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RELATION BETWEEN THE PROFILE OF MAJOR FRACTIONS OF UNSATURATED FATTY ACIDS IN COMMON CARP MEAT (*CYPRINUS CARPIO* L.) IN THE SECOND YEAR OF LIFE AND THEIR PROFILE IN ZOOPLANKTON

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Key words: common carp, fatty acids, natural food, pond fertilization

The aim of the study was to research the correlation of the level of PUFA (polyunsaturated fatty acids), PUFA-3, PUFA-6, EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid), CLA (conjugated linoleic acids), MUFA (monounsaturated fatty acids), MUFA+PUFA and the proportion of PUFA-6/PUFA-3 in the profile of higher fatty acids of carp meat in the second year of life to the respective fractions in the HFA (higher fatty acids) profile of zooplankton in ponds fertilized using various doses of cow and sheep dung. It was found that the content of PUFA-3, EPA, PUFA-6 and PUFA depended on the increase of the content of these fractions in the HFA profile of zooplankton. The content of DHA and MUFA fractions depended on water temperature: the content of the first of the fractions dropped with a temperature increase, whilst the content of the second fraction increased. The variations in PUFA-6/3 proportions and PUFA + MUFA content in the HFA profile were seasonal in character: these values were always higher in the autumn than in the spring. The high fertility of the breeding environment of carp ponds, combined with a moderate density of stocking material, had a positive impact for consumers on the HFA profile of carp meat bred in a 2-year cycle.

INTRODUCTION

The nutritional value of sea fish is currently discussed in terms of the share in the higher fatty acids profile of their tissues of some fractions of PUFA (polyunsaturated fatty acids), such as PUFA-3, and in particular the EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) and PUFA-6 fractions, and in particular the CLA (conjugated linoleic acids) contained in it and the proportions of PUFA-6/3 [Kuza *et al.*, 2006]. In the case of freshwater carp (*Cyprinus carpio* L.), as with sea fish, the HFA profile of the food consumed has high impact on the HFA profile of its meat [Hadjinikolowa, 2004]. In the conditions of semi-natural breeding of cyprinoids in ground ponds, the HFA profile of their meat depends on the fish's access to natural food, such as phytoplankton and zooplankton rich in unsaturated fatty acids [Domaizon *et al.*, 2000). The aim of the study was to research the correlation of the level of PUFA (polyunsaturated fatty acids) PUFA-3, PUFA-6, EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid), CLA (conjugated linoleic acids), MUFA (monounsaturated fatty acids), MUFA+PUFA and the proportion of PUFA-6/PUFA-3 in the profile of higher fatty acids of carp meat in the second year of life to the respective fractions in the HFA profile of zooplankton in ponds fertilized using various doses of cow and sheep dung. Moreover, the study covered the relationship of the above-mentioned fractions of the HFA profile in carp meat and in zooplankton to the load of organic components in pond water, measured using Chemical Oxygen Demand (COD, mg O_2/L) values, and to water temperature in the carp breeding season, from June to September.

MATERIAL AND METHODS

The two-year study was conducted for three ground ponds of the Roztropice carp breading complex, which is part of the IZ Grodziec Śląski Experimental Station, each pond covering an area of approx. 2.5 ha, stocked in spring with fry after wintering, at a density of 1000 fish/ha. The fish were fed with barley meal, and the relatively low density of the fish population ensured access to natural food in the form of zooplankton, among others, as well as a high rate of growth. In the first year of study, one pond was fertilized with 75 q/ha of cow dung, the second pond with 150 q/ha of cattle manure and the third one, not fertilised with dung, served as a control and was supplied with water with a high biogenic content. In the second year, sheep dung was used for the fertilization of these same ponds. In late spring and in autumn, test samples of 10 carps from each pond were taken, in order to determine the HFA profile in meat. Moreover, until late summer, collective tests of zooplankton were done. The higher fatty acids profile was determined using gas chro-

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TABLE 1. The profile of major fractions of unsaturated higher fatty acids (HFA) in the meat of carps in the second year of life and in zooplankton from the ponds fertilized with cow and sheep dung.

Component (%)	Type of test	Dung dose					
		0 q/ha (control)		75 q/ha		150 q/ha	
		cattle manure	sheep manure	cattle manure	sheep manure	cattle manure	sheep manure
PUFA-3	carp spring	30.4^{A}	8.5^{B}	28.0^{A}	10.1^{B}	24.3^{A}	12.4^{B}
	carp autumn	21.2^{A}	3.2^{B}	13.4^{A}	3.7^{B}	19.0^{A}	3.2^{B}
	zooplankton	46.9	24.8	40.6	36.5	45.9	37.4
EPA	carp spring	9.4^{A}	2.0^{B}	8.1^{A}	2.4^{B}	7.1^{A}	3.1^{B}
	carp autumn	5.9^{A}	0.7^{B}	3.7^{A}	0.8^{B}	5.1^{A}	0.7^{B}
	zooplankton	23,4	5.6	21.4	11.9	25.7	11.4
DHA	carp spring	11.4^{A}	2.5^{B}	13.1^{A}	2.4^{B}	8.0^{A}	2.8^{B}
	carp autumn	11.2^{A}	1.2^{B}	6.0^{A}	1.2^{B}	10.1^{A}	1.2^{B}
	zooplankton	3.8	1.3	0.9	2.8	2.8	0.5
PUFA-6	carp spring	23.4^{A}	11.4^{B}	24.5^{A}	11.4^{B}	19.6^{A}	12.7^{B}
	carp autumn	31.1^{A}	9.0^{B}	31.9^{A}	9.5^{B}	36.6^{A}	9.1^{B}
	zooplankton	14.6	10.1	17.3	8.7	16.2	9.8
CLA	carp spring	3.3^{A}	1.6^{B}	2.7^{a}	1.5^{b}	2.9^{A}	1.2^{B}
	carp autumn	2.8^{A}	2.1^{B}	2.6	2.0	2.9^{a}	2.0^{b}
	zooplankton	0.5	0.8	0.3	0.3	0.4	0.2
PUFA	carp spring	57.1^{A}	18.3^{B}	55.2^{A}	23.0^{B}	45.8^{A}	26.2^{B}
	carp autumn	55.1^{A}	14.3^{B}	47.9^{A}	15.1^{B}	58.2^{A}	14.3^{B}
	zooplankton	61.9	35.7	58.1	45.5	62.5	47.3
PUFA-6/3	carp spring	0.8^{A}	1.4^{B}	0.9^{A}	1.1^{B}	0.9	1.0
	carp autumn	1.6^{A}	2.9^{B}	2.4	2.8	2.0	2.5
	zooplankton	0.3	0.4	0.4	0.2	0.4	0.3
MUFA	carp spring	19.0^{A}	46.1^{B}	14.7^{A}	43.7^{B}	23.8^{A}	38.8^{B}
	carp autumn	21.6^{A}	58.0^{B}	25.6^{A}	57.0^{B}	19.8^{A}	56.0^{B}
	zooplankton	17.5	27.9	19.5	18.2	16.1	22.3
MUFA + PUFA	carp spring	76.2^{A}	68.9^{B}	70.4^{A}	66.7^{B}	69.6^{A}	65.0^{B}
	carp autumn	76.7^{A}	72.3^{B}	74.0	72.1	78.0^{A}	70.2^{B}
	zooplankton	79.4	63.6	77.6	63.7	78.7	69.6

The differences between the composition of the meat of carps from ponds fertilized with two types of dung: A, B-highly significant differences ($p<0.01$) and a, b – significant differences ($p<0.05$)

matography, assaying the acids in the form of methyl esters. Test samples were prepared based on Folch's method [Folch, 1957] consisting in homogenisation of the sample in a mixture of chloroform and methanol (2/1) and vapourisation of the solvent and then saponification (0.5 N NaOH in methanol) and esterification (BF_3 in methanol) of the matter remaining after vapourisation. The fatty acid methyl esters created this way were assayed in hexanoic extracts on a VARIAN 3400 gas chromatograph. The Chemical Oxygen Demand (COD) values were assayed using the bichromate method [Hermanowicz *et al.*, 1999]. A variance and single-factor regression analysis was conducted using the Statgraphics software.

RESULTS

The percentage shares of the individual fractions of unsaturated fatty acids in the tested HFA profiles fluctuated in a wide range of values (Table 1). However, the shares of the sum of all the unsaturated fatty acids (MUFA + PUFA) in these profiles were high at all times and in carp meat ranged from 65.0% do 78.0%, and in the zooplankton from 63.6% to 79.4%.

In carp meat from spring and autumn tests, the percentage shares of PUFA-3, including EPA and DHA as well as PUFA-6, including CLA, as well as the shares of the entire PUFA fraction and sums of PUFA + MUFA in the ponds fertilized using cow dung and in the control pond in the first year of the study were at all times higher than in the corresponding ponds fertilised using sheep manure, in the second year of testing. These were highly significant statistical differences ($p<0.01$), with the exception of CLA and the sum of PUFA + MUFA, where statistically significant differences ($p<0.05$) or no differences were also found. Conversely, the percentage shares of MUFA and proportions of PUFA-6/3 were in all the cases lower than in the ponds fertilised using cow dung and in the control pond than in the corresponding ponds fertilised using sheep dung. In the carp meat from spring tests, the percentage shares of PUFA-3 and EPA in the HFA profile were at all times higher than in carp meat from autumn tests, whilst the proportion of PUFA-6/3 in the spring was always lower. On the other hand, the share of the MUFA fraction in the HFA profile was in general higher in autumn than in spring. Based on the analysis of single-factor regression using all the results from the two years of study, it has been determined that there was a statistically highly significant or significant correlation between the increase of the percentage share of PUFA-3, EPA, PUFA-6 and PUFA in the HFA profile of carp meat and the increase of the share of these same HFA fractions in zooplankton (Table 2). In the pond fertilized with cow dung at the dose of 150 q/ha per year, the fertility of water measured with COD values was higher by 18.0% than in the pond fertilized with cow dung at the dose of 75 q/ha, and

TABLE 2. The results of the single-factor regression analysis of the composition of the fractions studied in the HFA profile of carp meat (Y) to the content of these fractions in zooplankton (x).

HFA fraction	Equation	r	r^2	$p^{*)}$
PUFA-3				
spring	Y = 1.0 x - 19.8	0.83	0.69	0.040
autumn	Y = 0.9 x - 23.0	0.84	0.71	0.040
EPA				
spring	Y =0.4 x - 0.8	0.92	0.85	0.009
autumn	Y =0.3 x - 1.8	0.94	0.88	0.006
PUFA-6				
spring	Y = 1.5 x - 2.5	0.94	0.88	0.006
autumn	Y = 3.5 x - 23.0	0.96	0.92	0.002
PUFA				
spring	Y = 1.5 x - 38.9	0.92	0.85	0.009
autumn	Y = 1.9 x - 63.6	0.93	0.86	0.006

*) $p< 0.01$ for highly significant results and $p<0.05$ for significant results

relations between the increase of COD value and the increase of shares of PUFA-3, EPA, PUFA-6 and PUFA in the HFA profile of carp meat (Table 4). The share of the above-mentioned fractions, and in addition DHA in the HFA profile of carp meat from autumn tests decreased statistically highly significantly and significantly with the increase of water temperature, whilst the share of MUFA increased. In the HFA profile of zooplankton, with the increase of the average seasonal COD value of pond water, the share of PUFA and the sum of PUFA + MUFA increased, whilst with an increase of temperature, the share of EPA, PUFA-6 and PUFA decreased (Table 5).

DISCUSSION

The average percentage of basic PUFA fractions in the higher fatty acids profile of carp meat from the study was presented in Table 6 as compared to additional species of freshwater fish

TABLE 3. Average values of COD (mg O_2/L) and temperature (°C) for pond water in the period from June to September.

Manure dose						Average	
0 q/ha (control)		75 q/ha		150 q/ha		cow dung 2004	sheep dung 2006
cow dung 2004	sheep dung 2006	cow dung 2004	sheep dung 2006	cow dung 2004	sheep dung 2006		
COD (mg O_2/L)							
41.4	20.9	35.2	20.8	41.4	27.2	39.4	22.9
Water temperature (°C)							
19.0	20.9	18.7	20.2	19.7	21.1	19.1	20.7

TABLE 4. The results of the single-factor regression analysis of the composition of the fractions studied in the HFA profile in the meat of 2-year old carp to the average seasonal COD value and water temperature.

HFA fraction (Y)	Environmental factor (x)	Regression equation	r	r^2	$P^{*)}$
PUFA-3	COD	Y= 0.9x - 23.0	0.84	0.71	0.040
	temperature	Y= - 6.8x + 147.0	-0.80	0.64	0.050
EPA	COD	Y= 0.3x - 1.8	0.94	0.88	0.006
	temperature	Y= - 2.0x + 42.4	-0.82	0.90	0.050
DHA	COD	no significant correlations			
	temperature	Y= - 4.6x + 99.2	-0.95	0.90	0.004
PUFA-6	COD	Y= 3.5x - 23.0	0.96	0.92	0.002
	temperature	Y= 0.9x +249.2	-0.85	0.72	0.030
PUFA	COD	Y= 1.9x - 63.6	0.93	0.86	0.006
	temperature	Y= -18.6x + 405	-0.84	0.71	0.040
MUFA	COD	no significant correlations			
	temperature	Y= 12.5x – 217.7	0.91	0.83	0.010

*) $p< 0.01$ for highly significant results and $p<0.05$ for significant results

when sheep dung was used, the difference was 31.0% (Table 3). The highest organic matter load was found in the non-fertilized control pond, due to a flood dump of water rich in biogenes. In the first year of the study, average seasonal water temperatures in the experimental ponds were lower by 1.4°C-1.9°C than in the same ponds in the second year. On the other hand, there were no statistically significant correlations between COD and water temperature. However, there were statistically highly significant bred in Poland [Bieniarz *et al.*, 2000]. That Table shows that in conductive trophic conditions, which were determined in the first year of the study, the average share of PUFA in the HFA profile of the meat of omnivorous carp can exceed 50% and be even higher in this respect than the quality of the meat of salmonids (rainbow trout) and other predator fishes (European catfish and pike), and distinctly higher than the quality of meat of herbivorous fishes (grass carp) benthos-eating fish (ide and

TABLE 5. The results of the single-factor regression analysis of the composition of the fractions studied in the HFA profile in zooplankton to the average seasonal COD value and water temperature.

HFA fraction (Y)	Environmental factor (x)	Regression equation	r	r^2	P*)
EPA	COD	no significant correlations			
	temperature	Y= - 6.7x + 150.5	- 0.82	0.67	0.050
PUFA-6	COD	no significant correlations			
	temperature	Y= - 0.84x + 75.8	- 0.84	0.71	0.040
PUFA	COD	Y= 1.1x + 18.5	0.95	0.90	0.003
	temperature	Y= -8.8 + 226.5	- 0.80	0.64	0.050
PUFA + MUFA	COD	Y= 1.9x -63.6	0.93	0.86	0.006
	temperature	no significant correlations			

*) $p< 0.01$ for highly significant results and $p<0.05$ for significant results

TABLE 6. The average percentage of basic PUFA fractions in the higher fatty acids profile of carp meat from the study compared to additional species of freshwater fish bred in Poland [Bieniarz *et al.*, 2000].

The results of tests of common carp meat in the second year its life			The results of tests of meat of other freshwater fish [Bieniarz *et al.*, 2000]						
	Study year		Fish species						
	first year	second year	Rainbow trout	European catfish	Pike	Grass carp	Crucian carp	Ide	Tench
PUFA	53.2	18.6	34.7	31.0	38.4	17.6	26.2	18.1	22.7
PUFA-3	22.7	6.9	24.7	21.9	29.8	8.7	14.7	7.7	14.2
PUFA-6	27.9	10.5	10.0	9.1	8.6	8.9	11.5	10.4	8.5
PUFA-6/3	1.2	1.5	0.4	0.4	0.3	1.0	0.8	1.4	0.6

tench). The same observation was related to particularly important, from a dietary point of view, content of PUFA-3 fraction in carp meat. The relation of PUFA-6 to PUFA-3 in carp meat was 3 to 4 times higher than with predator fish. However, in the unfavourable trophic conditions which were determined in the second year of the study, when the experimental ponds were fertilized using sheep dung, the share of PUFA in HFA profile dropped 3 times, because when compared to the first year of the study, the share of PUFA in the natural food of the carp dropped on average from 61% to 43% and the fertility of the pond, measured with COD values, decreased by 40%. Because this phenomenon was also observed in relation to the control pond, which was not fertilized with dung, it is hard to establish the superiority of pond fertilization with cow dung over their fertilization with sheep dung. The comparison of the quality of the meat of carp and its natural food with the sea fishes of moderate and arctic zone which also find their way to the Polish market works out less advantageously than in relation to other freshwater fish. The sum of PUFA + MUFA in the HFA profile of crustaceans, which are the food of arctic fishes (*Notothenia coriiceps* and *Lepidonotothen nudifrons*), amounted to 80% in the HFA profile [Kamler & Rakusa-Suszczewski, 2000], whilst in the tested zooplankton from carp ponds, the same value amounted from 65.6% to 78.6% on average. When with the above-mentioned arctic fish, the sum of EPA and DHA was 32% of the HFA profile, with carp this value was as little as from 3.5% to 16.7%. The sums of EPA and DHA in the meat of tested carps was only from 14.0% to 31.2% of the PUFA fraction, contrary to the results for herring [Kuza *et al.*, 2006], with which the sum of these fatty acids was 60-80% of PUFA. The results of statistical analyses, included in Tables 2, 4-5 proved the presumptions of other authors that the variance of the share of the studied fractions of unsaturated fatty acids in the HFA profile of carp meat was caused by the influence of the microclimate factor on the physiological functions of fish, that is water temperature [Yeo *et al.*, 1997; Rasoarahona, 2004], the HFA profile of zooplankton being the carp's natural food, the phenological factor, that is the breeding season period and the fertility of the aquatic environment [Ligaszewski *et al.*, 2006]; the most susceptible to the phenological factor, the degree of fertility and the temperature of water as well as the HFA profile of zooplankton, were such fractions of the HFA in carp meat as PUFA-3 and EPA, next, PUFA-6 and PUFA not susceptible only to the phenological factor. The MUFA fraction was dependent on the phenological factor and water temperature. According to Kamler & Rakus-Suszczyński [2000], in the meat of sea fish, the high share of the sum of PUFA + MUFA in the HFA profile was related to low water temperature and high content of this fraction in natural food of the fish; in relation to the discussed results concerning the quality of carp meat, this model proved right only as regards PUFA, because no correlation was found between the share of MUFA in the HFA profile of carp meat and zooplankton, whilst the share of this fraction increased with an increase in water temperature (Table 4). DHA content increased with an increase in water temperature (Table 4), which was the expression of acclimatisation adaptation as claimed Yeo *et al.* [1997]. The proportion of PUFA-6/3 and the sum of MUFA + PUFA in the study depended on the phenological factor only.

CONCLUSIONS

Among the eight studied fractions of higher fatty acids in the HFA profile of common carp meat in the second year of life, only the content of PUFA-3, EPA, PUFA-6 and PUFA fractions depended on the increase of these fractions in the HFA profile of zooplankton. The content of DHA and MUFA fractions was dependent on water temperature only and the content of the first of the fractions decreased with a temperature increase, whilst the share of the second one increased. Changes in the proportion of PUFA-6/3 and the content of the sum of unsaturated fatty acids (PUFA + MUFA) in the HFA profile were seasonal in character; these values were always higher in autumn than in spring. The high fertility of the breeding environment of carp ponds in the first year of studies, combined with the moderate density of stocking material, had a positive for consumers impact on the HFA profile of the meat of carps bred in a two-year cycle.

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ZALEŻNOŚĆ POMIĘDZY PROFILEM GŁÓWNYCH FRAKCJI NIENASYCONYCH KWASÓW TŁUSZCZOWYCH W MIĘSIE KARPIA (*CYPRINUS CARPIO* L.) W DRUGIM ROKU ŻYCIA, A PROFILEM TYCH KWASÓW W ZOOPLANKTONIE

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Badano zawartości procentową w profilu wyższych kwasów tłuszczowych (WKT) mięsa karpia hodowanego w dwuletnim cyklu produkcyjnym najważniejszych frakcji nienasyconych, wyższych kwasów tłuszczowych. Wyniki dwuletnich badań obejmują wartości średnie z prób wiosennych i jesiennych ryb pobieranych ze stawów nawożonych różnymi dawkami obornika bydlęcego i owczego. Odpowiednie badania przeprowadzono też na próbach zooplanktonu z tych stawów.

Analizując dane szczegółowe stwierdzono, że wzrost udziału frakcji PUFA-3, EPA, PUFA-6 i PUFA w profilu WKT mięsa karpi zależał od wzrostu udziału tych frakcji w profilu WKT zooplanktonu. Udziały frakcji DHA i MUFA były zależne tylko od temperatury wody, przy czym udział pierwszej frakcji malał ze wzrostem temperatury, podczas gdy udział drugiej frakcji rósł. Proporcje PUFA-6/3 oraz udział PUFA + MUFA w profilu WKT zawsze były większe w jesieni, niż na wiosnę. Wysoka żyzności stawów karpiowych mierzona wartościami ChZT w pierwszym roku badań, w połączeniu z umiarkowanym zagęszczeniem w nich materiału zarybieniowego wpłynęła na korzystne dla konsumentów ukształtowanie się profilu WKT mięsa karpia hodowanego w cyklu 2-letnim.