CHIRONOMUS PLUMOSUS (DIPTERA, INSECTA) LARVAE AS A SOURCE OF ESSENTIAL FATTY ACIDS IN FEED OF CARP FRY

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LARVE *CHIRONOMUS PLUMOSUS* (DIPTERA, INSECTA) IZVOR ESENCIJALNIH MASNIH KISELINA ZA ISHRANU ŠARANSKE MLAĐI

Abstrakt

U cilju ispitivanja koliko su larve *Chironomus plumosus*-a pogodne za ishranu gajenih slatkovodnih riba, pre svega šarana, izvršena je analiza hemijskog i masnokiselinskog sastava larvi koje su prikupljene na kraju tromesečnog perioda gajenja šaranske mlađi u dva eksperimentalna ribnjačka bazena u Centru za ribarstvo i primenjenu hidrobiologiju Poljoprivrednog fakulteta Univerziteta u Beogradu.

Udeo sirovih proteina u larvama *Chironomus plumosus*-a iznosio je 6,61 u jezeru L1 odnosno 6,18% u jezeru L2, što predstavlja vrednost adekvatnu za rast svih slatkovodnih vrsta riba. Sadržaj lipida bio je 0,49 odnosno 0,73%, što je energetski povoljno za sve ribe koje žive u toplim vodama. Prirodnu hranu (larve *Chironomus plumosus*) karakteriše i visok sadržaj vode: 88,95 u L1 i 89,62% u L2, a što ih čini pogodnom za ishranu šaranske malađi.

Lipidnu frakciju larvi Chironomidae u jezeru L1 je činilo 45.36% zasićenih i 53.96 nezasićenih masnih kiselina. U lipidnoj frakciji larvi Chironomidae iz jezera L2 nađeno je 53.47% zasićenih i 46.42% nezasićenih masnih kiselina.

Od polinezasićenih (esencijalnih) masnih kiselina nađenih u hironomidama u jezeru L1, najveći deo je pripadao ω-6 linolnoj kiselini (21,37%), zatim ω-3 linolenskoj (3,21%) i eikozopentanskoj ω-3 kiselini (1,27%). Sadržaj linolne kiseline u larvama Chironomidae iz jezera L2 je bio niži i iznosio je 9,78%, eikozopentanska ω-3 kiselina je zastupljena sa 0,45%, a sadržaj linoleinske kiseline je bio viši i iznosio 7,78%.

Nedostatak PUFA sa 22 C atoma je verovatno povezan sa slabom enzimatskom sposobnošću larvi Chironomidae za sintezu ovih kiselna iz njihovih prekursora PUFA sa 18 C atoma.

Izmerena količina ω-3 i ω-6 masnih kiselina u larvama *Chironomus plumosus* je iznosila 0,21 u L1, a u jezeru L2 0,81, zadovoljava nutritivne zahteve šarana.

Kjlučne reči: *Chironomus plumosus, zasićene i nezasićene masne kiseline, ω-6 linolna, ω-3 linolenska i ω-3 eikozopentanska kiselina*.

INTRODUCTION

Chironomidae larvae are widely distributed animal group, inhabiting various types of water basins. In still waters they are dominant component of benthic organisms, since 80% of bottom fauna biomass represents this animal group (Janković, 1966). Chironomidae larvae serve as a rich source of food for many benthophagous fish (larval stage lasts from several weeks to one year). Some Chironomidae species are very important component for fish nutrition because of their suitable biochemical composition (Janković, 1966a).

Nutritional value of farm-raised fish depends on the chemical composition of natural and supplement (industrial) food. Although industrial food for carp fry (Bogut *et al.,* 2007) tends to be similar to natural, containing 10-15% of carbohydrates (without remarkable fiber content) and 12-15 % of lipids (including the necessary essential fatty acids), there are mutiple advantages of nature food. Compared to the industrial food, adventages of natural food are: high digestibility (particulary of proteins), high water content (85-95%), soft and elastic food structure which allows its deformation short after ingestion, and the food movability, allowing fish to react on the „food" motions (Bogut *et al.,* 2007). Further, compared to natural food, unconsumed industrial food, containing high dry matter content, contaminates water manifold. Therefore, it is better to use natural food for fish diet, especially in nutrition of younger fish (Jirasek, 2001).

The aim of this study was to evaluate nutritive value and suitability of *Chironomus plumosus* larvea for carp fry nutrition, by determination of its primary chemical composition and fatty acid content.

MATERIAL AND METHODS

Sampling

Chironomidae larvae were collected from two fish ponds (L1 and L2) of the same area (650 m^2 in bottom base). Ponds are placed side by side, within an experimental fish farm of the Center for Fishery and Applied Hydrobiology, Faculty of Agriculture, University of Belgrade. 1200 carp individuals were placed in each pond (22.07.2010). They are genetically identical (family 27/86), aged two months and individual weight of 5,69±0.02g in the pond L1 and 5,71±0.02g in the pond L2, originated from the breeding program which takes place at the Center for Fishery and Applied Hydrobiology. In the next three months (july-october 2010) carps were fed with extruded (pond L1) and pelleted (L2) feed.

In order to analyze chemical and fatty acid composition of *Chironomus plumosus* larvae, 374 individuals were collected from the pond L1 and 262 individuals from the pond L2 (fourth and fifth larval instar). Until the laboratorical researches, samples were kept on the temperature of -18 ºC.

Chironomus plumosus larvae were sampled with modified Ekman-Birge's grab, addapted for usage in carp farms, grasping area of 87,55cm^2.

Chemical composition analysis of Chironomidae laravae

Crude protein content was determined by Kjeldahl method (N x 6,25). Water content was determined by drying the samples at 103 ± 2ºC, until the constant weight (SRPS ISO 1442/1998). Total lipid content was determined by extraction of fat in Soxhlet apparatus with 200 mL of 30-50°C boiling petroleum ether (SRPS ISO 1443/92). Ash content was determined by dry-ashing in porcelain crucibles in a muffle furnace at 550± 25 ºC (SRPS ISO 936/1999)

Extraction of lipids by ASE

Total lipids for fatty acids determination were extracted from *Chironomus plumosus* larvae tissues by accelerated solvent extraction (ASE 200, Dionex, Sunnyvale, CA). Homogenate of sample mixed with diatomaceous earth was extracted with a mixture of n-hexane and iso-propanol (60:40 v/v) in 33 ml extraction cell at 100°C and nitrogen pressure of 10.3 MPa. The extracts were collected and the solvent was removed under stream of nitrogen in Dionex Solvent Evaporator 500 at 50°C until dryness. The fat extract was further used for fatty acids determination.

FA analysis by capillary gas chromatography (CGC)

Fatty acid methyl esters (FAME) were prepared by transesterification using trimethylsulfonium hydroxide, according to EN ISO 5509:2000 procedure. The GC instrument Shimadzu 2010 (Kyoto, Japan) used for FAME determination was equipped with a split/splitless injector, fused silica cianopropyl HP-88 column (length 100 m, i.d. 0.25 mm, film thickness 0.20 μm, J&W Scientific, USA), and flame ionization detector. The column temperature was programmed. Injector temperature was 250°C and detector temperature was 280°C. The carrier gas was nitrogen at a flow rate of 1.33 ml/min and injector split ratio 1:50. Injected volume was 1 μl and total analysis time 50.5 min. The chromatographic peaks in the samples were identified by comparing relative retention times of FAME peaks with peaks in a Supelco 37 Component FAME mix standard (Supelco, Bellefonte, USA).

RESULTS AND DISCUSSION

Our results of *Chironomus plumosus larvae* chemical composition (table 1) are in accordance with previously published data (Bogut *et al.,* 2007, Steffens, 1986), provided it was noticed slightly lower protein (6,61 pond L1 and 6,18 pond L2) and lipid (0,49 or 0,73%) conntet, higher water content (88.95 or 89,62%), while the ash content in *Chironomus plumosus* larvae was nearly same 1,12% (pond L1) and 1,10% (pond L2). Also, lower lipid content was observed during three-month study period conducted by Mardsen and associates (Marsden *et al*., 1992).

Table 1. Chemical composition of *Chironomidae* larvae in g/100g fresh weight.

Chemical parameter	Pond L1	Pond L2
Protein content, %	6.61	6.18
Water content, %	88.95	89.62
Total lipid content, %	0.49	0.73
Ash content, %	1.12	1.10
Dry matter content, %	11.05	10.38

Most of the essential fatty acids in *Chironomus plumosus* larvae are present in quantities sufficient for the nutrition of most freshwater fish species, including carp. In the pond L1 lipid fraction of Chironomidae larvae was consisted of 45,36% saturated and 53,96 unsaturated fatty acids (Table 2). 53,47% saturarated and 46,42% unsaturated fatty acids were found in the lipid fraction of Chironomidae larvae from the pond L2. These percentage of fatty acids as well as 14 identified fatty acids in *Chironomus plumosus* larvae, fully confirm earlier researches that these organisms are almost ideal food source for carp fry.

Palmitic acid dominated (26,15 i.d. 32,67%), among saturated fatty acids in *Chironomus plumosus* larvae (Table 2), followed by stearic acid (10,75% in L1 i 10,82% in L2).

Oleic acid was most common among monounsaturated fatty acids (12,63% in L1 and 15,96% in L2), followed by palmitoleic acid (6,86 ie. 4,20%, Table 2).

Among polyunsaturated (essential) fatty acids found in Chironomidae larvae in the pond L1 (table 2), the highest concentration was measured for ω-6 linoleic acid (21,37%), followed by ω-3 linolenic acid (3,21%) and ω-3 eicosapentaenoic acid (1,27%). Linoleic acid (9,78%) and ω-3 eicosapentaenoic acid (0,45%) content was lower in Chironomidae larvae collected from the pond L2 (Table 2), while the content of linolenic acid was higher amounted to 7,78% High concentration of linolenic acid is probably associated with *Chironomus plumosus* nutrition, because they consume phytobenthic green algae as a food source.

Ratio of ω-3 i ω-6 fatty acids in *Chironomus plumosus* larvae was 0,21 in the L1, respectively 0,81 in L2 (Table 2).

Comparison of total fatty acids in Chironomidae larvae from the L1 and L2 ponds showed that these larvae do not contain PUFA with 22 carbon atoms. These data are in correspondence with previous results of Sushchik et al. (Sushchik *et al.,* 2003). The lack of PUFA with 22 carbon atoms is probably related to low enzimatic activity of Chironomidae larvae for the synthesis of these acids from their precursor, PUFA with 18 carbon atoms.

Chironomidae larvae in the pond L1 had lower saturated fatty acid content (45,36%) but higher unsaturated content (53,96%) compared to those in L2 pond (Table 2).

In our reseaches amount of saturated fatty acid was higher (45,36 i.d. 53,47%) which differates from the results of Bogut and associates (26,12 %, Bogut *et al*., 2007).

It should be noticed that in the research mentioned above, *Chironomus plumosus* larvea lacked of stearic acid, while in our research it's amount is 10,75 i.d. 10,82% (Table 2). Content of monostaurated fatty acid in our survey was somewhat lower (28,11% in L1 and 28,04% in L2) in accordance to results published by Bogut and associates

(30,42%, Bogut *et al.*, 2007) as well as the content of polyunstaurated fatty acid 25,85% i.d. 18,38% (34,03%, Bogut *et al.,* 2007).

As it was shown in the research by Bogut and associates (Bogut *et al.,* 2007), n-3-/n-6 ratio was 0,81% which was equal to obtained results from *Chironomus plumosus* larvae in the L2 pond, while that ratio was far lower (0,21%) because of the higher content of n-3 linolenic acid.

Table 2. Fatty acid composition of Chironomidae larvae (% of total fatty acids, given as mean±standard deviation)

Fatty acid	**Chironomidae L1**	**Chironomidae L2**
C14:0	2.34±0.09	4.79±0.10
C15:0	1.67±0.08	1.62±0.02
C16:0	26.15±0.41	32.67±0.23
C16:1	6.86±0.38	4.20±0.01
C17:0	2.37±0.04	2.08±0.25
C18:0	10.75±0.33	10.82±0.45
C18:1cis-9	12.63±0.08	15.96±0.29
C18:1 cis-11	7.02±0.23	7.00±0.26
C18:2n-6	21.37±0.93	9.78±0.11
C20:0	2.09±0.03	1.91±0.13
C18:3n-6	-	-
C18:3n-3	3.21±0.12	7.78±1.32
C20:1n-9	1.60±0.06	0.89±0.25
C20:2n-6	-	0.27±0.06
C20:3n-6	-	<0.1
C20:3n-3	-	-
C22:1n-9+C20:4n-6	0.70±0.08	<0.1
C20:5n-3	1.27±0.03	0.45±0.06
C22:5n-3	-	-
C22:6n-3	-	-
SFA	45.36±0.19	53.47±0.64
MUFA	28.11±0.59	28.04±0.78
PUFA	25.85±0.84	18.38±1.25
N-6	21.37±0.93	10.16±0.01
N-3	4.48±0.09	8.22±1.26
N-3/N-6	0.21±0.01	0.81±0.13

Concentration of ω-3 fatty acid (4,48 i.d. 8,22%, Table 2) found in *Chironomus plumosus* is significantly higher compared to other animals and it represents an important source of essential fatty acids not only for fish, but also for humans (Bogut *et al*., 2007).

What is more important is the fact that larvae of cyprinid fish have higher needs for ω-6 fatty acids (ca. 1% dry feed) than ω-3 (0,05-0,10%, Csengreri, 1993; Takeuchi,

1993; Steffens, 1993; Radünz-Neto etal., 1996). It also important to notice that these larvae are an exceptional source of fatty acids.Our researches have confirmed this fact and showed that *Chironomus plumosus* larvae are enriched with ω-6 fatty acids (21,37 in L1 and 10,16 in L2%) and represents suitable natural food source for carp fry.

Having in mind there are data which show lower ratio value of ω-3 and ω-6 fatty acid in cultured fish compared to fish found in natural habitat (Van Vliet and Katan, 1990), stimulating development of natural feed in carp farms – like *Chironomus plumosus* larvae, which are enriched with not only with essential fatty acids but also in most amino acids – can play very important role in high quality meet production.

CONCLUSIONS

Crude protein content was 6,61% (pond L1) and 6.18% (pond L2) in *Chironomus plumosus* larvae. Fat content was 0,49% and 0,73%. Water content was 88,95% in L1 and 89,62% in L2.

Fat content of the *Chironomidae* larvae from the pond L1 contained 45.36% saturated and 53.96% unsaturated fatty acids, while fat content of larvae from the pond L2 contained 53.47% saturated and 46.42% unsaturated fatty acids.

Among the polyunsaturated (essential) fatty acids isolated from the Chironomidae larvae, the most abundant was ω-6 linoleic acid (21,37%), followed by ω-3 linolenic (3,21%) and ω-3 eikosapentaenoic acid. Linoleic acid content in chironomidae larvae from the pond L2 was lower - 9,78%, ω-3 eicosapentaenoic acid content was 0,45%, and linolenic acid content was higher – 7,78%.

Among saturated fatty acids, plamitic (26,15% i.d. 32,67%) following stearic (10,75% in L1 and 10,82% in L2) acid dominated in *Chironomus plumosus* larvae.

Oleic acid was most common among monounsaturated fatty acids (12,63% in L1 and 15,96% in L2), followed by palmitoleic acid (6,86 ie. 4,20%)

The lack of PUFA with 22 carbon atoms is probably related to low enzimatic activity of Chironomidae larvae for the synthesis of these acids from their precursor, PUFA with 18 carbon atoms.

The measured quantity of ω-3 and ω-6 fatty acids from *Chironomus plumosus* larvae was 0,21 in the pond L1 and 0,81 in the pond L2, which is sufficient for the nutritional requirements of carp.

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