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Determination of Fatty Acid Compositions in Eggs of Commercial Aquafeed Fed Broodstocks Rainbow Trout (*Oncorhynchus mykiss)* and Brown Trout *(Salmo trutta spp.)*

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ABSTRACT: The experiment was carried out in a 6-month period prior to spawning of the broodstock rainbow and brown trout groups fed by commercial fish feed. The study aimed to determine the changes of fatty acid composition in eggs obtained from broodstock fish which were spawning for the first time. The fish in each group were selected from the same age and hatching eggs. The amount of PUFAs between the experimental groups was not different, while SFA and MUFA amounts were significantly different. The amounts of oleic acid (C18: 1n9c), linoleic acid (C18: 2n6c) and palmitic acid (C16: 0) were the highest among the fatty acids detected in broodstock fish egg groups and their feeds. Total omega-3 (n-3) content was considerably different between rainbow trout (4.3%) and brown trout (2.89%). The percentages of palmitic acid, palmitoleic acid, heptadecanoic acid and EPA were significantly different between the egg groups. Broodstock fishes need to be fed with good quality feed to give quality eggs in suitable environment conditions. For this reason, it has been shown that it reflected in the fatty acids of fish eggs while the breeding fish consume sufficient amounts of essential fatty acids on the diet. As a result of this situation, it can be ensured that the fish are healthier and have a higher percentage of life.
Keywords – *Fatty acid, Rainbow trout, Brown trout, egg, n-3, n-6, EPA*

1. Introduction

Aquaculture is considered as the fastest growing food sector in the world by the World Food Agriculture Organization. Aquaculture is the basic industry meeting the majority of nutrient requirements of the world. In addition, aquaculture is an important production and science area that has a relationship with various disciplines and sectors. Aquaculture provides controlled and semi-controlled optimum environmental conditions according to biological development stages of plants and animals in aquatic environments, preserves the natural environment and stocks by balancing water resources and conserving the ecological structures and reduces hunting pressure in natural stocks and also takes economic principles into account (Altun and Kubilay, 2009).

The United Nations reports that the demand for animal products will increase by two-fold in 20 years, with the assumption that the world population will be approximately 8 billion by 2030 by an average increase of 78 million per year (Anonymous, 2018a). Twenty percent of animal protein demands are met by fish (Deutscha *et al*., 2007). Aquaculture production of Turkey in 2017 was 276502 tons and of which 103705 tons was from trout farming in inland waters. Production of Turkey is only 0.3% of world aquaculture production (Anonymous, 2018b).

Fish cannot be able to synthesize essential fatty acids which must be supplied with feed. Marine fish meet fatty acid requirements from algae and phytoplankton, which are rich in EPA, DHA, linoleic and linolenic acid. Essential fatty acids are supplied with feed in cultured fish. The fatty acids taken from feed are the only sources of essential energy and essential fatty acids for fish in aquaculture (Sargent *et al*., 2002).

The amount of n-3 also varies depending on the type of fish. The n-3 ratio is higher especially in the fish that live in deep seas and have black meat. Salmon, sardines, mackerel, and tuna fish are quite rich in n-3; however, n-3 level is slightly lower in culture fish. Nevertheless, the amount of unsaturated fatty acids is high in culture fish fed by n-3 rich feed (Hepgul, 2002). In a study conducted on *Rachycentron canadum* fish reported that higher the rate of unsaturated fatty acids in the fatty acid content of the eggs causes higher survival rates (Faulk and Holt 2005).

Trouts are members of carnivorous feeders; therefore both high-quality feeds and animal-based substances should be included in their rations (Hoyer, 1975). Studies clearly indicated that feed is very important in egg production, fish population dynamics and determination of production potential of different fish (Woodhead, 1960; Nicolskii, 1969; Bagenal, 1973).

The most important factor on the egg production and quality of the cultured trout is the characteristics of feed. Regular feeding with good quality artificial feeds accelerates the development, thus young and large female fish can be obtained. Broodstocks which give the generations that efficiently utilize the artificial feed can be determined by selection among the fish fed regularly (Atay and Tatar, 1980).

This study was aimed to determine the fatty acid compositions affecting the quality of two different broodstock trout eggs.

2. Material and Methods

The research was carried out in concrete pool and fiberglass tanks located in Fisheries Research and Application Center of Tokat Gaziosmanpaşa University. Three-year-old 20 female and 10 male rainbow and brown trout were used as fish material. Commercial brood stock trout feed was used in the study. The feed used is trout grow (9 mm) feed and the basic nutritional values and fatty acid profiles were presented in Table 1 and Table 2.

The experiment was carried out six-month prior to spawning period of rainbow and river trout. The fish were fed twice a day (09:00 and 16:00) by hand feeding method. Feeding request criteria were taken into account and 1% of their weight was given in each feeding. Movements of fish were observed during feeding and feed was given accordingly. Feeding was continued for 6 months in natural lighting environment, feed was not given 1 day prior to spawning and on spawning day. Ten brood stock rainbow and 10 brown trout were spawned by double person method, and eggs were mixed and 3 samples were taken from each of them. Rainbow trout eggs, different from brown trout, were fertilized with sperm obtained from 3 male brood stocks after spawning, and 3 samples were taken from these eggs to determine the fatty acid composition.

Table 1. Nutrient composition of experimental feed, 100% D.M. (Dry Matter)

Proximate composition (%) of the Experiment feeds	Fish feed
CP % (Crude Protein)	44
CF % (Crude Fat)	20
CC % (Crude Cellulose)	3
CA % (Crude Ash)	12
Moisture %	12
Nitrogen-free extracts %	9
Total Energy Kj/g	19.85
Total Energy kcal/kg	4868
P\E mg CP/kcal	90.39
PE/TE	0.51
Calcium (Ca)	1.3
Phosphorus (P)	1.05
Sodium (Na)	0.3

The oils were saponified by the standard IUPAC methods (IUPAC, 1988). Fatty acids were esterified by 10% (v/v) BF_3-MeOH (methanolic boron tri fuoride) as reagent. The fatty acid methyl esters (FAMEs) of total lipids were obtained by transmethylation (AOAC, 1990). The temperature of the injector was 250 °C and the detector was 260 °C. Gas chromatographic (GC) analyses were performed using a Perkin Elmer Clarus 500 Series GC system, in split mode, 50:1, equipped with a flame ionization detector (FID) equipped with TR-FAME (Thermo Scientific) apolar capillary column (30 m x 0.25 mm and 0,25 m ID). Helium (0,5 mL/min) was used as carrier gas. The injector temperature was set at 250 °C and the FID was operated at 260 °C. An initial column oven temperature of 100 °C was elevated to 220 °C at a rate of 2 °C/min and held for 0 min. Fatty acid components were identified with the comparison of their retention times with those of authentic standards (Supelco 37 Comp. Fatty acid Mix, 18919). The relative peak area percentages of compounds were calculated based on the FID data. Fatty acid profiles of experimental fish feeds were presented in Table 2. All data on growth, feed conversion ratio, survival and fatty acid assessments of fish were expressed as means and ± standard error. Data from each treatment diet for each sampling period were analyzed by one-way ANOVA, and significant differences (if present) were ranked with Tukey's multiple comparison test at the %5 level of significance by using the MINITAB Release 13.1 Statistical Analysis Software Program for Windows, Version 10.0.1 (Minitab Inc., Chicago, Illinois, U.S.A.).

Table 2. Fatty Composition (%) of Experimental Fish Feed

Fatty acid numbers	Trivial name	%
C12:0	Lauric acid ME	0.095± 0.01
C14:0	Myristic acid ME	2.675± 0.01
C15:0	Pentadecanoic acid ME	0.260± 0.01
C16:0	Palmitic acid ME	16.025± 0.06
C17:0	Heptadecanoic acid ME	nd
C18:0	Stearic acid ME	5.125± 0.01
C20:0	Arachidic acid ME	0.530± 0.03
C21:0	Heneicosanoic acid ME	0.740± 0.01
C22:0	Behenic acid ME	0.010± 0.001^{b}
C24:0	Lignoceric acid ME	nd
Total SFA		**25.365 ± 0.042**
C14:1	Myristoleic acid ME	0.065± 0.007
C16:1	Palmitoleic acid ME	3.930± 0.014
C17:1	Heptadecenoic acid ME	0.165± 0.007
C18:1n9c	Oleic acid ME	36.375 ± 0.021
C22:1n9	Eruric acid ME	0.255± 0.007
Total MUFA		**40.785±0.049**
C18:2n6c	Linoleic acid ME	22.920± 0.071^{b}
C18:2n6t	Linoelaidic acid ME	nd
C18:3n6	γ-Linoleic acid ME	0.395± 0.007^{b}
C20:3n6	cis-8,11,14-eicosatrienoic acid ME	0.190± 0.014
C20:2	cis-11,14-eicosadienoic acid ME	0.990± 0.001
C22:2	cis-13,16-Docosadienoic acid ME	3.585± 0.007
Total n-6		**28.08± 0.057**
C18:3n3	Linolenic acid ME	4.78± 0.14^{b}
C20:3n3	cis-11,14,17-eicosatrienoic acid ME	0.650± 0.001
C20:5n3	cis-5,8,11,14,17-eicosapentanoic acid ME	Nd
C22:6n3	cis-4,7,10,13,16,19-docosahexanoic acid ME	0.250± 0.001
Total n-3		**5.680 ±0.141^{b}**
Total PUFA		**33.760 ±0.085**
n-3/n-6		**0.202±0.006^{b}**
Total FA		**100**

nd: not detected

Values in the same column with different superscripts significantly different (one-way ANOVA and Tukey's multiple-range test; *p<0.05).*

3. Results

Average water temperature, dissolved oxygen and pH levels were not different among the experiments throughout the feeding experiment. The fatty acid profiles (% composition) of the eggs obtained from fish (broodstock) and fish feed were presented in Table 3. The highest saturated fatty acid (SFA) concentration was obtained in brown trout as 33.197.

The C16:0 (palmitic acid) (17.923%) and C18:0 (Stearic) acids (7.27%) were the most common species of SFA defined in brown trout eggs.

Table 3. Fatty acid compositions (%) in fish feed and eggs of rainbow trout (*Oncorhyncus mykiss)* and Brown trout *(Salmo trutto spp.)*

Fatty acid numbers	**Trivial name**	**Brown trout egg**	**Rainbow Trout egg**	**Fertilized Rainbow Trout egg**	**Fish Feed**
C12:0	Lauric Asit ME	0.000± 0,00	0.000± 0,00	0.000± 0,00	0.095± 0.01
C14:0	Myristic acid ME	2.080± 0.38^{ab}	1.443± 0.32^{a}	1.590± 0.03^{a}	2.675± 0.01^{b}
C15:0	Pentadecanoic acid ME	0.000± 0.00	0.00± 0.00	0.233± 0.01^{a}	0.260± 0.01^{b}
C16:0	Palmitic acid ME	17.923± 4.43^{a}	14.210± 0.7^{a}	13.013± 0.19^{a}	16.025± 0.06^{a}
C17:0	Heptadecanoic acid ME	3.220± 1.02^{a}	0.887± 0.50^{b}	0.333± 0.02^{b}	0.000± 0.00
C18:0	Stearic acid ME	7.270± 3.51	4.580± 0.24	4.063± 0.16	5.125± 0.01
C20:0	Arachidic acid ME	0.000± 0.00	0.00± 0.00	0.000± 0.00	0.530± 0.03
C21:0	Heneicosanoic acid ME	0.000± 0.00	0.820± 0.67	1.173± 0.02	0.740± 0.01
C22:0	Behenic acid ME	2.703± 0.58^{a}	1.833± 0.41^{ab}	1.677± 0.05^{b}	0.010± 0.001^{c}
Total SFA		**33.197± 3.99**^{a}	**23.773± 0.73**^{ab}	**22.083± 0.19**^{b}	**25.365± 0.03**^{ab}
C14:1	Myristic acid	0.000± 0.00	0.000± 0.00	0.000± 0.00	0.065±0.01
C16:1	Palmitoleic acid ME	3.650± 0.15^{a}	4.157± 0.14^{bc}	4.263± 0.01^{b}	3.93± 0.01^{ac}
C17:1	Cis-10 Heptadekonoik acid	0.000± 0.00	0.000± 0.00	0.000± 0.00	0.165±0.01
C18:1n9c	Oleic acid ME	31.477± 2.73^{a}	35.087± 0.63^{ab}	32.837± 0.22^{ab}	36.375± 0.02^{b}
C22:1n9	Eruric acid ME	1.707± 0.29^{a}	2.320± 0.41^{a}	2.403± 0.09^{b}	0.255± 0.01^{b}
Total MUFA		**36.833± 1.70** ^{a}	**41.563± 0.48** ^{b}	**39.503± 0.17** ^{ab}	**40.785± 0.04** ^{ab}
C18:2n6c	Linoleic acid ME	24.793± 3.38	27.503± 0.10	26.050± 0.25	22.920± 0.07
C18:3n6	γ - Linoleic acid	0.000± 0.00	0.000± 0.00	0.000± 0.00	0.395±0.001
C20:3n6	cis-8,11,14-eicosatrienoic acid ME	2.290± 0.41^{a}	2.327± 0.24^{a}	2.403± 0.07^{a}	0.190± 0.0 ^{b}
C22:2	cis-13,16-Docosadienoic acid ME	0.000± 0.00	0.000± 0.00	0.000± 0.00	3.585± 0.01
Total n-6		**27.083± 2.17**	**29.830± 0.51**	**28.453± 0.17**	**27.090± 0.04**
C18:3n3	Linolenic acid ME	0.000± 0.00	0.000± 0.00	4.197± 0.07^{a}	4.780± 0.14^{b}
C20:3n3	Eikosadionik acid	0.000± 0.00	0.000± 0.00	0.000± 0.00	0.655±0.001
C20:5n3	cis-5,8,11,14,17-eicosapentanoic acid EPA	2.893± 0.50^{a}	4.357± 0.82^{b}	5.273± 0.18^{b}	0.000± 0.00
C22:6n3	Dokosaheksaenoik acid DHA	0.000± 0.00	0.000± 0.00	0.000± 0.00	0.250±0.01
Total n-3		**2.893± 0.29**^{a}	**4.357± 0.47**^{b}	**9.470± 0.07**^{c}	**5.680± 0.10**^{b}
Total PUFA		**29.977± 2.39**^{a}	**34.187± 0.06**^{ab}	**37.923± 0.17**^{b}	**32.770± 0.6**^{ab}
n-3/n-6		**0.107± 0.01**^{a}	**0.147± 0.02**^{a}	**0.333± 0.01**^{b}	**0.210± 0.01**^{c}
Total FA		**100**	**100**	**100**	**100**

Values are expressed as mean ± SD (n= 3) on a dry weight basis.
Values in the same column with different superscripts significantly different (one-way ANOVA and Tukey's multiple-range test; *p<0.05).*

The amount of monounsaturated fatty acids (MUFA) was significantly higher in rainbow egg than in brown trout ($p<0.05$). The oleic acid (C18: 1n9c) was the most common fatty acid in MUFA and it was 36.375% in feed.

The highest polyunsaturated fatty acid (PUFA) concentration was detected in fertilized rainbow trout eggs (37.923%) followed by rainbow trout eggs (34.187%), feed (32.77%) and brown trout eggs (29.977%) ($p<0.05$). The highest linoleic acid concentration (27.503%) was obtained in the rainbow trout eggs.

The highest total amount of n-6 was found in rainbow trout with 29.83%. This was followed by fertilized rainbow eggs (28.453%), feed (27.09%) and brown trout eggs (27.083%) (Table 3). The highest total amount of n-3 was found in fertilized rainbow eggs as 9.47%, and followed by rainbow trout (4.357%) and brown trout (2.893%). The feed used for feeding contained 5.68 % n-3.

When the eggs were examined, it was determined that EPA could not be detected in feed, 5.273 in rainbow fertilized eggs, 4.357 in rainbow eggs and 2.893 in brown trout eggs. The n-3/n-6 ratio of experimental groups and feed was in the order of fertilized rainbow trout eggs (0.333), (0.21), rainbow trout eggs (0.147) and brown trout eggs (0.107) (Table 3).

4. Discussion and Conclusion

The main goal in fish production is to obtain the highest quantity and quality of eggs and larvae. Egg quality parameters defined as egg characteristics, play a decisive role in the development of primitive egg cells, fertilization, embryonic development and survival rate of the larvae. The production of high quality eggs is an important problem for many fish species that are cultured and/or attemped to be cultured. Good quality juvenile fish production is only possible in hatcheries with high quality broodstocks, under the most favorable conditions, and proper feeding of broodstocks using feed especially high in n-3 fatty acids and compound feeds balanced for other nutrient levels. Feed quality and essential fatty acid levels concentration of feed used in broodstock feeding are the major criteria. The feeding of broodstock fish with n-3 series fatty acid feeds is of great importance in obtaining high number and quality of eggs, embryos, larvae and juvenile fish.

Despite the significant impact on the reproductive physiology of the food, studies on the effects of these changes on the quality of eggs and larvae are inadequate (Hardy, 1985; Watanabe, 1985; Bromage *et al*., 1992). The n-3 series fatty acids found in especially in PUFAs and their derivatives including DHA, EPA, linoleic and linolenic acids, vitamins (especially vitamins A, C and E), carotenoids and various trace elements have been effective on larval quality reproductive performance (Bromage and Roberts, 1995).

Energy requirement during embryonic development period and larval period is obtained from monounsaturated fatty acids, while fatty acids of the PUFA group especially the n-3 group have very important physiological functions for cell membrane. PUFAs are the main components of the phospholipids found in the membrane structure (Tocher *et al*., 1985).

Although there were no differences in PUFA values between experimental groups, statistically significant differences were found in SFA and MUFA values. The EPA in n-3 PUFAs was found the most in fertilized rainbow eggs. Bulut (2004) reported lower SFA ratio compared to that of MUFA fatty acids and PUFA fatty acids in fatty acids found in eggs of Dicentrachus labrax (*Dicentrachus labrax*). This result is in parallel with the amount of SFA determined in our study. In general, the ratio of unsaturated fatty acids was higher than the saturated fatty acids. Bulut (2004) also indicated that fatty acids of EPA, DHA, AA, PUFA, HUFA, MUFA, n-3 and n-6 which are important for egg composition should be at sufficient level in egg composition due to the reducing the survival rate of larvae in future stages.

Determining the composition of fat and fatty acids in broodstock feeds affects the success of reproduction and the survival rate of larvae. They have emphasised that high unsaturated fatty acids (HUFA) with carbon number twenty or more directly affect egg laying metabolism and HUFA's diet increases egg productivity, fertilization and egg quality in some fish species (Izquierdo *et al*., 2001; Kobayashi, *et al*., 2008; Xu *et al*., 2015).

Ballestrazzi *et al.,* (2003) reported the ∑n-3 series fatty acids of trout eggs between 27.60 and 40.35%, the ∑n-6 series fatty acids between 6.31 and 6.72% and the ∑n-3/∑n-6 ratio between 4.04 and 6.69 which are higher than those foun in our study. In a different study, Haliloğlu *et al.,* (2003) determined the ∑n-3 series fatty acids as 26.04%, the ∑n-6 series fatty acids as 15.72% and the ∑n-3/∑n-6 ratio as 1.63. All three of the data reported by Haliloğlu *et al*., (2003) are higher than those reported in our study.

Fish cannot be able to synthesize essential fatty acids which must be supplied with feed. Marine fish meet fatty acid requirements from algae and phytoplankton, which are rich in EPA, DHA, linoleic and linolenic acid. Essential fatty acids are supplied with feed in cultured fish. The fatty acids taken from feed are the only sources of essential energy and essential fatty acids for fish in aquaculture (Sargent *et al*., 2002).

Individuals can survive healthy from egg stage to broodstock stage by using essential fatty acids taken from the feed. Broodstock fish should be fed with quality feed to provide high quality eggs in appropriate conditions. Therefore, it was reported that the levels of essential fatty acids in the feed consumed by broodstock fish were reflected in fatty acids in fish eggs. Fish should be healthy to give healthy and environmentally resistant individuals. Fish must contain sufficient quantities of some essential fatty acids in their bodies. Sufficient amount of essential fatty acids in fish is highly important to produce healthy individuals and increase the high percentage of survival rate (Bromage *et al*., 1992; Sargent *et al*., 2002).

Fish flour and oil should be included in fish feed, and rations should be prepared accordingly. Many studies reported that fish oil is sufficient for feeding fish in terms of n-3 and n-6. Healthier fish can be obtained from eggs and larvae of broodstock fish fed with the feed prepared in this way. Healthy individuals will certainly reflect the fatty acids taken by feed in their structures. The results clearly revealed that healthy individuals can be obtained from broodstock fish fed with high quality feed.

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