



## **High Stocking Density Impairs Mineral Content of Brown Trout**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author MB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors GA and AB managed the analyses of the study. Author AB managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

The aim of the present study was to determine the effects of high stocking density on mineral content of muscle and liver in brown trout. Juvenile fish were stocked in experimental tanks as triplicates for 90 days. Initial stocking density was 10 kg m<sup>-3</sup> for control and 50 kg m<sup>-3</sup> for high stocking density group. Atomic absorption spectrophotometry was used for the indication of mineral concentrations. Stocking density was calculated by dividing the weight of the fish in the tank water volume (kg m<sup>-3</sup>). The average values of P, K, Mg, Na, Ca, Fe, Zn, Cu, and Mn were found 2012.3, 958.0, 560.0, 506.1, 60.3, 31.5, 10.6, 1.61 and 0.27 mg kg<sup>-1</sup>, respectively, in muscle of fish reared high stocking density and the values for same minerals were found 2463.6, 1594.8, 866.2, 762.5, 155.7, 65.0, 20.0, 1.96, and 0.37 mg kg<sup>-1</sup>, in muscle of control group. However, P, K, Mg, Na, Ca, Fe, Zn, Cu, and Mn values were 1612.3, 1501.1, 530.0, 266.8, 129.2, 52.7, 0.82, 0.32, and 0.12 mg kg<sup>-1</sup> in liver of fish reared in high stocking density and 2270.4, 2063.6, 1300.8, 644.6, 189.6, 80.1, 1.59, 0.39 and 0.24 mg kg<sup>-1</sup> in liver of control group. It was concluded that that overcrowded condition causes to decrease amount of liver and muscle mineral content in brown trout.

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**Keywords:** Fish welfare; high stocking density; *Salmo trutta*; stress; mineral metabolism.

## 1. INTRODUCTION

Brown trout (*Salmo trutta*) is one of the most valuable salmonid species in the world and commonly used for restocking of freshwaters, as well as very popular for anglers. It distributes into rivers and lakes of Asia, Europe and North America [1]. Consumers prefer consumption of brown trout to that of rainbow trout because of taste and texture of the meat. Because of combined effects of all these factors, it is expected to steady increase in global production of farmed brown trout, at least for the next few years.

Stocking density depends on the fish size and the oxygen substance of water and it can applied 25 kg m<sup>-3</sup> to 45 kg m<sup>-3</sup> depend on fish size if good quality water in 15°C water temperature. But, this density made removable 80-90 kg m<sup>-3</sup> in case of increasing dissolved oxygen concentration in pool [2].

Fish are naturally low in calories and high in protein as well as contain a sufficient quantity of almost all mineral deposits. Although fishes can obtain minerals from water [3], their mineral contents are influenced by some factors such as biological differences, nutrition, season and environmental conditions. Fish is a mineral provider for human nutrition such as phosphorus (P), calcium (Ca), magnesium (Mg), sulfur (S), potassium (K), sodium (Na), iodine (I), chloride (Cl) and the other minerals such as manganese (Mn), zinc (Zn), copper (Cu), selenium (Se) and iron (Fe) [4]. Minerals that constitute approximately 4% of human body are substances essential for growth and health. Some fish species are perfect source of Ca and P in range from 100 to 400 mg per 100 g of fish; others are good source of K, which plays a catalytic role in carbohydrates and protein metabolism, and Mg that has catalyst role in enzyme system [5-7].

Growth parameters, survival and fish welfare are affected by stocking density [8,9]. There is very limited study on the effects of stress on mineral metabolism in brown trout, even in fish. Bayir et al. [10] suggested that lower mineral content was seen in high stocking density group compared to control group in juvenile rainbow trout. For this reasons, we determined the effect of high stocking density on the mineral content of muscle and liver in brown trout.

## 2. MATERIALS AND METHODS

### 2.1 Fish and Experiment

Brown trout juveniles (about 5 g) were weighted and stocked separately in six experimental tanks (three tanks were used for high stocking density group and others were for control group) in Trout Research Center of Ataturk University, Turkey. 10 kg m<sup>-3</sup> and 50 kg m<sup>-3</sup> initial stocking densities were applied for 90 days for control and crowded groups, respectively. The study was carried out as triplicates. Both of experimental groups were fed with commercial trout feed four times a day during acclimation period (15 days) and trial. Average water temperature was 9.0±1.0°C during the trial.

### 2.2 Analysis

Fish samples were prepared for mineral analysis following the standard method 937.07 [11]. Six fish liver and muscle samples were used from each group (control and high stocking). The samples were homogenized and transferred to plastic bags and kept in -86°C until analysis.

For the mineral matter analysis, firstly the working solution was prepared using 5:3 ratio of concentrated nitric acid (HNO<sub>3</sub>) and concentrated perchloric acid (HClO<sub>4</sub>). This solution was waited on a water bath for three hours and then left for cooling at room temperature. Finally, filtered into a standard flask and used for analysis [12]. Atomic absorption spectrophotometer was used for mineral matter analysis.

### 2.3 Statistical Analysis

Results were evaluated by statistical analysis and given as means±standard deviation (SD). One-way analysis of variance and Duncan's multiple range test was used to analyze differences between high stocking density and control group (p<0.05) [13].

## 3. RESULTS AND DISCUSSION

The mineral contents of fish muscle and liver are given in Table 1. The differences were found to be significant (p<0.05) except from Cu content of liver in the studied stock densities.

**Table 1. Mineral content of muscle and liver in brown trout reared different stocking densities**

Mineral content (mg kg <sup>-1</sup> )	Muscle		Liver	
	Control	High stock density	Control	High stock density
Calcium	155.7±4.2 <sup>b</sup>	60.3±2.5 <sup>d</sup>	189.6±2.7 <sup>a</sup>	129.2±9.6 <sup>c</sup>
Copper	1.96±0.2 <sup>a</sup>	1.61±0.2 <sup>b</sup>	0.39±0.0 <sup>c</sup>	0.32±0.0 <sup>c</sup>
Iron	65.0±2.3 <sup>b</sup>	31.5±0.7 <sup>c</sup>	80.1±5.4 <sup>a</sup>	52.7±2.4 <sup>c</sup>
Potassium	1594.8±62.7 <sup>a</sup>	958.0±25.1 <sup>d</sup>	2270.4±15.3 <sup>a</sup>	1501.1±21.9 <sup>c</sup>
Magnesium	762.5±24.0 <sup>b</sup>	560.0±28.4 <sup>c</sup>	1300.8±8.5 <sup>a</sup>	530.0±5.1 <sup>c</sup>
Manganese	0.37±0.0 <sup>a</sup>	0.27±0.0 <sup>b</sup>	0.24±0.0 <sup>b</sup>	0.12±0.0 <sup>c</sup>
Sodium	866.2±8.3 <sup>a</sup>	506.1±7.5 <sup>c</sup>	644.6±4.8 <sup>b</sup>	266.8±7.3 <sup>d</sup>
Phosphorus	2463.6±31.0 <sup>a</sup>	2012.3±128.4 <sup>b</sup>	2063.6±28.0 <sup>b</sup>	1612.3±128.4 <sup>c</sup>
Zinc	20.0±1.9 <sup>a</sup>	10.6±0.9 <sup>b</sup>	1.59±0.1 <sup>c</sup>	0.82±0.0 <sup>d</sup>

(a-b-c-d): Different letters mean statistically different from each other ( $p < 0.05$ )

Minerals, especially Ca and P, are directly related to the development of fish skeletal system [14]. However, there are differences among studies that focused on Ca content of fish. For example, the average Ca content was detected to 155.7 and 60.3 mg kg<sup>-1</sup> in the muscle of brown trout, which reared uncrowded and crowded condition, respectively in this study. Polak-Juszczak [15] reported that different Ca content for different fish species including oily fish (59.3 mg kg<sup>-1</sup>), Pangasius catfish (73.9 mg kg<sup>-1</sup>), African catfish (105.8 mg kg<sup>-1</sup>), Nile perch (178.0 mg kg<sup>-1</sup>). Gokoglu et al. [16] reported that raw fillet of rainbow trout has very high Ca (632 mg kg<sup>-1</sup>) inversely Łuczyńska et al. [17] determined a lower Ca content in rainbow trout and salmon (137 mg kg<sup>-1</sup> and 90 mg kg<sup>-1</sup>, respectively) bought on Polish markets. This study found that both Ca and P content in liver and muscle of brown trout decreased in high stocking density. Data also showed that Na : K ratio are about 1:6 and 1:8 in muscle and 1:3 and 1:2 in liver of brown trout in control and high density groups, respectively. This finding is similar to Valverde et al. [6] who reported that the Na : K ratio is 1:2-1:10 in rainbow trout muscle. The Fe content was found about 65 mg kg<sup>-1</sup> in the muscle and 80.1 mg kg<sup>-1</sup> in the liver and these both values were higher than high stocking density groups. However, high stocking density did not affect hepatic Cu content. In a previous study, it was found that high stocking density had no effect on hepatic Mg and Mn content in juvenile rainbow trout [10]. In that study, Ca, Cu, Fe, K, Na, P and Zn amount was negatively affected by high stocking density and this was in agreement with present research.

It is known that mineral composition of fish meat shows different value depend on some factors including feeding, farming and environmental conditions. In addition these factors, breeding

conditions is the most important effect on chemical composition of fish [18,19]. Sadler and Lynam [20] suggested that pH effects mineral metabolism only in starved brown trout, not in fed fish.

Siemianowska et al. [19] carried out a study to compare the muscle minerals of rainbow trout which are reared in recirculation system and water flow-through system and they reported that some minerals (P, Mg and K) are not effected the farming although Na, Ca, Fe, Cu and Zn of rainbow trout muscle are influenced the rearing. Similar to this study, it was found that stocking brown trout at high density caused to decrease content of mineral matters in the muscle and liver. On the other hand, fish is also very important for human nutrition because of its high mineral content as well as the other nutritional content like oil and protein [21]. Ca is necessary for bone formation. Mg and K are also components of bones [22-24]. Na and K are good for muscle functioning and these two minerals are important for sugar metabolism and transmission of impulses in the nerves [24]. However, Zn is essential mineral for some metalloenzymes, which are necessary for cellular growth and gene expression [24]. Mn is needed for many enzyme activities. Even though fish has low Fe content compared to other animal meats; its absorption by human intestine is quite high. Therefore, it has been clearly indicated that bioavailability of Fe coming from fish muscle is high in human.

#### 4. CONCLUSION

As a general result of the present study, it has been found that mineral content of muscle and liver (except Cu) in juvenile brown trout was decreased dramatically by high stocking density. It was also reported that high stocking density

enhances reactive oxygen species in salmonids [9]. Therefore, it has been concluded that overcrowding negatively affects both nutritional quality (mineral content) and welfare of juvenile brown trout.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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