

Effects of acclimation to seawater salinity on some blood parameters in wild Caspian brown trout, *Salmo trutta caspius*

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Abstract In the present study, some hematological indices were assayed after salinity challenge in smolts obtained from wild populations of Caspian brown trout. The experiment was designed into three treatments and one control group containing 90 fish per treatment. In experimental treatment, fish were exposed to salinity challenge (12 mg/l salinity) for 48 h. Fish of control group were not challenged and held in freshwater with 0.37 g/l salinity. After salinity challenge in seawater, the higher values of plasma cortisol and glucose were found in fish acclimated to seawater compared to control group ($P < 0.05$). No significant differences were found between control group and fish before salinity challenge in terms of plasma glucose and cortisol levels ($P > 0.05$). Plasma potassium and sodium concentrations were statistically similar between experimental groups and control group ($P > 0.05$). Also, plasma protein levels were lower in fish exposed to salinity challenge compared to control ($P < 0.05$). The values of red blood cells (RBC), white blood cells (WBC), and hemoglobin (Hb) increased significantly after salinity challenge ($P < 0.05$). In addition, there were no significant differences between control group and fish before salinity challenge in terms of plasma RBC, WBC, and Hb ($P > 0.05$). In conclusion, the results of the present study confirm the ability of the

Caspian brown trout smolts in controlling osmoregulation by alternation of some hematological parameters.

Keywords Salinity · Challenge · Potassium · Sodium · Protein · Wild Caspian brown trout

Introduction

Caspian brown trout are known as one of the ecologically and commercially important fish species of the Caspian Sea. The natural populations of Caspian brown trout are declining due to the many cues including overfishing and loss of natural environments in sea and related rivers (Kiabi et al. 1999; Hajirezaee et al. 2010a, b). Therefore, this fish was considered as an endangered species and thus thousands of smolts are annually produced by hatcheries and restocked in nature (Kiabi et al. 1999; Hajirezaee et al. 2010a, b).

Adaptation to seawater salinity is the important stage for released smolts of Caspian brown since fish must be able to be adapting with increased salinity. Generally, an appropriate understanding on physiological bases of this adaptation may help to identify the biomarkers of osmoregulation in Caspian brown trout. In addition, previous studies reported that different salinity levels lead changed hematological and biochemical parameters in fish. Hematology, a valuable tool for determination of fish physiological condition and blood parameters, is commonly used as a physiological index against internal and external changes (Garcia-Navarro 1994; Cataldi et al. 1998; Serpunin and Likhatchyova 1998; Ranzani-Paiva et al. 1999; Fazio et al. 2012, 2013, 2017; Toorchi et al. 2012).

In teleosts including the Caspian brown trout, osmoregulation and components which participate in this process have been well recognized. In this regard, the study of chloride cells and activity of Na^+/K^+ -ATPase are common (Hoar 1988;

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Evans 1993). However, little data is available regarding hematological parameters such as the number of red blood cells, white blood cells, hemoglobin, proteins, etc. Therefore, the present study was aimed to study the effects of salinity challenge on hematological and biochemical components of wild Caspian brown trout, with two major goals including obtaining data on plasma biochemical parameters and assaying how plasma biochemistry is alerted following salinity challenge.

Materials and methods

A total of 360 healthy fish (6.2 ± 0.13 g) were prepared from Kelardasht Salmonids Reproduction Centre (KSRC), Iran. Then, fish were randomly distributed into 12 tanks in groups of 30 with three replicates (totally 12 tanks). Three groups were considered as experimental treatments and one group was considered as control. After 3 days adaptation to tank condition, in experimental treatment, fish were exposed to water with 12 mg/l salinity for 48 h. Fish of control group were not introduced to salinity acclimation. Fish were not fed throughout the experimental period (48 h). The water salinity (12 mg/l) was provided by addition of sea salt. The aeration of tanks was carried out to maintain the levels of dissolved oxygen at 7.1 ± 0.13 mg L⁻¹, and also water temperature and pH during the experiment were maintained at 17.1 ± 0.11 °C and 7.5 ± 0.12 , respectively. Water was exchanged in order to prevent ammonia toxicity and other wastes in the tanks every 12 h.

Fish sampling and analytical methods

The blood samples were collected from the caudal vein with syringes in two steps: (1) before acclimation to seawater (SW) and (2) 48 h after acclimation to SW. Before blood sampling, fish were anesthetized with MS222 (200 mg L⁻¹ for 5–10 min). Then, an aliquot of blood samples was used for determination of RBCs, WBCs, and Hb. The remainders were centrifuged and plasma samples were stored immediately at -80 °C until assay of cortisol, glucose, potassium (K⁺), sodium (Na⁺), and total protein. All biochemical and hematological

Table 1 Changes of organic parameters in the serum of Caspian brown trout smolts after 48 h acclimation in seawater. Means with different letters are statistically different

Organic parameters	Control	Before SW-adapting	After SW-adapting
Total protein (mg/ml)	18.1 ± 1.1 ^a	17.3 ± 0.9 ^a	4.5 ± 0.8 ^b
Glucose (mg/dl)	8.5 ± 1.2 ^a	9.1 ± 1.3 ^a	13.4 ± 1.7 ^b
Cortisol (ng/l)	7.5 ± 0.3 ^a	9.1 ± 2.1 ^a	14.1 ± 0.9 ^b

Table 2 Changes of ions in the serum of Caspian brown trout smolts after 48 h acclimation in seawater. Means with different letters are statistically different

Organic parameters	Control	Before SW-adapting	After SW-adapting
Na ⁺ (mM/l)	131.5 ± 11 ^a	142.1 ± 21 ^a	125.2 ± 20 ^a
K ⁺ (mM/l)	0.9 ± 0.1 ^a	1.1 ± 0.4 ^a	0.8 ± 0.3 ^a

components were assayed according to Sahkar et al. (2015). In this regard, RBC counting was performed by using hemocytometers. The concentration of Hb was measured by using a spectrometer according to the cyanmethemoglobin method at a wavelength of 550 nm. Plasma cortisol was assayed with the method of radioimmunoassay. Plasma glucose was assayed with an assay kit. Plasma K⁺ and Na⁺ values were measured using a flame photometer.

Data analysis

The data of the present study was analyzed by using SPSS software. The data normality was determined with Kolmogorov–Smirnov test before analysis of variance. Then, one-way analysis of variance (ANOVA) was employed to compare the means. After determining the *F* values, the Tukey test was used to determine which groups were different.

Results

The plasma cortisol (Table 1) and glucose (Table 1) were higher in fish exposed to salinity challenge compared to control group ($P < 0.05$). No significant differences were found between control group and fish before salinity challenge in terms of plasma glucose and cortisol levels (Table 1, $P > 0.05$). There were no significant differences between experimental groups and control group in terms of plasma levels of potassium (Table 2) and sodium (Table 2) ($P > 0.05$). Also, plasma protein levels were lower in fish exposed to salinity challenge compared to control (Table 1, $P < 0.05$). The values of RBC (Table 3), WBC

Table 3 Changes of hematological parameters in the serum of Caspian brown trout smolts after 48 h acclimation in seawater. Means with different letters are statistically different

Hematological parameters	Control	Before SW-adapting	After SW-adapting
RBC ($\times 10^8$)	2.7 ± 1.2 ^a	1.8 ± 0.9 ^a	4.3 ± 1.1 ^b
WBC ($\times 10^8$)	2.5 ± 0.7 ^a	2.1 ± 0.5 ^a	4.4 ± 0.4 ^b
Hb (g/100 cm ³)	5.1 ± 1.2 ^a	4.5 ± 1.3 ^a	9.1 ± 2.1 ^b

(Table 3), and Hb (Table 3) increased significantly after salinity challenge ($P < 0.05$). There were no significant differences between control group and fish before salinity challenge in terms of plasma RBC, WBC, and Hb (Table 3, $P > 0.05$).

Discussion

There are some studies regarding the effects of different salinity levels on hematological and biochemical parameters in fish. Hematology, a valuable tool for determination of fish physiological condition and blood parameters, is commonly used as a physiological index against internal and external changes (Garcia-Navarro 1994; Cataldi et al. 1998; Serpunin and Likhatchyova 1998; Ranzani-Paiva et al. 1999; Fazio et al. 2012, 2013, 2017; Toorchi et al. 2012).

In our study, plasma cortisol and glucose were higher in fish exposed to 12 g/l salinity compared to control group. The role of cortisol in osmoregulation has been well known in teleosts (Reviewed by McCormick 2001).

However, stress caused hormonal responses especially the elevation of plasma cortisol and catecholamines, regulating the osmoregulation ability of fish in relation to environments with high osmotic condition such as seawater (Pickering and Pottinger 1995). Cortisol increases the tolerance of fish to high osmotic pressure through its effects on the permeability of the cell membrane, increasing glycogenolysis in the liver and further elevations in plasma glucose. Also, this hormone elevates the activity of Na^+/K^+ -ATPase and the number and size of chloride cells (Reviewed by McCormick 2001). Thus, the elevation of cortisol and glucose in our study may be in relation to the key role of cortisol in acclimation to water salinity in Caspian brown trout smolts.

Cortisol provides glucose for energetic requirements of osmoregulation in seawater (Krumnschnabel and Lackner 1993; Plaut 1998). During osmotic regulation of euryhaline fish species, the energy costs are usually provided by metabolic hydrolysis of glycogen stores in the liver through cortisol action and further increases in glucose (Shahkar et al. 2015). In Persian sturgeon, the plasma cortisol levels increased clearly after 24 h salinity challenge, probably confirming the key role of this corticosteroid in stimulating *glycogenolysis* in the liver, subsequently releasing glucose into the blood and thus providing the energy for *hyperosmotic* shock.

In the present study, plasma levels of potassium and sodium were similar in all groups, indicating the high ability of smolts to regulate plasma ionic levels during 48 h salinity challenge. The lower plasma values of proteins in fish exposed to water with 12 g/l salinity compared to control group may be in relation to the role plasma proteins as energy

sources for osmoregulation as reported by Huang et al. (2006). Proteins are modulated towards production of energy when glucose and lipids are not so enough for providing the energetic costs of osmoregulation (Lin 1999; Huang et al. 2006; Tseng and Hwang 2008; Shahkar et al. 2015). In the present study, plasma levels of RBC and Hb increased significantly after acclimation to seawater. This result may be due to decreases in the water content of the blood which this decrease in water returns to the effects of hyperosmotic (Plaut 1998). Loss of water results increases in the concentrations of blood-cell components.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All experiments were conducted under a protocol for the care and use of animals approved by the committee of ethics of the faculty of sciences of the University of Tehran (357; 8 November 2000).

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