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ORIGINAL PAPER

CONTENT OF MINERALS IN MUSCLE TISSUE OF DIPLOID RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) IN FRESHWATER (DERBENT DAM) AND SEAWATER (THE BLACK SEA)

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Abstract

Trouts are commonly farmed in many countries of the world. Minerals are required for normal life processes, and fish need these inorganic elements. Fish may derive these minerals from a diet and from ambient water. This study aimed to research differences in some of the mineral values in muscle tissues of diploid rainbow trouts (*Oncorhynchus mykiss*) cultured in freshwater and seawater. The study material consisted of twenty diploid rainbow trouts (*Oncorhynchus mykiss*), ten farmed in seawater and ten farmed in freshwater, with a weight of about 0.8-1 kg and average age of 17-19 months. Supernatant was prepared from muscle tissues of the fish. Concentrations of major minerals (Na, K, Ca, Mg, Cl, P) were investigated in supernatant from fish muscle tissues with an autoanalyzer. pH temperature, oxygen amount, salinity amount and saturation ratio of the trouts were found to be 8.40, 12°C, 9.57 mg dl^{-1}, 18‰, 88.77% for seawater and 7.60, 11°C 7.40 mg dl^{-1}, 0.68 ‰, 67.26% for freshwater fish. It was found that P, K and Mg levels in the muscle tissue of diploid rainbow trouts (*Oncorhynchus mykiss*) farmed in seawater were statistically significantly higher than in those farmed in freshwater (P<0.001), and Ca levels in trouts farmed in seawater were also higher than in those farmed in freshwater, although not statistically significantly different. It was determined that Na and Cl levels were slightly higher in rainbow trouts farmed in freshwater than in those farmed in seawater, but it was not a statistically significant difference (P>0.05). This study showed that concentrations of minerals in trout muscle tissue can be affected by changes in water quality. It was concluded that muscle tissue of diploid rainbow trouts (*Oncorhynchus mykiss*) farmed in seawater is a better source of minerals in human diet than trouts farmed in freshwater.

Keywords: freshwater, rainbow trout, seawater, minerals.

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INTRODUCTION

Minerals or inorganic elements are required for normal life processes by all terrestrial and aquatic organisms, including fish. Fish may derive the minerals from a diet or directly from water (Lall 2002). Calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), and chloride (C) have critically important physiological and biochemical functions in biological systems, and both their deficiency or excess can lead to some disturbance of metabolism and consequently to various diseases.

In mammals and aquatic animals, Ca is considered as one of the most important minerals. Among aquatic animals, fish can absorb Ca from the surrounding water to satisfy part or the entire metabolic Ca requirement (Lall 2002). Compared with other minerals, calcium absorbance to the body is relatively inefficient. In general, only about 25% to 30% of dietary calcium is effectively absorbed (FAO Agriculture and Consumer Protection Department, 2002). Fish and other aquatic animal food products are a rich source of calcium (Martinez-Valverde et al. 2000). Unlike calcium, the main source of phosphorus is the food ingested by fish. Phosphorus in fresh and sea water is either very scarce or not present at all. If there is enough ^{32}P in water, it is suggested that fish can absorb this form of phosphorus from water. The Ca/P ratio is the most important indicator of good bone health.

Chloride was originally described by Keys and Willmer (1932) to be present in the gills of seawater adapted teleosts. Sodium, potassium and chlorine have important functions in the osmoregulation of fish. To maintain the normal neuromuscular response of the body, sodium must appear in appropriate proportions with other electrolytes (P, Ca) – Dunham 2004. Transition of sodium into cells or loss of sodium from the body affect the extracellular fluid volume, circulation, kidney function, and central nervous system function (Murray et al. 1993). In contrast to Na, Mg plays an important role in physiological processes. Magnesium also plays a vital part in the reversible association of intracellular particles and in the binding of macromolecules to subcellular organelles (Barat et al. 2013). Mg deficiency can cause abnormalities in fish (Çelik 2006). A negative relationship has been reported between Mg deficiency and weight gain, specific growth rate (SGR) and feed evaluation rates, FCR (Dabrowska et al. 1991).

As the most abundant intracellular ion in all animals, K participates in basic physiological functions, including maintenance of membrane potentials and cellular volume, acid/base balance, cardiac function and nerve impulse transmission. In fish, potassium additionally plays a critical role in osmoregulation (Dunham 2004, Marshall, Grosell 2006).

People obtain the amounts of minerals they need by eating a wide variety of foods, including fish. This study was aimed to research the effects of freshwater and seawater on some of the major minerals in muscle tissues of diploid rainbow trouts (*Oncorhynchus mykiss*).

MATERIALS AND METHODS

Material

The study material consisted of twenty rainbow trouts (*Oncorhynchus mykiss*) with 2n chromosomes, which were farmed in freshwater (Derbent Dam) and seawater (the Black Sea). The fish were aged between 17-19 months and weighed approximately 0.8-1 kg.

The pH level of the water where the rainbow trout were farmed, i.e. the dam water and the Black Sea water, was measured with a pH meter in December 2018. The oxygen amount, oxygen saturation and temperature level of the water in the same water bodies were determined with an YSI oxygen meter (YSI PRO 20 Polarographic Do Field Electrode Portable Oxygen Meter 10). The water salinity was determined with a refractometer. pH, temperature, oxygen content, salinity and saturation rate of freshwater and seawater where rainbow trouts were farmed are presented in Table 1.

Table 1

pH, temperature, oxygen contents, and saturation rates of dam water and seawater

Specification	pH	Temperature (°C)	Oxygen (mg dl^{-1})	Saturation (%)	Salinity (‰)
Seawater	8.40	12	9.57	88.77	18
Dam water	7.60	11	7.40	67.26	0.68

Trouts farmed in seawater were maintained in HDPE cages, 22 m in diameter with 15±1 depth nets, and supplied a ready-to-feed pellet feed (crude protein 45%, crude fat 20%, crude ash 9.5%, crude cellulose 1.6%, calcium 1.8%, phosphorus 0.7%). They were kept 3.5 m nautical miles away from Yakakent port (Samsun, Turkey) until the time of harvest.

Trouts farmed in fresh water in a dam were maintained in HDPE cages and fed with a ready-to-feed pellet feed (crude protein 45%, crude fat 20%, crude ash 9.5%, crude cellulose 1.6%, calcium 1.8%, phosphorus 0.7%) until the time of harvest. Rainbow trouts farmed in sea and freshwater were not fed for 24 h before sampling, and at harvest, muscle tissue between the lateral line and the dorsal fin was removed from each diploid rainbow trout.

Preparation of tissue samples

For biochemical analyses, muscle tissues were homogenized in phosphate buffered saline (PBS) in a 1/2 weight/volume (w/v) homogenizer for 3 min on ice, and after that the homogenate was centrifuged at 15 000 x g for 20 min at +4°C (De Boeck et al. 2003). Mineral levels were examined in the supernatants obtained.

Determination of major mineral levels in fish muscle tissue

The levels of Mg, Ca, P and Na, K, Cl in muscle tissue supernatants were measured with a spectrophotometric method (Mann, Yoe 1957, Bohuon 1962, Michaylova, Illkova 1971, Daly, Ertingshausen 1972, Gamst, Try 1980, Tietz 1987) in an autoanalyser (Beckman Coulter Inc., Brea, CA, USA) using Beckman kits. The following kits were used: P – Beckman Coulter OSR6122; Mg – Beckman Coulter OSR6189; Ca – Beckman Coulter OSR60117, Na, K, and Cl – Beckman Coulter 66320, as described by the manufacturer.

Statistical analysis

The resulting data were analyzed using a one-way ANOVA procedure in SPSS software (IBM SPSS ver. 20 software package, Ondokuz Mayis University, Samsun, Turkey). The data were tested for normality with the Kolmorov-Smirnov Test and for homogeneity – with the Levene Test and Mann-Whitney *U* test. A *t*-test was also performed. Differences were considered significant at $P<0.05$. All variables were expressed as mean and standard error, SE (Rao 1973).

RESULTS

The levels of Na, K, Cl, P, Mg, and Ca in diploid rainbow trout muscle tissues farmed in freshwater and seawater are presented in Table 2.

Na and Cl levels in muscle tissues of rainbow trouts farmed in seawater and freshwater were found to be 416.1±9.38 (mmol g^{-1} tissue), 423.3±16.15 (mmol g^{-1} tissue), and 385.8±9.5 (mmol g^{-1} tissue), 391.5±13.13 (mmol g^{-1} tissue), respectively. It was found that the levels of Na and Cl were slightly lower in the muscule tissue of rainbow trouts cultured in seawater than in muscle tissue of the fish farmed in freshwater, but the difference was not statistically significant ($P>0.05$).

Table 2

Mineral levels in the muscle tissue of rainbow trout farmed in seawater and dam water

Minerals	Seawater	Dam water	*P*
Na (mmol g^{-1} tissue)	416.1±9.38	423.3±16.15	0.239
K (mmol g^{-1} tissue)	74.88±6.73	48.2±7.21	0.0001***
Cl (mmol g^{-1} tissue)	385.8±9.5	391.5±13.13	0.281
P(mg g^{-1} tissue)	129.96±3.71	120.48±5.63	0.0001***
Mg (mg g^{-1} tissue)	17.58±1.43	9.45±1.95	0.0001***
Ca (mg g^{-1} tissue)	6.09±1.38	4.77±3.63	0.298

*** $P<0.001$

K levels and P levels in muscle tissues of rainbow trouts farmed in seawater and freshwater were found to be 74.88±6.73 (mmol g^{-1} tissue), 48.2±7.21 (mmol g^{-1} tissue), and 129.96±3.71 (mg g^{-1} tissue), 120.48±5.63 (mg g^{-1} tissue), respectively. It was found that potassium and phosphorus levels in muscle tissue of rainbow trouts farmed in seawater were significantly higher than in those of rainbow trouts grown in freshwater (P<0.001).

Ca levels and Mg levels in muscle tissues of rainbow trouts farmed in seawater and freshwater were found to be 6.09±1.38 (mg g^{-1} tissue), 4.77±3.63 (mg g^{-1} tissue), and 17.58±1.43 (mg g^{-1} tissue), 9.45±1.95 (mg g^{-1} tissue), respectively. It was found that magnesium (P<0.001) and calcium (P>0.05) levels in muscle tissue of rainbow trout which were cultured in seawater were significantly higher than in those farmed in freshwater.

DISCUSSION

Fish are important aquatic organisms that appear in human diet (Dore 1984) because consumption of fish meat lowers the low risk of coronary cardiac disease, hypertension and cancer (Mendil et al. 2010). The rainbow trout is an economically important species, commercially farmed in many countries throughout the world. Sea food has a wide variety of nutrients that are easily absorbed in the digestive system, which makes it one of the best dietary sources of alimentary constituents (Bourre, Paquotte 2008). Minerals are essential nutrients and contribute to the growth of fish (Glover, Hogstrand 2002). Most people obtain the amount of minerals they need by eating a wide variety of foods. The five major minerals in the human body are Ca, P, K, Na, and Mg. The levels of these minerals are commonly used to determine the physiological characteristics, toxicity and health status of fish (Percin et al. 2010).

Water quality influences the composition, fertility, abundance of species and physiological status of aquatic species. Since dam lakes are the type of an environment constantly receiving water from the external sources, they are particularly exposed to environmental pollution. Such pollution negatively affects the water living organisms, reaching humans via food chains (Yilmaz 2004). The pH of water can also affect fish health. For most freshwater species, a pH range between 6.5-9.0 is optimal, but most marine animals typically cannot tolerate as wide a range pH as freshwater animals, thus the optimum pH for the former fish is usually between pH 7.5 and 8.5 (Boyd 1990). In our study, it was found that pH was 8.4 in seawater and 7.6 in freshwater. Decrease in the amount of dissolved oxygen in the blood leads to an increase in the amount of carbon dioxide and consequently to a decrease in the pH of blood. It has been reported that a decrease in pH in blood may limit the vital functions of a living organism and even cause death (Çelik et al. 2008). Below pH 6.5, the growth of some species, expressed by certain

physical parameters, slows down. At lower pH, an organism's ability to maintain its salt balance is affected and reproduction ceases (Lloyd 1992). At approximately pH 4.0 or below and pH 11.0 or above, most species die (Lawson 1995). In our study, it was determined that the pH of seawater is higher and it is a better environment for fish.

Salinity is expressed as the total amount of salt dissolved in water. Salinity in water is caused by chloride, mainly sodium chloride (Cirik, Gökpinar 1993). The amount of oxygen detected in water at any given time depends on the temperature of water, the partial pressure of atmospheric gas on the water surface, the concentration of dissolved salt in the water and biological events at that time. An increased salt concentration in water has been reported to decrease the amount of dissolved oxygen (Cirik, Gökpinar 1993). In our study, salinity in seawater was found to be 18‰, while salinity in dam water was 0.68 ‰.We found that both salinity and oxygen content in seawater were higher than in dam water.

Oxygen in water is available to fish in a dissolved state. Dissolved oxygen is considered as one of the most important aspects of aquaculture. It is needed by fish to respire and to perform metabolic activities. In our study, the oxygen content of seawater was found to be 9.57 mg dl^{-1}, while the oxygen content of freshwater was 7.40 mg dl^{-1}. In general, a saturation level of at least 5 mg L^{-1} is required. Values lower than this can put undue stress on fish, and levels reaching less than 2 mg L^{-1} may result in death but 3 mg L^{-1} to some species (Lloyd 1992, Svobodova et al. 1993). Thus, low levels of dissolved oxygen are often linked to fish death incidents. On the other hand, optimum levels can result in good growth and consequently lead to high production yield.

Calcium is considered as one of the most important minerals, essential for normal growth, skeletal development and several physiological processes in aquatic species (NRC 1993, Lall 2002). The absorption of Ca by fish in different farming environments varies. For example, fish in freshwater can absorb Ca through the gills and fins, while marine fish absorb calcium from their bodies by drinking seawater. Fish in seawater tend to gain ions such as sodium and chloride through diffusion and to lose water by osmosis. Since whole-body calcium uptake correlates positively with environment (Ca^{2+}), salinity (as a result of (NaCl) and drinking, the relative contribution of each of these variables to calcium uptake is uncertain (Guerreiro et al. 2004). Marine fish regulate their plasma osmotic value at approximately one third of that of the seawater; their main problem is counteracting the body water loss due to osmosis. For this purpose, seawater fish drink seawater that is deionised in the oesophagus and intestine under strict endocrine control (Takei 2008). In our study, it was found that the Ca level was higher in rainbow trout farmed in seawater when compared with fish farmed in dam water (P>0.05).

Magnesium deficiency has a pronounced effect on calcium homeostasis

in mammals (Welsh, Weaver 1988) and fish (Cowey et al. 1977). Experimental and observational studies have shown that higher Mg intake can exert beneficial effects on CV risk factors by improving glucose and insulin metabolism, enhancing endothelium-dependent vasodilation, ameliorating lipid profile and by its actions as an antihypertensive and anti-inflammatory agent (Shechter 2010). It was found in our study that magnesium levels in the muscle tissues of rainbow trouts farmed in seawater were significantly higher when compared with those of rainbow trouts farmed in freshwater ($P<0.001$).

Like in all animals, potassium is the most abundant intracellular ion in fish and plays many important physiological roles, including the maintenance of cellular volume and membrane potentials and the generation of nerve impulses (Epstein et al. 1980). In fish, potassium plays additional critical roles in osmo-and iono-regulation and acid/base balance (Marshall, Bryson 1998, Evans et al. 2005). Potassium deficiency causes overall muscle weakness, resulting in intestinal distention, weakness of cardiac and respiratory muscles, and their ultimate failures (Lall 2002, Roy et al. 2007). In our study, we found that the level of potassium and phosphorus in the muscle tissue of rainbow trouts farmed in sea water was significantly higher than that of the rainbow trout farmed in fresh water ($P<0.001$). In a study conducted in Poland, the minerals (Ca, Mg, Na, Zn, P, K, Fe, Cu) in muscle tissue of 40 rainbow trouts farmed in technological stream flow systems, allowing the use of excess water the autumn and spring, were assessed in 2010-2011. It was reported that phosporus and potassium levels were higher in the muscle tissue of rainbow trouts cultured on farms in which a RAS system was used (Fallah et al. 2011, Siemianowska et al. 2016).

While sodium is essential for normal human functions, current sodium intakes far exceed recommendations for good health (Brown et al. 2009). Excessive sodium intake is associated with an increase in blood pressure, which is a major cause of cardiovascular diseases (He, Macgregor 2010). Excess sodium consumption has also been associated with numerous other negative health effects, including gastric cancer (Tsugane et al. 2004), decreased bone mineral density (Devine et al. 1995) and possibly obesity (He, Macgregor 2008). In our study, it was found that Na and Cl levels in the muscle tissues of rainbow trouts farmed in seawater were slightly lower than those farmed in freshwater, but it was not statistically significant ($P>0.05$).

CONCLUSION

In our study, we found that muscle mineral contents of fish vary depending on the environment in which they were farmed. The levels of calcium, magnesium, potassium and phosphorus were higher in the muscle tissues

of the rainbow trouts which were farmed in seawater, while the sodium and chlorine levels were lower when compared with fish farmed in freshwater. It was concluded that muscle tissue of diploid rainbow trouts (*Oncorhynchus mykiss*) farmed in seawater is a better source of minerals in human diet when compared with trouts farmed in freshwater.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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