OPEN ACCESS
International Journal of Aquatic Science
ISSN: 2008-8019
Vol. 5, No. 2, 167-180, 2014

IJAS

Variations of fatty acids in Rainbow trout (*Oncorhynchus mykiss*) during eyed egg and larval development stages

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Received: 16 July 2013 **Accepted:** 4 September 2013 **Published:** 30 June 2014

Abstract: In the present study, fatty acid composition was determined in eyed egg embryo and larval development stages in Rainbow trout (*Oncorhynchus mykiss*). All fatty acids revealed significant differences between eyed egg and 10-days larvae ($p<0.05$) except for docosahexaenoic acid (DHA)/eicosapentaenoic acid (EPA), eicosapentaenoic acid (EPA)/docosahexaenoic acid (DHA), total unsaturated fatty acids (∑ USFA)/ sum of unsaturated fatty acids (∑ SFA). However, in both larvae before (19, 22, 24, 27, 29) and after complete absorption of the yolk sac (34, 39, 44, 49, 58), there was an apparent preference in the utilization of polyunsaturated fatty acids (PUFA) followed by monounsaturated fatty acids (MUFA). Palmitic acid (C16:0), oleic acid (C18: 1n-9) and DHA (C22: 6n-3) were the main SFA, MUFA and PUFA, respectively, which were utilized and significantly decreased ($p< 0.01$) from 12-day larvae (day 19, with 80% yolk sac) to 22-day larvae as well as from 27-day larvae to 51-day larvae and then reached to the minimum levels in 22 and 51-day larvae. In larvae, these 3 nutrients were also the most fatty acids utilizing as energy source and possibly as precursors for others monounsaturated fatty acids biosynthesis as well. During the same period and among (n-6) polyunsaturated fatty acids, linoleic (18:2n-6); LA) and arachidonic (20:4n-6); AA) acids contents significantly decreased ($p< 0:05$) from 12-day larvae to 22-day larvae as well as from 27-day larvae to 51-day larvae. DHA is generally spared for physiological functions.

Key Words: eyed egg, fatty acids, larval development, *Oncorhynchus mykiss*, yolk sac

Introduction

In most of species, growth and energy supply during embryogenesis and larval development is depend on endogenous yolk reserves transferred by broodstocks (Watanabe, 1993; Abi-Ayad *et al*., 2000) so maternal nutrition affects the egg nutrient composition (Lavens and Sorgeloos, 1991; Peleteiro *et al*., 1995). In the case of fatty acids, it is showed that DHA should be provided in sufficient level (0.6% of D.W.) in the broodstock diet to produce healthy larvae (Abi-Ayad *et al*., 2000). After yolk resorption, larval growth and survival

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depend on the availability of exogenous food in sufficient quantity and of adequate quality (Heming and Buddington 1988). It is established that biochemical composition varied during embryonic and larval development (Tocher *et al*., 1985a, b; Dabrowski *et al*., 1991). Proteins and amino acids, lipids and fatty acids as well as carbohydrates are used differently and selectively by different fish species as energy resources (Sargent *et al*., 1989).

Catabolism increases with body growth during fish development as such larvae have more catabolism than embryo, probably due to increased activity and the resultant higher energy requirements following hatch (Heming and Buddington 1988). In fact, several studies (Fiogbe 1996; Kestemont *et al*., 1999) showed a relationship between embryo and larval quality with biochemical composition (fatty acids and amino acids).

In the last decade, it has been demonstrated extensively that highly unsaturated fatty acids are an essential nutrient for early larval development (Fraser *et al*., 1987; Watanabe, 1993; Peleteiro *et al*., 1995).

Embryos of trout catabolize proteins, lipids and carbohydrates to satisfy their energy requirements (Boulekbache 1981, Watanabe, 1982; Izquierdo *et al*., 2000). Lipids and fatty acids constitute a major energy source for fish and polyunsaturated fatty acids are considered as a structural components during organogenesis (muscles, brain, retina, etc) (Bell and Tocher 1989; Navarro and Sargent 1992; Sargent 1995) and precursors of physiologically active molecules such as prostaglandins and other eicosanoids (Goetz *et al*., 1989a, b; Bell *et al*., 1992; Sargent 1995; Abi-Ayad *et al*., 2000).

Since fish egg should contain all the nutrients required for the normal development of embryo and endogenous feeding larvae, the study of the evolution of fatty acid composition in eggs and larvae would contribute to determining their first feeding requirements (Izquierdo, 1996).

It is also recognized that environmental factors such as temperature (Farkas *et al*., 1980; Henderson, 1996; Olsen *et al*., 1999), salinity (Borlongan and Benitez, 1992; Tocher *et al*., 1994), light (Ota and Yamada, 1971) and or the type of food available (Watanabe, 1982) exert an effect on lipid contents and fatty acid composition of aquatic organisms (Olsen and Skjervold, 1995; Jobling *et al*., 1998; Zenebe *et al*., 1998). These environmental changes primarily affect metabolic processes (Sheridan, 1989), causing accumulations which may vary in different organs of aquatic organisms (Farkas *et al*., 1980; Sheridan, 1989; Borlongan and Benitez, 1992; Dantagnan *et al*., 2007)

The aim of the present study was to determine the variations of fatty acids, particularly of linolenic (18:3n-3; LNA), eicosapentaenoic (20:5n-3; EPA) and

docosahexaenoic (22:6n-3; DHA) acids in eyed egg and larval development stages of Rainbow trout (*Oncorhynchus mykiss*) as these nutrients and particularly DHA have a vital role in predatory fish (Tocher *et al*., 1992; Abi-Ayad *et al*., 2000).

Material and methods

Facilities and fish

1500 eyed eggs were obtained from Haraz Rainbow trout reproduction center and transported to incubate in horizontal trays (215×42×12cm) with 12 l/min flow rate after temperature adaptation. Horizontal trays divided to 3 equal parts for preparing of 3 experimental replicates. After hatching (6-7 days after eyed eggs were stocked) and absorption of about 80% of yolk sac (active swimming), larvae were fed by commercial diet (Biomar, France, diameter 0.5mm) containing 52% protein; 13% lipid; 0.4% fiber and 6/9% ash for 12 times per day from 6 am to 6 pm (fatty acids composition of larval feed, see Table 1). This period was started from day 19 (12-day larvae). During experiment, factors including temperature, pH (by pH meter, model: WPA CD 500, UK), dissolved oxygen (DO) (Oxy meter, model: Aqualytic Multimeter, AL15, Germany) and ammonia (Tetra test kit, D-49304 Melle, Germany) were measured. Light/dark cycle and light intensity were 10:14h and 400 lux (Light meter, model, Ml-120: Testoon SAF, Chatinnon, French), respectively. This research was done for 58 days.

Sampling for fatty acid analysis

Samples of egg and larvae (1 gr) were collected once at starting stage (day 1, eyed egg) and once before active feeding of larvae (10-day larvae, day 17), 5 times after active feeding of larvae with yolk sac from 12-day larvae to 22-day larvae (19, 22, 24, 27, 29) and 5 times, without yolk sac from 27-day larvae to 51-day larvae (34, 39, 44, 49, 58). Then samples were kept in -80°C for fatty acid analysis.

Fatty acid profile determination

Lipid content was extracted according to the procedure of Folch *et al* (1957). The Fatty acid methyl esters (FAME) were separated and quantified by using a Shimadzu GC-17 gas chromatograph (Shimadzu, Japan) equipped with a flame ionization detector (250°C) and column BPX70 (50 m · 0.32 mm ID) (SGE, Australia). Nitrogen was used as carrier gas and the oven initial temperature was 125°C for 1 min, followed by an increase at a rate of 4°C min) 1 to a final temperature of 215°C. Individual FAME was identified by reference to authentic standards and to well characterize fish oil (Cronin *et al*., 1991; Hernandez *et al*., 2003., Yeganeh *et al*., 2009).

Analysis of data

All data were expressed as the mean ± SD (n: 3 replicates) and statistically compared by

One-Way Analysis of Variance (ANOVA). Data from different fatty acid measurements were subjected to Duncan multiple range test ($p<0.05$).

Tab. 1: Fatty acids composition of dry feeds distributed to *Oncorhynchus mykiss* larvae.

Fatty acids (%)	Dry feed	Fatty acids (%)	Dry feed
C14:0	3.7	C20:4 n-6	0.94
C16:0	17.24	C20:5 n-3	7.2
C18:0	2.4	C22:6 n-3	12.7
C20:0	Nd	∑PUFA n-3	24.73
∑SFA	23.34	∑PUFA n-6	5.64
C14:1 n-5	0.85	∑LC-PUFA n-3	20.23
C16:1 n-7	6.9	∑LC-PUFA	21.17
C18:1	17.2	∑USFA	62.52
C20:1 n-9	7.2	n-3/ n-6	4.38
∑ MUFA	32.15	DHA/EPA	0.57
C18:2 n-6	4.7	EPA/DHA	1.76
C18:3 n-3	4.5	∑USFA/∑SFA	2.68
C20:3 n-3	0.33		

∑SFA: Total Saturated Fatty Acid; ∑MUFA: Total Monounsaturated Fatty Acid; ∑PUFA n-3= Total Poly Unsaturated Fatty Acids n-3; ∑PUFA n-6: Total Poly Unsaturated Fatty Acids n-6; ∑LC-PUFA n-3: long chain PUFA n-3; ∑LC-PUFA: long chain PUFA; ∑ USFA: Total Unsaturated Fatty Acid; (n-3/ n-6=∑PUFA n-3/∑PUFA n-6), (DHA/EPA= C22:6 n-3/ C20:5 n-3)

Results

Environmental parameters were temperature: 15.02 ± 1.84°C, pH: 7.62±0.62, DO: 8.36±0.92 (mg/l) and Total Ammonia: 0.6±0.25 (mg/l).

Before active feeding

In eyed egg embryos (EEE), polyunsaturated fatty acids (PUFA n-3+ PUFA n-6, 5.45%) were more than monounsaturated (MUFA, 4.37%) or saturated (SFA, 3.39%) fatty acids. Also, in 10-days larvae (10DL, day 17) polyunsaturated (9.92%) fatty acids were more than monounsaturated (9.80%) or saturated (6.78%) fatty acids (Table 2). All fatty acids detected significant differences between eyed egg and 10-days larvae ($p<0.05$) except DHA/EPA, EPA/DHA, ∑ USFA/∑ SFA. Fatty acids in 10-day larvae were more than eyed egg stage except arachidic acid (C20:0), eicosatrienoic acid (C20: 3n-3), and n-3/ n-6 ratio.

Tab. 2: Fatty acid measurements in Eyed egg embryo (day 1) and 10-day larvae (day 17) samples of Rainbow trout (*Oncorhynchus mykiss*)

Fatty acids (%)	EEE	10DL	Fatty acids (%)	EEE	10DL
C14:0	0.007±0.01[a*]	0.17±0.01[b]	C20:3 n-3	0.11±0.08[a]	0.05±0[a]
C16:0	2.03±0.02[a]	4.50±0.05[b]	C20:4 n-6	0.62±0.01[a]	1.05 ±0.01[b]
C18:0	1.03±0.01[a]	2.07±0.02[b]	C20:5 n-3	0.31±0.01[a]	0.56±0[b]
C20:0	0.07±0.02[a]	0.01±0.01[b]	C22:6 n-3	2.59±0.87[a]	4.59±0.14[b]
∑ SFA	3.39±0.01[a]	6.78±0.05[b]	∑PUFA n-3	3.16±0.02[a]	5.51±0.14[b]
C14:1 n-5	0[a]	0.01±0.01 [b]	∑PUFA n-6	2.29±0.03[a]	4.41±0.01[b]
C16:1 n-7	0.38±0.01[a]	0.90±0.01[b]	∑LC-PUFA n-3	3.01±0.01[a]	5.20±0.14[b]
C18:1 n-7	0.45±0.01[a]	0.97± 0[b]	∑LC-PUFA	3.62±0.02[a]	6.25±0.14[b]
C18:1 n-9	3.20±0.04[a]	7.26±0.06[b]	∑USFA	9.82± 0.1[a]	19.74±0.11[b]
C20:1 n-9	0.28±0.01[a]	0.55±0.01[b]	n-3/ n-6	1.38±0.01[a]	1.25±0.03[b]
∑MUFA	4.37±0.06[a]	9.80±0.03[b]	DHA/EPA	8.47±0.23[a]	8.20±0.23[a]
C18:2 n-6cis	1.33±0.02[a]	2.69±0.01[b]	EPA/DHA	0.11±0.01[a]	0.12±0[a]
C18:2 n-6tra	0.07±0[a]	0.12±0[b]	∑USFA/∑SFA	2.90±0.04[a]	2.91±0.03[a]
C18:3 n-3	0.15±0.01[a]	0.31±0[b]	other	0.73±0.1[a]	1.41±0.06[b]
C20:2 n-6	0.27±0.01[a]	0.54±0[b]			

[*]a) Average of three lots analysed; values reported are means ± SD; Different letters show significant difference in each row (P< 0.05). EEE: eyed egg embryo, DL: day larvae.

Fed Larvae (active feeding)

SFA, MUFA, PUFA and most fatty acids showed a significant decreasing trend from 12-day larvae (12 DL, day 19, with 80% yolk sac) to 22-day larvae (22DL, day 29, absorbing yolk sac completely), and their highest levels with all fatty acids except rachidic acid, myristoleic acid (C14: 1n-5), and palmitoleic acid (C16: 1n-7) obtained in 27-day larvae (27 DL, day 34, after absorbing yolk sac) and afterwards the levels decreased until final examination day ($p<0.05$, Table 3). In all experimental days (day 19 -58) polyunsaturated fatty acids were more than monounsaturated or saturated fatty acids. Palmitic (C16:0), oleic acid and DHA were the main SFA, MUFA and PUFA, respectively, which were utilized and decreased significantly ($p< 0.01$) from 12-day larvae (day 19, with 80% yolk sac) to 22-day larvae (day 29) as well as from 27-day larvae (34) to 51-day larvae (58) and then reached to the minimum values in 22 and 51-day larvae. Before complete absorption of the yolk sac (from 19 to 29) and thereafter (from 34 to 58) PUFA were utilized higher than MUFA and/or SFA.

Among (n-6) PUFA, contents of linoleic and arachidonic acids decreased significantly ($p<0.05$) from day 19 to 29 (from 2.49 to 1.09,

from 1.19 to 0.31%, respectively) and day 34 to 58 (from 3.6 to 0.9, from 1.45 to 0.06%, respectively) and reached to a minimum value at the end of experiment (day 58).

Tab. 3: Fatty acid measurements of Rainbow trout (*Oncorhynchus mykiss*) larvae before (12, 22, 24, 27, 29) and after absorption of yolk sac completely (34, 39, 44, 49, 58)

Fatty acids (%)	12 LBYR	22 LBYR	24 LBYR	27 LBYR	29 LBYR	34 LAYR	39 LAYR	44 LAYR	49 LAYR	58 LAYR
C14:0*	0.16 (0.02)^{a}	0.09 (0.01)^{b}	0.24 (0.01)^{b}	0.2 (0.02)^{ab}	0.11 (0.02)^{a}	0.4 (0.02)^{e}	0.25 (0.04)^{bc}	0.35 (0.01)^{d}	0.67 (0.08)^{a}	0.3 (0.03)^{c}
C16:0	4.80 (0.14)^{e}	2.67 (0.06)^{b}	4.52 (0.06)^{d}	4.71 (0.08)^{de}	1.85 (0.1)^{a}	6.52 (0.38)^{f}	3.64 (0.12)^{c}	4.53(0.01)^{d}	4.76 (0.2)^{de}	1.77 (0.04)^{a}
C18:0	1.91 (0.01)^{a}	1.06 (0.01)^{b}	1.54 (0.01)^{c}	1.73 (0.01)^{d}	0.63 (0)^{e}	2.17 (0.04)^{f}	1.22 (0.01)^{g}	1.45 (0.01)^{h}	1.17 (0)^{k}	0.39 (0)^{l}
C20:0	0.03 (0.02)^{a}	0.08 (0)^{b}	0.01 (0.01)^{a}	0.02 (0)^{a}	0.09 (0.01)^{b}	0.02 (0.01)^{a}	0.08 (0.01)^{b}	0.02 (0.01)^{a}	0.02 (0)^{a}	0.01 (0)^{a}
∑SFA	7 (0.12)^{h}	4.1 (0.08)^{c}	6.36 (0.08)^{e}	6.77 (0.13)^{gh}	2.82 (0.11)^{b}	9.17 (0.45)^{k}	5.36 (0.10)^{d}	6.4 (0.11)^{efg}	6.68 (0.27)^{f}	2.49 (0.69)^{a}
C14:1 n-5	0^{a}	0.003 (0.01)^{a}	0^{a}	0.003 (0.01)^{a}	0^{a}	0.003 (0.01)^{a}	0.01 (0)^{b}	0^{a}	0.01 (0)^{b}	0.01 (0)^{b}
C16:1 n-7	0.81 (0.03)^{f}	0.4 (0.01)^{ab}	0.68 (0.01)^{cdf}	0.69 (0.02)^{ef}	0.47 (0.37)^{abc}	0.71 (0.17)^{ef}	0.57 (0.07)^{bcd}	0.64 (0.01)^{cdf}	0.84 (0.04)^{f}	0.35 (0.01)^{a}
C18:1 n-7	0.86 (0.01)^{a}	0.47 (0.01)^{b}	0.72 (0)^{c}	0.79 (0)^{d}	0.29 (0)^{e}	0.96 (0.02)^{f}	0.57 (0.01)^{g}	0.67 (0)^{h}	0.69 (0.01)^{k}	0.25 (0)^{l}
C18:1 n-9	7.20 (0.06)^{a}	3.57 (0.01)^{b}	5.62 (0.02)^{c}	5.90 (0.02)^{d}	2.35 (0)^{e}	7.39 (0.2)^{f}	4.40 (0.02)^{g}	5.31 (0.01)^{h}	4.83 (0.04)^{k}	1.76 (0.12)^{l}
C20:1 n-9	0.39 (0.04)^{g}	0.21 (0)^{b}	0.31 (0)^{e}	0.34 (0.01)^{f}	0.11 (0)^{a}	0.4 (0.02)^{g}	0.23 (0.01)^{b}	0.3 (0.01)^{de}	0.53 (0.01)^{h}	0.28 (0.01)^{c}
∑MUFA	9.37 (0.1)^{a}	4.69 (0.04)^{b}	7.5 (0.05)^{c}	7.84 (0.06)^{d}	3.03 (0.01)^{e}	10.03 (0.24)^{f}	5.84 (0.01)^{g}	7.27 (0.01)^{h}	7.49 (0.1)^{c}	3 (0.01)^{e}
C18:2 n-6cis	2.49 (0.03)^{a}	1.32 (0)^{b}	2.34 (0.01)^{c}	2.4 (0.01)^{d}	1.09 (0.01)^{e}	3.6 (0.11)^{f}	1.86(0)^{g}	2.81 (0.01)^{h}	2.57 (0.02)^{k}	0.9 (0.01)^{l}
C18:2 n-6tra	0.11 (0.01)^{cd}	0^{a}	0.09 (0)^{c}	0.11 (0)^{cd}	0^{a}	0.16 (0.01)^{e}	0.02 (0.04)^{b}	0.11 (0)^{cd}	0.12 (0)^{d}	0.04 (0)^{b}
C18:3 n-3	0.3 (0.01)^{a}	0.16 (0.01)^{b}	0.26 (0)^{c}	0.28 (0)^{d}	0.12 (0)^{e}	0.42 (0.01)^{f}	0.23 (0)^{g}	0.36 (0.01)^{h}	0.39 (0.01)^{k}	0.15 (0)^{l}
C20:2 n-6	0.45 (0.02)^{a}	0.23 (0.01)^{b}	0.3 (0.01)^{c}	0.33 (0.01)^{d}	0.1 (0)^{e}	0.38 (0.01)^{f}	0.18 (0.01)^{g}	0.22 (0.01)^{b}	0.18 (0)^{g}	0.06 (0)^{h}

Tab. 3: Continued

Fatty acids (%)	12 LBYR	22 LBYR	24 LBYR	27 LBYR	29 LBYR	34 LAYR	39 LAYR	44 LAYR	49 LAYR	58 LAYR
C20:3 n-3	0.07 (0.02)^{ab}	0.06 (0.06)^{ab}	0.04 (0.01)^{b}	0.04 (0.01)^{b}	0.02 (0)^{b}	0.11 (0.01)^{a}	0.05 (0)^{b}	0.04 (0.02)^{b}	0.11 (0.01)^{a}	0.05 (0)^{b}
C20:4 n-6	1.19 (0.04)^{a}	0.63 (0.01)^{b}	1 (0)^{c}	1.13 (0.01)^{d}	0.31 (0.01)^{e}	1.45 (0.02)^{f}	0.72 (0.01)^{g}	0.73 (0)^{g}	0.18 (0)^{h}	0.06 (0)^{k}
C20:5 n-3	0.58 (0.05)^{a}	0.38 (0.01)^{b}	0.74 (0)^{c}	0.8 (0)^{d}	0.31 (0.01)^{e}	1.59 (0.03)^{f}	0.72 (0.01)^{c}	0.79 (0.01)^{d}	1.27 (0.01)^{g}	0.48 (0.01)^{h}
C22:6 n-3	5.18 (0.5)^{de}	2.88 (0.15)^{b}	4.77 (0.03)^{cd}	5.11 (0.16)^{de}	1.88 (0.11)^{a}	10.76 (0.83)^{f}	4.38 (0.23)^{c}	4.72 (0.08)^{cd}	5.44 (0.17)^{e}	2.07 (0.11)^{a}
∑PUFA n-3	6.13 (0.57)^{e}	3.52 (0.09)^{b}	5.81 (0.02)^{de}	6.23 (0.17)^{e}	3.03 (0.01)^{a}	10.03 (0.24)^{g}	5.38 (0.25)^{c}	5.93 (0.05)^{e}	7.22 (0.16)^{f}	2.76 (0.12)^{a}
∑PUFA n-6	4.24 (0.03)^{a}	2.17 (0.02)^{b}	3.74 (0.01)^{c}	3.96 (0.02)^{d}	1.51 (0.01)^{e}	5.6 (0.09)^{f}	2.8 (0.03)^{g}	3.88 (0)^{h}	3.44 (0.01)^{k}	1.19 (0.01)^{l}
∑LC-PUFA n-3	5.84 (0.56)^{d}	3.36 (0.1)^{b}	5.55 (0.02)^{cd}	5.95 (0.16)^{d}	2.21 (0.12)^{a}	12.46 (0.85)^{f}	5.15 (0.24)^{c}	5.56 (0.06)^{cd}	6.83 (0.17)^{e}	2.67 (0.12)^{a}
∑LC-PUFA	7.02 (0.6)^{b}	3.98 (0.1)^{a}	6.55 (0.02)^{b}	7.07 (0.18)^{b}	2.53 (0.12)^{a}	15.58 (3.75)^{c}	5.88 (0.25)^{b}	6.29 (0.06)^{b}	7.38 (0.18)^{b}	2.77 (0.12)^{a}
∑USFA	19.73 (0.66)^{a}	10.43 (0.08)^{b}	17.05 (0.08)^{c}	18.02 (0.27)^{d}	6.87 (0.1)^{e}	28.7 (0.55)^{f}	14.03 (0.23)^{g}	17.07 (0.05)^{c}	18.14 (0.25)^{d}	6.95 (0.13)^{e}
n-3/ n-6	1.44 (0.12)^{ab}	1.62 (0.03)^{c}	1.56 (0)^{bc}	1.57 (0.03)^{bc}	1.54 (0.07)^{bc}	2.28 (0.19)^{f}	1.91 (0.11)^{d}	1.53 (0.01)^{abc}	2.09 (0.06)^{e}	2.31 (0.1)^{f}
DHA/EPA	8.84 (0.34)^{f}	7.43 (0.26)^{e}	6.43 (0.03)^{cd}	6.42 (0.18)^{cd}	6.09 (0.21)^{bc}	6.79 (0.4)^{d}	6.1 (0.2)^{bc}	5.95 (0.17)^{b}	4.28 (0.1)^{a}	4.31 (0.18)^{a}
EPA/DHA	0.11 (0.01)^{a}	0.13 (0.01)^{b}	0.15 (0.01)^{cd}	0.15 (0.01)^{cd}	0.16 (0.01)^{d}	0.14 (0.01)^{bc}	0.16 (0.01)^{d}	0.16 (0.01)^{d}	0.23 (0.01)^{e}	0.23 (0.01)^{e}
∑USFA/∑SFA	2.82 (0.13)^{de}	2.52 (0.06)^{ab}	2.67 (0.02)^{bcd}	2.63 (0.09)^{bcd}	2.38 (0.13)^{a}	3.05 (0.21)^{e}	2.59 (0.09)^{bc}	2.66 (0.17)^{bcd}	2.66 (0.15)^{bcd}	2.75 (0.13)^{cde}
Other	1.41 (0.16)^{d}	0.45 (0)^{b}	1.05 (0.16)^{c}	1.06 (0.11)^{c}	0.22 (0.01)^{a}	1.84 (0.06)^{e}	0.47 (0.13)^{b}	0.93 (0.03)^{c}	1.01 (0.03)^{c}	0.49 (0.05)^{b}

*different letters shows significant difference (at P< 0.05) in each row. LBYR: larvae before yolk resorption, LAYR: larvae after yolk resorption.

With respect to (n-3) PUFA, DHA, EPA and LNA contents decreased significantly (p<0:05) from 19 to 29 (from 5.18 to 1.88, from 0.58 to 0.31, from 0.3 to 0.12%, respectively) and 34 to 58 (from 10.76 to 2.07, from 1.59 to 0.48, from 0.42 to 0.15%, respectively) and then

reached to the minimum value at the end of experiment (day 58).

DHA/EPA and ∑ USFA/∑ SFA ratios also decreased significantly (p< 0:05) from 19 to 29 (from 8.84 to 6.09, from 2.82 to 2.38, respectively) and 34 to 58 (from 6.79 to 4.31, from 3.05 to 2.75, from 0.42 to 0.15%, respectively) and reached to the minimum level at the end of experiment (day 58).

n-3/ n-6 and EPA/DHA ratios increased from19 to 29 (from 1.44 to 1.54 (p> 0:05), from 0.11 to 0.16 (p< 0:05), respectively) and 34 to 58 (from 2.28 to 2.31 (p> 0:05), from 0.14 to 0.23 (p< 0:05), from 0.42 to 0.15%, respectively) and reached to the maximum level at the end of experiment (day 58).

Discussion

Broodstock diet could have been important effect on fatty acid composition of the eggs and embryos (Peleteiro *et al*., 1995; Dantagnan *et al*., 2007). Based on variations of fatty acids in eyed egg and 10-day larvae of Rainbow trout (*Oncorhynchus mykiss*), it is likely that these nutrients were not used as energy sources by embryos and larvae except arachidic acid (C20:0), and eicosatrienoic acid (C20: 3n-3). It is possible that like in turbot (*S. maximus*) (Planas *et al*., 1990), embryos of Rainbow trout spared lipids and particularly PUFA for new cell constitution and organogenesis rather than for energy production, which is probably supplied by proteins and carbohydrates, as in winter flounder (*Pseudopleuronectes americanus*) and turbot (*S. maximus*) (Cetta and Capuzzo 1982; Planas *et al*., 1989). In some freshwater fish (*Nothobranchius quenteri*), embryonated eggs use lipids and fatty acids as an emergency energy source, but only when hatching is delayed (Sargent *et al*., 1989). On the contrary, embryos of trout, red drum (*Sciaenops ocellata*), Atlantic salmon (*Salmo salar*) and cod (*Gadus morhua*) catabolize fatty acids during the last stage of embryogenesis (Cowey *et al*., 1985; Fraser *et al*., 1988). During both embryo development and yolk sac absorption in the present experiment, the most abundant saturated fatty acid was 16:0, which is a main component of phospholipids, principally phosphatidylcholine and phosphatidyleth-anolamine for the majority of species (Tocher *et al*., 1985b; Mourente and Vazquez, 1996) and hence very important in membrane formation during embryogenesis. This pattern of conservation of saturated fatty acids during embryogenesis is typical of cold water fishes with long incubation periods (>20 days), such as Atlantic salmon *Salmo salar* L. (Cowey *et al*., 1985), herring *Clupea harengus* L. (Tocher *et al*., 1985b) and cod *Gadus morhua* L. (Fraser *et al*., 1988) and differs to the reduction found in warm water fish. PUFA were conserved during embryogenesis and larval development, denoting their importance and were used only at the end of the experimental period when starved larvae were exhausted (Dantagnan *et*

al., 2007). Clearly, there are many differences in lipid and fatty acid utilization during embryogenesis between fish species (Abi-ayad *et al*., 2000).

Our results exhibited that there is an apparent preference in the utilization of PUFA and monounsaturated fatty acids. Indeed, saturated fatty acids were not utilized by Rainbow trout larvae. The latter were in unfavorable nutritional conditions, especially during the last days of absorption yolk sac when energy reserves were depleted. This is in agreement with the b-oxidation of fatty acids from triacylglicerides found during the yolk sac absorption in other fish species larvae (Rainuzzo, 1993). Dabrowski *et al*. (1991) indicated that 16:0 and 18:0 acids did not vary during the ontogeny of yellow perch (*P. flavescens*). Atlantic herring (*Clupea harengus*) and pikeperch (*Stizostedion lucioperca*) larvae utilize all fatty acids classes (Tocher *et al*., 1985a; Abi-ayad *et al*., 2000).

Monounsaturated fatty acids and particularly, 18:1(n-9) were intensively utilized by *O. mykiss* larvae at the end of the present experiments. The elongation of 16:1(n-7), 18:1(n-9) and other monounsaturated fatty acids to 20:1(n-9), 20:1(n-7) does not fully explain the decrease of 16:1(n-7) and 18:1(n-9) and we suspect that they were also catabolized for energy by the larvae (Izquierdo, 1996; Dantagnan *et al*., 2007). Indeed, Takeushi and Watanabe (1982) and Csengeri and Dey (1995) revealed the utilization of monounsaturated fatty acids as an energy source by starved rainbow trout (*Oncorhynchus mykiss*) and fed carp (*C. carpio*) larvae, respectively. Moreover, monounsaturated fatty acids can be involved in brain development as described previously in turbot (*S. maximus*) by Mourente *et al*. (1991).

In both fed *O. mykiss* larvae before and after absorption of the yolk sac, (n-6) PUFA were intensively utilized. The contents of none of the (n-6) PUFA increased before and after absorption of the yolk sac in larvae. This suggested that this fatty acid class was essentially catabolized as an energy source. Note that in fed larvae, feed also constituted an important source of energy.

In both fed *O. mykiss* larvae before and after absorption of the yolk sac, the high utilization of (n-3) PUFA, especially DHA was observed. In fed larvae and during the first week, the high utilization of (n-3) PUFA, including DHA, was also observed in trout (*O. mykiss*) (Tocher and Sargent, 1990), pike perch (*S. lucioperca*) and Eurasian perch (*Perca fluviatilis*) (Abi-ayad *et al*., 2000). The intense utilization of (n-3) PUFA and especially of DHA during this period may be explained by the transition period from endogenous to exogenous feeding which is generally accompanied by high mortality rates as a result of decreased growth (Guyot *et al*., 1993). In other species, DHA is generally spared for

physiological functions such as membrane fluidity (Lovell 1988), enzymatic activities related to ionic transport (Di Costanzo *et al*., 1983; Bourre *et al*., 1988), and constitutes a component of brain and retina (Bell and Dick 1991; Mourente and Tocher 1992; Tocher *et al*., 1991, 1992) which are crucial for predatory fish species like *Perca fluviatilis*. To satisfy their physiological functions and a part of their energy requirements, fed trout (*O. mykiss*) larvae probably utilized fatty acids from diet.

Despite the variations in fatty acids before absorption of the yolk sac, the (n-3/n-6) ratio of larvae remained unchanged and larvae regulated their lipid metabolism to maintain a balance between (n-6) and (n-3) PUFA. This condition was recorded in starved perch larvae that regulate their lipid metabolism to maintain a balance between (n-6) and (n-3) PUFA similar to that observed during embryogenesis (Abi-ayad *et al*., 2000). However, the DHA/EPA ratio decreased and EPA/DHA increased as a result of more utilization of DHA when compared to EPA.

According to the present results, it seems that DHA play an important role in *O. mykiss* and must be included at an adequate level in trout diets to avoid problems related to embryonic and larval performance. Abi-ayad *et al* (1997 and 2000) reported that DHA play an important role in *P. fluviatilis* embryonic and larval performance.

In the present trail, n-3/ n-6 ratio of *O. mykiss* eggs was 1.38. In a previous research by De Silva *et al*., (1998), this ratio was reported for eggs from freshwater populations of fishes (0.5-3.8).

The increasing of almost fatty acids in the middle of experiment (in 27-day larvae, day 34) probably related to feed commercial pellet after absorption of the yolk sac completely, and also due to the development of intestine organs and digestive enzymes, followed by the ability of absorbing nutrients or replenish fatty acid utilization at early growth and its decrease afterwards probably might be implicated to more larval activity.

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