Ontogenetic changes in lipids, fatty acid, and body composition during larval stages of Caspian Kutum (*Rutilus frisii kutum*)

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Abstract

Body composition and fatty acid profile of the body tissue of Caspian kutum (*Rutilus frisii kutum)* fish larvae were determined from fertilized egg to 50 days post hatching (dph). Feeding with rotifer started from day 3 to day 7; thereafter they were fed with dry food until the end of the experiment. The changes observed in lipid content and the decrease in lipid levels during larval ontogeny reflect the utilization and mobilization of lipids during both embryogenesis and larvae development. During this study, palmitic and stearic acids were the most abundant saturated fatty acid (SAFA).In kutum larvae, no utilization of palmitic and stearic acids was observed until 20 dph. It thereafter was utilized as an energy source. Oleic and palmitoleic cacids were the predominant monounsaturated fatty acid (MUFA). Oleic and palmitoleic acids utilized until 10 and 25 dph, respectively; thereafter they increased. DHA, EPA and ARA were the major polyunsaturated fatty acids (PUFA). The decrease in levels of DHA and EPA denotes their utilization as an energy substrate by kutum larvae. ARA decreased during larval ontogeny, reflecting dietary values. With regard to reared kutum larvae in fresh water and the low levels of HUFA in larval diet, it can be said that kutum larvae possibly are quite capable of elongating and desaturating C18 to C20 PUFA. In case of proximate analysis, the percentage of body protein and ash increased trend during ontogeny while the percentage of body lipid and moisture decreased trend during larval growth.

Keywords: *Rutilus frisii kutum,* fatty acid*,* SAFA, MUFA, PUFA

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Introduction

Caspian Kutum *Rutilus frisii kutum* (Kamenskii, 1901; family Cyprinidae) is one of the economically important fishes of the south shores of the Caspian Sea. The sharp decline in its annual catch observed in 1970s and early 1980s was caused by overfishing and other factors (Ghaninejad et al., 2007) had instigated the Iran Fisheries Organization to collection of kutum Broodstock from their natural spawning grounds. Each year more than 200 million fry (average weight of 1 g) are released into the Caspian Sea for restoration purposes (www.shilat.com).

The feeding regime of kutum fry in rearing ponds consisted of zooplankton and an artificial diet until the time they reach a body weight of 1 g. Knowledge of the fatty acid biochemical composition of kutum larvae provides a better understanding of the nutritional requirements at the start of exogenous feeding. Such knowledge also enhances the quality and survival of larvae, achieved through investigation and understanding of the species' lipid and fatty acid requirements. Until now, there was no feed specifically formulated for kutum fishes, so knowledge of their nutritional requirements and fatty acid profiles during the early life stages of larvae can help to formulate diets most suitable for the growth of these larvae.

Lipids are among the most important nutritional factors affecting growth and survival in early life stages (Dantagnanet al., 2007), also they are the major energy substrates in fish (Sargent, 1995).

Various experiments demonstrated that the composition of nutrients in eggs and larval bodies change during the ontogeny of fishes (Tocher et al., 1985a; Abi-ayad et al., 2000).At the larval stage, utilization of fatty acids is different and selective among fish species and depends on yolk reserves transferred by Brood stock (Heming and Buddington, 1988).The fatty acid composition and the fat content of larval fish are not constant(Kalyoncu et al., 2009).

Essential fatty acid (EFA) requirements and fatty acid composition among fish species can vary because of diet, location, gender, and environmental conditions (Gruger, 1967), such as temperature (Farkas et al., 1980; Olsen et al., 1999), water salinity (Borlongan and Benitez, 1992; Tocher et al., 1994), light(Ota and Yamada, 1971), and seasonal or geographic changes (Jobling et al., 1998; Zenebe et al., 1998). These differences are more complex in fish than in mammals (Castell, 1979), in those these environmental changes primarily affect metabolic processes (Sheridan, 1989). The different study showed that larval fatty acid profiles were reflected in the dietary profiles (Lund et al., 2007).

The quality of maternal nutrition has a direct influence on egg quality and hence on embryo and larval development throughout the period of endogenous feeding (Czesny and Dabrowski, 1998). The PUFA are known as "essential fatty acids" (EFA) and they include categories of both the n-6 and n-3 series (Das, 2006), especially the n-3 series, since they are crucial for larval development (Watanabe et al., 1983; Lisac et al., 1986).These fatty acids such as docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3) and

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arachidonic acid (ARA, 20:4n-6) play important physiological roles in fish as components of membrane phospholipids and as precursors of biologically active eicosanoids (Sargent et al., 1995, 2002). Research showed that marine fish larvae contain eicosapentaenoic acid (EPA) and docosahexaenoic (DHA), which are considered to be essential, whereas as freshwater fish are capable of synthesizing these important highly unsaturated fatty acids (HUFAs) from their C18 precursors, such as linoleic acid (18:2n-6) and linolenic acid (18:3n-3) (Sargent et al., 1989).

Ontogenesis of fatty acid profiles have been studied in various species, such as gilthead seabream (*Sparus aurata*) (Naz, 2009); white seabream (*Diplodus sargus sargus*)(Cejas et al., 2004); pikeperch (*Sander lucioperca*) (Abi-ayad et al., 2004); trout cod and Murray cod (*Maccullochella macquariensis* and *M. peelii peelii*) (Gunasekera et al., 1999); Caspian Sea carp *(Cyprinus carpio)* (Farhoudi et al., 2011).

At present, however, there is no previous study of the fatty acids profiles of *R. frisii kutum*. Consequently, determining the fatty acid profiles of this species is important. The aim of the present study is to investigate ontogenetic changes in lipids and fatty acid during larval stages. We hope that the present study will aid our understanding of nutrient requirements and fatty acids' physiological role in the development in kutum larvae, and give practical information for the formulation their diet, in order to increase their survival and growth.

Materials and methods

Caspian kutum larval culture was conducted at the Shahid Rajaie Restocking Center (Sari, Mazandaran, north of Iran; lat 36˚37´ N, long 53˚05´ E) in May 2010. In April and May, Broodstock were selected from wild breeders from four rivers: the Shirod, Tonekabon, Tajan, and Goharbaran. Caught Broodstock were stripped for propagation to obtain egg. The mean weight of Broodstock males and females were 0.68–0.70 kg, and 1.18–1.20kg, respectively. During propagation, the temperature was 24–25ºC. Spawned eggs after fertilization were immediately transferred to the Shahid Rajaee Restocking Center, and egg incubation and larval rearing were conducted in this center. The fertilized eggs transferred to vase incubator. The water temperature, oxygen levels, and pH were maintained at 18−19ºC, >5.1 mg l^{-1}, and 7.9, respectively, during incubation.

Buoyant viable eggs were daily separated from sinking dead eggs. Hatching occurred after 5–7 days. When yolk sacs were determined to be almost completely absorbed, marking the onset of exogenous feeding, newly-hatched larvae were transferred from incubators to the one hectare pool at 3 dph at the density of 100 larvae/m^2. The pools were supplied with freshwater. Throughout the experimental period, the water temperature, oxygen levels, and pH were maintained at 24–26ºC, 5.5–7 mg l^{-1}, and 8, respectively.

At early of exogenous feeding, fish larvae were fed rotifers during 3–7 dph, and then larvae were fed artificial diet (SFK) during 7–50 dph in this experiment (Table 3).The artificial diet

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was commercial kutum feed (SFK) produced by Mazandaran Feed Manufacturing Company.

Larvae (L) samples were randomly collected from the vase incubator and pool at days egg, L1, L3, L7, L10, L15, L20, L25, L30, L35, L40 and L50 corresponding to 0 (fertilized egg), 1 (hatching), 3 (onset of exogenous feeding), 7, 10, 15, 20, 25, 30, 35, 40, and 50 days post hatching (dph) for fatty acid analysis, respectively.

1 g of sample for each larval stage was washed with distilled water. After removing water with a filter paper, samples were frozen in liquid nitrogen at -190 °C and stored at -80˚C, awaiting proximate composition and fatty acid analysis. Growth measurements were obtained from a pool of 30 larvae at the sampling days.

The crude protein(CP), lipids, moisture, and ash of the larvae, live foods (rotifer),and artificial diet (SFK) were measured in triplicates using the standard methods of AOAC (1995). Protein was measured by the Kjeldahl method using a conversion factor of 6.25. Lipid was measured by chloroform extraction using the Foss 2050 (Soxtec^{TM} Systems, Hillerød, Denmark, DK-3400). Moisture content by drying the samples at 105°C for 24 h and Ash by combustion at 550 °C for 12 h were determined.

Total lipids of homogenized diets (rotifer, SFK) and samples of 50-day-old larvae were extracted in chloroform/methanol (2:1, v:v) using the method of Folch et al. (1957). The extracts were mixed and separated by the addition of water. Crude lipid extracts were evaporated under nitrogen.

Fatty acid methyl ester (FAME) was prepared according to Metcalfe and Schmitz (1961).The crude lipid extract was saponified with NaOH in methanol and fatty acid methyl esters were prepared by transmethylation with BF_3, in methanol. BF_3 has been used for the conservation of lipid to FAME. Fatty acid methyl esters were obtained on a Varian Gas Chromatograph (CP3800, Walnut Creek, Netherlands), equipped with a BPX 70 SGE capillary column (60 m× 0/25 mm ID× 0/25 µm film thickness) and a FID detector. Injection temperature and detection temperature were 230 and 260ºC, respectively. Oven temperature was initially 150ºC, rising to 190 ºC at a 2ºC min^{-1} rate; then rising to 245 ºC at a 20ºC min^{-1} rate. Pure nitrogen was used as the carrier. Peak areas were characterized using Varian software.

Results are expressed as means±standard deviation. Statistical comparisons were conducted using SPSS 11.5 for Windows software. A one-way Analysis of Variance (ANOVA) was performed to statistical comparisons. Duncan's tests were used to discover differences between means. Differences were considered statistically significant when $p<.05$.

Results

The study of growth of Caspian kutum was conducted for 50 dph. Results of kutum growth in terms of the length and weight of this fish were studied (Fig. 1). An exponential relationship (R^2=0.96, y=2.799x+6.057) and (R^2=0.96, y=49.08x-106.9) describes Caspian kutum growth, expressed in total length (TL) and wet body weight, respectively. In this

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experiment the mean initial weight and length (1 dph) and final body weight and total length (50 dph) of this fish were 4.02±0.01mg, 8.47±0.02mm and 483.33±1.11mg, 35.34±0.41mm, respectively (Table 1).

The chemical analysis of kutum larvae body, rotifer, and artificial diet is presented in Table 2. The crude protein and ash content of fish larvae ranged from 63–76% and 5–15% during the 50 dph, respectively. This result showed that crude protein and ash content increased during larval development (body weight).

A decrease in the protein content was observed from hatching to mouth opening (3 dph) ($p<.05$), and thereafter an increased trend was observed to the end of experiment ($p<.05$).The lipid and moisture contents ranged from 3.72–12.33% and 89–81% during the 50 dph, respectively. Content percentage of lipid decreased significantly with body weight ($p<.05$) except in first 3 dph. In addition, content percentage of moisture decreased insignificantly during larval ontogeny.

In Caspian kutum fish, 13 fatty acids were identified and quantified for all developmental stages (Table 4). The fatty acid composition of the kutum larvae at 50 dph reflected the dietary content (rotifer and dry food) (Table 3). Fatty acids were divided into three broad categories: saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA), and polyunsaturated (PUFA).

The sum of saturated fatty acids (∑SAFA) increased until 20 dph and reached 33.59%, Thereafter, the sum significantly decreased ($p<.05$).

The predominant saturated fatty acids in kutum larvae (SAFA) are 16:0, 18:0 during ontogeny. Among fertilized eggs until 20 dph the major saturated fatty acid (palmitic acid 16:0 and stearic 18:0 acids) contents increased Then they were utilized ($p<.05$) by kutum larvae until the last day of the experiment (50 dph). The components of saturated fatty acid, such as pentadecanoic acid (15:0) and heptadecanoic acid (17:0) were not significantly changed ($p>0.05$).

The sum of monounsaturated fatty acid (∑MUFA) increased in ontogeny and reached a maximum at 50 dph (35.84%) ($p<.05$). Dominant monounsaturated fatty acid (MUFA) was 18:1n-9 and 16:1n-7. Palmitoleic acid (16:1n-7) decreased from 3 dph to 25 dph($p<.05$), then increased until the end of experiment ($p>.05$).Oleic acid (18:1n-9) decreased from the fertilized egg stage and reached a minimum at 10 dph and then increased to end of the experiment ($p<.05$).

The long chain n-3, n-6 fatty acid is referred to as polyunsaturated fatty acids, PUFAs. The most important polyunsaturated fatty acids (PUFA) were docosahexaenoic acid (DHA, 22:6 n-3),eicosapentaenoic acid (EPA20:5n-3), and arachidonic acid(ARA, 20:4 n-6),which in aquaculture are often termed highly unsaturated fatty acids (HUFA, PUFA with ≥C20 and ≥3 double bonds). The sum of PUFAs and HUFAs (ARA, EPA, and DHA) increased during yolk resorption and reached a maximum at 3 dph (30.03% and 27.70%, respectively); thereafter they decreased and reached a minimum at the end of experiment (50 dph) (21.33 % and 9.07%) ($p<.05$).

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The highest PUFA was 22:6n-3. Among n-6 polyunsaturated fatty acids (n-6PUFA), linoleic acid (LA, 18:2n-6) was utilized by kutum larvae between the fertilized egg stage and 3 dph ($p>.05$), then increased and reached a maximum at 50 dph ($p<.05$), while the most important n-6 PUFA, arachidonic acid (ARA, 20:4n-6),increased from fertilized egg stage to 3 dph; then a significant decrease ($p<.05$) in the ARA was found after yolk sac absorption (3dph) during larval development.

Among n-3polyunsaturated fatty acids (n-3 PUFA), linolenic acid (LNA, 18:3n-3), eicosapentaenoic (EPA, 20:5n-3), and docosahexaenoic (DHA, 22:6n-3) were utilized during ontogeny and decreased. DHA increased between fertilized eggs to 3dph (yolk sac absorption) and then were markedly reduced ($p<.05$). EPA increased between 3–7 dph, then decreased markedly at the end of study (50 dph) ($p<.05$).

LNA content decreased and reached a minimum at 15 dph, then increased until the end of experiment (50 dph) ($p<.05$).

At yolk sac absorption (3dph), DHA content was the highest, comparatively, to EPA and LNA. At the end of experiment, the content of DHA was again higher than the content of LNA and EPA. The ratios of DHA/EPA and AA/EPA increased through larval growth and reached maximum at 50dph ($p<.05$). The decrease in EPA gave place to increases in AA/EPA and DHA/EPA ratios. The n-3 to n-6 (n-3/n-6) ratio during ontogeny declined from fertilized egg stage to 50 dph, ranging between 3.39%–0.66% ($p<.05$).

In addition, fatty acid composition of food is important. Fatty acid composition varied among rotifer and dry food (SFK) (Table 3).

The highest percentage of saturated fatty acid occurred in dry food (SFK) that were mainly attributable to palmitic acid, which in dry food and rotifer represented 23.50% and 16.48% respectively. Dry food (SFK) had the highest percentage (30.72%) of monounsaturated fatty acid oleic acid, while the rotifer proportion was 9.09%. Rotifers showed a lower proportion of PUFA compared to dry food (SFK). Differences in proportions of total PUFA were mainly attributable to linoleic acid (LA, 18:2n-6) which were 6.64% in rotifer and 14.12% in dry food. The ratio of DHA/EPA was 3.75 and 3.47 in dry food and rotifer, respectively. The ratio of ARA/EPA was 0.89 and 0.67 in dry food and rotifer, respectively. In two diets, ARA had the lowest percentage of the PUFAs that proportions of ARA were below 1%.

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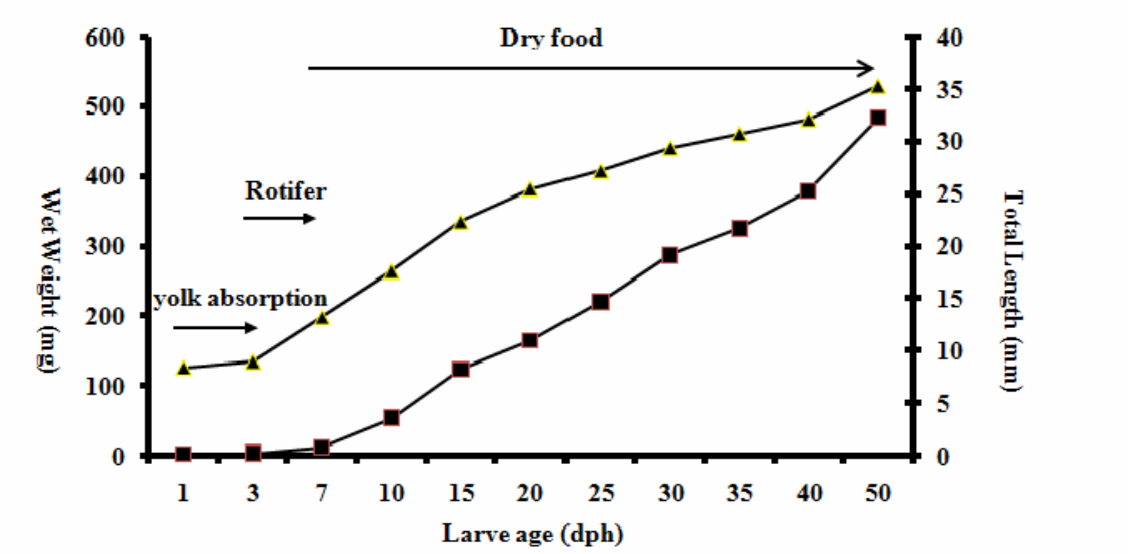


Figure 1: ***Rutilus frisii kutum*** **growth in wet weight (■) and total length (▲) during the first 50 days post hatched (dph).**

Table 1: Mean wet weight and total length of ***Rutilus frisii kutum***

Age(dph)	wet weight	total
L-1	4.02 ± 0.01^{j}	8.47 ± 0.02^{j}
L-3	4.37 ± 0.02^{j}	9.04 ± 0.02^{j}
L-7	12.49 ± 0.12^{i}	13.27 ± 0.02^{i}
L-10	54.72 ± 1.03^{h}	19.37 ± 0.23^{h}
L-15	104.30 ± 1.2^{g}	22.18 ± 0.17^{g}
L-20	175.60 ± 1.06^{f}	25.54 ± 0.2^{f}
L-25	221.28 ± 1.08^{e}	27.23 ± 0.02^{e}
L-30	287.14 ± 0.9^{d}	29.45 ± 0.19^{d}
L-35	325.83 ± 0.83^{c}	30.81 ± 1.0^{c}
L-40	379.58 ± 0.89^{b}	32.19 ± 1.0^{b}
L-50	483.33 ± 1.11^{a}	35.34 ± 0.41^{a}

Each mean [mean± S.D (n = 3)] is a pool of 30 larvae. Different letters indicate significant differences ($p<.05$).

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Table 2: Proximate composition of Caspian kutum larvae, rotifer, artificial diet (%Dry weight)

	EGG	L-1	L-3	L-7	L-10	L-15	L-20	L-25	L-30	L-35	L-40	L-50	Rotifer	Dry food
Protein	66.93^{f}	65.39^{g}	63.89^{h}	67.83^{ef}	67.35^{f}	69.00^{e}	70.76^{d}	71.83^{d}	72.12^{cd}	73.46^{bc}	74.15^{b}	76.16^{a}	57.48	38.54
Lipid	12.33^{a}	11.91^{a}	11.61^{a}	10.27^{b}	8.90^{c}	8.80^{cd}	7.84^{de}	6.68^{ef}	6.28^{fg}	5.66^{gh}	4.89^{h}	3.72^{i}	11.65	12.30
Moisture	85.52^{b}	88.62^{a}	89.61^{a}	85.76^{b}	85.43^{b}	85.57^{b}	85.62^{b}	83.92^{b}	83.73^{b}	82.40^{b}	82.11^{b}	81.56^{b}	92.28	9.11
Ash	5.10^{g}	5.84^{g}	6.68^{fg}	8.10^{f}	10.08^{e}	10.88^{de}	12.11^{cd}	12.84^{bc}	13.41^{bc}	13.97^{ab}	14.61^{ab}	15.56^{a}	10.60	11.60

Proximate composition are expressed in terms of dry weight (D.W). All values represent the mean ± S.D(n=3). Different letters indicate significant differences ($p<.05$).

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Table 3: Fatty acid composition of diet fed to kutum larvae (% of total fatty acids)

	Rotifer	Dry food
C15:0	0.04±0.73	0.10±0.42
C16:0	0.20±16.48	0.15±23.50
C17:0	0.17±1.54	0.02±0.65
C18:0	0.04±7.84	0.15±5.55
∑SAFA	0.12±26.59	0.14±30.12
C16:1n-7	0.71±4.83	0.28±4.91
C18:1n-9	0.12±9.09	0.23±30.72
C18:1n-7	0.1±2.90	0.12±11.91
C20:1n-9	0.46±1.65	0.03±0.34
∑MUFA	0.9±18.49	0.61±47.89
C18:2n-6	1.31±6.64	0.36±14.12
C18:3n-3	0.57±2.14	0.04±1.21
C20:4n-6	0.1±0.67	0.05±0.65
C20:5n-3	0.11±1.00	0.02±0.72
C22:6n-3	0.95±3.51	0.15±2.71
∑PUFA	0.78±13.97	0.16±19.42
∑HUFA	1.15±5.19	0.21±4.08
∑ n-3	0.56±6.66	0.15±4.65
∑ n-6	1.21±7.31	0.31±14.77
n-3/n-6	0.24±0.93	0.01±0.31
DHA/EP	0.60±3.47	0.19±3.75
AA/EPA	0.05±0.67	0.05±0.89
∑F.A.M.	1.54±59.06	0.43±97.45

Results represent means ±S.D.

∑-Total; ∑SAFA: total saturated fatty acids. ∑MUFAt: total monounsaturated fatty acids. ∑PUFA:total polyunsaturated fatty acids ∑ (n-3) and ∑ (n-6): total (n-3) and (n-6) fatty acids series. 6 FAME: total fatty acids methyl esters.

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Table 4: Fatty acid composition of larval stages of *Rutilus frisii kutum* at different periods of development (% of total fatty acid; means ±S.D. (n=3))

Fatty acid	Egg	L-1	L-3	L-7	L-10	L-15	L-20	L-25	L-30	L-35	L-40	L-50
C15:0	0/57±0/01^{b}	0/63±0/03^{b}	0/65±0/03^{b}	0/66±0/03^{b}	0/65±0/03^{b}	0/42±0/04^{e}	0/46±0/03^{cde}	0/45±0/07de	0/59±0/01^{bc}	0/58±0/05^{bcd}	0/65±0/05^{b}	0/85±0/21^{a}
C16:0	17/16±0.02	18/65±0/2^{g}	20.09±0/03^{e}	20/95±0/01	20/77±0/01^{d}	21/37±0/02^{b}	21/65±0/01^{a}	18/79±0/05^{f}	17/04±0/03^{i}	16/88±0/01^{j}	16/15±0/01^{k}	14/92±0/01^{l}
C17:0	0/66±0/05^{e}	0/71±0/01de	0/73±0/03de	0/79±0/02^{cd}	1/11±0/02^{a}	0/95±0/11^{b}	0/84±0/08^{bc}	0/73±0/12de	0/81±0/02^{cd}	0/86±0/03^{bc}	0/94±0/03^{b}	1/16±0/03^{a}
C18:0	5/85±0/01^{j}	6/39±0/01^{j}	7/77±0/15^{g}	9/17±0/08^{d}	9/53±0/04^{c}	10/04±0/16^{b}	10/63±0/23^{a}	8/60±0/02^{e}	8/23±0/11^{f}	7/68±0/07^{g}	6/86±0/02^{h}	4/96±0/34^{k}
∑SAFA	24/24±0/01	26/38±0/1^{gh}	29/24±0/11^{e}	31/57±0/11	32/06±0/04^{c}	32/79±0/ 3^{b}	33/59±0/20^{a}	28/58±0/65^{f}	26/68±0/08^{g}	26/02±0/05^{h}	24/60±0/08^{i}	21/90±0/31^{j}
C16:1n-7	4/24±0/8^{b}	4/53±0/34^{ab}	3/10±0/03^{c}	2/48±0/06de	2/94±0/04^{cd}	1/78±0/13^{f}	2/10±0/08^{ef}	2/50±0/27de	4/47±0/21^{ab}	4/19±0/22^{b}	4/67±0/22^{ab}	4/95±0/08^{a}
C18:1n-9	12/50±0/05	13/67±0/02^{g}	13/43±0/02^{h}	12/05±0/03^{j}	11/52±0/01^{k}	15/36±0/01^{f}	16/52±0/02^{e}	20/30±0/03^{d}	25/54±0/01^{c}	26/85±0/13^{b}	26/92±0/07^{b}	27/84±0/04^{a}
C18:1n-7	5/25±0/8^{a}	5/60±0/4^{a}	5/52±0/23^{a}	4/32±0/07^{b}	3/99±0/12^{b}	2/88±0/04^{c}	2/57±0/02^{c}	2/48±0/24^{c}	2/81±0/12^{c}	2/75±0/07^{c}	2/68±0/13^{c}	2/61±0/06^{c}
C20:1n-9	0/13±0/02^{f}	0/15±0/02^{f}	0/24±0/01^{cde}	0/19±0/02^{ef}	0/20±0/01^{def}	0/23±0/06^{cde}	0/25±0/08^{cde}	0/26±0/01^{cd}	0/29±0/01^{bc}	0/33±0/01^{b}	0/40±0/01^{a}	0/44±0/06^{a}
∑MUFA	22/13±0/91	23/96±0/36^{e}	22/30±0/23^{f}	19/05±0/13^{i}	18/65±0/14^{j}	20/20±0/1^{h}	21/45±0/09^{g}	25/55±0/19^{d}	33/12±0/28^{c}	34/13±0/26^{b}	34/69±0/11^{b}	35/84±0/23^{a}
C18:2n-6	1/35±0/16^{f}	1/13±0/05^{f}	0/97±0/03^{f}	1/63±0/02^{f}	2/97±0/04^{e}	5/42±1/76^{d}	6/16±0/04^{d}	8/20±0/86^{c}	10/12±0/17^{b}	10/50±0/48^{ab}	10/91±0/20^{ab}	11/31±0/44^{a}
C18:3n-3	1/16±0/03^{b}	1/29±0/02^{a}	1/36±0/03^{a}	0/96±0/03^{c}	0/84±0/02^{f}	0/36±0/04^{i}	0/47±0/05^{h}	0/67±0/05^{g}	0/83±0/14^{f}	0/89±0/03^{cde}	0/85±0/02de	0/95±0/06^{cd}
C20:4n-6	5/15±0/01^{d}	5/32±0/01^{c}	5/72±0/01^{b}	4/99±0/02^{e}	6/10±0/01^{a}	4/41±0/01^{f}	2/81±0/01^{g}	1/94±0/01^{j}	2/29±0/03^{h}	2/10±0/01^{i}	1/75±0/01^{k}	1/54±0/01^{l}
C20:5n-3	2/30±0/02^{d}	2/36±0/01^{c}	2/20±0/01^{e}	3/30±0/02^{a}	2/70±0/01^{b}	1/84±0/01^{f}	1/52±0/01^{g}	1/22±0/01^{h}	0/90±0/01^{i}	0/79±0/01^{j}	0/67±0/02^{k}	0/51±0/01^{l}
C22:6n-3	18/14±0/01	18/25±0/01^{b}	19/78±0/02^{a}	18/24±0/01	17/38±0/01^{d}	17/04±0/01^{e}	14/23±0/02^{f}	13/40±0/02^{g}	9/09±0/01^{h}	8/27±0/01^{i}	7/83±0/03^{j}	7/02±0/01^{k}
∑PUFA	28/11±0/16	28/36±0/07^{b}	30/03±0/05^{a}	29/13±0/05	30±0/04^{a}	29/07±1/7^{ab}	25/20±0/01^{c}	25/43±0/82^{c}	23/23±0/27^{d}	22/56±0/48de	22/01±0/21^{ef}	21/33±0/05^{f}
∑HUFA	25/59±0/01	25/93±0/02^{d}	27/70±0/01^{a}	26/53±0/01	26/18±0/01^{c}	23/29±0/03^{f}	18/56±0/01^{g}	16/56±0/01^{h}	12/28±0/01^{i}	11/16±0/01^{j}	10/25±0/02^{k}	9/07±0/00^{l}
∑n-3	21/60±0/02	21/90±0/02^{c}	23/34±0/03^{a}	22/50±0/03	20/92±0/02^{e}	19/24±0/06^{f}	16/22±0/06^{g}	15/29±0/04^{h}	10/82±0/15^{i}	9/95±0/04^{j}	9/35±0/03^{k}	8/48±0/05^{l}
∑n-6	6/50±0/15^{d}	6/45±0/06^{d}	6/69±0/03^{d}	6/62±0/02^{d}	9/07±0/05^{bc}	9/83±1/75^{bc}	8/97±0/04^{c}	10/14±0/86^{b}	12/41±0/18^{a}	12/60±0/49^{a}	12/66±0/21^{a}	12/85±0/45^{a}
n-3/n-6	3/32±0/07^{a}	3/39±0/03^{a}	3/48±0/01^{a}	3/39±0/01^{a}	2/30±0/01^{b}	2/00±0/39^{c}	1/80±0/01^{c}	1/51±0/13^{d}	0/87±0/01^{e}	0/79±0/03^{e}	0/73±0/01^{e}	0/66±0/01^{e}
DHA/EPA	7/88±0/03^{h}	7/73±0/02^{h}	8/99±0/04^{g}	5/52±0/02^{j}	6/43±0/02^{i}	9/26±0/04^{f}	9/36±0/06^{f}	10/98±0/09^{c}	10/10±0/1^{e}	10/47±0/12^{d}	11/68±0/16^{b}	13/76±0/28^{a}
AA/EPA	2/23±0/01^{f}	2/25±0/00^{f}	2/60±0/01^{c}	1/51±0/00^{i}	2/25±0/01^{f}	2/39±0/00^{e}	1/84±0/01^{g}	1/59±0/01^{h}	2/54±0/03^{d}	2/65±0/04^{b}	2/61±0/03^{bc}	3/02±0/07^{a}
∑FAME	74/34±1/1^{c}	79/35±2/1^{b}	81/64±0/14^{b}	79/73±0/4^{b}	80/92±0/24^{b}	81/81±2/96^{b}	80/07±0/74^{b}	80/16±1/8^{b}	89/26±0/46^{a}	89/94±0/80^{a}	88/26±1/89^{a}	88/87±1/6^{a}

Mean values with different superscripts are significantly different from each other. (Duncan significance level is defined as $p>.05$). ∑-Total; ∑SAFA: total saturated fatty acids. ∑MUFAt: total monounsaturated fatty acids. ∑PUFA: total polyunsaturated fatty acids ∑ (n-3) and ∑ (n-6): total (n-3) and (n-6) fatty acids series. ∑FAME: total fatty acids methyl esters.

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Discussion

During the period of investigation, the growth rate in terms of length and weight showed an increasing trend in this study. Larvae were showed an insignificant increase in weight and length from 1 to 3 dph ($p>.05$).The average larval weight and length increased significantly with exogenous feeding (after day 3) reached a maximum at 50 dph, indicating that the growth of larvae was significantly influenced by diet.

Results from the current study indicated that body composition varied during larval development. In kutum larvae, protein content decreased from fertilized egg to mouth opening (3dph) (endogenous feeding) ($p<.05$), that such a trend was not observed in total lipid during endogenous feeding ($p>.05$). This can be explained by the fact that, kutum larvae utilize protein as main energy substrate than lipid. This result is found in Caspian sea carp (*Cyprinus carpio*) (Farhoudiet al., 2011), trout cod (*Maccullochella macquariensis*), and Murray cod (*Maccullochella peelii peelii*) (Gunasekera et al., 1999).

The decrease in protein content from fertilized egg to one-day-old larvae protein may be related to shedding of the chorion and perivitelline fluids (Tulli and Tibaldi, 1997).

From 3 dph until the end of the experiment, protein content increased with larvae development and reached 76.16% observed after the fish started to feed on rotifer and SFK. This suggests that the larvae were not utilizing protein as energy substrates during ontogenesis and they probably utilized other sources (lipid and carbohydrates) for development. Similar findings were also reported for Caspian Sea carp (Farhoudi et al., 2011), sea bass (Southgate et al., 1994), and dentex (*Dentex dentex*) (Tulli and Tibaldi, 1997).

Ash content is higher in larger fish (Niimi, 1972), so ash content increased with increasing body weight (Barziza and Gatlin, 2000).

The quantity of lipid decreased with larval development. It seems kutum larvae utilize lipids predominantly as the main metabolic fuel and energy during ontogeny. Similar findings are reported by Farhoudiet al. (2011). After hatching reduction in total lipid content reflects the utilization of lipid as an energy source by the larvae (Sargent, 1995).
Fish larvae tend to have higher water content than juveniles and adults (Machiels and Henken, 1986). Consequently, a gradual decrease in water content (% dry weight) was observed during the length of the experiment, consistent with Ehrlich (1974).

Larval fatty acid profiles were reflected in dietary profiles (Lund et al., 2007). Furthermore, it is known that dietary fat affects gene expression leading to pronounced changes in metabolism (Jump and Clarke, 1999).

Long-term absence of EFA from the diet leads to deficiency symptoms that, in fish, most often include reduced growth and increased mortality (Glencross, 2009).

According to Abedian-Kenari et al. (2009),the most dominant saturated and monounsaturated fatty acids in Beluga fish were palmitic acid (C16:0), oleic acid (C18:1), and docosahexaenoic acid (C22:6 n-3) was common among polyunsaturated fatty acids which is similar to our results.

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Saturated fatty acids (SAFA), especially 16:0 and 18:0, are generally substrates for energy production (Sargent, 1995). A main component of phospholipids, principally phosphatidylcholine and phosphatidylethanolamine (Tocher et al., 1985b; Mourente and Vazquez, 1996) is 16:0, and hence very important in membrane formation during embryogenesis (Dantagnan et al., 2007).

In the bodies of kutum larvae, palmitic and stearic acid content increased from the stage of fertilized eggs to 20 dph, and there was no utilization of these saturat ed fatty acids (16:0 and 18:0) in this period; furthermore, an important accumulation of these nutrients (especially of palmitic acid) was observed. According to Abi-ayad et al. (2004), in fed and starved pikeperch *(Sander lucioperca)*,there was an increase of saturated fatty during the first 3 days of larvae probably due to bioconversion processes (de novo synthesis by bioconversion processes is an important source of SAFA and MUFA in fishes,(Henderson and Sargent,1985), which was in agreement with our result. In addition, the increase of 16:0 and 18:0 contents in larvae can be explained by feed intake (Abi-ayad et al., 2000).

The decrease in contents of SAFA (16:0 and 18:0) from 20 dph to 50 dph can be explained by the fact that saturated fatty acids probably were utilized as energy substrate. Catabolism of saturated fatty acids can be due to feed intake. This result is similar to Caspian sea carp (Farhoudi et al, 2011), and Atlantic herring brain (Mourenteand Tocher,1992).

Monounsaturated fatty acids (MUFAs) constitute a main energetic source for development and growth in marine and freshwater fish, especially during the larval stage when fish need energy for organogenesis, metamorphosis, fast growth, and basal metabolism (Abi-ayad et al., 2004). Oleic acid (18:1n-9) and Palmitoleic acid 16:1(n-7) were the dominant monounsaturated fatty acid that catabolized and utilized by kutum fish as energy substrates until 10 and 25 dph, respectively. The decrease in content of 16:1(n-7) and 18:1(n-9), reflects the utilization of these fatty acids as an energy source by kutum larvae.

The decrease in monounsaturated fatty acids is found in rainbow trout (Takeuchi and Watanabe, 1982), carp (Csengeri and Dey, 1995), Caspian Sea carp (Farhoudi et al., 2011). There was an important accumulation of 18:1n-9 from 15 to 50 dph and 16:1n-7 from 25 to 50dph. It seems that the major monounsaturated fatty acids (MUFA) were not preferentially used to provide energy (Abi-ayad et al., 2004).

The contents of dietary fatty acids affected PUFA retention in the larval body. PUFA, particularly C20 PUFA, have unique roles in controlling and regulating cellular metabolism and animal physiology.

Fatty acids profiles of Broodstock diets are closely reflected in egg lipids (Watanabe et al., 1978). The fertilized eggs and yolk sac larvae were rich in n-3 and n-6 fatty acids, EPA (20:5n-3) and DHA (22:6n-3), ARA (20:4n-6). An increase of ∑PUFA and ∑HUFA during the first 3 days (from egg to 3 dph) were related with the increase in n-3/n-6, denoting their importance in tissue and in physiological processes.

After yolk sack absorption (transition from endogenous to exogenous) stage PUFAs (except C18:2n-6 and EPA) were reduced in the larvae during ontogeny, suggesting their

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use as an important source of energy for the larvae, which have high energy requirements. The major effect of the diet (rotifer and SFK) on larval fatty acid composition was an increase in the percentage of total n-6 (particularly 18:2n-6). This increase was accompanied by a decrease in the percentage of n-3 fatty acids (especially 20:5n-3 and 22:6n-3).

Therefore, decreased ∑PUFA and ∑HUFA contents after the yolk sac absorption stage were related with the decrease in n-3/n-6. The most important n-6 fatty acids were arachidonic acid (ARA, 20:4n-6) and linoleic acid (LA, 18:2n-6).

Unlike marine fish, almost all freshwater fish have an innate ability to convert 18:2n-6 (linoleic acid, LA) to 20:4n-6 (arachidonic acid, ARA) and 18:3n-3 (linolenic acid, LNA) to 20:5n-3 (eicosapentaenoic acid, EPA), and ultimately to 22:6n-3 (docosahexaenoic acid, DHA). (Sargent et al., 2002), reflecting the abundance of C18 PUFA (particularly) in larval diet. This process could be much more important when fish are fed low levels of n−3 HUFA (Caballero et al., 2002).

With regard to kutum larvae reared in freshwater and low levels of HUFA (DHA,EPA,DHA) in the larval diet (rotifer and SFK) can be explained by the fact that reared kutum larvae in fresh water are well capable of elongating and desaturating 18:2n-6 and 18:3n-3 to their respective C20 and C22 end-product PUFA.

In n-6 PUFA, the increase of ARA content coincided with the decrease of LA during endogenous feeding. This may be indicative of biosynthesis of 20:4(n-6) during endogenous feeding (from egg to 3dph) of kutum larvae. The conservation of ARA in this period might be due to the importance of ARA in brain, retina, and infants' growth (Osman et al., 2001). From 3 dph onwards C18:2n-6 increased to the end of experiment, while ARA was consumed. ARA and EPA are known as the main eicosanoid precursors in cell membranes, including prostaglandins, leukotrienes and hydroxyeicosatetraenoic acids (Plante et al., 2007; Bell and Sargent, 2003) and are involved in numerous physiological processes, including stress reactions, development of immune system and hatching and early larval performance (Sorbera et al., 1998; Fountoulaki et al., 2003). In the case of PUFA and HUFA from the (n-3) series, DHA, EPA and LNA showed a significant decrease during the ontogeny of kutum larvae.

Decreased PUFA contents such as C18:2n-6, 20:5n-3 during yolk sac absorption in this fish, as an important source of energy, might be driven by β-oxidation of fatty acids from triacylglycerides during yolk sac absorption (Rainuzzo, 1993).

According to Awaiss et al. (1996), EPA synthesis for the cellular membrane of organs such as the liver and the swim bladder of fin fishes larvae is necessary, so the increased EPA in swim-up fry during 3–7 dph (yolk sac absorption and larvae feeding on rotifer), can be due to the larvae requirement to major cellular structural component of the swim bladder.

The increase of DHA content in early ontogeny (fertilized egg to 3 dph) may be due to the importance of this fatty acid in vitellogenesis (Sargent, 1995) and larval development (Koven et al., 1993; Rodriguez et al., 1997, 1998).

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DHA and EPA markedly decreased from 3 dph onwards. It seems they were used as source of energy in physiological functions. In white seabream larvae, DHA was not totally conserved and utilized as an energy substrate (Cejas et al., 2004). EPA is utilized as energy source in rainbow trout (Tocher and Sargent, 1990).

DHA is as a structural component in cell membranes especially in the processes of synaptogenesis and retinogenesis (Sargent, 1995; Feller, 2008; Wassell and Stillwell, 2008).

EPA and DHA play a critical role in growth and development of fish larvae especially in marine fish (Watanabe et al., 1983). EPA also plays an important role in the formation of eicosanoids as it competes for the enzyme systems producing eicosanoids derived from ARA.

The increase in DHA/EPA and ARA/EPA ratio in kutum larvae were related with utilization of EPA compared to DHA and ARA.

HUFA content (especially ARA) is low in the kutum larvae diet (rotifer and SFK). For this reason, dietary ratios of ARA/EPA and also of DHA/EPA can be considered important factors for optimizing diet formulation (Rodríguez et al., 2004).

The n-3 and n-6 PUFAs are also considered essential for larval growth and development (Kalyoncuet al., 2009).The decrease in the ratio of n-3/n-6 PUFA decreases the availability of n-3 PUFA or increases the availability of n-6 PUFA, which are beneficial for human health (Bell et al., 2001).The high ratio of n-3/n-6 PUFA in time of endogenous feeding (especially 3 dph) can result from the lowest of content of 18:2n-6 during ontogeny, and the decrease of ratio of n-3/n-6 PUFA in the time of exogenous feeding, because of the low level of this ratio in larval food (rotifer and dry food).

Compared with marine fishes, the freshwater fishes generally have high levels of n-6PUFA (Rahman et al., 1995). With regard to kutum larvae reared in freshwater , it now can be suggested that these larvae require high level of n-6during their rearing in freshwater .

This study has shown that with regard to reared kutum larvae in freshwater and the levels minimum of HUFA in larval diet, it can said that these larvae probably are capable of elongating and desaturating essential fatty acids and utilizing fatty acids as energy substrates during ontogeny. Saturated fatty acids were not utilized but were consumed at the end of the experiment. Instead, monounsaturated fatty acids were utilized as energy source during development; thereafter were conserved at the end of larval growth.

DHA and EPA as the major polyunsaturated fatty acids were reduced during the experiment and used as a fuel source for larval development and structural and functional roles. The proportion of HUFAs (DHA, EPA and especially ARA) was low in the larval diet. This can have an influence on the fatty acids in kutum larvae and may have an adverse effect on larvae health and growth. Therefore, the fatty acid ontogeny in egg and larval development stages can provide a new way for overcoming problems in larvae stage of kutum, and suggests that further studies are needed in order to explain the lipid requirements.

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