Magnesium content, total antioxidant status and lipid peroxidation in rainbow trout (*Oncorhynchus mykiss* Walbaum)

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**Abstract.** The present study was aimed at evaluating magnesium content, ferric reducing antioxidant power (FRAP) and lipid peroxidation in selected tissues of rainbow trout during their development. For mineral and biochemical assay, samples of liver, kidney, gills and blood were taken from fish. Magnesium concentration ranged between 35.5 and 249.2 mg·kg⁻¹ wt/wt. Most magnesium was found in the gills and the less in kidneys. FRAP values in the examined fish varied from 0.85 to 4.64 nmol Trolox Eq·mg⁻¹ protein. The highest FRAP was observed in the kidneys and the lowest in the gills. Concentration of malondialdehyde (MDA) in the examined tissue homogenates averaged 4.16-11.36 nmol·mg⁻¹ protein. We observed that levels of analyzed parameters increased during growth of the fish.

**Key words:** fish, rainbow trout, magnesium, lipid peroxidation, antioxidant status

Rainbow trout is one of the most important freshwater fish reared in the waters of America and Europe. Trout demands water saturation with oxygen not lower than 80%. They are also poorly resistant to elevated water temperature. The fish grow best at water temperatures within 13-18°C and an oxygen content above 5 mg·dm⁻³. They cannot survive prolonged periods of temperatures above 21°C, and even short term occurrence of temperatures above 25°C makes water useless for trout culture. Rainbow trout are able to use various kinds of natural and artificial feeds. For fish farmers, the most important fish features are high rates of growth and adaptation to changeable rearing conditions [1, 2].

Magnesium is one of the most abundant elements by mass in the vertebrata body and is essential to all living cells, where it plays a major role in manipulating important biological polyphosphate compounds like ATP, DNA, and RNA. Many enzymes require the presence of magnesium ions for their catalytic action, including all enzymes utilizing or synthesizing ATP, or those which use other nucleotides to synthesize DNA and RNA [3, 4]. Magnesium plays a regulatory role in oxidative processes [4-7].

This study aimed to estimate the magnesium content, total antioxidant capacity and lipid peroxidation in tissues of rainbow trout and the relationship between these parameters during their growth.

**Materials and methods**

**Animals**

The study was performed on rainbow trout (*Oncorhynchus mykiss* Walbaum), which were grown in privately owned fish breeding pounds in West-Pomeranian Province, Poland. The study involved 40 fish, from 4- to 8-months-old. The fish were grown in a commercial trout farm (Goleniów, West-Pomeranian Province, Poland). For this research we obtained the agreement of the Local Ethics Committee (nr 9/05). The fish were collected three times, in spring and summer, from April till August. Each time, 10-15 individuals
were collected (table 1). During the growing period, the fish were fed an Aller Aqua 576 pelleted feed (Aller Aqua Polska Co. Ltd., Nożycko-Czarna Dąbrówka, Poland), containing 37% proteins and 12% lipids, the detailed composition is presented in table 2. This feed is an intensified energy feed used in normal trout breeding conditions. The daily food ration was 2.4 ± 0.2 g per fish. Fish were fed twice a day.

**Water parameters**

Water was monitored throughout the experiment (figure 1), including temperature (12.83 ± 7.52°C), dissolved oxygen content (7.81 ± 0.35 mg L⁻¹), oxygen saturation (77.81 ± 2.55%), pH (7.88 ± 0.55) and magnesium concentration (0.54 ± 0.8 mg L⁻¹).

**Tissue sampling**

From each individual, samples of blood, liver, kidneys and gill lamellae were collected for assays. Before examination, fish were in 5 m x 20 m fish breeding ponds with a water temperature of 12 - 16°C. Prior to tissue dissection, the fish were transferred to a separate tank, which was gradually cooled to induce fish hibernation. In order to hibernate the fish they were transferred to a separate tank a the water temperature of 10°C. After 20 minutes the fish were transferred to a new tank with a water temperature of 4-5°C. Directly after the blood collection, the fish were decapitated and dissected. No anaesthetics were used, as they affect biochemical parameters [8]. Blood was sampled from the caudal vessel (a. et v. caudalis) into a heparinised syringe (50 IU sodium heparin per 1 mL blood). Directly after the blood collection, the fish were decapitated and dissected. When dissecting the fish, anatomical observations of the organs and tissues were recorded. Fish behaviour was observed throughout the study period. The fish showed no changes in behaviour and external appearance, and neither did their food consumption change.

**Table 1.** Body weights and lengths of fish during the experimental period.

<table>
<thead>
<tr>
<th>Age of fish (month)</th>
<th>Body weight (g)</th>
<th>Length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4th months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring (April and May)</td>
<td>182.4 ± 21.3</td>
<td>24.7 ± 1.9</td>
</tr>
<tr>
<td>6th months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring/Summer (June and July)</td>
<td>266.4 ± 19.2</td>
<td>21.9 ± 1.3</td>
</tr>
<tr>
<td>8th months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer (July and August)</td>
<td>377.5 ± 23.5</td>
<td>38.4 ± 2.8</td>
</tr>
</tbody>
</table>

Values are means ± SD.

**Figure 1.** Changes of water temperature, pH, oxygen content and oxygen saturation in pools during the experiment.

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8. 0.1x + 7.32 R² = 0.183

9. y = -0.27x + 8.71 R² = 0.985

10. y = -0.27x + 8.71 R² = 0.985
Biochemical parameters
For the ferric reducing antioxidant power (FRAP) and malondialdehyde (MDA) measurements the samples were frozen and kept at -80°C until analysed. Tissue homogenates and erythrocyte lysates were prepared according to Rice-Evans et al. [9]. The FRAP method is based on the reduction of iron (III) ions [10]. The determination involved a mixture of acetate buffer (300 mM) with solutions of 10 mM 2,4,6-tripyridyl-S-triazine (TPTZ) and FeCl₃ × 6H₂O (20 mM). Absorbance was measured at 593 nm. The results have been converted to nmol of Trolox Eq·mg⁻¹ of proteins and haemoglobin. The FRAP values in tissues were calculated from relevant calibration curves after correcting the data with blank results. Lipid peroxidation was measured by determination of MDA concentrations and expressed per milligram of protein (nmol·mg⁻¹ protein). The absor- bance was measured at 595 nm. The MDA method is based on the reaction of a chromogenic reagent, N-methyl-2-phenylindole, with MDA at 45°C.

Wesel, Germany) were quantitative (104.4 ± 10.3%). Experimental values averaged 366.5 ± 39.5 mg·kg⁻¹ wt/wt, while the certified value was 348.5 ± 21.3 mg·kg⁻¹ wt/wt.

Haematology
Erythrocyte counts (RBC) in 1 μL blood were counted in the Bürker chamber, following 200 x dilutions in the Hayem fluid [12]. The erythrocyte counts are expressed as T·L⁻¹. Leukocyte count (WBC) in 1 μL blood was counted in the Bürker chamber, following 20 x dilutions in the Türck’s fluid. The leukocyte counts are expressed as G·L⁻¹ [12].

Statistics
The results are given as arithmetic mean values (mean) and standard deviations (SD). The data obtained were subjected to statistical treatment involving analysis of variance (ANOVA) at the significance level of p ≤ 0.01, and comparison of correlation coefficients (R²). Statistical analysis was performed using Statistica® 6.0 software.

Results
All the examined fish significantly gained body weight and length during the study period, which indicated good ingestion of the feed (table 1). A positive correlation was observed between fish growth rate and the experiment duration. Body weight increased by 14.4% (R² = 0.898), and body length by 25.3% (R² = 0.868). Anatomical and histological examination was conducted in order to eliminate potentially sick individuals. Autopsy revealed no disorders or disease symptoms. Moreover, the good health condition of the fish was evidenced by basic haematological tests (RBC, WBC) [13, 14]. Haematological parameters in rainbow trout collected from both rearing sites examined were within the reference limits for the fish species, and did not differ significantly between the sites. Levels of erythrocytes in the blood of rainbow trout were within the range 3.2-5.2 T·L⁻¹ wt/wt. Levels of leukocytes in the blood of rainbow trout were within the range 3.1-8.2 G·L⁻¹ wt/wt. Between 4th and 8th month of life erythrocyte counts decreased by 29.57% (R² = -0.982), while leukocyte counts increased by 12.88% (R² = 1.000). The concentration of magnesium in various tissues of rainbow trout was within the range 38.7-255.2 mg·kg⁻¹ wt/wt (figure 2A-D). Most mag- nesium was found in the gills (201.4-255.2 mg·kg⁻¹ wt/wt).
wt/wt; figure 2D), while there was less in the blood (38.7-45.5 mg·kg⁻¹ wt/wt; figure 2A).

The highest FRAP values were detected in the kidney (2.7 ± 0.8 nmol Trolox Eq·mg⁻¹ protein⁻¹), and the lowest in the gills (1.17 – 1.60 nmol Trolox Eq·mg-protein⁻¹; figure 3C, D). In the blood and the gills, no significant changes in FRAP values were observed with fish age. MDA concentrations in the rainbow trout tissue homogenates averaged from 4.50 to 11.36 nmol·mg⁻¹ protein⁻¹ (figure 4A-D). The highest MDA concentration was found in the gills - 5.34 to 11.52 nmol·mg-protein⁻¹ (figure 4D), and the lowest in the blood - 2.04 to 7.12 nmol·mg-protein⁻¹ (0.75 to 5.23 nmol·mg-haemoglobin⁻¹) (figure 4A). MDA concentrations increased along with fish age (figure 4A, C, D).

**Discussion**

In this experiment, fish had been kept in pools from April till August, when water temperatures ranged from 6 to 14°C, which was slightly below the optimum temperature range for trout culture. A gradual increase in water temperature by 4°C during 5 months did not induce any adverse symptoms in the fish, whereas a decrease in oxygen levels during the last two months of the experiment (July, August) reduced the rate of body weight gain by 5-8%. During the experiment, an increased rate of body weight gain was observed, similar to the data from the literature [2, 15, 16]. This increased growth rate can probably be attributed to a favourable combination of the environmental conditions and adequate feed. Blood physiological values (RBC, WBC) in fish are highly dependent on individual variability, age, rearing method, diet and season of the year [14, 15]. Erythrocyte counts in rainbow trout fluctuate during the year, around 1.4 ± 0.8 T·L⁻¹ [15]. Blood parameters typical for healthy fish may vary in a wide range, therefore determination of adequate physiological reference values is much more difficult than in case of warm-blooded animals [14, 15]. The blood parameters examined were within the reference values [16, 17].

The results of our study indicate that the magnesium content in the blood of rainbow trout remained relatively constant for the observed four months, while, in the organs examined, magnesium levels increased along with age. The highest concen-
The concentration of magnesium was in the gills and the least in the kidney. Knox et al. [15] studied how diets with different magnesium contents affected the growth of the rainbow trout. These results show that the magnesium requirement of rainbow trout is met by a diet containing 0.5 g magnesium/kg diet [18]. Oikari et al. [18] have shown that infusion of magnesium salt into the body cavity of rainbow trout affects the magnesium concentration in the plasma.

Magnesium plays a regulatory role in oxidative processes [3, 7, 17, 19]. A decrease in magnesium levels in the body leads to a reduction in glutathione levels, especially in the erythrocytes [20]. This mechanism has never been fully explained; however, magnesium is considered an essential cofactor of GSH synthesis. Magnesium deficiency also intensifies production of ROS by phagocytic cells [19, 21, 22]. Thus, it is of interest to correlate Mg status and oxidative stress parameters [3, 4, 22]. A wide range of oxygen tolerance was observed among fish. Cold-adapted fish, like rainbow trout, usually need high oxygen levels, while cyprinid species can survive from nearly full anoxia to hyperoxia [22, 23]. We observed that 8-month-old rainbow trout had higher FRAP values than 4- and 6-month-old specimens. FRAP levels were lower when the dissolved oxygen content was higher. However, in contrast to our results, data from the literature indicate that FRAP in rainbow trout decreases with age [16, 17, 23, 24]. Riotola et al. [23, 25] showed that water over saturated with oxygen boosted antioxidant enzyme activities in the liver and gills of rainbow trout. According to Wdzieczak et al. [17] that fish have fluctuations in antioxidant enzyme activities, which

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**Figure 3.** Ferric reducing antioxidant power (FRAP) in selected tissues of rainbow trout: A) blood, B) liver, C) kidney, D) gills; significant differences in FRAP (p ≤ 0.01) in fish from different age groups: a) between 4- and 6-months-old, b) between 4- and 8-months-old, c) between 6- and 8-months-old (values are means ± SD).
could be due to high rates of free-radical generation. Thus, this discrepancy between our results and those from the literature could be explained by differences in water temperature and dissolved oxygen content.

In conclusion, our work provides interesting observations of the evolution with age of Mg status and oxidative stress parameters in trout. It could be hypothesized that an adequate Mg status may help to prevent oxidative damage during specific periods of trout life. This needs to be evaluated in future works.

Acknowledgments

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References


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**Figure 4.** The degree of lipid peroxidation (MDA) in selected tissues of rainbow trout: **A**) blood, **B**) liver, **C**) kidney, **D**) gills; significant differences in MDA concentrations (p ≤ 0.01) in fish from different age groups: a) between 4- and 6-months-old, b) between 4- and 8-months-old, c) between 6- and 8-months-old (values are means ± SD).


