Elimination of copper in tissues and organs of rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) following dietary exposure

Ayse Gündogdu, 1 F. Burcu Harmantepe, 2 Zafer Karslı, 1 Gaye Dogan 1
1 Fisheries Faculty, Sinop University, Turkey
2 Yumurtalık Vocational High School, Çukurova University, Adana, Turkey

Abstract

Copper (Cu) elimination was investigated in the tissue and organs of rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792), after Cu-free diets exposure. In the current study, fish were fed to satiation on diets containing 0.022 (Group 1; Control), 0.043 (Group 2), 0.123 (Group 3), 0.424 (Group 4) g Cu*kg*–1* diet for 60 days before elimination experiment. A total of 288 fish (mean weight 84.28±1.05 g) were randomly transferred to 12 fibreglass tanks. The fish were fed the Cu-free diet twice daily, until apparent satiation, during 60 days. Subsequently, the experiment was established for a period of elimination, during which samples were taken at days 15, 30, 45 and 60. Cu concentration in the muscle, gill tissue, digestive system, liver and whole body of fish were determined after 60 days depuration.

Cu concentrations in tissues of rainbow trout decreased during depuration period, and the order of Cu elimination in tissue and organs of rainbow trout was: digestive system (73.1 %), then gill (41.1 %), muscle (31.5 %) and liver (17.2 %) for group 2; digestive system (74.1%), then muscle (65.8%), gill (60.0%) and liver (34.6%) for group 3; and digestive system (85.8%), then muscle (80.8%), liver (50.5%) and less/equal in gill (56.2%) for group 4. In statistical analysis, both groups and time were significant factors (P<0.05) on elimination rate. Moreover, significant interaction between groups and time were identified on elimination rate. Digestive system showed the fastest elimination rates of Cu at all groups compared with other tissues.

Introduction

Copper (Cu) is an essential metal in cellular metabolism (Cousins, 1985) but also potentially highly toxic to fish (Cusimano et al., 1986; Grossel et al., 1997). Despite of its essentiality, Cu requirements differ among species and even within different life stages of a single species (Clearwater et al., 2002). Cu is also a very potent toxicant when allowed to accumulate in excess of cellular needs (Harris, 1991; Pena et al., 1999; Kamunde et al., 2002; Shiu and Ning, 2003; Bielmyer et al., 2005). Toxicity to Cu as well as other trace elements depends on species, age and diet, a reflection of variation in efficiency of absorption (Uauy et al., 1998; Campbell et al., 2005; Hoyle et al., 2007). Metal effects in aquatic organisms are considered important issues in the evaluation of possible risk of environmental contamination. Usually, the first measurable changes due to contaminant exposure in the aquatic organisms are biochemical responses known as cellular and histological biomarkers (Hinton et al., 1992, Marijic and Raspor, 2007). Heavy metals are generally accumulated in fish metabolic active organs in higher concentrations than in other tissues and organs (Karakoc and Kargin, 1999; Berntssen et al., 1999; Gündogdu et al., 2009).

Metal elimination studies are important for health protection, allowing the determination of the self-cleansing ability of contaminated organisms (Kim et al., 2006). Several factors influence the elimination of metals from the fish tissues. These include time, temperature, interacting agents, age and metabolic activity of fish, and the biological half life of the metal (Douben, 1989; Heath, 1995; Kargin, 1996; Kim et al., 2006). Bremmer et al. (2005) study was related on how the sub-cellular partitioning of metals changed in liver during the depuration phase, what excess Cu rate associated with this subcellular fraction would decrease during the depuration phase, and examined elimination kinetics in individual organs. Lanno et al. (1987) suggested that the storage of Cu within these granules might be important in the elimination of excess cellular Cu via bile.

Industrial activity often results in increased disposal of toxic trace metals into the environment. Whatever the source of uptake, fish accumulate Cu in their tissues. Fish are an important food resource and a major ecosystem component, making therefore important to assess the effect of Cu in fish (Larsson et al., 1985; Kim et al., 2006). The rainbow trout is an economically important food fish in Turkey, cultured in cages and pool. Despite its importance, relatively little information is available on the effect of Cu, especially through dietary exposure. Therefore, the aim of present study was to investigate Cu elimination in rainbow trout of dietary Cu exposure.

Materials and methods

Experimental design and test diet

Rainbow trout (*Oncorhynchus mykiss*) were obtained from a local fish farm (Karacasu Trout Farm, Sinop, Turkey). Fish were fed to satiation on diets containing 0.022 (Group 1; control), 0.043 (Group 2), 0.123 (Group 3), 0.424 (Group 4) g Cu*kg*–1* diet for 60 days before elimination experiment started. After 60 days, 72 fish were selected from each Cu-diet group (0.022, 0.043, 0.123, 0.424 g Cu*kg–1* diet) and 288 fish in total were randomly transferred to 12 fiberglass tanks (250 L) with triplicate groups of 24 fish each. The fish used in the Cu elimination experiment had an average weight of 84.28±1.05 g. The water used for the experiment had an average temperature of 16.99±0.11°C, O2 of 7.08±0.12 mgL–1, pH of 7.42±0.12, NO3*–* of 1.43±0.08 mgL–1, NO2*–* of 0.11±0.07 mgL–1, NH3 of 0.14±0.07 mgL–1, PO43*–* of 0.71±0.04 mgL–1 and Cu of 0.5±0.14 μgL–1.

The fish were fed the Cu-free diet twice daily, until apparent satiation, for 60 days. The formulation of the Cu-free diet is shown in Table 1. All dry ingredients were well mixed for 15 min to prepare diets, then fish oil was added. The diet were pelleted after adding approximately 35% water pelleted, then dried at 70°C to obtain approximately 90% dry mat-
paper with a pore size of 0.45 μm. The samples were analyzed by atomic absorption spectrophotometry (An air-acetylene flame atomic absorption spectrophotometer: FAAS UNICAM Model 929) using the method described by Bernhard (1976). The data were presented in μg of sample dry weight (DW). Chemical analyses of diets and fish muscle tissue were done according to AOAC (1990) guidelines as follows: dry matter after drying in oven at 105 oC for 24 h until constant weight, protein (Nx6.25) by the Kjeldahl method after acid digestion, lipids by ethyl ether extraction in a Soxhlet System, ash by incineration in a muffle furnace at 550°C for 12 h, while nitrogen free extract was calculated by difference. All analyses were conducted in triplicate.

Results

Growth and nutritional performance

Table 2 presents the initial-final weight, mean weight gain (WG), SGR, FCR, PER, and percent survival rate of rainbow trout. No mortality was recorded during the experiment. The overall performance of trout was good during the whole period. The best FCR and PER were obtained from group 4, but there was no significant difference (P<0.05) in WG, SGR, FCR and PER.

Table 2 presents FCR of rainbow trout fed the Cu-free diet over 60 days. Time was a significant factor (P<0.05) on FCR, which increased with increasing time. However, no significant difference was found in FCR among groups in each period. The best FCR was obtained at the 15-day point in all group. As shown in Table 3, no significant interaction in both groups and time was identified in FCR of rainbow trout fed the Cu-free diet.

The lowest nitrogen (N) intake and the highest N accumulation and retention were obtained from group 4, which includes the highest rate of Cu in tissue and organs. The highest lipid (L) intake and lipid loading, and the lowest L retention were obtained from group 2. However, there was no significant difference (P>0.05) in N intake, N accumulation, N loading, N retention, L intake, L accumulation, L loading and L retention (Table 4).

As shown in Table 5, time was a significant factors among groups using the Bartlett test. The data were subjected to one and two-way analysis of variance (ANOVA) to test the effects of accumulation and growth performance. If significant (P<0.05) differences were found, Tukeys range test was used to rank the groups using the SPSS program Version 11.5 for Windows.

Table 1. Ingredient composition and proximate analysis of the experimental diet.

| Ingredients | Diet |
|-------------|------|
| Fish meal, g kg⁻¹ | 390 |
| Soybean protein, g kg⁻¹ | 100 |
| Soybean meal, g kg⁻¹ | 180 |
| Fish oil, g kg⁻¹ | 120 |
| Semolina flour, g kg⁻¹ | 206.5 |
| Vitamin premix°, g kg⁻¹ | 2 |
| Mineral premix, g kg⁻¹ | 1.5 |

Proximate composition

| Moisture, g kg⁻¹ as fed | 78 |
| Crude protein, g kg⁻¹ DM | 463 |
| Crude lipid, g kg⁻¹ DM | 222 |
| Crude ash, g kg⁻¹ DM | 71 |
| Nitrogen-free extracts, g kg⁻¹ DM | 244 |
| Cu, mg/g DM | 0.022 |

Vitamin premix (mg or U/kg of diet): vitamin A, 6250 U; vitamin D₃, 250 U; vitamin E, 100 mg; vitamin K₃, 5 mg; vitamin B₆, 7.5 mg; vitamin B₁₂, 12.5 mg; niacin, 100 mg; Calcium pantothenate, 12 mg; vitamin B₆, 10 mg; vitamin B₁₂, 0.01 mg; folic acid, 4 mg; vitamin C, 185 mg; inositol, 100 mg; d-biotin, 0.25 mg. *Mineral premix (mg kg⁻¹ of diet): Mn, 10 mg; Zn, 37.5 mg; Cu, 25 mg; Cobalt, 2.5 mg; I, 1.5 mg; Se, 0.15 mg.

Table 2. Growth performance and feed utilization by rainbow trout fed Cu-free diet over 60 days°.

| Group | 1 (Control) | 2 | 3 | 4 |
|-------|-------------|---|---|---|
| Initial weight, g | 84.42±0.956 | 82.05±0.849 | 85.13±1.408 | 81.22±1.713 |
| Final weight, g | 228.86±3.506 | 226.66±3.195 | 216.53±5.640 | 219.85±6.721 |
| Weight gain, %/day | 171.25±7.164 | 171.52±6.711 | 154.4±7.994 | 171.20±13.247 |
| Specific growth rate, %/day | 1.66±0.045 | 1.66±0.041 | 1.56±0.052 | 1.66±0.084 |
| Feed conversion ratio | 1.16±0.072 | 1.17±0.065 | 1.11±0.072 | 1.09±0.081 |
| Protein efficiency ratio | 1.87±0.040 | 1.86±0.061 | 1.95±0.019 | 1.99±0.056 |
| Survival, % | 100 | 100 | 100 | 100 |

Values are means of triplicate groups±SE. ANOVA results were performed by transformation method (arcsin).
factor (P<0.05) on dry matter, crude protein, crude lipid, crude ash. Muscle dry matter and crude lipid were significantly increased with increasing time. The highest dry matter and total lipid contents were observed at the 60-day point. However, no significant difference was found in dry matter and crude lipid content of fish among groups. Muscle crude protein and crude ash were significantly (P<0.05) decreased with increasing time. The lowest crude protein and crude ash content were observed in the 60-day point. Muscle crude protein and crude ash were not affected by Cu in tissue and organs of fish.

**Target organs of dietborne copper**

At the beginning of the experiment, Cu accumulation was evaluated over the 60-day exposure period in the tissues of rainbow trout after dietary Cu exposure (0.022, 0.043, 0.123, 0.424 g Cu kg⁻¹). Then, Cu elimination was investigated during the 60-day depuration period in muscle, gill, digestive system, liver and whole body. Experimental periods of 15, 30 and 60 days were determined. Elimination rate (between the values after 60 days of accumulation (0-day) and values after the 60-day elimination period) are shown in the Table 6. Copper concentrations in tissues decreased sharply varying (P<0.05) decreased with increasing time. The lowest crude protein and crude ash content were identified on elimination rate during the depuration period. As shown in Table 6, both groups and time were significantly factors (P<0.05) on elimination rate. On the other hand, significant interaction among groups and time was identified on elimination rate. Elimination of metal level in all tissues and organs was depending on time and decrease of Cu accumulation.

After dietary exposure, fish fed low dose diets only in the whole body had the lowest elimination rate during the depuration period. The elimination rates at the end of depuration periods were 0.55% for group 1 (Control; 45-day), 35.98% for group 2, 21.4% for group 3 and 48.26% for group 4 (60-day) diet group (Table 6). Intestine showed fastest elimination rates of Cu at all concentrations compared to the muscle, gill, digestive system, liver and whole body (Table 6). During 60 days of depuration, Cu concentrations decreased sharply varying from 3.64±0.13 to 0.98±0.06 µg g⁻¹ (elimination rate: 73.1%), from 4.49±0.05 to 1.16±0.01 µg g⁻¹ (74.1%), and from 8.77±0.74 to 1.25±0.06 µg g⁻¹ (85.8%) at group 2, group 3 and group 4 Cu diet groups compared to the Cu concentration, respectively. At the end of the depuration period, the Cu concentration was similar to the one in the control.

Liver showed lowest elimination rates of Cu

**Table 3. Feed conversion ratio of rainbow trout fed Cu-free diet over 60 days**

| Group | 1 (Control) | 2 | 3 | 4 |
|-------|-------------|---|---|---|
| 15 days | 0.97±0.06⁶ᵇ | 1.01±0.08⁵³ᶜ | 0.89±0.10²ᵃ | 0.91±0.02⁵ᵇ |
| 30 days | 1.11±0.04¹ᵇ | 1.16±0.02²ᵈ | 1.12±0.06⁵ᵇ | 0.97±0.00⁸ᵇ |
| 45 days | 1.27±0.04⁴ᵈ | 1.16±0.04⁶ᵈ | 1.23±0.07³ᵏ | 1.31±0.09⁴ᵈ |
| 60 days | 1.27±0.03¹ᵈ | 1.33±0.05⁶ᵈ | 1.17±0.04⁹ᵇ | 1.16±0.09⁹ᵇ |

Two-way ANOVA (P value)

| Groups | | Days | | Interaction |
|--------|---|-----|---|-----------|
| 0.0000 | | 0.295 | | 0.420 |

*Values are means of triplicate groups±SE; **ANOVA results were performed by transformation method (arcsin); #BW, body weight.

**Table 4. Nitrogen and lipid budget per unit body weight gain and nutrient retention in rainbow trout fed Cu-free diets over 60 days**

| Group | 1 (Control) | 2 | 3 | 4 |
|-------|-------------|---|---|---|
| Nitrogen, g kg⁻¹ BW gain | | | | |
| Intake | 85.67±1.81 | 86.41±2.79 | 81.91±0.77 | 80.62±2.31 |
| Accumulation | 28.31±0.28 | 29.09±1.89 | 27.23±0.73 | 29.78±4.44 |
| Loading | 57.35±1.59 | 57.32±4.68 | 54.69±0.45 | 50.84±6.66 |
| Lipid, g kg⁻¹ BW gain | | | | |
| Intake | 256.73±5.41 | 258.96±8.37 | 245.48±2.32 | 241.59±6.94 |
| Accumulation | 118.05±8.35 | 89.45±1.36 | 103.50±10.49 | 101.82±8.24 |
| Loading | 138.67±5.62 | 169.51±7.45 | 141.96±5.18 | 139.77±12.32 |
| Retention, % of intake | 33.07±0.47 | 33.88±3.36 | 33.23±0.65 | 37.29±5.54 |
| Nitrogen | 46.02±3.30 | 34.59±0.89 | 42.12±4.03 | 42.28±4.08 |

*Values are means of triplicate groups±SE; **ANOVA results were performed by transformation method (arcsin); BW, body weight.

**Table 5. Muscle proximate composition of rainbow trout fed the Cu-free diet over 60 days**

| Group | Days | Dry matter, % | Crude protein, % | Crude lipid, % | Crude ash, % |
|-------|------|---------------|------------------|----------------|-------------|
| 1 (Control) | 0 | 25.82±0.81ᵃ | 72.65±0.78ᵃ | 22.52±0.52ᵃ | 4.69±0.05ᵃ |
| 30 days | 30.03±1.07ᵇ | 63.51±2.02ᵇ | 31.81±2.31ᵇ | 4.09±0.08ᵇ |
| 60 days | 32.69±0.59ᵇ | 61.06±1.10ᵇ | 33.61±1.34ᵇ | 4.03±1.99ᵇ |
| 2 | 0 | 25.59±0.26ᶜ | 71.18±0.45ᶜ | 24.01±0.54ᶜ | 4.67±0.19ᶜ |
| 30 days | 27.56±0.76ᶜ | 69.25±0.06ᶜ | 26.73±0.74ᶜ | 3.91±0.16ᶜ |
| 60 days | 31.23±1.08ᶜ | 65.46±0.62ᶜ | 30.25±1.03ᶜ | 3.93±0.16ᶜ |
| 3 | 0 | 25.29±0.98ᵈ | 69.34±0.72ᵈ | 26.16±0.42ᵈ | 4.49±0.01ᵈ |
| 30 days | 28.94±0.75ᵈ | 66.28±0.09ᵈ | 29.69±0.37ᵈ | 3.90±0.06ᵈ |
| 60 days | 31.33±2.63ᵈ | 64.59±0.43ᵈ | 31.38±0.78ᵈ | 3.95±0.16ᵈ |
| 4 | 0 | 25.65±0.60ᵉ | 67.68±1.33ᵉ | 27.14±0.15ᵉ | 4.99±0.02ᵉ |
| 30 days | 29.67±0.79ᵉ | 63.87±1.47ᵉ | 31.71±1.79ᵉ | 3.98±0.18ᵉ |
| 60 days | 32.29±1.16ᵉ | 63.32±0.81ᵉ | 32.29±0.71ᵉ | 3.81±0.13ᵉ |

Two-way ANOVA (P value)

| Groups | | Days | | Interaction |
|--------|---|-----|---|-----------|
| 0.479 | | 0.053 | | 0.205 |
| 0.0001 | | 0.0000 | | 0.0000 |
| 0.928 | | 0.072 | | 0.107 |
| 0.955 | | 0.380 | | 0.000 |

*Values are means of triplicate groups±SE; **ANOVA results were performed by transformation method (arcsin); ᵃᵇᶜᵈᵉµ means with different superscripts are significantly different (P<0.05).
at all concentrations compared to the muscle, gill, digestive system, liver and whole body (Table 6). During first 15 days of depuration, Cu concentrations decreased slowly varying from 202.91±4.71 to 197.78±4.41 µg g⁻¹ (elimination rate: 2%), from 249.16±1.75 to 248.45±2.56 µg g⁻¹ (0.3%) and from 396.67±3.70 to 292.94±10.06 µg g⁻¹ (26.1%) at group 2, group 3 and group 4 Cu diet groups compared to the Cu concentration, respectively.

After that, Cu decrease sharply reaching 67.96±2.36 µg g⁻¹ (elimination rate: 17.2%), 162.89±3.74 µg g⁻¹ (34.6%) and 196.19±8.58 µg g⁻¹ (50.5%) at the end of the experiment. In control, the Cu concentration was not similar to that in the control. The order of Cu elimination in organs during the depuration period was:

digestive system > gill > whole body > muscle > liver for group 2;
digestive system > muscle > gill > liver > whole body for group 3;
digestive system > muscle > liver > gill > whole body for group 4.

As can be seen from Table 6, the elimination levels of Cu in the group 1 (Control), both depending on the time and the concentrations of Cu to diet was evaluated. There were no statistical differences in elimination levels in control group along time, thus having gill on day 0 (initial), digestive system on day 0, 15, 30 and 60 and whole body on day 30 and 60 by the end of the experiment (ANOVA, P>0.05). In addition, for the three remaining groups, the levels of Cu elimination in fish tissue and organs were compared statistically by taking the time factor into account, and the statistical differences were determined to be at a P value less than 0.05.

**Discussion**

**Growth and nutritional performance**

Lett et al. (1976), DeBoeck et al. (1997) and Berntssen et al. (1999) reported that lipid content decreased with increment of Cu concentration in diets. Berntssen et al. (1999) reported that protein catabolism appears to be the primary source for release of stored energy in dietary Cu stressed fish. In this study, the highest lipid retention and lipid content in muscle were observed in group 1 (control), including a low Cu rate in tissue and organs; the lowest nitrogen intake and the highest nitrogen retention were obtained from group 4, including the highest Cu rate. Furthermore, WG, SGR and FCR were similar in groups, while FCR values obtained in this study are similar to FCR values obtained from the feedings of different researchers (Cheng et al., 2002; Bureau et al., 2000; Gomes et al., 1999; Krogdahl et al., 1994; Morales et al., 1994). These results can be explained by disappearance of the metal stress and toxic effects, with reduction of Cu concentrations in tissues and organs of fish.

**Copper elimination**

Elimination of heavy metals depend on the biological half life of the metals and differences among various species of water animals (Kargin and Cogun, 1999; Kraemer et al., 2005). Additionally, these include time, environmental conditions, interacting agents, age of fish. Similar assessments were made by many authors (Douben, 1989; Heath, 1995; Kargin, 1996; Kim et al., 2006). Elimination routes of metals from fish are generally through bile, urine, gill, skin, and mucus (Varanasi and Markey, 1978; Heath, 1995; Kim et al., 2004). Metal elimination routes are more numerous than uptake routes, however metal accumulation is more rapid than elimination, presumably due to the presence of metal binding proteins in tissues (Kargin and Cogun, 1999).

As it can be seen from Table 6, Cu accumulation were investigated in the tissues and organs of fish, after different dietary Cu exposure (60 days). Copper accumulation rates were significantly different than those of control. At the same time, the order of Cu accumulation in each groups was differently showed in tissues and organs of fish. As for the accumulation, the different elimination levels of metals in different tissues may be primarily due to different metabolic activities (P<0.05). At the end of the depuration period, Cu concentration in the muscle and digestive system decreased immediately following the end of the exposure period, Cu concentration being similar to that in the control. In the muscle and digestive system, the elimination rates were determined to be 63.8% and 74.1% for 0.123 g Cu kg⁻¹ exposure, 80.8% and 85.8% for 0.424 g Cu kg⁻¹ exposure, respectively (Table 6). Kim et al. (2006) observed a 63.1% elimination in the intestine of cadmium (Cd) concentration during 30 days of depuration, after exposition to 125 mg kg⁻¹

### Table 6. Changes of Cu concentration (µg Cu g⁻¹) in muscle, gill, digestive system, liver and whole body of rainbow trout during the elimination period of 60 days°. Day 0, start of the experiment, after rainbow trout exposed to dietary copper (0.022, 0.043, 0.123, 0.424 g Cu kg⁻¹) for 60 days°.

| Group | Days | Muscle | Gill | Digestive system | Liver | Whole body |
|-------|------|--------|------|-----------------|-------|------------|
| 1 (Control) | 0 | 0.98±0.06 | 1.82±0.03 | 1.49±0.12 | 98.06±5.11 | 2.02±0.13 |
| | 15 | 0.88±0.02 | 1.33±0.07 | 1.24±0.12 | 96.81±5.06 | 1.97±0.18 |
| | 30 | 0.96±0.05 | 1.59±0.35 | 1.16±0.30 | 92.32±3.70 | 2.67±0.49 |
| | 45 | 0.79±0.11 | 1.38±0.16 | 1.09±0.06 | 86.68±3.66 | 2.01±0.12 |
| | 60 | 0.77±0.04 | 1.39±0.05 | 1.15±0.04 | 94.21±5.28 | 2.69±0.32 |
| 2 | 0 | 1.45±0.07 | 2.85±0.19 | 3.64±0.13 | 202.91±4.71 | 4.12±0.51 |
| | 15 | 1.15±0.08 | 2.86±0.14 | 3.12±0.17 | 198.78±4.41 | 3.84±0.62 |
| | 30 | 1.12±0.09 | 2.69±0.17 | 3.10±0.16 | 193.05±4.08 | 3.56±0.23 |
| | 45 | 1.03±0.14 | 2.14±0.08 | 2.11±0.11 | 180.70±3.50 | 2.98±0.43 |
| | 60 | 1.00±0.06 | 1.68±0.06 | 0.98±0.06 | 167.96±2.36 | 2.64±0.10 |
| 3 | 0 | 2.69±0.38 | 4.04±0.21 | 4.49±0.05 | 249.16±1.75 | 5.22±0.82 |
| | 15 | 2.11±0.12 | 3.69±0.07 | 3.11±0.04 | 248.45±2.55 | 5.20±0.23 |
| | 30 | 1.28±0.11 | 2.89±0.13 | 2.22±0.14 | 224.80±1.77 | 5.02±0.06 |
| | 45 | 1.16±0.12 | 2.09±0.06 | 1.44±0.17 | 210.55±1.80 | 4.22±0.16 |
| | 60 | 0.92±0.02 | 1.62±0.07 | 1.16±0.01 | 182.89±1.74 | 4.10±0.14 |
| 4 | 0 | 4.72±0.42 | 4.39±0.11 | 8.77±0.74 | 396.67±3.70 | 8.41±0.69 |
| | 15 | 3.36±0.32 | 3.87±0.07 | 5.80±0.41 | 292.94±10.68 | 6.35±0.38 |
| | 30 | 1.74±0.11 | 3.38±0.15 | 4.17±0.09 | 267.83±2.63 | 5.16±0.36 |
| | 45 | 1.08±0.02 | 2.29±0.19 | 1.99±0.06 | 215.28±9.40 | 4.42±0.59 |
| | 60 | 0.91±0.02 | 2.19±0.07 | 1.25±0.06 | 196.19±8.58 | 4.35±0.20 |

**Two-way ANOVA (P value)**

| Groups | Days | Interaction |
|--------|------|-------------|
|         | 0.0000 | 0.0000 |
|         | 0.0000 | 0.0000 |
|         | 0.0000 | 0.0000 |
|         | 0.0000 | 0.0003 |

*Values are means of triplicate groups±SE; a,b,c,d,e,f,g,h,imeans with different superscripts are significantly different (P<0.05).
Cd in rainbow trout for 60 days. Harrison and Klaverkamp (1989) found that Cd concentration in intestine exposed dietary Cd, in rainbow trout and white fish, dropped sharply during the depuration periods. Besides, they suggested that this result is due to enteric excretion and intestine is major elimination route for Cd (Kim et al., 2006).

In the present experiment, digestive system showed fastest elimination rates compared to other tissues, and the order of Cu elimination (in percentage) in whole body and organs of rainbow trout during depuration period was:

digestive system > muscle > liver > whole body for groups 3 and 4 (except gill).

Similar results of heavy metals elimination in tissues and organs were shown in many aquatic animal species (Viarengo et al., 1985; Baden et al., 1999, Das and Jana, 1999; Kim et al., 2004). During the trial, it has been observed that Cu concentration in the whole body slightly increased during depuration phase. This can be explained, during the elimination period, by the fact that the accumulated Cu may be transferred from gill, digestive system, muscle and liver to whole body. Kuroshima (1987) reported that cadmium, once taken up in a body, is hardly excreted but is redistributed among tissues and organs. Besides, Cd elimination in tissue was biphasic, which may be composed of the rapid elimination of Cd bound weakly to ligands and the slow elimination of Cd bound strongly to ligands (Kuroshima et al., 1993; Kim et al., 2006).

In the present study, gill showed different elimination rates of Cu at all concentrations compared to the muscle, digestive system, liver and whole body.

The order of Cu elimination in tissue and organs of fish was digestive system (73.1%) >gill (41.1%) >muscle (31.5%) >liver (17.2%) for group 2; digestive system (74.1%) >muscle (65.8%) >gill (60.0%) >liver (34.6 %) for group 3; and digestive system (85.8%) >muscle (80.8%) >liver (50.5%) >gill (50.2%) for group 4 (at 60 days).

In this case, Cu elimination in the gill varies not only according to fast metabolic activity of organs having direct contact with the aquatic environment, but also to accumulation levels. Moreover, several authors have emphasized that metal, once taken up in a body, is hardly excreted but is redistributed among tissues (Kuroshima, 1987; Kim et al., 2004; Kim et al., 2006). Copper is accumulated in the liver to be excreted via the bile, even though gills and kidneys also participate in its excretion (Grosell et al., 1998; Mazon and Fernandes, 1999; Carvalho and Fernandes, 2008).

**Conclusions**

The findings of the present study revealed that elimination of metals is relatively low in the liver at the end of the depuration period, as the Cu concentration in the liver was not similar to that in control group (Kraemer et al., 2005). Copper in the liver was lost mainly from the organelle and cellular debris fractions, suggesting that vesicles such as lysosomes were expelled from these cells. Many authors have emphasized that there is a slow elimination of metal that is strongly bound to ligands, probably due to the presence of metal binding proteins in tissues (Roesijadi, 1992; Kargin and Cogun, 1999; Kalay and Canlí, 2000). Kraemer et al. (2005) examined how the sub-cellular partitioning of metals changed in the liver during the depuration phase. Also, this is probably because liver is the organ of metal storage and detoxification (Kargin and Cogun, 1999; Kalay and Canlí, 2000; Carvalho and Fernandes, 2008). These results also support the findings of the present study.

**References**

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