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Scientific Article

Changes in common carp (*Cyprinus carpio*) sperm quality and membrane lipid composition related to cryopreservation by *Tribulus terrestris*

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ABSTRACT

Docosahexaenoic acid (DHA) was the major polyunsaturated fatty acid (PUFA) in the sperm plasma membrane, and the levels of PUFAs decreased in treated groups during the cryopreservation process. The supplementation of TT (*Tribulus terrestris*) to *Cyprinus carpio* sperm at 200 μg·L^{-1} positively affected the maintenance of motility parameters and FAs levels after the freeze-thaw cycle, and TT can be used as an herbal medicine instead of synthetic pharmaceuticals to improve the productive performance in aquaculture.

Keywords: *Tribulus terrestris*; Aromatic plant; Sperm quality; Fatty acid; Common carp; *Cyprinus carpio.*

Alterações na qualidade do esperma e na composição lipídica da membrana da carpa comum (*Cyprinus carpio*) relacionadas à criopreservação por *Tribulus terrestris*

RESUMO

Ácido docosa-hexaenoico (DHA) foi o principal ácido graxo poli-insaturado (AGPI) na membrana plasmática dos espermatozoides, e os níveis de AGPI diminuíram nos grupos tratados durante o processo de criopreservação. A suplementação de *Tribulus terrestris* (TT) ao esperma de *Cyprinus carpio* a 200 μg·L^{-1} afetou positivamente a manutenção dos parâmetros de motilidade e dos níveis de AG após o ciclo de congelamento-descongelamento, e o TT pode ser usado como um fitoterápico em vez de produtos farmacêuticos sintéticos para melhorar o desempenho produtivo na aquicultura.

Palavras-chave: *Tribulus terrestris*; Planta aromática; Qualidade do esperma; Ácido graxo; Carpa-comum; *Cyprinus carpio*.

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INTRODUCTION

The methods of sperm cryopreservation provide the effective transport of samples from spawning locations to far-off hatcheries or research facilities and also allow for sample manipulation to overcome unbalanced sex ratios and the male-female spawning asynchrony in captivity (Díaz et al., 2019; 2021; Huang et al., 2019; Mylonas & Zohar, 2001). The cryopreservation of semen causes numerous irreversible damages at the structural, functional, and molecular levels due to cold shock, osmotic stress atmospheric oxygen, and cryoprotectant toxicity (Cabrita et al., 2010; Figueroa et al., 2016; Magnotti et al., 2018; Türk et al., 2022). Furthermore, fatty acids in the membrane structure of sperm cells are adversely affected by the processes of cooling, freezing, and thawing in aquatic species (Mandal et al., 2014; Türk et al., 2022). The variations in the lipid composition of the spermatozoan plasma membrane play a crucial role in the differing freeze tolerance of spermatozoa (Kutluyer et al., 2014; Maldjian et al., 2005).

An important marker of cold sensitivity, viability, motility, and membrane integrity in sperm is the lipid composition of the plasma membrane (Bozkurt & Yavaş, 2021; Hammerstedt et al., 1990; Robinson et al., 2006; Yildiz et al., 2015; Zaniboni et al., 2006). In this regard, polyunsaturated fatty acids (PUFAs) play a crucial role in increasing the fluidity of the sperm plasma membrane and strengthening sperm resilience to cold shock (Bozkurt & Yavaş, 2021; Stubbs and Smith, 1984; Wathes et al., 2007). Moreover, it is widely recognized that a substantial amount of PUFAs is necessary to maintain sperm membrane fluidity and ensure the sperm's fertilization capacity. The content of fatty acids (FAs) in gametes is species-specific and crucial for determination the quality and cryotolerance potential of sperm cells (Bozkurt & Yavaş, 2021).

The Zygophyllaceae family has 24 genera and approximately 275 species in the world. It has been widely used in traditional medicine from ancient times to the present as an aphrodisiac, free-radical scavenging, diuretic, anti-inflammatory, antibacterial, anti-cancer, analgesic, antiurolithic, and antidiabetic. *Tribulus terrestris* (TT) is an annual and herbaceous plant belonging to the Zygophyllaceae family and native to the Mediterranean Region. It grows in many parts of the world, *e.g.*, Turkey, especially in Asia, Africa, and America. It can grow spontaneously in empty areas, fields and roadsides, and grows horizontally on the soil surface (Ma et al., 2017; Tekin et al., 2024).

Studies have identified more than 20 saponins from TT plant. This plant is rich in steroidal saponins (mainly protodioscin and protogracilin), and saponins have effects on farm animals such as reducing egg cholesterol content, ammonia binding, reducing tension in the stomach and intestines, reducing urease activity, antiprotozoal, antioxidant, antibacterial, antifungal and hormonal system stimulating effects in animals (Akram et al., 2011; Duru, 2024; Hammoda et al., 2013; Kamenov et al., 2017; Ma et al., 2017; Ölçülü et al., 2022). In addition, it can raise testosterone levels, along with those of its precursors and luteinizing hormone in animals and humans (Asadmobini et al., 2017; Neychev & Mitev, 2016; Omitoyin et al., 2013; Ölçülü et al., 2022; Yeganeh et al., 2017). TT is widely distributed in Turkey and has been consumed in different ways for centuries by people in places such as China and India to treatment of sexual dysfunction and desire problems and increase sexual potency and fertility. However, nowadays it is also used by athletes as a performance enhancer (Tekin et al., 2024).

Therefore, numerous studies have examined factors such as growth, survival rates, feed efficiency, masculinization, reproductive success, and hematological, immunological, and biochemical markers, histopathological changes in various fish species (Cek & Turan, 2007; Cek et al., 2007; Gültepe et al., 2014; Hassona et al., 2020; Kavitha & Subramanian, 2011; Omitoyin et al., 2013; Ölçülü et al., 2022; Yilmaz et al., 2014). Ölçülü et al. (2022) demonstrated that supplementation of *T. terrestris* to activation medium increased the sperm motility of *Oncorhynchus mykiss*. *Cyprinus carpio* is the most extensively farmed freshwater fish species for economic purposes globally (FAO, 2022; Yao et al., 2024). To the best of our knowledge, there is no evidence about the effect of TT on quality and FA composition of *C. carpio* sperm after cryopreservation up to now. In this context, this study aimed to assess the effects of different concentrations of TT on changes in quality parameters and FAs composition of *C. carpio* sperm induced by freeze-thawing process.

MATERIALS AND METHODS

Sperm collection

Mature male fish (n = 6, aged 3^{+} years old) were selected from individuals cultured under natural reproductive conditions. The ethics committee of Munzur University (Tunceli, Turkey) approved this study (Protocol No.: 2024/38-03). Males were anesthetized using a 1:3,000 aqueous solution of 2-phenoxyethanol prior to hormone treatment. A single dose of ovopel (1 pellet per kg of body weight) was administered to males showing detectable sperm flow upon gentle abdominal massage. Twenty-four hours post-injection, the urogenital pore was carefully dried, and sperm was collected directly into tubes

and then stored on ice (2–4°C) until further use. Precautions were taken to avoid contamination of the sperm with urine, feces, blood, mucus, or water.

Extraction of *Tribulus terrestris*

Tribulus terrestris (TT) was collected during the summer from its natural habitat in Osmaniye (Turkey). The fruits and aerial parts were cleaned, rinsed with water, and dried in the shade. After drying, the material was ground into a powder (25 g), and ethanol (70%, 50 mL) was used for extraction in a Soxhlet apparatus as described by Ahmed et al. (2009). The extract was then evaporated using a rotary evaporator under reduced pressure at 45°C, followed by storage in a freezer at -20°C until the experiments were conducted (Ölçülü et al., 2022).

Sperm cryopreservation and motility assessment

An immobilization medium consisting of 0.3 M glucose, 10% dimethyl sulfoxide (DMSO), and 10% egg yolk were used to dilute the pooled sperm at a 1:5 ratio (semen to medium) (Kutluyer & Aksu, 2019). The resulting mixture was then pipetted into individual 15-mL Falcon tubes for further processing. Various concentrations of TT extract [0 mM (Control), 200 $\mu g \cdot L^{-1}$, 400 $\mu g \cdot L^{-1}$, 600 $\mu g \cdot L^{-1}$] were incorporated into the extenders. These specific concentrations were chosen based on initial experimental findings and a published study by Ölçülü et al. (2022). To assess sperm quality, parameters such as curve speed (VCL), oscillation index (WOB), linear speed (VSL), velocity of the average path (VAP), amplitude of lateral head displacement (ALH), and linearity (LIN) were analyzed using the Sperm Class Analyzer system (Microptic S.L., Barcelona, Spain). The motility of sperm (progressive motility—PRG) was determined as a percentage of actively moving sperm, and a stopwatch was used to measure the movement duration.

Sperm cryopreservation procedure

The diluted sperm samples were kept in an icebox at 4°C for 10 minutes to achieve equilibration. During the freezing process, filled straws (0.5 mL) were placed horizontally on a tray inside an insulated box, positioned 5 cm above the liquid nitrogen (LN) surface (approximately -180°C). The straws were then immersed in LN and stored for 14 days further analyses. For motility analysis, the straws were thawed by placing them in a 30°C-water bath for 15 seconds before the evaluation (Kutluyer & Aksu, 2019).

Analysis of fatty acid

The lipid extraction from sperm cells was performed according to the procedure described by Hara and Radin (1978). Samples were homogenized with a hexane-isopropanol mixture (10 mL), followed by vortexing and centrifugation at 8,000 *rpm* × g three times to remove non-lipid components. To synthesize fatty acid methyl esters (FAMEs), the samples were incubated with 2% methanolic sulfuric acid for 16 hours at 55°C. After the addition of sodium chloride and hexane, the samples were vortexed and shaken. Phase separation was achieved by adding 2% $KHCO_3$, and the upper organic layer was collected, pooled, and concentrated under nitrogen gas. The hexane phase evaporated using nitrogen flow, and the resulting lipid extract was re-dissolved in 1 mL of heptane. This solution was then distributed into 2-mL autosampler vials for further analysis (Christie, 1992). The FAMEs were analyzed on a Shimadzu GC 17 gas chromatograph equipped with a temperature-programmable injector and autosampler. FAME separation was achieved using a Rtx 2330 GC column (30 m, 0.25 mm ID, 0.25 µm df). The chromatographic program began with an initial temperature of 148°C for 1 minute, followed by a ramp of 5°C/min to 200°C, then 4°C/min to 218°C. Data analysis was performed using Lab Solution 5.67 (Kyoto, Japan), and if necessary, chromatogram adjustments were made manually. FAMEs were identified and quantified by matching their retention times and peak areas to those of known standards (Supelco 37 Component FAME Mix), allowing for the determination of fatty acid concentrations. The relative amounts of each FA were calculated using the area normalization mode.

Statistical analysis

The data were analyzed using IBM Statistical Package for the Social Sciences Statistics version 27.0 for Windows, with the results expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was applied to assess the differences, followed by Duncan's multiple range test for post hoc mean comparisons. To investigate the relationships between variables, principal component analysis (PCA) and Pearson's correlation analysis were performed by the Past 4.03 program. Statistical significance was set at $p < 0.05$.

PCA was performed to evaluate the relationships between sperm quality parameters and FA composition in fresh and frozen-thawed sperm samples of *C. carpio*. Before performing PCA, the normality of the data was verified using the Shapiro–Wilk's test. For variables showing normal distribution, Pearson's correlation analysis was applied, while Spearman's rank correlation was used for non-normally distributed variables. Prior to analysis, all data were standardized (mean-centered and scaled to unit variance) to eliminate the effect of different measurement units. The assumptions of linearity and multicollinearity were evaluated

through correlation coefficients and the Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy. The PCA was carried out using the correlation matrix to reduce the dimensionality of the dataset and to identify the main variables responsible for variation among groups. The first two principal components (PC1 and PC2) were used to construct the biplot.

RESULTS

Sperm quality parameters

The motility rate (%) and duration (s) of fresh and post-thaw sperm are given in Fig. 1. The highest sperm motility rate and duration was in T1 group (200 µg·L^{-1}) with a mean of 58.00 ± 2.74% and 139.00 ± 4.14 s, respectively. A significant increase was determined in motility rates and durations of 200 µg·L^{-1}, 400 µg·L^{-1}, and 600 µg·L^{-1} concentrations compared to control ($p < 0.05$). The motility parameters of fresh and post-thaw sperm are presented in Fig. 2. There were significant differences in sperm parameters (VCL, VSL, VAP, LIN, straightness index, and beat cross frequency) between different treatments ($p < 0.05$).

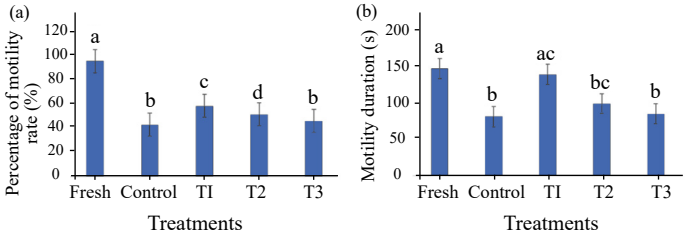


Figure 1. The mean values for (a) motility rate (%) and (b) duration (s) in fresh and frozen-thawed *Cyprinus carpio* sperm with and without (control) different concentrations of *Tribulus terrestris*.

Changes in fatty acid levels

The proportions of FAs in fresh and post-thaw sperm are presented in Table 1. Figure 3 demonstrates the area graph of the FA profile of fresh and post-thaw sperm in *C. carpio*. A total of 12 SFAs were determined in fresh and post-thaw sperm. C16:0 (Palmitic acid) and C18:0 (stearic acid) were at high levels. No significant difference was in fresh and post-thaw sperm in terms of SFAs.

A total of 6 MUFAs were determined in fresh and post-thaw sperm. C18:1 n-9 (oleic acid), C18:1 n-11 (vaccenic acid) and C22:1 n−9 (erucic acid) were at high levels. No significant difference was seen in fresh and post-thaw sperm in terms of MUFAs.

A total of 8 PUFAs were determined in fresh and post-thaw sperm. C22:6 n−3 (docosahexaenoic acid, DHA), C18:2 n−6 (linoleic acid, LA) and C20:4 n−6 (arachidonic acid, ARA)

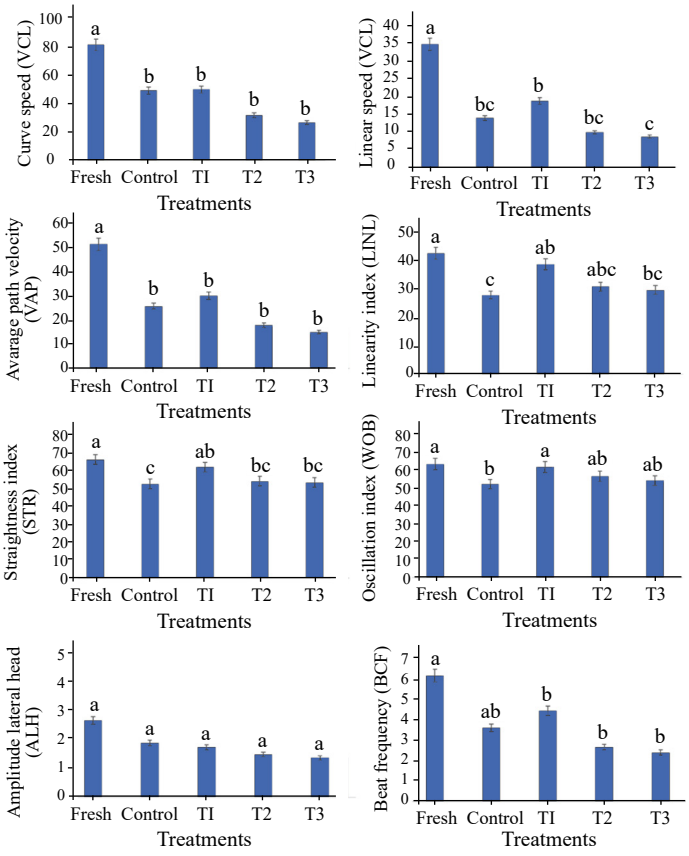


Figure 2. The mean values for motility parameters in fresh and frozen-thawed *Cyprinus carpio* sperm with and without (control) different concentrations of *Tribulus terrestris*.

A (C8:0), B (C10:0), C (C11:0), D (C12:0), E (C14:0), F (C15:0), G (C16:0), H (C17:0), I (C18:0), J (C20:0), K (C22:0), L (C24:0), M (C15:01), N (C16:1 n−7), O (C18:1 n-9), P (C18:1 n-11), R (C20:01), Q (C22:1 n9), Q (C18:2 n−6), S (C20:4 n−6), T (C20:4 n−6), U (C20:5 n−3), V (C22:4 n−6), X (C22:5 n−3), Z (C22:6 n−3).

Figure 3. Area graph of the fatty acid composition (% of total fatty acids) of fresh and frozen-thawed sperm in *Cyprinus carpio*.

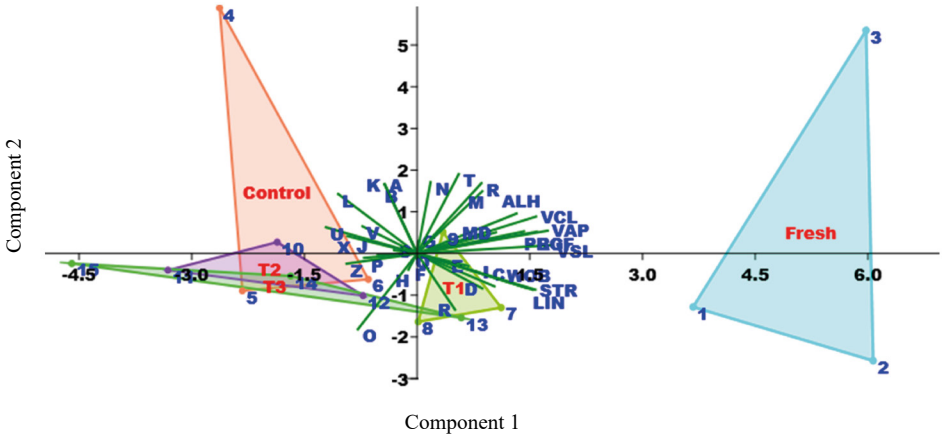
Table 1. The fatty acid composition (% of total fatty acids) of fresh and frozen-thawed sperm in *Cyprinus carpio*.

	Treatments					
Fatty acids	**Fresh**	**Control**	**T1**	**T2**	**T3**	***P*-value**
SFA						
C8:0 (Caprylic acid)	0 ± 0	0.22 ± 0.38	0 ± 0	0 ± 0	0 ± 0	0.406
C10:0 (Decanoic acid)	0 ± 0	0.31 ± 0.53	0 ± 0	0 ± 0	0 ± 0	0.406
C11:0 (Undecanoic acid)	2.44 ± 4.23	0 ± 0	0.43 ± 0.60	0 ± 0	0 ± 0	0.519
C12:0 (Lauric acid)	2.41 ± 3.00	1.22 ± 1.08	1.16 ± 1.41	0 ± 0	0.70 ± 1.00	0.507
C14:0 (Myristic acid)	2.52 ± 1.99	1.44 ± 0.65	0.56 ± 0.39	0.39 ± 0.56	2.14 ± 1.26	0.130
C15:0 (Pentadecanoic acid)	1.61 ± 2.78	0.53 ± 0.93	0 ± 0	1.64 ± 2.38	1.62 ± 2.34	0.849
C16:0 (Palmitic acid)	7.44 ± 2.59	8.02 ± 5.43	6.05 ± 4.27	11.37 ± 10.16	7.11 ± 4.02	0.947
C17:0 (Heptadecanoic acid)	1.10 ± 1.91	2.36 ± 4.08	0 ± 0	0.19 ± 0.37	0 ± 0	0.669
C18:0 (Stearic acid)	21.10 ± 6.53	13.80 ± 4.50	9.63 ± 6.00	14.21 ± 7.61	17.81 ± 9.47	0.312
C20:0 (Arachidic acid)	0 ± 0	0.84 ± 1.45	0.58 ± 0.72	0 ± 0	1.49 ± 2.13	0.669
C22:0 (Behenic Acid)	0 ± 0	0.26 ± 0.45	0 ± 0	0 ± 0	0 ± 0	0.406
C24:0 (Lignoceric acid)	0 ± 0	3.81 ± 1.61	2.47 ± 1.12	2.37 ± 1.61	3.57 ± 1.78	0.580
MUFA						
C15:1 (Pentadecylic acid)	1.80 ± 1.94	2.48 ± 1.42	0 ± 0	1.32 ± 1.90	0 ± 0	0.134
C16:1 n−7 (Palmitoleic acid)	0 ± 0	0.76 ± 1.31	0 ± 0	1.10 ± 1.91	0.36 ± 0.62	0.849
C18:1 n-9 (Oleic acid)	24.50 ± 1.22	27.14 ± 6.77	32.25 ± 6.88	32.23 ± 4.93	30.26 ± 6.10	0.234
C18:1 n-11 (Vaccenic acid)	1.39 ± 1.37	4.27 ± 2.03	4.51 ± 0.69	4.12 ± 1.73	2.81 ± 2.44	0.321
C20:1 (Eicosenoic acid)	0 ± 0	0.21 ± 0.36	0 ± 0	0.47 ± 0.69	0 ± 0	0.669
C22:1 n−9 (Erucic acid)	2.19 ± 3.79	1.99 ± 2.58	6.09 ± 10.54	4.37 ± 3.88	3.17 ± 2.88	0.934
PUFA						
C18:2 n−6 (Linoleic acid)	10.79 ± 8.20	7.38 ± 3.48	12.60 ± 4.10	9.63 ± 3.45	8.08 ± 1.96	0.586
C18:3 n−6 (γ-Linolenic acid)	0 ± 0	0 ± 0	0 ± 0	0.47 ± 0.81	0 ± 0	0.406
C18:3 n−3 (α-Linolenic acid)	0 ± 0	0 ± 0	0 ± 0	0.39 ± 0.68	0 ± 0	0.158
C20:4 n−6 (Arachidonic acid)	2.87 ± 2.93	6.01 ± 2.68	4.29 ± 1.21	3.34 ± 1.89	5.31 ± 1.15	0.154
C20:5 n−3 (Eicosapentaenoic acid)	1.19 ± 2.05	2.80 ± 1.45	2.39 ± 2.10	2.10 ± 1.01	1.49 ± 1.29	0.048*
C22:4 n−6 (Adrenic acid)	0 ± 0	0.53 ± 0.92	0.40 ± 0.69	0 ± 0	0 ± 0	0.232
C22:5 n−3 (Docosapentaenoic acid)	1.82 ± 3.16	1.05 ± 1.82	2.81 ± 0.31	1.67 ± 1.97	1.38 ± 1.19	0.448
C22:6 n−3 (Docosahexaenoic acid)	14.84 ± 4.73	12.58 ± 3.98	8.41 ± 2.26	8.59 ± 4.37	12.71 ± 4.62	0.586
ΣSFA	38.61	32.81	20.90	30.18	34.44	
ΣMUFA	29.88	36.85	42.84	43.62	36.60	
ΣPUFA	31.51	30.35	30.89	26.20	28.96	
Σn−3	17.85	16.43	13.60	12.76	15.58	
Σn−6	13.66	13.92	17.29	13.44	13.39	
n−3/n-6	1.31	1.18	0.79	0.95	1.16	

Data expressed as mean ± standard error; *significantly different at $p < 0.05$.

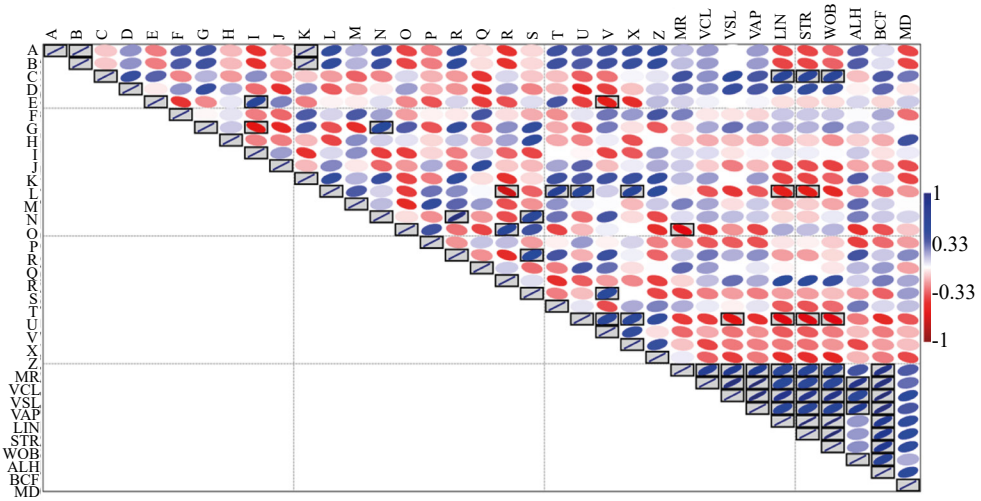
were at high levels. There were significant differences in the proportion of C20:5 n−3 (eicosapentaenoic acid, EPA) between different treatments ($p < 0.05$).

The analysis of PCA showed relationships between the FAs composition and sperm quality parameters of fresh and post-thaw sperm. The PRG was strongly related to motility duration, VCL, VSL, VAP, ALH, beat cross frequency, C16:0, C15:01, C16:1 n−7, C20:01 and C20:4 n−6. C17:0 and C18:1 n-9, C18:1 n-11, and C22:6 n−3 have negative scores featuring fresh and post-thaw sperm (Figs. 4 and 5). Correlation analysis of variables (sperm quality parameters and fatty acids) in fresh and frozen-thawed sperm in *Cyprinus carpio* is described in Kocabaş (2026).



A: C8:0; B: C10:0; C: C11:0; D: C12:0; E: C14:0; F: C15:0; G: C16:0; H: C17:0; I: C18:0; J: C20:0; K: C22:0; L: C24:0; M: C15:01; N: C16:1 n−7; O: C18:1 n-9; P: C18:1 n−11; R: C20:01; Q: C22:1 n9; R: C18:2 n−6; S: C20:4 n−6; T: C20:4 n−6; U: C20:5 n−3; V: C22:4 n−6; X: C22:5 n−3; Z: C22:6 n−3; BCF: beat cross frequency; STR: straightness index; LIN: linearity; VAP: average path velocity; VCL: curve speed; VSL: linear speed; ALH: amplitude of lateral head displacement; WOB: oscillation index; MD: motility duration; PRG: progressive motility.

Figure 4. Biplot of principal component analysis (PCA) analysis of variables (sperm quality parameters and fatty acids) in fresh and frozen-thawed sperm in *Cyprinus carpio*.



A: C8:0; B: C10:0; C: C11:0; D: C12:0; E: C14:0; F: C15:0; G: C16:0; H: C17:0; I: C18:0; J: C20:0; K: C22:0; L: C24:0; M: C15:01; N: C16:1 n−7; O: C18:1 n-9; P: C18:1 n−11; R: C20:01; Q: C22:1 n9; R: C18:2 n−6; S: C20:4 n−6; T: C20:4 n−6; U: C20:5 n−3; V: C22:4 n−6; X: C22:5 n−3; Z: C22:6 n−3; BCF: beat cross frequency; STR: straightness index; LIN: linearity; VAP: average path velocity; VCL: curve speed; VSL: linear speed; ALH: amplitude of lateral head displacement; WOB: oscillation index; MD: motility duration; PRG: progressive motility.

Figure 5. Correlation analysis of variables (sperm quality parameters and fatty acids) in fresh and frozen-thawed sperm in *Cyprinus carpio*.

DISCUSSION

Numerous approaches for the cryopreservation of fish sperm have been created for a range of teleost species, focusing on optimizing sample quality and increasing sperm viability following cryopreservation process (Cabrita et al., 2009; 2014; Díaz et al., 2021; Müller et al., 2018). Sperm cells show notable ultrastructural changes in the membrane and shifts in FA composition after the freeze-thaw process (Díaz et al., 2019; 2021). Therefore, we examined the impact of supplementation TT on the post-thaw functional parameters of *C. carpio* spermatozoa. To the best of our knowledge, this is the first study about the effect of TT on quality and FA composition of *C. carpio* sperm after cryopreservation.

Due to its aphrodisiac effects and ability to scavenge free radicals, TT plays a beneficial role in the treatment of various diseases and disorders (Fernández-Lázaro et al., 2022; Hammoda et al., 2013; Keshtmand et al., 2014; Neychev & Mitev, 2016; Singh et al., 2012). Despite the lack of literature regarding use of TT in enhancing cryotolerance of spermatozoa in *C. carpio*, Ölçülü et al. (2022) demonstrated that the addition of TT to the activation solution increased spermatozoa motility rate and duration at 400 $\mu g \cdot L^{-1}$ in *O. mykiss*. These results are in line with the report by Ölçülü et al. (2022). Our results demonstrated that TT supplementation improved the sperm movement parameters (VCL, VSL, VAP, LIN, straightness index, and beat cross frequency) and motility duration compared to control group at 200 $\mu g \cdot L^{-1}$. The increment can be explained by the presence of protodioscin, a compound classified as a steroidal saponin, through protection against oxidative stress (Ölçülü et al., 2022). The generation of free oxygen radicals at lower levels might be provided by TT due to its components [polyphenols, H_2O_2, DPPH (2,2-di-(4tert-octylphenol)-1-picrylhydrazyl)]. Additionally, its zinc and calcium content may play a role. The inhibition of the enzyme phosphodiesterase by CA^{++} could enhance sperm motility by preventing the degradation of cyclic adenosine monophosphate (cAMP) (Asadmobini et al., 2017; Keshtmand et al., 2014; Nassar et al., 1998; Ölçülü et al., 2022). Zinc may contribute to the enhancement of sperm motility by promoting protein synthesis and stabilizing nuclear chromatin (Asadmobini et al., 2017).

This study demonstrated that supplementation of any concentration of TT to *C. carpio* sperm before freezing had no impact on the levels of FAs following thawing. Total PUFA decreased in treated groups compared fresh sperm, while total SFA and MUFA were slightly increased during cryopreservation as compared to fresh spermatozoa. PUFAs represent a substantial fraction of membrane lipids and play a crucial role in protecting sperm cell membranes from the detrimental effects of oxidative stress during cryopreservation, primarily through modulation of membrane fluidity (Kaeoket et al., 2008; Wathes et al., 2007). According to Maldjian et al. (2005), DHA was the predominant PUFA in the sperm plasma membrane, and PUFA levels decreased due to LPO during cryopreservation (Cerolini et al., 2001). These results are in line with the findings of previous studies. This can be explained by the oxidative damage in sperm induced by reactive oxygen species through the attack on PUFAs bound to phospholipids, resulting in lipid peroxidation (LPO) and reduction in motility (Klaiwattana et al., 2016).

Four-dimensional PCA score plots were used to detect the variations in sperm movement parameters and FA profiles across the five sperm sample groups (fresh, control, and treated groups). As illustrated in Fig. 4, samples from different groups showed segregation into two clusters with a few intermixed samples in control and treated groups. The data in the current study indicated that PRG was strongly related to motility duration, VCL, VSL, VAP, ALH, beat cross frequency, C16:0, C15:01, C16:1 n−7, C20:01, and C20:4 n−6 in the fresh sperm and treated groups. C17:0 and C18:1 n-9, C18:1 n-11, and C22:6 n−3 had negative scores featuring the fresh sperm and treated groups. The correlation between the variables may be explained with used TT concentrations.

To conclude, the supplementation of TT improved post-thaw sperm movement parameters in *C. carpio* at a dose of 200 $\mu g \bullet L^{-1}$ during cryopreservation, while FAs composition was influenced slightly. The findings of this study indicated that adding TT to the cooling/freezing diluents could be a viable alternative for protecting *C. carpio* sperm. With its low-side effects, easy availability, affordability, and safe application, the study provide insights in evaluating the impact of TT on short-term sperm preservation and cryopreservation in other fish species. Further experiments are required to understand the mechanisms and effects of TT on fertility and subsequent development and to increase reproduction efficiency.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

Additional data is available at Figshare. https://doi.org/10.6084/m9.figshare.32545428.v1

AUTHORS' CONTRIBUTION

Conceptualization: Ölçülü, A., Özçiçek, E., Kocabaş, F. K.; **Formal Analysis:** Kocabaş, F. K.; **Investigation:** Ölçülü, A., Özçiçek, E., Kocabaş, F. K., Kocabaş, M., Kızak, V., Yilmaz, Ö.; **Resources:** Kocabaş, F. K.; **Supervision:** Ölçülü, A., Özçiçek, E., Kocabaş, F. K.; **Validation:** Ölçülü, A., Özçiçek, E., Kocabaş, F. K., Kocabaş, M., Kızak, V., Yilmaz, Ö.; **Data curation:** Özçiçek, E., Kocabaş, F. K., Yilmaz, Ö.; **Writing – original draft:** Özçiçek, E., Kocabaş, F. K., Kocabaş, M., Kızak, V., Yilmaz, Ö.; **Writing – review & editing:** Ölçülü, A., Özçiçek, E., Kocabaş, F. K., Kocabaş, M., Kızak, V., Yilmaz, Ö.; F. K., Kocabaş.; **Final approval:** Kocabas, F. K.

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DECLARATION OF USE OF ARTIFICIAL INTELLIGENCE TOOLS

We declare that no artificial intelligence tools were used in the preparation, writing, data analysis, or review of this manuscript.

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