The Antioxidant Vitamin (A, C, E) and the Lipid Peroxidation Levels in Some Tissues of Juvenile Rainbow trout (*Oncorhynchus mykiss*, W. 1792) at Different Oxygen Levels

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Abstract

In this study, the malondialdehyde (MDA) levels in the muscle, liver and kidney tissues and the antioxidant vitamin (A, C, E) levels in the muscle tissue of juvenile rainbow trout were investigated under different oxygen levels (3.5 mg O_2/l, 4.5 mg O_2/l and 7 mg O_2/l) at the end of the 8 hour trial. Fish (initial weight and length, 30.19±2.2 g and 13.15±0.22 cm, respectively) were distributed into 9 fiberglass rectangular tanks. The highest MDA level of all tissues was obtained in 3.5 mg O_2/l ($p<0.05$). Different oxygen levels did not affect muscle vitamin A concentration statically ($p>0.05$). But the highest muscle vitamin C and E concentrations were obtained in 7 mg O_2/l. ($p<0.05$), followed by 3.5 mg O_2/l, while the lowest muscle vitamin C concentration was obtained in 4.5 mg O_2/l. ($p<0.05$).

Keywords: Rainbow trout, Hypoxia, Stress, Antioxidant vitamins, Lipid peroxidation, Tissue

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IntrodIuction

Hypoxia, or dissolved oxygen below saturation, is considered a major threat to the value of estuarine habitats as nurseries for ecologically and economically important fishes (Stierhoff, 2009). Acute decreases in water oxygen concentrations may occur in intensive fish farming, especially when fishes are reared at high densities and insufficient water flow. 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 (Pichavant et al., 2000). All aerobic organisms depend on oxygen presence in the environment, using it primarily for energy generation via oxidative phosphorylation (Storey, 1996). The generation of various by-products of oxygen metabolism, so-called reactive oxygen species (ROS), is the other side of the coin. They include superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (^-OH) and others (Lushchak and Bagnyukova, 2006). If antioxidant defenses are effective in detoxifiying ROS, then no harmful consequence will result in the tissues (Fang et al., 2002; Quiles et al., 2002). However, if the ROS attack is severe, then antioxidant defense systems may be overwhelmed, resulting in inhibition of antioxidant enzymes and oxidative damage to lipid, protein, DNA and other key molecules. ROS are generated by normal physiologic processes and are, in fact, essential to normal cellular function (Lushchak and Bagnyukova, 2006). Some vitamins possessing an antioxidant activity protect the cells from the damage caused by the free radicals and by preventing free radical formation they play an important role in the antioxidant defense. The most significant antioxidant vitamins are vitamins E, A and C (ascorbic acid) (Zhang and Omley, 2001). Among these smaller molecules, vitamin E and vitamin A, is regarded as the primary lipid-soluble antioxidant that operates synergistically with vitamin C to protect lipids against per oxidative damage (Choi et al., 2004). The most widely used assay for lipid peroxidation is the MDA formation. Malondialdehyde is the final product of lipid peroxidation. The concentration of MDA is the direct evidence of toxic processes caused by free radicals (Talas et al., 2009).

Hypoxia is a common event in aquatic environments. Reductions of oxygen concentration (O_2) in water have resulted in significant changes in antioxidant defense systems in fish. In recent years, physiology and biochemical effects of the hypoxic stress on fish created by various methods have been investigated (Randal, 1982). But, the effects of the hypoxic stress occurring with the decreasing flow rate in the cultivation of rainbow trout have not been reported to date. Therefore, this study was designed to determine the effects of different oxygen levels (3.5, 4.5 and 7 mg O_2/l) on antioxidant vitamins (A, C, E) in the muscle and lipid peroxidation levels in tissues of juvenile rainbow trout.

Materials and methods

Fish material

The study was conducted in the Keban Dam Lake General Directorate of the State

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Hydraulic Works Laboratory (DSI, Elazig). Following the acclimation, fish were selected and randomly stocked. A total of 90 juvenile rainbow trout (initial weight and length, 30.19±2.2 g and average total length 13.15±0.22 cm, respectively) were distributed into 9 fiberglass rectangular (200 cm × 40 cm × 40 cm) tanks with a 3×3 experimental design (3 dissolved oxygen (DO) treatment × 3 replicate groups) with a density of 10 fish per tank.

Experimental design

At waters of flow rate 0.9 l/min, 0.3 l/min 0_2 and 2.1 l/min, the 0_2 concentrations were 4.5 mg O_2/l, 3.5 mg O_2/l and 7 mg O_2/l respectively. Dissolved oxygen and temperature values were recorded with a portable YSI Probe (55 Model 51/12) during the research (Table 1). The feeding of the fish was determined 12 hours 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 and Ozdemir, 2010). The preliminary analysis indicated that the commencing of death cases is after 8 hours in the 3.5 mg O_2/l treatment. Therefore this study duration was planned to be 8 hours. The tissue samples were taken from 45 fish; 15 from each one of the groups, during the autopsy right after the anesthetic process (15 mg/l Quinaldin) and the samples were stored in the freezer at -20^{o}C until analysis.

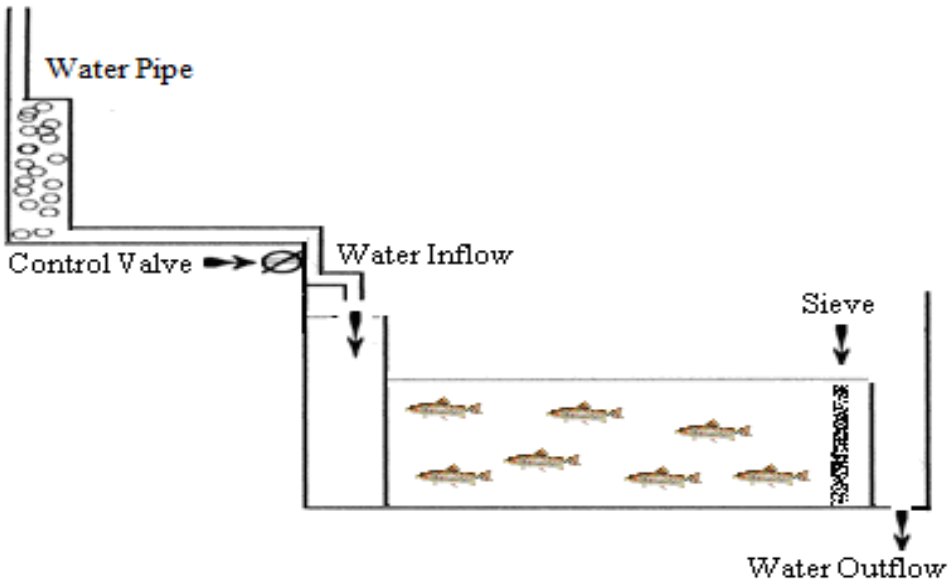


Figure 1: Schematic representation of the fish tanks used in the study.

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

DO treatment ($mg\ O_2$ /l)	Flow rate (l/min)	DO ($mg\ O_2$ /l, Mean±SD)	Temparature (°C)	pH
7.0	2.1	7.16±0.33	9.3	8.5
3.5	0.3	3.54±0.25	9.3	8.5
4.5	0.9	4.56±0.36	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

Tissue sampling

Tissue samples (liver, muscle, kidney) were rinsed with saline to remove the blood. The homogenization of tissues was carried out in a teflon-glass homogenizer with a buffer containing 1.15% KCl to obtain 1:10 (wv^{-1}) whole homogenate (Ateşşahin et al., 2011). The homogenates were centrifuged to determine malondialdehyde (MDA), and vitamin A, C and E concentrations.

Determination of MDA and vitamin C levels in tissues

Fish tissue samples were homogenized. The homogenization mixture consisted of 0.5 ml $HClO_4$ (0.5 M), 4.5 ml distilled water and 100 ml-500 ppm 2(6)-di-tart-butyl-p-cresol (BTH). Then, the samples were centrifuged at 4500 rpm for 5 min and the supernatants were injected into the HPLC. The mobile phase was 30 mM KH_2PO_4-methanol (82.5+17, v/v %, pH 3.6) mixture and the flow rate was 1.2 ml min^{-1}. The chromatograms were detected at 250 nm and the injection volume was 20 ml (Karatepe, 2004).

Determination of vitamin A and E levels in muscle

The homogenized muscle tissues were transferred into polyethylene tubes and 2 ml of ethanol was added to the tubes. Following the addition of 0.3 ml n-hexane that is required for the vitamin extractions into the tubes, they were centrifuged. This step was repeated two times. N-hexane in the tubes was evaporated using nitrogen. Then the residues were dissolved in the mobile phase (methanol: acetonitrile:chloroform; 47:42:11,v/v/v). The chromatographs were monitored at 326 and 296 nm for vitamins A and E, respectively and the injection volume was set to 50 ml. Techsphere ODS-2 packed column (5 mm particle, 250× 4.6 ID) was used and the flow rate was 1.0 ml min^{-1} ((Miller et al., 1984).

Statistical analysis

Biochemical data were analyzed with SPSS 11.5 for Windows using One-Way Analysis of Variance (ANOVA) and significant means were subjected to a multiple comparison test (Duncan) at

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P<0.05. (SPSS 11.0, SPSS Ltd. Working, Surrey, UK). A Student's t-test was used to test in comparison for muscle Vitamin E concentration and muscle MDA levels in juvenile rainbow trout at different DO treatments (3.5, 4.5 and 7 mg O_2/l.).

Results

MDA levels of fish tissues were negatively affected by hypoxic conditions (3.5 mg O_2/l and 4.5 mg O_2/l). MDA levels in tissues were found to be significantly higher in 3.5 mg O_2/l than 7 mg O_2/l and 4.5 mg O_2/l ($p<0.05$). Liver and muscle MDA levels were similarly affected by 7 mg O_2/l and 4.5 mg O_2/l; however kidney MDA levels decreased in 7 mg O_2/l compared to other experimental groups (Fig. 2).

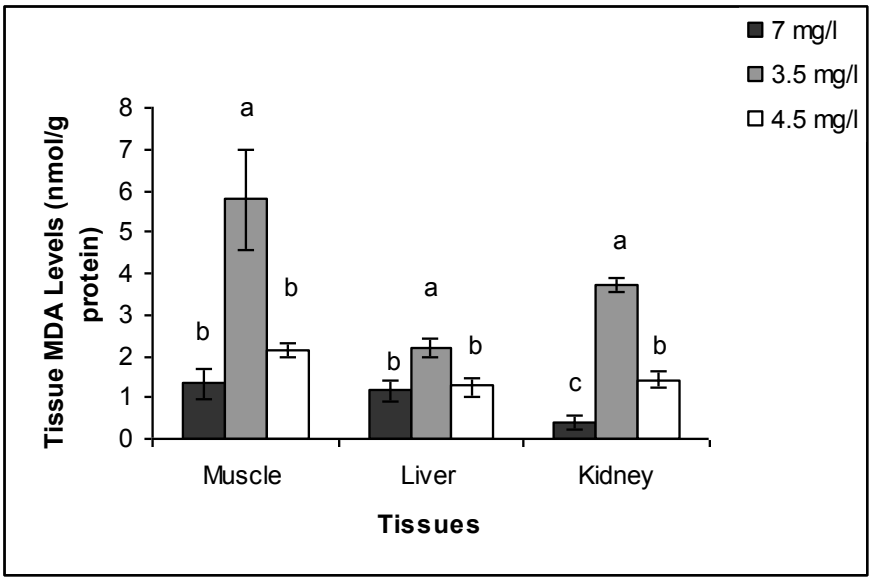


Figure 2: Change of muscle, liver and kidney MDA levels of juvenile rainbow trout in relation to O_2 concentration: 3.5, 4.5 and 7 mg O_2 l^{-1}. Data are presented as means ±*SE* (n=3 replicate). Bars show the standard error. [a,b,c] Different letters indicate significant differences (p<0.05, ANOVA)

Vitamin A level in the muscle did not differ among all the groups ($p>0.05$, Fig. 3). However the lowest muscle vitamin C and E concentrations were obtained in 4.5 mg O_2/l. ($p<0.05$), the highest muscle vitamin E concentration was obtained in 7 mg O_2/l and 3.5 mg O_2/l ($p<0.05$, Fig. 3).

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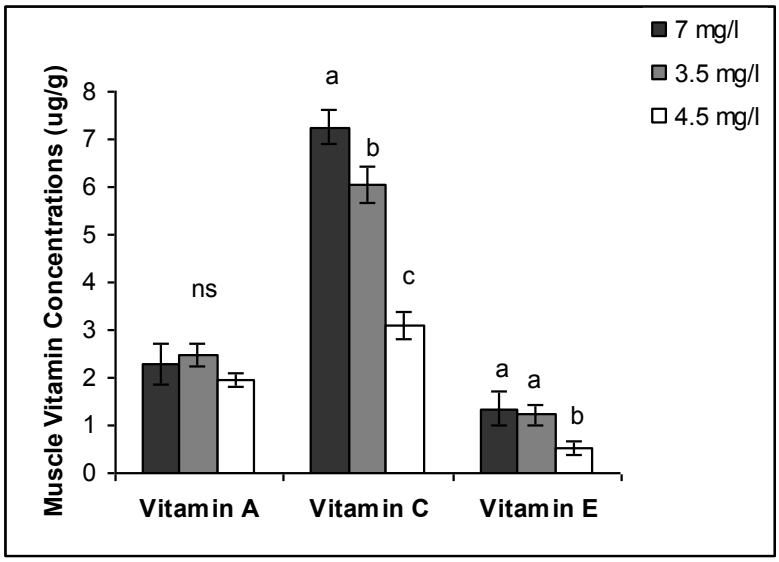


Figure 3: Change of Vitamin A, C and E levels of the muscle tissue of juvenile rainbow trout in relation to O_2 concentration: 3.5, 4.5 and 7 mg O_2 l^{-1}. Data are presented as means ±*SE* (n=3 replicate). Bars show the standard error. [a,b,c] Different letters indicate significant differences, p<0.05 (ANOVA, Duncan multiple comparison test). ns: no significant difference, p>0.05.

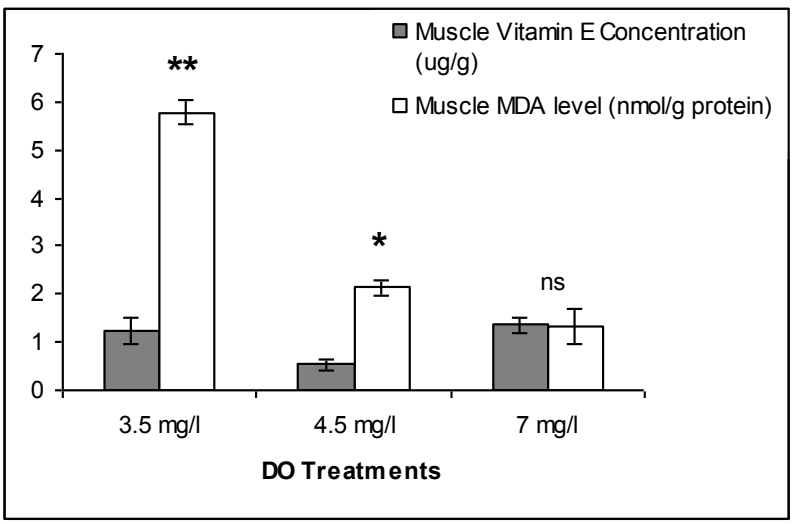


Figure 4: A Student's t-test was used to test in comparison for muscle vitamin E Concentration and muscle MDA levels in juvenile rainbow trout at different DO treatments (3.5, 4.5 and 7 mg/l). Vertical bars: ± S.E. (n = 3 replicate). *P<0.05 ** P<0.01, ns=no significant difference, P > 0.05.

The results of Students' t-test showed that muscle MDA levels were negatively affected by hypoxic conditions (Fig. 4). Muscle MDA levels were found higher than muscle vitamin E concentration in 3.5 and 4.5 mg O_2/l (highly significant p<0.01

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and significantly $p<0.05$, respectively), but in 7 mg O_2/l, among muscle MDA levels and vitamin E concentrations of fish did not differ ($p>0.05$, Fig. 4).

Discussion

Hypoxia has been recognized as one of the main mass mortality factors in aquatic organism populations (Paerl et al., 1999). Fish exposed to hypoxic conditions have also reduced developmental rates (Taylor and Miller, 2001). Stress leads to generation of free radicals, such as O_2^- and HO. These free radicals can damage cell membranes by inducing lipid peroxidation of polyunsaturated fatty acids in the cell membrane (Sorg, 2004). Salmonid tissues are characterized by high concentrations of polyunsaturated fatty acids compared with most mammalian tissues, and fish may, therefore, be particularly susceptible to lipid per oxidative cellular damage (Gülçin et al., 2009).

Hypoxic stress increased lipid peroxidation as a consequence of increased free radical generation (Dabrowski et al., 2004; Tuna Keleştemur and Seven, 2012). The rise of lipid peroxidation increases the MDA level in blood and tissues (Ates et al., 2006). The requirements of non-enzymatic antioxidants, for example tocopherols, ascorbic acid, vitamin A and carotenoids increase with stress. The increase in lipid peroxidation decreases antioxidants such as vitamin C and vitamin E in tissues (Wejil et al., 1997; Choi 2004; Fang et al., 2002). Similarly, in our study, lipid peroxidation increased and vitamin C and E decreased in tissues under hypoxic stress.

If the oxygen level is at the critical level that is required for the fish or below that hypoxia (oxygen deficiency) occurs and if the oxygen is completely used by the body tissues and consumed, anoxia (oxygen deprivation) occurs (Lushchak and Bagnyukova, 2006). The presence of hypoxia is a condition which could be surmounted by the provision of the necessary oxygen whereas the presence of anoxia results in the loss of function in the tissues followed by death. The inability to prevent increases in the MDA levels of the rainbow trout that are affected by the stress factors, which is caused by the lipid peroxidation formed as a result of possible metabolic deficiencies in the cell, causes death through the formation of functional deficiencies in many tissues and organs, mainly on the immune system of the fish (Dabrowski et al., 2004).

In conclusion, 3.5 mg O_2/l and 4.5 mg O_2/l caused hypoxic stress in juvenile rainbow trout. Therefore, antioxidant parameters in tissues of fish were negatively affected by hypoxic stress. Especially, in 3.5 mg O_2/l, it was determined that MDA levels increased more in tissues; it was followed by 4.5 mg O_2/l. Also in 4.5 mg O_2/l, vitamin C concentration in muscle was significantly affected by hypoxic stress; it was followed by vitamin E concentration. But vitamin A concentration in muscle of fish was not

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affected by hypoxic stress. It should be considered that sudden decrease or depletion of the dissolved oxygen concentrations in water adversely affects the developmental stages of fish, additionally the nutrient uptake and the resistance against diseases and infections in the rainbow trout juveniles leads to deterioration of the product quality and therefore the necessary measures to prevent it should be taken. Further investigations are required to elucidate the effects of oxygen consumption on the antioxidant defence system in tissues in rainbow trout under different stress conditions.

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