



Improvement of Sperm Motility of Rainbow Trout (*Oncorhynchus mykiss* W., 1792) by Supplementation of L-Arginine

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ARTICLE INFO	ABSTRACT
<p>Research Article</p> <p>Received : 26/02/2019 Accepted : 05/08/2019</p> <p>Keywords: L-arginine Sperm Quality <i>Oncorhynchus mykiss</i> Rainbow Trout Amino Acid</p>	<p>In this study, the effect of supplementing amino acid-precursor of nitric oxide (NO) L-arginine were examined on sperm motility of rainbow trout <i>Oncorhynchus mykiss</i>. Different concentrations [0 mM (Control), 1 mM, 2 mM, 4 mM and 8 mM] of L-arginine were used in the study. To assess the effects on percentage of motile sperm and longevity, L-arginine was added to activation medium containing NaCl (52 mM). The higher L-arginine concentration (4mM) has promoting-effect on sperm motility. The treatments containing L-arginine caused significant effect on percentage of motile sperm and longevity. Overall, the findings of the present study indicated that supplementation of L-arginine may have improving effects on sperm motility of rainbow trout.</p>

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Introduction

In aquaculture and aquatic life, fertilizing ability and hatching success are correlated with sperm motility (Kutluyur et al., 2015, 2016; Kocabaş et al., 2011; Kocabaş and Kutluyur, 2017a, b, c). The extinction or loss of populations and reducing productivity might be due to poor sperm quality (Kocabaş et al., 2018). In multiple biologic and psychological processes, amino acids have an important role (Kutluyur et al., 2016). Due to their antioxidant properties, structure of membrane is protected by amino acids. L-arginine being the main precursor of nitric oxide (NO) is needed in living organism for protein synthesis (Santana et al., 2016; Salgueiro et al., 2017). Superoxide and hydrogen peroxide is decreased by NO. Thus, protection to free radical damage is provided by L-arginine (Scott and Bolton, 2000). Under *in vitro* conditions, studies in different species (e.g. pig, humans, buffalo, rabbits, bovine, goat and mouse) have been conducted on the sperm motility (Herrero et al., 1994; Hellstrom et al., 1994; Aydin et al., 1995; Patel et al., 1998, 1999; Zhang and Zheng, 1996; Yeoman et al., 1998; Srivastava et al., 2006). Literature in these studies stated that L-arginine addition provided decreasing lipid peroxidation and improving sperm capacitating

parameters. In fish (Çoruh trout, *Salmo coruhensis*) spermatozoa, only a study has been performed about *in vitro* impact of L-arginine on sperm motility by Kocabaş et al. (2018). Within this context, experiments were realized to determination the optimal concentration of L-arginine for improved sperm motility of rainbow trout.

Material and Method

Semen Collection and Processing

Collection of sperm was made from three-year-old 6 mature rainbow trout's in a commercial fish farm. Sperm was collected during the 2017 breeding season. The water temperature was at 6.9±0.1°C, pH around 8.34, and dissolved oxygen 8.5±0.3 mg l⁻¹ with natural photoperiod. Fish were anesthetized with 2-phenoxyethanol (0.6 ml L⁻¹) and abdominal massage technique was applied for stripping. The sperm contaminated with urine, water, blood or feces were disused in the experiments. Sperm was stripped into a collection 50 ml vial and kept on ice transport purposes until sperm analysis commenced in less than 30 min after collection.

Sperm Quality Assessment

Different concentrations [0 mM (Control), 1 mM, 2 mM, 4 mM and 8 mM] of L-arginine were added to activation solution (NaCl, 52 mM). After supplementation, percentage and longevity of motile sperm cells were determined. Progressive motility was recorded using the SCA (Sperm Class Analyzer) system. Duration of progressive motility was described as time from activation initiation to sperm stop move. The selected sperm samples (normal pH, volume and motility >80%) were pooled for experiments. Thoma cell hemocytometer was used for determination of spermatozoa density. Spermocrit (%) was determined using the method of Rurangwa et al. (2004).

Statistical Analysis

Data obtained from measurements are given as the mean \pm standard deviation. For comparison of data, One-way ANOVA was used with Duncan Multiple Range Test. A minimum significance level of $P < 0.05$ was accepted. Data were analyzed using SPSS 24.0 software.

Results and Discussion

Table 1 summarizes sperm quality parameters (mean \pm SD) for all analysed samples and sperm motility rate (%) and motility duration (s) of groups in the *O. mykiss* applied L-arginine, were given in the Table 2. In fresh spermatozoa, the motility percent and longevity were 88.33 \pm 2.89 % and 45.99 \pm 1.34 s, respectively. Comparable

results about sperm quality parameters in previous studies and the present findings are presented in Table 3. The results of the present study differs from former studies due to different factors (e.g.; age and weight of broodstock, term and method of stripping, habitat of broodstock) (Piironen and Hyvarinen, 1983; Suquet et al., 1994; Tekin et al., 2003; Kocabaş et al., 2012; Kocabaş and Kutluyur, 2017b).

Former studies in humans and animals demonstrated that L-arginine supplementation had a positive impact on the motility of spermatozoa because of deactivation superoxide through NO (Herrero et al., 1994; Hellstrom et al., 1994; Aydin et al., 1995; Patel et al., 1998, 1999; Zhang and Zheng, 1996; Yeoman et al., 1998; Srivastava et al., 2006; Buzadzic et al., 2015). Our results are in line with previous reports. Consistent with previous studies, longevity and percentage of motility were increased by L-arginine (Figure 1 and 2). The maximum increase was obtained from the treatment containing 4 mM L-arginine. Significant increased motility (91.25 \pm 2.50 %) ($P=0.041$, $P < 0.05$) and longevity (52.94 \pm 3.03 s) ($P=0.028$, $P < 0.05$) of rainbow trout spermatozoa was in the high concentration of L-arginine. The arginine-induced NO production might be reason of the increment in cells. NO production cause to increase in protein phosphorylation and modifications through the activation of soluble guanylyl cyclase and S-nitrosylation of cysteine residues (Lefièvre et al., 2007; Santana et al., 2016). NO production through L-arginine can be prevented oxidative damage in sperm cells due to stimulate the sperm chemotaxis and reaction of acrosome (Revelli et al., 2001; Hassanpour et al., 2010).

Table 1 Summary of standard sperm quality parameters (mean \pm SD) assessed for *O. mykiss* (n=6).

Parameters	Mean	Range
Colour	White	
Volume (ml)	8.60 \pm 4.31	2.5-13.0
pH	7.75 \pm 0.14	7.54-7.88
Spermocrit (%)	19.27 \pm 0.24	8.57-40.00
Sperm density ($\times 10^9$)	9.27 \pm 0.56	9.17-9.37
Sperm motility (%)	88.33 \pm 2.89	85-90
Motility duration (s)	45.99 \pm 15.45	29-59

Table 2 Sperm motility rate (%) and motility duration (s) of groups in the *O. mykiss* applied L-arginine (Mean \pm SD)

Treatments (mM)	Sperm Motility Rate (%)	Motility Duration (s)
Control (0)	88.33 \pm 2.89	45.99 \pm 15.45
1	88.33 \pm 2.89	46.65 \pm 5.60
2	90.50 \pm 6.14	52.08 \pm 5.86
4	91.25 \pm 2.50	52.94 \pm 3.03
8	67.50 \pm 9.57	46.33 \pm 4.76

Table 3 Some sperm quality characteristics in Salmonids reported from present study and former studies.

Species	SV	pH	SP	SD	Researcher
<i>Salmo salar</i>				3.5 – 17.9	Aas et al. (1991)
<i>Oncorhynchus mykiss</i>				8.9	Ciereszko and Dabrowski (1993)
<i>Salmo trutta caspius</i>			28.8-45.6	6.02	Hajirezaee et al (2010)
<i>Salmo trutta macrostigma</i>	13.93 \pm 0.84	7.53 \pm 0.20	55.6 (24-72)	0.8 – 5.3	Bozkurt et al. (2011)
<i>Salmo cettii</i>	0.2-5		63.2	6.5-14.7	Iaffaldano et al. (2016)
<i>Oncorhynchus mykiss</i>	7.33 \pm 0.18	7.17 \pm 0.34	40.00 \pm 0.18	3.81 \pm 0.24	Kocabaş and Kutluyur (2017a)
<i>Salmo rizeensis</i>	7.00 \pm 0.25	7.76 \pm 0.22	55.33 \pm 0.24	9.27 \pm 0.56	Kutluyur and Kocabaş (2017)
<i>Salmo coruhensis</i>	6.67 \pm 0.53	7.71 \pm 0.14	50.00 \pm 0.35	6.18 \pm 0.52	Kocabaş and Kutluyur (2017b)
<i>Salmo coruhensis</i>	4.25 \pm 0.95	7.60 \pm 0.13	31.25 \pm 0.24	7.25 \pm 0.17	Kocabaş et al. (2018)
<i>Oncorhynchus mykiss</i>	8.60 \pm 4.31	7.75 \pm 0.14	19.27 \pm 0.24	9.20 \pm 0.15	In this study

SV: Sperm volume (ml), SP: Spermocrit (%), SD: Sperm density ($\times 10^9$)

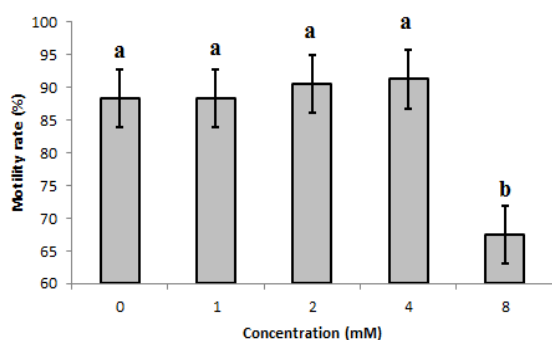


Figure 1 Effect of different levels of L-arginine on percentages of motility in *O. mykiss* sperm. Values at the different parameters marked with the same letter are not significantly different at $P < 0.05$. The bars and vertical lines represent mean \pm SD, $n = 6$

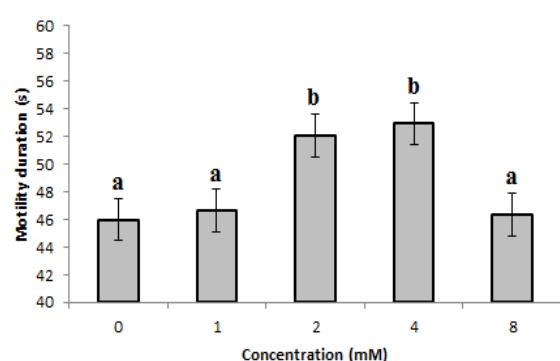


Figure 2 Effect of different levels of L-arginine on motility duration of *O. mykiss* sperm. Values at the different parameters marked with the same letter are not significantly different at $P < 0.05$. The bars and vertical lines represent mean \pm SD, $n = 6$

Furthermore, triggering the ATP production and the metabolism of glucose are stimulated by NO (ShikhMaidin et al., 2014). Especially, the ATP production affects movement flagella of sperm cells with succinate dehydrogenase enzyme providing respiratory electron transfer chain and citric acid cycle and being important for mitochondrial activity

Previous reports demonstrated that the increasing doses of L-Arginine caused the decrease in the sperm quality (İnan et al., 2004; Hassanpour et al., 2007; Hassanpour et al., 2010; Wilson-Leedy and Ingermann, 2011; ShikhMaidin et al., 2014). Contrary to the above-mentioned studies, Kocabaş et al. (2018) reported positive impact of L-Arginine with increasing concentration on sperm motility and longevity of *S. coruhensis*. In this work, the used highest concentration (8 mM) reduced motility rate and duration of sperm cells. Hence, researches are required for assessment threshold dose of L-arginine for *O. mykiss* spermatozoa.

There is no published literature on the use of L-arginine in rainbow trout sperm for comparison. In the one report available on the use of L-arginine in fish (*S. coruhensis*) *in vitro* usage, the supplementation of L-arginine at different concentrations (0, 2, 4 and 8 mM) improved motility and survival of spermatozoa (Kocabaş et al., 2018). In this

former study, the highest concentration (8 mM) exhibited motility enhancement effect in *S. coruhensis*. Contrary to the findings in our former work, the effective concentration of L-arginine was 4 mM in rainbow trout. The reason of the results may be species difference.

Conclusions

In summary, these results showed that L-arginine was the motility-promoting agent and improving sperm motility can be provided by *in vitro* addition of L-arginine in rainbow trout. This study would be useful to evaluate the L-arginine effect on spermatozoa of other fish species. To clarify its involvement and the mechanism, complementary tests and analysis are needed on subsequent fertilization and hatching success, and larval development.

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