

# Original Research Article

## High Stocking Density Impairs Mineral Content of Brown Trout

### 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.

**Keywords:** Fish welfare, High stocking density, *Salmo trutta*, Stress, mineral metabolism

26 **1. INTRODUCTION**

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

33 Stocking density depends on the fish size and the oxygen substance of water and it  
34 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  
35 temperature. But, this density made removable 80-90 kg/m<sup>3</sup> in case of increasing dissolved  
36 oxygen concentration in pool [2].

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

48 Growth parameters, survival and fish welfare are affected by stocking density [8,9].  
49 There is very limited study on the effects of stress on mineral metabolism in brown trout, even  
50 in fish. Bayir et al. [10] suggested that lower mineral content was seen in high stocking

51 density group compared to control group in juvenile rainbow trout. For this reasons, we  
52 determined the effect of high stocking density on the mineral content of muscle and liver in  
53 brown trout.

54

## 55 **2. MATERIAL and METHODS**

### 56 **2.1. Fish and Experiment**

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

64

### 65 **2.2. Analysis**

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

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

74

75

## 76 2.3. Statistical Analysis

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

80

## 81 3. RESULTS and DISCUSSION

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

84 Minerals, especially Ca and P, are directly related to the development of fish skeletal  
85 system [14]. However, there are differences among studies that focused on Ca content of fish.  
86 For example, the average Ca content was detected to 155.7 and 60.3 mg kg<sup>-1</sup> in the muscle of  
87 brown trout, which reared uncrowded and crowded condition, respectively in this study.  
88 Polak-Juszczak [15] reported that different Ca content for different fish species including oily  
89 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  
90 perch (178.0 mg kg<sup>-1</sup>). Gokoglu et al. [16] reported that raw fillet of rainbow trout has very  
91 high Ca (632 mg kg<sup>-1</sup>) inversely Łuczyńska et al. [17] determined a lower Ca content in  
92 rainbow trout and salmon (137 mg kg<sup>-1</sup> and 90 mg kg<sup>-1</sup>, respectively) bought on Polish  
93 markets. This study found that both Ca and P content in liver and muscle of brown trout  
94 decreased in high stocking density. Data also showed that Na : K ratio are about 1:6 and 1:8 in  
95 muscle and 1:3 ad 1:2 in liver of brown trout in control and high density groups, respectively.  
96 This finding is similar to Valverde et al. [6] who reported that the Na : K ratio is 1:2-1:10 in  
97 rainbow trout muscle. The Fe content was found about 65 mg kg<sup>-1</sup> in the muscle and 80.1 mg  
98 kg<sup>-1</sup> in the liver and these both values were higher than high stocking density groups.  
99 However, high stocking density did not affect hepatic Cu content. In a previous study, it was  
100 found that high stocking density had no affect on hepatic Mg and Mn content in juvenile

101 rainbow trout [10]. In that study, Ca, Cu, Fe, K, Na, P and Zi amount was negatively affected  
102 by high stocking density and this was in agreement with present research.

103 It is known that mineral composition of fish meat shows different value depend on  
104 some factors including feeding, farming and environmental conditions. In addition these  
105 factors, breeding conditions is the most important effect on chemical composition of fish  
106 [18,19]. Sadler and Lynam [20] suggested that pH effects mineral metabolism only in starved  
107 brown trout, not in fed fish.

108 Siemianowska et al. [19] carried out a study to compare the muscle minerals of  
109 rainbow trout which are reared in recirculation system and water flow-through system and  
110 they reported that some minerals (P, Mg and K) are not effected the farming although Na,  
111 Ca, Fe, Cu and Zn of rainbow trout muscle are influenced the rearing. Similar to this study, **it**  
112 **was** found that stocking brown trout at high density caused to decrease content of mineral  
113 matters in the muscle and liver. On the other hand, fish is also very important for human  
114 nutrition because of its high mineral content as well as the other nutritional content like oil  
115 and protein [21]. Ca is necessary for bone formation. Mg and K are also components of bones  
116 [22-24]. Na and K are good for muscle functioning and these two minerals are important for  
117 sugar metabolism and transmission of impulses in the nerves [24]. However, Zn is essential  
118 mineral for some metalloenzymes, which are necessary for cellular growth and gene  
119 expression [24]. Mn is needed for many enzyme activities. Even tough fish has low Fe content  
120 compared to other animal meats; its absorption by human intestine is quite high. Therefore, it  
121 has been clearly indicated that bioavailability of Fe coming from fish muscle is high in  
122 human.

123

124

125

#### 126 4. CONCLUSION

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

129 It was also reported that high stocking density enhances reactive oxygen species in salmonids  
130 [9]. Therefore, it has been concluded that overcrowding negatively affects both nutritional  
131 quality (mineral content) and welfare of juvenile brown trout.

132

#### 133 REFERENCES

- 134 1. Geldiay R, Balık S. Türkiye Tatlısu Balıkları, (Book). No.: 46, Ege University Publications,  
135 Izmir, Turkey. 1996. Turkish.
- 136 2. Logan SH, Johnston WE. Economics of commercial trout production. *Aquaculture*,  
137 1992;100:25-46.
- 138 3. Lall SP. The minerals, Ln: Halver, JE, Hardy RW. (eds.), *Fish Nutrition*. Third Edition.  
139 Academic Press San Diego, 2002.
- 140 4. Tilami SK, Sampels S. Nutritional value of fish: Lipids, proteins, vitamins, and minerals.  
141 *Reviews in Fisheries Science and Aquaculture*, 2017;26:1-11.
- 142 5. Dean LM, Nutrition and preparation. Ln Martin RE, Flick GJ, (eds.), *The seafood industry*.  
143 Chap.16. Published Van Nostrand Reinhold, New York, 1990.
- 144 6. Valverde IM, Periago MJ, Santaella M. The content and nutritional significance of minerals  
145 on fish flesh in the presence and absence of bone. *Food Chemistry*, 2000;71:503-509.
- 146 7. Turan H, Kaya Y, Sönmez G. Balık etinin besin değeri ve insan sağlığındaki yeri. E.U.  
147 *Journal of Fisheries & Aquatic Sciences*, 2006;23:505-508. Turkish.
- 148 8. Braun N, De-Lima RL, Baldisserotto B, Dafre AL, De-Oliveira Nuñez AP. Growth,  
149 biochemical and physiological responses of *Salminus brasiliensis* with different  
150 stocking densities and handling. *Aquaculture*, 2010;301:22-30.

- 151 9. Bayir M, Bayir A. A comparison of growth, antioxidant levels and fatty acid metabolism in  
152 juvenile rainbow trout (*Oncorhynchus mykiss*) reared in two different stocking  
153 densities: low and high. *Marine and Freshwater Behaviour and Physiology*,  
154 2017;50:345-357.
- 155 10. Bayir M, Bayir, A, Cakir, O. Effects of high stocking density on liver and muscle mineral  
156 content in rainbow trout. *Aquaculture Europe 2015 (Symposium)*. October 23-23, 2015.  
157 Rotterdam, The Netherlands.
- 158 11. AOAC. Official Methods of Analysis of Association of Analytical Chemist. 17th Ed.  
159 Gaithersburg, Maryland. 2003.
- 160 12. Gagophein PO, Nwajei JE. Distribution of heavy metals in the sediment of Lagos Lagoon.  
161 *Pakistan Journal of Scientific and Industrial Research*, 2000;43:338-340.
- 162 13. SAS. SAS User's Guide: Statistics, Version 9th. Statistical Analysis System Institute Inc.,  
163 Cary, NC, 2002.
- 164 14. Lall SP, Lewis-McCrea LM. Role of nutrients in skeletal metabolism and pathology in  
165 fish an overview. *Aquaculture*, 2007;267:3-19.
- 166 15. Polak-Juszczak L. Chemical characteristics of fishes new to the Polish market. *Acta*  
167 *Scientiarum Polonorum, Piscaria*, 2007; 6(2): 23-32.
- 168 16. Gokoglu N, Yerlikaya P, Cengiz E. Effects of cooking methods on the proximate  
169 composition and mineral contents of rainbow trout (*Oncorhynchus mykiss*). *Food*  
170 *Chemistry*, 2004;84:19-22.
- 171 17. Łuczyńska J, Tońska E, Borejszo Z. Content of macro- and microelements, and fatty acids  
172 in muscles of salmon (*Salmo salar* L.), rainbow trout (*Oncorhynchus mykiss* Walb.),  
173 and carp (*Cyprinus carpio* L.). *Żywność. Nauka. Technologia. Jakość*, 2011;3:162-172.

- 174 18. Tkaczewska J, Migdał W. Comparison of slaughter field, contents of basic nutrients, and  
175 levels of heavy metals in muscles of rainbow trout (*Oncorhynchus mykiss*) originating  
176 from various regions in Poland. Food Science Technology Quality, 2012;5:177-186.
- 177 19. Siemianowska E, Barszcz AA, Skibniewska KA, Markowski A, Polak-Juszczak L,  
178 Zakrzewski J, Woźniak M, Szarek J, Dzwolak W. Mineral content of muscle tissue of  
179 rainbow trout (*Oncorhynchus mykiss* Walbaum). Journal of Elementology, 2016;  
180 21:833-845.
- 181 20. Sadler K, Lynam S. Some effects of low pH and calcium on the growth and tissue mineral  
182 content of yearling brown trout, *Salmo trutta*. Journal of Fish Biology, 1986;29:313-  
183 324.
- 184 21. Gladyshev M, Sushchik NN, Makhutova ON, Kalachova GS. Content of essential  
185 polyunsaturated fatty acids in three canned fish species. International Journal of Food  
186 Sciences and Nutrition, 2008;60:224-30
- 187 22. Larsen T, Thilsted HS, Kongsbak K, Hansen M. Whole fish as a rich calcium source.  
188 British Journal of Nutrition, 2000;83:191-196.
- 189 23. Roos N, Wahab AM, Chamnan C, Thilsted SH. The role of fish in food-based strategies to  
190 combat vitamin A and mineral deficiencies in developing countries. Journal of  
191 Nutrition, 2007;137:1106-1109.
- 192 24. Mogobe O, Mosepele K, Masamba WRL. Essential mineral content of common fish  
193 species in Chanoga, Okavango Delta, Botswana. African Journal of Food Science,  
194 2015;9:480-486.

195

196

197

198

199 **Table1.** 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
<b>Calcium</b>	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>
<b>Copper</b>	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>
<b>Iron</b>	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>
<b>Potassium</b>	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>
<b>Magnesium</b>	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>
<b>Manganese</b>	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>
<b>Sodium</b>	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>
<b>Phosphorus</b>	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>
<b>Zinc</b>	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>

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

201

202

203

204

205

UNDER PEER REVIEW