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CHIRONOMUS PLUMOSUS LARVAE - A SUITABLE NUTRIENT FOR FRESHWATER FARMED FISH

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SUMMARY

Crude protein, fat, water, ash, dry matter and essential amino and fatty acids were analyzed from freshly collected Chironomus plumosus larvae in order to evaluate their suitability as a component for farmed fish diet. The analyses were performed in intact living organisms and in the dry matter. Essential amino acids were determined by LKB 4101 automatic analyzer, while fatty acids were determined by Chronopack CP 9000 chromatograph with flame ionization detector. Crude protein content was 7.6% and 55.7% in fresh larvae and dry matter, respectively, being a value adequate for growth needs of all freshwater fish sorts and categories. Most essential amino acids in fresh larvae and dry matter are present in quantities adequate for feeding majority of omnivorous and carnivorous freshwater fish species. Crude fat content was 1.3% and 9.7% in fresh larvae and dry matter, respectively, being energetically sufficient for all warm-water living fish. The crude fat contains 26.12% saturated, 30.42% monounsaturated and 34.03% polyunsaturated fatty acids (PUFA). Among the highly unsaturated fatty acids (HUFA), the most abundant is linolenic (7.21%), followed by eicosapentanoic (4.36%), docosahexaenoic (2.49%) and docosapentaenoic (1.16%) acid. The measured quantity of ω-3 and ω-6 fatty acids (essential for omnivorous fish), as well as ω-6 to ω-3 fatty acid ratio (0.81), completely meets nutritional requirements of carp and tench.

Key-words: Chironomus plumosus, freshwater fish diet, fatty acids, amino acids, PUFA, MUFA

INTRODUCTION

Nutritional value of farm-raised fish depends on the chemical composition of the fish diet. The industrial fish food tends to mimic the natural food, containing approximately 50% of proteins (including all the essential amino acids), 10 – 15% of carbohydrates (without remarkable fiber content) and 12 – 15% of lipids (including the necessary essential fatty acids). Yet, some authors (Jirásek, 2001) prefer and recommend natural food for fish diet, especially in nutrition of younger fish. The outlined advantages of the natural food compared to the industrial one are: a high digestibility (particularly 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. Additionally, unconsumed industrial food, containing high dry matter content, contaminates water manifold, compared to natural food.

Among the possible source of natural food, in our geographical region (east-north Croatia, Europe) *Chironomus plumosus,* a member of the chironomids *(Diptera: Chironomidae)* is present. Chironomids are a common group of aquatic insects living in running and still waters represented by a large number of individuals. Duration of larval phase lasts from several weeks up to one year, depending on water temperature. At pupae stage and as an adult fly they live only for a few days. Chironomid larvae have elongated cylindrical body varying in size between 1 and 25 mm. They move in a characteristic twitch, resembling "S" letter. Their feeding on green algae or detritus is species diversified (Kerovec 1986).

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The aim of our research was to evaluate the nutritional quality and suitability of *Chironomus plumosus* for fish diet, by determination of its basic chemical composition and essential amino and fatty acids content.

MATERIAL AND METHODS

Pond operating conditions. Oxygen concentration in the pond water, measured in the morning was 6.5-7 mg×L^{-1}, while in the afternoon it raised to 9-10 mg×L^{-1}. Value of pH ranged from 7.5 to 8.2. Water transparence ranged from 25 – 45 cm, and the water color (slight green) indicated an adequate phytoplankton quantity. The concentration of nitrogen and phosphate in the water used to fill up the pond was 0.5 and 0.3 mg×L^{-1}, respectively, and it remained constant throughout the experiment.
Larvae sampling. *Chironomus plumosus* larvae of different growth stages were sampled at the end of July of the year 2003. Sediment samples containing zoobenthos were taken using the Ekman dredge from several sites at the fishpond, stocked with the fry of carp. The samples were rinsed and sieved with 1 mm mesh net, and inspected using a stereo dissecting microscope. Larvae of *Chironomus plumosus* were kept in cold at temperature of -18 ˚C prior to chemical analyses. All, 216 larvae were collected. Out of this pool, 10 individual samples were prepared and subjected to chemical analysis.
Chemical analysis. Water content in larvae was determined by drying the samples at 105^{o}C until the constant weight. Ash content was determined by burning the larvae at 550^{o}C for 4 hours in the muffle furnace. Crude protein content was determined by Kjeldahl method using the Kjel-Foss 16200 type of the nitrogen analyzer, and crude lipid content by extraction according to Soxhlet. Amino acids quantification in the fresh larvae and dry matter hydrolysates was performed by the LKB 4101 (LKB Biochrom, England) automatic analyzer using the Merck standards (Csapo et al., 1986). Lipid for fatty acids analysis was extracted by the method of Folch et al. (1957), while fatty acids were determined with Chrompack CP 9000 chromatograph, using the flame ionization detector. Each sample was analyzed in triplicate. The percent of essential fatty acids was calculated according to Csapo et al. 1986a.

RESULTS AND DISCUSSION

The results of *Chironomus plumosus* larvae chemical composition are presented in Table 1. The obtained water content in fresh larvae (87.9%) is in accordance with the values reported in other chironomid species. The larvae protein content expressed on fresh (7.6%) and dry (55.2%) weight, mineral content in fresh (1.1%) and dry (8.2%) weight, crude fat content in fresh weight (1.1%) and N-free extractive substances in dry weight (28.4%) are similar as the values reported by Steffens (1986) for natural fish food sources. In our samples, slightly higher content of N-free extractive substances in fresh weight, and lower content of crude fat in dry matter (3.8% and 8.2%, respectively) were detected, when compared with the above cited author. Nevertheless, the differences in fat and N-free extractive substances could be explained by the sampling dynamics as well as by the larvae age. Analyzing quarterly the chironomid larvae during one-year study period, Mardsen et al. (1991) recorded higher protein content and lower fat and dry matter content in younger larvae.

Table 1. Chemical composition of *Chironomus plumosus,* expressed as percents in fresh and dry weight (mean value ± standard deviation, N = 10)

Component	%	
	Fresh weight	Dry weight
Water	87.9 ± 0.26	-
Crude protein	7.6 ± 0.29	55.7 ± 0.28
Crude fat	1.3 ± 0.18	9.7 ± 0.3
N-free extractive substances	2.1 ± 0.64	26.4 ± 0.48
Ash	1.1 ± 0.25	8.2 ± 0.36

In Table 2, the results of essential amino acid content analysis expressed on fresh and dry weight of *Chironomus plumosus* larvae are given. Protein and amino acids utilization depend on water temperature and dissolved oxygen concentration, as well as on feeding schedule. Fish needs for essential amino acids depend on the energy-to-protein ratio in their food, essential amino acid bioavailability, fish weight category, the quantity of other amino acids, but also on the partial lack of some particular essential amino acid. The essential amino acids for fish organism as well as deficiency symptoms are well identified and documented (Halver, 1989).

Table 2. Amino acids content in *Chironomus plumosus* larvae (mean value ± standard deviation, expressed as % in fresh and dry weight; N = 10)

Amino acid	*Chironomus plumosus* (%)	
	Fresh weight	Dry weight
Arginine	0.29± 0.008	2.12± 0.003
Histidine	0.14 ± 0.005	1.02 ± 0.003
Isoleucine	0.27 ± 0.010	1.98 ± 0.005
Leucine	0.34± 0.005	2.49± 0.005
Valine	0.27± 0.009	1.99± 0.003
Lysine	0.33 ± 0.006	2.48 ± 0.003
Phenylalanine	0.37± 0.008	2.76 ± 0.004
Methionine	0.30 0.007	2.19± 0.004
Threonine	0.27 ± 0.008	2.01 ± 0.003
Tryptophan	0.19± 0.006	1.39± 0.004

Obtained values for essential amino acids quantities in larvae dry matter are further compared with average requirements of omnivorous and carnivorous fish (Steffens, 1986; Halver, 1989). The most essential amino acids are present in larvae in a marked excess; yet, Arg and His needs in carnivores are slightly higher than larvae content. Anyway, some of essential amino acids can be in small quantities synthesized by fish, among them also Arg.
Chironomus plumosus larvae fat analyses revealed 26.12% of saturated and 64.45% unsaturated fatty acids (Table 3).

Table 3. Fatty acid composition of *Chironomus plumosus* larvae (% of total fat, mean values, N = 10).

Fatty acid group		Fatty acid	%	Total (%)		ω–6 : ω–3
Saturated		Myristic. 12:0	1.17	26.12		
		Palmitic. 16:0	19.43			
		Arachidic. 20:0	0.21			
Monounsaturated		Palmitoleic	7.24	30.42		
		Oleic	21.51			
		Eicosanoic	0.41			
		Neuronic	1.26			
PUFA	ω – 6	Linoleic. 18:2	13.76	18.81	34.03	0.81
		Eicosadienic. 20:2	0.88			
		Arachidonic. 24:4	4.17			
	ω – 3	Linolenic. 18:3	7.21	15.22		
		Eicosapentaenoic. 20:5	4.36			
		Docosapentaenoic. 22:5	1.16			
		Docosahexaenoic. 22:6	2.49			
Others (unidentified)			9.43	9.43		

Among saturated, palmitic acid dominated (19.43%) when compared with myristic (1.17%) and arachidic fatty acid (0.21%). Out of unsaturated fatty acids, the greatest portion belongs to monounsaturated (30.42%), followed by ω-6 (18.81%) and ω-3 (15.22%). Oleic acid is present in highest proportion among monounsaturated fatty acids (21.51%), followed by palmitoleic acid (7.24%) and others detected in minor quantities. In ω-6 group, the highest concentration was measured for linoleic (13.76%) followed by arachidonic fatty acid (4.17%), while in ω-3 group, the greatest quantity belongs to linolenic (7.21%), followed by eicosapentaenoic (4.36%) and docosahexaenoic

(2.49%) fatty acid. Moreover, ω-6 to ω-3 ratio has a value of 0.81, which can be acceptable, although it depends largely on fish sort and growth stage.

Fish fatty acid composition is very important, because fish comprise a highly recommended and significant part of human diet. It is well-known that essential ω-3 fatty acids have a beneficial influence on human health, primarily in prevention and healing of cardiovascular diseases, but also as important factors in neurological development (Arts, 2001). In fish, the deficiency of essential fatty acids can induce a number of disorders: fatty liver degeneration, myocarditis, low hemoglobin concentration in erythrocytes as well as their deformation, changes in cell membranes permeability, fast breathing, bad food utilization, growth lagging, slower growth rate of fins, and a greater susceptibility to stress (Bogut, 1996).

Commonly cultured fish sorts such as rainbow trout *(Oncorhynchus mykiss)*, common carp *(Cyprinus carpio)*, Channel catfish *(Ictalurus punctatus)* and European catfish *(Silurus glanis)* require between 0.5 and 1.0 % HUFAs and PUFAs (in total lipids) for optimal growth (Bogut 1995; Robinson, 1984; Takeuchi, 1982).

It is obvious that the great quantity of ω-3 fatty acids (15.22%) detected in *Chironomus plumosus* larvae, being significantly higher compared to other animals, represents a rich source of essential fatty acids, not only for fish, but also for humans. For example, the requirements of rainbow trout for highly unsaturated fatty acids (HUFA) are 1% of linolenic or 0.5% of eicosapentaenoic plus 0.5% of docosahexaenoic fatty acid (Takeuchi, 1982), while carp needs 0.5% HUFA or 1% of linoleic and 1% of linolenic fatty acid (Takeuchi, 1977). The needs of channel catfish for ω-3 fatty acids are less than 1% (Robinson, 1984), while the requirements of other fish sorts are similar (Bogut, 1995). Therefore, we can conclude that *Chironomus plumosus* larvae completely meet the needs of all fish sorts for essential fatty acids. Typically, farmed fish have higher lipid content than their counterparts caught from the wild area. Anyway, there are reports showing a lower ratio of ω-3 to ω-6 fatty acids in cultured than in wild fish (Van Vliet, 1990). Although the lack of essential fatty acids in these fish is not presumable because of high total lipid content, the addition of natural food (like *Chironomus plumosus* larvae), very rich not only in essential fatty acids but also in most amino acids, can have beneficial effect on fish growth, condition and health. This can also be the way to improve the fish quality and enrich this part of human diet with essential fatty and amino acids.

Our final conclusion is that *Chironomus plumosus* larvae represent a potential suitable natural component of farm fish diet.

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