EFFECTS OF β-CAROTENE AND VITAMIN - C ON BLOOD ELECTROLYTE LEVELS OF JUVENILE RAINBOW TROUT (ONCORHYNCHUS MYKISS W. 1792) UNDER HYPOXIC STRESS

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ABSTRACT

In this study, the effect of dietary supplementation of β-carotene and vitamin C on blood electrolytes (Na⁺, K⁺, Ca²⁺, Mg²⁺, Cl⁻) and pH of juvenile rainbow trouts under hypoxic stress (4.5 mg/l O₂) was investigated. Three diets were prepared differing only in the type of supplementation as follows: Bc dietary group with 70 mg/kg β-carotene, Aa dietary group with 250 mg/kg vitamin C and control (C) dietary group with no β-carotene and Vitamin C. Based on statistical evaluations, it was observed that serum Na⁺ level of Aa group was significantly higher than Bc and C groups (p<0.05) and the least serum Na⁺ level was found in C group. Serum K⁺ and Cl⁻ levels of Aa and Bc groups were determined to be significantly higher than C group (p<0.05). Serum Ca²⁺ level of C group was determined to be significantly higher than the other groups (p<0.05) whereas serum Mg²⁺ level of C group was found to be significantly lower than other groups (p<0.05). Serum pH level was not statistically different among the groups (p>0.05).

Key words: Rainbow trout, Vitamin C, β-carotene, Blood electrolytes, Stress, Hypoxia.

INTRODUCTION

Hypoxia in aquatic ecosystems refers to waters where there is a reduction of dissolved oxygen concentration due to natural means (eutrophication, water movements and seasonal biologic, chemical or physical changes etc.) or human-based sources (chemical and biologic wastes). The reduction in oxygen levels limits the vital functions such as growth, development, reproduction and nutrition of aquatic animals, especially fish and causes death in chronically ongoing conditions (Kramer and McClure, 1982; Chapman et al., 2010).

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 (Conte, 2004; Johannessen and Dahl, 1996). The regulatory mechanisms of the organism must maintain optimum cellular pH and osmolarity. Osmoregulation is maintained with homeostasis of intracellular and extracellular ions (Natochin et. al., 1985; Johnston and Cheverie, 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 parameters 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 (Mg²⁺) 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 is essential in absorption of calcium ion from intestines (Karnaky, 1998; Railo et al., 1985).

As in all living beings, the main two factors which affecting acid-base balance in fish are the nutrition and environment. In intensive cultivation, any interventions made to maintain this balance must improve the immune system (Moeller and
Many studies showed that supplementation of vitamin A, C, E and β-carotene into the rations of cultured rainbow trouts for the purpose of eliminating the stress factors was proven to be beneficial for the development of fish (Tuna Keleºtemur and Özdemir, 2011). Dietary levels of these vitamins have been reported to influence antioxidant defenses of fish (Zhang and Omley, 2001; Redge et al., 1997; Choi et al., 2004; Bray, 2000).

This study aims to determine the blood electrolytes such as Na⁺, K⁺, Ca⁺², Mg⁺², Cl⁻ and blood pH of juvenile rainbow trout fed the β-carotene and vitamin C supplemented diets under hypoxic stress at the level of 4.5 mg/l dissolved oxygen.

MATERIALS AND METHODS

**Experimental fish**

Rainbow trout (Oncorhynchus mykiss) were produced at the Çircir Hatchery (Elazig, Turkey) were transferred Keban Dam Lake General Directorate of State Hydraulic Works Laboratory. After the acclimation, fish were selected and randomly stocked. Fish (initial average weight, 42.27±2.13 g; initial length, 15.39±1.01 cm) were distributed into nine fiberglass rectangular tanks (200×40×40 cm) in triplicate groups at density of 10 fish/tank.

**Experimental design**

Experimental fish were randomly distributed in each tank as (capacity of tank is 360 L with a dimension of 200 cm long, 40 cm height and 40 cm wide) at a groups of 10 fish. Experiments were conducted in a tank, supplied with well water flow rate at 0.9 l min⁻¹. At water flow rate 0.9 l/min, O₂ concentrations were as seen 4.5±0.3 mg/l. The flow rates were stabilized through the use of the water inflow control valves (Tuna Keleºtemur and Özdemir, 2010). Water temperature ranged from 9.3±0.5 ⁰C and water pH ranged from 8.4±0.5. The pH of the water in the tanks where the fish were situated was determined by a portable Checker brand pH meter and the dissolved oxygen and the temperature values were recorded through the use of a portable YSI 55 Model 51/12 oxygen probe throughout the duration of the research. The study was initiated after 1 week-adaptation period of fish to the hypoxic condition at the level of 4.5 mg/l dissolved oxygen.

**Diets**

In this study, experimental diets were prepared as follows: Bc diet with 70 mg/kg β-carotene, Aa diet with 250 mg/kg Vitamin C (Synthetic Rovimix β-carotene, 10 % and Synthetic L-ascorbic acid 99 %) and C₀ (control) diet with no β-carotene and vitamin C. Rovimix β-carotene and vitamin C (L-ascorbic acid) were supplied by Turkey DSM nutritional productions firm. The amounts of supplemented β-carotene and Vitamin C in the rations were determined by taking the recommended amounts for fish exposed to stress into consideration (Hilton et al., 1977). Composition of the basal diet (NRC, 1993) is shown in Table 1.

Dry matter (105⁰C, overnight), ash (650⁰C, 6 h), crude protein (nitrogen×6.25), ether extract, crude fibre of diets was analyzed by methods of AOAC (1990). The experimental fish were fed two times a day at 09.00 and 17.00 hours for 4 weeks. Daily feed allowance was 3% body weight per day.

**Blood Samples**

At the end of the experiment, 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 Quinaldine (15 mg/l Quinaldin). The blood samples were centrifuged (3500 rpm for 7 min) to obtain serum and serum samples were stored -20 °C until analysis (Sahan et al., 2003). The electrolyte values of the blood samples were determined using Autoanalyzer (Roche Hitachi Cobas 6000) device in in vitro laboratory.

**Statistical analysis**

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).

**RESULTS AND DISCUSSION**

Serum Na⁺, K⁺, Ca⁺², Mg⁺², Cl⁻ levels, and pH values of fish fed with the various experimental diets are presented in Figure 1. The results of one-way ANOVA test showed that blood ion levels of fish were positively affected by dietary supplementation of β-carotene and vitamin C. Serum Na⁺ level was significantly affected by 250 mg/kg vitamin C supplemented diet group (Aa), followed
by the 70 mg/kg β-carotene supplemented diet group (Bc) (p<0.05). The highest serum K+, Cl and Mg²⁺ levels were obtained vitamin C supplemented group and β-carotene supplemented group (p<0.05), while the lowest K⁺, Cl and Mg²⁺ levels were obtained in the C group (p<0.05). Serum Ca²⁺ level was higher in the C group (p<0.05) than other diet groups. pH value of rainbow trout under hypoxic stress was not statistically different among all dietary treatments.

Diminished oxygen availability to cells and tissues (hypoxia) and the complete absence of oxygen (anoxia) are physiological stressors that can cause a significant and rapid reduction in the production and supply of ATP (Randall and Hungy, 2004; Tuvikene et al., 2001). There follows a consequent loss of cellular homeostasis and membrane integrity, which subsequently leads to cellular degradation and death (Chapman et al., 2010). Several researchers have reported that the concentration of the dissolved oxygen in water should not be allowed to fall below 5 mg/l and that the optimal concentration of dissolved oxygen varies between 7 and 9 mg/l in trout stock farming (Clark, 2003). Petersen (1987) has determined that the natural oxygen requirement for the trout is 8 mg/l that at concentrations of 4 mg/l difficulties in breathing appeared and that at concentrations of 3 mg/l slow deaths occurred. In this study, effect of β-carotene and vitamin C supplementation into the rations of juvenile rainbow trout on blood electrolytes (Na⁺, K⁺, Ca²⁺, Mg²⁺, Cl⁻) was investigated under hypoxic conditions.

**TABLE 1: Composition and proximate analysis of the experimental diets**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percent of dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish (anchovy) meal</td>
<td>50</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>23.1</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>19.8</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>6</td>
</tr>
<tr>
<td>Antioxidant a</td>
<td>0.10</td>
</tr>
<tr>
<td>Vitamin premix b</td>
<td>0.90</td>
</tr>
<tr>
<td>Mineral premix c</td>
<td>0.10</td>
</tr>
</tbody>
</table>

**Proximate composition**

- Dry matter: 80.35
- Crude protein: 44.48
- Crude fat: 8.02
- Crude fibre: 4.12
- Crude ash: 13.10

**a**Antioxidant (mg/kg dry diet): Butilen Hydroxytoluene (BHT); 125.000 mg/kg

**b**Vitamin premix (IU or mg/kg dry diet): Menadion 3.000, Riboflavin 6.000, Pridoksin 5.000, kobalamin 15, Niasin 25.000, Biotin 40, Folik asit 1.000, Kolin klorid 300, Kalsiyum D-pantothenat 8.000, Kalsiferol 2.000.000, Vitamin A 1000, Vitamin E 1.000.000 IU.

**c**Mineral premix (mg/kg dry diet): Mn 80.000, Cu 5.000, Zn 50.000, Fe 35.000, I 2.000, Co 400, Se 150.

It was reported that Na⁺ plays an important role in maintenance of acid-base balance of blood, function of nerve and muscle, pH of blood. Serum Na⁺ levels are found to be between 138.7 and 158.3 mmol/l. It was reported that serum K⁺ levels of a rainbow trout obtained from natural environment was about 2.50-3.17 mmol/l. Serum Cl⁻ levels of rainbow trout found in natural environments were determined as 127-133 mmol/l. Researchers found that serum Ca²⁺ and Mg²⁺ levels of rainbow trout were 2.72-4 mmol/l and 0.8-0.9 in average, respectively (Çelik, 2006).

Various stressors affect blood ion concentrations of fish. Lylytkainena et al. (2002) were determined that there were no statistical differences in blood calcium, sodium and chloride concentrations of Arctic charr (Salvelinus alpinus) 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 these conditions (p<0.05). Xugang et al. (2009) were determined that the blood Na⁺, Ca²⁺ and Cl⁻ levels of Chinese sturgeon (Acipenser sinensis) increased significantly after direct transfer from fresh water to brackish water.

In this study, serum Na⁺ levels of C, Bc and Aa dietary groups were determined as 141±2.23 mmol/l, 149±3.45 and 157±4.77 mmol/l, respectively. Serum Na⁺ levels of Aa group fed the 250 mg/kg vitamin C was determined to be higher than the other groups. In this study, serum K⁺ levels of juvenile rainbow trout fed the β-carotene and vitamin C were determined to be significantly higher than the C group (p<0.05). Serum K⁺ levels of Bc and Aa groups were determined as 3.88±0.34 mmol/l and 3.25±0.67, respectively whereas serum K⁺ levels of C group was determined as 1.53±0.45 mmol/l. Serum Cl⁻ levels of juvenile rainbow trout
FIGURE 1: Serum Na (a), K (b), Ca (c), Mg (d), Cl (e), pH (f) values of the rainbow trouts fed with C, Bc and Aa diets under hypoxic stress during four weeks. a,b,c indicates a significant differences with respect to controls (P<0.05). (Hypoxia = 4.5 mgO2/l). All data points are the average of n = 12 with ±SD.
fed the Bc and Aa rations were determined to be as
127±3.27mmol/l and 133±3.15 mmol/l, respectively
and are significantly higher (p<0.05) than C group
(119±4.12 mmol/l). Highest Ca+2 level of fish under
hypoxic stress was observed in C group (5.27±0.12
mmol/l) whereas serum Ca+2 serum levels of Bc and
Aa groups were determined as 2.35±0.07 mmol/l
and 2.33±0.04 mmol/l, respectively (p<0.05) thus
statistically lower than C group. Serum pH level did
not statistically differ among the groups (p>0.05).
However, the lowest pH level was observed in C
group (Fig.1).

In conclusion, this study determined that
feeding juvenile rainbow trout with 250 mg/kg
Vitamin C and 70 mg/kg β-carotene diets promotes
preservation of blood electrolytes such as Na+, K+,
Mg+2 and Cl- in fish under hypoxic stress at the level
of 4.5 mg/kg oxygen. It was found that 250 mg/kg
vitamin C supplementation was more effective than
70 mg/kg beta carotene in preservation of serum Na+
level. Also serum Ca2+ level of C group was observed
to be significantly higher than the other groups. The
reason for serum Ca2+ level of C group being higher
than other groups is postulated as a result of decrease
of Mg+2 levels in blood causing Ca2+ transfer from
tissues to blood. 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. It was determined that
supplementation of vitamin C and β-carotene which
are antioxidant vitamins into the rations of juvenile
rainbow trout has positive effects in preservation of
blood electrolyte levels of fish which provides acid-
base balance during homeostasis period.

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