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Early ontogenic development of the fatty acid composition in seabass (*Dicentrarchus labrax*) eggs and juveniles from captive broodstocks.

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ABSTRACT

In the present study, effects of different diets on the fatty acid composition of hatched seabass (*Dicentrarchus labrax*) at after fertilization of the egg (E), and yolk sac (Y) stages have been investigated. In the study, the fish were fed with Artemia (A), Artemia-microparticulate mix (AMD) and microparticulate diet (M). According to the results of the study, the levels of total PUFAs in the total lipid (TL) were significantly higher in eggs (E) ($P<0.05$) compared to the other groups. 14:1, 15:1, 17:1 and 22:1n 9 and also aracidonic acid and docosaksaneoic acid could not be detected in Artemia but detectable in E and Y groups. During the development stages, polyunsaturated fatty acids (PUFA), arachidonic (20:4n6 ArA), eicosapentaenoic (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) were decreased significantly ($P<0.05$) till the AMD stage. ArA, EPA and DHA were used as energy sources by *Dicentrarchus labrax* larvae. Total n-3 content showed the highest levels at the AMD stage, which was followed by the M, E, Y and A stages, respectively. EPA/DHA ratio was the highest in the AMD group ($P>0.05$). In the study, seabass fatty acid composition at different growth stages was affected by diet composition. After feeding stage especially in the AMD and M groups, essential fatty acids were provided from diets.

Introduction

Today's aquaculture in Turkey is focused on three main fish species, namely, seabass (*Dicentrarchus labrax*), seabream (*Sparus aurata*) and rainbow trout (*Oncorhynchus mykiss*), where seabass (*Dicentrarchus labrax*) has the highest production value in marine aquaculture among the cultured fish species (TUIK 2010). Hatchery production of marine finfish, including seabass, is highly variable and routinely suffers from heavy losses during early ontogenic stages presumably due to both nutritional deficiencies and disease outbreaks (Shields 2001). Especially in Turkey, in seabass hatcheries, success rate varies from year to year. Factors affecting the success rate such as diseases, diet composition, water quality, genetic effects, broodstock management and production protocols are among the main problems. The dietary fatty acid composition and broodstock management have the key role in the marine fish hatcheries. In marine hatcheries in Turkey, cultured fishes are mainly used as broodstock and there is a variety of commercial fish feed types in the industry.

Fatty acids are important structural and physiological components of cell membranes and thought to play important roles in permeability, enzyme activity and other functions in polar lipids of biomembranes (Bell et al. 1986; Lee 2001) and plays a significant role in fish nutrition (Cejas et al. 2003). Highly unsaturated fatty acids (HUFA) are considered as essential fatty acid (EFA) required in marine fish diets for normal growth and survival (Henderson and Tocher 1987; Castell et al. 1994; Sargent et al. 2002). Marine fish species need docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) as these cannot be biosynthesized. Arachidonic acid (ArA) which is an essential fatty acid for marine fish is also important for the regulation of fish cells of numerous physiological processes (Mustafa and Srivastava 1989; Sorbera et al. 1998). Lipids

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and their constituent fatty acids are, along with proteins, the major organic constituents of fish and they play major roles as sources of metabolic energy for growth including reproduction and movement, including migration (Tocher 2003).

Diet composition for larvae and juvenile seabass has been mentioned in earlier studies (Skalli and Robin 2004; Skalli et al. 2006) as well as fatty acid composition of seabass broodstock and juveniles (Bell et al. 1997; Bruce et al. 1999; Navas et al. 1998). However, in the present study, it is aimed to determine the changes in lipid composition class and fatty acids occurring in seabass (*Dicentrarchus labrax*), which were obtained from wild caught broodstocks, from fertilization of eggs to microparticulate diet feeding stage in Marmara region. With the present study, it is intended to bring new approach to the nutritional requirements of seabass juveniles.

Material and methods

Experimental material

Seabass (*D. labrax*) with an average weight of 800-1000 g were hatched in captivity and used as broodstock for the experiment. The broodstock were randomly stocked at the rate of 3 fish/m^3 for each of three spawning tanks (30 m^3) maintaining a 2:1 male-to-female sex ratio. The tanks were supplied with seawater (30 L/min, salinity ‰27) and kept under ambient water temperature (19.0-23 °C). Fish were fed a commercial European seabass diet (Pınar Co. Inc., Turkey) once a day (2-3% of biomass day^{-1}). Commercial pellets were analyzed in triplicate for proximate and fatty acid compositions.

The eggs used for the present study were collected in March. Eggs from the same spawn were collected from each tank overflow in semi-submerged 500-Am baskets. Floating eggs were transferred to 400-L cylindroconical incubation tanks. Tanks were supplied with filtered seawater and continuous soft aeration to maintain eggs and larvae suspended in the water column. Dead eggs were removed daily. Larvae were started feeding on the 7th day with AF Artemia (Salt Lake) and 20 days after the start of feeding with Artemia, the fish were fed with a feed mix of artemia and microparticulate diet.

Proximate analysis

The chemical composition of microparticulate diet and artemia such as moisture, crude ash and total lipid were analyzed in Canakkale Onsekiz Mart University, Faculty of Marine Science and Technology, Laboratory of Feed Nutrition according to AOAC (2000) procedures.

Fatty acid

The extraction of lipids from microparticulate diet, artemia, egg and larvae were carried out with a mixture of chloroform and methanol (2:1, v/v) (Folch et al. 1957) containing 0.01% BHT. The lipid extract was dried out overnight under vacuum following the subtraction of solvent by evaporation under a stream of nitrogen. After being weighed, the lipid extract was redissolved at a known concentration in chloroform/methanol (2:1, v/v) containing 0.1 g kg^{-1} BHT and stored at −20°C prior to analysis. Fatty acid methyl esters (FAME) were prepared according to the procedure of Miyashita et al. (1999) and were purified with the Seppak® cartridge (Furuita et al. 2003). Analytical conditions for analysis of FAME by gas liquid chromatography (GC-17A, Shimadzu Corp., Kyoto, Japan) is described in Furuita et al. (2003).

Table 1. Moisture (%), total lipid (% dry weight), crude ash (%) and fatty acid composition of the artemia and commercial microparticulate diet.

Component	Artemia	Microparticulate Diet
Moisture (%)	86.9±1.34	8.25±1.33
Crude Ash (%)	8.85±0.72	8.96±0.1
Lipid (%)	9.49±1	14.47±0.64
Fatty Acids		
C14:0	0.72 ± 0.2	5.89± 0.1
C15:0	N/A	0.46± 0.1
C16:0	20.97 ± 0.1	26.08± 0.1
C17:0	16.68± 0.1	0.34± 0.1
C18:0	32.81± 0.1	8.04± 0.1
C20:0	2.41 ± 0.1	0.26± 0.1
C21:0	N/A	0.05± 0.1
C22:0	2.21± 0.1	4.58± 0.1
C23:0	N/A	0.1± 0.1
C24:0	2.2± 0.1	8.6± 0.1
∑SFA	78± 0.1	54.4± 0.1
C14:1	N/A	0.18± 0.1
C15:1	N/A	0.09± 0.1
C16:1	3.18± 0.1	6.74± 0.1
C17:1	N/A	0.04± 0.1
C18:1n9c	7.15± 0.1	14.57± 0.1
C20:1n9	4.67± 0.1	0.36± 0.1
C22:1n9	N/A	0.14± 0.1
C20:2	1.05± 0.1	1.21± 0.1
C22:2	1.76± 0.1	0.41± 0.1
∑MUFA	17.81± 0.1	23.74± 0.1
C18:2n6c	2.08± 0.1	15.6± 0.1
C18:2n6t	N/A	0.05± 0.1
C18:3n6	N/A	0.2± 0.1
C18:3n3	0.57± 0.1	0.04± 0.1
C18:4n-3	0.59± 0.1	1.98± 0.1
C20:3n6	N/A	0.07± 0.1
C20:3n3	N/A	0.25± 0.1
C20:4n6	N/A	0.66± 0.1
C20:5n3	0.95± 0.1	0.33± 0.1
C22-5n3	N/A	0.14± 0.1
C22:6n3	N/A	2.04± 0.1
∑PUFA	4.19± 0.1	21.36± 0.1
∑ n-6	2.08± 0.1	16.59± 0.1
∑ n-3	2.12± 0.1	4.78± 0.1
∑n-3/∑n-6	1.02± 0.1	0.29± 0.1
EPA ⁄ DHA	N/A	0.16± 0.1

Data are expressed as mean±SD (*n*=3). SFA; Saturated Fatty Acids, MUFA; monounsaturated fatty acids, PUFA; polyunsaturated fatty acids, HUFA; 20-22 Highly Unsaturated Fatty Acids, AA; arachidonic acid (20:4n-6), EPA; eicosapentaenoic acid (20:5n-3), DHA; docosahexaenoic acid (22:6n-3). N/A; not available.

Statistical analyses

All variables were tested by two-way analysis of variance for the effects of dietary protein and dietary lipid. Differences between means were determined by Tukey's multiple range test and were reported to be significant if $P<0.05$. All statistical analyses were performed using SAS program.

Results

Moisture (%), total lipid (% dry weight) and crude ash (%) content of the Artemia and commercial microparticulate diet, together with the fatty acid composition are given in Table 1. The levels of certain fatty acids in Artemia such as 15:0, 21:0, 23:0 were not detected. In addition, some MUFA's such as 14:1, 15:1, 17:1 and 22:1n 9 and also 18:2n6t, 18:3n6, 20:3n6, 20:3n3, 20:4n6 and docosahexaneoic acid and docosapentaenoic acid could not be detected in Artemia.

Total SFA of the fish at different stages was recorded as the highest in the A stage of the study ($P<0.05$), and lowest SFA content was found in the E stage. Total MUFA ratio was similar among the E, Y and AMD groups compared to the A and M groups. The lowest total MUFA level was observed in the M stage.

Table 2: Fatty acid composition of seabass larvae at different growth stages

Fatty Acid	Egg	Yolk Sac	Artemia	Artemia and Microparticul Diet	Microperticul Diet
C14:0	1.71±0.12^{a}	1.31±0.04^{b}	0.49±0.01^{c}	0.07±0.01^{d}	0.16±0.01^{e}
C15:0	0.27±0.17^{c}	0.55±0.03^{a}	N/A	0.16±0.01^{d}	0.46±0.01^{b}
C16:0	14.51±1.01^{b}	26.15±1.22^{a}	13.77±1.75^{b}	11.30±1.51^{c}	24.45±1.98^{a}
C17:0	0.40 ±0.02^{b}	3.92±.057^{b}	8.19±1.78^{a}	0.35±0.12^{d}	0.67±0.01^{c}
C18:0	5.83±0.22^{e}	13.89±1.1^{b}	16.60±2.1^{a}	9.59±1.1^{c}	7.68±2.1^{d}
C20:0	1.58± 0.17^{a}	0.73±0.3^{d}	0.90±0.2^{c}	1.21±0.11^{b}	0.27±0.12^{e}
C21:0	1.38±0.12^{b}	N/A	N/A	4.10±0.22^{a}	0.01±0.01^{c}
C22:0	0.48±0.01^{d}	0.29±0.01^{e}	0.94 ± 0.01^{b}	0.76±0.01^{c}	4.16±0.14^{a}
C23:0	0.33±0.02^{d}	0.38±0.01^{d}	4.04±0.1^{a}	0.75±0.01^{c}	0.69±0.01^{b}
C24:0	N/A	1.94±0.025^{b}	7.53 ± 0.2^{a}	N/A	N/A
ΣSFA	26.51±1.15^{e}	49.16±1.87^{b}	52.47±2.35 ^{a}	28.29±2.22^{d}	38.55±2.78^{c}
C14:1	0.08± 0.01^{c}	5.47±1.1^{a}	0.83±0.1^{b}	0.49±0.1^{c}	0.21±0.02^{c}
C15:1	0.06± 0.01^{a}	0.05±0.01^{a}	N/A	0.23±0.1^{a}	0.14±0.12^{a}
C16:1	5.08±0.51^{b}	7.30 ±1.1^{a}	2.64±0.14^{c}	0.10±0.01^{d}	5.27±1.1^{b}
C17:1	0.70 ±0.02^{b}	0.88±0.01^{a}	N/A	0.45±0.12^{b}	0.11±0.01^{b}
C18:1n9c	27.23±0.2^{a}	9.77±1.1^{c}	5.67±0.45^{d}	3.78±0.81^{e}	17.81±2.17^{b}
C20:1n9	0.15±0.02^{c}	2.99±0.57^{a}	1.88±0.25^{b}	0.20±0.01^{c}	0.25±0.14^{c}
C22:1n9	1.51±0.01^{a}	0.68±0.2^{b}	N/A	N/A	0.09±0.01^{c}
C20:2	0.56 ±0.04^{c}	0.53±0.12^{c}	1.35±0.1^{a}	0.59±0.12^{c}	1.26±0.31^{b}
C22:2	0.29 ±0.02^{d}	2.69±0.3^{c}	17.30±2.1^{b}	25.15±2.22^{a}	0.86±0.22 ^{d}
Σ MUFA	35.66±4.21^{a}	30.37±3.28^{a}	29.68±2.84^{ab}	30.99±3.1^{a}	26.01±2.22^{b}
C18:2n6c	3.52±0.25^{c}	4.17±0.2^{b}	1.87±0.14^{d}	3.13±0.86^{c}	12.80±1.1^{a}
C18:2n6t	12.34±1.24^{a}	1.22±0.35^{b}	N/A	0.10±0.1^{c}	0.05±0.001^{c}
C18:3n6	0.18±0.1^{b}	0.39±0.1^{a}	N/A	0.33±0.1^{a}	0.30±0.13^{a}
C18:3n3	1.25±0.25^{c}	1.38±0.41^{b}	1.52±0.54^{b}	12.6±1.14^{a}	0.05±0.001^{d}
C18:4n-3	0.44±0.1^{b}	0.59±0.14^{ab}	N/A	1.95±0.65^{a}	0.10±0.1^{b}
C20:3n6	0.37±0.2^{b}	1.11±0.24^{a}	N/A	1.12±0.21^{a}	0.06±0.1^{b}
C20:3n3	1.11±0.21^{b}	2.15±0.38^{a}	1.08±0.1^{b}	0.93±0.3^{bc}	0.31±0.02^{c}
C20:4n6	0.59±0.14^{a}	0.51±0.14^{a}	0.33±0.12^{b}	0.13±0.12^{c}	0.04±0.11^{c}
C20:5n3	4.77±0.85^{a}	2.31±0.14^{b}	0.40±0.13^{c}	0.12±0.1^{c}	0.70±0.1^{c}
C22-5n3	0.01±0.001^{c}	0.21±0.01^{b}	0.69±0.15^{a}	0.61±0.03^{a}	0.14±0.12^{b}
C22:6n3	13.07±1.35^{b}	3.91±0.59^{d}	4.98±1.1^{c}	13.51±1.24^{b}	20.43±2.1^{a}
Σ PUFA	37.66±3.1^{a}	17.96±2.1^{d}	10.86±1.58^{b}	34.56±2.5^{b}	34.99±3.1^{b}
Σ n-6	17.01±2.21^{a}	7.41± 1.47^{ab}	2.20±1.02^{b}	4.82± 1.1^{b}	13.24 ±2.1^{a}
Σ n-3	20.65±2.35^{c}	10.55±0.21^{d}	8.66±2.1^{e}	29.74±2.57^{a}	21.74±2.14^{b}
Σn-3/Σn-6	1.21±0.14^{e}	1.42±1.01^{d}	3.95±1.35^{b}	6.17±1.7^{a}	1.64±0.7^{c}
DHA / EPA	2.74±0.85^{d}	1.69± 0.21^{e}	12.55±0.21^{c}	113.08±.045^{a}	29.05±2.89^{b}
EPA/AA	8.08±1.02^{b}	4.52±1.23^{c}	1.2±0.2^{d}	0.92±0.1^{d}	17.5±2.74^{a}

Means followed by different letters are significantly different ($P<0.05$); the values are expressed as mean±SD. SFA; Saturated Fatty Acids. MUFA; monounsaturated fatty acids. PUFA; polyunsaturated fatty acids. HUFA; 20-22 Highly Unsaturated Fatty Acids. AA; arachidonic acid (20:4n-6). EPA; eicosapentaenoic acid (20:5n-3). DHA; docosahexaenoic acid (22:6n-3). N/A; not available.

The levels of total PUFA's in the total lipid (TL) were significantly higher in eggs (E) ($P<0.05$) compared to those in yolk sac (Y), artemia stage (A), artemia and microparticulate stage (AMD) and microparticulate diet (M) stages of the study (Table 2). Total n-3 content showed the highest levels at the AMD stage and M, E, Y and A stages respectively. Total n-6 content was found as the highest at the E and M stage but opposite trend was seen at the A and AMD stages. Highest level of ArA observed in the A and Y stages have been decreased steadily. Similar results were observed in EPA levels. However, DHA level was found as the highest during the M stage. These tendencies gave rise to a high DHA /EPA ratio at the AMD stage of the study.

Discussion

The lipid composition of different stages of *D. labrax* eggs and larvae showed differences depending on the growth stage. In previous reports, eggs containing high amount of total lipid (Cejas et al. 2004) have also been observed in red drum (Vetter et al. 1983) and sea bream (Ronnestad et al. 1994). This high lipid level is an indication of resorbing yolksack following hatching when fish become free-swimming. In the study, TL content was found to be higher in the E group and steadily decreased in Y, A, AMD and M groups, respectively. Although, our results are comparable with the findings of Cejas et al. (2004), fatty acid content of the E stage group was found lower than that reported by Cejas et al. (2004). This could be attributed to the differences of the broodstock diets in both studies.

Previous studies have pointed out the physiological importance of maintaining right proportions of EPA, ArA and DHA fatty acid in the phospholipids of the cell membrane bilayer (Bruce et al. 1999; Sargent et al. 1999). Higher EPA content was found in the E and Y stage compared to the other stages in the study. This could be a result of metabolic and physiological functions of EPA. Therefore, the high EPA/ArA ratio observed in the M group also supports this statement.

It is widely accepted that the fatty acid composition of fish tissue reflects the fatty acid composition of the fish diet (Watanabe et al. 1978). Taking into account that the majority of marine organisms are poor in 18:2n-6, higher percentage of this fatty acid at the M stage is notable. During larval development, of the essential fatty acids, ArA was conserved, possibly due to the importance of this fatty acid as the major eicosanoid precursor in fish cells (Bell et al. 1994; Van Der Kraak and Biddiscombe 1999). However, ArA decreased during the ongoing feeding stages in the present study.

The higher percentage of DHA was detected at the E stage, while a decrease was observed at the Y and A stages. DHA may be used as an metabolic energy source, and the lack of DHA in artemia based diet may explain this outcome. DHA rich microparticulate diet showed higher value of this fatty acid, at the AMD and M stages. This result is in agreement with the findings of Watanabe et al. (1978).

As a conclusion, it is suggested to consider not only the individual levels of these fatty acids but also the correct ratio among them (AA/EPA/DHA) for an assessment of efficient feeding regime during early larval stages after hatching.

Conclusion

In conclusion, the present results indicate that, seabass fatty acid composition was affected by diet composition. ArA, EPA and DHA was used as an energy source in the early larval stages. Right after the start of feeding, the fish provide the fatty acid from diets. Further studies on the accurate definition of fatty acid composition of the broodstock are encouraged.

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