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SHORT COMMUNICATION

Effect of water salinity on enzymatic and hormonal indices of rainbow trout (*Oncorhynchus mykiss*) fingerlings

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Abstract Salinity is one of the stressful environmental factors which affects life, metabolism, and distribution of aquatic animals. In this study, the effects of different levels of water salinity were investigated on serum biochemical parameters in rainbow trout (*Oncorhynchus mykiss*) fingerlings. For this, the fish (5.53±0.057 g) were exposed to 0, 5, 13, 20 or 30 ppt water salinities for 60 days; then blood samples were taken from all treatments for hormonal and enzymatic studies. All fish died at 30 ppt water salinity, during adaptation. The results showed that there was a tendency to triiodothyronine (T3) elevation along with water salinity levels, although the changes were not statistically significant (P >0.05). Increase in water salinity significantly increased serum thyroxine (T4), cortisol, alkaline phosphatase (ALP) and protease levels. Among the water salinities, 20 ppt led to a significant elevation in serum alanine aminotransferase (ALT) activity. There was no significant difference in serum glucose and aspartate aminotransferase (AST) levels in the fish reared at 5 and 13 ppt salinities; both treatments exhibited lower values compared to the 0 and 20 ppt salinities. Water salinity induced no significant changes in serum lipase activity. The Results indicate that increase in water salinity causes elevation in cortisol and thyroid hormones, which are necessary for energy supply and osmoregulation. Moreover, rainbow trout may face organ damage in saltwater, particularly at 20 ppt.

Keywords Saltwater . Stress . Rainbow trout . Blood . Biochemistry

Introduction

Rainbow trout (*Oncorhynchus mykiss*) is one of the most important salmonid species, which is cultured in many parts of the world and has high economic value (Khodadadi et al. 2018). Climate changes and population growth led to limitation in use of freshwater for aquaculture industry; thus about half of the aquaculture productions have been shifted to brackish and saline waters (Hoseini et al. 2019). Considering that Iran is among the semi-arid countries in the world, it is necessary to focus on marine fish species or those with wide range of salinity tolerance. In this case, rainbow trout is a good candidate for aquaculture in Iran because it is produced in the country about 170000 tons per year (Taheri Mirghaed et al. 2019) and some studies have shown this species has an ability to tolerate water salinity (Teskeredžić et al. 1989). Previous studies have shown that rearing rainbow trout in brackish water (20 ppt), induced stress, pathological damages and immunosuppression (Hoseini 2019; Hoseini et al. 2019; Hoseini et al. 2020). However, there is a need to assess further parameters of rainbow trout at different salinities to increase physiological performance of this species at different salinities.

Water physicochemical parameters are important stress factors in aquaculture (Person-Le Ruyet et al. 2008; Mazandarani and Hoseini 2017) and among them, water salinity is common one with great effects on fish (Hoseini and Hosseini 2010; Hosseini and Hoseini 2012; Ghelichpour and Taheri Mirghaed 2019). Saltwater exposure affects fish biochemical parameters that can be used as effective tools to monitor the fish health during the saltwater exposure (Akhtar et al. 2013; Hedayati et al. 2014; Abdel-Tawwab and Monier 2018). As a stressful condition, water salinity triggers stress axis in fish, leading to cortisol release from interrenal tissue (Hoseini and Hosseini 2010). Cortisol, in turn, increases circulating glucose levels by

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activating gluconeogenesis to provide enough energy to cope with stress (Barton 2002). Thus, blood cortisol and glucose have been considered as common humoral responses of fish to water salinity in different studies (Hoseini and Hosseini 2010; Hosseini and Hoseini 2012; Ghelichpour et al. 2018). Thyroid hormones have also important role in fish growth and metabolism (Walpita et al. 2007; Ghelichpour et al. 2019). Moreover, it has been shown that circulating levels of thyroid hormones increases during transfer from freshwater to saltwater in salmonids (Redding et al. 1984; Prunet et al. 1989); thus, it is necessary to monitor these hormones during saltwater adaptation in the fish.

Blood enzymes are suitable indicators of fish health that can be used as indicators of different diseases and disorders (Simakani et al. 2018; Ghelichpour et al. 2019). Alanine aminotransferase (ALT) is found at high concentrations in fish liver and hepatocyte damage leads to leakage of the enzyme into circulation; thus, the level of the enzyme in circulation is an indicator of liver health and function (Yousefi et al. 2018b; Yousefi et al. 2018a). Aspartate aminotransferase (AST) is found in various tissues and is used to monitor histopathological damages in fish (Taheri Mirghaed et al. 2018). Alkaline phosphatase (ALP) is abundant in erythrocyte and biliary ducts and elevated level of the enzyme in fish blood is an indicator of hemolysis and/or biliary duct damages (Taheri Mirghaed et al. 2017). Lipase is produced in pancreas and is responsible for fat digestion in fish gut; however, pancreatic damages lead to lipase leakage into circulation, which is used to diagnose pancreas health (Firat and Kargın 2010). Proteases are diverse molecules and found in different cell types. These enzymes have various functions, one of them is activation of pro-proteins. Therefore, blood protease activity may increase when fish needs more proteins (hormones, enzymes and acute phase proteins) (Torrissen and Torrissen 1985). It has been found that rainbow trout may face stress and pathological damages in different tissues, when reared in salt water (Hoseini 2019; Hoseini et al. 2019a); therefore, monitoring of the aforementioned enzymes may help to understand the effects of water salinity on rainbow trout health. Considering the interest for rearing rainbow trout in salt water, the present study was conducted to investigate its efficacy under different water salinity by monitoring blood biochemical and enzymatic parameters.

Materials and methods

Experimental design

In the present study, 450 rainbow trout (5.53 ± 0.057 g) were classified into five groups reared at 0, 5, 13, 20 and 30 ppt water salinity for 60 days. Each salinity treatment contained three tanks (100×50×50 cm) and 30 fish were stocked in each tank. Water was supplied to the tanks through a freshwater well. Water salinity was adjusted by adding salt and measured by a salinity meter device (Cond 330i model). Water salinity increased 3 ppt per day to reach the aforementioned values. After acclimation with the experimental conditions, the fish were fed by a commercial feed during eight weeks. The feed contained 50% protein, 15% fat, 18% carbohydrate, 10% moisture, 3% fiber, and 4% ash. Feeding was carried out according to the feed manufacturer; the fish were fed near 3% biomass at 8:00, 12:00, and 16:00. Fish wastes were daily siphoned and the tanks water was daily exchanged (50%) to maintain water quality, and then shortly followed by the salt adjustment per tank for each salinity group. The tanks were aerated by an air pump and air stones to keep dissolved oxygen at accepted levels. The average dissolved oxygen, temperature, and pH were measured by a portable apparatus (Hach multiparameters meter, USA), determined at 7-8 mg L^{-1}, 17-19 °C, and 7.5-8.8, respectively. Water nitrite, nitrate, and total ammonia levels were measured by Palintest apparatus (Model 7500, England), measured at 0.08, 5.5 and 1.6 mg L^{-1}, respectively.

Enzymatic and hormonal assay

At the end of the experiment, blood samples were taken from all treatments. For this, three fish were caught from each tank (nine fish per treatment) and anesthetized by 0.5 g L^{-1} clove solution (Hoseini 2011). After anesthesia, blood samples were taken by syringe from the fish caudal vein and collected in non-heparinized tubes. Serum was obtained by 10 min centrifugation (3000 rpm) and kept at -20 °C until analysis.

Serum glucose content was measured by AutoAnalyzer device (Eurolyser model, Belgium) using Pars Azmun Co. (Tehran, Iran) kit based on glucose oxidase method (Mazandarani et al. 2017). ALT, AST and

Table 1 Serum T3, T4, glucose and cortisol levels in rainbow trout at different water salinities (mean ± SD; n = 9)

Water salinity (ppt)	T3 (ng mL^{-1})	T4 (ng mL^{-1})	Glucose (mg dL^{-1})	Cortisol (ng mL^{-1})
0	2.27±0.67^{a*}	6.81±1.18^{c}	95.0±0.27^{a}	6.84±0.40^{c}
5	2.61±0.28^{a}	9.03±0.50^{b}	55.7±0.43^{b}	9.21±0.18^{b}
13	2.40±2.05^{a}	9.49±0.98^{b}	49.9±9.48^{b}	8.92±0.71^{b}
20	3.59±0.24^{a}	12.3±0.99^{a}	120±8.73^{a}	15.2±0.69^{a}

*Different letters within a column indicate significant difference (P <0.05).

ALP activities were determined kinetically using a commercial kit (Pars Azmun Co., Tehran Iran) by auto-analyzer device (Eurolyser model, Belgium) according to Shahsavani et al. (2010). Contents of serum cortisol, triiodothyronine, and thyroxine were measured by radioimmunoassay (RIA) method. Assessment of protease activity was carried out using AZOcasein substrate solution 1.5% into 50 mM Tris/HCl buffer at pH=7.5 (García-Carreño and Haard 1993). Furthermore, serum lipase activity was determined by p-nitrophenyl myristate hydrolysis method and spectrophotometry (Iijima et al. 1998).

Statistical analysis

The data normality and variance homogeneity were checked by Shapiro-Wilk and Leven tests. Accordingly, the data of serum glucose, ALP, ALT, and AST were log-transformed before ANOVA. The data were analyzed by SPSS software (version 16). One-way ANOVA test was used to find significant effects of water salinity on the fish blood parameters. Significant differences among the treatments were determined by Duncan's multiple range tests at P <0.05.

Results

All fish died in salinity of 30 ppt during the adaptation period; thus, comparison was made among the rest of treatments. Increase in water salinity led to significant decrease in the fish growth rate. Specific growth rates (SGR) of the fish were 2.30 ± 0.24, 1.94 ± 0.12, 1.56 ± 0.05, and 1.54 ± 0.05 at water salinities of 0, 5, 13, and 20 ppt, respectively (P < 0.05). Moreover, mortality rates were 4.00 ± 2.26, 8.00 ± 1.73, 5.33 ± 2.52, and 6.66 ± 1.15, at water salinities of 0, 5, 13, and 20 ppt, respectively (P > 0.05).

There was no significant difference in serum T3 levels among the treatments (P >0.05) (Table 1). Serum T4 showed significant (P <0.05) increase along with water salinity elevation, as the lowest and highest levels were observed in salinity 0 and 20 ppt, respectively (Table 1). Serum glucose showed significant (P <0.05) differences among the treatments as the highest levels were observed in the fish reared in 0 and 20 ppt salinity and the lowest was related to those reared in 5 and 13 ppt water (Table 1). Serum cortisol showed significant (P <0.05) increase along with water salinity elevation, as the lowest and highest levels were observed in salinity 0 and 20 ppt, respectively (Table 1).

The highest and lowest serum ALP activities were related to salinity 0 and 13 ppt, respectively (P <0.05); the fish of 5 and 20 ppt salinities had similar ALP activities (Table 2). There were no significant differences

Table 2 Serum ALP, ALT, AST, protease and lipase activities in rainbow trout at different water salinities (mean ± SD; n = 9)

Water salinity (ppt)	ALP (IU L^{-1})	ALT (IU L^{-1})	AST (IU L^{-1})	Protease (IU L^{-1})	Lipase (IU L^{-1})
0	171±20.8^{c*}	32.5±20.8^{b}	551±42.6^{a}	258±18.3^{c}	21.7±3.05^{a}
5	246±7.50^{b}	38.4±4.92^{b}	120±9.07^{b}	385±6.24^{b}	20.7±1.52^{a}
13	385±6.24^{a}	38.4±4.92^{b}	126±3.82^{b}	389±3.05^{b}	21.2±1.32^{a}
20	294±44.3^{b}	51.2±10.4^{a}	572±2.64^{a}	464±15.9^{a}	23.3±1.52^{a}

*Different letters within a column indicate significant difference (P <0.05)

in serum ALT activities among the fish reared at 0-13 ppt salinities; the highest enzyme's activity ($P < 0.05$) was related to the fish reared at 20 ppt salinity (Table 2). Serum AST activities showed significant ($P < 0.05$) differences among the treatments as the highest levels were observed in the fish reared at 0 and 20 ppt salinities and the lowest values were related to those reared in 5 and 13 ppt water (Table 2). Serum protease activity exhibited significant ($P < 0.05$) increase along with water salinity elevation, as the lowest and highest levels were observed at salinity 0 and 20 ppt, respectively (Table 2). There was no significant difference in serum lipase activities among the treatments (Table 2).

Discussion

Water salinity alters hydromineral homeostasis and induces osmotic stress in fish, making them to expend energy to cope with the stress (Abdel-Tawwab and Monier 2018). Cortisol is the main hormone in hyperosmotic homeostasis in fish that elevates in freshwater fish during saltwater exposure (Shirangi et al. 2019). By elevating circulating levels of cortisol, Na^+/K^+-ATPase activity increases in fish gill to excrete excessive ions from internal fluids (Ghelichpour et al. 2020). Similar to the present results, Hoseini (2019) found increase in plasma cortisol levels of rainbow trout during rearing in saltwater ponds. Thyroid hormones are responsible for fish metabolism regulation, moreover, they have roles in osmoregulation (Shrimpton and Mccormick 1999). The increase in thyroid hormone levels along with salinity elevation in the present study is in line with previous studies on this species. Thyroid hormones were found to increase gill chloride cell number, cortisol receptors and ion-pumping capability in rainbow trout in saltwater (Leloup and Lebel 1993; Shrimpton and Mccormick 1999). Elevation of blood glucose is an indicator of stress and increase in energy expenditure, when freshwater fish are transferred to saltwater (Hoseini et al. 2019b). The results of serum glucose levels were not in this manner, which are different compared to the previous studies on rainbow trout and other freshwater fish (Jalali et al. 2010; Hoseini 2019; Ghelichpour et al. 2020). However, the results are in line with Albrektsen and Torrissen (1988) that found decrease in adult rainbow trout circulating levels of glucose in brackish and saltwater compared to freshwater. The reasons for such controversies is not clear based on the parameters studies in the present research; thus, further studies are needed to assess rainbow trout bioenergetics at different salinities in order to better understanding of the present results.

ALP is found at high amounts in biliary system and erythrocytes, therefore, damage to these cells leads to leakage of the enzyme into circulation (Banaee et al. 2013; Taheri Mirghaed et al. 2017). According to the results, increase in the water salinity might induce biliary system and erythrocyte damage in the fish. A previous study has shown increase in water salinity leads to hepatic damage in rainbow trout (Hoseini et al. 2019); moreover, stressful conditions such as osmotic disturbance lead to oxidative stress and formation of reactive oxygen species (Ghelichpour et al. 2019). These molecules may induce hemolysis and elevation in blood ALP (Taheri Mirghaed et al. 2018). Blood ALT is the specific marker of liver health and hepatic damages lead to increase in blood ALT (Ghasemi et al. 2017). Thus, the fish presumably experienced hepatic damage in water salinity of 20 ppt, in the present study. The results are in line with a previous study that showed rainbow trout exhibited hepatic damages when reared at water salinity around 20 ppt (Hoseini et al. 2019a). AST is found in many cell types and is non-specific indicator of tissue damages (Ghasemi et al. 2017). A previous study on rainbow trout showed that salinity around 20 ppt induced damage to different tissues of the fish and elevation in blood AST (Hoseini 2019; Hoseini et al. 2019a), which is not in line with the present results. On the other hand, study on *Schizothorax zarudnyi* (Shahriarimoghaddam et al. 2018) and *Huso huso* (Rajabipour et al. 2010) showed that water salinity had no significant effects on blood AST. The changes in blood AST were similar to those of the blood glucose in the present study; thus, the changes in blood AST might not likely be related to tissue damages, but energy metabolism. AST is gluconeogenic enzymes that participate in glucose production from amino acids in fish liver (Tejpal et al. 2014). Further studies on rainbow trout energy metabolism at different salinities may address such a change in blood AST and its relationship with blood glucose.

Blood proteases are involved in activation of pre-proteins. It has been suggested that fish may need certain proteins under stressful conditions (e.g. acute phase proteins and peptide hormones) (Torrissen and Torrissen 1985). Therefore, it is speculated that increase in the fish blood protease activity at the higher salinities in the present study might be due to increased demand for protein molecules to cope with the

saltwater stress. In this case, Hoseini and Tarkhani (2013) showed that saltwater exposure led to increase in plasma protein levels in goldfish (*Carassius auratus*). There are no studies on the effects of water salinity on blood protease activity in fish for precise comparison. Lipases are secreted by pancreas into fish gut for fat digestion and damage to the gland leads to the enzyme leakage into circulation (Firat and Kargın 2010). The present results suggest water salinity might have no detrimental effects on the fish pancreas. There are no similar studies in fish for comparison.

In conclusion, the present study demonstrates that increase in water salinity leads to activation of hormonal mechanisms to facilitate adaptation to the osmotic stress. Elevations in cortisol and thyroid hormones are necessary for energy production and increase in ionoregulation capacity. On the other hand, based on the results, water salinities of 5-20 ppt probably caused organ damages in rainbow trout, but the damages seem to milder at 5 ppt compared to 20 ppt.

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

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