

Content of basic nutrients, amino acids and fatty acids in the benthos of carp ponds

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Abstract. Benthos is the most important ingredient of natural food of fish in carp ponds. Its chemical composition and profile of fatty acids has not been studied up to now. The aim of this research was to determine these parameters for the benthic organisms that carp feeds on, which are a source of fatty acids and cholesterol, as well as amino acid composition and fatty acid profile of these organisms. Benthos was sampled from 2 ponds with Ekman-Birge sediment sampler in July and October. Benthos from the bottom of drained ponds was also collected after the harvest. The content of basic nutrients was determined in the benthos samples using AOAC procedures. Amino acids and fatty acids profiles were also determined. It has been found that benthos in carp ponds contains a high amount of protein and it is rich in the essential amino acids. The limiting amino acids in benthic protein are the sulphur amino acids: cystine and methionine as well as tyrosine. The fat content of the benthos is at intermediate level with a high content of essential unsaturated fatty acids.

Key Words: benthos, carp ponds, fatty acids, amino acids.

Streszczenie. Bentos to najważniejszy składnik pokarmu naturalnego ryb w stawach karpowych. Skład chemiczny i profil kwasów tłuszczowych bentosu nie był do tej pory badany, dlatego też celem pracy było określenie tych parametrów dla organizmów bentosowych będących pokarmem i źródłem kwasów tłuszczowych oraz cholesterolu dla karpia. Bentos do badań pobierano z dwóch stawów przy użyciu chwytacza osadów Ekmana-Birge'a w lipcu i październiku, a po odłowach także z dna odwodnionych stawów. W pobranych próbkach zawartość podstawowych składników pokarmowych oznaczono metodą AOAC. Określono też zawartość aminokwasów i profil kwasów tłuszczowych. Stwierdzono, że bentos to pokarm dla ryb o wysokiej zawartości białka oraz o bogatym składzie aminokwasowym, w szczególności bogaty jest w aminokwasy egzogenne. Aminokwasami ograniczającymi w białku bentosu są aminokwasy siarkowe: cystyna i metionina oraz tyrozyna. Zawartość tłuszczu w bentosie kształtuje się na średnim poziomie, przy dużej zawartości niezbędnych nienasyconych kwasów tłuszczowych.

Słowa kluczowe: bentos, stawy karpowe, kwasy tłuszczowe, aminokwasy.

Rezumat. Bentosul este ingredientul cel mai important al hranei naturale a crapilor din bazinele de pământ. Compoziția lui chimică și profilul acizilor grași nu a fost studiat până în prezent. Scopul acestui studiu a fost determinarea acestor parametri ai organismelor bentonice cu care crapii se hrănesc și care sunt surse de acizi grași și colesterol. Bentosul a fost prelevat din două bazine cu unealta de recoltare a sedimentelor Ekman-Birge în Iulie și Octombrie. Bentosul a fost cules și după golirea bazinelor în perioada de recoltare. Conținutul nutrienților de bază în probele de bentos au fost determinate prin procedeele AOAC. Aminoacizii și profilul acizilor grași au fost de asemenea determinate. S-a constatat că bentosul bazinelor cu crap conține cantități importante de proteină și este bogat în aminoacizi esențiali. Aminoacizii aflați în cantități limitate în proteinele bentosului sunt cei bogați în sulf: cistină și metionină, precum și tirozină. Conținutul în grăsime al bentosului este la un nivel intermediar, dar bogat în acizi grași esențiali nesaturați.

Cuvinte cheie: bentos, bazine de creșterea crapului, acizi grași, aminoacizi.

Introduction. The natural food of fish in carp ponds has been adequately studied (Kownacki 2003), but chemical composition of the benthos and profile of fatty acids, according to our data, has never been published. Research on chemical composition of meat and profile of fatty acids is essential because benthos is the most important ingredient of natural food (Wunder 1949; Merla 1958) and thus a source of fatty acids

and cholesterol to carp. As reported by Matlak & Matlak (1976), carp larvae can already feed on Chironomidae larvae on the third day after hatching.

The latest evidence on species composition of the benthos in 10 carp ponds belonging to the Fisheries Experimental Station of the University of Agriculture in Kraków is reported in a doctoral thesis of Fałowska (2007), who collected samples at 3-week intervals and divided the macrozoobenthos obtained into 3 groups: oligochaetes (Oligochaeta), larvae of chironomids (Chironomidae) and others, which included Hirudinea, Crustacea, other Insecta and Gastropoda. She found the presence of 96 taxons, including 45 Chironomidae, 18 Oligochaeta and 33 taxons of the other benthic organisms. The largest proportion in benthos composition was formed by oligochaetes, which ranged from 79.16 to 94.63% according to the pond category and stocking density. The proportion of Chironomidae ranged from 13.1 to 19.42%, and the organisms representing the other groups constituted 0.44-4.1% (Fałowska 2007). Among Oligochaeta, the dominant species was *Limnodrilus hoffmeisteri* (18.9-44.7%). Among Chironomidae, the largest proportion was made up by *Chironomus* spp. (35-50%). The group of the other benthic organisms was dominated by Ceratopogonidae dipterans (23.7 to 76%).

The objective of this study was to determine chemical composition of the benthic organisms of carp ponds, as well as amino acid composition and fatty acid profile of the organisms that are the natural food of carp in ponds. The authors were guided by Bieniarz & Kołdras (2000), Bieniarz et al (2000) and Bieniarz et al (2001), who reported that fish reared only on natural food are higher in polyunsaturated fatty acids compared to undesirable saturated fatty acids, which is evidence of the health-promoting benefits of their meat.

Material and Methods. Benthos samples were collected from 2 ponds of the Fisheries Experimental Station of the University of Agriculture in Krakow (control pond and experimental pond) during a study investigating the effect of using a mixture of sunflower and linseed oils on the essential fatty acid content, amino acid composition and the level of total cholesterol of carp meat (Epler et al 2009a). The samples were obtained with a 225 cm² Ekman-Birge sediment sampler in July and October. Benthos from the bottom of drained ponds was also collected after the harvest. Both during the growing season and after the harvest, the collected benthos was rinsed and frozen. A total of 100 g of benthic organisms was obtained.

The content of basic nutrients was determined in the benthos samples using AOAC procedures (2005). Acidic amino acids were determined after hydrolysis in 6N HCl and sulphur amino acids after oxidative hydrolysis with formic acid and with perhydrol by the ninhydrin method. The determinations were made using an AAA-400 amino acid analyser (Ingos) and an Ostion LG ANB ion exchange column (370 mm long).

Benthos samples were prepared for determination of fatty acid profile following the method of Folch et al (1975). Fatty acid profile was determined by a Varian 3400CX gas chromatograph with FID detector and column CP-WAX (50 m long, 0.53 mm in diameter). The chromatograph operating conditions were as follows: argon as a carrier gas, injector temperature 200°C, detector temperature 240°C for 12 min and 220°C for 20 min.

Results. The bottom of carp ponds belonging to the Fisheries Experimental Station complex is highly similar because they are all situated on heavy clay soils. With this in mind, it can be assumed that benthic samples collected by grab samplers will be of similar quality in all ponds. Species composition of the oligochaetes dominant in the bottom fauna was similar in all ponds investigated by Fałowska (2007). Oligochaetes are the main component of the bottom fauna in most ponds, also those with muddy and sandy bottoms and a belt of reeds and bulrushes (Zięba 1967; Zięba & Srokosz 1974). The second most dominant component of natural carp food, the chironomids, were numerically much less abundant than oligochaetes, although in other pond complexes with sandy bottoms, chironomid larvae may exceed 50% of the biomass of benthic food

(Szumiec 1966). These data lead us to suggest that oligochaetes and chironomids are the principal source of polyunsaturated fatty acids for pond carps.

The food value of benthos depends on the content of nutrients given in Table 1. The results obtained indicate that the benthos is relatively low in dry matter and high in ash, whose content exceeds 21% on a dry matter basis. In terms of protein content, benthos is considered a high-protein food because the content of this component exceeds 43% in the dry matter. Benthos is an energy food because it contains relatively high amounts of nitrogen-free extractives and crude fat, totaling 20.18% to 34.59% in the dry matter. Analysis of the chemical composition of benthos as natural food confirmed its high nutritive value and suitability as food for fish.

When feeding Cyprinidae it is important to consider not only the amount of crude protein but also the composition of amino acids in the benthos, because its content of amino acids (especially essential amino acids) determines the nutritive value. The amino acid composition of the analysed benthos is shown in Table 2. No clear differences in the amino acid composition were found between the benthos sampled from ponds 1 and 2. It is pertinent to note that benthos is rich in lysine, leucine and phenylalanine, the amino acids classified as essential. Analysis of the biological value of benthic protein revealed that cystine, methionine and tyrosine are the limiting amino acids. As a consequence, care must be taken to give fish feeds with higher amounts of these amino acids. Evaluation of Oser's essential amino acid index (personal communication) showed this protein to be of high biological quality, because this index was in the 52.59-54.76 range.

Currently, much attention is focused on the quality of fat contained in fish meat, especially the content of essential unsaturated fatty acids (EUFA) (Vesa et al 2008, 2009). It is well known that the content of these acids in fish meat can be modified to a considerable extent through nutrition (Epler et al 2009ab).

Table 3 presents the fatty acid profile of fat from the pond benthos. The fatty acid profile of fat from the benthos of ponds 1 and 2 was the same. C₁₈ acid, including C_{18:2} (n-6), is found in the highest proportion in the fatty acid profile of benthos fat. The proportion of C_{16:0} and C_{16:1} acids in the fatty acid profile is also considerable. It should be particularly emphasized that benthos fat is rich in essential unsaturated fatty acids, which form over 71% of total acids, with a predominance of polyunsaturated acids. This is highly favourable because the intake of this food by fish will directly enrich fish fat in EUFA, especially since the content of saturated fatty acids is very low and does not exceed 28% of total acids.

Table 1

Chemical composition of natural food (benthos) - %

Item	Dry matter	Crude ash	Crude protein	Crude fat	N-free extractives
Control pond	14.92	3.20	6.56	0.95	4.21
	<i>100.00</i>	<i>21.44</i>	<i>43.97</i>	<i>6.37</i>	<i>28.22</i>
Experimental pond	13.92	3.61	7.50	0.79	2.02
	<i>100.00</i>	<i>25.93</i>	<i>53.83</i>	<i>5.67</i>	<i>14.51</i>

Discussion. Under natural conditions, benthos is the basic food of fish. Under carp farming conditions, it is an important supplement to fish diets containing cereals or pelleted mixtures (Bieniarz et al 2001). An understanding of benthos composition enables the diets of pond carps to be fine-tuned by selecting appropriate grains and their mixtures or pelleted mixtures with well-matched feed components (Przybył et al 1991).

Chemical analysis showed that benthos foods contain large amounts of protein and medium amounts of fat, which are essential components of fish nutrition. These values are similar to those reported by Steffens (1981, 1986). From the animal's (fish's) perspective, not only the amount but also the amino acid composition of dietary protein is important, especially its content of essential amino acids. Today the diets of some farm animal species, including fish, specify not only the protein but also the amino acid requirement (Bologa 2002; Filipiak 1997). This particularly concerns essential amino

acids, which have to be provided in foods because fish are unable to synthesize these amino acids from other compounds (Filipiak 1997). Like animal-origin protein, benthic protein is considered a rich source of essential amino acids with high biological value that exceeds 52% as expressed by the essential amino acid index (Bęza 1967). Analysis (Chemical Score) showed that benthic protein is relatively poor in cystine, methionine and tyrosine, the essential amino acids for every organism including fish. This fact should be taken into account when providing fish with supplemental feeds such as cereals and seeds, which should be high in these amino acids when fed to fish. Only the appropriate balancing of these amino acids in carp food will ensure good growth and weight gains of fish from all feeding groups (Filipiak & Trzebiatowski 1992; Conway 1994).

Table 2

Amino acid content of natural food (benthos) – g/kg

No.	Amino acids	Natural food (benthos)	
		Pond 1	Pond 2
1	asp	5.01	5.17
2	thr	2.10	2.25
3	ser	2.05	2.26
4	glu	5.83	5.96
5	pro	1.58	1.47
6	gly	2.14	2.21
7	ala	3.35	3.73
8	val	2.79	3.11
9	ile	2.18	2.25
10	leu	3.69	3.84
11	tyr	1.54	1.54
12	phe	3.02	3.45
13	his	1.76	1.94
14	lys	3.67	4.12
15	arg	3.31	3.36
16	cys	0.32	0.29
17	met	0.68	0.64
	Σ AA	46.02	47.59
Chemical Score		cystine, methionine, tyrosine	cystine, methionine, tyrosine
Essential Amino Acid Index		54.76	52.59

An essential component of fish diets is fat, especially the fatty acids it contains. The most important acids in fat are essential unsaturated fatty acids (EUFA), which play major roles in the body. They are precursors of some hormones, a component of cytoplasmic membrane structure, are involved in cholesterol transport and metabolism, and stimulate non-specific immunity in carp (Bartnikowska & Kulasek 1994). They have to be supplied through diet because fish bodies mostly do not synthesize these acids. In the analysed benthos, the amount of saturated acids was very high (over 71% of total acids), with low amounts of saturated acids. As a natural food, benthos is a good source of EUFA for fish, which may have a direct effect on the deposition of these acids in fish fat or contribute to "de novo" formation of EPA or DHA acids, which serve important physiological functions in animal bodies (Stickney & Hardy 1989). Considering the inadequate amounts of

natural food in carp ponds, EUFA have to be provided through supplemental diets in the form of fats and oils to meet 100% of the demand for these acids (Epler et al 2009a; Epler et al 2010; Song 1994).

Table 3

Fatty acid profile of benthos fat [% of total acids]

Type of acid	Natural food - benthos	
	control pond	experimental pond
C _{14:0}	1.91	1.32
C _{14:1}	0.14	0.21
C _{16:0}	21.47	21.80
C _{16:1}	10.79	10.20
C _{18:0}	4.20	4.50
C _{18:1}	31.15	29.11
C _{18:2} (n-6)	20.15	23.57
C _{18:3} (n-3)	4.03	3.49
C _{20:0}	0.14	0.12
C _{20:1}	0.61	0.54
C _{20:2}	0.41	0.35
C _{20:3} (n-6)	0.52	0.55
C _{20:3} (n-3)	3.13	2.80
C _{20:4}	0.16	0.10
C _{20:5}	0.18	0.20
Unidentified acids	1.01	1.14
Total saturated acids	27.72	27.74
Total monounsaturated acids	42.69	40.06
Total polyunsaturated acids	28.58	31.06
Total unsaturated acids	71.27	71.12

Conclusions

1. Benthos is a fish food containing high amounts of protein and rich in amino acids, especially the essential amino acids.
2. The limiting amino acids in benthic protein are the sulphur amino acids cystine and methionine as well as tyrosine.
3. The fat content of the benthos is at intermediate level with a high content of essential unsaturated fatty acids.

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Received: 10 February 2010. Accepted: 20 July 2010. Published online: 20 July 2010.

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How to cite this article:

Epler P., Borowiec F., Sokołowska-Mikołajczyk M., Fałowska B., 2010 Content of basic nutrients, amino acids and fatty acids in the benthos of carp ponds. *AAFL Bioflux* **3**(2):125-131.

