Fatty acid composition of *Hypophthalmichthys molitrix* during embryogenesis and larval development

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Abstract: Changes in fatty acid composition were investigated during the early development stages (eggs, 12h after fertilization, yolk sac larvae, yolk-sac absorption larvae and larvae feed on egg suspension) of Silver carp (*Hypophthalmichthys molitrix*). Oleic 18:1(n-9) acid was the preferentially monounsaturated fatty acids and such as monounsaturated fatty acid catabolized as energy during embryonic and larval development. During development, the increase in levels of polyunsaturated fatty acids (PUFA), eicosapentaenoic (20:5n-3, EPA) and docosahexaenoic (22:6n-3, DHA) acid was observed that stated their structural components. Our results can give information about the requirements during early and late ontogeny of silver carp larvae at the start of endogenous and exogenous Feeding.

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1. Introduction

In recent years, propagation and raring of fish and particularly native fish has gained special attention in the world. Silver carp, *Hypophthalmichthys molitrix*, is one of the most important native farmed species which lives in the basin of Amur River. This species is an economically farmed fish, so it is necessary to know and understand its physiological characteristics. One of the main objectives and development approaches for fisheries management is the propagation of Iranian native fish. Understanding of the various physiological and biological aspects of such species particularly in the early stages of development is prerequisite for the successful and commercial propagation and raring of native fish that limited information is available about them, because embryonic and larval stages are the most sensitive and vulnerable stages of aquatic organisms (Abi-Ayad *et al*., 2004).

During embryonic and larval development in most fish species, growth and supply of energy depend on yolk reserves transmitted by breeders. In addition, growth and survival of larva depend on access to external food in sufficient quantity and good quality after the yolk sac is absorbed (Springat & Bromage, 1984; Abi-Ayad *et al*., 2004). Given the amount and composition of yolk fats, time and amount of fat metabolism, and fat groups used to burn or generate tissue; the role of fatty acids varies (Abi-Ayad *et al*., 2004). Several studies have been done on the fatty acid changes during embryonic and larval stages of freshwater fish, such as *Perca fluviatilis* (Abi-Ayad *et al*., 2000), *Oncorhynchus mykiss* (Cejas et al., 2006), *Diplodus sargus* (Zengin & Akpinar, 2004), and *Hippocampus guttulatus* (Narciso & Faleiro, 2010).

Given the role of fatty acids in growth, survival, and larval quality, understanding of changes in fatty acid composition during early development of silver carp is necessary to find an appropriate diet with a desirable quality for active feeding stage and also for breeders

2. Material and Methods

This study was carried out at shahid malalki aquaculture complex (Ahvaz, Khozestan) in Iran, Eggs and sperm samples were obtained from two females and five males aged 4 and 3 years, respectively, on May 2000. Eggs were transferred into 200 lit zug jars. The water temperature was 25 ± 1.2°C and was maintained on the photoperiod of LD 16:8. Before the eggs were placed in the incubators, the samples for biochemical analyses were taken. Samples of 2 g of fertilized eggs were taken on 12hour after fertilization. Samples of 2 g of hatched larvae (yolk-sac stage) were taken on the 12hour of incubation. Samples of2 g of larvae (absorbed yolk-sac; start feeding stage) was taken on the 12hour of incubation. Finally, sample of 5 g of larvae were fed with eggs suspension in the jar were taken on the 72 hour of incubation. Samples (2 g) were collected and stored at- 30 C for total lipid and fatty acid analysis (Abi-Ayad et al., 2000). Total lipids were extracted by the method of Folch et al. (1957) and measured gravimetrically. The formation of FAME was carried out according to the procedure described by Desvilettes et al. (1994). The sample was saponified with methanol sodium hydroxide and the fatty acids were esterified with methanol sulfuric acid. FAME were analyzed with a 6890 N GC–FID (Agilent Technologies, Wilmington, DE, USA) fitted with a J&W DB-Wax capillary column (30m, 0.25 mm i.e., 0.25 mm film thickness), a split–split less injector with Agilent tapered liner (4mm id) and flame ionization

detector. The initial column temperature was maintained at 100 °C for 1 min and then raised at 25° C/min to 190 °C and held for 10 min and then raised to 220 °C and held for 5 min. Nitrogen was used as carrier and makeup gas, at flow rates of 1.0 and 45 mL/min, respectively. The injector and detector temperature were held at 250 and 260 °C, respectively. ChemStation software was used for online data collection and processing. Individual FAME was identified by comparison with known standards (Sigma, Chemical Co. St. Louis).

1.2. The statistical analysis of data

The mean and standard deviation were calculated for all parameters. Results were subjected to one-way analysis of variance followed by Duncan's entire comparison test (P<0.05), using a software SPSS 16.0.For all statistical to determine significant differences among treatment means. All data are presented as the mean ±SD.

3. Results

Among the saturated fatty acids, palmitic acid (C16:00) has the highest value at this stage of life in silver carp. This fatty acid dramatically reduces in the early stages of life and falls from 18.26% of all fatty acids to 3% (p<0.05). But it significantly increases in the larval stage (p<0.05). Stearic acid (C18:00) has a dramatic increase at the stage 12 hours after fertilization (p<0.05), decreases in the next stage (p<0.05), and dramatically increases when the larva with yolk sac appears (p<0.05). Total saturated fatty acids (SFA) change has an alternating trend- from beginning to the end it may be increasing or decreasing. Among the monounsaturated fatty acids, oleic acid (C18:1n-9) has the highest value in silver carp. Changes of this fatty acid in embryonic stages is s not significant (p<0.05). Oleic acid significantly increases when the larva with yolk sac appears (p<0.05), then dramatically increases after the yolk sac is absorbed (p<0.05), and decreases when larvae are fed with egg suspension (p<0.05). Total monounsaturated fatty acids (MUFA) undergo insignificant changes during the larval stages (p<0.05), and then significantly decreases after the larval stages to the end. In other words, total MUFA decreases at the larval stages (p<0.05). Among the polyunsaturated fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are of the most important essential fatty acids. These two fatty acids have a constant and upward trend. The ratio of these two fatty acids (DHA-EPA) has a descending trend and significantly decreases from the beginning to the end (p<0.05).

Table1: Fatty acid changes in larval and emberyogenesis stage of (*Hypophthalmichthys molitrix)* (ngd individual_1)

Egg feed larva	Yolk sac larvae absorbed	Larvae with yolk sac	Egg 12hour after fertilization	Fertilized egg	Fatty acids
9/51^{b}±0/84	4/42^{a}±1/98	15/08^{c}±0/61	16/99^{c}±0/9	8/66^{b}±1/04	C14:0
0/47^{a}±0/02	0/61^{ab}±0/08	0/59^{ab}±0/26	0/75^{b}±0/00	0/46^{a}±0/05	C14:1n-5
22/05^{c}±0/042	24/44^{d}±0/97	21/26^{c}±0/14	3/00^{a}±0/18	18/62^{b}±2/08	C16:0
2/71^{a}±2/08	4/63^{b}±0/15	5/71^{b}±0/93	5/81^{b}±1/01	4/62^{b}±0/9	C16:1n-7
12l03^{d}±0/3	10/99^{c}±0/16	7/8^{a}±0/91	12/44^{d}±0/19	8/62^{b}±0/06	C18:0
14/81^{a}±0/33	18/92^{b}±0/78	16/14^{a}±0/16	22/5^{c}±0/14	23/71^{c}±3/05	C18:1n-9
2/84^{a}±0/12	3/65^{ab}±0/28	4/18^{bc}±0/79	4/6^{c}±0/15	4/77^{c}±0/61	C18:2n-6
0/36^{a}±0/01	0/75^{c}±0/1	0/34^{a}±0/12	0/55^{b}±0/01	0/52^{b}±0/02	C18:3n-3
0/03^{a}±0/01	0/02^{a}±0/00	0/16^{b}±0/08	0/15^{b}±0/01	0/06^{a}±/0/02	C20:0
0/63^{a}±0/21	0/77^{a}±0/14	0/69^{a}±0/1	0/97^{a}±0/17	0/77^{a}±0/35	C18:3n-6
0/71^{ab}±0/05	0/45^{a}±0/38	0/45^{a}±0/31	1/08^{b}±0/06	0/79^{ab}±0/36	C18:4n-3
0/01^{a}±0/00	0/02^{ab}±0/01	0/05^{bc}±0/03	0/06^{c}±0/01	0/01^{a}±0/00	C22:0
0/7^{ab}±0/05	0/76^{b}±0/1	0/45^{a}±0/22	0/6^{ab}±0/2	0/66^{ab}±0/09	C20:3n-6
0/25^{a}±0/35	0/68^{ab}±0/55	0/87^{ab}±0/21	1/08^{b}±0/06	0/82^{ab}±0/32	C20:3n-3
0/01^{a}±0/00	0/02^{a}±0/00	0/07^{b}±0/01	0/11^{c}±0/00	0/05^{b}±0/02	C20:4n-6
5/34^{c}±0/09	4/03^{b}±0/31	3/17^{ab}±0/81	3/28^{ab}±0/13	2/78^{a}±0/7	C20:5n-3
3/45^{ab}±0/11	2/90^{a}±0/61	2/80^{a}±0/46	4/11^{b}±0/09	3/90^{b}±0/37	C22:5n-6
0/40^{ab}±0/04	0/23^{a}±0/00	0/29^{a}±0/08	0/49^{b}±0/20	0/26^{a}±0/05	C22:5n-3
21/38^{c}±0/58	18/51^{b}±1/01	16/07^{a}±0/15	17/67^{b}±1/87	17/96^{ab}±0/33	C22:6n-3
0/19^{a}±0/06	0/14^{a}±0/01	0/33^{a}±0/21	0/35^{a}±0/2	0/22^{a}±0/1	C24:0
8/17^{d}±0/26	6/29^{b}±0/13	6/99^{c}±0/22	4/00^{a}±0/18	6/31^{b}±0/26	Total lipids
43/84^{d}±1/15	40/05^{c}±2/76	44/7^{d}±1/59	33/01^{a}±0/72	36/21^{b}±1/19	SFA
18/00^{a}±0/35	24/18^{b}±0/61	22/44^{b}±0/85	29/04^{c}±0/94	28/8^{c}±2/11	MUFA
36/10^{a}±0/35	32/79^{b}±0/35	22/43^{b}±0/85	34/58^{c}±0/94	33/32^{c}±2/11	PUFA
28/46^{c}±0/54	24/67^{b}±0/88	21/22^{a}±0/72	24/17^{b}±1/60	23/15^{ab}±1/55	n-3
7/64^{b}±0/48	8/12^{b}±0/70	8/21^{b}±0/43	10/41^{a}±0/39	10/41^{a}±0/73	n-6
3/72/^{c}±0/22	3/04^{b}±0/17	2/58^{a}±0/05	2/32±^{a}0/08	2/29^{a}±0/32	(n-3/n-6)
4/00^{b}±0/06	4/62^{ab}±0/60	5/28^{ab}±1/25	5/40^{ab}±0/76	6/76^{a}±1/90	DHA/EPA
97/30^{ab}±1/05	97/04/^{ab}±1/00	96/59^{a}±0/52	96/68^{a}±0/36	98/35^{b}±0/35	FAME

4. Discussions

Palmitic acid has the highest amount among the saturated fatty acids in all larval and embryonic stages. Abundance of this fatty acid common carp of the Caspian Sea (Syprinus carpio) (Farhoudi & Abedinkenari), cod (Macullochellam acquarensis) (Gunasekera et al., 2002), and pikeperch (Sander lucioperca) (Abi-Ayad et al., 2004) has been reported. Abundance of these fatty acids can be attributed to the content of phospholipids and mainly phosphatidylcholine and phosphatidylethanol (Tocher et al., 1985; Murente and Vasquez, 1996). Hence, this fatty acid plays a role in membrane formation during embryonic stage (Dantaganon et al., 2006). Total saturated fatty acids have an alternating trend from the beginning to the end, as sometimes increases and sometimes decreases. Significant reduction of these fatty acids in the stage of developed larvae (Larvae that have absorbed their yolk sac and those that are feeding with egg yolk), compared with larvae with yolk sac, is probably due to the consumption of these fatty acids as an energy source. Total monounsaturated fatty acids (MUFA) have shown insignificant changes in the early stages of life ($p>0.05$). Then, they significantly decrease after entering the larval stages ($p>0.05$) and this reduction continues until the stage of developed larva fed with egg yolk. This can be attributed to the decreasing trend of palmitoleic acid and oleic acid that are of the most important monounsaturated fatty acids. This decline is probably due to the energizing applications of these fatty acids in the early development stages in silver carp. In freshwater and marine fish, these two fatty acids are considered as a structural component of the cell membrane to control membrane fluidity, ion transport in gill and kidney, enzymatic activities, synapse generation processes in brain, and also in the structure of retina (Ishizaki et al., 2007; Mourente & Ocher, 1995). Generally, DHA and EPA are used for physiological functions (D costanzo et al., 1983). This constant and increasing trend of these fatty acids has been reported in Carasius auaratus and Oncorhynchus mykiss (Haliloglu & Zengin, 2006). Arachidonic acid is a ployunsaturated fatty acids (PUFA) which has a significant increasing trend in the embryonic stage ($p>0.05$). Entering the stage of larva with yolk sac, it stars a significant decreasing trend that continues until the end of the experiment. This fatty acid is also of essential fatty acids for this fish. This decreasing trend in larval stages indicates that this fatty acid is consumed to generate energy in this stage of silver carp life. This is inconsistent with the findings reported from cod (*Macullochellam acquarensis*) (Gunasekera *et al* ., 2002), and white fish (*Rutilus frisii kutum*) but consistent with the results from pikeperch (*Sander lucioperca*) (Abi-Ayad *et al*., 2004) and perch (*Perca fluvitilis*) (Abi-Ayad *et al*., 2000). Constant and increasing trend of this fatty acid has been mentioned in some studies which is due to the role of this fatty acid in production of eicosanoids (Vander K raak and BiddiScobe, 1999). Moreover, the significant decrease in arachidonic acid in this fish can be attributed to the physiological functions of this fatty acid (Sargent *et al*., 1999). This fatty acid seems to be used for energy generation in the embryonic stage.

Linoleic acid (18:2n-6) had a decreasing trend during the 5 stages of trial. These two fatty acids, as a precursor, can be changed into EPA (20:5n-3) and arachidonic acid (20:4n-6) through metabolism. These results were also observed in the embryonic stages of *Diplodus sargus* (Cejas *et al*., 2002).

It can be found from the results that DHA and EPA are of essential fatty acids which have a constant and increasing trend and also have a structural function. In addition, arachidonic acid and monounsaturated fatty acids (MUFA) and have a role in energy generation. Function of producing eicosanoids was also proved for Linoleic acid and linoleic acid.

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