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In vitro Effect of L-Tryptophan on the Quality and Fertilizing Capacity of Sperms of Endangered Species of Trouts

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ABSTRACT

The present study was focused on determining the effect of L-tryptophan on quality of sperm and its fertilizing capacity in endangered trout *Salmo coruhensis*, Anatolian trout *Salmo rizeensis* and rainbow trout *Oncorhynchus mykiss*. Different activation media (NaCl, 0.3%; $NaHCO_3$, 1%) were supplemented with L-tryptophan [Control (0), 0.5, 1, 2, 3, 4 and 5 mM] for assessing sperm motility and its duration, fertility and hatching rate of eggs. The results from the present study indicated that addition of L-tryptophan increased the motility rate and duration in *O. mykiss*, *S. rizeensis* and *S. coruhensis* compared to control group. Highest sperm motility rate and its duration, and fertility and hatching rate of eggs of *O. mykiss*, *S. rizeensis* and *S. coruhensis* were 0.5 mM, 5 mM and 2 mM, respectively. To conclude, sperm quality and fertility were positively affected by quantitative changes different concentrations of L-tryptophan. In addition, L-tryptophan can be used in activation medium for *O. mykiss*, *S. rizeensis* and *S. coruhensis*.

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INTRODUCTION

Oncorhynchus mykiss and *Salmo trutta* are the most important Salmonid fish species owing to its aquaculture potential, economic value and wide consumer demand (Kocabas *et al*., 2011; Kocabas and Bascinar, 2013). *Salmo trutta* forms resident populations in the upper streams of rivers and occurs in North Africa, Europe, West Asia and Anatolia (Kuru, 2004; Kottelat *et al*., 2007; Kocabas and Bascinar, 2013). In addition, it is an important potential species for recreational fishery. Recently, *S. t. labrax* and *S. t. macrostigma* ecotype have been described by Turan *et al*. (2009) as *S. coruhensis* and *S. rizeensis* (Can *et al*., 2012; Seyhaneyıldız Can *et al*., 2014). In addition, *S. coruhensis* is an endemic anadromus fish and only distributed in the rivers of Eastern Black Sea Region (Kocabas and Bascınar, 2013). In particular, populations of the species are affected by natural hybridization, the local devastation in water sources through habitat fragmentation and modification, water eutrophication and contaminations, environmental instability and global warming (Costedoat *et al*., 2007; Roberts *et al*., 2009; Crego-Prieto *et al*., 2012; Šimková *et al*., 2015). Sperm motility is the essential functional parameter for successful fertilization in fish (Islam and Akhter, 2011; Öğretmen *et al*., 2016). Sperm cells in most fish species immotile in seminal fluid and require to release into the water in order to trigger motility and become metabolically active (Dzyuba *et al*., 2010; Öğretmen *et al*., 2016). Therefore, characteristics of activation media are crucial in terms of initiation and progression of sperm motility (Öğretmen *et al*., 2016). In addition, determination of suitable activation media is crucial in terms of increasing the fertilization rate (Le and Pham, 2016).

Amino acids are the building blocks of proteins and have a crucial role for biologically and psychologically process (Kutluyer and Kocabas, 2016; Husan *et al*., 2017). Thus far, several studies about sperm cryopreservation in different fish species (*Dicentrarchus labrax*, *Sparus aurata, Oncorhynchus mykiss, Salvelinus fontinalis, Pagrus major, Carassius auratus*) (Lahnsteiner *et al*., 2011; Martínez-Páramo *et al*., 2013; Liu *et al*., 2014; Ekici *et al*., 2014; Rani *et al*., 2014; Kutluyer *et al*., 2014, 2015; Öğretmen *et al*., 2015) or dietary effect of amino acid on sperm quality (*O. mykiss*) (Canyurt and Akhan, 2008) have been published over the past decade the latest available literature.

L-tryptophan (TRP) is aromatic amino acid, and research and clinical trials have been widely conducted due to critical role in metabolic functions (Richard *et al*., 2009). The essential amino acid L-tryptophan (TRP) is needed for the biosynthesis of proteins (Zhao *et al*., 2010).

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As far as the authors of this work are aware, no attempt has been made to use of L-tryptophan in sperm activation medium of fish species. Within this context, the aim of this study was to examine effect of supplementation of different activation mediums (NaCl, 0.3%; $NaHCO_3$, 1%) with different L-tryptophan concentrations (0.5 mM; 1 mM; 2 mM; 3 mM; 4 mM; 5 mM) on endangered trout *S. coruhensis*, Anatolian trout *S. rizeensis* and rainbow trout *O. mykiss* sperm.

MATERIALS AND METHODS

Mature endangered trout males (1589.17±0.13 g, 44.13±3.25 cm as mean±SD), rainbow trout (1357.36±0.46 g, 42.36±1.89 cm as mean±SD) and Anatolian trout (1445.10±0.18 g, 43.37±3.87 as mean±SD) were obtained from commercially trout farm, Trabzon, Turkey for sperm collection. Males were anesthetized (Benzoacaine, 50 mg/L) before stripping. Caution was exercised to prevent contamination of the semen with urine, feces, blood, mucus or water. The sperm was collected by a gentle abdominal massage, collected into glass vials and stored on ice (2-4°C) until use.

The pH of semens was measured with a pH meter (Thermo Scientific Orion 5-Star Plus pH meter, USA). The spermatocrit is defined as the ratio of white packed material (sperms) volume to the total volume of semen × 100 (Rurangwa *et al*., 2004). Microhaematocrit capillary tubes (75 mm in length and 1.1–1.2 mm in diameter) were used for spermatocrit measurement. Microhaematocrit capillary tubes filled with semen were centrifuged at 3000 rpm for 10 min in a LD5-2B centrifuge (Beijing Shiningsun Technology, Japan) and then spermatocrit was calculated on the basis of the ratio of spermatozoa volume (white part) to total volume of semen × 100.

L-tryptophan was separately added to the activation mediums (NaCl, 0.3%; $NaHCO_3$, 1%) (one per experimental group): Control (0) (C), (a) 0.5 mM (T1), (b) 1 mM (T2) (c) 2 mM (T3), (d) 3 mM (T4), (e) 4 mM (T5), (f) 5 mM (T6). Motility parameters were measured using an automated system, SCA (Sperm Class Analyzer v. 4.0.0. by Microptic S.L., Barcelona, Spain). The spermatozoa movement was monitored using a camera (Basler A312fc, with sensor type CCD) at 50 Hz mounted on a Nikon Eclipse 50i microscope, coworking with SCA. The duration of motility was determined as the time until forward movement stops. The negative phase contrast lens of 10 magnification and intermediate optic 2.5 were used to trace sperm head. Half-second films (25 frames) were recorded at 7, 10 and then at each 5 s intervals until the motility completely stopped. Undiluted milt (1–2 µl) was added to 400 µl while diluted milt (2 µl) was added to 50 µl of the activating solution in a polyethylene Eppendorf. After rapid pippeting mixing, a portion of 1.2 µl of this mixture was placed directly in one well of a 12-well multi-test glass slide with coverslip (MP Biomedicals LLC, Germany). The activation and film recording of undiluted milt was repeated triplicate. These operations of diluted milt were repeated five times in order to capture at least 50 sperm at beginning of recording. Sperm motility parameters were chosen for analysis: MOT (percent of motile sperm, %), VCL (curvilinear velocity, >20 µ ms^{-1}), VAP (average path velocity, µ ms^{-1}), VSL (straight line velocity, µ ms^{-1}), LIN (linearity, %), STR (straightness), and BCF (beat cross frequency, Hz) (Kutluyer *et al*., 2014).

Fertilization experiments were conducted at 8-10°C. One homogenous egg pool was used for the fertilization experiments. From the eggs the ovarian fluid was drained off and the eggs were placed in fertilization solution a ratio of 1:2 (eggs: solution), then the semen was added and the components were mixed with each other. 100 ± 5 eggs were fertilized with 100 µl sperm (sperm to egg ratio: $X10^5$: 1). Three to 5 minutes after fertilization the eggs were rinsed in hatchery water and incubated in flow incubators at water temperature of 9 ± 0.5 °C. The experimental success was determined as the percentage of eyed embryos in relation to the total number of eggs 28 to 30 d after fertilization (Kutluyer *et al*., 2014).

Statistical analysis was performed using the software package SPSS 14.0 for Windows and results were expressed as means ± Standard deviation. Differences among the treatments were tested by one-way ANOVA. The Duncan test was used for all *post-hoc* comparisons. Significance was set at $p<0.05$.

Table I.- Sperm parameters (Mean±SD) of *Salmo coruhensis*, *Salmo rizeensis*, *Oncorhynchus mykiss*.

Species	Semen volume (ml)	pH	Spermatocrit (%)	Sperm density* ($\times10^9$)
S. coruhensis	6.67±0.53	7.71±0.14	50.00±0.35	6.18±0.52
S. rizeensis	7.00±0.25	7.76±0.22	55.33±0.24	9.27±0.56
O. mykiss	7.33±0.18	7.17±0.34	40.00±0.18	3.81±0.24

* Sperm density is density/ml

RESULTS

Sperm parameters (mean ± SD) are presented in Table I. Effect of L-tryptophan on the motility rate and duration, fertility and hatching rate of *O. mykiss* sperm was shown in Figure 1. The results of the present study indicated that differences in the motility rate and duration

of *O. mykiss* sperm were significant among the treatments ($p<0.05$).Highest motility rate (96.67%) and duration (110 s), fertility (95.19%) and hatching rate (85.12%) were at concentration 0.5 mM in activation medium (NaCl). Highest motility (100%) and duration (38 s), fertility (93.57%) and hatching rate (83.07%) were at concentration 0.5 mM in activation medium ($NaHCO_3$).

Effect of supplementation of L-tryptophan to activation medium on motility and duration for *S. rizeensis* is presented in Figure 2. The trials in this study indicated that differences in the motility rate and duration of *S. rizeensis* sperm were significant among the treatments ($p<0.05$). Highest motility rate (100%) and duration (60 s), fertility (95.19%) and hatching rate (85.19%) were at concentration 5 mM in activation medium (NaCl). Highest motility (100%) and duration (47 s), fertility (94.05%) and hatching rate (84.07%) were at concentration 5 mM in activation medium ($NaHCO_3$).

Effect of addition of L-tryptophan to activation medium on motility and duration for *S. coruhensis* is presented in Figure 3. The data in this study indicated that differences in the motility rate and duration of *S. coruhensis* sperm were significant among the treatments ($p<0.05$). Highest motility (90.00%) and duration of motility (80.33 s), fertility (92.19%) and hatching rate (82.09%) were at concentration 1 mM in activation medium (NaCl). Highest motility (94.05%) and duration of motility (84.07 s) were at concentration 2 mM in activation medium ($NaHCO_3$).

Effect of L-tryptophan on the motility parameters (VCL, VSL, VAP, LIN, STR and BCF) of sperm is presented in Figure 4. Highest VCL, VSL, VAP, LIN, STR and BCF were at concentration 0.5 mM for *O. mykiss*, 5mM for *S. rizeensis*, 1 mM (NaCl) and 2 mM ($NaHCO_3$) for *S. coruhensis*.

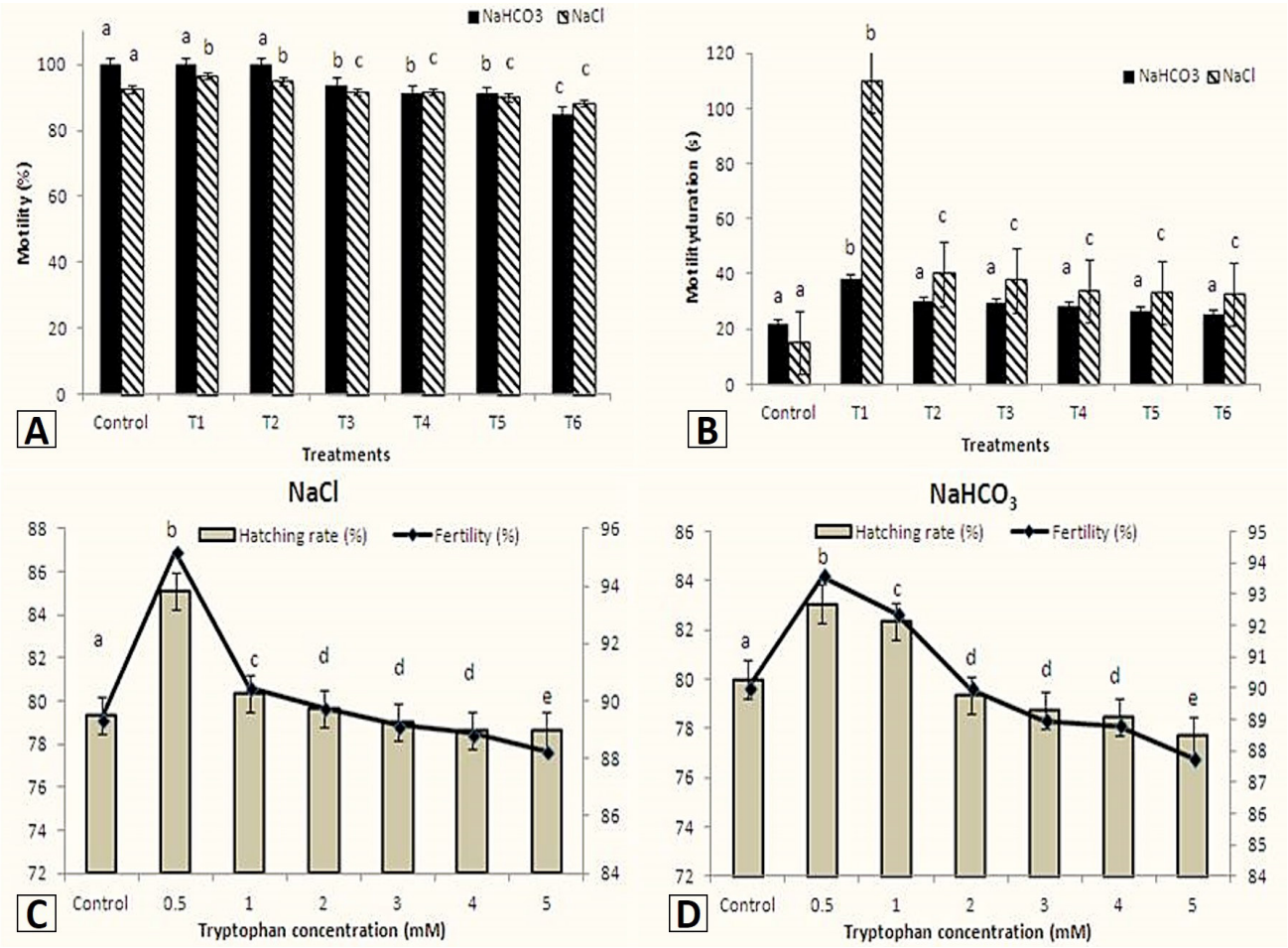

Fig. 1. Effect of supplementation of L-tryptophan to different activation mediums (NaCl and $NaHCO_3$) on the sperm motility (A), its duration (B), fertility (C) and hatching rate of eggs (D) of *O. mykiss* (n=6). Different letters show differences between treatments ($p<0.05$). T1; 0 mM, T2; 1 mM, T3; 2 mM, T4; 3 mM, T5; 4 mM, T6; 5 mM.

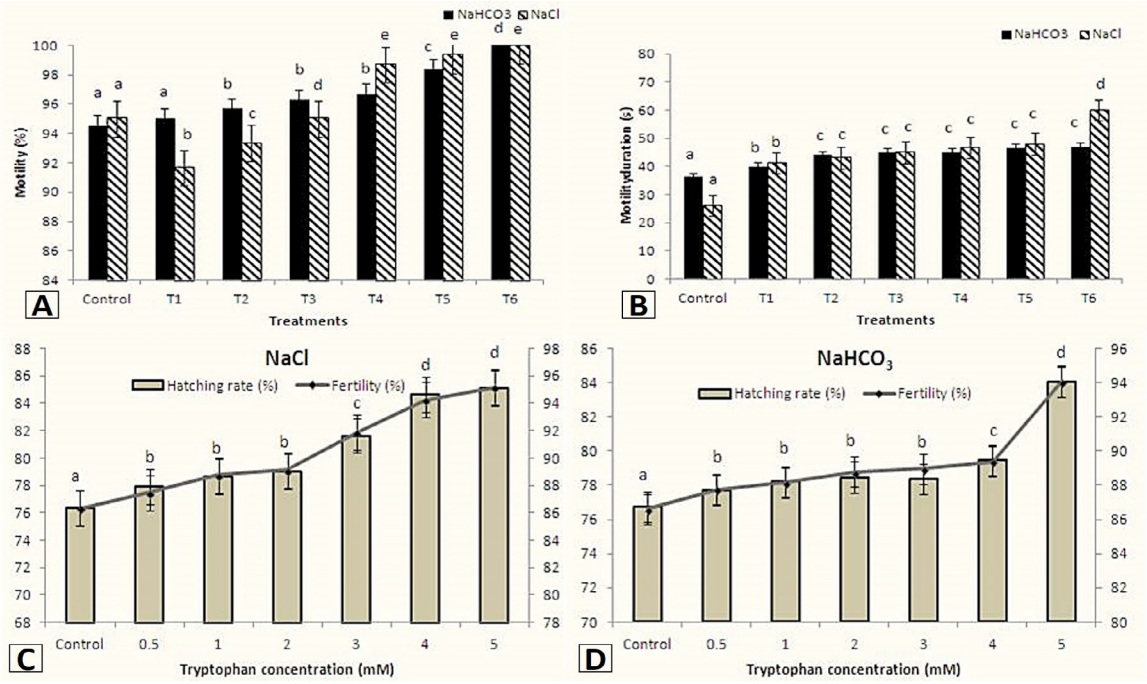

Fig. 2. Effect of supplementation of L-tryptophan to different activation mediums (NaCl and $NaHCO_3$) on sperm motility (A), its duration (B), fertility (C) and hatching rate of eggs (D) of *S. rizeensis* (n=6). Different letters show differences between treatments ($p<0.05$).

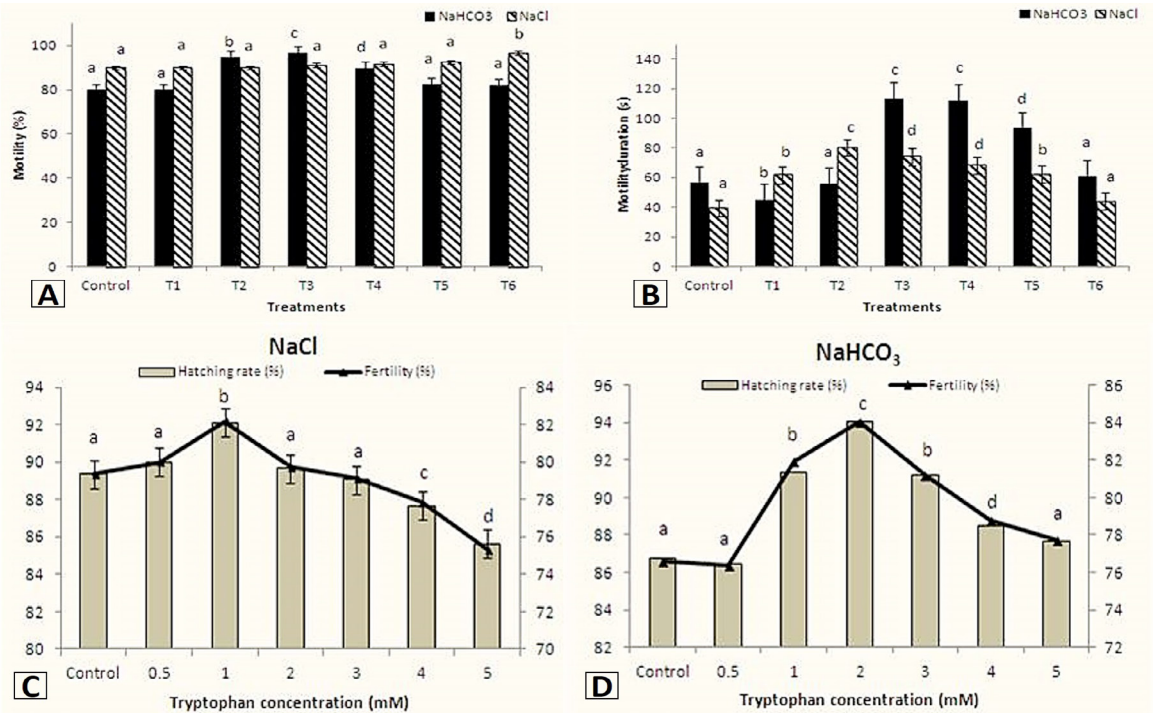

Fig. 3. Effect of supplementation of L-tryptophan to different activation mediums (NaCl and $NaHCO_3$) on sperm motility (A), its duration (B), fertility (C) and hatching rate of eggs (D) of *S. coruhensis* (n=6). Different letters show differences between treatments ($p<0.05$).

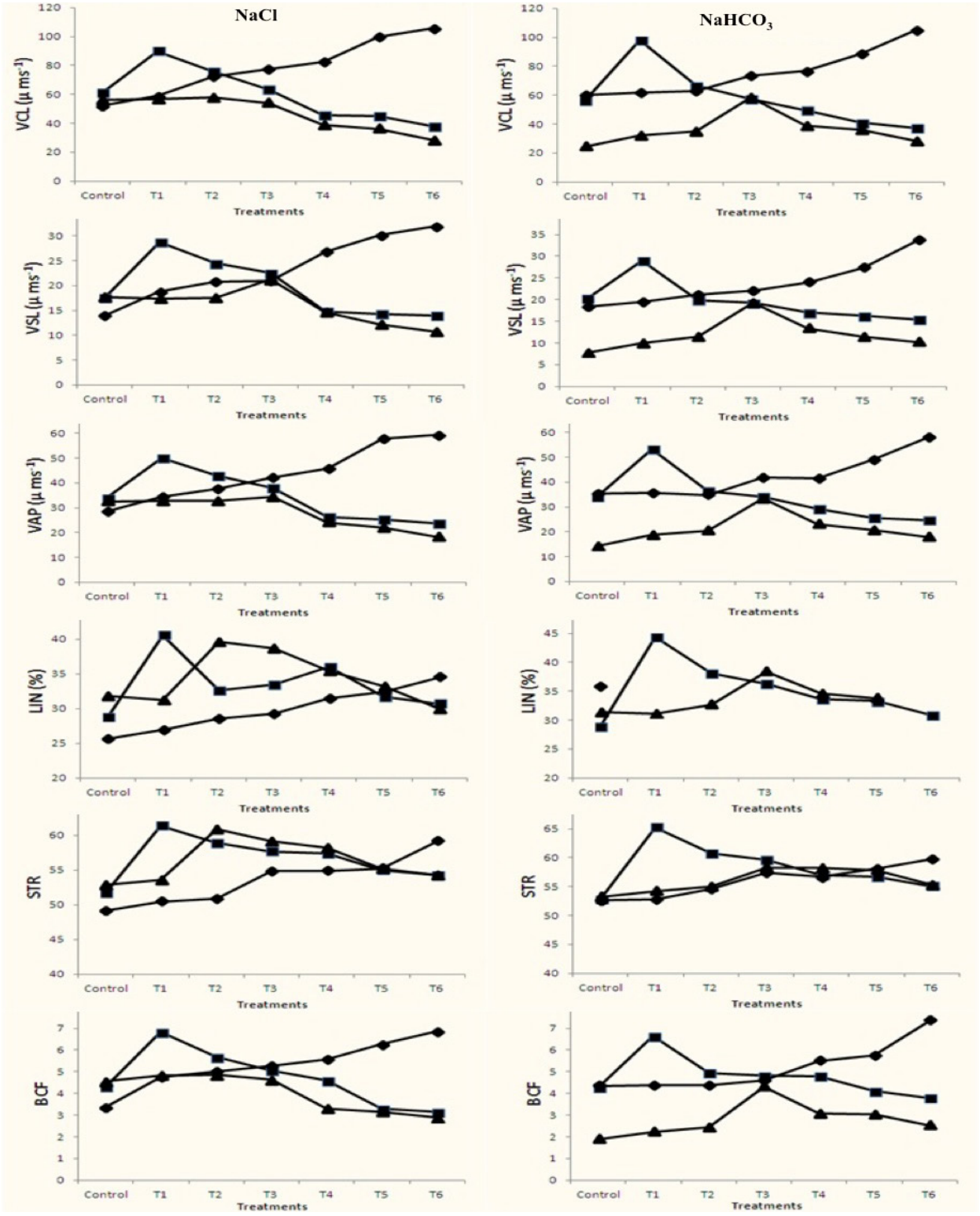

Fig. 4. Effect of L-tryptophan on the motility parameters of rainbow trout *O. mykiss*, *S. rizeensis*, *S. coruhensis* sperm for different activation mediums (NaCl, $NaHCO_3$). BCF, beat cross frequency; STR, straightness; LIN, linearity; VAP, average path velocity; VCL, curvilinear velocity; VSL, straight line velocity.

DISCUSSION

To the best of our knowledge, this is apparently the first report on effect of sperm activation medium supplemented with L-tryptophan on *O. mykiss*, *S. coruhensis* and *O. mykiss* sperm, although studies have been conducted about sperm cryopreservation in different fish species (*Dicentrarchus labrax*, *Sparus aurata, Oncorhynchus mykiss, Salvelinus fontinalis, Pagrus major, Carassius auratus*) (Lahnsteiner *et al*., 2011; Martínez-Páramo *et al*., 2013; Liu *et al*., 2014; Ekici *et al*., 2012; Rani *et al*., 2014; Kutluyer *et al*., 2014, 2015; Öğretmen *et al*., 2015) or dietary effect of amino acid on sperm quality (*O. mykiss*) (Canyurt and Akhan, 2008).

In this study, we demonstrated the usefulness of L-tryptophan in different activation mediums for three species sperm. Supplementation of L-tryptophan in activation media was increased motility rate and duration. The best concentration of L-tryptophan changed depending on species. Using L-tryptophan in different activation mediums resulted in high sperm motility rate of *S. coruhensis* (2 mM), *O. mykiss* (0.5 mM) and *S. rizeensis* (5 mM) for different concentrations. In *O. mykiss*, motility rate and duration decreased after concentration 0.5 mM. In *S. rizeensis*, an increase in the concentration of L-tryptophan in activation media caused a significant increase in the motility rate and duration of sperm. In *S. coruhensis*, a remarkably decrease was observed in motility duration after concentration 3 mM. This may be due to antioxidant property of amino acids. However, the mechanisms and role of amino acids on sperm quality have yet to be fully explored, and thus future studies on this question are required.

Sperm velocities play a crucial role for success of fertilization (Rudolfsen *et al*., 2008; Skjæraasen *et al*., 2009; Butts *et al*., 2010). In previous studiess, it was reported that success of fertilization correlated with sperm motility velocities (VCL, VSL, VAP, LIN, STR and BCF) in turbot (*Psetta maxima*) (Dreanno *et al*., 1999), African catfish (*Clarias gariepinus*) (Rurangwa *et al*., 2001), carp (*Cyprinus carpio*) (Martínez-Páramo *et al*., 2009), streaked prochilod (*Prochilodus lineatus*) (Viveiros *et al*., 2010) and rainbow trout (*Oncorhynchus mykiss*) (Lahnsteiner, 2000; Kutluyer *et al*., 2014). In present study, sperm from treatments containing L-tryptophan had higher fertility, hatching rate, VCL, VSL, VAP, LIN, STR and BCF. The present results may be due to protection the cells against damage of free radicals and oxidative stress of L-tryptophan (Kutluyer and Kocabas, 2016).

In conclusion, based on the data obtained within the context of this study, best concentrations of L-tryptophan changed depending on species. L-tryptophan was used efficiently in activation medium for rainbow trout *O. mykiss*, Anatolian trout *S. rizeensis*, endangered trout *S. coruhensis* sperm. Determination of suitable activation solution is important due to be increasing the fertilizing capacity (Le and Pham, 2016). Therefore, our study provides new insights related to use of L-tryptophan on fish sperm quality. The knowledge of effects of L-tryptophan and its mechanism of action might be helpful for both research and commercial use. Further studies would be needed to evaluation the precise mechanisms.

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Statement of conflict of interest

The authors declare that they have no conflict of interest.

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