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RESEARCH PAPER

Evaluation of the in vitro effect of cystein on sperm quality of Çoruh trout (*Salmo coruhensis*)

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Abstract

In this study, trials were conducted to assess the impact of cysteine addition to activation solution on sperm motility of Çoruh trout (*Salmo coruhensis*). In the trial, we used to different concentrations [0 mM (Control), 1 mM, 2 mM and 4 mM] of cysteine. Sperm motility characteristics and motility were determined. The current study revealed that the presence of cysteine resulted in an increase in sperm motility. The increases in duration (39.00±6.98 s) and motility rate (96.67±5.77%) at 2 mM were statistically significant ($p<0.05$). Overall, supplementation of cysteine to activation solution can increase the sperm motility of Çoruh trout and high doses of cysteine cause reduction on sperm motility.

Introduction

Sulfur-containing amino acids (cysteine, methionine, taurine and homocysteine) play an important role in the structure of proteins containing the sulfhydryl (thiol) group, in metabolic reactions and in the immune system (Margeret, 2001). As a proteinogenic amino acid, cysteine ($C_3H_7NO_2S$) is found in structure of proteins and plays crucial roles in metabolism and the antioxidant defense system (Demirkol et al., 2004; Kusmierek and Bald, 2008). Therefore, cysteine is necessary for biological events due to its important roles in primary and secondary metabolism (Wirtz and Droux, 2005). Sperm quality is important for production in aquaculture and has an important role in the success of fertilization and hatching (Kutluyer et al., 2015,2016; Kocabas and Kutluyer, 2017a, b, c). Decreased sperm quality may result in reduction in populations of fish. The penetration of sperm cells into the eggs and the initiation of fertilization depend on the activation of sperm in fish species with external fertilization (Dzyuba and Cosson 2014; Liu et al., 2018). Recently, studies have been conducted on the utilization of antioxidant properties and the supplementation of amino acids to diluents in different fish species (*Cyprinus carpio, Sparus aurata, Dicentrarchus labrax, Oncorhynchus mykiss*) (Öğretmen et al., 2015). As a sulfur-containing amino acid, cysteine prevents free radical formation through interactions with chemicals

(Bilodeau et al., 2001; Cocco et al., 2005). Regarding fish, information on the use of cysteine-containing extenders is limited. Stejskal et al. (2008) investigated the impact of cysteine in *Acipenser baerii*, *Perca fluviatilis Sander lucioperca* and *Acipenser ruthenus* spermatozoa. Kledmanee et al. (2013) suggested that L-cysteine addition positively affected sperm movement parameters of carp (*C carpio*) preserved sperm. In this context, we aimed to determine the effect of cysteine supplementation to activation solution on motility and duration of Çoruh trout (*S. coruhensis*) spermatozoa.

Material and Methods

Sperm were obtained from the broodstock (2-3 years old) in the İsina Trout Production Facility (Rize, Turkey). The males were anesthetized using clove oil (50 mg/lt) and sperm were collected with the abdominal massage method. Care was taken not to mix urine or feces with sperm in order to prevent contamination during stripping. The color, volume (ml) and pH parameters of the semen were examined. Concentration of spermatozoa was determined by hemocytometric method and data were expressed as $x10^9$/ml. Cysteine was added to the activation solution (50 mM/L $NaHCO_3$ and 60 mM/L Tris, pH 9) at different concentrations [Control (0), 1 mM, 2 mM, 4 mM] and its *in vitro* effect was assessed. Motility, which is expressed as the ratio of spermatozoa that move in one direction and strongly, to spermatozoa that are motionless and show other forms of movement, was determined using a x 40 magnification (%) in light microscope with SCA (Sperm Class Analyser) (Microptic S.L., Barcelona, Spain). The survival time of the spermatozoon was recorded until the last motile spermatozoon lost its viability in the sperm sample placed on the microscope table. Obtained data are presented as mean±SD. Statistical analyses were performed with SPSS 14.0 software. Kruskal Wallis analysis of variance and Duncan test were applied to analyze the differences between different concentrations on sperm motility characteristics. Significance level was determined as $p<0.05$.

Results

In the study, as the main spermatological features, sperm volume, spermatozoa motility, sperm pH and color were macroscopically determined for the sperm of Çoruh trout (Table 1). In all sperm samples, the color was determined as white-cream and the mean of pH was 7.60±0.13. After the spermatological examination of the sperm samples, the sperm quality parameters (motility percentage and duration) obtained from activation solution with added cysteine are given in Figures 1 and 2. Our data indicated that the supplementation of cysteine to the activation solution enhanced the percentage and duration of motile cells in Çoruh trout (*S. coruhensis*) sperm ($p<0.05$). The best results were obtained at the 2 mM concentration. When the data were evaluated, there was a decrease in motility time and percentage after 2 mM concentration.

Table 1. Sperm motility parameters (mean ± SD) of Çoruh trout (*S. coruhensis*) (n=6).

Parameters	Mean
Colour	White
Volume (mL)	5.18±1.07
pH	7.60±0.13
Spermatocrit (%)	21.92±1.89
Sperm density (×10^9)	6.69±0.54

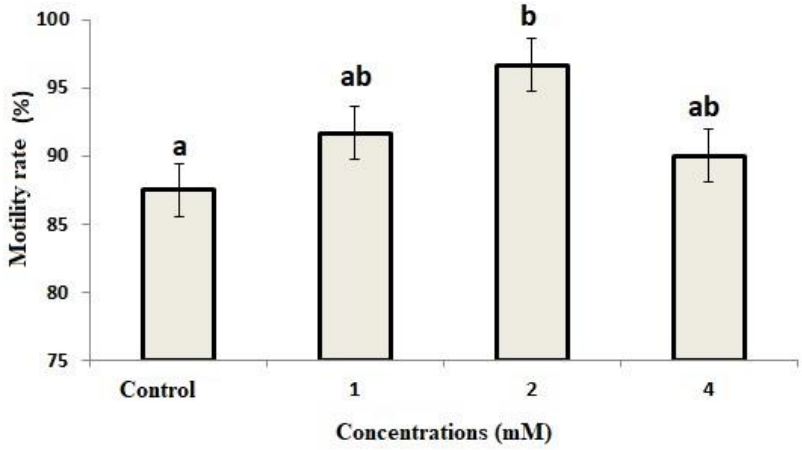

Figure 1. The effect of cysteine on motility rate (%) of *S. coruhensis*.

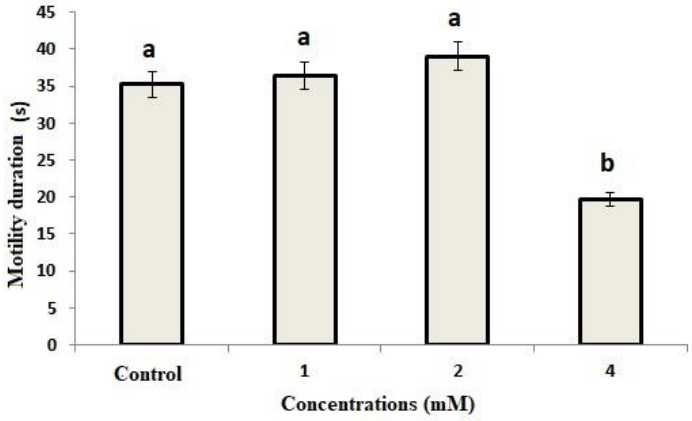

Figure 2. The effect of cysteine on motility duration (s) of *S. coruhensis*.

Discussion

The values obtained from this study are different from previous studies when sperm quality parameters are compared (Table 2). The reason for this difference may be ecology, breeding period and time, age and weight of the broodstocks (Piironen and Hyvarinen 1983; Suquet et al., 1998; Tekin et al., 2003; Kocabaş and Kutluyer 2017b).

Antioxidants are compounds that can stabilize a molecule by reducing its reactivity with free radicals, thus preventing or delaying the oxidation of biomolecules in the region where reactive species are formed and, the Reactive Oxygen Species (ROS) formation is prevented by antioxidants (Partyka et al., 2013). As a proteinogenic amino acid, cysteine is non-essential and acts as a building block for protein synthesis. Cells use L-cysteine for the synthesis of glutathione (GSH) (Demirkol et al., 2004; Kusmierek and Bald, 2008). Some cysteine-containing organosulfide compounds such as diallyl sulfide, diallyl disulfide, N-acetyl cysteine, S-allyl cysteine, S-methyl cysteine, S-ethyl cysteine and S-propyl cysteine have reducing power, metal chelating ability and superoxide ion scavenging ability. They are effective scavengers of reactive oxygen species and useful in preventing oxidative stress (Wirtz and Droux, 2005). Recently, studies have been conducted on supplementation amino acids with antioxidant properties to diluents in different fish species (Valdebenito et al., 2010; Cabrita

et al., 2011; Ekici et al., 2012; Rani and Munuswamy, 2014). In particular, studies on the use of cysteine-containing extenders in fish species are limited (Stejskal et al., 2008). Studies on supplementing extenders with amino acids have reported that sperm motility parameters (straight-line velocity, curvilinear velocity, total motility), membrane integrity and DNA integrity in sperm cells are protected by amino acids (Öğretmen et al., 2015). In consequence of cysteine supplementation, the enhancement in sperm movement parameters and motility duration, fertility and hatching rate were reported by Kledmanee et al. (2013). Our results are in agreement to these reports. In this study, the supplementation of cysteine to the activation solution enhanced the percentage and duration of motility. The increase may be explained with the synthesis of glutathione (GSH) and electron transfer reactions by cysteine (Piste et al., 2013).

Table 2. Sperm features of different Salmonid species obtained from the literature.

Species	Sperm volume (ml)	pH	Spermatocrit (%)	Sperm concentration ($\times 10^9$/ml)	References
Oncorhynchus mykiss	7.33±0.18	7.17±0.34	40.00±0.18	3.81±0.24	Kocabaş and Kutluyer (2017a)
Salmo rizeensis	7.00±0.25	7.76±0.22	55.33±0.24	9.27±0.56	Kocabaş and Kutluyer (2017b)
Salmo coruhensis	6.67±0.53	7.71±0.14	50.00±0.35	6.18±0.52	Kocabaş and Kutluyer (2017c)
Salmo coruhensis	8.60±4.3	7.75±0.14	19.27±0.24	9.27±0.56	Danabaş et al. (2019)
Salmo coruhensis	5.18±1.07	7.60±0.13	21.92±1.89	6.69±0.54	Present study

For efficiency of amino acids, determination of the best concentration and combination is important to obtain good results in sperm quality. Earlier studies reported that higher doses of amino acids adversely affect sperm movement parameters owing to hypertonicity and osmotic toxicity (White, 1993; Trimèche et al., 1999; Funahashi and Sano, 2005; Khlifaouia et al., 2005; Kaeoket et al., 2010). Kledmanee et al. (2013) worked different doses of L-cysteine (0, 0.5, 1, 1.5 and 2 mM) in cryopreservation of carp (*C carpio*) sperm and reported that the increase in L-cysteine concentration caused low sperm characteristics due to its toxic impact. Contrary to this result, Öğretmen et al. (2015) obtained higher post-solution percentage and motility time, fertilization and hatching rate at 20 mM concentration, and determined that DNA damage of carp (*C carpio*) sperm was improved with increasing concentrations. Similar to previous studies, in this study, sperm motility percentage and duration was reduced after the concentration of 2 mM.

Conclusions

In conclusion, cysteine is important for fish and has positive effects on sperm quality. In this study, the different concentrations of cysteine affected sperm quality and the best results were obtained at the 2 mM concentration. This study will be useful and beneficial for future studies and commercial production.

Ethical approval

The experiments were approved by the Institutional Animal Care and Use Committee at Karadeniz Technical University in Turkey.

Data availability statement

The authors declare that data are available from authors upon a reasonable request.

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