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# COMPARATIVE STUDY ON THE FATTY ACID COMPOSITION OF THE TISSUE LIPIDS IN THE FISH *CYPRINUS CARPIO L.*

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Numerous papers have been published on the fatty acid composition of fishes. Earlier reports were reviewed by HILDITCH (1956). More recently the exact chemical characterization of the fatty acids in fish (STOFFEL and AHRENS 1960), the metabolic pathways of their synthesis (MEAD et al. 1960) and the dietary factors influencing the fatty acid composition (KELLY et al. 1958) have been reported.

Many of these works were restricted to the study of the total lipids of a single organ or of the whole animal and did no throw light on the role of the different organs in the lipid metabolism of fish. In this communication we aimed at studying the fatty acid composition of both the total and different fractions of lipids from various organs hoping that our findings will form a step in the proper metabolic studies of lipids in fishes.

## Material and methods

The fish studied was a two year old carp *(Cyprinus carpio L.)* of half kilogram weight, caught from the fish pond of Soponya (Hungary). The blood was collected in a heparinized tube by cutting the caudal vein. The plasma was separated. Plasma lipids were extracted with BLOOR'S mixture (ethanol-ether 3 : 1). The fish was then dissected and 1 gr liver, muscle and intestinal adipose tissue were excized, homogenized and extracted immediately with chloroform-methanol 2 : 1 after FOLCH. The extracts were divided into two portions. One portion was evaporated representing the total lipids. The other portion was fractionated by thin layer chromatography on silica gel. The developing mixture consisted of heptane — diethylether-acetic acid (40 : 10 : 0.5). The total lipids and the different lipid fractions without being eluted from the silica gel were interesterified by a rapid method as described by SZŐKE et al. (1965).

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*Table 1.*

The fatty acid composition of total lipids of the various tissues of the fish

| | 14 : 0 | 14 : 1 | 16 : 0 | 16 : 1 | 17 : 0 | 18 : 0 |
|---|---|---|---|---|---|---|
| Plasma — Plazma ..... | 2.2 | 0.6 | 26.4 | 12.5 | 0.5 | 4.1 |
| Adipose tissue — Zsírszövet | 2.8 | tr | 19.0 | 11.5 | tr | 5.3 |
| Liver — Máj ..... | 1.1 | 0.2 | 22.0 | 14.0 | tr | 4.6 |
| Muscle — Izom ..... | 0.7 | 0.5 | 14.6 | 9.4 | tr | 4.8 |

*Table 2.*

The fatty acid composition of the cholesterol ester fraction of the various tissues of the fish

| | 10 : 0 | 12 : 0 | 14 : 0 | 14 : 1 | 16 : 0 | 16 : 1 | 17 : 0 |
|---|---|---|---|---|---|---|---|
| Plasma — Plazma | 7.0 | tr | 7.6 | 0.5 | 14.2 | 9.5 | tr |
| Adipose tissue — Zsírszövet | tr | tr | 0.5 | 0.3 | 17.6 | 8.7 | 1.6 |
| Liver — Máj ..... | tr | tr | 0.4 | 0.5 | 15.0 | 6.4 | 0.9 |
| Muscle — Izom | 0.3 | 0.6 | 0.3 | tr | 13.3 | 10.7 | 1.3 |

*Table 3.*

The fatty acid composition of the triglyceride fractions of the various tissues of the fish

| | 14 : 0 | 14 : 1 | 16 : 0 | 16 : 1 | 17 : 0 | 18 : 0 |
|---|---|---|---|---|---|---|
| Plasma — Plazma | 2.2 | 0.7 | 21.9 | 14.7 | 1.4 | 3.2 |
| Adipose tissue — Zsírszövet | 1.9 | 0.3 | 19.9 | 10.4 | tr | 4.2 |
| Liver — Máj | 0.6 | 0.2 | 17.4 | 12.7 | tr | 2.2 |
| Muscle — Izom | 0.7 | 0.2 | 14.1 | 13.0 | tr | 3.9 |

The methyl esters were then analysed by an Aerograph GLC apparatus Type 90, using hydrogen as a carrier gas on a column packed with diethylene glycol-succinate coated on Chromosorb W, 30—60 mesh. The column temperature was 180° C. The flow rate was 60 ml/min. The fatty acid distribution was determined by measuring the relative areas under the curves. For the identification of the peaks the National Heart Institute fatty acid standards were used. The data in the Tables represent the mean values of three analyses.

*1. táblázat*

Az összzsiradék zsírsav összetétele a hal különböző szöveteiben

| 18 : 1 | 18 : 2 | 18 : 3 | 20 : 0 | 20 : 2 | 20 : 3 | 20 : 4 | 20 : 5 | 22 : 6 |
|---|---|---|---|---|---|---|---|---|
| 33.6 | 5.6 | 1.4 | 2.6 | 0.8 | 1.1 | 2.1 | 4.7 | 1.8 |
| 47.1 | 11.5 | tr | tr | tr | 2.8 | tr | tr | tr |
| 42.0 | 7.1 | 0.3 | 4.1 | 0.5 | tr | 1.8 | 1.4 | 0.9 |
| 45.5 | 10.8 | 0.5 | 1.9 | tr | 0.7 | 8.7 | | 1.9 |

*2. táblázat*

A koleszterinészter frakció zsírsav összetétele a hal különböző szöveteiben

| 18 : 0 | 18 : 1 | 18 : 2 | 18 : 3 | 20 : 0 | 20 : 2 | 20 : 3 | 20 : 4 | 20 : 5 | 22 : 6 |
|---|---|---|---|---|---|---|---|---|---|
| 3.5 | 36.4 | 6.7 | 3.1 | 6.2 | tr | tr | 3.1 | 2.2 | tr |
| 6.5 | 54.4 | 6.9 | 1.6 | 1.1 | tr | tr | 0.8 | tr | tr |
| 3.2 | 54.5 | 9.1 | 2.3 | 6.8 | 0.9 | tr | tr | tr | tr |
| 6.3 | 50.5 | 9.5 | 1.6 | 4.4 | tr | 1.2 | tr | tr | tr |

*3. táblázat*

A triglicerid frakció zsírsav összetétele a hal különböző szöveteiben

| 18 : 1 | 18 : 2 | 18 : 3 | 20 : 0 | 20 : 2 | 20 : 3 | 20 : 4 | 20 : 5 | 22 : 6 |
|---|---|---|---|---|---|---|---|---|
| 38.5 | 7.1 | 2.7 | 3.6 | tr | tr | 2.2 | 1.7 | 0.3 |
| 50.7 | 8.1 | 0.6 | 2.2 | tr | 1.7 | tr | tr | tr |
| 50.5 | 12.0 | tr | 4.4 | tr | tr | tr | tr | tr |
| 52.0 | 12.1 | 1.2 | 1.2 | tr | 0.7 | 0.9 | tr | tr |

## Results and discussion

Fishes, as compared to land animals, are characterized by high percentages of palmitoleic and $C_{20}$, $C_{22}$ fatty acids. From *Table 1* it is evident that the total lipids of all of the tissues examined show these characteristics.

However the $C_{20}$, $C_{22}$ polyenoic acid levels were lower in this carp than in fishes in general. This can be explained by the fact, that in the pond where

*Table 4.*

The fatty acid composition of the diglyceride fraction of the various tissues of the fish

| | 14 : 0 | 14 : 1 | 16 : 0 | 16 : 1 | 17 : 0 | 18 : 0 |
|---|---|---|---|---|---|---|
| Plasma — Plazma | tr | tr | 26.0 | 10.4 | tr | 23.5 |
| Adipose tissue — Zsírszövet | 1.2 | 0.2 | 17.5 | 10.3 | tr | 6.1 |
| Liver — Máj | 0.9 | 0.2 | 30.1 | 8.5 | 0.7 | 5.0 |
| Muscle — Izom | 0.5 | tr | 18.7 | 7.0 | 3.1 | 12.4 |

*Table 5.*

The fatty acid composition of the phospholipid fraction of the various tissues of the fish

| | 14 : 0 | 14 : 1 | 16 : 0 | 16 : 1 | 17 : 0 | 18 : 0 |
|---|---|---|---|---|---|---|
| Plasma — Plazma | 0.8 | 0.4 | 38.5 | 10.3 | 0.4 | 5.4 |
| Adipose tissue — Zsírszövet | 0.9 | 0.2 | 18.0 | 12.8 | 0.4 | 7.8 |
| Liver — Máj | 0.5 | 0.3 | 28.3 | 9.3 | tr | 11.7 |
| Muscle — Izom | 0.5 | 0.5 | 25.2 | 6.7 | 0.2 | 6.0 |

our fish was caught the crustacean plankton does not contribute much to the diet of fishes. Such plankton has been claimed to be the main source for the $C_{20}$, $C_{22}$ polyenoic acids in fish (FARKAS and HERODEK 1964.)

On the other hand, we observed a high level of palmitoleic acid in all tissues examined, indicating that this fatty acid in fish lipid is mainly of endogenous origin, i. e. the conversion of palmitic to palmitoleic acid is very intensive in the fish.

In the muscle lipids there were less palmitic somewhat less palmitoleic and stearic but more linoleic, arachidonic and docosahexaenoic acids. Perhaps this drop in palmitic and stearic acids may be explained as a preferential utilisation of these acids by the muscle.

The plasma lipids had a low content of oleic acid and were rich in all polyenoic acids.

The differences existing between the different tissues are due in part to the disproportionality of the different lipid-franctions in the tissues examined and in part due to differences within the individual fractions. *Tables 2—5* compare the fatty acid composition of the different fractions from various organs.

In human (LINDGREN et al. 1961), and rat (GÖRANSON and OLIVECRONA 1964) sera the linoleic and arachidonic acids respectively constitute about half of the fatty acid components of cholesterol esters. This finding could lead

*4. táblázat*

A diglicerid frakció zsírsav összetétele a hal különböző szöveteiben

| 18 : 1 | 18 : 2 | 18 : 3 | 20 : 0 | 20 : 2 | 20 : 3 | 20 : 4 | 20 : 5 | 22 : 6 |
|---|---|---|---|---|---|---|---|---|
| 32.3 | 5.2 | tr | 2.6 | tr | tr | tr | tr | tr |
| 53.1 | 7.8 | tr | 3.4 | tr | 0.4 | tr | tr | tr |
| 41.2 | 4.6 | 0.9 | 3.9 | 1.1 | tr | 2.5 | 0.4 | tr |
| 42.0 | 6.2 | 2.3 | 2.3 | tr | 1.6 | 3.9 | tr | tr |

*5. táblázat*

A foszfolipid frakció zsírsav összetétele a hal különböző szöveteiben

| 18 : 1 | 18 : 2 | 18 : 3 | 20 : 0 | 20 : 2 | 20 : 3 | 20 : 4 | 20 : 5 | 22 : 6 |
|---|---|---|---|---|---|---|---|---|
| 25.0 | 4.7 | 1.1 | 1.8 | tr | 2.3 | 1.5 | 5.1 | 2.7 |
| 48.0 | 6.3 | tr | 1.5 | tr | 2 1 | 2.0 | tr | tr |
| 28.5 | 4.7 | 0.7 | 4.7 | 1.9 | tr | 9.4 | tr | tr |
| 31.8 | 8.9 | 0.2 | 3.7 | tr | 4.2 | 8.9 | 3.2 | tr |

to some hypothesis on the role of these acids in cholesterol metabolism. According to *Table 2* neither of these fatty acids are present in significant amounts in the cholesterol esters of our fish. The fatty acid pattern of the cholesterol ester franction of liver, muscle and adipose tissue is almost the same, and differs considerably from that of the plasma. The latter is characterized by a high percentage of $C_{10:0}$, $C_{14:0}$ and the polyenoic acids, while the oleic acid content is less than that in other tissues.

Concerning the triglyceride fraction *(Table 3)* we found no important differences between the various tissues. The only one to be mentioned is the lower oleic and higher polyenoic acid content of the plasma.

The fatty acids of diglyceride fractions *(Table 4)* showed great similarity in all tissues with the exception of the unexpected high level of stearic acid in both plasma and muscle diglycerides.

The fatty acid composition of the phospholipids are shown in *Table 5*. They differ significantly from those of the neutral lipids in that they contain higher saturated and polyenoic acid levels, and a lower monoenoic acid level. This can be markedly seen in *Fig. 1.*

The fatty acid composition of the liver and plasma phospholipids are similar. This points to the liver as the origin of plasma phospholipids. On the other hand the adipose tissue phospholipids show a picture which is very similar to that of neutral lipids, indicating that the phospholipids of the adipose

tissue are synthesized in situ. The pattern of muscle phospholipid franction lies in between that of liver and plasma and adipose tissue. From *Fig. 2* it is evident that the neutral fraction contained much less stearic and palmitic

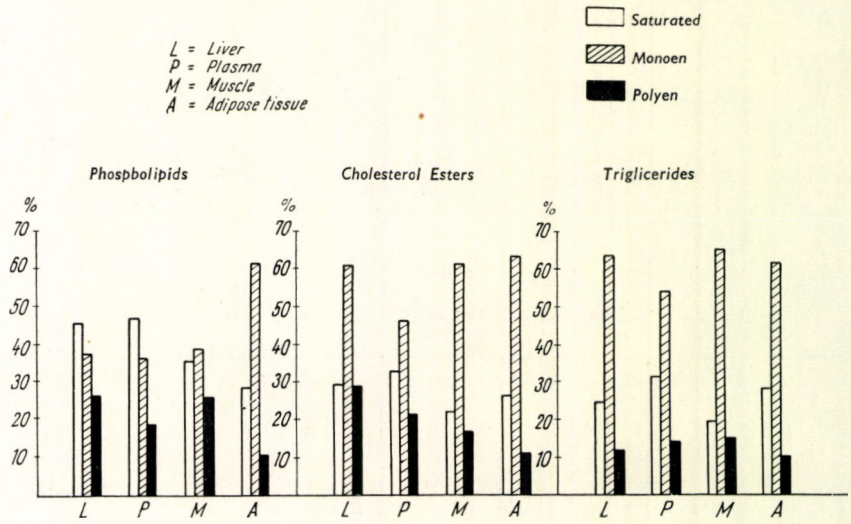


*Fig. 1.* The percentage of saturated, monoenoic and polyenoic fatty acids in the different tissues and fractions

*1. ábra* A telített, egyszer és többszörösen telítetlen zsírsavak százaléka a különböző szövetekben és frakciókban.

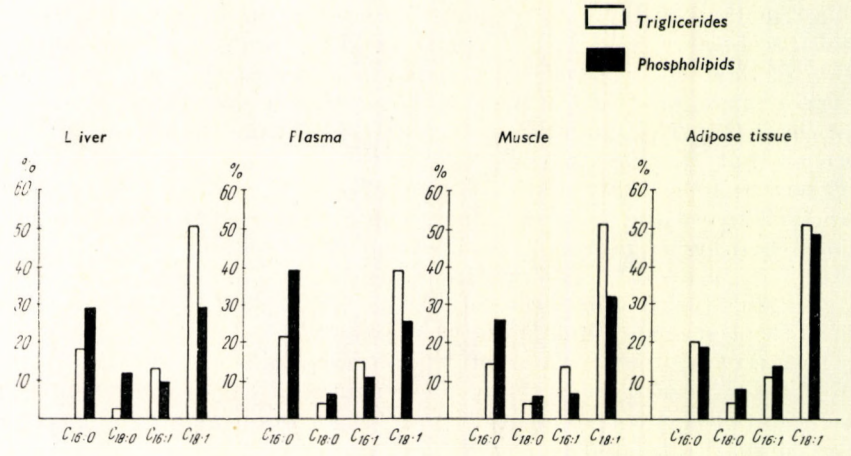


*Fig. 2.* The percentage of palmitic, stearic and the corresponding monoenoic acids in the triglyceride and phospholipid fractions of the different tissues

*2. ábra* A palmitin, sztearin és a megfelelő egyszer telítetlen zsírsavak százaléka a különböző szövetek trigliceridjeiben és foszfolipidjeiben.

acids than did the phospholipids. On the other hand the palmitoleic and oleic acid contents are higher in the neutral fractions than in the phospholipids. This picture clearly demonstrates a preferential incorporation of saturated fatty acids — palmitic, stearic — into the phospholipids and incorporation of the corresponding monoenoic acids — palmitoleic, oleic — into the neutral lipids. This relation is manifested in all tissues investigated except the adipose tissue.

Similar observations were reported recently for the stearic- oleic acid distribution in rats (GÖRANSSON and OLIVECRONA 1964).

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## Summary

The lipids of liver, plasma, muscle and adipose tissue were separated by thin layer chromatography. The fatty acid compositions of the cholesterol esters, triglyceride, diglyceride and phospholipid fractions were determined by gasliquid chromatography.

In our fish the percentage of $C_{20}$,$C_{22}$ polyenoic acids was lower than in fishes in general due to the lack of planktonic crustaceans as food constituents. The concentration of palmitoleic acid was high in the lipid franctions of all tissues.

The plasma cholesterol esters in the carp, in contrast to mammals, contain no significant amounts of linoleic or arachidonic acids. The concentrations of palmitic and stearic acid were higher in the phospholipids than in the triglicerides while with the corresponding monoenoic acids there was an inverse relationship.

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# ÖSSZEHASONLÍTÓ VIZSGÁLATOK A PONTY *(CYPRINUS CARPIO L.)* KÜLÖNBÖZŐ SZÖVETEIBEN TALÁLHATÓ LIPIDFRAKCIÓK ZSÍRSAV-ÖSSZETÉTELÉN

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## Összefoglalás

Egy ponty májának, vérplazmájának, izmának és zsírszövetének zsiradékait rétegkromatográfia segítségével szétválasztottuk. A koleszterin észter, triglicerid, diglicerid és foszfolipid frakciók zsírsavösszetételét gázkromatográfia segítségével meghatároztuk. A vizsgált halban a $C_{20}$, $C_{22}$ többszörösen telítetlen zsírsavak százalékos mennyisége alacsonyabb volt, mint a halakban általában, amit az okoz, hogy jelen esetben a Crustacea plankton nem szerepel jelentős mértékben a táplálékban. A halakra jellemző palmitoleinsav viszont nagy koncentrációban volt jelen minden vizsgált szövet összes lipid frakciójában. Az emlősökkel szemben ebben a pontyban a plazma koleszterinészterek nem tartalmaztak jelentősebb mennyiségű linol vagy arachidonsavat. A palmitin és sztearinsav koncetnrációja magasabb volt a foszfolipidekben, mint a trigliceridekben, míg a megfelelő egyszer telítetlen zsírsavak fordított viszonyt mutattak.

# СРАВНИТЕЛЬНОЕ ИССЛЕДОВАНИЕ СОСТАВА ЖИРНЫХ КИСЛОТ ЛИПИДНЫХ ФРАКЦИЙ РАЗЛИЧНЫХ ТКАНЕЙ КАРПА

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С помощью тонкослойной хроматографии фракционировали липиды жировой ткани, мышцы, сыворотки крови и печени карпа. Определяли посредством газовой хроматографии состав жирных кислот следующих фракций: эфиры холестерина, триглицериды, диглицериды и фосфолициды. У изученного вида процентное содержание насыщенных жирных кислот ($C_{20}$ и $C_{22}$) ниже в сравнении с другими рыбами, это объясняется отсутствием планктонных ракообразных в пище карпа. Пальмитиновая кислота, характерная для рыб, в большом количестве присутствует во всех липидных фракциях всех исследованных тканей. В отличие от млекопитающих, эфиры холестерина плазмы карпа не содержат значительных количеств линолевой и арахидоновой кислот. Концентрация пальмитиновой и стеариновой кислот была выше в фосфолипидах, чем в триглицеридах, а насыщенные жирные кислоты проявляли обратную зависимость.