The effects of dietary cadmium on growth, antioxidant defence system and feed evaluation performance of rainbow trout (*Oncorhynchus mykiss*)

Ayşe GÜNDOĞDU¹, Fatma Burcu HARMANTEPE²

ABSTRACT

The present study was carried out to determine the effect of dietary cadmium exposure on growth performance, changes in manganese, zinc, copper, calcium, magnesium, iron, selenium, and cadmium metals in liver and muscle tissue, liver antioxidant enzymes, and the histology of the fish. Rainbow trout (*Oncorhynchus mykiss*) with weights of 39.45 ±1.13 g was used in the experiment conducted in 2 groups and three replicates. The Control group was fed a cadmium-free diet, and the Cadmium group was fed a diet containing 5.03 µg/kg of cadmium twice a day until satiation. It was determined that cadmium intake through the diet affected growth rate and the feed evaluation performance negatively. In fish exposed to cadmium, manganese, zinc, copper, calcium, magnesium, iron, selenium, superoxide dismutase, catalase and glutathione peroxidase values in both muscle and liver tissues were significantly (p<0.05) decreased, whereas cadmium levels in muscle and liver and malondialdehyde levels in liver were significantly (p<0.05) increased. The histopathological examination of the liver revealed that cadmium caused liver damage. These results showed that rainbow trout exposed to dietary cadmium were highly sensitive to the metal, and the decreased levels of metals such as copper, zinc, manganese, and selenium in the liver tissue, which are involved in the antioxidant defence system, can be considered an indicator of the weakening of the antioxidant defence system.

**Keywords:** *Oncorhynchus mykiss*, Cadmium, Feed, Growth, Antioxidant defense system, Histology

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Introduction

Cadmium (Cd) is a metal that does not have any biological function in living organisms (van Dyk et al., 2007) and is also a metal in the group of heavy metals that cause pathogenic and biochemical (enzymatic) changes (Liu et al., 2011; Noor et al., 2020). Cd enters the bloodstream through the respiratory gills and digestive system, reaching all tissues and organs (Squadro et al., 2013). Many factors, such as the concentration of the metal, contact time, fish species, size, and physiological properties of the water, as well as the route of entry of the metal into the body, are effective in the accumulation and distribution of the metal in the tissues of fish (Dang & Wang, 2009; Gündoğdu et al., 2009; Yeşilbudak & Erdem, 2014; Li et al., 2018; Shekh et al., 2018; Özen & Pak, 2020; Varol et al., 2022; Kaçar, 2024). Entering the body through the digestive system, Cd is absorbed from the stomach and small intestine (Ojo & Wood, 2007). The blood is transported to organs by binding to metallothioneins (MT), albumin, and proteins with thiol groups (Thevenod, 2003). Cd is mostly stored in the metabolically active liver and kidney, bound to MTs and thiol groups of proteins (Kargın, 1996). MTs are fish's primary Cd-binding and storage proteins (Mceer et al., 2012; Genchi et al., 2020). Liver MT protein synthesis results in a high accumulation of Cd in the liver due to MT's rich thiol group content (Gündoğdu et al., 2009).

Oxidative stress is a condition that occurs due to the decrease in antioxidant enzymes in parallel with the increase in free radicals in the cell (Özcan et al., 2015). Following exposure to Cd, metals such as zinc (Zn), calcium (Ca), copper (Cu) and iron (Fe) in enzymes are replaced by Cd, causing the release of these metals. Increasing metal concentration accelerates the formation of free radicals. In addition, Cd binds to thiol and sulphydryl groups of enzymes that capture free radicals to inhibit antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX), increasing free radicals (Matović et al., 2015). Free oxygen radicals (ROS) oxidise PUFAs (polyunsaturated fatty acids) in cell membranes as a result of insufficient of the antioxidant system, leading to the disruption of the cell membrane structure, breaks in mitochondrial and nuclear DNA, as well as the breakdown of proteins and loss of enzymatic activity (Romero et al., 2011; Özcan et al., 2015). For this reason, the concentration of CAT, SOD, and GPX enzymes in fish exposed to heavy metals is measured in order to evaluate the condition of the antioxidant defence system, thereby determining the degree of toxicity (Talas et al., 2008; Almeida et al., 2009; Pan et al., 2018; Li et al., 2018).

Malondialdehyde (MDA) levels in tissues and blood are important oxidative stress and antioxidant capacity indicators. MDA is an end product of the oxidation reaction of PUFAs in cells. Increased MDA levels brought on by oxidative stress indicate that organs and tissues have been damaged. Therefore, in heavy metal toxicity studies conducted with fish, in addition to histological analyses on tissue samples obtained to determine the potential damage in tissues and organs resulting from exposure to heavy metals, MDA levels in tissues are examined as well (Pan et al., 2018).

It has been reported that information about oxidative stress and antioxidant enzyme activities can be obtained from the concentrations of specific metals in living tissues (Stephanie Fontagné-Dicharry et al., 2015; Eken, 2017). These metals include Fe, which mediates the formation of HO from H2O2 or lipid hydroperoxides via the Fenton reaction, and copper, Zn, manganese (Mn) and selenium (Se), cofactors for some antioxidant enzymes. Cu, Zn and Mn are cofactors of SOD enzyme and Se is a cofactor of GPX enzyme. These minerals are critical for the antioxidant defence system to function properly and serve many other essential purposes in living things, including fish. Examples include involvement in bone and tissue formation, cellular respiration, cellular homeostasis, immune system, oxygen transport, energy metabolism, electron transport, Ca absorption, fat, and carbohydrate metabolism (Lall & Kaushik, 2021).

Energy is produced in the mitochondria within the cell. Cd can accumulate in mitochondria, disrupting electron flow, affecting aerobic respiration, and reducing adenosine triphosphate (ATP) production (Genchi et al., 2020). Structural and functional alterations have been reported in the mitochondria of aquatic organisms exposed to Cd. Studies on fish have also reported that mitochondrial respiration decreases in parallel with increased Cd concentration (Dai et al., 2020; Qiu et al., 2021).

The growth performance of fish is an important parameter indicating its overall health. Therefore, when assessing Cd toxicity, growth rate must be taken into account in addition to Cd accumulation in tissues (Dang & Wang, 2009). Several studies concerning the toxicity of Cd also determined growth and feed evaluation parameters (Abdel-Tawwab & Wafeek, 2014; Li & Xie, 2019; Hu et al., 2022). The objective of this study was to assess the impact of dietary Cd on the growth of rainbow trout, feed evaluation performance, Cd accumulation in the tissues of the muscles and liver, the distribution of vital minerals (Fe, Cu, Zn, Mn, Se, and Ca), liver antioxidant enzyme activity, and liver histology.
Materials and Methods

Trial Planning and Sampling

The experiment was conducted at Sinop University, Faculty of Fisheries, Research and Application Center. Rainbow trout (Oncorhynchus mykiss) fry obtained from a private trout enterprise (Kuzey Su Ürünleri San. and Tic. LTD. STI) were used in the experiment. Rainbow trout fry, which did not contain Cd in their tissues, were used in the experiment. The fry used in the experiment was fed with commercial trout feed in stock tanks for 4 weeks to adapt to their new environment. After the acclimatisation period, the fish were placed in cylindrical fiberglass tanks with a capacity of 350 L. In each tank, 30 rainbow trout with an average weight of 39.45±1.13 g were placed (180 fish). Six tanks were used in the experiment set up in 2 groups and three replicates. The fish were fed twice daily (9:00–16:00) until they were satiated for 48 days during the 52-day experiment. Fish were starved for 24 hours before sampling. Three fish from each tank were sampled to determine the amount of cadmium accumulated in the liver and muscle tissue of the fish at the beginning and 12-day periods of the experiment, its metal composition, and also to determine the nutritional content of the fish meat at the beginning and end of the experiment. At the end of the experiment, three fish samples were taken from each tank for liver antioxidant enzyme parameters and histopathological examination of liver tissue. High doses of anaesthetic (400 mg/L, MS-222, Fluka, Sigma-Aldrich, USA) were administered to the fish during sampling. Water temperature, pH (WTW 3110 SET2), and O2 (YSI Multiparameter Professional Plus) values were 15.97 ±0.47°C, 8.41 ±0.02 and 8.14 ±0.12 mg/L, respectively.

The control group was fed with commercial pellet feed (Gümüşdoğa Feed), and the Cd group was fed with commercial pellet feed containing 5.03 mg/kg cadmium (Cd). For this purpose, cadmium chloride (CdCl2) was added to the pellet feed, the nutrient composition of which is given in Table 1. While preparing the feeds containing CdCl2, 00 mL/kg oil-water mixture (20 mL water + 80 mL sunflower oil to 1 kg feed) and 9.79 mg/kg CdCl2 were mixed into the spray bottle until CdCl2 dissolved in oil. The mixture was sprayed on the pellet feed. The feeds were mixed for 10 minutes to ensure a homogeneous distribution of CdCl2 in the pellet feed. To avoid any differences in the nutrient composition of the feeds, 100 mL/kg of an oil-water mixture was sprayed and combined with the feed of the control group using a spray bottle. The prepared feeds were packaged and kept at +4 °C until used.

<table>
<thead>
<tr>
<th>Nutrient composition</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Moist (%)</td>
<td>7.3</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>47.7</td>
</tr>
<tr>
<td>Crude lipid (%)</td>
<td>20.7</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>12.1</td>
</tr>
<tr>
<td>NFE + crude fibre</td>
<td>19.5</td>
</tr>
<tr>
<td>Cd (µg/kg)</td>
<td>5.03</td>
</tr>
<tr>
<td>Cu (mg/kg)</td>
<td>13.80</td>
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<tr>
<td>Zn (mg/kg)</td>
<td>163.57</td>
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<tr>
<td>Mn (mg/kg)</td>
<td>68.81</td>
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<tr>
<td>Se (mg/kg)</td>
<td>1.18</td>
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<tr>
<td>Fe (mg/kg)</td>
<td>258.48</td>
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<tr>
<td>Ca (mg/kg)</td>
<td>33947.89</td>
</tr>
<tr>
<td>Mg (mg/kg)</td>
<td>1846.38</td>
</tr>
</tbody>
</table>

Cd: Cadmium; Cu: Copper; Zn: Zinc; Mn: Manganese; Se: Selenium; Fe: Iron; Ca: Calcium; Mg: Magnesium

*Nitrogen free extract (NFE) + Crude fiber = 100 – (Crude protein + Crude lipid + ash)

Growth Performance

Condition Factor (CF), Specific Growth Rate (SGR), and Feed Conversion Ratio (FCR) were calculated using the formulas given below.

CF= (Fish weight/Cube of fish length) x 100
SGR, % = (Ln (Final weight)- Ln (Initial weight)/day) x 100
FCR = Feed consumption, g/ Weight gain, g

Nutrient Analysis

The AOAC (1995) (Association of Official Analytical Chemists) protocols were applied to determine the nutrient composition of the fish muscle and experimental diets. Crude protein (Nx6.25) was determined by the Kjeldahl method, dry matter content was determined by drying the samples at 105°C for 12 hours, crude ash was determined by incineration in a muffle furnace at 550°C for 12 hours, and crude lipid content was determined by ether-extraction using the Soxhlet system.

Metal Analysis

Metal concentrations of the samples were determined by an Inductively Coupled Plasma Mass Spectrophotometer (Agilent Technologies / 7700X ICP-MS Systems, Santa Clara, U.S.) using the EPA 200.3 method. Multi-element standard solutions supplied by Agilent (27-element mix: 8500-6940 2A and 8500-6940 Hg) were used for calibration curves. Agilent-supplied multi-element standard solutions (27-element...
Liver Lipid Peroxidation and Antioxidant Enzyme Analysis

After being rinsed with a physiological saline solution containing 0.59% NaCl, the liver tissue was stored at -80 °C until biochemical analyses were performed; then, it was diluted 1/10 (w/v) in 0.1M sodium-phosphate buffer containing 1.17% KCl (pH 7.4) and homogenised on ice for 4 min (Ultra Turax T-18). After centrifugation at 16000 rpm for 10 min at +4 °C, supernatants were obtained (Nuve NF 800R).

Superoxide Dismutase (SOD) values were determined by the method developed by Sun et al. (1988), and Catalase Activity (CAT) was determined according to the method of Paglia & Valentine (1967). Total protein levels of tissue supernatants were determined by Lowry's method (Lowry et al., 1951), and Malondialdehyde (MDA) levels were determined according to the method of Ohikawa et al. (1979).

Histopathological Analysis

Histopathological analysis was performed as described in Dai et al. (2020). Liver samples fixed in 10% neutral buffered formaldehyde were dehydrated in ethanol. The dehydrated tissues were washed with xylene, embedded in paraffin, and cut into 5 μm sections. Sections were stained with hematoxylin and eosin, and the stained preparations were observed under a light microscope.

Statistical Analysis

Growth, feed evaluation parameters, nutrient composition of fish meat, lipid peroxidation and antioxidant enzyme activities in liver tissue were tested by T-test, Cu, Zn, Mn, Se, Fe, Ca, and Mg concentrations in muscle and liver tissues were tested by a two-way ANOVA. A one-way ANOVA tested muscle and liver Cd concentrations—Tukey’s test assessed group differences in cases where metal concentrations in liver and muscle tissues differed significantly. Statistical analyses were executed using the SPSS Statistics 26 software. The values obtained were expressed as mean ± standard deviation, and differences below p<0.05 were considered significant.

Results and Discussion

Growth and feed evaluation data are given in Table 2. The end-of-trial weight, CF, and SGR of rainbow trout-fed Cd-containing feed were significantly (p<0.05) lower than those of the control group. The best FCR values were found in the control group (p<0.05).

Table 2. Growth and feed utilisation

<table>
<thead>
<tr>
<th></th>
<th>Initial</th>
<th>Control</th>
<th>Cd Group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial CF</td>
<td>1.13 ±1.16</td>
<td>91.36 ±4.56a</td>
<td>79.31 ±4.51b</td>
<td>0.009</td>
</tr>
<tr>
<td>FW</td>
<td>1.40 ±0.03a</td>
<td>1.36 ±0.07b</td>
<td>1.46 ±0.04b</td>
<td>0.000</td>
</tr>
<tr>
<td>SGR</td>
<td>1.74 ±0.01a</td>
<td>1.46 ±0.04b</td>
<td>1.07 ±0.10a</td>
<td>0.047</td>
</tr>
<tr>
<td>FCR</td>
<td>0.92 ±0.13b</td>
<td>1.07 ±0.10a</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CF: Conditional factor; FW: Final weight; SGR: Specific growth rate; FCR: Feed conversion ratio. Values represent the mean and standard deviation. Different superscript letters indicate the significant differences (P<0.05) between the treatments.

In studies conducted with copper, a heavy metal, negative effects on growth and feed evaluation have been determined. In one of these studies, Gündoğdu et al. (2009) recorded a decline in growth and feed evaluation parameters in rainbow trout (Oncorhynchus mykiss) fed with Cu-added feed compared to the control group. According to Ali et al. (2003), when the copper concentration in the water increased, the Nile tilapia (Oreochromis niloticus) growth parameters and feed intake significantly decreased. This decrease in growth was associated with a decrease in food intake. It was further discovered that copper exposure slowed muscle activity while speeding up the metabolism in the liver and gills. According to reports, the decline in muscle activity may have been a reaction to a decrease in energy demand, and the metal may have impacted the nervous system of the fish. Furthermore, it was reported that because of the linear relationship between growth and metabolic rate, changes in growth rate are expected to cause changes in the metabolism as well. In a similar study, Giacomini et al. (2018) reported a decrease in
feed intake or feed evaluation rate following exposure to Cd, bringing the possibility that Cd may reduce metabolism or directly affect brain hormones that regulate hunger and satiety in addition to possibly impacting the palatability of the diet.

Deterioration in feed evaluation ratio means lower body weight is attained with the consumed feed. The deterioration detected in feed evaluation raises the first concern regarding nutrient absorption from the feed. For the nutrients to be processed by the fish body (energy production, synthesis of new tissues, etc.), the digested nutrients must pass through the intestines and enter the bloodstream, reaching the cell and its organelles. Transmission is impossible in case of structural defects in the intestines or within cells and organelles, particularly in the cell membrane. Previous studies have observed tissue damage following metal exposure (Liu et al., 2011; Pan et al., 2018; Noor et al., 2020). PUFAs, which make the cell membrane permeable, are known to oxidise and interfere with cell permeability (Özcan et al., 2015). The inability to utilise nutrients as they enter the cell and mitochondria leads to a failure to adequately produce the energy required for growth, development, and survival. Moreover, the energy generated may have been used to produce antioxidant defence enzymes (SOD, CAT, GPx) to neutralise ROS released after exposure to the metal and MTs, which enable the metal to be eliminated from the body. These processes could also be contributing factors to the decline in growth.

Table 3 shows the nutrient composition of fish meat. At the end of the experiment, there was no significant difference (p>0.05) in dry matter, crude protein, and crude ash values, but lipid content in fish meat was determined to be higher (p<0.05) in the Cd-treated group in comparison to the control group. Similar to this study, Liu et al. (2011) reported that the amount of lipids in the total body and liver tissue increased with Cd exposure, and the reason for this increase was impaired lipid metabolism following Cd exposure.

Heavy metal concentrations in fish tissues are known to increase with length of exposure (Gündoğdu et al., 2009; Yeşilbudak & Erdem, 2014; Özen & Pak, 2020). Through their gills and gastrointestinal openings, fish absorb Cd from their food and the water environment they live in. Cd is taken into the body and spreads to different organs and tissues (Squadro et al., 2013; Li et al., 2018). Cd is accumulated mostly in liver and kidney tissue in fish (Bustamante et al., 2003; Cirillo et al., 2012; Mashroofeh et al., 2013; Squadro et al., 2013; Li et al., 2018). Most recent studies have focused on determining the effects of heavy metals incorporated into the aquatic habitat (Abdel Tawwab & Wafeek, 2014; Li et al., 2018). Fewer studies have been conducted on the accumulation rate of heavy metals from dietary intake in tissues, along with fish's growth and feed evaluation performance. This study demonstrated a significant (p<0.05) increase in Cd concentrations in muscle and liver tissues of rainbow trout when exposed to Cd through dietary intake for 48 days (Table 4). The highest Cd concentration was detected in liver tissue. In previous studies conducted with rainbow trout and other fish species, Cd concentration in liver tissue was higher than in muscle tissue in all sampling periods (Romeo et al., 2000; Kondera et al., 2014; Özen & Pak, 2020). The higher accumulation of Cd in the liver can be explained by the metabolically active nature of the liver, the presence of MTs in the liver and the binding of Cd to MTs (Vallee, 1995; Thevenod, 2003). During the experiment, the control group showed no Cd in either muscle or liver tissue.

Table 3. Proximate composition of fish muscle

<table>
<thead>
<tr>
<th></th>
<th>Initial</th>
<th>Control</th>
<th>Cd Group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Matter</td>
<td>25.12</td>
<td>26.17 ±0.10a</td>
<td>25.99 ±0.16a</td>
<td>0.251</td>
</tr>
<tr>
<td>Crude protein</td>
<td>20.42</td>
<td>18.43 ±0.41a</td>
<td>18.28 ±0.20a</td>
<td>0.341</td>
</tr>
<tr>
<td>Crude lipid</td>
<td>2.49</td>
<td>5.05 ±0.18a</td>
<td>4.81 ±0.28b</td>
<td>0.045</td>
</tr>
<tr>
<td>Ash</td>
<td>2.21</td>
<td>2.69 ±0.10a</td>
<td>2.89 ±0.11a</td>
<td>0.184</td>
</tr>
</tbody>
</table>

Values represent the mean and standard deviation. Different superscript letters indicate the significant differences (P<0.05) between the treatments.
A decrease in Cu, Zn, Mn, Se, Fe, Ca and Mg values in the muscle and liver tissues of the Cd group was recorded in the experiment (Table 4). Cu concentration in the muscle tissue of the Cd group was significantly lower (p<0.05) at all periods compared to the control group. In comparison, the Zn level in the muscle tissue of the control group showed a significant difference (p<0.05) on the 48th day, while the Zn level in the liver tissue was found to be the highest (p<0.05) beginning on the 30th day. Mn level in muscle tissue showed a significant (p<0.05) decrease from day 24, while Mn level in liver tissue significantly (p<0.05) decreased from day 36 compared to the control group. Se level in the muscle tissue did not change over time in the control group, whereas it showed a significant (p<0.05) decrease in the Cd group compared to the control group. Although Se levels in liver tissue decreased with time, a significant (p<0.05) difference was detected at day 36. The Fe level in muscle tissue started to decrease on the 12th day (p<0.05), and the lowest Fe value was obtained on the 48th day in the Cd-fed groups compared to the control group. The Fe level in the liver tissue did not change with time in the control group, while a significant decrease (p<0.05) was detected in the Cd group at the 24th sampling period, and the lowest Fe value was detected on the 48th day. The Ca content in muscle tissue showed a significant decrease (p<0.05) starting from the 24th sampling period in comparison to the control group, while the significant (p<0.05) decrease in liver tissue started at the 12th sampling period and the lowest Ca value was determined on the 48th day. Mg levels in muscle tissue were significantly lower (p<0.05) in the Cd group compared to the control group in all periods. While liver Mg concentration increased with time (p<0.05) in the control group, it was found to be significantly (p<0.05) lower and showed a decrease (p<0.05) with time (p<0.05) in the Cd-fed group compared to the control group.

Table 4. Metal composition of muscle and liver of rainbow trout (units μg/kg ww for Cd, mg/kg ww for other metals)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Cd</th>
<th>Cu</th>
<th>Zn</th>
<th>Mn</th>
<th>Se</th>
<th>Fe</th>
<th>Ca</th>
<th>Mg</th>
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<tr>
<td><strong>MUSCLE</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>12. day</td>
<td>0.64±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.57±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.38±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.66±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.99±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>379.38±4.77&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>350.32±3.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24. day</td>
<td>0.69±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.15±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.35±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.70±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.08±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>398.27±3.77&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>381.43±1.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
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<tr>
<td>36. day</td>
<td>0.63±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.99±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.35±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.70±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.28±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>361.32±3.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>365.37±2.57&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>48. day</td>
<td>0.80±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.11±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.40±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.69±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.76±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>391.56±2.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>350.98±3.45&lt;sup&gt;b&lt;/sup&gt;</td>
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**Cd Group**

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<tr>
<th></th>
<th>Control</th>
<th>Cd</th>
<th>Cu</th>
<th>Zn</th>
<th>Mn</th>
<th>Se</th>
<th>Fe</th>
<th>Ca</th>
<th>Mg</th>
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<tbody>
<tr>
<td>12. day</td>
<td>3.20±0.15&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.54±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.78±0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.34±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.47±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.08±0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>385.47±5.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>326.91±1.38&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>24. day</td>
<td>5.90±0.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.39±0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.08±0.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.19±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.42±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.24±0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>335.98±6.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>309.14±8.69&lt;sup&gt;d&lt;/sup&gt;</td>
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</tr>
<tr>
<td>36. day</td>
<td>10.03±0.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.40±0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.79±0.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.19±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.39±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.25±0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>282.11±2.92&lt;sup&gt;d&lt;/sup&gt;</td>
<td>291.83±3.66&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>48. day</td>
<td>14.23±0.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.39±0.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.36±0.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.15±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.39±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.03±0.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>120.62±17.30&lt;sup&gt;e&lt;/sup&gt;</td>
<td>258.80±6.48&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

**P Value**

<table>
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<tr>
<th></th>
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<th>Group</th>
<th>Time X Group</th>
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<tr>
<td>Control</td>
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<td>0.000</td>
</tr>
<tr>
<td>LIVER</td>
<td>0.000</td>
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<td>0.000</td>
</tr>
<tr>
<td>Time X Group</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Values represent the mean and standard deviation. Different superscript letters indicate the significant differences (P<0.05) between the treatments.
In previous studies conducted with fish species other than rainbow trout, decreases in Cu, Zn, Mn, Se, Fe, Ca, and Mg values in fish tissues were detected at various times following exposure to Cd. For example, Castaldo et al. (2020) reported that Mg content in the liver of common carp (Cyprinus carpio) decreased at the end of 1 week following exposure to a mixture of Cu, Zn, Cd metal, while Mg content in the muscle did not show any change. Paul et al. (2021) did not detect a decrease in Ca, Cu, Fe and Zn values in muscle, liver, and kidney tissues of channel catfish (Ictalurus punctatus) after exposure to Cd in their sampling at three months, while they detected a decrease in Ca, Cu, Fe and Zn values in muscle and liver tissues at six months. Liu et al. (2021) reported that Na content decreased in the carcass of catfish (Silurus meridionalis) following exposure to Cd, while Ca content was not affected by Cd exposure. According to the study results, the type of fish could significantly impact the duration of the decline in metal levels in tissues. Hence, Sheikh et al. (2018) reported in their study conducted with rainbow trout and sturgeon that life stage and fish species were important factors influencing Cd uptake, while trout were more Cd sensitive than sturgeon. The same study also reported that Cd intake significantly decreased the total body Ca in trout but did not have the same effect in sturgeon. Previous studies on fish have found that gastrointestinal uptake of Cd occurs in all parts of the stomach and intestines (Ojo & Wood, 2007; Megeer et al., 2012). Kwong et al. (2010) reported in their studies with trout that Cd follows the same pathway as Fe uptake in the intestine, leading Cd to interfere with Fe uptake.

Free radicals cause lipid peroxidation (Nam, 2011). Although Cd does not directly produce ROS, it indirectly causes the production of free radicals by affecting the mitochondrial electron transfer chain or increasing glutathione consumption (Romero et al., 2011). Aldehydes formed by lipid peroxidation in the presence of free radicals form cross-links with proteins, nucleic acids and lipids. MDA is the most important aldehyde among the degradation products of lipid peroxides (Ahmed et al., 2024). MDA is a widely used indicator of oxidative damage in cells and tissues (Zengin, 2018). In the current study, a significant (p<0.05) increase (Table 5) was detected in the MDA value in samples taken from the liver tissue of rainbow trout exposed to Cd in comparison to the control group. Another study with rainbow trout reported that MDA value in the liver increased after exposure to Cd (2 ppm) for one week (Talas et al., 2008). According to one of the studies conducted with different fish species, Li et al. (2018) reported that exposure to Cd (62.5, 125, 250 and 500 µg Cd/L) for 56 days caused an increase in the MDA value in the tissues of southern catfish (Silurus meridionalis) and the highest MDA value was detected in the liver, Hu et al. 2022 reported that exposure of zebrafish to Cd (5, 10, 20 Microg/L) for 48 days increased the MDA value. Similar increases were reported in studies where the experimental period was kept shorter. Souid et al. (2013) reported a significant increase in MDA levels in the liver of sea bream (Sparus auratus) exposed to Cd (0.5 mg/L) for 24 hours, while Zheng et al. (2016) reported that MDA value increased in zebrafish (Danio rerio) following exposure to Cd (1 mg/L) for 96 hours. Examining previous studies, the MDA value increased in all conditions independent of Cd doses, metal exposure method and metal exposure time. This indicates an acceleration of lipid peroxidation by Cd in a very short time.

| Table 5. Lipid peroxidation and antioxidant defence system enzymes |
|----------------------|-----------------|-----------------|-----------------|-----------------|
|                     | MDA (nmol/mg protein) | SOD (U/mg protein) | CAT (U/mg protein) | GPx (U/mg protein) |
| Control             | 0.52 ±0.04 b     | 22.32 ±0.32a     | 30.87 ±0.29a     | 20.98 ±0.56a     |
| Cd Group            | 10.02 ±0.15a     | 12.21 ±0.29b     | 4.986 ±0.15b     | 15.06 ±0.23b     |
| P value             | 0.009            | 0.037            | 0.002            | 0.009            |

Values represent the mean and standard deviation. Different superscript letters indicate the significant differences (P<0.05) between the treatments.

Fish have an antioxidant system that prevents cell damage caused by ROS following exposure to heavy metals. It is known that ROS are balanced by enzymatic (SOD, CAT, GPx) and non-enzymatic (GSH, C vit, Se) antioxidant barriers (Genchi et al., 2020). The findings of this study revealed that Cd significantly (p<0.05) decreased the activity of SOD, CAT and GPx enzymes and decreased the antioxidant capacity compared to the control group (Table 5). Similar to the results of this study, Talas et al. (2008) found that CAT, SOD and GPx activities decreased in rainbow trout (Oncorhynchus mykiss) after one week. The study conducted by Pan et al. (2018) showed that Cd decreased SOD and GPx activity in zebrafish at the end of 30 days, Li et al. (2018) found that SOD activity decreased in southern catfish (Silurus meridionalis) at the end of 56 days, while CAT activity remained constant, Souid et al. (2013) reported that CAT activity increased at the end of 24 hours, SOD activity peaked at the 4th
hour. It decreased at the 24th hour in Sea bream (*Sparus auratus*). Dabas et al. (2012) reported that in Freshwater mussels (*Channa punctatus*) exposed to Cd (6.7, 13.4 and 20.1 mg/L) for 96 hours, GPx activity increased at all concentrations, SOD activity increased depending on concentration and time, while the highest CAT activity was detected in the liver.

Considering the results of this study along with the results of the study conducted by Talas et al. (2008), the determination of decreases in SOD, CAT and GPx values in weeks compared to other fish species suggests that the trout has a lower antioxidant defence system capacity. A low antioxidant defence system may be related to the genetic structure of rainbow trout. Rainbow trout may have a less developed antioxidant defence system against pollutants like heavy metals because of their capacity to live and breed in clean waters.

Due to liver tissue damage, the liver's detoxification ability is significantly reduced (Liu et al., 2022). Histopathological evaluation of the liver sections examined under a light microscope revealed that the liver sections in the control group had a normal histologic structure. Hepatocytes around the central veins were found to have one or two large nuclei, morphologically smooth contours, normal sinusoidal spaces, and no pathologic lesions (Figure 1a). In the liver sections of Cd-treated fish, it was determined that there were necrotic areas throughout the section, mainly around the central vein, numerous foci of mononuclear cell infiltration, enlargement of sinusoids and vacuolar degenerations in hepatocytes (Figure 1b, c, d). Similar to the results of this study, van Dyk et al. (2007) observed hyalinisation, congestion of blood vessels, increased vacuolation associated with lipid accumulation, and cellular swelling in the liver of Tilapia (*Oreochromis mossambicus*) exposed to Cd and Zn metals, while Dai et al. (2020) observed hydropic degeneration and necrosis in the liver of Procypris merus, a carp species, and Noor et al. (2020) detected lesion and sinusoids in the liver cells of goldfish (*Carassius auratus*) exposed to Cd through their diet and water environment. The results from the studies indicate that exposure to Cd through their aquatic environment and diet causes liver damage in fish.

**Figure 1.** Light microscopic image of liver sections of fish in control and cadmium groups (a: control group, b, c, d: cadmium groups). Black arrow: mononuclear cell infiltration, red arrow: sinusoidal expansion, star: necrotic area, triangle: vacuolar degeneration. Hematoxylin-Eosin, x40
Conclusion

In the present study, dietary exposure of rainbow trout to Cd caused decreased growth and feed evaluation performance, decreased Se in the non-enzymatic antioxidant system of fish, decreased Mn, Zn, Cu as cofactors in the structure of antioxidant enzymes and Ca, Mg, Fe in biochemical reactions, decreased liver SOD, CAT and GPx activities and liver damage. All of these findings demonstrated that trout are highly sensitive to Cd toxicity. The decrease in Mn, Zn, Cu and Se metals, in parallel with the decrease in liver enzyme activity, showed that the amount of these metals could also be used as biomarkers when evaluating the antioxidant defence system capacity of the rainbow trout.

Compliance with Ethical Standards

Conflict of interest: The authors declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: This study was conducted following the ethical protocol (2020/03) of Sinop University Animal Experiments Control Council.

Data availability: Data will be made available on request.

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Disclosure: -

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https://doi.org/10.1016/S1546-5098(11)31025-4


