Pakistan J. Zool., pp 1-8, 2022. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20211112131153

Evaluation of Relationship between Sperm Cell Velocities and Fatty Acids Contents of Semen Seminal Fluid in the Two Trout Fish Species

Mustafa Erkan Özgür[1]*, Selim Erdoğan[2], Songül Aydemir[3] and Hatice Yumuşakbaş[2]

[1]*Department of Aquaculture, Vahap Kücük Vocational High School, Malatya Turgut Özal University, 44210, Malatya, Turkey*
[2]*Department of Analytical Chemistry, Faculty of Pharmacy, İnönü University, 44530, Malatya, Turkey*
[3]*Department of Biology, Faculty of Arts and Sciences, İnönü University, 44530, Malatya, Turkey*

Article Information
Received 12 November 2021
Revised 28 November 2021
Accepted 07 December 2021
Available online 06 May 2022
(early access)

Authors' Contribution
MEÖ and SE conceived and supervised the study. SE and HY performed analytical analyses for gas chromatography. All authors collected and analyzed data, contributed to the critical revision of the manuscript and have read and approved the final version.

Key words
Fatty acids, Semen seminal plasma, Sperm cell velocity, *Oncorhynchus mykiss*, *Salmo trutta fario*

ABSTRACT

In this study, the fatty acid compositions of semen seminal plasma in rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta fario*) were analyzed in fresh semen by gas chromatography (GC) and investigated the relationships with the sperm cell velocities. According to our results, total levels of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) for *Salmo trutta fario* occurred in higher quantities than *Oncorhynchus mykiss* but the quantities of total polyunsaturated fatty acids (PUFA) were less. Palmitic and stearic acid for SFA, oleic and nervonic acids for MUFA and linolenic, arachidonic, eicosapentaenoic and docosahexaenoic acids for PUFA of semen seminal plasma were main. Semen volume and the levels of SFA (myristic, palmitic and margaric acid), MUFA (palmitoleic, ginkgolic, oleic and nervonic acid) and PUFA (linoleic, gama-linolenic and eicosadienoic acid) were significantly different ($p<0.05$) between both species. Especially, the levels of eicosapentaenoic (EPA) and docosahexaenoic (DHA) determined no significant different ($p>0.05$). Additionally, we found negative correlation between the straight line velocity (VSL) and pentadecanoic acid ($r = -0.78$, $p<0.05$), stearic acid ($r = -0.76$, $p<0.05$) and nervonic acid ($r = -0.89$, $p<0.01$) for rainbow trout, while there was negative correlation ($r = -0.96$, $p<0.05$) between angular path velocity (VAP) and EPA for brown trout. Finally, it can be concluded with these results that the fatty acids of sperm seminal plasma affect the velocities of sperm cells and they would also improve semen quality and fertilization protocols.

INTRODUCTION

The control of performance in brood stock fishes in hatchery stations of fish farms is very important for their gametes quality. So, the semen quality is a very base issue for the aquaculture industry and biotechnological researches (Bobe and Labbé, 2010; Cabrita *et al*., 2014).

Many biotic and abiotic factors affect egg and semen quality and yield. Among the fatty acids, docosahexaenoic (DHA) and eicosapentaenoic acids (EPA) in polyunsaturated fatty acids (PUFA), vitamins (especially Vitamin A, C and E), carotenoids and various trace elements are effective on reproductive performance (Hardy, 1996). However, palmitic, arachidonic and linoleic acid in semen seminal plasma of rainbow trout effected positively on sperm cell viability, motility and average, also fertility rate path velocity when sperm cells activated (Lahnsteiner *et al*., 2009; Mansour *et al*., 2011). However, the sperm cells are immobilized in the testis and seminal fluid, and the initial of motility depends on the conditions of fertilization environment in teleost fish. The semen seminal plasma has very important ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, HCO_3^- etc.) which support the viability, motility or immobilization of sperm cells (Alavi and Cosson, 2006; Browne *et al*., 2015; Dzyuba and Cosson, 2014). On the other hand, it was also determined that the levels of PUFA and highly unsaturated fatty acids (HUFA) are more than the level of saturated fatty acids (SFA) in the fish semen and they effected the motility of sperm cells in the wild burbot (*Lota lota*) (Blecha *et al*., 2018).

This study aims (a) to determine the levels of fatty acids in the semen seminal plasma of fish fed with standard commercial feeds and (b) to investigate and understand the effects of fatty acids in semen seminal plasma on the kinematic characteristics of sperm cells in rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta fario*). We hope that our results contribute to assess reproductive capacities of rainbow trout commercial farms.

* Corresponding author: mustafa.ozgur@ozal.edu.tr
0030-9923/2022/0004-0001 $ 9.00/0

MATERIALS AND METHODS

Broodstocks care

Male brood stocks of rainbow trout (*Oncorhynchus mykiss* 1485.42±150.23 g) and brown trout (*Salmo trutta fario* 1350.00±281.65 g) were obtained from commercial farm in Malatya province, Turkey. For this study, it was noted that the average weights of both species were close to each other. Ten males of both species were stocked into 400-L fiberglass tank with a constant flow of well water. Fish of experiment adapted were in this tank for 2 weeks.

Fish were fed a commercial pellet diet (BioAqua, İzmir, Turkey) containing 46% raw protein, 16% raw fat, and 2% raw fibre. The digestible energy was 23.3 MJ/kg. Feeding was stopped two days before stripping and then semen samples were collected from males. The weight of the male fish was measured with a digital balance (1 g precision). The temperature (°C), pH and dissolved oxygen (DO) (mg/L) of water in tank was measured daily by Hatch Lange HQ40D multi-meter.

The sperm quality parameters

The semen samples of both species were collected in January 2018 in Malatya, Turkey. The semen from male fish was obtained by massage from the front to back of the fish abdomen without anesthesia. Fresh semen samples collected from seven individuals of *Oncorhynchus mykiss* and *Salmo trutta fario*. 10 µL of fresh semen samples used for sperm cell kinematic parameters, other part of fresh semen used for seminal plasma fatty acid analysis. The semen samples were kept on the ice until investigation.

Semen samples were diluted with inactivation solution (NaCl, 103 mmol/L; KCl, 40 mmol/L; CaCl2, 1 mmol/L; MgSO4, 0.8 mmol/L; Hepes, 20 mmol/L; pH 7.8; ratio 1:100, Semen:IS) (Özgür, 2018). The sperm cells were activated to determine the kinematic parameters with activation solution (CaCl2 1mM; Tris 20 mM, Glycine 30 mM, NaCl 125 mM; pH 9) (Özgür, 2018) at ratio 1:20 (Semen:AS) under microscope. The kinematic parameters of sperm cells were analyzed by the computer assisted semen analysis system, BASA-Sperm Aqua which has Olympus BX31 microscope (200x magnification) and CCD camera (30 fbs) by Merk Biotechnology Ltd. Co. in Turkey. The following setting were used in the picture settings and parameters of BASA Sperm Aqua: Acquired time delay of 0, an image field maximum of 60; images per record of 90, frame per second minimum of 30, track immotile level of 5, track motile level of 25, velocity maximum of 500. The values of motility parameters such as VSL (straight line velocity, µm/s), VCL (curvilinear velocity, µm/s), VAP (angular path velocity, µm/s), LIN (linearity, %, (VSL/VCL)*100), BCF (beat cross frequency, Hz) and ALH (amplitude of lateral displacement of the sperm cell head, µm) (Özgür *et al*., 2019) are examined in the study.

Fatty acids analysis

The fatty acid contents in semen seminal plasma were investigated in fresh semen from seven mature males for both species. Semen suspensions were stored in 10 ml tubes at 4°C for 48 h. Each subsample was centrifuged at 300× g for 10 min. Lipids were extracted from semen seminal plasma into a chloroform:methanol mixture (2:1/v:v) (Bligh and Dyer, 1959). After extraction, the extraction solution was evaporated under a stream of nitrogen and the lipid was stored at -20°C until analysis.

For saponification and esterification of semen samples, 60 mg of extracted oil was added 10 ml n-hexane (Merck, Darmstadt, Germany) and then was saponified with 0.5 mL of methanolic NaOH (0.5 M) solution by refluxing for 10 min. Then, the upper hexane layer containing the fatty acid methyl esters (FAME) was placed in mini-vials (Metcalfe *et al*., 1966; Sahari *et al*., 2013).

The fatty acid methyl esters were analyzed by GC-FID-QP2010 Ultra (Shimadzu Corporation, Kyoto, Japan) GC fitted with a AOC series auto-sampler, Restek Rt-2560 capillary column (100 m×0.25 mm i.d.×0.2 µm film thickness), FID detector, and integrated with Shimadzu GC Solution software. The operating conditions were that oven temperature was 140°C for 5 min then warming to 240°C at a rate of 25°C/min, 240°C for 15 min; injection port temperature in 250°C; detector temperature in 250°C; split injection volume with Helium (>99.996%) was used as carrier gas (25 cm/s) in 1:1 rate; split ratio: 50:1. Methyl ester standards (Supelco 37 Comp. Fame Mix 10 mg/ml in CH_2CI_2) were used to identify peaks. The results were reported as a proportion of total fatty acids by using correction factors (Ackman, 2002).

Statistical analysis

It was used to nonparametric Mann–Whitney U test and to define differences and pearson correlation to determine the relationship between sperm cell kinematics and fatty acid contents of both fish species. Normality test was done by sample Kolmogorov/Smirnov test as nonparametric test. However, it was used principal component analyses (PCA) for sperm cells kinematic and fatty acid contents of sperm seminal plasma to reduce the number of correlated variables. The varimax with Kaiser Normalization method was used for rotation in PCA analysis. Results are shown as a mean ± standard deviation (Mean ± SD) indicated with $p<0.05$ and $p<0.01$.

RESULTS

In experimental water quality parameters of this study, the water temperature as 10.5±0.1°C, pH

as 7.8±0.2 and dissolved oxygen as 9.9±0.43 mg/L were calculated and they were insignificant ($p>0.05$) statistically. For fatty acids of semen seminal plasma in both species, chromatographic profiles in the optimum Gas Chromatography (GC) conditions recorded at 280 nm of extracts obtained from samples is illustrated in Figure 1. Our results which are sperm kinematic parameters and fatty acids concentrations (% of total fatty acids) in both species are shown in Tables I and II, respectively. There was significant ($p<0.05$) difference as statistically in semen volume between species.

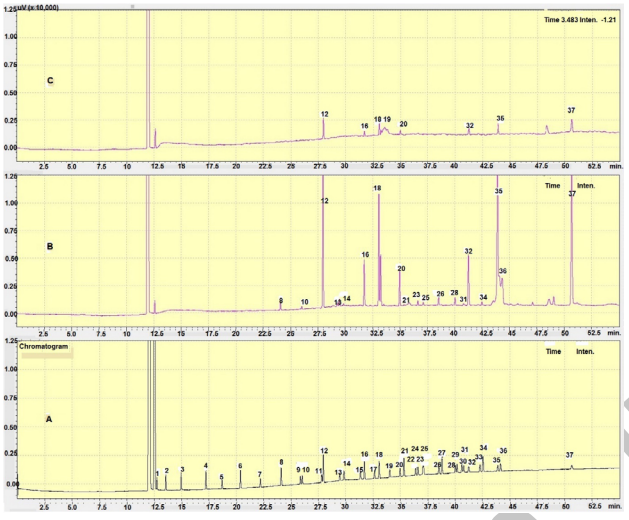

Fig. 1. Fatty acid chromatogram of the standards (A) and semen seminal plasma of Rainbow trout (B) and Brown trout (C) with GC-FID. 1- C4:0, 2- C6:0, 3- C8:0, 4- C10:0, 5- C11:0, 6- C12:0, 7- C13:0, 8- C14:0, 9- C14:1, 10- C15:0, 11- C15:1, 12- C16:0, 13- C16:1, 14- C17:0, 15- C17:1, 16- C18:0, 17- C18:1n9t, 18- C18:1n9c, 19- C18:2n6t, 20- C18:2n6c, 21- C20:0, 22- C18:3n6, 23- C20:1, 24- C18:3n3, 25- C21:0, 26- C20:2, 27- C22:0, 28- C20:3n6, 29- C22:1n9, 30- C20:3n6, 31- C23:0, 32- C20:4n6, 33- C22:2, 34- C24:0, 35- C20:5n3 (EPA), 36- C24:1, 37- C22:6n3 (DHA).

While total SFA and MUFA in semen seminal plasma of brown trout determined in higher quantities than rainbow trout but the quantities of total PUFA was less than brown trout. However, total PUFA levels of semen seminal plasma in both species were the highest. Palmitic and stearic acid were main SFA, oleic and nervonic were main MUFA, and linolenic, arachidonic, eicosapentaenoic and docosahexaenoic acid were main PUFA of semen seminal fluid. In the investigated fatty acids, while C16:0 was found in concentrations >20 % of total fatty acids in brown trout, it was less in rainbow trout. The levels of C18, C18:2n6c were found was found <5 % of total fatty acids for each species. The levels of C24:1 was found less <5 % of total fatty acids for only rainbow trout but C24:1 for brown trout and C18:1n9c for each species were more amount >10 % of total fatty acids. However, while C14 and C18:3n6 were found in concentrations equal of 1 % of total fatty acids for only brown trout, C14, C15, C17, C21, C24, C16:1, C17:1, C20:1, C18:3n6, C20:2 and C20:3n6 in concentrations of <1 % of total fatty acids for each species. C20:5n3 (EPA) was found in concentrations between 5-10 % of total fatty acids for each species. The levels of C22:6n3 (DHA) was found >20 % of total fatty acids for rainbow trout, but it was >10 % of total fatty acids for brown trout (Table II).

Table I. Semen quality parameters (Mean±S.D.) of rainbow trout and brown trout.

Parameters	Rainbow trout (n=7)	Brown trout (n=7)	Sig. (2-tailed)
Volume of semen (ml)	30.25±15.35	12.45±4.74	0.023*
Duration of sperm cell (second)	17.20±3.88	15.86±4.35	0.774
VSL (µm/sn)	48.82±13.19	57.14±4.12	0.260
VCL (µm/sn)	114.16±6.66	121.06±16.13	0.334
VAP (µm/sn)	51.53±8.60	61.16±2.95	0.062
LIN (%)	19.66±2.46	30.37±7.75	0.072
BCF (Hz)	10.52±1.55	9.20±4.36	0.474
ALH (µm)	21.85±2.76	27.46±10.36	0.380
pH of sperm	7.75±0.23	7.35±0.73	0.528
Weight of male broodstock, g	1485.42± 150.23	1350.00± 281.65	0.225
Length of male broodstock, cm	46.35±1.20	47.34±5.32	0.366

* Significant difference as statistically, $p<0.05$. VSL, straight line velocity; VCL, curvilinear velocity; VAP, angular path velocity; LIN, linearity; BCF, beat cross frequency; ALH, amplitude of lateral displacement of the sperm cell head.

In this research, the levels of SFA (myristic acid, palmitic acid and margaric acid), MUFA (palmitoleic, ginkgolic acid, oleic acid and nervonic acid) and PUFA (C18:2, C18:3 and C20:2) was significantly different ($p<0.05$) between both fish species. However, SFA (C15:0, C18:0, C21:0, C24:0), MUFA (C20:1) and PUFA (C20:3n6, C20:4n6, C20:5n3 and C22:6n3) in semen seminal plasma of both fish species determined no significant ($p>0.05$) different as statistically. However, in this study, it was determined significant ($p<0.05$) correlation between the straight line velocity (VSL) and C15:0, C18:0 and C24:1 fatty acids of semen seminal plasma in rainbow trout. However, there was a significant ($p<0.05$) correlation

Table II. Fatty acids levels (Mean±S.D. with ranges) in seminal plasma of rainbow trout and brown trout.

Fatty acids, (%)	Rainbow trout (n=7)	Brown trout (n=7)	Sig. (2-tailed)
C14:0 (Myristic acid)	0.66±0.15 (0.43-0.91)	1.07±0.13 (1.00-1.27)	0.0016*
C15:0 (Pentadecylic acid)	0.17±0.02 (0.14-0.20)	0.18±0.03 (0.15-0.22)	0.5322
C16:0 (Palmitic acid)	19.31±5.37 (9.68-25.01)	28.28±4.82 (22.21-33.84)	0.0223*
C17:0 (Margaric acid)	0.13±0.02 (0.10-0.14)	0.25±0.01 (0.24-0.27)	0.0000*
C18:0 (Stearic acid)	4.35±1.70 (2.36-7.41)	6.51±3.69 (3.89-11.97)	0.2088
C21:0 (Heneicosylic acid)	1.02±1.29 (0.26-3.29)	0.76±0.26 (0.49-0.99)	0.7079
C24:0 (Lignoceric acid)	1.49±1.22 (0.19-3.77)	0.35±0.04 (0.32-0.41)	0.1022
C16:1 (Palmitoleic acid)	0.53±0.12 (0.33-0.69)	0.85±0.12 (0.68-0.94)	0.0024*
C17:1 (Ginkgolic acid)	1.05±0.06 (0.98-1.16)	0.81±0.13 (0.66-0.96)	0.0026*
C18:1n9c (Oleic acid)	10.37±2.70 (5.46-12.97)	13.15±1.31 (11.68-14.74)	0.042*
C20:1 (Gondoic Acid)	0.42±0.11 (0.23-0.57)	0.59±0.12 (0.49-0.70)	0.07
C24:1 (Nervonic acid)	4.33±1,81 (2.31-6.79)	9.72±0.85 (8.65-10.80)	0.0004*
C18:2n6c (Linoleic acid)	3.58±1.01 (1.60-4.62)	5.62±1.03 (4.14-6.34)	0.0109*
C18:3n6 (gamma-linolenic acid)	0.85±0.26 (0.53-1.32)	2.34±0.87 (1.07-3.04)	0.0018*
C20:2 (Eicosadienoic acid)	0.72±0.16 (0.43-0.92)	0.93±0.07 (0.83-1.00)	0.0310*
C20:3n6 (Eicosatrienoic acid)	0.69±0.25 (0.42-2.40)	0.75±0.09 (0.63-0.85)	0.6476
C20:4n6 (Arachidonic acid)	5.40±1.77 (2.40-7.65)	5.43±2.23 (2.69-8.07)	0.9829
C20:5n3 (EPA, Eicosapentaenoic acid)	12.67±4.72 (6.00-20.76)	11.87±2.99 (9.34-15.61)	0.7677
C22:6n3 (DHA, Docosahexaenoic acid)	36.75±17.96 (20.54-67.58)	20.16±4.79 (13.50-24-50)	0.1098
∑SFA	27.13±6.96	37.4±10.36	
∑MUFA	16.27±4.24	25.12±5.98	
∑PUFA	60.66±13.10	47.1±7.05	

* Significant difference as statistically, p<0.05. SFA, saturated fatty acids; MUFA, mono unsaturated fatty acids; PUFA, poly unsaturated fatty acids.

Table III. Correlation coefficients between fatty acid levels of semen seminal plasma and velocities of sperm cells in rainbow trout and brown trout.

Fatty acids	Rainbow trout			Brown trout		
	VSL (µm/s)	VCL (µm/s)	VAP (µm/s)	VSL (µm/s)	VCL (µm/s)	VAP (µm/s)
C14:0	-.628	-.138	.479	-.244	-.288	.801
C15:0	-.784(*)	-.304	.232	.354	.336	.296
C16:0	-.612	.484	.491	-.582	-.692	.888
C16:1	.289	.214	.106	.639	.513	-.081
C17:0	-.490	-.253	-.275	.276	.088	-.025
C17:1	.052	-.432	-.106	-.227	-.265	-.345
C18:0	-.757(*)	.135	.353	-.713	-.570	.303
C18:1n9c	-.506	.548	.470	.149	.005	.431
C18:2n6c	-.506	.408	.551	-.695	-.818	.748
C18:3n6	-.723	-.040	.479	.698	.550	-.325
C20:1	-.228	-.136	-.023	-.606	-.623	.034
C21:0	-.365	-.555	-.077	.125	.291	-.549
C20:2	-.177	.649	.349	.008	.134	-.591
C20:3n6	-.609	-.062	.075	.151	.113	-.659
C20:4n6	-.694	.278	.367	.408	.569	-.362
C24:0	.275	-.528	-.452	.624	.755	-.781
C20:5n3 (EPA)	-.674	.339	.527	.909	.905	-.964(*)
C24:1	-.893(**)	-.291	.423	.594	.434	-.404
C22:6n3 (DHA)	.731	-.315	-.538	-.188	-.004	.111

** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed); For abbreviations see Table I.

between angular path velocity (VAP) and C20:5n3 (EPA) fatty acid of semen seminal plasma in brown trout (Table III). Other hand, in this study, we had analysis 2 fish groups, 14 individuals which have 728 data. These data was reduced and to extract a small number of latent factors (PCs) by PCA analysis. PCA analysis produced six principal components (with eigenvalue > 1) (Table IV). According to component plot in rotated space graphic, the first (PC1) and second (PC2) components were related with more of unsaturated fatty acids of sperm seminal plasma and the value of VCL and VAP in sperm cell velocities. Although third component (PC3) was related C20:5n3 (EPA), C20:4n6 and C20:3n6 of the main unsaturated fatty acids and the VSL value of sperm cell velocities (Fig. 2).

Table IV. Rotated component matrix in PCA for fatty acid contents in sperm seminal plasma and sperm kinematics in rainbow trout and brown trout.

Fatty acids and kinematic parameters	Components					
	PC1	PC2	PC3	PC4	PC5	PC6
C18:2n6c	0.93	-0.17	0.17	-0.19		0.12
C24:1	0.92	0.16		0.22	0.15	
C17:0	0.91	0.18	-0.20	0.18		
C14:0	0.89				0.36	0.14
C16:0	0.89	-0.13	0.29	-0.12		0.14
C18:3n6	0.86	0.33			0.13	-0.34
C16:1	0.82	0.29	-0.28		0.11	-0.11
C18:1n9c	0.79		0.49	-0.18		
C22:6n3 (DHA)	-0.75		-0.62	0.13		-0.13
C20:2	0.73	0.30	0.15		-0.24	0.37
VAP	0.70		0.10	-0.10		0.26
C17:1	-0.68	-0.36	0.15	0.12	-0.35	-0.13
C24:0	-0.65	-0.19	-0.34	0.57		-0.21
C20:1	0.63	-0.15	-0.15	0.56	-0.32	0.18
VCL	0.22	0.92	0.15	-0.11		-0.13
ALH	0.12	0.92	0.15	0.11	0.15	
BCF		-0.91		0.15	-0.30	
C20:5n3 (EPA)		0.33	0.88	-0.10		
VSL	0.13	0.44	-0.76	-0.23	-0.30	-0.15
C20:4n6		0.39	0.60		0.30	0.54
C21:0	-0.17	-0.16		0.92	0.14	
C203n6	0.23		0.54	0.71	-0.17	
MAD		0.45	-0.36	0.68	-0.12	0.11
C15:0	0.32	0.16	0.21		0.86	
LIN	0.37	0.59	-0.13		0.67	0.16
C18:0	0.41	-0.20	0.14	0.20		0.84
Eigenvalue	10.94	4.01	3.25	2.71	1.59	1.13
% of variance	42.10	15.43	12.50	10.43	6.10	4.33

Extraction method: PCA, principal component analysis; Rotation method: Varimax with Kaiser Normalization. For abbreviations see Table I.

DISCUSSION

In the study, we investigated the relationship between fatty acids contents of sperm seminal plasma and kinematics of sperm cells with other some semen quality parameters (volume and pH of semen, duration of motility) in rainbow and brown trout. In this study, water quality parameters such as temperature, pH and dissolved oxygen was suitable according to FAO Fisheries and Aquaculture Technical Paper (Woynarovich *et al*., 2011). On the other hand, we looked for literatures, for example, Lahnsteiner *et al*. (2009) studied about fatty acids content of semen and its effects on sperm functionality in rainbow trout (*Oncorhynchus mykiss*). They determined that saturated fatty acids (SFA) value in semen seminal plasma and sperm cell were higher quantities than MUFA. While, the levels of MUFA in sperm cell were higher than the levels of PUFA, the levels in semen seminal plasma were found same level. Additionally, they found that C14:0, C16:0 and C18:0 were main of SFA, C18:1n9, C18:1n7, and C18:3 main of MUFA in spermatozoa and semen seminal fluid. While C18:2, C22:6 (DHA), C20:5 EPA), and C20:4 (ARA) were found in high concentrations in spermatozoa, C16:1 and C20:1 were in seminal fluid. They found that there is a positive correlation between the viability, motility and the average path velocity value (VAP) of spermatozoa and the levels of C16:0 in SFA, and C20:4 and C18:2 in PUFA of spermatozoa and semen seminal plasma of rainbow trout. However, they concluded that there was not any effect of the fatty acids on spermatozoa motility duration (Lahnsteiner *et al*., 2009).

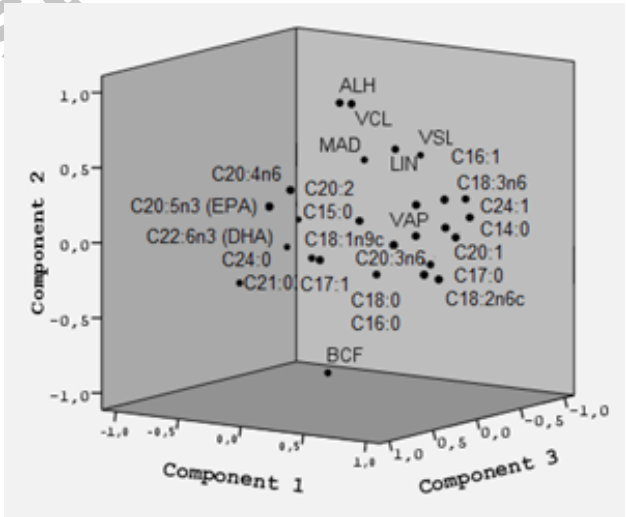

Fig. 2. Component plot in rotated space for measured parameters of fatty acids of sperm seminal plasma and sperm cells kinematics in rainbow and brown trout.

Mansour *et al*. (2011) investigated about the relationship between fertility and fatty acid profile of semen and eggs in Arctic char (*Salvelinus alpinus*). They determined less SFA values and more n-3 and n-6 value with a higher n-3/n-6 ratio in spermatozoa from the high fertility group compared the low fertility group. However, they found that the significant correlations between C15:0, total SFA, C22:5n-3 (DPA), C22:6n-3 (DHA), total n-3

fatty acids and the ratio of n-3/n-6 and fertility rate. But they did not found significant correlation between fatty acids profile of egg and fertility rate. So, they concluded that spermatozoa fertility is effected more by their fatty acid composition than that of the eggs in Arctic char (Mansour *et al*., 2011).

Hajiahmadian *et al*. (2016) investigated about semen quality parameters of rainbow trout (*Oncorhynchus mykiss*) which fed with diets with different vegetable fatty acid levels. They fed to fish with a commercial diet and ten formulated diets with different levels of vegetable fatty acids. Finally, they determined the highest sperm cell motility and duration in diet of formulated HUFA/MUFA= 0.0 and HUFA/SFA=0.25 ratios. the highest semen density and spermatocrit were calculated in diet of formulated HUFA/SFA= 0.0 and HUFA/PUFA= 0.37 ratios (Hajiahmadian *et al*., 2016). Harlıoğlu (2017) studied about fatty acid composition of gametes, embryos and larvae of rainbow trout, *Oncorhynchus mykiss*. Researcher found that C20:4 (Arachidonic acid), C22:6n-3 (DHA) and C20:5n3 (EPA) of PUFA were the highest value in fatty acids of the semen in rainbow trout. They determined that C16:0 (palmitic acid) and C18:0 were main of SFA in unfertilized eggs and semen, while C18:1n-9 was the main of MUFA in the unfertilized eggs and semen (Harlioglu, 2017). Blecha *et al*. (2018) studied about the sperm cell quality and semen lipid content in both intensively cultured and wild burbot (*Lota lota*). While they fed to RAS group fish with commercial pellets, WILD group were fed with live prey *Pseudorasbora parva*. They found that the sperm cell from the RAS group had a delay in activation, less volume of semen, less the level of SFA, PUFA n-6, especially 20:4(n-6) (ARA) compared to the WILD group. Additionally, they found that while the VCL value did not significant difference ($p>0.05$) at 10, 20, and 30 s post activation between groups but the WILD group had significant difference ($p <0.05$) less VCL value than the RAS group at 40 and 50 s post activation. They concluded that fish in natural conditions may be more suitable as brood stock (Blecha *et al*., 2018).

In this study, total SFA and MUFA in semen seminal plasma of brown trout determined in higher quantities than rainbow trout but total PUFA levels of semen seminal plasma in both species were the highest. While the C16:0 (palmitic acid) and C18:0 (stearic acid) were the main of SFA, the C18:1n9 (oleic acid) and C24:1 (nervonic acid) were for MUFA, and C18:2n6 (linoleic acid), C20:4n6 (arachidonic acid), C20:5n3 (EPA) and C22:6n3 (DHA) were for PUFA in both species (Fig. 3). Although the levels of C14:0 (myristic), C16:0 (palmitic), C17:0 (margaric) of SFA, C16:1 (palmitoleic), C17:1 (ginkgolic), C18:1 (oleic) and C24:1 (nervonic) of MUFA and C18:2 (linoleic), C18:3 (gama-linolenic) and C20:2 (eicosadienoic) of PUFA were significantly different ($p<0.05$) between semen seminal plasmaof both species, but the levels of C20:5n3 (EPA) and C22:6n3 (DHA) in semen seminal plasmaof both fish species determined no significant different ($p>0.05$). A similar situation has confirmed with the results of other researchers (Harlioglu, 2017; Lahnsteiner *et al*., 2009; Mansour *et al*., 2011). However, while there was a correlation between the straight line velocity (VSL) and C15:0, C18:0 and C24:1 of fatty acids in semen seminal plasma of rainbow trout, it was between angular path velocity (VAP) and C20:5n3 (EPA) fatty acid in semen seminal plasma of brown trout. These results which mean the relationship the velocity of sperm cells and some fatty acids in semen seminal plasma have supported by the results of other researchers (Blecha *et al*., 2018; Dzyuba *et al*., 2019; Hajiahmadian *et al*., 2016; Köprücü and Özcan, 2019; Lahnsteiner *et al*., 2009; Locatello *et al*., 2018).

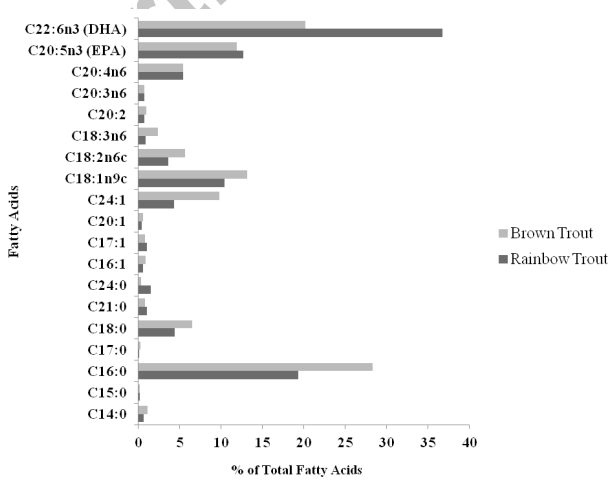

Fig. 3. Fatty acids levels in semen seminal plasma of rainbow and brown trout.

CONCLUSION

In conclude, we found that there is a strongly negative correlation between the straight line velocity (VSL) and C15:0 (pentadecanoic acid, $r = -0.78$, $p<0.05$), C18:0 (stearic acid, $r = -0.76$, $p<0.05$) and C24:1 (nervonic acid, $r = -0.89$, $p<0.01$) of semen seminal plasma in rainbow trout, while there was negative correlation ($r = -0.96$, $p<0.05$) between angular path velocity (VAP) and C20:5 (eicosapentaenoic acid, EPA) of semen seminal plasma in brown trout. So, we concluded that these results can be used to better understand the relationship between sperm cell kinematics and fatty acid contents of rainbow and brown trout in this study. This study wants to attract the

attention of fish farmers that fatty acids of food can improve semen quality in trouts. However, these results suggest and promote that next studies should be investigated about new fatty acids contents in fish food ratio to get good quality gametes of fish.

Data availability statement

All data and material for this study can be found in the manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Ackman, R.G., 2002. The gas chromatograph in practical analyses of common and uncommon fatty acids for the 21st century. *Anal. Chim. Acta,* **465**: 175–192. https://doi.org/10.1016/S0003-2670(02)00098-3

Alavi, S.M.H., and Cosson, J., 2006. Sperm motility in fishes. (II) Effects of ions and osmolality: A review. *Cell Biol. Int.,* **30**: 1–14. https://doi.org/10.1016/j.cellbi.2005.06.004

Blecha, M., Dzyuba, B., Boryshpolets, S., Horokhovatskyi, Y., Dadras, H., Malinovskyi, O., Sampels, S., and Policar, T., 2018. Spermatozoa quality and sperm lipid composition in intensively cultured and wild burbot (*Lota lota*). *Anim. Reprod. Sci.,* **198**: 129–136. https://doi.org/10.1016/j.anireprosci.2018.09.011

Bligh, E.G., and Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**: 911–917. https://doi.org/10.1139/o59-099

Bobe, J., and Labbé, C., 2010. Egg and sperm quality in fish. *Gen. Comp. Endocrinol.,* **165**: 535–548. https://doi.org/10.1016/j.ygcen.2009.02.011

Browne, R.K., Kaurova, S.A., Uteshev, V.K., Shishova, N.V., McGinnity, D., Figiel, C.R., Mansour, N., Agnew, D., Wu, M., Gakhova, E.N., Dzyuba, B., and Cosson, J., 2015. Sperm motility of externally fertilizing fish and amphibians. *Theriogenology,* **83**: 1–13.e8. https://doi.org/10.1016/j.theriogenology.2014.09.018

Cabrita, E., Martínez-Páramo, S., Gavaia, P.J., Riesco, M.F., Valcarce, D.G., Sarasquete, C., Herráez, M.P., and Robles, V., 2014. Factors enhancing fish sperm quality and emerging tools for sperm analysis. *Aquaculture*, **432**: 389–401. https://doi.org/10.1016/j.aquaculture.2014.04.034

Dzyuba, V., and Cosson, J., 2014. Motility of fish spermatozoa from external signaling to flagella response. *Reprod. Biol.,* **14**: 165–175. https://doi.org/10.1016/j.repbio.2013.12.005

Dzyuba, V., Sampels, S., Ninhaus-Silveira, A., Kahanec, M., Veríssimo-Silveira, R., Rodina, M., Cosson, J., Boryshpolets, S., Selinger, M., Sterba, J., and Dzyuba, B., 2019. Sperm motility and lipid composition in internally fertilizing ocellate river stingray *Potamotrygon motoro*. *Theriogenology,* **130**: 26–35. https://doi.org/10.1016/j.theriogenology.2019.02.029

Hajiahmadian, M., Sarvi Moghanlou, K., Agh, N., and Farrokhi A.F., 2016. Semen characteristics of rainbow trout (*Oncorhynchus mykiss*) following diets containing different vegetable fatty acid levels. *Reprod. Domest. Anim.,* **51**: 979–984. https://doi.org/10.1111/rda.12776

Hardy, R.W., 1996. Broodstock management and egg and larval quality. *Aquaculture,* **141**: 140–142. https://doi.org/10.1016/0044-8486(96)85211-2

Harlioglu, A.G., 2017. Comparative study of fatty acid composition of gametes, embryos and larvae of rainbow trout, *Oncorhynchus mykiss*. *Pakistan J. Zool.,* **49**: 1547-1936. https://doi.org/10.17582/journal.pjz/2017.49.5.1803.1808

Köprücü, K., and Özcan, S., 2019. Fatty acid, fat-soluble vitamin and sterol contents in testis and semen, testis-somatic index and spermatologic values of broodstock rainbow trout (*Oncorhynchus mykiss*) fed with different levels of omega-3 fatty acids under regular stripping conditions. *Aquac. Res.,* **50**: 2477–2485. https://doi.org/10.1111/are.14201

Lahnsteiner, F., Mansour, N., McNiven, M.A., and Richardson, G.F., 2009. Fatty acids of rainbow trout (*Oncorhynchus mykiss*) semen: Composition and effects on sperm functionality. *Aquaculture,* **298**: 118–124. https://doi.org/10.1016/j.aquaculture.2009.08.034

Locatello, L., Bertotto, D., Cerri, R., Parmeggiani, A., Govoni, N., Trocino, A., Xiccato, G., and Mordenti, O., 2018. Sperm quality in wild-caught and farmed males of the European eel (*Anguilla anguilla*). *Anim. Reprod. Sci.,* **198**: 167–176. https://doi.org/10.1016/j.anireprosci.2018.09.016

Mansour, N., Lahnsteiner, F., McNiven, M.A., Richardson, G.F., and Pelletier, C.S., 2011. Relationship between fertility and fatty acid profile of sperm and eggs in Arctic char, *Salvelinus alpinus*. *Aquaculture,* **318**: 371–378. https://doi.org/10.1016/j.aquaculture.2011.05.023

Metcalfe, L.D., Schmitz, A.A., and Pelka, J.R., 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Anal. Chem.,* **38**:

514–515. https://doi.org/10.1021/ac60235a044

Özgür, M.E., 2018. The effects of different sperm activator medias on the velocity and movement style brown trout (*Salmo trutta linnaeus*, 1792) sperm cells. *Aquacult. Stud.,* **18**: 75-78. https//doi.org/10.4194/2618-6381-v18_2_01

Özgür, M.E., Okumuş, F., and Kocamaz, A.F., 2019. A novel computer assisted sperm analyzer for assessment of spermatozoa motility in fish; BASA-Sperm Aqua. *El-Cezeri Fen ve Mühendislik Derg.,* **6**: 208 - 219 https://doi.org/10.31202/ecjse.486342

Sahari, M.A., Farahani, F., Soleimanian, Y., and Mokhlesi, A., 2013. N-3 Fatty acid distribution of commercial fish species components. *J. Am. Oil Chem. Soc.,* **90**: 1167–1178. https://doi.org/10.1007/s11746-013-2258-4

Woynarovich, A., Hoitsy, G., and Moth-Poulsen, T., 2011. Small-scale rainbow trout farming. FAO fisheries and aquaculture technical paper no. 561. *FAO Fish. Aquac.,* pp. 81.