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Study of Body Composition, Lipid and Fatty Acid Profile During Larval Development in Caspian Sea Carp (*Cyprinus carpio*)

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ABSTRACT

This study was aimed to gain knowledge on ontogeny of lipid and fatty acid profile in feral carp larvae and to determine nutritional requirements with a view to improving product quality. Real-time fatty acid profile was investigated in 1-33 day old larvae of *Cyprinus carpio*. This study was carried out at the governmental warm water fish aquaculture center of Shahid Rajaee in Sari, Mazandaran, Iran. *Cyprinus carpio* larvae obtained from breeders captured in an estuarine environment were analyzed for lipid/fatty acid composition. Feeding with rotifer started from day 3 to day 7 and then larvae were fed with dry diet from day 8 onwards. Decreased total lipid and fatty acid profile alterations indicated energy-directed preferential metabolism of studied nutrients within certain intervals. During early larval stages, reduced monounsaturated fatty acids showed that these fatty acids were utilized as an energy source ($p<0.05$). Increased contents of docosahexaenoic acid and eicosapentaenoic acid well proved that these polyunsaturated fatty acids were not energy-generating substrates ($p<0.05$) instead, were preserved as structural components for physiological processes. Marine carp larvae apparently metabolized dietary linolenic acid to eicosapentaenoic acid and docosahexaenoic acid and dietary linoleic acid to Arachidonic acid. Arachidonic acid was clearly insignificantly utilized ($p>0.05$). The percentage of body protein andash increased with body weight but percentage of body lipid and moisture decreased.

Key words: *Cyprinus carpio*, lipid, larval development, polyunsaturated fatty acids, high unsaturated fatty acids

INTRODUCTION

Cyprinus carpio, commonly called 'marine carp' in Iran, habituates in Southern Caspian Sea. Feral carp is one of the most economically important species in aquaculture and stock enhancement of Caspian Sea. Eastern south coasts of the Caspian Sea, an estuarine environment, with salinity ranged from 12 to 15 ppt, are the best habitats for feral carp. Feral carp is an anadromous fish. Indeed, brood fishes of these populations migrate to spawn in a freshwater environment after maturation in an estuarine environment. There is lack of about changes of fatty acids during early developmental phases of Caspian Sea feral carp. Studies on fatty acid profiles during early life history stages are very informative as they show the metabolism of energy substrates during

ontogeny; hence, allowing the estimation of nutritional requirements of larvae in exogenous feeding phase (Gunasekera *et al*., 1999).

Lipids constitute the predominant energy source for fish and have key role in the growing larvae (Ebrahimnezhadarabi *et al*., 2011). The mechanisms by which fish allocate energy from lipids for metabolism, development, growth and reproduction are critical for understanding key life-history strategies and transitions (Sutharshing and Sivashanthini, 2011). Growth and energy supplement strictly depend on endogenous yolk reservoir in most bred fish species during embryogenesis and larval development (Abi-Ayad *et al*., 2000; Cejas *et al*., 2004). Larvae metabolized egg lipid reservoir to energy and structural components required for membrane biogenesis as they develop (Tulli and Tibaldi, 1997; Sargent, 1995). Fish tissue is the main source of long chain polyunsaturated fatty acids especially the omega-3 and omega-6 (Tawfik, 2009). Researches indicate that marine fishes generally have higher levels of n-3 PUFA than that of fresh water fishes. Fishes need PUFA to adapt themselves to the lower water temperature (Kalyoncu *et al*., 2009). In general, warm water fishes require polyunsaturated n-6 fatty acids or a mixture of n-3 and n-6 fatty acids, while coldwater species require n-3 forms (Webster and Lim, 2002). Lipids play an important role in fish nutrition as they supply energy and Essential Fatty Acids (EFA) (Parpoura and Alexis, 2001; Skallia and Robin, 2004). On the other hand, researches have shown that finfish requires n-3 Highly Unsaturated Fatty Acid (n-3 HUFA), particularly eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA) for normal growth (Skallia and Robin, 2004) and as structural components during organogenesis such as cell membranes (muscles, brain, retina) and precursors of eicosanoids such as prostaglandins, thromboxans and leucotrienes (Abi-Ayad *et al*., 2004).

All freshwater fish are able to convert 18:2n-6 (linoleic acid, LA) to 20:4n-6 (arachidonic acid, ArA) and 18:3n-3 (α-linolenic acid, LnA) to 20:5n-3 (eicosapentaenoic acid, EPA) and 22:6n-3 (docosahexaenoic acid, DHA) (Sargent *et al*., 2002). LA and LnA are Essential Fatty Acids (EFA) as they can not be synthesized *de novo* by fishes and should be provided in the diet. These EFA can be converted to their respective long-chain Highly Unsaturated Fatty Acids (HUFA) *in vivo* through desaturation and elongation (Turchini *et al*., 2006). Linolenic acid (18:3n-6) is the precursors of prostaglandins (E2 and F2) which possess potent vasodilators, antiflammatory and antiggrgatory properties as well as may be useful to correct defects occur in metabolism of essential fatty acid and imbalance of eicosanoids formation (Abd El-Baky *et al*., 2004).

Ontogenesis of fatty acid profiles have been well documented in several species, such as white seabream (*Diplodus sargus*), (Cejas *et al*., 2004); freshwater salmon (*Salmo salar* L.), (Murzina *et al*., 2009); pikeperch (*Sander lucioperca*) (Abi-Ayad *et al*., 2004); pike (*Esox lucius* L.), (Desvilettes *et al*., 1997).

No research has yet led to typically determine nutritional requirements of marine carp within its on-growing phase aimed at achieving economically optimal growth for carp farms. Thus, this preliminary study investigates ontogeny of lipid and fatty acid profile in feral carp larvae and it will provide useful information for diet formulation with a view to improving product quality.

MATERIALS AND METHODS

Broodstock spawning and larval transfer: The research was carried out in Shahid Rajaie aquaculture complex (Sari, Mazandaran, Iran) from May 2009. Fertilized eggs of wild carp were obtained from wild broodstocks captured from freshwater environment. Broodstock spawned

between April and May. Caught broodstock were transferred to the farm and were kept in spawning tanks (average water depth was 1 m). Water temperature ranged 24.4-25.7°C. Males (0.7-0.8 kg weigh) and females (1-1.2 kg weigh) were stripped; eggs were transferred to vase incubator. Water temperature and pH in vase incubator were stabilized between 19.6-22.8°C and 7-8.5, respectively during the study. Dead eggs were daily removed to prevent fungal contamination. Eggs hatched after 6-7 days and Larvae were transferred to the pools 3 days after hatching. Larvae were reared in freshwater. Water temperature ranged 24.2-25.5°C. Larvae started to feed on rotifer between third (L-3 stage) to seventh days (L-7 stage). Dry food was applied on eighth day onwards. The experiment lasted 33 days.

Sample preparation: Total lengths of 20 larvae were measured in each period. Body weight of larvae (n = 20) were measured after removing water with filter paper. Larvae were collected randomly from different pools at days L1, L3, L7, L11, L15, L19, L 26 and L 33 corresponding to 1, 3, 7, 11, 15, 19, 26 and 33 days post hatch (dph), respectively. The first 24 h after hatching was considered as day 1. One gram of sample for each larval stage were washed to remove excess salts and frozen in liquid nitrogen and were stored at-80°C for proximate composition and fatty acid analysis.

Chemical analysis: Crude protein, ash, moisture and lipid of the larvae, rotifer and dry food were determined according to standard methods (AOAC, 1995); results are shown in Table 1. Crude protein was calculated through converting the nitrogen content (6.25×N). Ash was determined by combustion at 550°C for 12 h. Moisture content was analyzed by oven drying at 105°C for 24 h. Lipid content was determined by chloroform extraction using Foss 2050 (Soxtec™, Sweden).

Lipid and fatty acid analysis: Total lipid was extracted from larvae and diets according to Folch *et al.* (1957) with chloroform/methanol (2:1, v/v). The extracts were mixed and divided into distinct layers by adding water. Crude lipid extracts were evaporated under nitrogen. Fatty Acid Methyl Esters (FAME) were prepared according to Metcalfe and Schmitz (1961). Crude lipid extract was esterified in 5 mL of methanolic NaOH (2%); fatty acid methyl esters were then prepared by transmethylation with BF_3 in methanol. Boron fluoride has been used for the conservation of lipids to Fatty Acid Methyl Esters (FAME). Then, 1 mL normal hexane was added and shaken; finally 1 mL NaCl saturated solution (300 g NaCl in 1 L distilled water) was added. The upper phase (heptane layer containing FAME) was removed after decanting and 1 μL was injected onto chromatograph. Samples of larvae triplicate determinations were carried out on each sample. FAME were obtained on a Varian gas chromatograph (CP3800, Walnut Creek, Netherlands) equipped with an 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 260°C, respectively. Oven temperature was initially 150°C, rising to 190 at 2°C min^{-1} rate; then rising to 245 at 20°C min^{-1} rate. Pure nitrogen (99.9999%) was used as the carrier. Peak areas were characterized using Varian software.

Statistical analysis: Data are expressed as Mean±S.D. Significant differences between groups were determined by a One-way analysis of variance (ANOVA) followed by Duncan test. Statistical analysis was performed using the SPSS for Windows software, version 11.5 (SPSS Inc., Chicago, IL, USA). Mean values were considered significantly different at $p<0.05$.

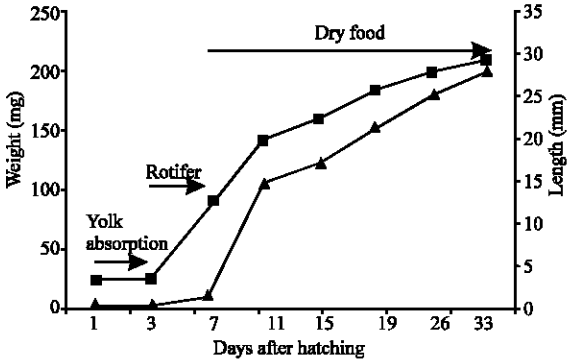

Fig. 1: Growth in weight (▲) and length (■) of *Cyprinus carpio* during larval development. Larval growth in weight and standard length followed an exponential curve (y = 32.063x-46.55, R^2= 0.939 and y = 4.0987x-0.3954, R^2= 0.935, respectively)

Table 1: Proximate composition of marine carp larvae, rotifer and dry food (% D.W)

	L-1	L-3	L-7	L-11	L-15	L-19	L-26	L-33	Rotifer	Dry food
Prptein	68.3±0.02^{bc}	66.9±0.01^{c}	70.5±0.00^{abc}	72.9±0.02^{ab}	72.5±0.03^{ab}	73.2±0.02^{ab}	74.1±0.01^{a}	75.0±0.03^{a}	36.3±0.06	40.0±0.00
Lipid	10.8±0.01^{a}	10.2±0.01^{a}	8.8±0.00^{b}	8.3±0.03^{b}	7.1±0.01^{b}	6.7±0.04^{c}	5.8±0.03^{c}	4.2±0.00d	11.2±0.00	12.0±0.00
Moisture	85.4±0.00	88.4±0.01	84.5±0.01	84.5±0.04	83.1±0.02	82.3±0.01	82.9±0.06	82.0±0.03	94.4±0.01	8.2±0.01
Ash	7.6±0.00^{a}	8.7±0.04^{a}	11.0±0.03^{a}	14.0±0.00^{c}	14.2±0.01^{a}	14.6±0.02^{c}	15.5±0.02b^{c}	16.4±0.02a^{b}	11.8±0.04	10.1±0.03

Proximate compositions are expressed in terms of dry weight (D.W). All data are represented as a percentage (Mean±SD; n=3). Mean values with different superscripts are significantly different from each other. Values without superscripts have no significant differences. (Duncan significance level is defined as p>0.05)

RESULTS

Marine carp larval growth as weight (mg) and standard length (mm) is presented in Fig. 1. Larval growth in weight and standard length followed an exponential curve (y = 32.063x-46.55, R^2= 0.939 and y = 4.0987x-0.3954, R^2= 0.935, respectively). The weight of larvae in the beginning and at the end of experiment was 3.8±0.11 and 199.5±0.02 mg, respectively. The length of larvae in initial and at the end of experiment was 3.36±0.34 and 29.09±0.17 mm, respectively.

The percentage of body protein and ash increased with body weight, but percentage of body lipid and moisture decreased. Protein content decreased from hatching to mouth opening (3 dph) ($p<0.05$); then increased onwards ($p<0.05$). Reported levels of protein ranged from 68 to 75% of dry weight in developing marine carp larvae (Table 1). In this study, body lipid decreased during larval development ($p<0.05$). Present results showed that moisture percentage decreased during development ($p>0.05$). Ash content increased in developing marine carp larvae ($p<0.05$).

The duration of yolk absorption in *Cyprinus carpio* was short, about 4 days. In marine carp larvae, 14:0, 16:0 and 18:0 were the predominant saturated fatty acids (SAFA). The Saturated fatty acids increased from L-1 to L-7 in the larvae bodies and then decreased ($p<0.05$). On day 1 to day 3, 14:0 and 16:0 levels remained stable and then, 14:0 gradually decreased between L-7 and L-33. While 16:0 increased from L-7 to L-15 and then decreased. 18:0 increased between L-1 and L-7 and gradually decreased to the end of the experiment ($p<0.05$). 18:1n-9 and 16:1n-7 were the most important monounsaturated fatty acid. Increases in total monounsaturated fatty acids are in consequence of accumulation of 18:1n-9. Content of 16:1n-7, more or less, remained stable to the end of the experiment. 18:1n-9 decreased from L-1 to L-11 and then increased to the end of the experiment ($p<0.05$). In the case of Σn-6, their levels increased ($p<0.05$), due to high accumulation of 18:2n-6, from L-15 to the end of the experiment. Among n-6 fatty acids, 18:2n-6 increased, while

Table 2: Fatty acid composition of larval stages of *Cyprinus carpio* at different periods of development (% of total fatty acids; Mean±SD; n=3) and fatty acid composition (in % of total fatty acids) of live and dry feeds distributed to carp larvae

Fatty acid	L-1	L-3	L-7	L-11	L-15	L-19	L-26	L-33	Rotifer	Dry food
C14:0	0.62±0.01	0.54±0.01	1.08±0.00	0.80±0.01	0.96±0.02	0.91±0.00	0.67±0.00	0.63±0.2	3.14	1.50
C15:0	0.66±0.00	0.75±0.04	1.17±0.02	1.19±0.04	1.10±0.04	0.85±0.00	0.84±0.01	0.58±0.00	1.22	0.33
C16:0	21.40±0.34	21.15±0.03	22.98±0.19	23.26±0.07	23.54±0.48	22.91±0.02	21.14±0.01	19.01±0.13	28.58	24.38
C17:0	0.83±0.00	0.91±0.01	1.28±0.5	1.39±0.05	1.24±0.03	0.91±0.01	0.82±0.01	0.65±0.02	1.60	0.68
C18:0	8.22±0.02	9.37±0.00	14.62±0.30	13.65±0.11	13.43±0.04	12.53±0.02	12.38±0.02	10.12±0.06	8.11	11.06
C24:0	1.71±0.02	1.94±0.01	0.99±0.00	1.00±0.00	1.22±0.03	0.92±0.01	1.25±0.01	0.47±0.00	-	0.14
∑SAFA	33.46±0.33^{f}	34.68±0.08^{e}	42.16±0.51^{a}	41.32±0.26^{b}	41.52±0.54^{b}	39.05±0.06^{c}	37.12±0.03^{d}	31.49±0.02^{g}	39.62	36.12
C16:1n-7	3.46±0.01	2.68±0.07	2.45±0.01	3.37±0.15	3.90±0.00	3.48±0.02	2.91±0.02	3.49±0.02	3.52	4.66
C18:1n-9	15.83±0.02	13.70±0.14	10.48±0.00	9.64±0.20	12.61±0.07	18.73±0.01	18.74±0.01	24.83±0.09	9.61	12.16
C18:1n-7	4.19±0.00	3.89±0.02	3.33±0.02	4.00±0.00	3.54±0.02	3.26±0.01	2.99±0.09	2.85±0.02	3.10	30.62
C20:1n-9	1.07±0.00	0.82±0.01	0.63±0.00	0.60±0.00	0.65±0.01	0.69±0.01	0.71±0.01	0.85±0.01	4.28	0.64
∑MUFA	24.57±0.03^{d}	21.11±0.23^{e}	16.91±0.03^{g}	17.02±0.36^{g}	20.06±0.1^{f}	25.72±0.41^{b}	25.12±0.44^{c}	32.03±0.17^{a}	20.64	48.10
C18:2n-6	1.92±0.02	1.31±0.03	2.33±0.08	3.86±0.09	5.33±0.02	8.31±0.03	9.19±0.02	13.63±0.06	5.70	15.89
C18:3n-3	0.27±0.01	1.41±0.01	1.65±0.04	1.62±0.07	1.20±0.00	0.98±0.01	0.63±0.01	0.89±0.01	15.92	1.19
C20:4n-6	7.34±0.01	8.63±0.03	5.05±0.03	4.41±0.03	4.67±0.03	5.64±0.10	6.34±0.04	4.74±0.03	1.52	2.75
C20:5n-3	2.42±0.02	2.23±0.03	5.73±0.07	4.88±0.06	3.93±0.00	3.14±0.03	2.13±0.01	1.07±0.00	2.75	0.72
C22:6n-3	16.33±0.17	18.65±0.27	15.71±0.11	18.82±0.01	17.16±0.17	13.55±0.00	13.15±0.03	9.20±0.03	2.73	2.46
∑HUFA	26.09±0.14^{d}	29.52±0.33^{a}	26.5±0.14^{c}	28.12±0.08^{b}	25.77±0.20^{e}	22.34±0.09^{f}	21.63±0.01^{g}	15.02±0.06^{h}	10.53	5.93
∑PUFA	28.30±0.11^{f}	32.24±0.35^{b}	30.49±0.24^{d}	33.60±0.24^{a}	32.30±0.22^{b}	31.64±0.11^{c}	31.47±0.03^{c}	29.56±0.13^{e}	32.15	21.02
∑n-3	19.02±0.13	22.30±0.31	23.10±0.15	25.32±0.12	22.29±0.17	17.68±0.04	15.92±0.02	11.17±0.0	23.80	2.67
∑n-6	9.27±0.02	9.94±0.04	7.39±0.11	8.27±0.12	10.00±0.04	13.96±0.10	15.54±0.06	18.38±0.09	8.35	18.35
∑n-3/n-6	2.05±0.01	2.24±0.02	3.12±0.03	3.05±0.03	2.22±0.00	1.26±0.00	1.02±0.00	0.60±0.00	2.85	0.14
DHA/EPA	6.75±0.14	8.36±0.11	2.74±0.04	3.85±0.05	4.35±0.03	4.30±0.04	6.14±0.03	8.55±0.04	0.40	3.39
AA/EPA	3.03±0.03	3.87±0.58	0.88±0.00	0.90±0.00	1.18±0.00	1.79±0.041	2.96±0.03	4.41±0.02	0.24	1.03
ΣFAME	86.34±0.28^{f}	88.04±0.67^{e}	89.57±0.69^{d}	91.95±0.87^{c}	93.89±0.80^{b}	96.43±0.56^{a}	93.72±0.44^{b}	93.09±0.56^{b}	92.42	105.25

Results of fatty acid composition of larval stages of *Cyprinus carpio* represent Means±S.D. Results of fatty acid composition of live food and dry food are expressed as the Mean of three replicates. (n=3). Mean values with different superscripts are significantly different from each other. (Duncan significance level is defined as p>0.05).Σ-Total; ΣSAFA=total saturated fatty acids. ΣMUFA = total monounsaturated fatty acids. ΣPUFA = total polyunsaturated fatty acids. Σ(n-3) and Σ(n-6) = total (n-3) and (n-6) fatty acids series. ΣFAME = total fatty acids methyl esters

arachidonic acid (20:4n-6, AA) decreased during ontogeny ($p<0.05$), which had minor changes in the percentages and more and less, remained stable (Table 2). The Σn-3 increased from L-1 to L-11 and then decreased ($p<0.05$). The ratios of Σ(n-3)/(n-6) PUFA were decreased from the beginning of exogenous feeding to the end experiment ($p<0.05$). The decrease in Σn-3 gave place to decrease in ratios of Σ(n-3)/(n-6). The HUFA increased during L-1 to L-11 and then decreased at the end of the experiment ($p<0.05$). The polyunsaturated fatty acids (PUFA) were dominated by 22:6n-3, 18:2n-6, 20:5n-3 and 18:3n-3. Among n-3 fatty acids, linolenic acid (LnA, 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) were dominant. The decrease in n-3 fatty acids is a result of the reduction in contents of 20:5n-3 (Eicosapentaenoic, EPA) and 22:6n-3 (docosahexaenoic, DHA). The ratios of DHA/EPA and AA/EPA from the beginning of exogenous feeding to the end experiment increased. The reduction in EPA gave place to increases in DHA/EPA and AA/EPA. AA/EPA ratio decreased from L-1 to L-11 and increased from L-15 to L-33.

DISCUSSION

Larvae were showed an insignificant increase in length from 1 to 3 dph and weight from 1 to 7 dph. A significant increase in length and weight was recorded from 3 to 11 dph and 7 to 11 dph, respectively. From 11 day after hatching to the end of the experiment a substantial increase in length and weight occurred.

Body composition changes have been extensively investigated during developmental stages of fish larvae (Heming and Buddington, 1988). Protein levels have a correlation with body weight (despite the various factors such as strain, feed requirement, water temperature, etc.) (Dumas *et al*., 2007). In marine carp, protein content decreased ($p<0.05$) during endogenous feeding. Such a trend was not observed in total lipid. This indicates that marine carp utilizes protein as main energy source than lipid. The same has been shown for trout cod and Murray cod (Gunasekera *et al*., 1999). In this study, from L-3 to L-33 protein content increased. Protein content enhancement during ontogenesis showed that the larvae preferentially retained protein instead of lipid or carbohydrates (Aragao *et al*., 2004). The same has been shown for sea bass larvae by Southgate *et al*. (1994). In marine carp, ash content increased. It might be due to bone mass increment, associated with somatic growth (Dumas *et al*., 2007) which also has close similarity with Southgate *et al*. (1994). Barziza and Gatlin (2000) found ash content increases more allometrically with fish body size than wet weight. In this study, lipid percentage decreased in developing marine carp larvae. Lipid content is further affected by exogenous factors, specially feeding regime (Bureau *et al*., 2006). After hatching reduction in total lipid content reflects the utilization of lipid as an energy source by the larvae (Sargent, 1995). Cejas *et al*. (2004) reported that decrease in total lipid in white seabream indicates that this nutrient was used as energy source by the larvae. In marine carp percent water reduced during larval development. Similarly to our data, a decrease in percent water during development was found for herring larvae (*Clupea harengus*) (Ehrlich, 1974). The cause of these changes in water content in developing fishes is that dividing cells lead to high amounts of extracellular space and water content, both of which decrease whit growth (Love, 1974).

Tissue fatty acid composition of the feeding larvae reflects diets received, at all stages of the life cycle (Sheikh-Eldin *et al*., 1996; Sargent *et al*., 2002). In general, the fatty acid composition changes during larval development (Roustaian *et al*., 1999). Preferential utilization of some fatty acids during development has been shown in some species (Ronnestad *et al*., 1994; Finn *et al*., 1995; Rainuzzo *et al*., 1997; Abi-ayad *et al*., 2004).

Lipids stored in various sites of the fish, including the liver, muscle and perivisceral and subcutaneous adipose tissues (Hedayatifard and Yousefian, 2010). Among species lipid metabolism is different during larval development of fish. This differentiation is due to level and composition of lipids in the yolk, the time, level utilization and the class of lipids used for either combustion or tissue synthesis and the role of the different fatty acids (Cejas *et al*., 2004). Similarly to our previous result by beluga fish (Abedian-Kenari *et al*., 2009) it has found that the most abundant fatty acid at all ages was palmitic acid (C16: 0) and the other major fatty acid was oleic acid (C18:1n-9) and docosahexanoic acid (C22: 6n-3). Our results showed in marine carp larvae, body saturated fatty acids increased from hatching to L-15 and were not utilized as an energy source during early development. It seems that energy requirements were satisfied by diet in this period or this probably can be due to bioconversion processes (Abi-Ayad *et al*., 2004). The most important source of saturated fatty acid and monounsaturated fatty acids accumulation is *de nevo* synthesis especially in starved larvae (Abi-Ayad *et al*., 2004). Similarly to our data, Abi-Ayad *et al*. (2000) showed in fed and starved Eurasian perch (*Perca fluviatilis*) larvae saturated fatty acids are not utilized as energy source. Saturated fatty acids content in marine carp larvae decreased from L-19 onwards, suggested that SAFAs probably were utilized as an energy source, although this can be explained by feed intake. Mourente and Tocher (1992) reported that in Atlantic herring (*C.harengus*) brain, saturated fatty acids decreased.

General decrease in the MUFAs, from stage L-1 to L-3 reflects the utilization of these fatty acids as an energy source during non-feeding early larval development of marine carp. In general, decrease in monounsaturated fatty acids during larval development could be related with their importance as energy source and or as precursors for others monounsaturated fatty acids biosynthesis (Abi-Ayad *et al*., 2000). Monounsaturated fatty acids such as 16:1n-7 and 18:1n-9 are chain elongated to 18:1n-7, 20:1n-9, 22:1n-9 and others monounsaturated fatty acids (Tocher, 2003). In this study no reduction in 16:1n-7 and 18:1n-9 was observed accompanied with marked increase of 18:1n-7 and 20:1n-9. This indicates MUFAs were used for energy production. Csengeri and Dey (1995) showed decrease in content of monounsaturated fatty acids is related with utilization of monounsaturated fatty acids as an energy source by carp larvae (*Cyprinus carpio*). In marine and freshwater fish monounsaturated fatty acids are a suitable energy source, particularly for organogenesis, metamorphosis, basal metabolism, including respiration, swimming, etc., (Abi-Ayad *et al*., 2004).

The increase in eicosapentaenoic acid (20:5n-3) concentration occurred in swim-up fry, within stages L-3 to L-7 (transition from endogenous to exogenous feeding mode). Probably eicosapentaenoic acid (20:5n-3) would be required by the larvae for a cellular structural component of the swim bladder; hence, its biosynthesis was raised during L-3 to L-7. The inclusion of eicosapentaenoic acid (20:5n-3) into the cellular membrane of organs such as the liver, the swim bladder of fin fishes larvae has been reported (Awaiss *et al*., 1996). The most important n-6 fatty acids were arachidonic acid (ARA, 20: 4n-6) and linolenic acid (LA, 18:2n-6). The freshwater fin fish larvae may have further capability to convert linoleic acid (18:2n-6) and linolenic acid (18:3n-3) to higher polyunsaturated n-6 and n-3 fatty acids (Turchini *et al*., 2006). Low dietary concentration of EPA, ArA and DHA, compared with that of marine carp larvae, suggested the larvae are able to convert linoleic acid (18: 2n-6) to arachidonic acid (20:4n-6) and linolenic acid (18:3n-3) to eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3). Highly Unsaturated Fatty Acids (HUFA) are found in the tissue at unbalanced concentrations in the diet, suggesting that *Macrobrachium rosenbergii* larvae have a tremendous ability to elongate and desaturate fatty acids (Roustaian *et al*., 1999). Marine fish species require substantial levels of dietary eicosapentaenoic

acid (EPA) and docosahexaenoic acid (DHA), which are considered as essential fatty acid, whereas freshwater fish are capable of synthesizing these Highly Unsaturated Fatty Acids (HUFAs) from their C18 precursors, e.g., linoleic acid (18:2n-6) and linolenic acid (18:3n-3) (Sargent *et al*., 1989; Ling *et al*., 2006). The proportion of DHA decreases from L-11 to the end of the experiment, possibly due to utilization as an energy substrate and its physiological roles. Despite the importance of DHA as a structural component in cell membranes to maintain membrane fluidity, ionic transport in gills and kidney, enzymatic activities and in the processes of synaptogenesis in brain and retinogenesis, particularly in rod cell outer segment membranes and synaptosomal membranes (Bell and Dick, 1991; Cejas *et al*., 2004; Mourente, 2003; Masuda, 2009; Tocher *et al*., 1992; Mourente and Tocher, 1992) in marine carp, this fatty acid was not totally preserved. Probably, in the growing larvae, DHA and EPA are consumed as energy substrate (Ronnestad *et al*., 1994; Tocher and Sargent, 1990). Also, Cejas *et al*. (2004) reported that DHA is consumed as energy source by white seabream larvae. On contrary, Mourente and Vaquez (1996) showed in Senegalese sole larvae, DHA was preserved and not being utilized as an energy substrate. DHA increases fluidity of the cell membrane but more than EPA (Hashimoto *et al*., 1999). This fatty acid is retained in starved or low-EFA fed fish, due to the lower cell oxidation rates than other fatty acids (Madsen *et al*., 1999). Previous studies confirmed that both of EPA and DHA play a critical role in growth and development of fish larvae especially in marine fish (Watanabe *et al*., 1983). Watanabe (1993) reported that DHA and EPA increase growth and survival in marine fish larvae. EPA is important for larval growth, DHA requirement being more limiting for growth and survival than other n-3 HUFA (Izquierdo, 1996). Present results showed that marine carp larvae used EPA as energy substrate. Likewise, Tocher and Sergeant (1990) showed that EPA is utilized as energy source in rainbow trout. In this study, during larval development, of the HUFAs, ARA was consumed to a lower degree. Arachidonic acid (20:4n-6, AA) is known as the major precursor to hormone-like compounds (local hormones), eicosanoids (highly active compounds), including prostaglandins, thromboxans and leucotrienes (Van der Kraak and Biddiscombe, 1999). It has been shown that eicosanoids intervene in numerous physiological processes, including stress reactions, inflammatory response and development of immune system (Fountoulaki *et al*., 2003; Sargent *et al*., 1995; Sorbera *et al*., 1998; Tocher, 2003). Prostaglandins play a key role in the control of osmoregulatory processes and stress included hypothalamus-Pituitary-Interrenal (HPI) axis, which facilitates cortisol release which is the main corticosteroid in teleost (Gupta *et al*., 1985). Kanazawa (1997) reported that high DHA levels increased red sea bream larvae tolerance to stressful conditions. Gholami (2010) indicated that feeding the white fish (*Rutilus frisii kutum*) with live food containing high n-3 HUFA content increased larval resistance to pH stress. In this study, the ratio of AA/EPA increased as a result of reduction in EPA. The AA/EPA ratio is important as an indicator of competitive inhibition between EPA and ARA for the cyclooxygenase enzyme system that intervenes in prostaglandin synthesis (Plante *et al*., 2007).

CONCLUSION

In conclusion, these results indicate that *Cyprinus carpio* (marine carp) utilizes fatty acids as energy substrates during early larval development. It is also suggested that marine carp is able to elongate and desaturate essential fatty acids. Saturated fatty acids are not utilized during early larval development, but are consumed at the end of larval growth. Instead, monounsaturated fatty acids are utilized as energy substrates during early larval stages and consequently stored. EPA and DHA were conserved during early larval development, possibly due to their importance in physiological processes and their utilization as energy substrate. However, more efforts are to be made to explain fatty acid metabolism of marine carp during existence in freshwater environment.

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