https://doi.org/10.52973/rcfcv-e35565 Revista Científica, FCV-LUZ / Vol. XXXV

The profile of fatty acids in the gametes of brook trout (*Salvelinus fontinalis*) during the spawning period under culture conditions

Perfil de ácidos grasos en gametos de trucha de arroyo (*Salvelinus fontinalis*) cultivada en época de desove

Mehmet Kocabaş[1], Filiz Kutluyer-Kocabaş[2*], Nadir Başçinar[3], Esin Özçiçek[2], Görkem Kirmizikaya-Özmen[4], Ökkeş Yilmaz[4]

[1]Karadeniz Technical University Faculty of Forestry, Department of Wildlife Ecology and Management, Trabzon, Turkey.
[2]Munzur University, Fisheries Faculty, Tunceli, Turkey.
[3]Department of Fisheries Technology Engineering, Faculty of Marine Sciences, Karadeniz Technical University, Trabzon, Turkey.
[4]Faculty of Science, Department of Biology, Fırat University, Elazığ, Turkey.
*Corresponding author: filizkutluyer@hotmail.com

ABSTRACT

The fertilization and subsequently embryo and larvae development are linked to gamete quality and FA (fatty acid) content is one of the specific factors affecting egg or sperm quality. Hence, FA profile of gametes (egg and sperm) was documented in brook trout (*Salvelinus fontinalis*) under cultivation. FAs were detected and measured using gas chromatography. These results indicated that a total of 26 and 22 FAs were determined in egg and sperm, respectively. The levels of PUFAs were determined in nearly identical quantities in both egg (47.57%) and sperm (47.53%). DHA (Docosahexaenoic acid), LA (Linoleic acid), EPA (Eicosapentaenoic acid) and ARA (Arachidonic acid) were predominant PUFAs for egg and sperm. Interestingly, vaccenic acid (C18:1 n-11) (24.07%) was major FAs in egg. In conclusion, the analysis of fatty acids in broodstock gametes contributes valuable insights into the dietary requirements necessary for effective aquaculture breeding programs.

Key words: Brook trout; Salvelinus fontinalis; egg; sperm; fatty acid

RESUMEN

La fertilización y posterior desarrollo de embriones y/o larvas está relacionada con la calidad de los gametos y su composición en ácidos grasos (AG), siendo este uno de los factores específicos que afectan la calidad del óvulo o del esperma. Por lo tanto, evaluamos el perfil de AG de los gametos (óvulos y espermatozoides) en trucha de arroyo (*Salvelinus fontinalis*) en cultivo. Los AG se detectaron y midieron mediante cromatografía de gases. Nuestros resultados indicaron que se detectaton un total de 26 y 22 AG en óvulos y espermatozoides, respectivamente. Los niveles de AGPI se determinaron en cantidades casi idénticas tanto en óvulos (47,57%) como en espermatozoides (47,53%). DHA (ácido docosahexaenoico), LA (ácido linoleico), EPA (ácido eicosapentaenoico) y ARA (ácido araquidónico) fueron los PUFA (Ácido Graso Poliinsaturado) predominantes para óvulos y espermatozoides. Curiosamente, el ácido vaccénico (C18:1 n-11) (24,07%) fue el principal AG en el huevo. En conclusión, el análisis de los ácidos grasos en los gametos de los reproductores aporta información valiosa sobre los requisitos dietéticos necesarios para programas eficaces de cría en acuicultura.

Palabras clave: Trucha de arroyo; Salvelinus fontinalis; óvulo; esperma; ácido graso

Recibido: 09/11/2024 Aceptado: 31/01/2025 Publicado: 15/04/2025

INTRODUCTION

Fatty acids (FAs) are vital for life for movement, growth and reproduction as they serve as fundamental building blocks for cell membranes, provide energy, and act as crucial mediators in metabolic and signaling mediators [1 , 2] The FAs content in fish varies based on several factors such as the aquatic ecosystems, as well as the physico-chemical and biological properties of aquatic bodies. Moreover, the FAs content is influenced by seasonal variations, age and size of fish, migration behaviors, sexual maturity, reproductive cycles, species-specific characteristics, the type and amount of feed available, feeding preferences, and whether they are reared in aquaculture or inhabit natural environments [3 , 4 , 5 , 6 , 7]

As a key issue in aquaculture, gamete (sperm and egg) quality is a restrictive factor influencing reproductive success [8]. Gamet quality (viability and motility in sperm, reaction of acrosome, fluidity of membrane, and hatching and survival rate) is associated with fatty acid (FA) content [9 , 10 , 11]. The FAs in sperm membranes affect function and structure in sperm [11 , 12]. FAs bound to phospholipids form an essential part of cell membranes, playing a critical role in their structural integrity [13]. More importantly, the subsequent embryonic and larval development are ensured by essential FAs [8].

Salvelinus fontinalis (Mitchill, 1814) is a freshwater species cultivated in Canadian commercial aquaculture. In North America and Europe, trout production remains a significant industry with growth expectations although trout production is lower than that of other salmonids, such as rainbow trout and Atlantic salmon [14]. Brook trout is an introduced species in Turkey, brought from Europe primarily for aquaculture purposes [15]. Currently, brook trout are farmed in a few rainbow trout facilities in the eastern Black Sea region however, its production has not been established in Turkey commercially [15 , 16]. As far as we are aware, this is the first study to examine the fatty acid profile of gametes in brook trout, although research has been conducted on the FA content of muscle, yolk, and fry during the development of brook trout [16 , 17 , 18 , 19 , 20 , 21 , 22]. In this context, the main goal of this study was to investigate the FA profile of egg and sperm in sexually mature brook trout (*S. fontinalis*).

MATERIAL AND METHODS

Animals and sampling

Sperm and egg samples were collected from the mature brook trout under culture conditions (Karadeniz Technical University, Faculty of Sürmene Marine Sciences, Prof. Dr. İbrahim Okumuş Research Center, Trabzon, Turkey) between November and December, 2023. The Ethics Committee for the Use of Animals at the National Institute for Research, Munzur University (Tunceli, Turkey), granted approval for all animal experiments (the protocol number 2024/36-04). The sperm and egg collection involved anesthetizing the fish with benzocaine (0.5 mg/L) and then using soft abdominal pressure for stripping. Falcon tubes (50 mL) were used to collect sperm and egg from fish. To prevent sperm activation and contamination (such as blood, mucus, urine, or feces), the abdomen surface was dried using paper towels. Subsequently, the samples were kept on crushed ice for sperm analysis. Finally, the samples were placed into plastic containers and stored at -85°C in ULT Freezer (DW-86L728BPST, Qingda, China) until further analysis.

Fatty acid profile

After thawing, hexane-isopropanol mixture (10 ml) was used for homogenization of samples. The method for lipid extraction from sperm and egg samples followed the protocol described by Hara and Radin [23]. FAMEs (Fatty acid methyl esters) were synthesized via trans-methylation with 2% methanolic sulfuric acid and analyzed by gas chromatography (GC) (Shimadzu GC-2010 Plus, Tokyo, Japan) fitted with an Rtx® 2330 GC column (30 m, 0·25 mm ID, 0·25 µm df). The hexane phase was evaporated using a nitrogen flow, and the lipid extract was then re-suspended in 1 mL of heptane. Subsequently, the solution was divided into 2-mL aliquots and placed into autosampler vials, as outlined by Christie [24]. Prior to analyzing the FAs in the samples, the oven temperature was initially set to 148°C and held for 1 min. The temperature was then raised to 200°C at a rate of 5°C per minute and subsequently increased to 218°C at a rate of 4°C per minute. Prior to the analysis, the standard fatty acid methyl ester mixture (Supelco® 37 Component FAME Mix) was used to determine the retention times of the protective structural FAs. The data processing was facilitated by Class GC (10 software version 2.01). The Shimadzu GC 2010 Plus operated using the GC Solution operating program for sample and standard analysis. Calculation of results utilized the Lab Solution 5.67 program (Kyoto, Japan). The relative amounts of FAMEs were assessed using the external standard method. The Area Normalization mode was used for calculation of Relative values of each fatty acid within the total fatty acids.

Data analysis

IBM SPSS Statistics 27.0 for Windows was used for data analysis, and the results were presented as mean ± SD (standard deviation). Differences FAs content between egg and sperm were assessed using independent t-test with significance set at $P < 0.05$ (95% confidence level). XLSTAT software (version 2015.5) was used to perform PCA (Principal Component Analysis).

RESULTS AND DISCUSSION

By comparing egg and sperm, this study presents the first analysis of FAs composition in brook trout. In eggs, a total of 26 FAs were assessed whereas sperm analysis revealed a total of 22 FAs. Both the egg and sperm of brook trout exhibit high levels of PUFAs (polyunsaturated fatty acids) (mostly n-3 HUFA, egg: 30.13% and sperm: 33.86%).

TABLE I shows the FA profiles of egg and sperm of brook trout during the spawning season. The area graph of the FA profile of egg and sperm in brook trout is presented in FIG.1. Significant differences were observed in SFAs, C17:0, C18:0, C21:0, C22:0 and C23:0 between egg and sperm ($P < 0.05$). A total of 8 SFAs (C14:0, C15:0, C16:0, C17:0, C18:0, C21:0, C22:0 and C24:0) were assessed in egg while a total of 9 SFAs (C14:0, C15:0, C16:0, C17:0, C18:0, C21:0, C22:0, C23:0 and C24:0) was determined in sperm. The egg contained the highest concentration of PUFAs (47.57%), followed by MUFAs (29.81%) and SFAs (21.7%). In contrast, sperm showed the highest concentration of PUFAs (47.53%), with SFAs (35.82%) and MUFAs (16.83%) following in concentration. Palmitic acid (C16:0) constituted the predominant SFA in both egg (13.29%) and sperm (22.95%). Additionally, DHA (C22:6 n−3) emerged as the principal PUFA in both samples. The primary MUFA was vaccenic acid (C18:1 n-11) in egg (24.07%) while oleic acid (C18:1 n-9) dominated as the major MUFA in sperm (10.78%).

Previous studies on salmonid sperm have documented higher concentrations of unsaturated FAs in comparison to SFAs [25 ,26]. In accordance with earlier works, the PUFAs (47.53%) were higher quantities compared to SFAs (35.82%). In addition, the sperm of brook trout exhibited a similar rate for PUFAs (primarily n-3 highly unsaturated fatty acids, 30.13%) with egg (mostly n-3 PUFA, 33.86%).

Specific long-chain FAs, such as LA (C18:2 n−6), ARA (20:4 n-6), EPA (20:5 n-3), and DHA (22:6 n-3), are essential components of gametes, playing pivotal roles in their structural integrity and biological functions. They are involved in the production of biologically active molecules, including prostaglandins and other eicosanoids. These compounds actively participate in shaping reproductive development and are pivotal for achieving successful reproduction overall [27 ,28]. In present study, the egg and sperm of brook trout are characterized by high PUFA. DHA (egg: 21.03%, sperm: 18.99%), LA (egg: 14.31%, sperm: 8.56%), EPA (egg: 3.78%, sperm: 12.75%) and ARA (egg: 2.59%, sperm: 5.11%) were predominant PUFAs.

TABLE I. The fatty acid composition (% of total fatty acids) of egg and sperm in *Salvelinus fontinalis*

Fatty acids	Egg	Sperm	F value	P value
SFA (Saturated fatty acid)				
C14:0 (Myristic acid)	1.13±0.18	0.99±0.22	0,289	0,598
C15:0 (Pentadecanoic acid)	0.27±0.04	0.22±0.04	0,015	0,903
C16:0 (Palmitic acid)	13.29±1.44	22.95±2.19	0,129	0,723
C17:0 (Margarinic acid)	0.41±0.12	0.35±0.05	13,639	0,002*
C18:0 (Stearic acid)	4.04±0.64	10.01±10.70	4,807	0,043*
C21:0 (Heneicosanoic acid)	1.89±1.74	0.67±0.24	76,947	0.000*
C22:0 (Behenic acid)	1.62±1.31	1.10±0.39	13,605	0,002*
C23:0 (Tricosanoic acid)	0±0	0.40±1.26	4,53	0,048*
C24:0 (Lignoceric acid)	0.15±0.05	0.10±0.07	1,537	0,232
MUFA (Monounsaturated fatty acid)				
C14:1 (Myristoleic Acid)	0.03±0.02	0.02±0.06	0,589	0,454
C15:1 (Ginkgolic acid)	0.02±0.01	0.18±0.10	5,863	0,027*
C16:1 n−7 (Palmitoleic acid)	1.97±0.26	0±0	0,474	0,5
C17:1 (Heptadecenoic Acid)	0.14±0.06	0.16±0.05	2,174	0,159
C18:1 n-9 (Oleic acid)	0±0	10.78±3.35	8,81	0,009*
C18:1 n-11 (Vaccenic acid)	24.07±1.52	3.98±0.69	0,272	0,608
C20:1 (Eicosenoic acid)	1.77±0.56	0.21±0.22	4,539	0,048*
C22:1 n-9 (Erucic acid)	0.42±0.14	0±0	15,595	0,001*
C24:1 (Tetracosenic acid)	0.29±0.04	0.53±0.21	3,693	0,072
PUFA (Polyunsaturated fatty acid)				
C18:2 n−6 (Linoleic acid)	14.31±2.16	8.56±1.70	1,532	0,233
C18:3 n−3 (α-Linolenic acid)	1.87±0.46	0.59±0.17	3,659	0,073
C18:3 n−6 (γ-Linolenic acid)	0.54±0.30	0±0	7,717	0,013*
C20:3 (dihomo-gamma-linolenic acid)	1.58±1.70	0±0	354,126	0.000*
C20:3 n−3 (Eicosatrienoic acid)	1.08±1.17	0±0	400,835	0.000*
C20:4 n−6 (Arachidonic acid)	2.59±0.86	5.11±0.75	0,039	0,846
C20:5 n−3 (Eicosapentaenoic acid)	3.78±0.28	12.75±2.16	5,142	0,037*
C22:5 n−3 (Docosapentaenoic acid)	0.79±0.25	1.53±2.81	3,441	0,081
C22:6 n−3 (Docosahexaenoic acid)	21.03±1.07	18.99±6.90	3,305	0,087
ΣSFA	21.7	35.82		
ΣMUFA	29.81	16.83		
ΣPUFA	47.57	47.53		
Σn−3	30.13	33.86		
Σn−6	17.44	13.67		
n-3/ n−6	1.73	2.47		

Data expressed as mean ± standard error; *the same row are significantly different at P < 0.05

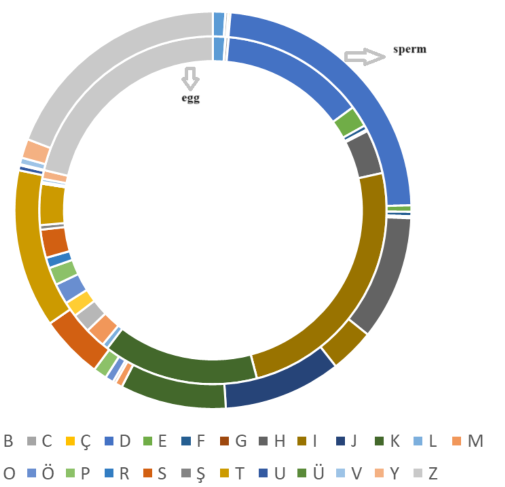

FIGURE 1. Area graph of fatty acid composition (%) of egg and sperm in Salvelinus fontinalis. A (C14:0), B (C14:1), C (C15:0), Ç (C15:1), D (C16:0), E (C16:1 n7), F (C17:0), G (C17:1), H (C18:0), I (C18:1 n9), J (C18:1 n11), K (C18:2 n6), L (C18:3 n6), M (C18:3 n3), N (C20:1), O (C20:3), Ö (C21:0), P (C22:0), R (C20:3 n3), S (C20:4 n6), Ş (C22:1n9), T (C20:5 n3), U (C23:0), Ü (C24:0), V (C24:1), Y (C22:5 n3). Z (C22:6 n3)

Palmitic acid and stearic acid constituted the majority of the total SFA content in the egg and sperm of brook trout in accordance with earlier findings in other Salmonid species [3 ,4 ,6 ,7 ,29 ,30 ,31 ,32 ,33 ,34 ,35 ,36 ,37 ,38]. Interestingly, Tricosanoic acid (C23:0) was not determined in eggs of brook trout.

The role of oleic acid (C18:1 n-9) on natural reproduction, gonadal maturation, and embryonic development processes have been emphasized by earlier studies [24 ,39]. In current work, oleic acid was assessed as the predominant monounsaturated fatty acid (MUFA) in sperm, exhibiting levels consistent with findings in eggs, sperm, and tissues of various other fish species [3 ,31 ,38 ,40 ,41 ,42 ,43 ,44 ,45]. Interestingly, oleic acid was not determined in egg of brook trout. Furthermore, the primary MUFA was vaccenic acid (C18:1 n-11) in the eggs (24.07%). In addition, the proportion of total MUFA in eggs (29.81%) in the current study was higher compared to sperm (16.83%).

In the current study, it was observed a higher n-3/n-6 ratio in sperm (2.47) than in eggs (1.73). This ratio in eggs plays a significant role in determining the hatching success and survival of larvae [9], with typical values ranging from 1 to 4 in freshwater fish eggs [3].

The n-3/n-6 ratio in the eggs of the studied brook trout was lower compared to values reported in other Salmonid fish species, namely Black Sea trout (7.56) [3], Arctic char (*Salvelinus alpinus*) (2.21-2.34) [46], rainbow trout (2.01) [31], and trout (*Salmo opimus*) (1.06-3.13) [35] and but it was similar with reported the n-3/n-6 ratio (1-21-1.88) in rainbow trout by Baki *et al.* [36]. In the sperm, n-3/n-6 ratio of the studied brook trout was lower compared to values demonstrated in other Salmonids such as *Oncorhynchus mykiss* (4.4% and 4.69%) [32 ,38], *Salmo trutta fario* [38], *Salvelinus alpinus* (4.7-5.3) [41], but it was higher level than reported the n-3/n-6 ratio (1.13%) in *O. mykiss* by Lahnsteiner *et al*. [30].

PCA analysis demonstrates alterations in the FAs compositions of egg and sperm. According to FIG.2, PC1 (the primary principal component: 44.24%) and PC2 (the second principal component: 18.09%) collectively explained 62.33% of the variance. C18:3 n-6 was strongly related to C18:3 n-3 and C22:1 n-9. C4:0 was strongly related to C6:0, C8:0, C18:0, C20:2 and C22:5 n–6. C15:1, C16:0, C18:1 n11, C20:4 n-6, C20:5 n-3 and C24:1 have negative scores featuring the gametes. PCA was conducted to investigate relationships among variables. In eggs, γ-Linolenic acid (C18:3 n-6) demonstrated strong correlations with total Erucic acid (C22:1 n-9) and α-Linolenic acid (C18:3 n-3). In contrast, C15:1, C16:0, C18:1 n-11, C24:1, ARA, and EPA exhibited negative scores in sperm. These variations in correlations could be influenced by biological factors and individual differences [5 , 37].

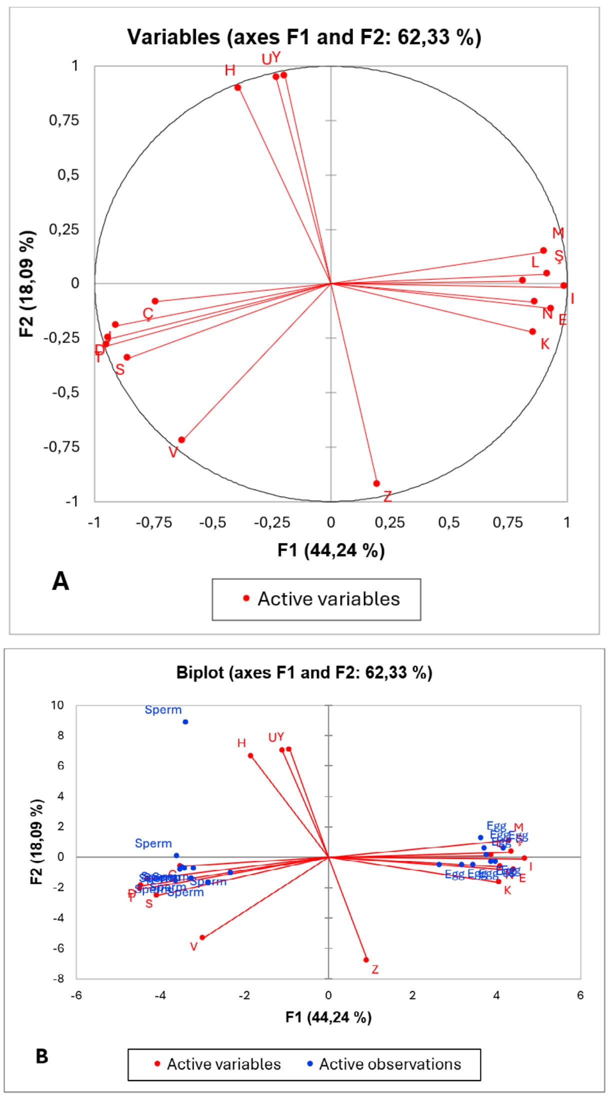

FIGURE 2. Illustration of scores (A) and loadings (B) from the Main Component Analysis of fatty acids of egg and sperm in Salvelinus fontinalis. A (C14:0), B (C14:1), C (C15:0), Ç (C15:1), D (C16:0), E (C16:1 n7), F (C17:0), G (C17:1), H (C18:0), I (C18:1 n9), J (C18:1 n11), K (C18:2 n6), L (C18:3 n6), M (C18:3 n3), N (C20:1), O (C20:3), Ö (C21:0), P (C22:0), R (C20:3 n3), S (C20:4 n6), Ş (C22:1n9), T (C20:5 n3), U (C23:0), Ü (C24:0), V (C24:1), Y (C22:5 n3). Z (C22:6 n3)

CONCLUSION

In conclusion, the egg exhibited notably higher percentages of DHA, LA (linoleic acid), palmitic acid, and vaccenic acid compared to other FAs while DHA, EPA, oleic acid, stearic acid and palmitic acid were the predominant FAs in sperm. The total quantity of n-3 FAs in sperm was higher than in eggs, whereas the total amount of n-6 FAs in eggs was higher than in sperm. This study presents the first comprehensive analysis of the complete FAs composition in the egg and sperm of brook trout. It will serve as a valuable resource for developing an optimal formulation for a specialized broodstock diet enriched with sufficient FAs, thereby maximizing productivity for fish farmers.

Ethics approval

All animal experiments were approved by the Ethics Committee for the Use of Animals of the National Institute for Research in the Munzur University (Tunceli, Turkey) (the protocol number 2024/36-04).

Conflict of Interest

The authors declare no conflict of interest.

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