of total PUFAs) whereas in autumn its level significantly decreased (23.00% and 10.20% of total PUFAs) (Table 1). A possible explanation is that nutrition spectrum of this fish species, which consumes only natural food, strongly depends on the availability of phyto – and zoo-plankton, which is rich on omega3 PUFA (DHA and EPA) [8, 9]. DHA and EPA metabolism strongly influenced by water temperature, which is higher in autumn, and this leads to decrease in their values [2]. For both season the C20:4 omega6 levels in studied fish species were lower than those of C22:6 omega3 (Table 1). The bighead carp feed predominantly with pelagic phytoplankton, which contains low levels of C20:4 omega6. This freshwater food chain from phytoplankton via zooplankton to fish is one of the main reasons for accumulation of significant amounts of omega6 PUFAs.

The studied spring bighead carp is characterized with relatively high levels of omega3 FA and low levels of omega6 FA (Table 2). This ratio changed in autumn's samples – omega6 PUFAs increases at the expense of reduction on omega3 PUFAs. These results are in agreement with Vujkovic et al., who observed similar changes in autumn silver carp and bighead carp [7].

Table 2. *PUFA/SFA and omega6/omega3 ratios, total sum of omega3 and omega6 FA content*

Fatty acid % of total FA	Bighead carp Spring 2010	Bighead carp Autumn 2010
Omega 6	14.10	14.13
Omega 3	23.02	9.65***
Omega 6/omega3	0.61	1.46
PUFA/SFA	1.08	0.61

*** $p<0.001$

The omega6/omega3 FA ratio has been suggested to be an useful indicator for comparing the relative nutritional value of a given fish. According to the UK Department of Health, a ratio within 0.20–1.50 would constitute a healthy human diet and values higher than 1.50 would be harmful and may promote cardiovascular diseases [13]. For studied freshwater fish the omega6/omega3 FA ratio (Table 2) was within the recommended range. Values for PUFA/SFA ratio greater than 0.45 are recommended [13]. Our results are in agreement with this requirement and highest and most balanced PUFA/SFA ratio was observed in spring fish samples whereas the lowest value was found for autumn's (Table 2).

Vitamins content

The results from the determination of vitamins A, D_3 and E content in both seasons' fish samples are given in table 3 as μg per 100g wet weight ($\mu g.100g^{-1}$ ww).

Table 3. *Fat soluble vitamins content in bighead carp fillets, $\mu g.100g^{-1}$ ww (mean ± SD)* +

Vitamin	Season	
	Spring	Autumn
Vitamin A	15.66±1.91	8.97±1.40**
Vitamin D_3	8.00±0.99	5.40±0.30***
Vitamin E	1097.03±44.06	1051.80±37.11

*** $p<0.001$ and ** $p<0.05$; + all samples analyzed in triplicate

The amounts of vitamin A ($p<0.005$) and vitamin D_3 ($p<0.001$) were higher in the spring sample of bighead carp fillets (table 3). Their contents correlated with PUFAs values. For vitamin E were observed the same relations but the differences in contents in two seasons are not significant. The three fat soluble vitamin contents did no correspond to the total lipid's amounts.

There is no information in the scientific literature about the content of vitamins in bighead carp edible tissue. The data for common carp (fish on the same family) about retinol, cholecalciferol and alpha-tocopherol contents in fish fillets given by others were of the same order of magnitude as ours [14, 15, 16, 17].

Karatas et al were measured 125 μg.100 g^{-1}ww retinol and 1200 μg.100 g^{-1}ww alpha-tocopherol in the edible tissue of common carp [14]; in the Czech food composition database for the same fish were given values of 44 μg.100g^{-1}ww for retinol and 630 μg.100g^{-1}ww for alpha-tocopherol in raw carp fish fillets [15]. Almost the same data was presented in the Slovak Food Composition Data Bank – 44 μg.100g^{-1}ww for vitamin A and 630 μg.100g^{-1}ww for Vitamin E in raw carp fish tissue [16].

The Whole Food Catalog database was given a value of 4 μg.100g^{-1}ww for retinol, 14 μg.100g^{-1}ww for cholecalciferol, which is in a good agreement with our findings, whilst a much higher value for alpha-tocopherol (2000 μg.100 g^{-1}ww) compared to our results was indicated [17].

CONCLUSION

Our study presented fatty acids composition and fat soluble vitamin contents on two season's bighead carp. The fatty acid and vitamins contents showed seasonal changes.

Spring bighead carp have lower SFA (37.5%) and MUFA (22.1%), and highest PUFA (40.4%), especially omega 3 fatty acids (23.0%). In contrast autumn samples showed higher SFA (40.5%) and MUFA (34.8%), and lower PUFA (24.6%), due to reduced omega 3 fatty acids (9.7%). Omega6/omega 3 ratio increased in autumn samples (up to 1.46) due to reduction of omega 3 PUFA during this period. More balanced PUFA/SFA ratio was obtained for the spring bighead carp (1.08).

All-trans retinol (15.7 μg.100g^{-1}ww) and cholecalciferol (8.0 μg.100g^{-1}ww) were found in higher amounts in spring fish samples. Similar differences were

not observed of alpha-tocopherol contents – 1097.0 μg.100 g^{-1}ww for spring samples and 1051.8 μg.100g^{-1}ww for autumn bighead carp.

Spring catches species are better sources of omega 3 FA and vitamin A and vitamin D_3. Bighead carp edible tissue in two seasons is rich on fat soluble vitamins and unsaturated fatty acids, which makes it a desirable item in the human diet.

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