

## Liposoluble Vitamins of Big Golomyanka (Comephoridae, Cottoidei)

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### Abstract

Original data that were obtained by means of microcolumn reversed-phase high performance liquid chromatography method have been presented on content of liposoluble vitamins (A, D, E, F) in muscles and liver of big golomyanka *Comephorus baicalensis*.

### INTRODUCTION

The application of methods of ecological-biochemical research allows one to succeed in solving the questions that deal with the study of characteristics of fishes, valuable nutrition objects for commercial animals and human beings [1]. It is common knowledge that fat of fishes contains in abundance important liposoluble vitamins A, D, E, and F that are necessary to keep human health. The content of vitamin A for fishes of the Baikal Lake has been determined only for omul, grayling, yellow-winged goby, and stone shirokolobka [2]. Determinations of a complex of biologically active compounds (vitamins A, D, E, and F) for Baikal aquatic organisms are yet to be performed.

Precinctive Baikal golomyankas (Comephoridae) dominate in their number and biomass over the pelagic region of the Baikal Lake and they are of considerable importance in formation of bioproduction as they serve as a food for commercial kinds of fish and for seal. A unique feature of big golomyanka *Comephorus baicalensis* (Pallas, 1776) consists in its high content of lipids [3–7]. The paper [3] gives insights into application of fat of the big golomy-

anka for medical purposes and a question has been also considered there regarding potential use of this species in fishing industry as a secondary commercial object for oil reduction. The purpose of this research is to determine the content of liposoluble vitamins in muscles and liver of the big golomyanka.

### EXPERIMENTAL

Muscles and liver of puberal fishes that were fished out in the region of a settlement of Bolshiye Koty (the southwest coast of the Baikal Lake) in August 2005 have been used as the subject of the investigation. Extraction of vitamins was conducted from the isolated muscle mass and liver. The samples were weighed, homogenised, extracted, and subjected to hydrolysis according to the classical scheme [8]. The produced evaporated extract was weighed for the subsequent determination of the content of lipids (fat content), and then it was dissolved in isopropanol and injected in a chromatographic column (5–10 µL).

Concentration of vitamins in the extract was determined by a HPLC method in a Mil-

ikhrom A-02 chromatograph (EcoNova Co., Novosibirsk) under the following conditions: a column  $2 \times 75$  mm with Silasorb C<sub>18</sub>; mobile phases: A – acetonitrile : water (75 : 15), B – acetonitrile; gradient elution from 75 to 100; the flow rate of 200  $\mu$ L/min; the column temperature of 35 °C; the UV detector wavelengths of 270 and 290 nm. The determination of vitamins was conducted by an absolute calibration method. Determination of a series of standard solutions of vitamins A, E, D (pharmaceutical preparations) in isopropanol was used as the calibrating solutions.

Muscles and liver were used to determine a content of fatty acids. Tissue samples were homogenised and extracted by chloroform–methanol mixture (2 : 1) at the rate of 20 mass parts of the extracting mixture per 1 mass part of the concentrated fraction. The cleanliness of the extracted lipide fractions was equal to 90 %.

To carry out GLC analysis, fatty acids were transformed to methylic ethers under the following schematic diagram. Benzene and methanol in 1 % NaOH solution was added to an extract of lipids, and hydrolysis was conducted on a water bath at a temperature of 55 °C over the course of 30 min. Then 5 % HCl solution was added to the sample and methylation was conducted over the course of 20 min. Extraction of methyl ethers of fatty acids (MEFA) was conducted with hexane with subsequent purification by thin layer chromatography method.

MEFA analysis was conducted in a Shimadzu GC-9A gas liquid chromatograph with a flame ionization detector, with the Chromatopac data processing base, in a Carbowax-20M capillary column of 45 m in length and with the inner diameter of 0.27 mm, efficiency of a column 2500 t.t./m. Gas carrier was helium. Parameters of the analysis: peripheral speed of gas carrier was 20 cm/s; the temperature of the evaporator was 240 °C, that of a thermostat – 210 °C, that of the detector – 240 °C.

## RESULTS AND DISCUSSION

Investigations that were made before have suggested that the total content of lipids in tissue of large golomyanka may be different (33.7 % [3], 44.3 % [9], and 38.8 % [10]) and

TABLE 1

Content of lipids in tissues of big golomyanka, % to the total mass of the tissue

Sampling date	Muscles	Liver
August 2005	13.4 $\pm$ 0.6	21.6 $\pm$ 1.4
October 1997 [10]	14.5 $\pm$ 0.5	23.5 $\pm$ 2.3

TABLE 2

Content of vitamins in tissues of big golomyanka, % of the mass of lipids

Vitamins	Muscles	Liver
A (retinol acetate)	1.05 $\pm$ 0.4	3.70 $\pm$ 0.9
D (calciferol)	0.22 $\pm$ 0.3	2.80 $\pm$ 0.4
E (tocopherol)	0.26 $\pm$ 0.6	1.45 $\pm$ 0.9

the value depends on the method of extraction of lipids and on the season when the samples were selected. The content of total lipids in our samples could amount from 19.4 to 26.3 %. The content of lipids in liver and muscles of the fishes that were fished out in August and October is approximately the same [10] (Table 1). Liver of big golomyanka contains approximately 1.5 times more lipids than its muscles.

Content of liposoluble vitamins A, D, E in liver of big golomyanka is higher as compared with that of muscles by the factor of 3.5, 12.7, and 5.6, respectively (Table 2).

It has been found as the result of the determination of the content of liposoluble vitamins in various fish tissues that liver contains more vitamin D at the expense of a decrease in the fraction of vitamin A, and the content of vitamin E of the liver and muscles is virtually the same, 17 and 18 %, respectively (Fig. 1).

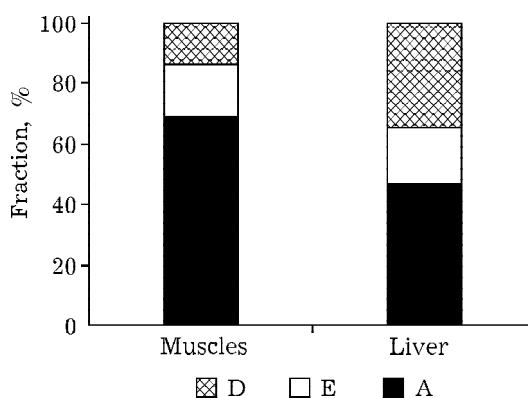


Fig. 1. Ratio of liposoluble vitamins in tissues of big golomyanka.

TABLE 3

Content of vitamin F in tissues of big golomyanka, % of the total mass of fatty acids (FA)

FA, code	Muscles	Liver
Linolic, 18:2 n-6	2.45±0.5	2.86±0.9
γ-Linolenic, 18:3 n-6	0.27±0.6	0.40±0.2
Arachidonic, 20:4 n-6	0.42±0.2	0.27±0.3

Total content of vitamin F in muscles of big golomyanka can be as high as 3 %, and that in the liver – 3.5 % from the total of fatty acids, which is 10 times lower by comparison to certain species of sea fish (25–45 %) [6]. Linolic acid constitutes the main fraction in the combination of acids of golomyanka muscles, whereas predominance of γ-linolenic acid is typical for the majority of sea species [6] (Table 3).

#### CONCLUSION

The big golomyanka can be assigned to valuable foodstuff based on its content of the main liposoluble vitamins (A, D, E, F). Taking into account the fact that golomyanka dominates in their number and biomass in the Baikal Lake, it is advisable to examine the potentials of their

use as a source of raw materials to yield medicinal preparations and bioadditives.

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