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Short communication

COMPARISON OF MICRO AND MACRO ELEMENT LEVELS IN THE MUSCLE TISSUES AND BLOOD ELECTROLYTES OF DIFFERENT SALMONID SPECIES

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ABSTRACT

The purpose of this study was to investigate changes in blood electrolytes levels (Na^+, K^+, Ca^{+2}, Mg^{+2} and P) and macro element (Na^+, K^+, Ca^{+2}, Mg^{+2} and P) and micro element (Cu, Fe, Mn, Ni, Zn, Cd and Pb) levels in muscle tissues of the rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*) grown in the same pond. In the study, 15 individuals of each species were randomly caught from the same pond. Na^+, K^+, Ca^{+2}, Mg^{+2} and P levels in blood fluid were determined by autoanalyzer Cobas C501 using commercial kits. Tissue samples were analyzed for Na^+, K^+, Ca^{+2}, Mg^{+2}, P, Cu, Fe, Mn, Ni, Zn, Cd and Pb levels, separately, by ICP-OES method. There was no significant difference between the blood electrolytes and the micro element levels of three different species. But, the significant changes in values of Na^+, Mg^{+2}, K^+, Ca^{+2} and P in muscle tissues of these species were observed ($p < 0.05$) Highest levels of macro, micro and blood electrolyte were obtained in the rainbow trout (except Mg^{+2} and K^+). The size and dominance of rainbow trout when compared to the other species may be the cause of changes in the macro element levels in muscle tissues.

Key words: Trout, tissue, blood electrolyte, micro element, macro element.

INTRODUCTION

Today, industrial and urban wastes are thrown directly into the aquatic environment, which leads to the bioaccumulation and bio-adhesion of heavy metals in fish. Fish are known as the best indicators of metal pollution in water systems depending on size and age (Burger *et al.,* 2002). Micro elements required for plant, animal and human life has beneficial or harmful effects depending on concentration and amount. Therefore, aquatic pollutants have a significant toxic potential (Gagnaire *et al.,* 2004). The inadequacy of mineral substances slows down the growth and feeding of the creatures quickly and leads to low yields in living things. There are a number of factors that affect mineral needs (Altıntaş, 2013). These factors can be listed as the level of production of the organism and the nature, age, chemical form and level of the elements, relation with other foods, mineral intake, adaptation of the living organism to nutrition and growth (Tchounwou *et al.,* 2014). Mineral insufficiency is supported by pathological and biochemical examinations as well as clinical diagnosis. Hemolysis, exercise, stress, ambient temperature and serum removal time are factors responsible for the high levels of minerals in the serum plasmas (Çelik, 2006). When these factors begin to increase their influence on metabolic activities, they activate regulatory mechanisms to reduce tissue damage the most that can occur in the immune system of living things (Conte, 2004). The organism's regulatory mechanisms are optimal cellular pH and osmolarity. Osmoregulation is achieved by the homeostasis of intracellular and extracellular ions (Tseng and Hwang, 2008). Determining changes in some parameters of blood tissue in parallel with physiological changes for the protection of homeostasis in metabolism may provide important information for evaluation of stress levels of living organisms (Keleştemur, 2012). These parameters, which are present in certain concentrations in blood plasma, are called blood electrolytes (Handy *et al.* 1999). Potassium (K^+) is the main ion of intracellular fluid, whereas sodium (Na^+) is the main ion of extracellular fluid. Na^+ and K^+ are important ions that provide the sustainability of the osmotic pressure of the body fluids and the acid-base balance. The second most abundant cation in the intracellular fluid is the magnesium ion (Mg^{+2}). It acts as an auxiliary factor in the functioning of many enzymes. In addition, the Mg ion plays an important role in neurochemical impulse transmission and muscle stimulation. Mg has an important role in the absorption of calcium ions from the intestines (Karnaky,1998; Railo *et al.,* 1985). As in all living beings, the main factors affecting acid-base balance in fish are nutrition and environment. In intensive farming, the immune system must be strengthened for the sustainability of the acid-base balance (Moeller and Robert, 2001; Moraes, 2004). In this study, it was aimed to investigate blood electrolytes (Na^+, K^+, Ca^{+2}, Mg^{+2} and P) and micro (Cu, Fe, Mn, Ni, Zn, Cd and Pb) and macro element (Na^+, K^+, Ca^{+2}, Mg^{+2} and P) levels of the rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*) grown in the same pond.

MATERIALS AND METHODS

Experimental animals and feeding: This study was carried out at Ataturk University, Faculty of Aquaculture, Inland Water Fish Research and Application Center (24.03.2010). In the study, 15 rainbow trout (*Oncorhynchus mykiss*), 15 brown trout (*Salmo trutta*) and 15 brook trout (*Salvelinus fontinalis*), 45 fish at total, grown in the same pond were used for each group. The average weight of the fish was 210±24.7 grams for the rainbow trout, 180±17.2 grams for the brown trout and 160±18.8 grams for the brook trout. Fish were fed twice daily with commercial trout with the same content, and fish were caught randomly from the same pool.

Determination of mineral levels in muscle tissue: The fish were made into a fillet after their internal organs were removed and a sample of 10 g muscle tissue was taken for each fish. Muscle samples were thoroughly disintegrated with the help of a bisturia, and the disintegrated muscle samples were allowed to dry at 105 ° C for one day. The dried samples were placed in airtight polyethylene bags after being powdered into the air. The mineral content of muscle tissues was determined by InductivelyCoupled Plasma-Optical Emission Spectroscopy (ICP-OES). The 2 gr weighing dried samples were finely crushed by using mortar and pestle. The tissues were digested overnight followed by hot plate method (120 °C) after mixing with 20 ml concentrated nitric acid and perchloricacid (2:1 v/v) (Merck) (Mertens, 2005b). All samples were completed to 50 mL with 20% conc. nitric acid indistilled water and then filtered with Whatman filter paper (11 ml). ICP-OES with radial torch equipped with argon saturation assembly was used for the determination of Na^+, K^+, Ca^{+2}, Mg^{+2}, Cu, Fe, Mn, Ni, Zn, Cd and Pb. High purity (99.99%) argon was used as plasma, auxiliary and nebulizer gas. Element concentrations were calculated in milligrams/kilogram (dry weight). Detailed information on ICP-OES can be found elsewhere (Karatas, 2014).

0.2 grams samples were taken from the muscle tissue of each fish and they were placed in a plastic balcony tube and they were allowed to stand for 10 minutes immediately after the addition of 2 ml of NO3 (nitrite oxide), the mixture. Then the mouths of the tubes were closed and subjected to a burning process in the microwave for half an hour. The burning process is carried out in 3 different steps (step 1: 5 minutes at 75% microwave power at 145ºC, step 2: 10 minutes at 90% microwave power at 180ºC and 10 minutes at 40% microwave power at 100ºC) in a pressurized microwave wet combustion unit (SpeedWave MWS-2 Berghof product + Instruments Harresstr.1. 72800 Enien Germany) resistant to 40 bar (Mertens,2005a). After the burning process, the lids of the tubes are slightly loosened to allow gas to escape from the tube. After the gas outlet is completed, the tubes are opened and the dissolved samples are passed through the filter paper and then are made ready to be read by adding distilled water over the total volume of 25 ml (Karatas, 2014).

Blood Samples and Serum: Blood samples were taken from the caudal vein of the fish using an injector and transferred to biochemical tubes, without any chemical, are used for serum separationand allowed to stand for 20 min for clotting. Then, blood samples were centrifuged at 3000 rpm for 10 minutes. Serum samples were analyzed with auto-analyzer Cobas C501 using commercial kits Karatas (2016).

Macro and Micro element levels of the water: Ca^{+2}, P, Mg^{+2}, Na^+, K^+, Cu, Fe, Mn, Ni, Zn, levels related to the water in which grown of the fish were determined as 49±5.10, 37±4.30, 7.11±0.8, 14.18±1.6, 3.4±0.40, 0.2±0.01, 0.05±0.01, 0.01±0.001, 0.03±0.002, 12±0.01 mg, respectively.

Statistical analysis: All data obtained from this study were analyzed by LSD-test. SPSS 15.0 statistical package program was used for this purpose. LSD-test was used to determine the differences between blood and muscle electrolyte levels (Ca^{+2}, P, Mg^{+2}, Na^+, K^+) in the rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*). $P<0.05$ was statistically considered significant Sun *et al.* (2014).

RESULTS AND DISCUSSION

The date related to the muscle and blood electrolytes levels of the rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*) are summarized in Table 1, Table 2 and Table 3. Potassium (K^+), the main cation of intracellular fluid is the main ion of sodium (Na^+) and Na^+ and K^+ are important ions that ensure the acid-base balance, permeability of cell membranes, functioning of nerves and muscles as well as the osmotic pressure of the body fluids (Lilly*et al.,* 2017). Sodium and potassium levels in the water are important to meet the basic needs of fish, but it should be supplemented with nutritionally best Na and K ratios in fish growth (Kalantarian *et al.,* 2013). Potassium deficiency in fish has been reported to cause loss of appetite (Kopp *et al.,* 2013). Respiration in hunger and toughness is necessary in maintaining mineral balance and maintaining vital functions (Salem *et al.,* 2007). In this study, there was no significant change in the sodium (155.0-186.8mg/kg) and potassium (0.99-1.35mg/kg) values of the blood fluid, respectively. But, the sodium (1351.7-2162.5 mg/kg) and potassium (3240.5-7465.2 mg/kg) levels in the muscle tissue of the rainbow trout were significantly different when compared to the brown and the brook trout (Table 2) ($p<0.05$). Karatas (2014) reported sodium valueof 1247.0 mg/kg and potassium value of 3052.1mg/kg of cultured rainbow trout. The results of this study were consistent with the findings of the Karatas (2014). Potassium values in studies conducted on different species of fish were determined as 289mg/100g in *Carangoides chrysophrys* (Lourenco *et al.,* 2009) and 414 mg/100g in *Carangoides chrysophrys* (Lilly *et al.,*2017), 99.60 mg/100g in french rose shrimp and 64.49 mg/100g in red shrimp (Oksuz *et al.,* 2009), 414 mg/100g in *Hemiramphus far* (Lilly *et al.,* 2017), 459.7mg/100g in sea bass and 393.8 mg/100g in sea bream (Erkan and Ozden, 2007).

Magnesium is the most abundant mineral after potassium in the cell, and it carries out many metabolic events including maintaining the correct distribution of sodium, potassium and calcium in the cell (Terech-Majewska *et al.,* 2015). Magnesium is involved in the formation of bones and teeth, in metabolism, in the synthesis of nucleic acids and proteins, and in thermoregulation (Lilly *et al.,* 2017). In this study, the magnesium levels of rainbow, brown and brook were in the range of 2.10-2.84mg/dL in the blood fluid ($p>0.05$) (Table 1) and were in the range of 133.7-232.6mg/kg in muscle tissues ($p<0.05$) (Table 2). Magnesium is found in low levels (range of 0.22- 0.33mg/100g) in some species such as *Cephalipholis boenak, Lutjanus fulvus, Parupeneus indicus* and high (range of 0.39 - 0.52 mg/100g) in some species like *Velamugilseheli, Dussumieria acuta, Carangoide schrysophrys, Hemiramphus far, Arius subrostratus, and Sardinella albella* (Lilly*et al.,* 2017).

Calcium has an important role in the regulation of muscle, nerve and heart functions as well as the development of bones and teeth (Lilly*et al.,* 2017).Calcium, one of the most abundant cations in the body of a fish, plays an important role not only in skeletal development but also in the realization of many physiological processes (Terech-Majewska*et al.,*2015).In this study, the calcium levels of rainbow, brown and brook were in the range of 12.32.-15.32mg/dL in the blood fluid ($p>0.05$) and were in the range of 148.6-330.3mg/kg in muscle tissues (Table 1 and 2) ($p<0.05$). Calcium levels in the muscle tissue of wild and cultured rainbow trout were in the range of 11.3-48.1 (Karatas, 2014). It was reported that calcium levels may vary according to fish species such as *Dussumieria acuta* (61.20 mg/100g), *Hemiramphus far* (28.30 mg/100g), Indian mackerel (1.51 mg/100g), *sixbar grouper* (3.03 mg/100g), *Japanese threadfin bream* (1.04 mg/100g) and *Spanish mackerel* (1.02 mg/100g) (Lilly *et al.,* 2017).

Phosphorus is a component of bones, teeth, high energetic compounds, nucleic acids, caffeine, lecithin, cell membranes and blood (Lilly *et al.,* 2017). Phosphorus is also a mineral that is responsible for balancing the normal pH value in intracellular and extracellular fluids. Contrary to calcium, phosphorus is the main source of nutrients (Terech-Majewska *et al.,*2015). In this study, the phosphorus levels of rainbow, brown and brook were in the range of 13.8-18.6mg/dL in the blood fluid ($p>0.05$) and were in the range of 2237.0-4412.7 mg/kg in muscle tissues (Table 1 and 2) ($p<0.05$). Phosphorus levels in the muscle tissue of wild and cultured rainbow trout were determined in the range of 2845.1-3357.2 mg/kg (Karatas, 2014). The results of this study were consistent with the results of Karatas (2014). The mean concentrations of the mineral are between 618.38 and 1534.80mg/100g according to FAOand World Health Organization (FAO/WHO, 2004).

Fe and Cu, which have an important role in biological systems of organisms and fish, are basic elements (Karatas, 2014). In this study, Fe, Cu and Mn levels of rainbow, brown and brook were found in the range of 29.3-36.9,4.88-6.22 and 0.96-1.28mg/kg in muscle tissues, respectively. Fe, Cu and Mn levels of the rainbow trout were higher than these values of brown and brook trout. Fe, Cu and Mn levels obtained in this study were different from values of Al-Arab River, Northwest Atlantic, Kuzgun Dam Lake, Atatürk Dam Lake and Yesilirmak River (Hellou *et al.,* 1992; Karadede *et al.,* 1997; Karatas, 2014; Karadede and Ünlü, 1998; Mendil *et al.,* 2010). According to the Turkish Food Codex, the maximum level of Cu is 20 mg / kg.

Zinc is present in most of the metabolic pathways in humans and zinc deficiency can cause loss of appetite, loss of growth, skin alterations and immunological abnormalities (Karatas, 2014). In this study, Zn levels of rainbow, brown and brook were in the range of 31.6-32.5 mg/kg in muscle tissues. The zinc levels of fish living in the Kuzgun Dam, Swan and Boeuf lakes have been reported as 17.46-34.75, 45-60.9 and 4.62-14.6 mg/kg (Karatas, 2014; Park and Presley, 1997; Aucoin *et al.,* 1999). The results of this study were consistent with the results obtained in the Kuzgun Dam Lake. According to the Turkish Food Codex, the maximum level of Zn is 50 mg / kg.

Lead disrupts the function of the kidney, bone, central nervous system, and hematopoietic system and causes harmful effects on biochemical, histopathological, neuropsychological, fetotoxic, teratogenic and reproduction (Eisler, 2009; Bilandžić *et al.,* 2018). In this study, Pb levels of rainbow, brown and brook were observed in the range of 0.89-1.19 mg/kg in muscle tissues. Pb levels were reported as 3 µg/kg (Copat *et al.,* 2013), 9 µg/kg (Guérin *et al.,* 2011) and 450 µg/kg for Atlantic mackerel (Tuzen, 2009).

Cadmium is known as one of the environmental pollutants and is among the most dangerous substances (ATSDR, 2012). While acute effects of CD lead to intoxication, pulmonary edema, hemorrhage, testicular damage and mortality, long-term exposure to Cd is observed nephrotoxicity, osteotoxicity and immunotoxicity (ATSDR, 2012). In this study, Cd levels of rainbow, brown and brook were in the range of 0.18-0.29 mg/kg in muscle tissues. The results of this study were lower than the results set out in the fish in Greece (3.7 µg/kg; Kalogeropoulos *et al.,* 2012). The mean Cd levels of Atlantic mackerel muscle were determined as 10 µg / kg (Bilandžić *et al.,* 2018). Tuzen (2009) and Copat *et al.* (2013) found that the levels of Cd in fish varied between 1.3 and 150 µg / kg.

As a result, it was found that micro, macro and blood electrolyte levels of rainbow trout (*Oncorhynchus mykiss)* (except Mg^{+2} and K^{+}) were higher than brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*) grown in the same pond. It can be said that these differences may be due to malnutrition, gender and stress as well as size and dominance. Further research is needed on the cultivation of different species of fish on the same conditions. This study may provide useful information in terms of providing insight for the future studies to be done.

Table 1. The blood electrolytes levels of the rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*).

Blood values (mg/dL)	Ca^{+2}	P	Mg^{+2}	Na^{+}	K^{+}
Rainbow Trout	15.32±12.8	18.6±15.6	2.84±2.39	186.8±68.7	1.25±0.22
Brown Trout	13.48±0.82	14.22±0.77	2.44±0.11	163.0±3.74	1.35±1.17
Brook Trout	12.32±0.82	13.8±0.58	2.10±0.18	155.0±5.0	1.02±0.72

The results were given as mean and standard deviation. Different letters indicate differences between groups

Table 2. Macro element levels in muscle tissues of the rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*).

Muscle Values (mg/kg)	Ca^{+2}	P	Mg^{+2}	Na^{+}	K^{+}
Rainbow Trout	330.3±166.7 ^{a}	4412.7±823.5 ^{a}	149.57±25.2 ^{a}	2162.5±1040.4 ^{b}	7465.2±1475.2 ^{a}
Brown Trout	173.2±79.8 ^{b}	3094.7±442.1 ^{b}	232.6±82.3^{b}	1372.0±84.6 ^{a}	5184.2±1094.4 ^{b}
Brook Trout	148.6±62.9 ^{b}	2237.0±1085.5 ^{b}	133.7±101.7 ^{a}	1351.7±120.0 ^{a}	3240.5±1706.3 ^{b}

The results were given as mean and standard deviation. Different letters indicate differences between groups

Table 3. Micro element levels in muscle tissues of the rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*).

Muscle Values (mg/kg)	Cu	Fe	Mn	Ni	Zn	Cd	Pb
Rainbow Trout	6.22±2.55	36.9±4.73	1.28±0.23	0.66±0.13	32.5±3.92	0.29±0.02	1.19±0.15
Brown Trout	5.40±1.33	36.6±7.18	1.20±0.95	0.50±0.08	31.7±17.2	0.20±0.27	1.10±0.10
Brook Trout	4.88±0.60	29.3±6.0	0.96±0.19	0.45±0.05	31.6±6.26	0.18±0.01	0.89±0.50

The results were given as mean and standard deviation. Different letters indicate differences between groups

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