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THE FATTY ACIDS COMPOSITION IN EGG LIPIDS OF PIKE, *Esox lucius* L. FROM THE PUCK BAY AND LAKES NEAR LIPUSZ

SKŁAD KWASÓW TŁUSZCZOWYCH W LIPIDACH IKRY SZCZUPAKA (*Esox lucius* L.) Z ZATOKI PUCKIEJ I JEZIOR OKOLIC LIPUSZA

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The composition of fatty acids was determined in eggs of pike inhabiting some inland lakes and the Baltic's Puck Bay. The acid percentage composition was found to vary in each lipid series. Additionally, considerable differences were revealed in quantities of the C-24:0; C-24:1, and C-24:2 acids present. In brackish water pike eggs no amount of the following acids was detected: C-20:1, C-23:1, and C-23:2 in phospholipids, C-18:0 in cholesterol esters, C-16:4 in triglycerides. The acids listed above are present, although in very small quantities, in eggs of pike from inland waters.

INTRODUCTION

In the brackish waters of Poland, e.g., in the Puck Bay, many freshwater fish species that have adapted their life habits to the ecological specificity of these waters can be encountered. The basic difference between inland waters and those of the Puck Bay lies in the presence of inorganic salts (6–7% as converted to NaCl) in the latter. Some of these "adapted" fish species, for instance perch (*Perca fluviatilis* L.) display a higher growth rate in their first year of life when compared to the species' representatives living in fresh waters (Jurkowski, 1973); other species, like pike (*Esox lucius* L.) show a lower

growth rate (Skóra, 1973). These variations in growth rates can be accounted for by different, both qualitative and quantitative, composition of organic substances controlling the embryonic development and contained in an egg cell ready to be fertilized. A very important group of egg organic substances is formed by lipids contained (as phospholipids) in cell plasmatic walls and – as triglyceridesproviding the major energy source for the developing embryo. Changes occurring in the egg lipids composition can severely affect growth rate in the first year of fish life. The present paper attempts to find an answer to the question of how the Puck Bay waters salinity influences the composition of fatty acids in egg lipids of pike dwelling in these waters. The results obtained were compared to the data collected from the same species inhabiting lakes in the vicinity of Lipusz, the lakes having no connection with the Puck Bay whatsoever so that any possible migration of fish is excluded.

MATERIAL AND METHODS

Eggs were obtained from fishes commercially caught in April 1975 in the Puck Bay and lakes near Lipusz. The individuals captured were at the gonad maturity stage VI (Meyer scale). The eggs were collected form live individuals by a light pressure applied to fish abdomens. In order to exclude variation in the biochemical composition resulting from individual characters, eggs derived from a few specimens were mixed together, 10-g samples being then withdrawn for lipid extraction.

The reagents used were: chloroform, methanol, ethyl ether, potassium dichromate ($K_2Cr_2O_7$), NaCl glacial acetic acid, concentr. sulphuric acid (all produced by BOCh, Poland), BF_3 in methanol, hexane, and cholesterol (BDH, England), lecithin (Koch-Light Ltd., England), and glycerol trioleinate (Fluka AG, Switzerland).

Lipids were extracted by Bligh and Dyer (1959) method using choloroform/methanol and separated into series by thin layer chromatography (TLC) using 10X20 cm glass plates covered with 2 mm-thick layer of silica gel (G. Merck). The developing mixture used consisted of 80:20:0.25 (v/v) hexane: ethyl ether:glacial acetic acid. The lipids were sprayed with 0.02% solution of rodamine 6G in 96% ethanol. Glycerol trioleinate, lecithin, and cholesterol were used as standards.

Four distinct fractions were obtained after treating the chromatograms with rodamine 6G solution; they were made up by cholesterol esters (CE), triglycerides (TG), free cholesterol (C), and phospholipids (PL). CE, PL, and TG were scraped off from the plates and extracted from the gel using 2:1 (v/v) chloroform: methanol for PL and the developing agent for CE and TG.

Methyl esters were obtained as in Metcalfe, Schnitz, and Pelka (1966). The gas chromatography of esters was performed in a flame ionization detector "Chromatoprep" chromatograph. 2mX4 mm (inner diameter) glass columns filled with 12.5% of 2:1 DEGS:PEGA (Ap. Sci. Lab. St. Col., USA) on Chromosorb W (Johns. Manville Inc., USA) were used. The temperatures of the oven, detector, and injector were 182, 200, and 230°C, respectively. Argon of 35 cm^3/min. flux ($1 \cdot 10^{-9}$ A amplification) served as the carrying gas.

The results obtained were compared to the similar data collected for the rape (*Brassica nupus*) oil, the latter being used as an internal standard containing fatty acids of 12–24 carbon atoms and 1–4 unsaturated bonds basing on these acids' methyl esters retention time logarithm.

RESULTS AND DISCUSSION

Table 1 illustrates the fatty acids weight percentage composition in separated lipid series obtained from pike eggs. Apart from quantitative differences in acid contents, the results obtained reveal that some quantitative variations do exist as well.

In egg lipids of the Puck Bay pike, the following acids were not detected: C–20:1, C–23:1, and C–23:2 in phospholipids, C–16:4 in triglycerides, and C–18:0 in cholesterol esters, all these acids being present in the freshwater pike eggs lipids. In view of the fact that the differences observed concern the acids present in very small quantities, it is difficult to offer an explanation of the differences, although the fish diet influences the fatty acid composition to a minimum degree only (Kluytmans and Zandee, 1973). Similarly to Aclman (1967) and Kluytmans and Zandee (1973) we found small amounts of linoleic and linolenic acids; these are not synthesized de novo by pike but are indispensable in the synthesis of highly unsanturated long-chain fatty acids.

In contrary to the results obtained by Kluytmans and Zandee (1973), pike of the two habilitats yielded eggs of a considerable content of the C–24:O and C–24:1 acids in all the lipid series and C–24:2 acid in CE. We also found two acid containing more than four unsaturated bonds, but due to the lack of suitable standards they were impossible to identify. In our opinion, the unidentified acids denoted as A and B (Table 1) are the C–20:5 and C–22:6 acids, respectively, which had been found in other works on the problem.

Similarly to lipids of male gonads (Kluytmans and Zandee, 1973), the eggs of pike of the two habitats were found to contain the C–23:1 acid and small amounts of the C–23:2 one, but the C–22:3 was not detected. Particularly large quantities of these acids were present in the cholesterol esters fraction of lipids.

Table 2 shows differences that exist between the percentage composition of fatty acids in lipids and their saturation degree. In lipids of both pike groups, acids of a single double bond prevail. More saturated acids are present in pike from lakes. Reverse in the case in highly unsaturated fatty acids with two and more double bonds; these make up 13.9 and as much as 29.7% of all the fatty acids in pike from lakes and from the Puck Bay, respectively. The fatty saturation degree and their amount in fish organisms depend, to a large extent, upon the temperature of the actual habitat of fish: the lower the water temperature, the higher percentage of synthesized highly unsaturated acids (Ackman, 1967). Although no relevant comparative studies of water temperatures in the Lipusz lakes and Puck Bay were carried out, both the fact of the Puck Bay pike spawning being delayed by ca 1 month as compared to the situation in the Lipusz lakes (own observation) and the data obtained on the quantities of highly unsaturated fatty acids

Table 1

Fatty acid composition in egg lipids of pike from fresh- and brackish waters (data expressed as weight percentages)

Methyl esters of acids	Egg lipids of pike from lakes				Egg lipids of Puck Bay pike			
	CE	TG	PL	Total	CE	TG	PL	Total
14:0	0.6	1.7	0.9	1.0	+	1.1	0.9	0.7
14:1	+	1.3	+	0.4	1.5	+	+	0.7
14:2	0.7	0.7	0.6	0.6	+	+	0.6	0.1
14:3	+	+	+	+	2.5	+	+	1.1
16:0	5.1	12.0	13.2	11.2	4.8	8.2	21.7	7.6
16:1 ,	5.0	19.9	10.0	11.3	1.4	12.0	9.3	7.3
16:2	1.3	2.4	1.9	2.0	4.7	3.0	2.3	3.5
16:3	+	2.4	1.2	1.4	1.9	3.3	1.1	2.4
16:4	+	+	+	+	+		0.7	+
18:0	1.5	2.9	7.6	2.5		5.2	3.4	2.8
18:1	12.0	25.5	13.3	16.5	4.8	19.9	15.5	12.6
18:2	3.3	6.0	4.3	4.8	2.0	4.5	1.6	3.2
18:3	+	2.2	1.0	1.2	3.6	1.7	+	2.2
18:4	0.9	1.4	+	0.5	4.3	3.6	0.9	3.3
19:1	2.1	3.9	2.4	2.5	5.0	1.9	0.8	3.2
20:0	+	1.9	0.9	1.0	1.5	1.2	+	1.1
20:1	+	0.5	+	+	2.0	1.9		1.9
20:2	+	+	+	+	4.9	1.2	+	2.6
20:3	1.4	+	0.7	1.2	4.2	+	+	2.4
20:5 ? (A)	7.3			1.3	3.6			1.6
21:1	0.9	1.9	6.4	0.5	+	2.0	+	+
22:0	3.0	0.9	1.1	4.1	1.7	1.8	2.8	2.1
22:1	9.4	2.9	8.1	7.4	3.9	6.8	7.3	5.8
22:2	+	+		+	+	+		+
22:6 ? (B)	7.3			1.3	3.6			1.6
23:1	2.1	+	+	0.5	3.4	+		1.3
23:2	2.0	+	+	0.4	2.7	+		1.0
24:0	9.3	1.4	1.7	4.0	3.8	2.3	3.0	3.1
24:1	30.7	8.3	24.6	23.1	16.8	18.3	27.6	20.1
24:2	1.4			0.3	1.7			0.7

CE – cholesterol esters
TG – triglycerides
PL – phospholipids

+ – acid amount less than 0.5%
unoccupied space in the table – fatty acids not found

Table 2

Fatty acids summarized in order of their unsaturation degree
(data extracted from Table 1)

Number of unsaturated bonds	Eggs from lake pike (%)	Eggs from Puck Bay pike (%)
0	23.8	17.4
1	62.2	52.9
2	8.1	11.1
3	3.8	8.3
4	0.5	3.3
5	1.3	1.6
6	0.2	5.4

point out the Puck Bay water temperature to be lower than that prevailing in shallow lakes.

When our data are compared to those collected by Kluytmans and Zandee (1973), the differences between the first and those reported by the above mentioned authors for pike ovaries clearly emerge. Unfortunately, no conclusion can be drawn from this comparison due to the lack of a gonad maturity stage being stated for the fishes examined by the authors. A relatively high percentage of 24 carbon atom acids occurring in egg lipids in the two groups of pike (27.4 and 23.9% in the lake and Puck Bay pikes, respectively) is an interesting fact. Similar acids as well as those of 25 carbon atoms were found by Glass, Krick, and Eckard (1974) who, however, reported no quantitative data obtained during their studies.

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SKŁAD KWASÓW TŁUSZCZOWYCH W LIPIDACH IKRY SZCZUPAKA (*Esox lucius* L.) Z ZATOKI PUCKIEJ I JEZIOR OKOLIC LIPUSZA

Streszczenie

Niniejsza praca przedstawia wyniki badań nad składem kwasów tłuszczowych w lipidach ikry szczupaka (*Esox lucius* L.) żyjącego wyłącznie w wodach słodkich (jeziora) lub słonawych (Zatoka Pucka). Materiał do badań uzyskano z dojrzałych samic złowionych w połowach przemysłowych w kwietniu 1975 r. Analizę lipidów przeprowadzono w oparciu o chromatografię cienkowarstwową, analizę kwasów tłuszczowych w oparciu o chromatografię gazową ich estrów metylowych. Znaleziono różnice w składzie kwasów tłuszczowych w lipidach ikry obu rodzajów szczupaka zarówno ilościowe jak i jakościowe. W lipidach ikry szczupaka z Zatoki Puckiej nie stwierdzono następujących kwasów tłuszczowych: w fosfolipidach C–20:I; C–23:I i C–23:2 w trójglicerydach C–16:4, w estrach cholesterolu C–18:0, które to kwasy występują w małych ilościach w odpowiednich lipidach ikry szczupaka z jezior. Na podstawie uzyskanych rezultatów trudno określić czym spowodowany jest brak ww. kwasów tłuszczowych. W obu rodzajach ikry znaleziono duże ilości kwasów tł. o 24 atomach węgla, wśród których dominuje ilościowo kwas o jednym wiązaniu nienasyconym, ponadto kwas C–24:2 znaleziono wyłącznie we frakcji estrów cholesterolowych. Uzyskane wyniki porównano z literaturą dotyczącą podobnych badań na szczupaku oraz innych gatunkach ryb.

СОСТАВ ЖИРНЫХ КИСЛОТ В ЛИПИДАХ ИКРЫ ЩУКИ (ESOX LUCIUS L.) ИЗ ПУЦКОЙ БУХТЫ И ОЗЁР РАЙОНА ЛИПУША

Резюме

В работе представлены результаты исследований состава жирных кислот в липидах икры щуки (Esox lucius L.) обитающей в пресноводных водоёмах (озёра) или солоноватых (Пуцкая бухта). Материал для исследований был взят из взрослых самок, выловленных в период промысла в апреле 1975 г. Анализ липидов был проведен на основе тонкослойной хроматографии, анализ жирных кислот – на основе газовой хроматографии сложных метиловых эфиров. В составе жирных кислот в липидах икры обоих видов щуки обнаружены как количественные, так и качественные различия. В липидах икры щуки из Пуцкой бухты не были обнаружены следующие жирные кислоты: в фосфолипидах – С-20:1;

C-23:1; C-23:2; в триглицеридах - C-16:4; в сложных эфирах холестерина - C-18:0; последние содержатся в небольших количествах в соответствующих липидах икры озёрной щуки.

На основе полученных результатов трудно определить, чем вызвано отсутствие вышеназванных жирных кислот. В икре обоих видов щуки обнаружено большое количество жирных кислот с 24 атомами угля, среди которых в количественном отношении преобладает однооснованая ненасыщенная кислота, а, кроме того, кислота C-24:2 обнаружена только во фракции сложных холестериновых эфиров.

Полученные результаты сравнили с литературными денными, касающимися подобных исследований щуки и других видов рыб.

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