www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 13: 397-405 (2013) DOI: 10.4194/1303-2712-v13_3_02

Comparison of Changes in Fatty Acid Composition of Starved and Fed Rainbow Trout, (*Oncorhynchus mykiss*) Larvae

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Received 21 June 2012
Accepted 7 June 2013

Abstract

Changes in the fatty acid (FA) composition of unfed and fed rainbow trout (*Oncorhynchus mykiss*) larvae were determined by gas chromatography. Total lipids were extracted from samples by homogenization in chloroform/methanol (2:1, v/v). Fatty acid methyl esters were prepared from total lipid. The sample was saponified with methanolic potassium hydroxide (KOH). The fatty acids esterified with boron trifluoride–methanol (BF_3–methanol) and fatty acid methyl esters were analyzed with a Shimadzu GCMSQP5000 gas chromatograph.In starved larvae, there was an apparent preference in utilization of oleic acid (C18:1ω-9) in monounsaturated fatty acids than in the fed larvae. In both starved and fed larvae, palmitoleic (C16:1ω-7) acid was preferentially kept during the same period. Larvae kept 29 days under starvation conditions exhausted linolenic acid (C18:3ω-6), eicosanoic acid (C20:0), docosanoic acid (C22:0) and docosatrienoic acid (C22:3ω-3). They utilized less eicosapentaenoic (C20:5ω-3; EPA) acid and conserved strongly docosahexaenoic (C22:6ω-3; DHA) acid.

Keywords: Feeding, starvation, embryogenesis, larval development, larvae.

Aç Bırakılan ve Beslenen Gökkuşağı Alabalığı (*Oncorhynchus mykiss*) Yavrularının Yağ Asidi Kompozisyonundaki Değişimlerin Karşılaştırılması

Özet

Aç bırakılan ve beslenen Gökkuşağı alabalığı (*Oncorhynchus mykiss*) yavrularının yağ asidi kompozisyonundaki değişimler gaz kromatografisi ile belirlenmiştir. Örneklerdeki total lipidler kloroform/metanol (2:1, v/v) karışımında homojenize edilerek elde edilmiştir. Yağ asit metil esterleri total lipitden hazırlanmıştır. Örnek metanollü potasyum hidroksit (KOH) ile sabunlaştırılmıştır. Yağ asitleri metanollü-Boron Trifluoride (metanollü-BF_3) ile esterleştirilmiş ve yağ asit metil esterleri Shimadzu GCMSQP5000 marka gaz kromatografisi ile analiz edilmiştir. Beslenen yavrulara kıyasla, aç bırakılan yavrularda, tek çiftbağlı doymamış yağ asitlerinden oleik asit (C18:1ω-9) kullanımı daha fazla tercih edilmiştir. Aynı periyod esnasında, hem aç bırakılan hem de beslenen yavrularda palmitoleik (C16:1ω-7) asit tercihli olarak korunmuştur. 29 gün aç bırakılan yavrularda linolenik asit (C18:3ω-6), eikosanoik asit (C20:0), dokosanoik asit (C22:0) ve dokosatrienoik asit (C22:3ω-3) tükenmiştir. Bu yavrular, eikosapentaenoik (C20:5ω-3; EPA) asidi az kullanmış ve dokosaheksaenoik (C22:6ω-3; DHA) asidi güçlü bir şekilde korumuşlardır.

Anahtar Kelimeler: Beslenme, açlık, embriyogenesiz, larval gelişim, larva.

Introduction

Fish are the major dietary source for humans of omega-3 (ω-3) polyunsaturated fatty acid (PUFA), eicosapentaenoic (C20:5ω-3; EPA) acid and docosahexaenoic (C22:6ω-3; DHA) acid (Holub and Holub, 2004). Linoleic acid (C18:2ω-6; LA), linolenic acid (C18:3ω-3; LNA) and their long-chain derivatives such as arachidonic (C20:5ω-3; AA) acid and C22:6ω-3 are important structural and physiological components of animal and plant cells and are thought to play important roles in permeability, enzyme activity and other functions in polar lipids of biomembranes (Simopoulos, 2000; Lee, 2001). Therefore, PUFA, especially of ω-3 PUFA (EPA and DHA) are considered as essential fatty acid (EFA) required in fish diets for normal growth and survival (Sargent *et al.,* 2002; Wilson *et al.*, 2007).

Lipids have been shown to be critical for embryonic and larval development, as they play an important role in a wide range of processes (Sargent

et al., 1999; Abi-Ayad *et al.*, 2004; Zengin and Akpınar, 2006). Lipids play important roles in the energy production in animal tissues. Alongside energy resource, lipids are essential structural components of cell membranes and precursors of bioactive compounds like eicosanoids. At the same time, they have very important physiological roles such as feeding, growth, behaviour and reproduction in fish (Parrish, 1999; Lee, 2001; Ling *et al.*, 2006; Hachero-Cruzado *et al.*, 2009).

In fish embryos and larvae, adequate nutrition is critical for proper development and survival to metamorphosis. During the period from spawning up until first-feeding, maternal nutrients delivered in the yolk must provide all the nutrients required for development and growth. The "first-feeding" or transition phase between endogenous and exogenous feeding has thus been identified as a "critical period" of the life-cycle where larvae are very susceptible to starvation if adequate food is not available and/or if maternally provided nutrition is inadequate (Navas *et al.*, 1997; Vassallo-Agius *et al.*, 2001; Gunasekera *et al.*, 2001; Li *et al.*, 2005; Johnston *et al.*, 2007).

Izquierdo and Fernandez-Palacios (1997) pointed out three ways to investigate the fatty acid (FA) requirement of fish larvae: the study of eggs and larval composition at different developmental stages, the comparison of the composition between fed and starved larvae at the same developmental stage and the use of feeding experiments controlling the FA composition of the delivered food.

Starvation studies can help to determine the nutrients most critical as energy reserves and those catabolized or conserved in the face of increasing food deprivation (Ritar *et al.*, 2003), and accordingly permit a better understanding of fish physiology and nutrient metabolism. This study will shed further light on our understanding of larval nutritional metabolism and also provide leads for commercial diet formulation for young of *O. mykiss*. Thus the aim of the present study was to compare the changes in fatty acid composition of starved and fed *O. mykiss* larvae which passed to free-swimming stage after embryonic and larval development.

Materials and Methods

Fish and Sampling

This study was carried out at a local and commercial fish farm in Sivas, Turkey. Eggs and sperm samples used in the present study were obtained from three females and males aged 4 and 3 years, respectively. Mature *O. mykiss* were artificially spawned and unfertilized eggs samples were taken from each of the three female *O. mykiss* during spawning on the farm and then the eggs were fertilized by conventional procedures and immediately transported to a hatchery. The oxygen, pH and the temperature of the water in the pools used throughout the experiment were measured periodically (3 measurements per month). Water temperature and oxygen level varied between 9 and 9.5°C and 8.8 and 8.5 mg L^{-1} respectively. The pH values of the water (7.5) were constant during the trial. The water flow was 28 $L.min^{-1}$. The pool in which the fish were reared had a flow-through water supply originating from an underground natural spring.

Fish were kept in the pool under natural environmental conditions. Eggs were fertilized on 1 January. The water temperature was 9°C during embryogenesis and yolk-sac larvae in January and february and was 9.5°C during feeding and starvation period in March. After one day post fertilization and every 7 days (days 7, 14, 21 and 29 post fertilization) until hatching, samples of fertilized eggs were taken. Eyed eggs were determined on 23th day of embryonic development. Hatching occurred 29 days after the fertilization and the yolk-sacs were completely absorbed 24 days after the embryonic development, the *O. mykiss* being at their free-swimming stage. Samples of yolk-sac larvae were taken at 5, 14 and 24 days. Just after yolk-sac absorption, free-swimming larvae were divided into two groups of 200 *O. mykiss* larvae. Each group was stocked in two pools (0.5m\2m\0.5m). The first group was starved until the fish died. When deaths were seen after 29th day in starved group, the trial was ended. In the second group, free-swimming larvae were fed manually on commercial diet contained 10% fat over a period of 29 days. The maintenance and feeding conditions were carried by the fish farm. Food was well distributed over the water surface and feeding observed. The feeding protocol is to feed the larvae with many meals separated by very short periods of time. Fish were fed at 20-25 min. intervals for 3–4 h in the morning. With this type of feeding schedule, fed larvae will very rapidly get food during the first meals. Samples of starved and fed larvae were collected at 9, 16, 23 and 29 days. One gram samples of commercial diet, used in the feeding of larvae, were also taken for analyses. All larvae were killed using strong MS-222 anaesthetic. All samples were taken as 1 g × 3 (all samples were taken as 1 g of 3 repetition) and immediately placed in chloroform-methanol (2:1, v/v) and stored at −84°C until analysis.

Lipid Extraction and Fatty Acid Analyses

Total lipids from the weighted samples were extracted from eggs, whole body larvae and feed after homogenization (Ultra-Turrax T25 homogenizer) using a modified chloroform/methanol (2:1, v/v) extraction procedure (Folch *et al.*, 1957). The fatty acids from eggs, whole body larvae and feed were methylated and extracted according to procedures described by Moss *et al*. (1974).

Total lipids extracted from the samples were

saponified with methanol (50%) containing 5% potassium hydroxide for 1 h, the fatty acids esterified with the standard Boron trifluoride-methanol (BF_3-methanol) and fatty acids methyl ester (FAME) extracted with hexane/chloroform (4:1, v/v). The samples were separated in gas chromatograph mass spectrometry (Shimadzu GCMSQP5000). At each time 1 µl sample was chromatographed on apolar DB-1capillary column (60 m × 0.25 mm × 0.25 mm) using hydrogen as carrier gas at 0.9 ml min^{-1}.

The injection and detection temperatures were 250°C and 280°C, respectively. The GCMS oven was operated at initial temperature of 100°C and was kept for 3 min. The oven was programmed to rise to 140°C at a rate of 5°C min^{-1} and was kept for 1 min, then programmed to 200°C at a rate of 2°C min^{-1} and was kept for 1 min. It reached the final temperature of 250°C at a rate of 2°C min^{-1} and the final temperature was maintained for 10 min. Total duration of an analysis took 78 min. Hexane/chloroform (4:1, v/v) was used as the solvent. Separated individual FAMEs were identified by comparison of retention times with known standards and quantified by percent values obtained from computer linked to the gas chromatograph.

Statistical Analysis

The statistical analyses were performed using a commercial statistical program (SPSS 15.0) for Windows. All analytical determinations were performed in triplicate and the mean values were reported. All data are statistically compared by one way variance analysis (ANOVA) and comparisons between means were performed with Tukey's test. Differences between means were reported as significant if $P<0.05$.

Results

Embryogenesis and Yolk-Sac Larvae

During embryogenesis, an important increase in the percentage of C14:0, C15:0, C16:1ω-7, C17:0, C18:0, C18:1ω-9, C20:0, C20:2ω-6, C20:4ω-6, C20:5ω-3, C22:3ω-3, C22:5ω-3, C22:6ω-3 and C24:1ω-9 fatty acids and a decrease in the percentage of C16:0, C18:2ω-6 and C18:3ω-6 of unfertilized eggs was observed in the fertilized eggs ($P<0.05$). The most significant depletion was observed in total saturated fatty acids (ΣSFA), which fell from 42.10% in unfertilized eggs to 32.19% in 21 day embryo (Table 1). The percentages of total monounsaturated fatty acid (ΣMUFA) were low (28.74 %) in the same unfertilized eggs. During embryonic developmental stages, the percentages of ΣMUFA increased significantly ($P<0.05$) due to increase in the content of the most abundant unsaturated fatty acid, C18:1ω9 and reached a maximum (32.88%) in 14 day embryo while the percentages of ΣSFA decreased

Table 1. Fatty acid composition of unfertilized and fertilized eggs, 7, 14 and 21 day embryos and 0 day yolk-sac larvae*

Fatty acids	unfertilized eggs	Fertilized eggs	7 day embryo	14 day embryo	21 day embryo	0 day Yolk-sac larvae
C14:0	2.92 ± 0.05^{a}	3.22 ± 0.04^{bc}	3.35 ± 0.05^{c}	3.34 ± 0.07^{c}	3.20 ± 0.08^{cd}	3.08 ± 0.03^{abd}
C15:0	2.70 ± 0.05^{a}	2.94 ± 0.02^{bc}	3.08 ± 0.03^{b}	3.04 ± 0.02^{b}	2.89 ± 0.05^{cd}	2.76 ± 0.04^{ad}
C16:0	24.28 ± 0.06^{a}	13.35 ± 0.03^{b}	14.02 ± 0.03^{c}	12.99 ± 0.05^{d}	12.67 ± 0.06^{e}	12.29 ± 0.05^{f}
C16:1ω-7	5.66 ± 0.04^{a}	6.35 ± 0.03^{b}	5.99 ± 0.04^{c}	6.40 ± 0.03^{b}	5.81 ± 0.02^{ac}	6.87 ± 0.03^{d}
C17:0	3.90 ± 0.05^{a}	5.06 ± 0.02^{b}	4.76 ± 0.01^{c}	5.69 ± 0.03^{d}	5.04 ± 0.05^{b}	4.48 ± 0.06^{e}
C18:0	2.78 ± 0.01^{a}	3.26 ± 0.03^{b}	2.82 ± 0.03^{a}	3.51 ± 0.02^{c}	2.63 ± 0.03^{d}	2.67 ± 0.03^{d}
C18:1ω-9	19.93 ± 0.04^{a}	22.23 ± 0.06^{b}	23.39 ± 0.05^{c}	22.93 ± 0.10^{d}	23.08 ± 0.07^{d}	23.10 ± 0.03^{d}
C18:2ω-6	5.58 ± 0.03^{a}	3.39 ± 0.04^{b}	3.11 ± 0.05^{c}	3.83 ± 0.06^{d}	5.71 ± 0.06^{ae}	5.83 ± 0.02^{e}
C18:3ω-6	4.02 ± 0.02^{a}	3.60 ± 0.06^{b}	4.11 ± 0.04^{a}	3.80 ± 0.03^{c}	3.84 ± 0.05^{c}	3.73 ± 0.06^{bc}
C20:0	2.57 ± 0.04^{a}	3.38 ± 0.06^{b}	2.72 ± 0.02^{cd}	2.83 ± 0.05^{c}	2.65 ± 0.06^{ad}	2.67 ± 0.06^{ad}
C20:2ω-6	2.91 ± 0.04^{ac}	3.09 ± 0.04^{b}	3.08 ± 0.05^{b}	3.13 ± 0.03^{b}	3.03 ± 0.07^{ab}	2.81 ± 0.04^{c}
C20:3ω-6	2.93 ± 0.02^{ac}	3.03 ± 0.03^{ab}	3.10 ± 0.04^{b}	3.10 ± 0.06^{b}	3.04 ± 0.04^{ab}	2.81 ± 0.06^{c}
C20:4ω-6	1.59 ± 0.03^{a}	2.08 ± 0.02^{b}	1.57 ± 0.06^{a}	1.66 ± 0.04^{a}	2.69 ± 0.05^{c}	2.76 ± 0.05^{c}
C20:5ω-3	2.36 ± 0.06^{a}	6.45 ± 0.04^{b}	6.55 ± 0.06^{b}	5.04 ± 0.08^{c}	6.02 ± 0.01^{d}	6.79 ± 0.03^{e}
C22:0	2.94 ± 0.04^{a}	3.03 ± 0.05^{ab}	3.19 ± 0.06^{b}	3.21 ± 0.06^{b}	3.12 ± 0.04^{ab}	2.93 ± 0.02^{a}
C22:3ω-3	2.92 ± 0.04^{a}	3.23 ± 0.06^{b}	3.41 ± 0.02^{c}	3.76 ± 0.06^{d}	3.28 ± 0.03^{bc}	3.10 ± 0.06^{b}
C22:5ω-3	2.20 ± 0.02^{a}	2.93 ± 0.05^{b}	2.76 ± 0.03^{cd}	2.65 ± 0.02^{c}	2.79 ± 0.06^{bc}	2.88 ± 0.08^{bd}
C22:6ω-3	4.65 ± 0.07^{a}	5.99 ± 0.03^{b}	5.52 ± 0.06^{c}	5.54 ± 0.06^{c}	5.21 ± 0.04^{d}	5.30 ± 0.03^{d}
C24:1ω-9	3.15 ± 0.04^{a}	3.41 ± 0.03^{bd}	3.46 ± 0.05^{bc}	3.56 ± 0.03^{c}	3.31 ± 0.02^{d}	3.13 ± 0.04^{a}
ΣSFA	42.10 ± 0.02^{a}	34.24 ± 0.03^{bc}	33.94 ± 0.06^{b}	34.61 ± 0.15^{c}	32.19 ± 0.13^{d}	30.89 ± 0.14^{e}
ΣMUFA	28.74 ± 0.01^{a}	31.98 ± 0.06^{b}	32.84 ± 0.08^{c}	32.88 ± 0.13^{cd}	32.20 ± 0.07^{b}	33.10 ± 0.01^{d}
Σ ω-3	12.14 ± 0.07^{a}	18.59 ± 0.06^{b}	18.23 ± 0.12^{c}	16.98 ± 0.08^{d}	17.30 ± 0.08^{e}	18.07 ± 0.10^{c}
Σ ω-6	17.03 ± 0.06^{a}	15.19 ± 0.06^{bc}	14.98 ± 0.08^{b}	15.53 ± 0.06^{c}	18.31 ± 0.03^{d}	17.94 ± 0.18^{d}
Σ ω-3/ω-6	0.71 ± 0.01^{a}	1.22 ± 0.01^{b}	1.22 ± 0.01^{b}	1.09 ± 0.01^{c}	0.95 ± 0.00^{d}	1.00 ± 0.01^{e}
EPA/DHA	0.51 ± 0.02^{a}	1.08 ± 0.01^{b}	1.19 ± 0.00^{c}	0.91 ± 0.00^{d}	1.16 ± 0.01^{c}	1.28 ± 0.01^{e}

*The data are expressed as percentages of total fatty acids. Each value is the mean±S.E. (standard error) of 3 repetitions. Superscripts in a row with different letters represent significant difference ($p < 0.05$). Σ: Total. ΣSFA: Total Saturated Fatty Acid. ΣMUFA: Total Monounsaturated Fatty Acid. Σω-3: Total ω-3 Fatty Acid. Σω-6: Total ω-6 Fatty Acid. EPA: eicosapentaenoic acid. DHA: docosahexaenoic acid.

significantly (P<0.05) due to decrease in the content of the most abundant saturated fatty acid, C16:0 (Table 1). During the study, total ω-3 (Σ ω-3) PUFA content suddenly increased in fertilized eggs from 12.14% in unfertilized eggs to 18.59% in fertilized eggs due to increased levels of C20:5ω-3, C22:3ω-3, C22:5ω-3 and C22:6ω-3.

Throughout the yolk sac stage, the PUFA were dominated by C20:5ω-3, C22:6ω-3, C18:2ω-6 and C18:3ω-6 (Table 2). Σω-3 PUFA values increased during the yolk sac stage and changed from 18.07% in 0 days yolk-sac larvae to 19.73% in 5 days yolk-sac larvae. C16:0, C17:0 and C14:0 were the most common saturated fatty acids. ΣSFA were at a minimum (30.86%) at 5 days yolk-sac larvae and reached a maximum (33.35%) at 24 days absorbed yolk-sac larvae of *O. mykiss*, mainly due to the increased percentage of C16:0. While the C20:0 saturated fatty acid contents showed a statistically insignificant decrease between 0 days yolk-sac larvae and 5 days yolk-sac larvae, this fatty acid was not detected in 14 days yolk-sac larvae and 24 days absorbed yolk-sac larvae during the experiment. The major constituents of monounsaturated fatty acid were C18:1ω-9 and C16:1ω-7. ΣMUFA composition of 5 and 14 day yolk-sac larvae and 24 days absorbed yolk-sac larvae decreased compared with 0 day yolk-sac larvae mainly due to the decreased percentage of C16:1ω7 (P<0.05) (Table 2).

Starved and Fed Larvae

It was noted that on the investigation of the fatty acid composition of the commercial feed used in the feeding of the fish, C18:0, C18:3ω-6, C20:3ω-6, C22:0, C22:6ω-3 and C24:1ω-9 were not present. C18:3ω-6, C20:0, C22:0 and C22:3ω-3 were not determined in the starved larvae (Table 3). C14:0 and C15:0 were determined to be in higher percentage in the feed than in the fed larvae. On comparison of the percentages of C14:0 and C15:0 of the starved larvae with the fed larvae, while the starved larvae showed a significant increase, the fed larvae showed no important difference during the feeding period from being 24 day absorbed yolk-sac larvae. The level of C16:0 (6.94%) was low in the feed. The increase of this fatty acid in the starved fish was noteworthy when compared to the fish fed with this fatty acid. The highest levels in the starved and fed fish were 19.08% and 17.29% respectively. C20:0 was not determined in all the development stages of the starved larvae, 14 day yolk-sac larvae and the 24 days absorbed yolk-sac larvae.

Among monounsaturated fatty acids, C18:1ω-9 was the major fatty acid catabolised as energy source in starved larvae and reached its lowest level

Table 2. Fatty acid composition of yolk-sac larvae 0 day, 5 and 14 days yolk-sac larvae and 24 days absorbed yolk-sac larvae*

Fatty acids	0 day yolk-sac larvae	5 days yolk-sac larvae	14 days yolk-sac larvae	24 days absorbed yolk-sac larvae
C14:0	3.08 ± 0.03^{a}	3.09 ± 0.06^{a}	3.39 ± 0.08^{b}	3.59 ± 0.08^{c}
C15:0	2.76 ± 0.04^{a}	2.76 ± 0.07^{a}	3.10 ± 0.08^{b}	3.39 ± 0.05^{c}
C16:0	12.29 ± 0.05^{a}	12.20 ± 0.05^{a}	14.26 ± 0.01^{b}	15.57 ± 0.02^{c}
C16:1ω-7	6.87 ± 0.03^{a}	5.82 ± 0.08^{b}	4.18 ± 0.12^{c}	4.84 ± 0.21^{d}
C17:0	4.48 ± 0.06^{a}	4.61 ± 0.07^{a}	3.96 ± 0.05^{b}	3.61 ± 0.09^{c}
C18:0	2.67 ± 0.03^{a}	2.72 ± 0.01^{a}	3.38 ± 0.07^{b}	3.44 ± 0.00^{b}
C18:1ω-9	23.10 ± 0.03^{a}	22.33 ± 0.12^{b}	24.03 ± 0.10^{c}	24.04 ± 0.15^{c}
C18:2ω-6	5.83 ± 0.02^{a}	5.65 ± 0.08^{a}	3.26 ± 0.04^{b}	3.05 ± 0.14^{c}
C18:3ω-6	3.73 ± 0.06^{a}	3.63 ± 0.06^{a}	4.06 ± 0.03^{b}	2.82 ± 0.08^{c}
C20:0	2.67 ± 0.06^{a}	2.54 ± 0.04^{a}	-	-
C20:2ω-6	2.81 ± 0.04^{a}	3.19 ± 0.06^{b}	3.29 ± 0.04^{b}	3.27 ± 0.06^{b}
C20:3ω-6	2.81 ± 0.06^{a}	2.81 ± 0.00^{a}	3.11 ± 0.05^{b}	3.65 ± 0.05^{c}
C20:4ω-6	2.76 ± 0.05^{a}	2.75 ± 0.02^{a}	3.61 ± 0.07^{b}	3.45 ± 0.15^{b}
C20:5ω-3	6.79 ± 0.03^{a}	8.02 ± 0.03^{b}	7.47 ± 0.13^{c}	6.07 ± 0.03^{d}
C22:0	2.93 ± 0.02^{a}	2.94 ± 0.06^{a}	3.37 ± 0.11^{b}	3.75 ± 0.10^{c}
C22:3ω-3	3.10 ± 0.06^{a}	3.21 ± 0.09^{a}	3.11 ± 0.05^{a}	3.65 ± 0.09^{b}
C22:5ω-3	2.88 ± 0.08^{a}	3.11 ± 0.09^{b}	2.91 ± 0.09^{a}	2.56 ± 0.04^{c}
C22:6ω-3	5.30 ± 0.03^{a}	5.39 ± 0.08^{ac}	5.79 ± 0.02^{b}	5.54 ± 0.08^{c}
C24:1ω-9	3.13 ± 0.04^{a}	3.23 ± 0.04^{a}	3.72 ± 0.04^{b}	3.71 ± 0.06^{b}
ΣSFA	30.89 ± 0.14^{a}	30.86 ± 0.14^{a}	31.45 ± 0.09^{b}	33.35 ± 0.34^{c}
ΣMUFA	33.10 ± 0.01^{a}	31.38 ± 0.08^{b}	31.93 ± 0.15^{c}	32.58 ± 0.06^{d}
Σ ω-3	18.07 ± 0.10^{a}	19.73 ± 0.07^{b}	19.29 ± 0.02^{c}	17.82 ± 0.01^{d}
Σ ω-6	17.94 ± 0.18^{a}	18.03 ± 0.13^{a}	17.33 ± 0.04^{b}	16.25 ± 0.34^{c}
Σ ω-3/ω-6	1.00 ± 0.01^{a}	1.09 ± 0.01^{b}	1.11 ± 0.00^{b}	1.10 ± 0.02^{b}
EPA/DHA	1.28 ± 0.01^{a}	1.49 ± 0.02^{b}	1.29 ± 0.03^{a}	1.09 ± 0.01^{c}

*The data are expressed as percentages of total fatty acids. Each value is the mean±S.E. (standard error) of 3 repetitions. Superscripts in a row with different letters represent significant difference (P<0.05). Σ: Total. ΣSFA: Total Saturated Fatty Acid. ΣMUFA: Total Monounsaturated Fatty Acid. Σω-3: Total ω-3 Fatty Acid. Σω-6: Total ω-6 Fatty Acid. EPA: eicosapentaenoic acid. DHA: docosahexaenoic acid.

Table 3. Fatty acid composition of 24 days absorbed yolk-sac larvae, 9, 16, 23 and 29 days starved and fed larvae of *Oncorhynchus mykiss* and diet*

Fatty acids	24 days absorbed yolk-sac larvae	9 day Starved larvae	16 day Starved larvae	23 day Starved larvae	29 day Starved larvae	Diet	9 day Fed larvae	16 day Fed larvae	23 day Fed larvae	29 day Fed larvae
C14:0	3.59 ± 0.08^{c}	3.91 ± 0.06^{b}	4.17 ± 0.03^{c}	4.20 ± 0.02^{c}	4.42 ± 0.10^{d}	7.34 ± 0.02^{k}	3.43 ± 0.05^{a}	3.55 ± 0.07^{a}	3.48 ± 0.02^{a}	3.42 ± 0.05^{a}
C15:0	3.39 ± 0.05^{c}	3.58 ± 0.08^{b}	4.01 ± 0.02^{c}	4.14 ± 0.04^{c}	4.03 ± 0.01^{c}	10.56 ± 0.03^{k}	3.34 ± 0.02^{a}	3.26 ± 0.11^{a}	3.24 ± 0.03^{a}	3.35 ± 0.04^{a}
C16:0	15.57 ± 0.02^{c}	16.73 ± 0.02^{d}	18.46 ± 0.01^{e}	18.77 ± 0.03^{f}	19.08 ± 0.20^{g}	6.94 ± 0.05^{k}	15.66 ± 0.10^{a}	15.46 ± 0.07^{a}	16.38 ± 0.07^{b}	17.29 ± 0.07^{c}
C16:1ω-7	4.84 ± 0.21^{d}	7.52 ± 0.04^{ce}	8.66 ± 0.06^{k}	8.54 ± 0.03^{k}	7.73 ± 0.18^{e}	8.59 ± 0.05^{k}	6.65 ± 0.02^{b}	7.45 ± 0.04^{c}	7.40 ± 0.04^{c}	6.79 ± 0.05^{b}
C17:0	3.61 ± 0.09^{c}	3.62 ± 0.03^{a}	4.66 ± 0.09^{d}	4.03 ± 0.06^{e}	3.88 ± 0.02^{e}	8.39 ± 0.02^{k}	4.48 ± 0.06^{b}	3.11 ± 0.03^{c}	2.96 ± 0.02^{c}	2.99 ± 0.05^{c}
C18:0	3.44 ± 0.00^{b}	3.97 ± 0.04^{e}	4.51 ± 0.05^{f}	4.37 ± 0.05^{g}	4.53 ± 0.03^{f}	-	4.15 ± 0.06^{b}	3.64 ± 0.09^{c}	3.52 ± 0.02^{ac}	3.38 ± 0.04^{d}
C18:1ω-9	24.04 ± 0.15^{c}	23.48 ± 0.07^{d}	18.74 ± 0.05^{e}	19.94 ± 0.04^{f}	20.74 ± 0.16^{g}	17.19 ± 0.09^{k}	23.05 ± 0.04^{b}	24.45 ± 0.05^{c}	24.03 ± 0.09^{a}	24.26 ± 0.19^{ac}
C18:2ω-6	3.05 ± 0.14^{c}	4.07 ± 0.10^{c}	4.20 ± 0.03^{c}	4.11 ± 0.06^{c}	4.64 ± 0.19^{d}	6.85 ± 0.05^{k}	3.56 ± 0.05^{b}	3.58 ± 0.04^{b}	3.49 ± 0.01^{b}	3.56 ± 0.06^{b}
C18:3ω-6	2.82 ±0.08^{c}	-	-	-	-	-	1.60 ± 0.07^{b}	1.70 ± 0.03^{b}	1.93 ± 0.02^{c}	1.94 ± 0.02^{c}
C20:0	-	-	-	-	-	8.41 ± 0.06^{k}	0.40 ± 0.03^{a}	0.57 ± 0.01^{b}	0.55 ± 0.01^{b}	0.52 ± 0.01^{b}
C20:2ω-6	3.27 ± 0.06^{b}	3.70 ± 0.05^{b}	4.07 ± 0.05^{c}	4.51 ± 0.06^{d}	4.78 ± 0.03^{e}	7.22 ± 0.06^{k}	3.23 ± 0.03^{a}	3.58 ± 0.08^{b}	3.30 ± 0.02^{a}	3.30 ± 0.01^{a}
C20:3ω-6	3.65 ± 0.05^{c}	5.47 ± 0.10^{b}	5.74 ± 0.20^{b}	4.92 ± 0.16^{c}	4.87 ± 0.19^{c}	-	5.88 ± 0.14^{b}	4.31 ± 0.06^{d}	4.82 ± 0.04^{c}	4.53 ± 0.28^{cd}
C20:4ω-6	3.45 ± 0.15^{b}	3.43 ± 0.02^{a}	3.71 ± 0.05^{d}	4.15 ± 0.03^{e}	4.30 ± 0.04^{e}	2.09 ± 0.05^{k}	2.83 ± 0.03^{b}	3.10 ± 0.05^{c}	3.25 ± 0.0^{c}	3.48 ± 0.03^{a}
C20:5ω-3	6.07 ± 0.03^{d}	3.92 ± 0.05^{d}	4.20 ± 0.04^{e}	3.84 ± 0.03^{d}	3.52 ± 0.04^{f}	7.33 ± 0.06^{k}	4.79 ± 0.03^{b}	5.02 ± 0.02^{c}	4.93 ± 0.07^{bc}	4.82 ± 0.10^{b}
C22:0	3.75 ± 0.10^{c}	-	-	-	-	-	0.80 ± 0.01^{b}	0.84 ± 0.01^{b}	0.87 ± 0.02^{b}	0.82 ± 0.03^{b}
C22:3ω-3	3.65 ± 0.09^{b}	3.06 ± 0.00^{d}	-	-	-	7.39 ± 0.06^{k}	3.49 ± 0.06^{b}	3.28 ± 0.02^{c}	3.40 ± 0.04^{b}	3.15 ± 0.02^{d}
C22:5ω-3	2.56 ± 0.04^{c}	2.23 ± 0.02^{c}	2.38 ± 0.04^{b}	2.10 ± 0.06^{cd}	2.03 ± 0.00^{d}	1.69 ± 0.05^{k}	2.40 ± 0.01^{b}	2.47 ± 0.07^{ab}	2.54 ± 0.05^{a}	2.58 ± 0.07^{a}
C22:6ω-3	5.54 ± 0.08^{c}	7.74 ± 0.00^{d}	8.40 ± 0.04^{e}	8.61 ± 0.02^{f}	8.60 ± 0.03^{f}	-	6.93 ± 0.07^{b}	7.11 ± 0.01^{c}	7.08 ± 0.06^{c}	7.01 ± 0.02^{cb}
C24:1ω-9	3.71 ± 0.06^{b}	3.69 ± 0.09^{ac}	4.07 ± 0.01^{a}	3.77 ± 0.02^{ad}	2.84 ± 0.02^{b}	-	3.33 ± 0.01^{c}	3.53 ± 0.01^{cd}	2.84 ± 0.45^{b}	2.81 ± 0.03^{b}
ΣSFA	33.35 ± 0.34^{c}	31.82 ± 0.07^{b}	35.81 ± 0.15^{e}	35.51 ± 0.08^{e}	35.94 ± 0.31^{e}	41.64 ± 0.06^{k}	32.26 ± 0.13^{b}	30.43 ± 0.02^{c}	31.00 ± 0.15^{d}	31.77 ± 0.01^{b}
ΣMUFA	32.58 ± 0.06^{d}	34.57 ± 0.12^{d}	31.48 ± 0.03^{f}	32.26 ± 0.02^{a}	31.32 ± 0.03^{f}	25.78 ± 0.12^{k}	33.03 ± 0.02^{b}	35.42 ± 0.03^{c}	34.27 ± 0.33^{d}	33.86 ± 0.16^{e}
Σ ω-3	17.82 ± 0.01^{d}	16.94 ± 0.06^{d}	14.98 ± 0.07^{e}	14.54 ± 0.10^{f}	14.15 ± 0.03^{g}	16.41 ± 0.05^{k}	17.61 ± 0.03^{bc}	17.88 ± 0.09^{a}	17.94 ± 0.09^{a}	17.56 ± 0.16^{c}
Σ ω-6	16.25 ± 0.34^{c}	16.68 ± 0.14^{ack}	17.72 ± 0.20^{b}	17.69 ± 0.15^{b}	18.59 ± 0.31^{d}	16.16 ± 0.13^{k}	17.10 ± 0.12^{bc}	16.27 ± 0.14^{ak}	16.79 ± 0.09^{ack}	16.81 ± 0.33^{ac}
Σ ω-3/ω-6	1.10 ± 0.02^{b}	1.02 ± 0.01^{k}	0.84 ± 0.01^{d}	0.82 ± 0.01^{d}	0.76 ± 0.02^{e}	1.02 ± 0.01^{k}	1.03 ± 0.01^{bk}	1.10 ± 0.02^{a}	1.07 ± 0.00^{ab}	1.05 ± 0.03^{kb}
EPA/DHA	1.09 ± 0.01^{c}	0.51 ± 0.01^{c}	0.50 ± 0.00^{c}	0.45 ± 0.00^{d}	0.41 ± 0.01^{e}	-	0.69 ± 0.01^{b}	0.71 ± 0.00^{b}	0.70 ± 0.01^{b}	0.69 ± 0.02^{b}

*The data are expressed as percentages of total fatty acids. Each value is the mean±S.E. (standard error) of 3 repetitions. Superscripts in a row with different letters represent significant difference ($p < 0.05$). Σ: Total. ΣSFA: Total Saturated Fatty Acid. ΣMUFA: Total Monounsaturated Fatty Acid. Σω-3: Total ω-3 Fatty Acid. Σω-6: Total ω-6 Fatty Acid. EPA: eicosapentaenoic acid. DHA: docosahexaenoic acid.

(18.74%) in the larvae starved for 16 days, whereas it reached its highest value (24.45%) in the larvae fed for 16 days ($P<0.05$). The value of C16:1ω-7 in starved larvae in the postlarval development stage was being higher than the fed larvae in the same developmental stage.

In the case of ω-6 polyunsaturated fatty acids, C18:2ω-6, whose highest values in the starved and fed larvae were 4.64% (in the 29 day starved larvae) and 3.58% (in the 16 day fed larvae) respectively showed a significant ($P<0.05$) increase in comparison with the beginning of the postlarval stage (3.05%). C20:4ω-6, found to be 2.09% in the feed, showed a regular and significant ($P<0.05$) increase in starved and fed larvae during their development from the 16th day. The highest values of C20:5ω-3 in the feed and absorbed yolk-sac larvae were 7.33±0.06 and 6.07±0.03 respectively. This fatty acid showed a significant ($P<0.05$) decrease in starved and fed larvae.

The most important ω-3 fatty acids, i.e. C20:5ω-3 and docosapentaenoic (C22:5ω-3; DPA) were progressively utilized by starved learvae. C22:6ω-3 was found to be at the highest level in the ω-3 PUFA fraction in the starved and fed larvae (8.61% and 7.11% respectively). It can be noted that the starved larvae displayed a higher value than the fed larvae.

Discussion

There are many differences in lipid and fatty acid utilization during embryogenesis between fish species (Mourente and Vazquez, 1996; Haliloğlu *et al*., 2003; Cejas *et al.,* 2004). In the present study, a significant ($P<0.05$) decrease in the percentage of total saturated fatty acids occurred in fertilised eggs and the embrionic development stage. This decrease in the percentage of total saturated fatty acids during embryogenesis of *O.mykiss* reflects the utilization of these nutrients as an energy source by embryos. The percentages of total ω-3 and total monounsaturated fatty acids showed an important increase with fertilisation (Table 1). Embryos of *O. mykiss* spared lipids and particularly PUFA for new cell constitution and organogenesis rather than for energy production. The results of Wiegand *et al.* (1991) and Abi-Ayad *et al.* (2000) support the findings of the present study.

After the hatching of the eggs, during the absorption of the egg sac, an increase in the content of total saturated fatty acids and changes in fatty acids were observed which reflects the use and movement of lipids in larvae. It can be seen on examination of sac absorbed larvae that a significant ($P<0.05$) decrease in saturated fatty acids, especially in C17:0, occurred and C20:0 was exhausted. On the other hand, the amounts of C14:0, C15:0, C16:0, C18:0 and C22:0 demonstrated a significant ($P<0.05$) increase, as seen in Table 2. C16:0, showing an important decrease during the embryonic development stage, while an important increase occured in the developmental stage of sac larvae and the fed and starved larvae. These increases are probably the result of the synthesis of fatty acids from acetic acid molecules obtained from proteins and carbohydrates (Abi-Ayad *et al.,* 2000). Along with the fluctuations in fatty acid content in the developing *O. mykiss*'s sac larvae showed an important decrease in total monounsaturated fatty acids. This decrease in the total monounsaturated fatty acids is probably due to the consumption of the C16:1ω-7 and the preservation of the C18:1ω-9 and C24:1ω-9 monounsaturated fatty acids. The conservation of C18:1ω-9 within the monounsaturated fatty acids is consistent with the previous findings (Mourente and Vázquez, 1996; Rinchard *et al.,* 2007). In the present study, an important fall was observed on comparison of total ω-3 and ω-6 fatty acids along with the absorption of their sacs. During fish development, catabolism increases with body growth. The increased catabolism is much higher during larval development than embryogenesis, probably due to increased activity and the resultant higher energy requirements following hatch (Gunasekera *et al.,* 2001; Johnston *et al.,* 2007).

In this study, on comparison of the composition of fatty acids of 24 day sac absorbed larvae (start of postlarval stage) with the fatty acid composition of starved 9 day larvae (Table 3), a significant ($P<0.05$) increase in the percentages of C14:0, C15:0, C16:0 and C18:0 fatty acids with the absorption of the sacs and a significant decrease ($P<0.05$) in the percentages of C18:1ω-9, monounsaturated fatty acid, and C20:5ω-3, C22:3ω-3 and C22:5ω-3, polyunsaturated fatty acids. Our results show that there is an apparent preference in utilization of PUFA and monounsaturated fatty acids. Saturated fatty acids were not used by *O. mykiss* larvae, especially by starved larvae (Dabrowski *et al.,* 1991; Abi-Ayad *et al.,* 2000). The results of Abi-Ayad *et al.* (2000) obtained on embryos and larvae of *Perca fluviatilis* support the results of the present study. In a similar manner, Dabrowski *et al.* (1991) determined that C16:0 and C18:0 did not change during ontogeny in *Perca flavescens.*

Also in this study the fact that C20:0 fatty acid in larvae starved from being 14 day sac larvae until death, C18:3ω-6 and C22:0 fatty acids in 9 day starved larvae and C22:3ω-3 fatty acid in 16 day starved larvae were not determined gives support to the idea that they were used as an energy source due to the high expansion of energy in the larvae passing into the free-swimming stage with the absorption of the yolk-sac (Cejas *et al.,* 2004; Gimènez *et al.,* 2008). In this study, it was observed that in starved larvae after the absorption of the yolk-sac, C20:0 was the first fatty acid to be consumed as an energy source during the starvation period, followed by C18:3ω-6 and C22:0 fatty acids and C22:3ω-3 was used during the first half of the starvation period.

Also in this study, it was observed in starved larvae that C20:0 was the first fatty acid to be consumed as an energy source during the starvation

period, followed by C18:3ω-6 and C22:0 fatty acids. C22:3ω-3 was used during the first half of the starvation period. This finding supports the idea that these fatty acids were used as an energy source due to the high expansion of energy in the larvae passing into the free-swimming stage with the absorption of the yolk-sac (Cejas *et al.*, 2004; Gimènez *et al.*, 2008).

In the development stage of the fed *O. mykiss* larvae, an important fluctuation with a decreasing trend in the percentages of total saturated fatty acids was observed. This fluctuation seen in the fed larvae, could be as a result of a significant ($P<0.05$) decrease in the amount of C18:0 in the fed larvae due to the lack of C18:0 in the feed and its use to produce energy. In contrast to C18:0, C16:0 which holds an important place in the formation of the membrane during embryogenesis and is a main component of phospholipids, (principally phosphatidylcholine and phosphatidylethanolamine) (Dantagnan *et al.*, 2007) showed a significant ($P<0.05$) increase in the starved and fed larvae (Table 3). Despite the fact that saturated fatty acids are generally considered as substrates for energy production (Sargent, 1995), catabolism of saturated fatty acids in starved larvae was low compared to that of monounsaturated and polyunsaturated fatty acids. There was, even, an increase of saturated fatty acids especially C14:0, C16:0, C17:0 and C18:0, probably due to bioconversion processes which is in accordance with other studies (Cejas *et al.*, 2004; Abi-ayad *et al.*, 2004). The predominant saturated fatty acids that occur naturally in animal fats, including fish lipids are C16:0 and C18:0, although a range of chain lengths from C12 to C24 can be found. The main products of fatty acid synthetase are the saturated fatty acids C16:0 and C18:0 , which can be biosynthesized *de novo* by all known organisms, including fish (Tocher, 2003).

On examination of the percentages of C16:1ω-7 and C18:1ω-9, monounsaturated fatty acids, in the feed in the present study shows that they were not used as energy sources in the fed larvae. The intensive use of C18:1ω-9, while conserving C16:1ω-7 during development was observed in starved larvae (Sargent, 1995). While C24:1ω-9 was conserved in the first three stages, showing an important fall in the final stage in which the larvae died, C16:1ω-7 and C24:1ω-9 were conserved in the starvation period until the final moment and C18:1ω-9 was used as a priority energy source. In starved larvae, there was an intense utilization of monounsaturated fatty acids, especially 18:1ω-9 pointing out the importance of this nutrient as energy substrates (Sargent, 1995; Abi-ayad *et al.*, 2000; 2004). Takeushi and Watanabe (1982) showed utilization of monounsaturated fatty acids as an energy source by starved rainbow trout (*Oncorhynchus mykiss*) larvae. Our data are in general in agreement with these observations.

In the present study, although the starved *O. mykiss* were under very difficult nutritional conditions, C18:2ω-6 and C20:4ω-6 acids were more strongly conserved in the starved larvae than in the fed larvae. On examination of ω-6 and ω-3 fatty acids, it was determined that the starved larvae, in comparison with the fed larvae, more exhausted C18:3ω-6 and C22:3ω-3**,** while using less C20:5ω-3, they more strongly conserved C22:6ω-3. This supports the view that C20:5ω-3 and C22:6ω-3 acids are generally spared for physiological functions as well for incorporation in specific tissues such as the brain and retina (Wiegand, 1996; Mourente and Vázquez, 1996; Rainuzzo *et al.*, 1997; Gunasekera *et al.*, 2001).

While the highest values of C22:6ω-3 in embryonic development stage, sac-larvae stage, starved and fed groups were determined as 5.99%, 5.79%, 8.61% and 7.11% respectively, the highest values of C20:4ω-6 were established as 2.69%, 3.61%, 4.30% and 3.48% respectively. It was determined that our data of both fatty acids in the free-swimming stage, in either starved or fed larvae, were high. It was also established that both fatty acids were higher in the starved larvae than in the fed larvae (Mourente and Vazquez, 1996). Selective retention in embryogenesis (Izquierdo, 1996) and starvation (Tandler *et al.*, 1989) of C22: 6ω-3 has been shown to be important during the embrionic and larval stages.

In conclusion, although the total ω-3 and total ω-6 (Table 3) amounts were close to each other (16.94 % and 16.68% respectively) on the ninth day of starvation in the starved larvae, a significant ($P<0.05$) fall of total ω-3 (14.15%) on the completion of starvation period (29th day) showed that ω-3 polyunsaturated fatty acids were more consumed as fuel. However, despite this decrease in amount of total ω-3, the amount of C22:6ω-3 was more strongly protected than the amount in the fed larvae.

Acknowledgements

We thanks the Research Fund of Cumhuriyet University (Sivas, Turkey) for supporting this research and the local and commercial Fish Farm in Sivas for providing the eggs and sperm samples.

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