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Research Article

Investigation of Changes in Fatty Acid Composition at Early Development Stages of Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract: Changes in fatty acid composition were investigated during the early development stages (egg, embryo, alevin and fry) of rainbow trout (*Oncorhyncus mykiss*). It was found that saturated and monounsaturated fatty acids, i.e., 14:0, 16:1n-7 and 18:1n-9, were utilized to a greater extent as energy substrates. Quantities of 18:3n-6, 18:4n-3, 20:2n-6 and 22:5n-3 fatty acids were significantly reduced ($P < 0.05$) during early development. The most significant depletion was observed in monounsaturated fatty acids, which fell from 33.89% to 23.41% during development while n-6 polyunsaturated fatty acids fell from 16% to 12.9%.

Key Words: Rainbow trout (*O. mykiss*), egg, embryo, alevin, fry, fatty acid

Gökkuşağı Alabalığının (*Oncorhynchus mykiss*) Yağ Asit Kompozisyonundaki Değişimlerin Erken Gelişim Safhalarında İncelenmesi

Özet: Gökkuşağı alabalığı (*Oncorhynchus mykiss*) yağ asidi kompozisyonundaki değişimler erken gelişme safhalarında (yumurta, embriyo, alevin ve fry) incelenmiştir. Enerji kaynağı olarak 14:0, 16:1n-7 ve 18:1n-9 gibi doymuş ve tekli doymamış yağ asitlerinin fazla miktarda kullanıldığı tespit edilmiştir. Erken gelişme süresince 18:3n-6, 18:4n-3, 20:2n-6 ve 22:5n-3 yağ asitlerinin miktarları önemli derecede azalmıştır ($P < 0,05$). Gelişme esnasında n-6 çoklu doymamış yağ asiti miktarı %16'dan %12,9'a düşerken, en yüksek azalma %33,89'dan %23,41'e düşen tekli doymamış yağ asitlerinde gözlenmiştir.

Anahtar Sözcükler: Gökkuşağı alabalığı (*O. mykiss*), yumurta, embriyo, alevin, fry, yağ asiti

Introduction

It has been reported that changes in the chemical composition of the egg or yolk sac may indicate nutritive needs during early feeding stages (1). A few pieces of research have been carried out related to the fatty acid compositions of larvae and fry in sea bass and turbot (2), Atlantic halibut (3), salmon (4), Senegal sole (5) and Japanese flounder (6).

The chemical composition of eggs has been examined to evaluate egg quality, as the egg must satisfy nutritional needs for embryonic and fry development (3,7). Therefore, the determination of the fatty acid composition of the eggs and fry would be a worthwhile study. Surprisingly, this problem has been examined in only a few studies concerned with Atlantic herring (8), cod (9), Senegal sole (5) and Japanese flounder (6).

The objective of the present study was to investigate the changes of fatty acid composition in the eggs and fry of rainbow trout from fertilization to first feeding stage. Furthermore, the aim was to establish whether the fatty acid composition of the eggs would have any impact on the fatty acid composition of fry at first feeding stage.

Materials and Methods

Eggs were determined from three-year-old rainbow trout broodstock raised intensively in the Research and Extension Center of Fishery Department at Atatürk University in Erzurum. Eggs from the same batch, at spawning (unfertilized) and 24 h after spawning (fertilized), were collected in a mesh screen, washed in distilled water and blotted on filter paper before being frozen in liquid nitrogen and stored in a freezer at –80 °C for analysis (5). The same treatment was repeated at the eyed stage (eyed embryo, 20 days after hatching), for alevins (yolk sac, 36 days after hatching) and for fry at the first feeding stages. The fatty acid profiles of sterile (unfertilized) eggs were also determined.

Lipids and the Analysis of Fatty Acid Methyl Esters (FAME)

Fatty acid analysis was carried out at the Biotechnology Application and Research Center, Atatürk University, Erzurum, Turkey. The preparation and analyses of FAMEs from all samples including feed were performed according to the method described by Microbial ID Inc. (10). Samples were added to 1 ml 1.2 M NaOH in 50% aqueous methanol with six glass beads (3 mm dia) in a screw cap tube, and then incubated at 100 ºC for 30 min in a water bath. The saponified samples were cooled at room temperature for 25 min. They were then acidified and methylated by adding 2 ml 54% 6 N HCl in 46% aqueous methanol and incubated at 80 ºC for 10 min in a water bath. After rapid cooling, methylated fatty acids (FA) were extracted with 1.25 ml 50% methyl-tert butyl ether (MTBE) in hexane. Each sample was mixed for 10 min and the bottom phase was removed with a Pasteur pipette. The top phase was washed with 3 ml 0.3 M NaOH. After mixing for 5 min, the top phase was removed for analysis. Following the base wash step, the FAMEs were cleaned in anhydrous sodium sulfate and then transferred into a gas chromatography sample vial for analysis. FAMEs were separated by gas chromatography (HP6890, Hewlett Packard, Palo Alto, CA) with a fused-silica capillary column (25 m x 0.2 mm) with cross-linked 5% phenylmethyl silicone. The operating parameters for the study were set and controlled automatically by a computer program. The chromatograms with peak retention times and areas were produced on the recording integrator and were electronically transferred to the computer for analysis, storage and report generation. Peak naming and column performance were achieved through the use of a calibration standard FA mix (Eucary Method 697110) containing nC9-nC30 saturated fatty acids. Fatty acids were identified on the basis of equivalent chain length data. FAME profiles of the tissues were identified by comparing the commercial Eucary database with the MIS software package (MIS ver. No 3.8, Microbial ID, Inc., Newark, Delaware).

Statistical Analysis

Results are presented as means ± SE. The sample size (n = 6) was chosen according to Vazquez et al. (5). Differences between parameters were analyzed by one-way analysis of variance (ANOVA), and significant means were subjected to a multiple comparison test (Tukey) at the $\alpha = 0.05$ level (11).

Results

Fatty acid compositions of the eggs, expressed as a percentage of total fatty acids, were determined during the different developmental stages of rainbow trout (*Oncorhyncus mykiss*) (Table). Significant differences were observed among 14:0, 16:0, 16:1n-7, 18:1n-9, 18:2n-6, 18:3n-6, 20:2n-6, 20:4n-6, 22:5n-3 and 22:6n-3 fatty acids from fertilization to first feeding ($P < 0.05$). No significant changes were determined among 15:0, 16:1n-9, 17:0, 17:1n-8, 18:0, 18:4n-3, 20:1n-9, 20:3n-6, 20:5n-3 fatty acids ($P > 0.05$). Total saturated fatty acids (SFA) were at a minimum (21.85%) at yolk sac stage and reached a maximum (25.1%) at exogenous feeding. Among the saturated fatty acids such as miristic (14:0) and stearic acid (18:0), palmitic acid was the most abundant (70%) whereas oleic acid (18:1n-9) was the most abundant in monounsaturated fatty acid (MUFA) and significant differences were observed at different stages ($P < 0.05$). The most significant depletion was observed in MUFA, which fell from 33.89% at the unfertilized egg stage to 23.41% at first feeding.

n-3 polyunsaturated fatty acid (PUFA) values gradually increased during the study and changed from 26.04% in eggs to 36.25% at first feeding. The quantity of n-6 PUFA reduced significantly from 16-17% to 12.9% ($P < 0.05$). The most important levels were 18:1n-9 in MUFA, 22:6n-3 in (n-3) PUFA and 18:2n-6 in (n-6) PUFA. These changes also showed a very similar pattern in n-3/n-6 ratio with (n-3) PUFA and (n-6) PUFA, which increased significantly from an initial 1.63% to 2.81% at first feeding ($P < 0.05$). The differences between EPA/DHA ratios were significant ($P < 0.05$) during the development of rainbow trout. It reduced by some 30% (0.23 – 0.16) from the initial stage to first feeding.

Discussion

It has been reported that during the process of development, the initial fatty acid content of the egg had been changed, e.g. in the Atlantic herring (8) and cod (9). It was evident that the rainbow trout utilized MUFAs to a greater extent and saturated fatty acids (SA) to a lesser extent than n-3 PUFA during early development (Table). Significant changes occurred in n-3 PUFA and n-6 PUFA from egg to first feeding. Mean values showed an upward trend in n-3 PUFA and a downward trend in n-6 PUFA.

Table. Fatty acid composition (%) of rainbow trout during early development stage. Superscripts in a row with different letters represent significant difference ($p < 0.05$). Each value is the mean ± SE of six individual observations.

Fatty acids	Unfertili. egg	Embryo	Eyed Embryo	Alevin	Fry
14:0	3.17 ± 0.23^{ab}	3.59 ± 0.3^{a}	2.77 ± 0.3^{ab}	3.03 ± 0.3^{ab}	1.95 ± 0.52^{ab}
15:0	0.56 ± 0.05	0.67 ± 0.07	0.58 ± 0.07	0.68 ± 0.07	0.4 ± 0.17
16:1n9	1.04 ± 0.05	1.16 ± 0.07	1.12 ± 0.07	0.87 ± 0.08	1.19 ± 0.12
16:1n7	5.94 ± 0.33^{a}	6.42 ± 0.43^{a}	5.38 ± 0.43^{ab}	4.21 ± 0.43^{bc}	3.18 ± 0.75^{c}
16:0	15.24 ± 0.44^{b}	16.11 ± 0.57^{b}	17.85 ± 0.57^{ab}	15.34 ± 0.57^{b}	18.89 ± 0.99^{a}
17:1n8	0.37 ± 0.02	0.4 ± 0.03	0.38 ± 0.03	0.45 ± 0.03	nd
17:0	0.28 ± 0.13	0.66 ± 0.17	0.33 ± 0.21	0.36 ± 0.17	nd
18:3n6	0.75 ± 0.05^{ab}	0.88 ± 0.06^{a}	0.56 ± 0.07^{ab}	0.48 ± 0.09^{b}	0.52 ± 0.11^{b}
18:4n3	0.71 ± 0.07	0.79 ± 0.08	0.64 ± 0.09	0.95 ± 0.08	0.72 ± 0.14
18:2n6	9.57 ± 0.36^{ab}	10.77 ± 0.46^{a}	9.62 ± 0.46^{ab}	7.55 ± 0.46^{bc}	5.45 ± 0.81^{c}
18:1n9	22.41 ± 0.52^{a}	22.14 ± 0.68^{a}	20.78 ± 0.68^{ab}	18.38 ± 0.68^{bc}	15.62 ± 1.18^{c}
18:0	3.09 ± 0.22	3.15 ± 0.28	3.32 ± 0.28	3.64 ± 0.31	3.98 ± 0.49
20:4n6	3.13 ± 0.14^{b}	3.26 ± 0.19^{b}	3.87 ± 0.19^{ab}	3.62 ± 0.21^{b}	4.61 ± 0.33^{a}
20:5n3	4.72 ± 0.2	5.29 ± 0.26	5.13 ± 0.26	5.47 ± 0.29	4.94 ± 0.46
20:3n6	1.53 ± 0.09	1.51 ± 0.12	1.43 ± 0.12	1.06 ± 0.15	0.97 ± 0.21
20:2n6	1.13 ± 0.07^{a}	1.05 ± 0.09^{ab}	0.99 ± 0.09^{ab}	0.85 ± 0.11^{ab}	0.63 ± 0.15^{b}
20:1n9	0.94 ± 0.17	0.91 ± 0.23	0.82 ± 0.28	1.33 ± 0.25	0.64 ± 0.4
22:6n3	20.71 ± 0.71^{b}	17.34 ± 0.92^{b}	20.18 ± 0.92^{b}	25.32 ± 1.01^{a}	29.43 ± 1.61^{a}
22:5n3	1.24 ± 0.06^{ab}	1.41 ± 0.08^{ab}	1.33 ± 0.08^{ab}	1.66 ± 0.09^{a}	1.17 ± 0.15^{b}
18:1n9t	3.01 ± 0.09^{a}	2.77 ± 0.11^{ab}	2.83 ± 0.11^{ab}	2.66 ± 0.12^{ab}	2.4 ± 0.2^{b}
SFA	21.92 ± 0.38^{b}	23.52 ± 0.49^{ab}	24.38 ± 0.49^{a}	21.85 ± 0.54^{b}	25.1 ± 0.86^{a}
MUFA	33.89 ± 0.71^{a}	33.61 ± 0.91^{a}	30.51 ± 0.91^{ab}	28.5 ± 1.01^{b}	23.41 ± 1.59^{c}
n3 PUFA	26.04 ± 0.81^{b}	25.01 ± 1.05^{b}	27.06 ± 1.05^{b}	33.48 ± 1.15^{a}	36.25 ± 1.83^{a}
n6 PUFA	15.72 ± 0.45^{abc}	17.46 ± 0.59^{a}	16.52 ± 0.59^{ab}	13.91 ± 0.64^{cb}	12.91 ± 1.02^{c}
n3/n6	1.63 ± 0.09^{b}	1.43 ± 0.11^{b}	1.64 ± 0.11^{b}	2.48 ± 0.12^{a}	2.81 ± 0.2^{a}
EPA/DHA	0.23 ± 0.01^{abc}	0.29 ± 0.01^{a}	0.25 ± 0.01^{ab}	0.21 ± 0.01^{bc}	0.16 ± 0.03^{c}

nd: not detected

This may indicate that n-6 PUFA was utilized more effectively than n-3 PUFA. The increase in n-3 may be caused by the synthesis of C_{18} fatty acids.

Major saturated and monounsaturated fatty acids such as 16:0, 16:1n-7 and 18:1n-9 were consumed from egg to first feeding stage for energetic purposes. PUFAs did present a different pattern of utilization. C_{18} fatty acids such as 18:0 and 18:4n-3 remained constant from egg to first feeding stage (Table). Although no significant differences were observed in the content of 18:0 and 18:4n-3 between egg and first feeding stage, mean values fluctuated in 18:4n-3 and increased in 18:0. This may indicate that the 18:4n-3 was not being utilized to a great extent either for energy purposes or as a precursor for desaturation or elongation to a longer chain. However, 18:0 may be more deposited by synthesis than used during development. Bautista and De la Cruz (12) found that 18:2n-6 was effective for good growth and survival of seawater-reared milkfish (1.5 g). Likewise, in the present study 18:2n-6 was found to be very important for development. Furthermore, 18:3n-6 and 18:1n-9 were reduced significantly.

Major C_{20} PUFAs showed two different patterns of utilization during development. n-3 C_{20} fatty acids such as 20:2n-6 and 20:4n-6 showed a significant decrease and increase, respectively. This may indicate that 20:2n-6 was being used for energetic purposes. There were significant differences in the content of 22:5n-3 and 22:6n-3 fatty

acids, which increased from egg to first feeding. This may indicate that this particular fatty acid may be the end product of the bioconversion of PUFA with high activity during rainbow trout development. This situation was confirmed by the findings of Sargent et al. (13). Prostaglandins in marine fish have been known to be synthesized primarily from 20:4n-6 rather than 20:5n-3 (14). Our findings, which were in agreement with the data presented by Fraser et al. (15) and Ostrowski and Divakaran, (16), strongly suggest that these fatty acids are important for the early development of rainbow trout.

Several studies have reported that docosahexaenoic acid (22:6n-3) was essential in marine fish for the development of the brain and retina (17-20) and a lack of it may cause behavioral abnormalities, possibly due to visual and neural impairment (21).

The significant decrease in 22:5n-3 content from egg to alevin indicates that little, if any, bioconversion or docosahexaenoic acis synthesis was occurring during non-feeding development. Thus, 22:5n-3 must be supplemented to the first feeding fry to achieve the good growth and performance of developing fry. This was in agreement with the results found in turbot larvae (22), in common sole larvae (23) and in plaice larvae (24), but was not in agreement with the data presented in *S. aurata* (25) or *C. hippurus* (16).

In conclusion, this investigation has shown that the composition of the total fatty acids of eggs changed significantly during the development stages. In addition, some fatty acids such as 18:3n-6, 18:4n-3, 20:2n-6 and 22:5n-3 should be supplemented to fish feeds. Further work may be needed to determine whole fatty acid requirements of fish at further production stages.

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References

1. Heming, T.A., Buddington, R.K.: Yolk sac absorption in embriyonic and larval fishes. In Hoar, W.S., Randall, D.J., Brett, J.R., (Eds.), Fish Physiology. Academic Press, New York, 1988; Part A, 11: 407- 446.
2. Devauchelle, N., Brichon, G., Lamour, F., Stephan, G.: Biochemical composition of ovules and fecund eggs of sea bass (*Dicentrarchus labrax*) and turbot (*Scopthalmus maximus*). In: Richter, C.J.J., Goos, H.J.Th. (Eds). Proceedings of International Symposium on Reproductive Physiology in Fish. Wageningen, The Netherlands 1982; 155-157.
3. Parrish, C.C., Brown, J.A., Daniel, E.S., Somerton, D.C.: Fatty acid composition of Atlantic halibut eggs in relation to fertilization. Bull. Aquacult. Assoc. Canada. 1994; 94: 36-38.
4. Hove, H.T., Grahl-Nielsen, O.: Fatty acid composition of start-feeding salmon (*Salmo salar*) larvae. Aquaculture. 1991; 96: 305-319.
5. Vazquez, R., Gonzalez, S., Rodriguez, A., Mourente, G.: Biochemical composition and fatty acid content of fertilized eggs, yolk sac stage larvae and first-feeding larvae of Senegal sole (*Solea senegalensis* Kaup), Aquaculture. 1994; 119: 273-286.
6. Furuita, H., Tanaka, H., Yamamato, T., Suzuki, N., Takeuchi, T.: Effects of high levels of n-3 HUFA in broodstock diet on egg quality and egg fatty acid composition of Japanese flounder, *Paralichthys olivaceus*. Aquaculture. 2002; 210: 323-333.
7. Bell, J.G., Farndale, B.M., Bruce, M.P., Navas, J.M., Carillo, M.: Effects of broodstock dietary lipid on fatty acid compositions of eggs from sea bass (*Dicentrarchus labrax*). Aquaculture. 1997; 149: 107-119.
8. Tocher, D.R., Fraser, A.J., Sargent, J.R., Gamble, J.C.: Lipid class composition during embriyonic and early larval development in Atlantic herring (*Cuplea harengus* L.). Lipids, 1985; 20: 84-89.
9. Barnung, T.N., Grahl-Nielsen, O.: The fatty acid profile in cod (*Gadus morhua* L.) eggs and larvae. Developmental variations and responses to oil pollution. Sarsia, 1987; 72: 415-417.
10. Microbiol ID Inc.: Sherlock Microbial Identification System, Version 4 MIS Operating Manual, Newark, USA, 2000.
11. SAS.: System on Microsoft Win, Release. 6.11, TS 20, SAS Ins., NC, USA. 1996.
12. Bautista, M.N., De la Cruz, M.C.: Linoleic and linolenic acids in the diet of fingerling milk fish (*Chanos chanos* Forsskal). Aquaculture. 1988; 71: 347-358.
13. Sargent, J.R., Henderson, R.J., Tocher, D.R.: The Lipids. In Halver, J.E. Editor, Fish Nut. Second Edition, Academic Press Inc., London 1989; 153-218.
14. Sargent, J.R., Henderson, R.J., Tocher, D.R.: Polyunsaturated fatty acids in marine and terrestrial food webs. In: J. Mellinger (Editor), Animal Nutrition and Transport Process. 1. Nutrition in Wild and Domestic Animals. Comparative Physiology, Basal Karger 1990; 5: 11-13.

15. Fraser, A.J., Gamble, J.C., Sargent, J.R.: Changes in lipid content, lipid class and fatty acid composition of developing eggs and unfed larvae cod (*Gadus morhua*). Mar. Biol., 1988; 99: 307 – 313.

16. Ostrowski, A.C., Divakaran, S.: Survival and bioconversion of n-3 fatty acids during early development of dolphin /*Coryphaena hippurus*) larvae fed oil-enriched rotifers. Aquaculture. 1990; 89: 273-285.

17. Bell, M.V., Dick, J.R.: Molecular species composition of the major diacyl glycero-phospholipids from muscle, liver, retina and brain of cod *(Gadus morhua)*. Lipids 1991; 26: 565-573.

18. Mourente, G., Rodriguez, A., Tocher, D.R., Sargent, J.R.: Specific accumulation of docosahexaenoic acid (22:6n-3) in brain lipids during development of juvenile turbot *S. maximus* L. Lipids, 1991; 26: 871- 877.

19. Tocher, D.R., Mourente, G., Sargent, J.R.: Metabolism of [1-^{14}C] docosahexaenoate (22:6n-3), [1-^{14}C] eicosapentaenoate (20:5n-3) and [1-^{14}C] linolenate (18:3n-3) in brain cells from juvenile turbot *Scophthalmus maximus* L. Lipids, 1992; 27: 494 –499.

20. Sargent, J.R., Henderson, R.J., Tocher, D. R.: Docosahexaenoic acid and the development of brain and retina in marine fish. In: Devon, C.A., Baksaas, J., Krokan, H.E. (Eds.), Omega-3 Fatty Acids: Metabolism and Biological Effects. Birkhauser Verlag, Basel, Switzerland. 1993; 39-149.

21. Navarro, J.C., Sargent, J.R.: Behavioural differences in starving herring *Clupea harengus* L. Larvae correlate with body levels of essential fatty acids. J. Fish Biol. 1992; 41: 509-513.

22. Minkoff, G.: The effects of secondarily enriched rotifers on growth and survival of marine fish larvae. Ph.D. Thesis, University of Stirling, 1987.

23. Tzoumas, A.S.: The nutritional value of freshly hatched and enriched *Artemia nauplii* for sole larvae (*Solea solea*) from the standpoint of their polyunsatured fatty acid content. M.Sc. Thesis, University of Stirling, 1988.

24. Dickey-Collas, M., Geffen, A.J.: Importance of the fatty acids 20:5n-3 and 22:6n-3 in the diet of plaice (*Pleuronectes platessa*) larvae. Mar. Biol., 1992; 113: 463-468.

25. Mourente, G., Rodriguez, A., Tocher, D.R., Sargent, J.R.: Effects of dietary docosahexaenoic acid (DHA; 22:6 n-3) on lipid and fatty acid composition and growth in gilthead sea bream (*Sparus aurata* L.) larvae during first feeding. Aquaculture, 1993; 112: 79-98.