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Research Article

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Dose dependent treatment with boric acid induces more changes in the sperm cells of endangered trout *Salmo coruhensis* and rainbow trout *Oncorhynchus mykiss*

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Abstract This study was designed to test the usefulness of boric acid on endangered trout *Salmo coruhensis* and rainbow trout *Oncorhynchus mykiss* sperm quality and fertility. Different activation solutions (NaCl, 0.3%; $NaHCO_3$, 1%) were supplemented with boric acid (0.5, 1, 2, 3, 4 and 5 mM). Sperm motility, duration, fertility and hatching rate were determined in sperm samples. Our results indicated that addition of boric acid (0.5 mM) to different activation solutions was increased motility rate, duration, fertility and hatching rate in endangered trout (*S. coruhensis*) and rainbow trout (*O. mykiss*). On the other hand, an increase in the concentration of boric acid caused a significant decrease the motility rate, fertility and hatching rate of sperm endangered trout (*S. coruhensis*) and rainbow trout (*O. mykiss*) ($p<0.05$) after concentration 2 mM. The boric acid concentrations in activation solution containing NaCl used for *O. mykiss* were too high to determine the dose response in the non-toxic range. Consequently, sperm quality was affected by quantitative changes different concentrations of boric acid and the best results were obtained at concentration 0.5 mM for two species. Additionally, further experiments would be realized for *O. mykiss* in activation solution containing NaCl to determine the dose response in the non-toxic range.

Keywords *Salmo coruhensis*; *Oncorhynchus mykiss*; boric acid; sperm cell.

Introduction

Oncorhynchus mykiss and *Salmo trutta* are the most important Salmonid fish species owing to its aquaculture potential, wide consumer demand and economic value [1]. *Salmo trutta* forms populations are distributed in the upper streams of rivers in Europe, North Africa, Anatolia and West Asia [2-3]. In addition, it is one of potential species in recreational fishery. Recently, *S. t. labrax* ecotype has been described by Turan *et al*. [4] as *S. coruhensis* [5-6]. In addition, *S. coruhensis* is an endemic anadromus fish and only distributed in the rivers of Eastern Black Sea Region [7]. 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 [7-11]. Sperm motility is the essential functional parameter for successful fertilization in fish [12-13]. 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 [13-14]. Sperm is activated by aqueous solution through traditional methods. However, activation rates can be insufficient and increased with supplementation of different chemical substances to activation solutions. Therefore, characteristics of activation solution are crucial in terms of progression and initiation of sperm motility [13].

Trace elements have a crucial role for the male reproductive process. Boric acid (H_3BO_3) is a bioactive beneficial element [14] and widely used in glass, ceramic, detergent, plastic, agricultural, textile, metallurgy,

nuclear and medicine industries owing to its excellent characteristics and it is produced from boron ore and salt lake brine [16-18]. The greatest majority of boric acid is produced by Eti Maden Works (Turkey) in the world [19]. Increasing production does not meet the demand due to using as extensive [20-21]. Several studies about the nutritional benefits [15, 20 22-23], metabolic functions [24-27], U shaped dose responses on growth of embryonic fish and frogs [28-29], and therapeutic applications [30-32] toxic effect on male reproduction system of different species (e.g. rat, rodent, dog, human) [33-36] of boron and its compounds have been published in the latest available literature. As far as the authors of this work are aware, no attempt has been made to use of boric acid on sperm quality and fertility of fish species. In this context, the aim of this study was to examine effect of supplementation of different activation solutions (NaCl, 0.3%; $NaHCO_3$, 1%) with different boric acid concentrations (0.5 mM; 1 mM; 2 mM; 3 mM; 4 mM; 5 mM) on endangered trout *S. coruhensis* and rainbow trout *O. mykiss* sperm quality and fertility.

Materials and Methods

Six mature endangered trout males (1652.71±0.52 g, 45.61±2.62 cm as mean±SD) and rainbow trout (1388.00±0.55 g, 44.52±2.62 cm as mean±SD) were randomly selected from a broodstock at natural photoperiod and temperature in Fish Production Station Meryemana, Trabzon, Turkey for sperm collection. Water temperature and dissolved oxygen were 5.0±1ºC and 8.6±0.4 mg l^{-1}, respectively. Males were anesthetized (Benzoacaine, 50 mg/L) before stripping. Caution was exercised to prevent contamination of the semen with feces, urine, mucus, blood or water. The sperm was collected by a gentle abdominal massage, collected into glass vials and stored on ice (2-4 °C) until use [36-39].

In this study, two activation solutions were prepared; a) NaCl (0.3%) and deionized water, b) $NaHCO_3$ (1%) and deionized water. Boric acid was separately added to the activation solutions (one per experimental group): (a) 0.5 mM, (b) 1 mM, (c) 2 mM, (d) 3 mM, (e) 4 mM, (f) 5 mM for pure water. Control groups of activation solutions were not supplemented with boric acid. Sperm samples was evaluated for the motility parameters using a light microscope with a digital image processing software connected to the computer (Eclipse E50; Nikon Corporation, Tokyo, Japan) to evaluate the percentage of spermatozoa motility and duration. The percentage of sperm motility was estimated as the cell performing progressive forward movement while the duration of motility was determined as the time until forward movement stops. Assessing the percentage of sperm motility was assessed using an arbitrary scale with 10% interval increments in which non motile represents 0% [13, 37-40]. Fertilization experiments were carried out 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 assessed as the percentage of eyed embryos in relation to the total number of eggs 28 to 30 d after fertilization [37].

Statistical analysis were performed using SPSS 14.0 software and values were reported as mean±SD. ANOVA (one-way) with Duncan *post hoc* tests was used for assessment differences among groups. The level of significance was set as 0.05.

Results

Effect of boric acid on the motility rate, duration fertility and hatching rate of sperm for *S. coruhensis* was shown in Figure 1, 2 and 3. The results of the present study indicated that differences in the motility rate, duration, fertility and hatching rate of *S. coruhensis* sperm were significant among the treatments ($p<0.05$). Highest motility (99%) was at concentration 2 mM in activation solution containing NaCl whilst highest duration of motility (65 s) was obtained from control group. Highest motility (98%) was at concentration 0.5, 1, 2 mM in activation solution containing $NaHCO_3$ while highest duration of motility (164 s), fertility (95.05%) and hatching rate (85.07%) were obtained at concentration 0.5 mM. After concentration of 2 mM, a remarkably decrease was observed in motility rate, fertility and hatching rate.

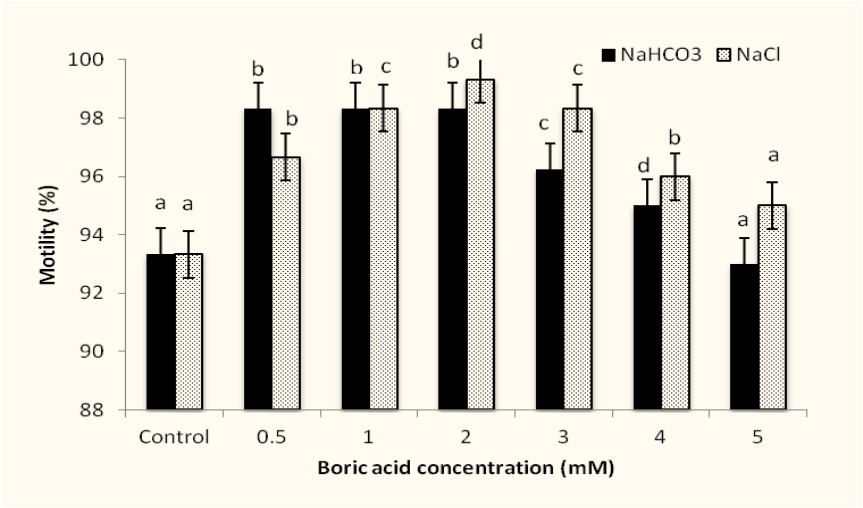

Figure 1: Effect of supplementation of boric acid to different activation solutions on the motility rate of S. coruhensis sperm (n=6). Different letters show differences between treatments (p<0.05).

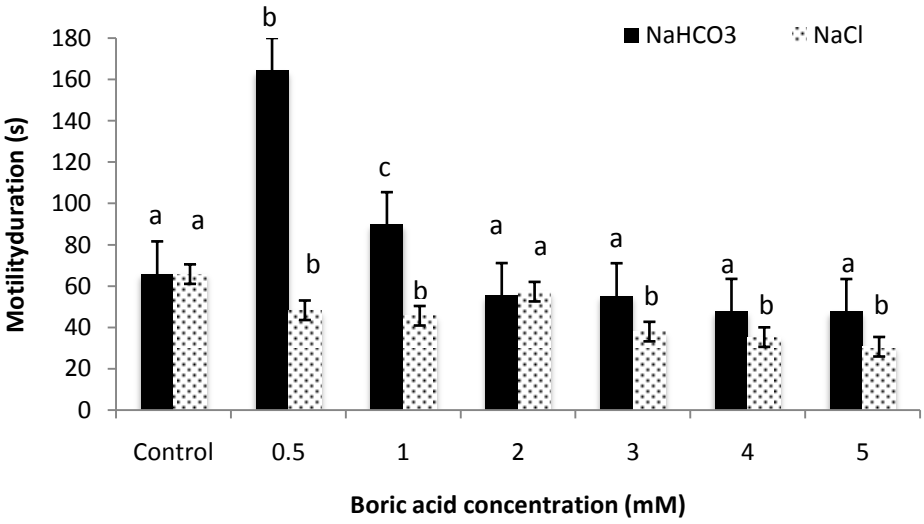

Figure 2: Effect of supplementation of boric acid to different activation solutions on the motility duration of S. coruhensis sperm (n=6). Different letters show differences between treatments (p<0.05).

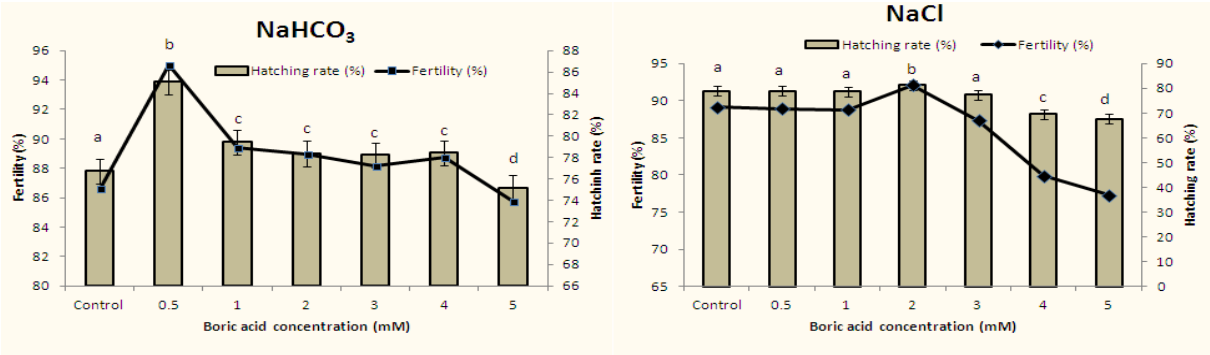

Figure 3: Effect of supplementation of boric acid to different activation solutions on the fertility and hatching rate of S. coruhensis sperm (n=6). Different letters show differences between treatments (p<0.05).

Effect of supplementation of boric acid to activation solution on motility, duration fertility and hatching rate for *O. mykiss* is presented in Figure 4, 5 and 6.

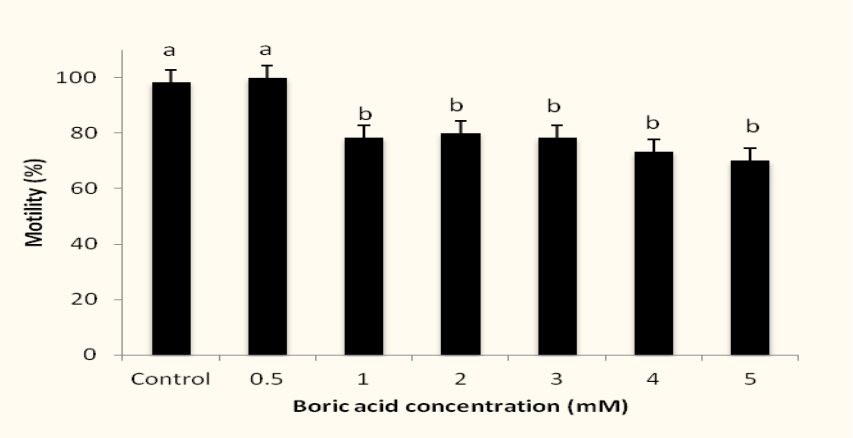

Figure 4: Effect of supplementation of boric acid to activation solution containing $NaHCO_3$ on the motility rate of O. mykiss sperm (n=6). Different letters show differences between treatments (p<0.05).

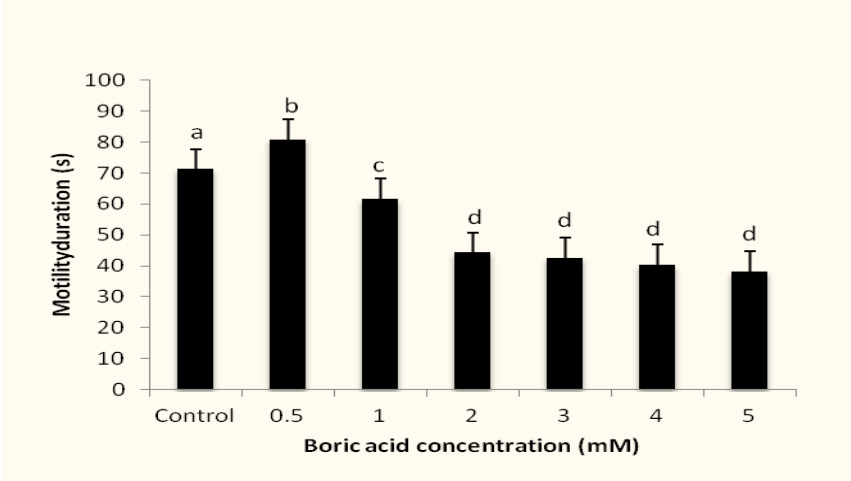

Figure 5: Effect of supplementation of boric acid to activation solution containing $NaHCO_3$ on the motility duration of O. mykiss sperm (n=6). Different letters show differences between treatments (p<0.05).

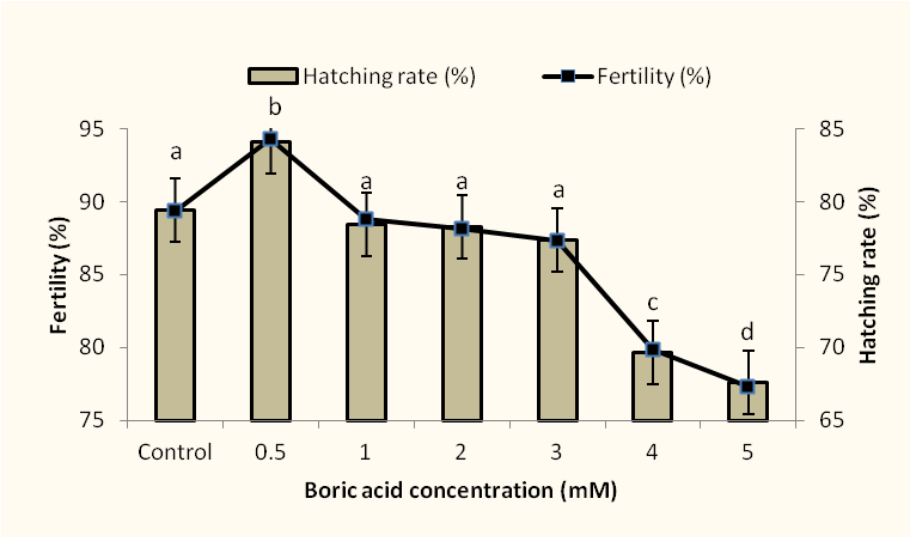

Figure 6: Effect of supplementation of boric acid to different activation solutions on the fertility and hatching rate of O. mykiss sperm (n=6). Different letters show differences between treatments (p<0.05).

The trials in this study indicated that differences in the motility rate and duration of *O. mykiss* sperm were significant among the treatments ($p<0.05$). Highest motility (100%), duration of motility (81 s), fertility (94.27%) and hatching rate (84.07%) were at concentration 0.5 mM in activation solution containing $NaHCO_3$. In particular, a significant decrease after concentration 0.5 mM was observed in motility rate and duration. Surprisingly, results from activation solution were not obtained when boric acid was added to activation solution containing NaCl.

Discussion

To the best of our knowledge, this is apparently the first report on effect of sperm activation solution supplemented with boric acid on *S. coruhensis* and *O. mykiss* sperm, although studies have been conducted about the nutritional benefits [15, 22, 23, 25], metabolic functions [24-27], U shaped dose responses on growth of embryonic fish and frogs [28-29], and therapeutic applications [30-32] toxic effect on male reproduction system of different species (e.g. rat, rodent, dog, human) [33-36] of boron and its compounds. In this study, we demonstrated the usefulness of boric acid in different activation solutions for two trout species sperm. Using 0.5 mM boric acid in activation solution resulted in high sperm motility rate (98.33%), duration (164.33 s), fertility (95.05%) and hatching rate (85.07%) of *S. coruhensis* while high sperm motility rate, duration fertility and hatching rate in *O. mykiss* were 100.00%, 81.00 s, 94.27% and 84.07, respectively. This may be due to the fact that boric acid is involved in a number of metabolic processes and interact with critical biological substances, including pyridoxine, polysaccharides, dehydroascorbic acid, riboflavin, and the pyridine nucleotides [24, 41-43]. However, increase in concentration of boric acid in activation media was decreased motility rate, duration, fertility and hatching rate. In particular, a significant decrease after concentration 2 mM was observed in motility rate, duration, and fertility and hatching rate. This may be due to toxic effect of boric acid. Interestingly, data from activation solution were not obtained when boric acid was added to activation solution containing NaCl. Reason of the result may be inhibition of motility rate, the sperm lipid peroxidation, disrupting membrane integrity, damage of DNA duration, low proportion of live cells and infertility owing to negatively reaction of boric acid and NaCl for *O. mykiss* sperm. In addition, the results showed a U-shaped response for boric acid. This finding is in agreement with results from previous studies: Rowe *et al*. [28] and Fort *et al*. [29].

Conclusion

In conclusion, based on these results, boric acid was used efficiently for *S. coruhensis* sperm. The boric acid concentrations in activation solution containing NaCl used for *O. mykiss* were too high to determine the dose response in the non-toxic range. Our study provides new insights related to use of boric acid on fish sperm quality and fertility. The knowledge of effects of boric acid and its mechanism of action might be helpful for both research and commercial use. Further studies would be needed to evaluation the precise mechanisms and to determine the dose response in the non-toxic range.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

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