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Ontogeny Changes in Fatty Acid and Amino Acid Profiles in Yellowfin Seabream (*Acanthopagrus latus*) Eggs and Larvae

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Abstract: This study was aimed to gain knowledge on ontogeny of fatty acid and amino acid profiles in *Acanthopagrus latus* larvae. The samples were collected randomly in triplicate at 5 stages. The samples for biochemical analyses were taken for the first stage (stage 1) before placing the eggs in the hatching buckets. After hatching, sampling was made every 24 hours as follows: at 0 time (stage 2), 24th (stage 3), 48th (stage 4) and 72th (stage 5) hours. Fatty acid results showed that the eggs and yolk sac larvae contain more monounsaturated and polyunsaturated fatty acids than saturated fatty acids. Among polyunsaturated fatty acids, linoleic acid (C18:2n-6), linolenic acid (C18:3n-3) and arachidonic acid (C20:4n-6) increased during development, but docosahexaenoic acid (C22: 6n-3) and eicosapentaenoic acid (C20:5n-3) decreased from stage 2 to stage 5. The changes observed in amino acid contents of the fertilized egg and yolk sac larvae were significantly different (P<0.05). The amounts of essential amino acids and nonessential amino acids decreased during development with the exception of phenylalanine, methionine, glutamic acid and serine (P<0.05). In conclusion, our results can give essential information for a better understanding of nutritional requirements at the start of exogenous feeding.

Key words: *Acanthopagrus latus* % Yellowfin seabream % Yolk-sac larvae

INTRODUCTION

Yellowfin seabream, *Acanthopagrus latus*, is a sparid with high potential for aquaculture in the Indo-Pacific region due to its high economic value [1] and easy adaptation to captivity [2]. Studies on the biochemical changes during early life history stages are indicative of the use of energy substrates during ontogeny, which allow the estimation of nutritional requirements for embryos and exogenous feeding larvae and thus can be used in improving broodstock condition [3].

It is well known that yolk sac stage represents an important developmental period for all fish larvae. At this stage, the significant changes in the larval body take place before exogenous feeding. Also, the energy in the yolk is used for growth, development and activity [4]. Both protein and lipid are major energy source during the embryonic and yolk sac stages of fish larvae [5-7].

Amino acids are critical biochemical compounds for living organisms and an important source of chemical energy for metabolic reactions. During early developmental stages, amino acids are important substrates for the synthesis a large number of bioactive molecules and proteins. The indispensable amino acid (IAA) profile seems to be as competent indicator for estimation of amino acids requirements in fish larvae [8].

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

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exogenous feeding phase [9]. The biochemical composition of yolk is made up of proteins, free amino acids, lipids and carbohydrates. Among of nutrients, lipids and fatty acids constitute the predominant energy source for fish and have key role in the growing larvae [10] and polyunsaturated fatty acids are considered as a structural components during organogenesis (muscles, brain, retina, etc) and precursors of physiologically active molecules such as prostaglandins and other eicosanoids [11].

An estimation of the utilization of endogenous nutrients from the yolk-sac that occurs during embryonic and early larval development can be a useful approach to the study of the nutritional requirements of fish larvae [12].

There is lack of about biochemical changes during early developmental phases of *Acanthopagrus latus*. The present study was instigated to determine the biochemical changes during early ontogeny in *Acanthopagrus latus*. Knowledge of the biochemical compositions of fertilized eggs and yolk sac larvae is important in understanding factors which influence larval survival such as the rate of utilization of endogenous energy reserves.

MATERIALS AND METHODS

Cultural Facilities and Broodstock Spawning: The research was carried out in South Iranian Aquaculture Research Center, Ahwaz, Iran. Nearly 7 months prior to the natural spawning season (February-early March) wild broodstock yellowfin seabream were caught with hooks from the northwest Persian Gulf from August to September 2011 and transferred in a fiberglass tank equipped with aeration to the center. The fishes maintained in concrete tanks (75 m^3) prior to the commencement of the trail.

The experiment was conducted in cylindrical polyethylene tanks (1139-L tank). The water supply to the system consisted of pumped seawater filtered through a 60 ìm filter. In addition, about 25 % of culture water was replaced daily with new filtered seawater. The tanks were equipped with a filter connected to an air stone for aeration and fluorescent artificial lights for adjusting ambient photoperiod of 11 h light: 13 h dark in the pre-spawning period and 8 h light: 16 h dark in spawning tanks [1].

Water temperature, salinity, dissolved oxygen and pH was recorded daily. Throughout the experimental period, the temperature was between 15 and 23°C. Salinity ranged between 40 % and 45 % (42±0.1). Average values for dissolved oxygen and pH were 7.5 mg/l and 7.9, respectively. Spawning started in late February (water temperature > 20°C). Spawning took place at night (between 02:00 and 05:00) and the eggs were collected the next morning from the tanks [1, 13]. Dead eggs were removed to prevent fungal contamination.

Sample Preparation: The samples were collected randomly in triplicate at 5 stages. Before the eggs were placed in the hatching buckets, the samples for biochemical analyses were taken (stage 1). After hatching, sampling was made at 0 time (stage 2, newly hatched larvae), 24^{th} (stage 3), 48^{th} (stage 4) and 72^{th} (stage 5, yolk-sac-resorbed larvae) hours. Egg and larvae samples were washed with distilled water and after removing freshwater with a filter paper, stored in liquid nitrogen and frozen at -80°C until further analysis.

Lipid and Fatty Acid Analysis: Total lipid was extracted from the fertilized egg and larvae according to Folch et al. [14] 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 [15]. Fatty acid methyl esters (FAME) were separated and quantified by Varian gas chromatograph (CP3800, Walnut Creek, Netherlands) equipped with a BPX 70 SGE capillary column (60m×0/25mm ID×0/25 Fm film thickness) and a FID detector. Injection temperature and detection temperature were 230°C and 260°C, respectively. Oven temperature was initially 150°C, rising to 190°C at 2°C minG¹ rate; then rising to 245°C at 20°C minG¹ rate. Pure Nitrogen (99.9999 %) was used as the carrier. Methyl esters were identified with the aid of authentic standard mixtures (Sigma Aldrich). Peak areas were characterized using Varian software.

Amino Acids Analysis: For determination of amino acid contents in the fertilized egg and larvae, Freeze-dried samples (Operon-Model: OPRFDU 7012, Korea) were hydrolysed in 6N HCl for 24 h at 110°C in glass vials replaced with nitrogen. Derivatization of amino acids in the samples, were used by *o*-phthaldialdehyde (OPA) as a pre-column derivatization reagent, followed by high pressure liquid chromatography (HPLC) with fluorescence detection and spherical type column, all parts were from Knauer Corporation, Berlin, Germany, using the method of Lindroth and Mopper [16] as modified by Flynn [17]. The fluorescence excitation and emission wavelengths were

set 330 nm and 450 nm, respectively. In this study tryptophan was not estimated, because this amino acid is destroyed by acid hydrolysis. Amino acids were identified by comparison of retention times against appropriate standards (Sigma, USA) and the concentration was calculated from the peak area of each standard. All determinations were carried out in triplicate.

Statistical Analysis: Data are expressed as mean ± SD. All data were tested for homogeneity of variances and normality of the data. All percentage data were arcsin ($x^{1/2}$) transformed. 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 $P<0.05$. All data is given as mean values with standard deviations (S.D.).

RESULTS

Table 1 shows the results of fatty acid (FA) composition of the fertilized egg and larvae of *Acanthopagrus latus* at different periods of development. Significant difference was observed in fatty acid contents of the fertilized egg and yolk-sac larvae ($P<0.05$), even if they were not very marked. In the present study, unsaturated fatty acid content was higher than saturated fatty acid content. Fatty acid results showed that the eggs and yolk-sac larvae contain more monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs) than saturated fatty acids (SFAs). Total SFAs reduced during development from 28.03 % at the stage 1 to 22.11 % by late stage 5 ($P<0.05$). MUFAs exhibited an increasing pattern from 31.92 % in the fertilized eggs to 36.18 % in yolk-sac-resorbed larvae ($P<0.05$). Significant difference was observed in level of PUFAs from stage 1 (30.91 %) to stage 5 (31.38 %). Among PUFAs, linoleic acid (C18:2n-6), linolenic acid (C18:3n-3) and arachidonic acid (ARA, C20: 4n-6) showed an increasing pattern during development ($P<0.05$), but docosahexaenoic acid (DHA, C22: 6n-3) and eicosapentaenoic acid (EPA, C20: 5n-3) decreased from stage 2 to stage 5 ($P<0.05$). G n-3, G n-6, G n-3 / G n-6 ratio and DHA / EPA ratio showed significant difference during development ($P<0.05$).

The amino acid (AA) profiles of the fertilized egg and larvae of *Acanthopagrus latus* are presented in Table 2. The changes observed in amino acid contents of the

Table 1: Fatty acid content of fertilized eggs and yolk sac larvae of *Acanthopagrus latus* during development (% of total fatty acids; Mean ± SD; n=3)

Fatty acid	Eggs (stage 1)	0 time (stage 2)	24th (stage 3)	48th (stage 4)	72th (stage 5)
C14:0	3.26±0.68^{c}	3.51±0.84^{a}	3.34±0.72^{b}	3.17±0.60^{d}	2.99±0.570^{e}
C15:0	0.38±0.13^{c}	0.5±0.22^{a}	0.49±0.21^{a}	0.42±0.19^{b}	0.33±0.11^{d}
C16:0	19.11±4.86^{a}	18.88±3.91^{b}	17.94±3.85^{c}	17.56±3.30^{d}	16.02±2.23^{e}
C17:0	0.79±0.14^{c}	0.86±0.80^{ab}	0.88±0.92^{a}	0.82±0.53^{bc}	0.69±0.11^{d}
C18:0	4.12±1.93^{a}	3.18±1.29^{b}	2.87±1.48^{c}	2.55±1.33^{d}	1.78±1.18^{e}
C 24:0	0.34±0.12^{a}	0.34±0.12^{a}	0.30±0.09^{ab}	0.29±0.09^{b}	0.27±0.12^{b}
SFA	28.03±2.91^{a}	27.28±1.52^{b}	25.82±3.49^{c}	24.82±2.22^{d}	22.11±1.35^{e}
C16:1n-7	6.65±2.31^{e}	6.97±3.19^{d}	7.29±4.02^{c}	7.95±4.14^{b}	8.17±1.97^{a}
C18:1n-9	20.31±1.11^{d}	20.20±0.99^{e}	21.18±1.22^{c}	22.93±3.62^{b}	24.23±3.51^{a}
C18:1n-7	4.03±0.97^{a}	3.96±1.03^{b}	3.35±0.83^{d}	3.55±1.04^{c}	2.99±0.91^{e}
C20:1n-9	0.91±0.19^{a}	0.82±0.22^{b}	0.69±0.17^{d}	0.76±0.31^{c}	0.78±0.11^{bc}
MUFA	31.92±5.12^{d}	31.96±4.17^{d}	32.52±5.05^{c}	35.19±4.91^{b}	36.18±6.34^{a}
C18:2n-6	8.96±1.09^{c}	7.76±3.31^{d}	8.98±2.57^{c}	9.14±4.37^{b}	9.57±4.09^{a}
C18:3n-3	2.56±1.01^{c}	2.03±2.05^{e}	2.34±1.14^{d}	2.75±0.97^{b}	2.94±0.88^{a}
C20:4n-6	0.87±0.15^{e}	1.31±0.10^{d}	1.64±0.09^{c}	1.73±0.09^{b}	1.76±0.11^{a}
C20:5n-3	2.44±0.82^{e}	3.92±1.05^{a}	3.76±1.03^{b}	3.56±0.79^{c}	3.42±0.55^{d}
C22:6n-3	16.07±5.43^{b}	18.53±6.23^{a}	15.45±4.14^{c}	14.74±4.01^{d}	14.94±3.29^{e}
PUFA	30.91±4.56^{e}	33.56±2.69^{a}	32.18±6.71^{b}	31.93±1.20^{c}	31.38±3.22^{d}
n-3	21.08±2.09^{c}	24.49±0.99^{a}	21.56±3.19^{b}	21.06±3.77^{c}	20.09±2.49^{d}
n-6	9.83±3.79^{d}	9.08±4.09^{e}	10.62±2.12^{c}	10.87±2.16^{b}	11.37±2.54^{a}
n-3/n-6	2.13±0.97^{b}	2.69±1.02^{a}	2.02±0.95^{c}	1.93±0.74^{d}	1.75±0.52^{e}
DHA/EPA	6.58±2.76^{a}	4.71±1.98^{b}	4.10±1.43^{d}	4.13±2.02^{c}	3.98±0.99^{e}

Results of fatty acid content of fertilized eggs and yolk sac larvae of *Acanthopagrus latus* are represented as means ± SD (n=3)

Mean values with different superscripts in each row are significantly different ($P<0.05$)

G- Total; *G SAFA* -total saturated fatty acids. *G MUFA*- total monounsaturated fatty acids. *G PUFA*- total polyunsaturated fatty acids. G (n-3) and G (n-6) - total (n-3) and (n-6) fatty acids series

Table 2: Amino acid content of fertilized eggs and yolk sac larvae of *Acanthopagrus latus* during development (g/100g protein; Mean ±SD; n=3)

Amino acid	Eggs (stage 1)	0 time (stage 2)	24th (stage 3)	48th (stage 4)	72th (stage 5)
Arg	7.23±3.77^{d}	7.42±4.95^{c}	7.41±3.29^{c}	7.62±3.24^{b}	7.81±2.58^{a}
His	9.32±3.16^{b}	9.20±1.98^{c}	9.41±2.01^{a}	9.41±2.65^{a}	9.00±1.17^{d}
Ile	6.62±2.24^{a}	6.52±3.17^{b}	6.62±3.76^{a}	6.41±1.44^{c}	6.20±2.92^{d}
Leu	5.70±1.87^{b}	5.81±2.56^{a}	5.61±2.11^{c}	5.71±2.33^{b}	5.52±2.07^{d}
Lys	9.82±1.90^{a}	9.61±0.99^{b}	9.32±0.84^{d}	9.52±1.07^{c}	9.62±1.09^{b}
Phe	10.07±4.54^{b}	10.04±3.22^{b}	9.82±2.69^{d}	9.92±1.21^{c}	10.15±4.56^{a}
Thr	4.92±1.01^{a}	4.90±0.97^{a}	4.82±0.72^{b}	4.53±1.08^{c}	4.11±0.82^{d}
Val	8.53±2.76^{b}	8.32±3.05^{d}	8.43±2.76^{c}	8.61±3.09^{a}	8.12±3.89^{e}
Met	3.72±0.92^{b}	3.42±1.02^{d}	3.54±0.88^{c}	3.21±1.22^{e}	3.80±1.10^{a}
TEAA	65.83±6.98^{a}	65.12±7.46^{b}	64.85±8.90^{c}	64.82±4.78^{d}	64.09±8.54^{e}
Glu	11.25±5.39^{d}	13.12±4.16^{b}	12.32±3.29^{c}	12.54±4.72^{c}	13.43±3.36^{a}
Ser	3.52±1.67^{c}	3.67±0.56^{b}	3.52±0.89^{c}	3.45±0.50^{d}	3.80±0.81^{a}
Asp	8.74±2.54^{a}	8.41±4.13^{cd}	8.43±2.13^{c}	8.36±1.93^{d}	8.53±3.81^{b}
Gly	7.63±3.07^{b}	7.83±1.09^{a}	7.53±2.32^{c}	7.23±2.44^{d}	7.47±3.09^{c}
Ala	2.13±0.88^{a}	0.98±0.71^{b}	2.11±0.92^{a}	2.02±1.07^{b}	2.03±0.99^{b}
Tyr	0.85±0.19^{a}	0.85±0.51^{a}	0.64±0.19^{b}	0.43±0.27^{d}	0.52±0.23^{c}
TNEAA	34.12±7.62^{d}	34.86±6.83^{b}	34.46±4.22^{c}	33.74±5.87^{e}	35.26±6.39^{a}
TAA	99.95±8.56^{a}	99.45±8.34^{b}	99.25±7.22^{c}	98.54±6.94^{d}	97.85±7.14^{e}

Results of amino acid content of fertilized eggs and yolk sac larvae of *Acanthopagrus latus* are represented as means ± S.D (n=3)

Mean values with different superscripts in each row are significantly different (P <0.05)

TEAA: Total Essential Amino Acids, TNEAA: Total Non Essential Amino Acids, TAA: Total Amino Acids

fertilized egg and yolk-sac larvae were significantly different (P<0.05). The amounts of total essential amino acids (TEAA) exhibited a decreasing pattern from 65.83 % at the stage 1 to 64.09 % by late stage 5. However, an increase trend was observed for arginine, phenylalanine and methionine. Similarly, the amount of total non essential amino acids (TNEAA) increased from 34.12 % to 35.26 % during development with the exception of aspartic acid, glycine alanine and tyrosine (P<0.05). The predominant AAs observed in the fertilized egg and yolk-sac larvae of *Acanthopagrus latus* were arginine, phenylalanine, histidine, lysine, glutamic acid and aspartic acid, whereas alanine and tyrosine presented the lowest relative AA levels. The most depleted essential amino acids (EAAs) until stage 5 were the threonine, Isoleucine and valine (P<0.05). Phenylalanine and methionine remained relatively constant through the experimental period (P>0.05). Among non essential amino acids (NEAAs), tyrosine and aspartic acid were determined as the most depleted NEAAs (P<0.05).

DISCUSSION

Preferential utilization of some fatty acids during development has been shown in some species [5, 18-21]. Fatty acid profile of *Acanthopagrus latus* changed significantly, which had only minor changes in the percentages of fatty acids during larval endogenous feeding. Significant difference in fatty acid contents of the fertilized egg and yolk-sac larvae has been reported in *Diplodus sargus* [12], *Maccullochella macquariensis* and *Maccullochella. peelii peelii* [9]. According to the results of the present study, MUFAs and PUFAs ratios in fertilized eggs of *Acanthopagrus latus* were higher than SFAs ratio. It is possible that like in Pseudopleuronectes americanus [22] and Perca fluviatilis [11] embryos of *Acanthopagrus latus* spared PUFA for new cell constitution and organogenesis rather than for energy production.

In present study, MUFAs were not preferentially used to provide energy. Nevertheless, in both marine and freshwater species, these nutrients constitute a good energetic substrate for development, especially during organogenesis, metamorphosis, fast growth and basal metabolism (respiration, swimming, excretion, etc.) [21]. Moreover, monounsaturated fatty acids can be involved in brain development as described previously in *Scophthalmus maximus* by Mourente *et al*. [23].

Saturated fatty acids content decreased in *Acanthopagrus latus* larvae from stage 1 onwards, suggested that SFAs probably were utilized as an energy source. These results are consistent with the previous findings in *Solea senegalensis* by Vázquez *et al*. [24].

Among essential fatty acids, during larval development, only arachidonic acid (ArA: C20: 4n-6) was conserved. Arachidonic acid is known as the major

eicosanoids precursor in fish cells, including prostaglandins, thromboxans and leucotrienes [12]. It has been shown that eicosanoids intervene in numerous physiological processes, including stress reactions, inflammatory response and development of immune system [25]. 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 fishes [26]. The proportions of DHA and EPA decrease throughout the experimental period, possibly due to utilization as an energy substrate and their physiological roles. Despite the importance of DHA as a structural component in cell membranes, especially in the processes of synaptogenesis and retinogenesis during early development of fish [12, 27], in *Acanthopagrus latus*, DHA was not totally preserved. Probably, in the growing larvae, DHA and EPA are consumed as energy substrate [28]. On contrary, Mourente and Vázquez [23] showed in Senegalese sole larvae, DHA was preserved and not being utilized as an energy substrate.

The present study is the first conducted on the changes in the amino acid profiles in relation to development in *Acanthopagrus latus*. Amino acid profile of *Acanthopagrus latus* changed significantly, which had only minor changes in the percentages of amino acids during larval endogenous feeding. These changes may implicate a change in the rates of different proteins synthesis in fish larvae growing. The AA profile of *Clarias gariepinus* larvae changed before the start of exogenous feeding [29]. Also, changes in the AA profile during larval endogenous feeding have been reported for *Maccullochella macquariensis* and *Maccullochella. peelii peelii* by Gunasekera *et al*. [30].

The yolk molecules are mainly made up of proteins, free amino acids, lipids and carbohydrates [3]. During the embryonic and yolk sac stages, yolk proteins are broken down into amino acids for organogenesis or energy production. Therefore, until first feeding developing larvae depend entirely on the nutritional material in the yolk [9]. Several authors have suggested that fish larvae may be able to discriminate between dispensable (DAA) and indispensable amino acids (IAA) [18, 31], favouring the catabolism of DAA for energy production and the retention of IAA for growth purposes. In *Acanthopagrus latus* larvae, from newly hatched larvae to yolk-sac resorbed, pre-feeding larvae, every EAA except arginine, phenylalanine and methionine and two NEAA (glutamic acid, serine) decreased with development. This indicates that amino acids are used for energy production and growth purposes during endogenous feeding stage in *Acanthopagrus latus*. Of the amino acids that compose proteins, arginine may have the most complex and important role in cell replication, protein deposition and collagen synthesis, all processes involved in fish growth [32]. Further more, arginine may play a central role in the regulation of hormone production such as growth hormone and thyroid hormones [33]. The decrease in total amino acids (TAA) following hatching (from stage 1 until stage 5) is consistent with the above observation. Conceição *et al*. [34] and Saavedra *et al*. [35] reported that AAs are an important energy source during fish larval stages. The same has been shown for alevins of cultured and wild Atlantic salmon *Salmo salar* [36], *Maccullochella macquariensis* and *Maccullochella peelii peelii* larvae [30].

CONCLUSION

Knowledge of the ontogeny changes of biochemical compositions of developing eggs and larvae are essential for a better understanding of nutritional requirements at the start of exogenous feeding. On the basis of the above-mentioned data, the results of fatty acid profiles indicate that *Acanthopagrus latus* utilizes fatty acids as energy substrates during early larval development. In general, the fatty acid composition changed during larval development. The composition of total amino acids changed significantly during ontogeny. In the present study, both EAA and NEAA decreased throughout the experimental period.

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