Effects of Hypoxic Stress on Electrolyte Levels of Blood in Juvenile Rainbow Trout (*Oncorhynchus mykiss*)

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Stressors that affect fish can be categorized into acute (short-term) or chronic (long-term) stressors. Acute stressors include handling, confinement, abrupt changes in water quality and improper acclimation, and chronic stressors include extended periods of poor water quality, improper stocking densities and improper diets. Severe stress might result in immediate mortality, presumably through ion loss (McDonald and McMahon, 1977). Internal physiological mechanisms responsible for adapting to a stressor include nervous, immunological and hormonal mechanisms (Selye, 1973; Barton and Iwama, 1991). These responses are often categorized as primary, secondary and tertiary stress responses. The primary response is the release of hormones into the circulatory system, which then trigger secondary responses that can include increases in heart rate, gill blood flows and metabolic rate, as well as decreases in plasma chloride, sodium and potassium. Acute decreases in water oxygen (O_2) concentrations may occur in intensive fish farming, especially when fishes are reared at high densities, insufficient flow, poor water and food waste. Considerable attention has been paid to oxygen, as low ambient O_2 concentrations are known to affect growth, food consumption and physiological state of fishes (Jobling, 1994). O_2 levels at which the decrease in growth is observed vary according to the species. In coho, *Oncorhynchus kisutch* (Walbaum) and sockeye salmon *O. nerka* (Walbaum), largemouth bass *Micropterus salmoides* (Lacépède) and common carp *Cyprinus carpio* (L.), growth is affected by O_2 concentrations (). In rainbow troutthe O_2 concentration threshold for growth is much higher, 7 mg/l (Petersen, 1987). Fish depend on aerobic metabolism. Oxygen is needed to oxidize food material to produce energy that is vital in all organism activity. Reduced oxygen supply to tissues causes a myriad of problems including reduced growth, abnormal protein synthesis, migration impairment, low fertility, and mortality. Fish respond to changes in environmental and blood oxygen content by adjusting biochemical, physiological, and behavioral processes to minimize disturbances in oxygen transfer to their tissues (Kramer and McClure, 1982; Wendalaar, 1997). The adjustments are designed to facilitate oxygen uptake at the

gas exchange surfaces and oxygen unloading to the tissues under both long- and short-term hypoxia exposure. Among these adjustments are variations in the functional properties of hemoglobin that result partly from its molecular structure and partly from changes caused by pH and heterotrophic factors like erythrocytic phosphates that bind to deoxyhemoglobin, decreasing

its affinity to oxygen (Chrousos, 1998; Weber et al., 2000). Acquisition of food and its digestion and assimilation are major energy expenditures (up to 60%) of fishes. Decreased oxygen availability is also considered a major factor in determination of food intake. Low dissolved oxygen is a type of stress frequently found in fish farms characterized by high fish densities and polluted fresh or marine waters (Natochin et al., 1985; Dam and Pauly, 1995).

The aim of this study is to determine the effect of hypoxia factor on the blood electrolyte [sodium (Na^{+}), potassium (K^{+}), calcium (Ca^{+2}), magnesium (Mg^{+2}), chloride (Cl^{-})] levels of rainbow trout fry at various flow rates (0.3, 0.9 and 1.4 l/min).

The study was conducted in the Keban Dam Lake General Directorate of the State Hydraulic Works Laboratory (DSI, Elazig). Following the acclimation, fish were selected and randomly stocked. Fish (initial total weight and length, 30.19±2.20 g and 13.15±0.22 cm, respectively) were distributed into 9 fiberglass rectangular (200 × 40 × 40 cm) tanks with a 3×3 experimental design (3 dissolved oxygen treatment × 3 replicate groups) with a density of 10 juvenile rainbow trout (*Oncorhynchus mykiss*) per tank. A total of 90 juvenile rainbow trout were used in this research.

At waters of flow rate 0.9, 0.3 and 1.4 l/min, the O_2 concentrations were 4.5, 3.5 and 7.0 mg O_2/l, respectively. Dissolved oxygen and temperature values were recorded with a portable YSI Probe (55 Model 51/12) duration of the research (Table 1). The feeding of the fish was determined 12 h prior to the experiment, thus preventing any environmental factors of stress that might have caused any complications. The flow rates that were determined for the experimental groups were stabilized through the use of the water inflow control valves that are shown in Figure 1 schematically (Tuna Keleştemur, 2011). The preliminary analysis indicated that the commencing of death cases after 8 h in the 3.5 mg O_2/l treatment.

Blood was obtained from the caudal vasculature of 10 fish per tank. Blood was obtained from the caudal vein of individual fish after anesthesia with Qinaldine (15 mg/l Quinaldin). The blood samples were centrifuged (3500 rpm for 5 min) to obtain serum and serum samples were stored -20°C until analysis (Atamanalp and Bayır, 2003). The electrolyte values of the blood samples were determined using Autoanalyzer (Roche Hitachi Cobas 6000) device in *in vitro* laboratory.

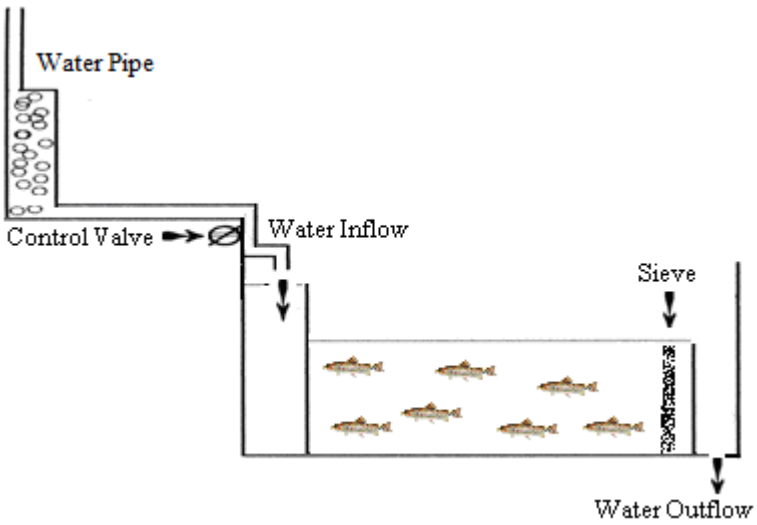


Figure 1: Schematic representation of the fish tanks used in the study

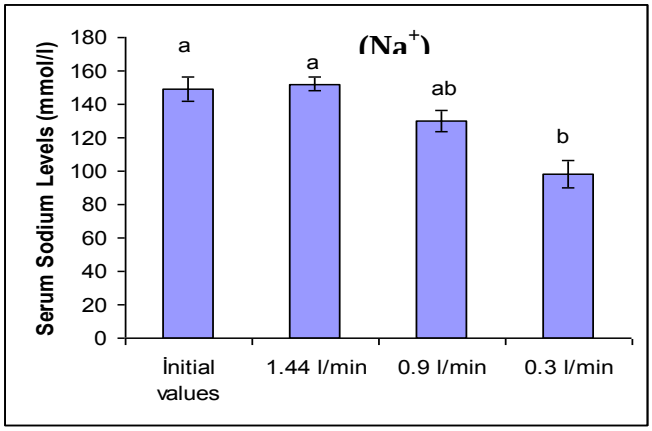
Table 1: Research used water flow rate, dissolved oxygen concentrations, temperature and pH values.

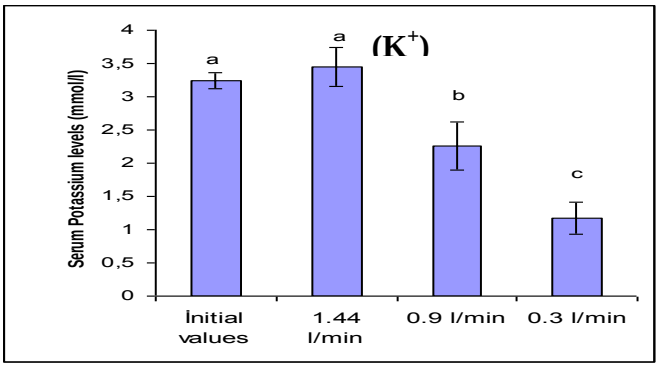
DO treatment (mg O_2 /l)	Flow rate (l/min)	DO (mg O_2 /l, Mean±SD)	Temperature (°C)	pH
7.0	**1.4**	**7.00±0.33**	**9.3**	**8.5**
4.5	**0.9**	**4.56±0.36**	**9.3**	**8.5**
3.5	**0.3**	**3.54±0.25**	**9.3**	**8.5**

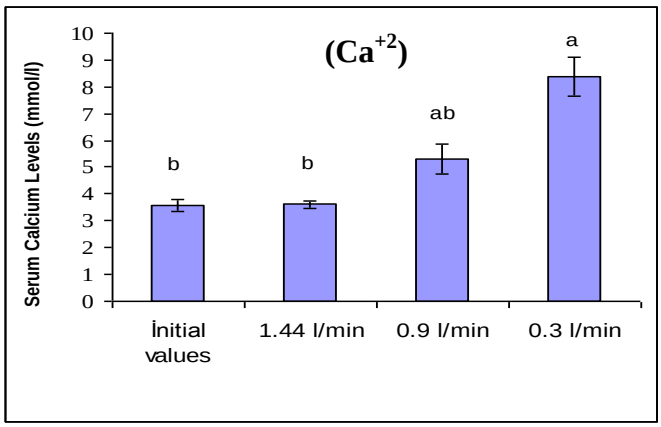
DO, temperature, pH and flow rate in each constant treatment was calculated from triplicate measurements taken every 10 minutes in each tank

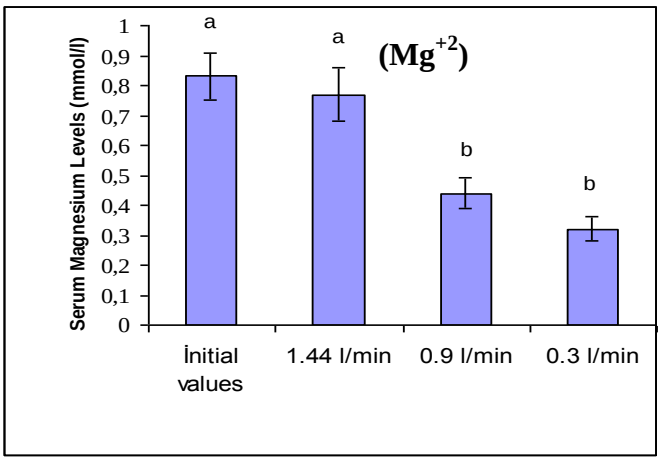
Biochemical data were analyzed with SPSS 11.5 for Windows using one-way analyses of variance (ANOVA) and significant means were subjected to a multiple comparison test (Duncan) at $P<0.05$ (SPSS 11.0, SPSS Ltd. Working, Surrey, UK).

At the end of 8 hours-long the study and beginning of the study (initial values), statistical comparisons of Na^+, K^+, Ca^{+2}, Mg^{+2}, Cl^- serums values of fish at different flow rate (under hypoxic stress) were illustrated in Figure 1. Initial value of experimental groups and 1.4 l/min flow rate groups Na^+ serum level was determined to be significantly higher than 0.3 l/min flow rate groups ($p<0.05$) and 0.9 l/min flow rate group Na serum levels were not significant other groups ($p>0.05$). Initial value of experimental groups and 1.4 l/min. flow rate groups Na^+ serum level was determined to be significantly higher than other groups whereas initial value and 1.4 l/min flow rate of serum Ca^{+2} levels was lower than other groups. Initial value of experimental groups and 1.4 l/min flow rate groups of serum Mg^{+2} and Cl^- levels was determined to be significantly higher than other groups ($p<0.05$).


(Na+)
Serum Sodium Levels (mmol/l)
180
160
140
120
100
80
60
40
20
0
a
a
ab
b
İnitial values
1.44 l/min
0.9 l/min
0.3 l/min


(K+)
Serum Potassium levels (mmol/l)
4
3,5
3
2,5
2
1,5
1
0,5
0
a
a
b
c
İnitial values
1.44 l/min
0.9 l/min
0.3 l/min


(Ca+2)
Serum Calcium Levels (mmol/l)
10
9
8
7
6
5
4
3
2
1
0
b
b
ab
a
İnitial values
1.44 l/min
0.9 l/min
0.3 l/min


(Mg+2)
Serum Magnesium Levels (mmol/l)
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
a
a
b
b
İnitial values
1.44 l/min
0.9 l/min
0.3 l/min

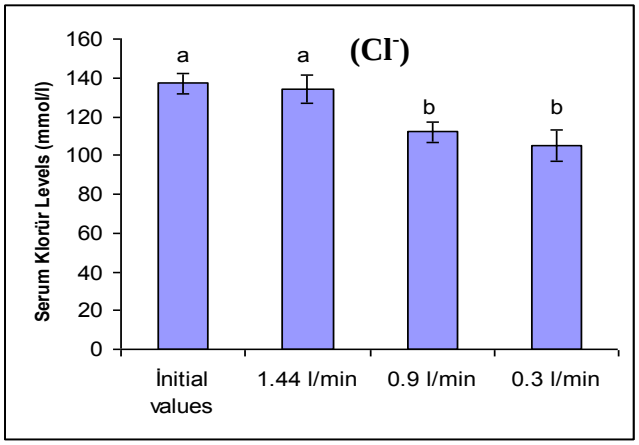


Figure 1: **Before the trial serum electrolyt (Na^{+}, K^{+}, Ca^{+2}, Mg^{+2}, Cl^{-}) of initial values and at the end of the trial serum electrolyt levels at a three different flow rate (respectively; 1.4 L/min., 0.9 L/min., 0.3 L/min.). ^{a,b,c} Indicates a significant differences with respect to controls (P<0.05). All data points are the average of *n* =12 with ±*SD*.**

When the stress factors start to have an effect on metabolic activities, the regulatory mechanisms of the organism attempts to minimize the damage as a result of an immune response caused by tissue damage (Johannessen and Dahl, 1996; Conte, 2004). The regulatory mechanisms of the organism must maintain optimum cellular pH and osmolarity. Osmoregulation is maintained with homeostasis of intracellular and extracellular ions (Johnston and Cheverie, 1985; Natochin et al., 1985). In parallel with physiological changes for the maintenance of homeostasis in metabolism, changes in some of the parameters of blood tissue provide important data for the evaluation of the stress levels. Those elements that exist in certain concentrations in blood plasma are blood electrolytes (Handy et al., 1999). Potassium (K^{+}) is the major cation of intracellular fluid whereas sodium (Na^{+}) and chloride (Cl^{-}) are the major ions of extracellular fluid. Na^{+}, K^{+} and Cl^{-} are important ions which provide the sustainability of the osmotic pressure of body fluids as well as acid-base balance. Magnesium ion (Mg^{+2}) is the second most abundant cation that exist in intracellular fluid. It acts as a co-factor in function of many enzymes. It also plays an important role in neurochemical impulse transmission and muscle excitability. Mg^{+2} is essential in absorption of calcium ion from intestines (Karnaky, 1998).

Lyytikainena et al. (2002) determined that there were no statistical differences in calcium, sodium and chloride concentrations between fluctuating and constant thermal conditions ($p>0.05$), the influence of stress on plasma calcium, sodium and potassium concentrations was similar under

fluctuating and constant conditions ($p>0.05$), the influence of stress on plasma potassium concentrations was statistically significant in this subset ($p<0.05$). The researchers were determined that there were, however, no differences between constant and fluctuating conditions, the decrease in plasma potassium concentration after acute stress appeared to be higher at 14°C than at 18°C but this effect was not statistically significant ($p > 0.05$) (Lyytikainena et al., 2002).

Keleştemur and Seven (2012) was determined that hypoxic stress effect of blood ion concentrations. Xugang et al. (2009) was determined that the blood Na^{+}, Ca^{+2} and $C1^{-}$ concentrations displayed similar patterns of change in relation to duration of brackish water exposure. Following direct transfer from fresh water to brackish water, serum Na^{+}, Ca^{+2} and $C1^{-}$ levels increased significantly to peak value during the first 24 h ($p<0.05$) and between 24 h and 216 h of brackish water exposure, serum Na^{+}, Ca^{2+} and $C1^{-}$ levels decreased significantly, reaching a plateau which was significantly higher ($p<0.05$) than the levels of freshwater control, which maintained its initial levels during the experiment, serum potassium levels of fish cultured at brackish water were unaffected by salinity at the beginning and end of the trial. Between 24 h and 216 h, a significant effect of salinity on serum Na^{+} level occurred; the increase was gradual and significant.

Results obtained in this study showed that hypoxic stress occurred on the juvenile rainbow trout because of the flow rate of the water in the tanks was reduced. Hence, the serum Na^{+}, K^{+}, Mg^{+2} and Cl^{-} levels decreased and Ca^{+2} values increased depending on the stress factor. Artificial water products, limited water areas used in their cultivation systems, (pool, aquarium, boat varieties etc.) stock density, feed and metabolic wastes are stress factors inevitably lead the formation of hypoxia in fish due to reduction in levels of oxygen in water. Therefore in trout farming should be controlled flowrate. In this way in trout farms could be protected from hypoxic stress. On the other hand, the response to any kind of stress sources such as hypoxia may differ in some fish, and therefore further work is needed to determine the responses of different fish species.

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