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# The Effects of Dietary Fatty Acids on the Fatty Acid Composition in the Phospholipid Fraction of *Gambusia affinis*

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**Abstract:** The present study represents an investigation into the effect of dietary fatty acids on the fatty acid composition of the phospholipid fraction of *Gambusia affinis*, a small freshwater fish living in a source located on the campus of Dicle University.

The fatty acid composition of the phospholipid fraction was analysed by gas chromatography for fish collected from the freshwater source, which were naturally fed, and also fish fed an artificial diet with a ω6/ω3 ratio of 5.5:1 for 30 days. The level of ω3 in the fish fed artificially for 30 days was higher than in those collected from the freshwater source. This indicates that the ω6/ω3 ratio in fish lipids is largely affected by ω6/ω3 ratio dietary lipids.

Key Words: *Gambusia affinis*, fatty acids, feed, ω6/ω3 ratio

## *Gambusia affinis*'in Fosfolipit Fraksiyonundaki Yağ Asidi Bileşimine Besinsel Yağ Asitlerinin Etkileri

**Özet:** Bu çalışmada, Dicle Üniversitesi kampus alanında bulunan küçük bir su kaynağında yaşayan, küçük bir tatlı su balığı *Gambusia affinis*'in fosfolipit fraksiyonundaki yağ asidi bileşimine, besinsel yağ asitlerinin etkileri araştırılmıştır. ω6/ω3 oranı 5.5:1 olan yapay balık yemleri ile 30 gün boyunca beslenen ve ayrıca bir tatlı su kaynağından toplanan balıkların fosfolipit fraksiyonundaki yağ asitleri bileşimleri gaz kromatografisi yöntemiyle analiz edilmiştir. Otuz gün süre ile yapay balık yemi ile beslenen balıkların fosfolipit fraksiyonundaki ω3 yağ asit yüzdelerinde, araziden getirilenler ile karşılaştırılınca bir artış gözlenmiştir.

Çalışma sonucunda, balık lipitlerinin ω6/ω3 oranlarının diyet lipitlerinin ω6/ω3 oranı tarafından büyük bir şekilde etkilendiği sonucuna varılmıştır.

Anahtar Sözcükler: *Gambusia affinis*, yağ asitleri, beslenme, ω6/ω3 oranı

## Introduction

Food is one of the environmental factors which affect the fatty acid composition in fish. Feeding tests have shown that dietary fatty acids directly affect the fatty acid composition in fish (1-3).

Dietary lipids are not only used in animal tissues as an energy source and in structural matters; they are also important for transporting food which does not contain fat, especially for fat soluble vitamins (4).

The effects of dietary lipids on the fatty acid composition in body lipids reveal a difference between triacylglycerids and phospholipids. The fatty acid composition in the phospholipid fraction is much more affected than that of triacylglycerids (4).

Fish convert fatty acids obtained either from dietary sources or via biosynthesis into saturated or polyunsaturated fatty acids (5). However, the percentage of polyunsaturated fatty acids depends on the food consumed by the fish. The synthesis of these fatty acids is low if it is taken in sufficient amounts in the diet (6).

The effects of dietary lipid composition on the fatty acid composition of the phospholipids of artificially fed fish are important. The present study aims to investigate the differences which may occur in the fatty acid composition in the phospholipid fraction in *Gambusia affinis* fed an artificial diet.

## Materials and Methods

### Collection of samples for fatty acid analysis

Mosquitofish, *G. affinis*, were collected from a freshwater source in the campus area of Dicle University and transferred to the laboratory. The fish were kept in the laboratory for 30 days in an aquarium containing tap water with a temperature of 20 ± 1 °C, pH 6-7 and 6.5-7 mg/l dissolved oxygen. The temperature of the water in the aquarium was the same as the natural water source. The fish were fed with an artificial fish diet with a ω6/ω3 ratio of 5.5:1 twice a day for 30 days.

The samples for fatty acid analysis from both the control and artificially fed fish were kept in a chloroform/methanol mixture (2/1, v/v).

### Fractionating total lipids and the preparation of fatty acid methyl esters

All the experiments were repeated three times. Total fish lipids were extracted by Bligh and Dyer's method (7). The autoxidation of unsaturated fatty acid was minimised by the addition of 50 µl butylated hydroxytoluene (2% in chloroform, v/v). For the analysis of fatty acids in the phospholipid class, total lipids were applied to thin layer chromatography plates (Silica Gel G, 20 x 20 cm, 0.25 mm gel thickness). The plates were developed in petroleum ether: diethyl ether: acetic acid (80:20:1, v/v) (8).

The fractions were visualised by spraying them with 2'7'-dichlorofluorescein and viewing them under UV light. Then, the band belonging to the phospholipid fraction was scraped into a reaction tube. The fatty acids in the phospholipid fraction were then transmethylated to fatty acid methyl esters by refluxing in acidified methanol for 9 min (8). The fatty acid methyl esters were extracted from the reaction mixture with hexane and then concentrated and analysed by gas chromatography as described below.

### Gas chromatography

The fatty acid methyl esters were concentrated and then analysed by gas chromatography. The methyl esters were chromatographed on a Quadrex 007-23 series capillary column (0.25 mm x 30 m, 0.25 µm film thickness), a flame ionisation detector and a Unicam 4815 recording integrator. All GC runs used temperature programming from 100 to 260 °C at 5 °C/min, with an initial 1 min hold period. Injections were made in split mode (40:1) and separations were carried out with $N_2$ as a carrier. The flow rates of gasses was: $N_2$, 30 ml/min; hydrogen, 33 ml/min; dry air, 330 ml/min. Components were identified by comparisons of retention times with authentic standards.

The fatty acid composition of the total lipid extracted from the artificial fish diet was also analysed by gas chromatography.

### Statistical analysis

The analysis was performed using a commercial statistical program (SPSS 8.0).

Percentages of fatty acids were compared using analysis of variance. The Multiple Range Test was used to determine the significance of the difference between the means.

## Results

### The fatty acid composition of the artificial fish diet

The fatty acid composition of artificial fish diet used for feeding the fish in the aquarium is given in Table 1. C16:0 (palmitic acid), C18:1 (oleic acid) and C18:2 (linoleic acid) had the highest percentages in this diet. C16:1 (palmitoleic acid), C18:0 (stearic acid) and C18:3 (linolenic acid) levels were 7.1%, 6.04% and 2.93%, respectively. C14:0 (myristic acid), C20:1 (eicosaenoic acid), C20:3 (eicosatrienoic acid), C20:4 (arachidonic acid), C20:5 (eicosapentaenoic acid), C22:1 (docosaenoic acid), C22:4 (docosatetraenoic acid) and C22:6 (docosahexaenoic acid) were the other fatty acids found in the diet. Total saturated, total ω9, total ω6 and total ω3 fatty acid levels were 26.91%, 43.61%, 25.25% and 4.23%, respectively. The ω6/ω3 ratio was calculated at 5.5:1.

### Fatty acid composition of the fish collected from a freshwater source

The fatty acid composition of the phospholipids of *G. affinis* which were naturally fed and collected from a freshwater source is given in Table 2. C16:0 and C18:1 represented the highest percentages in the fish. C18:0, C18:2, C20:4 and C22:6 followed the above-mentioned

Table 1. Fatty acid composition of the total lipid extracted from the artificial fish diet.

| Fatty Acids | |
|---|---|
| C14:0 | 1.13 ± 0.14 |
| C16:0 | 19.74 ± 1.22 |
| C16:1ω9 | 7.10 ± 0.86 |
| C18:0 | 6.04 ± 0.33 |
| C18:1ω9 | 34.55 ± 1.75 |
| C18:2ω6 | 23.11 ± 1.25 |
| C18:3ω3 | 2.93 ± 0.17 |
| C20:1ω9 | 1.61 ± 0.15 |
| C20:3ω6 | 0.43 ± 0.12 |
| C20:4ω6 | 1.08 ± 0.24 |
| C20:5ω3 | 1.05 ± 0.38 |
| C22:1ω9 | 0.35 ± 0.08 |
| C22:4ω6 | 0.63 ± 0.12 |
| C22:6ω3 | 0.25 ± 0.10 |
| ΣSAFA | 26.91 ± 1.52 |
| Σω9 | 43.61 ± 2.51 |
| Σω6 | 25.25 ± 1.65 |
| Σω3 | 4.23 ± 0.56 |
| Σω6/Σω3 | 5.5:1 |

Values are the mean ± S.D. of three replicates.
SAFA, saturated fatty acids

fatty acids. Total saturated fatty acids, total ω9 fatty acids, total ω6 fatty acids and total ω3 fatty acid levels were 32.1%, 24.84%, 23.32% and 19.72%, respectively. The ω6/ω3 ratio was calculated at 1.18:1.

### Fatty acid composition of the fish fed with an artificial diet

The fatty acid composition in the phospholipids of *G. affinis* fed on an artificial fish diet for 30 days is given in Table 2. C18:1, C20:4 and C22:6 had the highest levels in the fish. C16:0, C18:0 and C18:2 followed these fatty acids. Total saturated fatty acids, total ω9, total ω6 and total ω3 fatty acids were 19.31%, 22.04%, 27.21% and 31.43%, respectively. The ω6/ω3 ratio was calculated at 1:1.15.

### Comparison of the fatty acid compositions of fish collected from a freshwater source and those fed an artificial diet

When comparing the fatty acids in the phospholipids of fish fed artificially for 30 days, with those of fish from a freshwater source, increases in the ω3 fatty acids C20:5, C22:3, C22:5 (docosapentaenoic acid) and C22:6 and in the ω6 fatty acids C20:2 (eicosadienoic acid), C20:4 and C22:4 were observed. There was also an increase in total ω3 and ω6, and a decrease in total saturated and total ω9 fatty acids. The ω6/ω3 ratio in fish from the freshwater source was 1.18:1, out 1:1.15 in artificially fed fish.

Table. 2. Comparison of the fatty acid composition of fish collected from a freshwater source and those fed an artificial diet

| Fatty Acids | Fatty acid composition of fish collected from a freshwater source | Fatty acid composition of fish fed an artifical diet |
|---|---|---|
| C14:0 | 0.44 ± 0.21 a | 0.31 ± 0.14 a |
| C16:0 | 19.75 ± 0.59 a | 9.93 ± 0.01 b |
| C16:1ω9 | 3.52 ± 0.01 a | 1.77 ± 0.12 b |
| C18:0 | 11.91 ± 0.32 a | 9.06 ± 0.11 b |
| C18:1ω9 | 19.65 ± 0.50 a | 17.69 ± 0.59 a |
| C18:2ω6 | 9.97 ± 0.16 a | 7.80 ± 0.20 b |
| C18:3ω3 | 1.78 ± 0.18 a | 1.43 ± 0.01 a |
| C20:1ω9 | 0.58 ± 0.42 a | 1.11 ± 0.15 b |
| C20:2ω6 | 0.95 ± 0.01 a | 1.27 ± 0.11 b |
| C20:3ω6 | 0.52 ± 0.01 a | 0.60 ± 0.52 a |
| C20:4ω6 | 10.69 ± 0.21 a | 14.66 ± 0.52 b |
| C20:5ω3 | 2.09 ± 0.01 a | 2.81 ± 0.14 b |
| C22:1ω9 | 1.09 ± 0.11 a | 1.47 ± 0.01 b |
| C22:4ω6 | 1.19 ± 0.20 a | 2.88 ± 0.01b |
| C22:5ω3 | 3.03 ± 0.14 a | 4.16 ± 0.01 b |
| C22:6ω3 | 12.82 ± 2.40 a | 23.03 ± 1.79 b |
| Σ SAFA | 32.10 ± 1.05 a | 19.31 ± 0.12 b |
| Σω9 | 24.84 ± 0.98 a | 22.04 ± 0.84 b |
| Σω6 | 23.32 ± 0.44 a | 27.21 ± 0.94 b |
| Σω3 | 19.72 ± 0.25 a | 31.43 ± 1.89 b |
| Σω6/Σω3 | 1.18:1 | 1:1.15 |

Values are the mean ± S.D. of three replicates.
Means followed by the same letter (a and b) are not significantly different ($P < 0.05$).
SAFA, saturated fatty acids

## Discussion

The fatty acid composition of tissue lipids in freshwater fish is markedly influenced by the patterns of fatty acids in their dietary lipids. The fatty acid profile of lipids in wild fish reflects the availability of fatty acids in the aquatic food chain (9).

The ω6/ω3 ratio was lower in the fish fed an artificial diet for 30 days. The results obtained showed that the

fatty acid composition of the dietary lipids affected the ω6/ω3 ratio of fish lipids. The increase in ω6 fatty acids in the diet may cause a decrease in the ω6/ω3 ratio. It is known that an increase in ω6 fatty acids causes an increase in ω3 fatty acids (10).

Commercially available fish diets are often low in ω3 PUFA and high in ω6 fatty acids. It is important to consider the effect of dietary lipid composition on the fatty acid composition of fish fed artificial diets. The ω6/ω3 ratio of the fish lipids is greatly affected by the ω6/ω3 ratio of the dietary lipids (10).

Similar results have been obtained by others researchers. Mosquitofish and catfish were fed artificial diets with ω6/ω3 ratios of 2.75:1 and 18.13:1, respectively (11). These fish were able to alter the dietary ω6/ω3 ratio in favour of ω3 fatty acid incorporation into the body lipids.

The present study demonstrates that the lipid composition of the *G. affinis* is markedly influenced by dietary lipids.

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