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Effects of Dietborne Copper on Accumulation in the Tissues and Organs, Growth and Feed Utilization of Rainbow Trout (*Oncorhynchus mykiss*, Walbaum, 1792) Juvenile

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Abstract: The present experiment was conducted to study effects of elevated dietary Cu and establish upper limits of Cu in fish feed. Accumulation and growth of dietborne copper in the gill, muscle tissue, digestive system and whole body of rainbow trout (*Oncorhynchus mykiss*, Walbaum). Four experimental isonitrogenic (460 g kg^{-1}) and isolipidic (200 g kg^{-1}) diets were formulated. Diets were prepared by adding 0 (control), 0.1, 0.4 or 1.6 g kg^{-1} $CuSO_4.5H_2O$ feed, respectively. Each diet was randomly assigned to triplicate groups of 576 juvenile fish whose average weight 19.97±0.048 g. Fish were fed to satiation for experimental period. After 15, 30, 45 and 60 days, the fish were sampled and Cu accumulation in the gill, muscle tissue and the digestive system were determined. The data were presented in μg of sample Dry Weight (DW). At the end of the trial, with increasing dietary Cu concentration, Cu accumulation of gill, muscle tissue, liver and the digestive system were increased. However, accumulation of gill were not significant ($p>0.05$) in the fish fed with 0.4 (D 3) and 1.6 (D 4) g kg^{-1} $CuSO_4.5H_2O$ diets. Accumulation of muscle tissue were not significant ($p>0.05$) in the fish fed with 0 (D 1) and 0.1 (D 2) g kg^{-1} $CuSO_4.5H_2O$ diets. The accumulation of Cu in the digestive system increased with increasing dietary Cu concentration and at periyot were significant ($p<0.05$) in group fed with the highest Cu concentration diet.

Key words: Cu, rainbow trout, accumulation, diet, liver, muscle, digestive system, gill

INTRODUCTION

Aquatic organisms require Cu as an element and can obtain this micronutrient from water or their diet. Copper is essential for the survival of all organisms, including fish (Satoh *et al*., 1983). Despite of its essentiality, Cu requirements differ among species and even within different life stages of a single species (Clearwater *et al*., 2002). It is a cofactor for several proteins that carry out fundamental functions in growth and development (Fairweather-Tait, 1997; Uauy *et al*., 1998). Cu is important for iron metabolism, as a part of anti-oxidant enzymes and in enzymes in the electron transport chain. Cu is also needed for normal connective tissue metabolism and has functions within the central nervous system (Turnlund, 1994).

Although, beneficial at low levels, severe Cu deficiency is known to result in growth retardation, reduced reproduction and in bone and nerve disorders in mammals (Davis and Mertz, 1986; Lorentzen *et al*., 1998). 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*., 2001, 2002; Shiau and Ning, 2003; Bielmyera *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).

Tissue deposition of lipid, protein, carbohydrate and minerals is dependent on feed intake, metabolic use and intestinal absorption and these factors can all be influenced by elevated dietary Cu concentrations. On account of potential for toxic metal contamination is also notable that the concentration of metal in diet may also result in increased metallothionein in gill, muscle, digestive system and liver indicating that dietary metal levels might be excessive, from both fish health and environmental health viewpoints (Lanno *et al*., 1985; Kim *et al*., 2006). Heavy metals are generally accumulated metabolic active organs of the fish is much more than the accumulation rates in the other tissues and organs (Karakoc and Kargin, 1999; Berntssen *et al*., 1999; Gündogdu *et al*., 2009). Both Cu and metallothionein concentrations increased in the intestinal tissues and liver of fish that were fed the Cu contaminated diet. Metallothionein was probably associated with Cu detoxification in these organs. Growth was only slightly reduced in the treatment fish (Clearwater *et al*., 2002).

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It is known that determination of the effects of heavy metals on living organisms is not possible by only evaluating the species collected from natural environments. Conducting, these kinds of studies in laboratories enable us to obtain more detailed results and help to show the situation more clearly.

MATERIALS AND METHODS

Experimental design: Rainbow trout (*Oncorhynchus mykiss*) were obtained from a local fish farm (Karacasu Trout Farm, Sinop, Turkey) and held for 10 days in stock tank. The fishes were maintained on a commercial formulated trout diet (Trout A 45/20, BlackSea Feed, Sinop, Türkey) for 10 days prior to the experiment. After 10 days in the acclimating tanks, 576 fish with a mean weight of 19.97±0.048 g were randomly distributed into each of 12 fibreglass tanks (200 L). The tanks were supplied with running fresh water and continuous aeration. Water temperature, O_2, pH, NO_3^{-1}, NO_2^{-1}, NH_3 PO_4^{-3}-P were 14.23±0.067, 7.48±0.16, 7.09±0.02, 0.14±0.03, 1.20±0.05, 0.12±0.08, 0.064±0.06 °C, respectively, during the trial. Fish were hand fed twice daily at 9:00 and 16:00 h. Feeding activity was monitored carefully to ensure an even distribution of the feed offered among all experimental fish in each tank. The fish were fed the test diets for 60 days.

Test diets: The formulation of the experimental diets is shown in Table 1. The form of Cu used in the diets was copper sulfate pentahydrate, $CuSO_4.5H_2O$. Diets were prepared by adding 0 (control), 0.1, 0.4 or 1.6 g kg^{-1} $CuSO_4.5H_2O$ feed, respectively. The Cu-supplemented diets was prepared using the same formulation except that 0.1, 0.4, 1.6 g kg^{-1} of semolina flour was omitted to compensate for the mass of copper sulphate added to the food. All diets were isonitrogenous and isolipidic. To prepare diets, all dry ingredients were well mixed for 15 min. Then fish oil was added. To ensure a homogeneous blend, the $CuSO_4.5H_2O$ was dissolved in 300 mL of water and added before the feed was pelleted. Then dried at 70°C until obtained approximately 90% dry matter. After processing, the diets were packed into small bags and stored at -20°C until they were fed to the fish.

Sampling: In experiment, fish were starved for 48 h prior to sampling to allow all feed to be excreted. Six fishes were removed from each dose group 15 days during the 60 days experiment. Three fish were sampled for total whole Cu analysis. Three fish were sampled for liver, gill, intestine, muscle Cu analysis and muscle proximate analysis. Fish were anesthetized with a diluted solution of benzocaine in ethanol and killed. Weight was recorded for each individual and then the liver, gill, intestine, muscle and whole body were sampled. Samples of liver, gill, intestine, muscle and whole body were put into plastic bags immediately. All samples were kept at -21°C in a deep-freezer.

Table 1: Ingredient composition and proximate analysis of the experimental diets

	Diet (g kg^{-1})			
Ingredients	D1	D2	D3	D4
Fish meal[1]	400	400	400	400
Soybean protein	100	100	100	100
Soybean meal	170	170	170	170
Fish oil[2]	120	120	120	120
Semolina flour	206.5	206.4	206.1	204.9
Vitamin premix[3]	2	2	2	2
Mineral premix[4]	1.5	1.5	1.5	1.5
$CuSO_4.5H_2O$	-	0.1	0.4	1.6
Proximate composition (g kg^{-1} dry basis)				
Moisture	64	66	59	59
Crude protein	487	487	483	485
Crude lipid	192	192	193	194
Crude ash	63	64	63	63
Nitrogen-free extracts	258	257	261	258
Cu (mg g^{-1})	0.022	0.043	0.123	0.424

[1]Vitamin karmasi (mg veya IU kg^{-1} Yem); vitamin A, 6250 IU; vitamin D_3, 1250 IU; vitamin E, 100 mg; vitamin K_3, 5 mg; vitamin B_1, 7.5 mg; vitamin B_2, 12.5 mg; niacin, 100 mg; Calcium pantothenate, 12 mg; vitamin B_6, 10 mg; vitamin B_{12}, 0.01 mg; folic acid, 4 mg; vitamin C, 105 mg; inositol, 100 mg; d-biotin, 0.25 mg. Mineral karmasi (mg kg^{-1} Yem); Mn, 10 mg; Zn, 37.5 mg; Cu, 2.5 mg; Cobalt, 2.5 mg; I, 1.5 mg; Se, 0.15 mg

Chemical analysis: Samples of liver, gill, digestive system, muscle and total whole were dried in an oven at 105°C to constant dry weight. For the determination of Cu, dry samples were placed in flasks and 10 mL of concentrated HNO_3:$HClO_4$ (5:1) was added to each flask and the solutions were evaporated to dryness on a hot plate. After allowing the flasks to cool, 1 mL of HCl (37%; spurious) was added to bring the volume to 20 mL and filtered through a Whiteman filter 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 Benhard (1976). The data were presented in μ/g of sample Dry Weight (DW). Chemical analysis of diets and fish muscle tissue were done according to AOAC (1990) guidelines as follows: dry matter after drying in oven at 105°C for 24 h until constant weight, protein (N ×6.25) 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 NFE was calculated by difference. All analysis were conducted triplicate.

Statistical analysis: All data were tested for homogeneity of variances among groups using the Barttlett 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, Duncan's multiple range test was used to rank the groups using the SPSS program Version 11.5 for Windows.

RESULTS

To quantify dietary Cu in grouper, $CuSO_4.5H_2O$ was added to the basal diet at 0 (control), 0.1, 0.4 and 1.6 g kg^{-1}diet providing the actual dietary value of 0.022, 0.043, 0.123, 0.424 mg Cu g^{-1} diet, respectively.

Growth and nutritional performance: Data on growth performance are presented in Table 2. No mortality occurred as a result of dietary Cu exposure throughout experimental periods. Dietary Cu concentration significantly ($p<0.05$) affected Final Weight (FW), Specific Growth Rate (SGR) and Daily Feed Intake (DFI) of fish fed the experimental diets. FW and SGR decreased with increasing Cu concentration in diets. However, SGR was not statistically significantly ($p>0.05$) in D1 and D2. FW and DFI were not significantly ($p>0.05$) in D1 and D2, also D3 and D4. The best FCR and PER were obtained from the fish fed D1 and D2. With D1 and D2 group the difference between the D3 and D4 group significant ($p<0.05$) was found.

The intake, accumulation and loading rates of Cu, nitrogen and lipid are presented in Table 3. Cu accumulation and loading significantly increased ($p<0.05$) with increasing levels of Cu. The lowest Cu retention value was observed from the fish fed the D4. The highest N intake (71.02±0.94 g N kg^{-1} BW gain) and loading (44.18±1.29 g N kg^{-1} BW gain) were observed from the group fed the D4. N accumulation rate of fish fed D4 was significantly ($p<0.05$) lower than those fed D1. Lipid (L) intake and accumulation increased with increasing Cu concentration in diets. However, L intake and accumulation were not statistically significantly ($p>0.05$) in D1, D2, D3 and D4. The loading of dietary lipid did not show any significant ($p>0.05$) difference among the groups. The highest L retention value was observed from the fish fed the D3 and D4.

The relationships both between nitrogen concentrations (loading in muscle) and Cu concentrations (in diet) and between Cu accumulation (in muscle) and Cu concentrations (in diet) were analyzed. The results in the basic correlation analysis illustrated a positive linear relationship as follows; $y = 1.802x + 36.545$, $r^2 = 0.8959$ for loading of nitrogen and $y = 0.435x - 0.285$, $r^2 = 0.9299$ for accumulation of Cu, respectively. Nitrogen loading and Cu accumulation in muscle of fish increased with increasing Cu concentrations in diet. As can be seen from Table 4, crude protein (%) was reduced by the decrease of nitrogen in muscle.

Furthermore, the relationships both between lipid concentrations (accumulation in muscle) and Cu concentrations (in diet) and between Cu accumulation (in muscle) and Cu concentrations (in diet) were analyzed. The results in the basic correlation analysis illustrated a positive linear relationship as follows; $y = 6.055x + 54.765$, $r^2 = 0.862$ for accumulation of lipid, $y = 0.435x - 0.285$, $r^2 = 0.9299$ for accumulation of Cu, respectively. Lipid accumulation and Cu accumulation in muscle of fish increased with increasing Cu concentrations in diet.

The proximate compositions of muscle of rainbow trout at the end of the experiment are shown in Table 4. No significant differences was detected in moisture levels among all treatment groups. However, protein, lipid and ash content were affected by dietary treatments. Protein and lipid content of fish fed D3 and D4 were not significantly different. Ash content of fish fed D4 was significantly ($p<0.05$) higher than those fed D1, D2 and D3.

Target organs of dietborne copper: Final Cu concentrations in the experimental diets_were found to be: 0.022±0.002 (control-D1), 0.043±0.003 (D2), 0.123±0.003 (D3) and 0.424±0.017 (D4) mg g^{-1} DW, respectively. Experiment period 15, 30 and 60 days were determined. As can be shown from Table 5 at the end of each experimental period copper levels in the tissues and organs were measured for each metal concentration and their arithmetic means with Standard errors. Accumulation of metal level in all tissues and organs all the time and depending on the concentration of dietborne Cu increased.

Fish fed low dose diets only accumulated the lowest (exhibited elevated) concentrations of Cu in digestive system on day 15, 30 or 45 and muscle on day 60. However, fish fed D2 and D3 dose diets, accumulated the lowest concentrations of Cu in muscle on all days. Fish fed the highest dose diets, accumulated the lowest concentrations of Cu in muscle on day 15 or 30 and digestive system on day 45 or 60. The three dose (D2, D3 and D4) diets, accumulated the highest concentrations of Cu in digestive system on day 60, with the exceptions of liver.

Table 2: Growth performance and feed utilization by rainbow trout fed the experimental diets

	Experimental diets			
Parameters	D1 (0.022)	D2 (0.043)	D3 (0.123)	D4 (0.424)
Initial weight (g)	20.02±0.080^{a}	19.98±0.05^{a}	19.92±0.02^{a}	19.95±0.03^{a}
Final weight (g)	88.69±3.580^{a}	86.54±0.36^{ac}	82.59±0.33^{bc}	79.72±0.58^{b}
Weight gain (% day^{-1})	343.09±12.70^{a}	333.18±2.94^{a}	314.66±1.91^{b}	299.65±2.39^{b}
Specific growth rate[1] (SGR (%))	2.48±0.050^{a}	2.44±0.02^{a}	2.37±0.01^{b}	2.31±0.01^{c}
Daily Feed Intake[2] (DFI, g fish^{-1})	36.14±0.790^{a}	35.08±0.59^{ac}	34.45±0.64^{bc}	33.07±0.37^{b}
Feed Conversion Ratio[3] (FCR)	0.87±0.010^{a}	0.88±0.01^{a}	0.91±0.01^{b}	0.92±0.01^{b}
Protein Efficiency Ratio[4] (PER)	2.35±0.040^{a}	2.34±0.03^{a}	2.29±0.03^{ab}	2.26±0.03^{b}
Survival (%)	100.00±0.000	100.00±0.00	100.00±0.00	100.00±0.00

[1]SGR (ln (final weight)- ln (initial weight)/60 days)×100; [2]Daily feed intake (dry feed intake/number of fish)/60 days; [3]FCR, dry feed intake (g)/weight gain (g); [4]PER, weight gain (g)/protein intake (g)

Table 3: Cu, nitrogen and lipid budget per unit body weight gain and nutrient retention in rainbow trout fed diets containing different levels of Cu over 60 days

	Experimental diets			
Rates	D1 (0.022)	D2 (0.043)	D3 (0.123)	D4 (0.424)
Cu (g Cu kg^{-1} BW gain)				
Intake[1]	19.03±0.29^{a}	37.60±0.43^{b}	111.53±1.48^{c}	387.46±5.12^{d}
Accumulation[2]	0.29±0.06^{a}	0.43±0.05^{a}	0.91±0.25^{b}	1.58±0.15^{c}
Loading[3]	18.74±0.31^{a}	37.17±0.38^{b}	110.62±1.25^{c}	385.85±5.22^{d}
Nitrogen (g N kg^{-1} BW gain)				
Intake	68.01±1.03^{a}	68.39±0.78^{a}	69.97±0.93^{ab}	71.02±0.94^{b}
Accumulation	29.84±0.82^{a}	27.49±0.51^{b}	29.05±1.28^{a}	26.84±0.41^{b}
Loading	38.17±1.08^{a}	40.93±1.21^{b}	40.92±0.37^{b}	44.18±1.29^{c}
Lipid (g L kg^{-1} BW gain)				
Intake	167.22±2.54^{a}	168.63±1.93^{a}	174.66±2.32^{b}	177.28±2.34^{b}
Accumulation	61.81±3.95^{a}	63.47±1.18^{a}	76.77±2.88^{b}	77.56±2.26^{b}
Loading	105.41±4.50^{a}	105.16±0.75^{a}	97.89±0.95^{a}	99.71±4.59^{a}
Retention[4] (intake (%))				
Cu	1.53±0.34^{a}	1.14±0.13^{ab}	0.81±0.21^{ab}	0.41±0.04^{b}
Nitrogen	43.88±1.17^{a}	40.22±1.14^{bc}	41.48±1.27^{b}	37.82±1.03^{c}
Lipid	36.98±2.41^{a}	37.63±0.27^{a}	43.93±1.11^{b}	43.80±1.87^{b}

[1](Feed intake (g fish^{-1}) × Cu, Nitrogen or lipid concentration in diet (%)/100)/(Mean body weight gain (g))× 1000; [2]{Final mean body weight (g) × Final muscle Cu, Nitrogen or Lipid concentration (%)/100) -(Initial mean body weight (g) × Initial muscle Cu, Nitrogen or Lipid concentration (%)/100)}/(Mean body weight gain (g)) ×1000; [3]Cu, Nitrogen or Lipid intake (g kg^{-1} body weight gain)-Cu, Nitrogen or Lipid accumulation (g kg^{-1} body weight gain); [4]Cu, Nitrogen or Lipid accumulation (g kg^{-1} body weight gain) × 100)/Nitrogen or Lipid intake (g kg^{-1} body weight gain)

Table 4: Muscle proximate composition of rainbow trout fed diets containing different levels of Cu over 60 days

		Final			
Composition (%)	Initial	D1 (0.022)	D2 (0.043)	D3 (0.123)	D4 (0.424)
Moisture (wet weight)	24.85	25.82±0.81^{a}	25.93±0.26^{a}	25.29±0.98^{a}	25.65±0.60^{a}
Crude protein	76.72	72.65±0.78^{a}	71.18±0.45^{a}	69.34±0.72^{b}	67.68±1.33^{b}
Crude lipid	18.43	22.52±0.52^{a}	24.01±0.54^{b}	26.16±0.42^{c}	27.14±0.15^{c}
Crude ash	4.85	4.69±0.05^{a}	4.67±0.19^{a}	4.49±0.01^{a}	4.99±0.02^{b}

In liver, digestive system, gill and muscle, the highest Cu concentrations were observed in rainbow trout fed the high dose diet for 60 days. Mean Cu concentrations (DW) in these tissues on day 60 was as follows: Liver (396.67±3.70 μg g^{-1})>digestive system (8.77±0.74 μg g^{-4})> muscle (4.72±0.42 μg g^{-1})>gill (4.39±0.11 μg g^{-1}). However, the other diet groups is reviewed in these tissues on day 60, the order of Cu accumulation in each groups was: Liver>digestive system>gill>muscle (Table 5). If we review all the diet groups, the highest levels of copper were detected in the liver of fish (at D4 group: 396.67±3.70 μg g^{-1}), whereas accumulation was lowest in the digestive system of fish (at D1 group: 0.59±0.03 μg g^{-1}).

As can be seen from Table 5, the accumulation levels of Cu in the control group both depending on the time and the concentrations of Cu to diet was evaluated. There were no statistical differences in accumulation levels to control groups between on the time, which expect, gill on day 45 and 60 by the end of the experiment (ANOVA, $p>0.05$). However, in all tissues and organs control groups were statistical differences from other groups (ANOVA, $p<0.05$). In addition, other groups except the control group in the evaluation results; the fish tissue and organ levels of accumulation by taking the time to consider, which between each of the three groups, as compared statistically, differences were determined to be at $p<0.05$.

Table 5: Accumulation of copper (μg Cu g^{-1}) by tissues and organs of rainbow trout fed diets containing different levels of Cu g kg^{-1} over 60 days

	Diet Cu concentrations (mg g^{-1})			
Days	D1 (0.022)	D2 (0.043)	D3 (0.123)	D4 (0.424)
Muscle				
15	1.37±0.05^{a}	1.34±0.01^{a}	2.36±0.19^{b}	3.50±0.21^{d}
30	1.12±0.15^{a}	1.21±0.02^{a}	2.15±0.15^{bc}	4.14±0.47^{de}
45	1.02±0.13^{a}	1.49±0.03^{ac}	2.48±0.05^{b}	4.36±0.24^{e}
60	0.98±0.06^{a}	1.45±0.07^{a}	2.69±0.58^{b}	4.72±0.42^{e}
Gill				
15	1.18±0.17^{a}	2.59±0.11^{bd}	3.77±0.09^{ef}	3.99±0.26^{f}
30	1.15±0.08^{a}	2.21±0.18^{bc}	3.91±0.24^{f}	4.31±0.48^{f}
45	1.76±0.05^{ac}	3.03±0.07^{de}	4.13±0.56^{f}	4.24±0.46^{f}
60	1.82±0.03^{ac}	2.85±0.19^{bd}	4.04±0.21^{f}	4.39±0.11^{f}
Digestive system				
15	0.66±0.01^{a}	2.61±0.25^{b}	3.51±0.48^{bd}	4.84±0.39^{e}
30	0.59±0.03^{a}	2.72±0.62^{bc}	3.48±0.69^{bd}	7.12±0.19^{f}
45	0.85±0.02^{a}	2.92±0.26^{bc}	3.73±0.13^{cd}	8.38±0.29^{g}
60	1.49±0.11^{a}	3.64±0.13^{bd}	4.49±0.05^{de}	8.77±0.74^{g}
Liver				
15	88.57±4.40^{a}	120.30±6.94^{b}	137.33±5.94^{c}	170.93±2.17^{h}
30	91.30±2.80^{a}	146.94±5.30^{e}	186.74±3.41^{e}	250.84±2.23^{g}
45	92.56±4.57^{a}	146.80±2.69^{e}	216.60±5.23^{f}	372.69±5.56^{i}
60	98.06±5.11^{a}	202.91±4.71^{d}	249.16±1.75^{g}	396.67±3.70^{j}

Data are presented as mean±SE; SE: Standard Error; a-j show differences ($p<0.05$) among groups (dry weights)

DISCUSSION

This study gives data on the chronic dietary toxicity of Cu to rainbow trout. Overall, we show that the fish accumulate Cu in the digestive system, gill, muscle tissue and liver with only marginal reductions in growth rate that are associated with reduced food intake. Cu may also be potentially toxic at elevated concentrations, studies have also shown that overloading of Cu in fish caused toxic syndrome (Lanno *et al*., 1985; Lundebye *et al*., 1999; Shiau and Ning, 2003).

Copper accumulation: No mortalities was observed by elevated dietary Cu concentrations during the experiment. However, the fish tissues and organs shown an increase in Cu content during exposure. Some authors worked in similar concentrations in the experiment also; same results were obtained (Shaw and Handy, 2006; Hoyle *et al*., 2007). Metal accumulation in tissues of aquatic animals is dependent upon exposure concentration and periods as well as some other factors such as salinity, temperature, pH and metabolic activity of tissue (Kargin and Erdem, 1991; Lloyd, 1992; Hoyle *et al*., 2007). Moreover, it is also, known that metal accumulation in tissues of fish is dependent upon the rate of uptake, storage and elimination (Karakoc, 1999; Clearwater *et al*., 2002; Ureña *et al*., 2007). In experiment, Cu accumulation in gill, intestine, liver and muscle of juvenile fish were increased with exposure periods and concentration during 60 days of exposed to dietary Cu. Cu accumulation in the internal organs varies not only according to the route of uptake and level of dietary Cu exposure, but also over time (Handy *et al*., 1999; Clearwater *et al*., 2002; Kamunde *et al*., 2002). Rainbow trout dosed with Cu in the gut initially accumulated new Cu in the intestinal tissues and the liver (Clearwater *et al*., 2002). In mammals, Cu absorbed by the gut is released into the plasma, transported as a complex with albumin and absorbed by the liver. Cu is incorporated into ceruloplasmin in the liver, then released to the plasma again to be transported to other internal organs (Harris, 1991; Clearwater *et al*., 2002; Kamunde *et al*., 2002). The heavy metal were important accumulation in the intestine. Cu is transported from the gastrointestinal tract to blood, where it is bound to erythrocytes, plasma metallothionein and then transported to other organs such as liver (Swiergosz *et al*., 1998; Kim *et al*., 2006). Similar patterns of Cu accumulation and other heavy metal were also shown in other studies carried out with aquatic animals (Berntssen *et al*., 1999; Kim *et al*., 2006; Ureña *et al*., 2007; Lin *et al*., 2008).

In this study, usually, the order of Cu accumulation in each groups was shown: Liver>digestive system>gill>muscle. In the other study, the liver and gall bladder are the first internal organs to accumulate high Cu concentrations, followed by the gill and muscle (Clearwater *et al*., 2002). Theses same results were observed the another studies of Cu dietary exposure e.g., Nile tilapia, Shaw and Handy (2006), rainbow trout, Handy *et al*. (1999), Kamude and Wood (2003) and *Atlantic salmon*, Berntssen *et al*. (1999). The order of metallothionein content was kidney>liver>gills both in farmed and in wild eel. A highly significant positive correlation was found between metallothionein concentration and copper in liver of wild eel (Urena *et al*., 2007). Generally, the liver (as high metabolic activity in the tissues and organs) in fish tends to concentrate metals and play a major role in detoxification and excretion of metals through induction of metal-binding proteins such as metallothioneins (Roesijadi and Robinson, 1994; Kim *et al*., 2006; Minghetti *et al*., 2008).

At the same time (before 45 days), Cu levels in the liver shown a rapid rise, but, the liver also, shown a small increase in Cu content after 45 days in present study and similar assessments were mad for between 20 and 30 days in previous studies (Shaw and Handy, 2006; Hoyle *et al*., 2007). This station can be explained by decrease capacity to bind metal to reach the saturation level of metal-binding proteins such as metallothioneins in the liver.

In the present experiment, exposure to copper via the oral route was confirmed by large increases in the copper

content of the digestive system, muscle and liver compared to fish on the control diet, while contamination in the gill remained low. Dietary copper exposure was confirmed by elevated Cu concentrations (0.424 g kg^{-1}) in the digestive system (6.9 fold), muscle (4.8 fold) liver (4 fold) and gill (2.4 fold) of Cu-exposed fish compared to controls after 60 days (ANOVA, $p<0.05$; Table 5). In the other study, dietary copper exposure caused elevated Cu concentrations in the digestive system (20 fold), liver (5 fold) and gills (4 fold) of Cu-exposed fish compared to controls after 30 days to those determined by Hoyle *et al.* (2007). In the another study, Cu levels in the intestine, liver and gills increased 30 fold, 3 fold and 2.7 fold, respectively, compared to controls after 42 days (Shaw and Handy, 2006). The three trial, the increased level of accumulation fold, is ranked the same as it is seen (intestine>liver>gill). This metabolic activity of the organs of the fish is compatible with. The reasons for the determination of the different fold which can be explained by the influence of many factors (fish species and size, water quality, the amount of feed consumed, the amount of Cu added to diet and trial period). Additionally, it is also known that metal accumulation in tissues of fish is dependent upon the rate of uptake, storage and elimination (Roesijadi and Robinson, 1994; Kim *et al.*, 2006).

In the present experiment, levels accumulation of Cu in the muscle tissue was lower than levels of accumulation in the gill tissue and digestive system. To slow the metabolic activity of muscle tissue that reduces the bind capacity of the heavy metals. This case, the normal conditions of muscle tissue metallothionein and other low molecular weight metal-binding proteins contain less and this protein synthesis capacity of metal under the influence, can be explained by limited (Kalay and Karatas, 1999; Clearwater *et al.*, 2002; Urena *et al.*, 2007). Many fish species and rainbow trout were taken from the area of metal pollution and the textural analysis; the muscle tissue from other tissues and organs were determined to be the lowest in the metal accumulation (Kim *et al.*, 2006; Urena *et al.*, 2007; Lin *et al.*, 2008).

Metal accumulation in the digestive system, according to the accumulation of metals in the gill tissue were determined to be more, even at the highest concentration; the difference between the two tissues that has been found that approximately two-fold (Table 5).

Fish metabolism, the digestive system's activity compared with activity in the gill tissue is more active, so the accumulation of metals in the digestive system is more than is usual. In addition, because of the gill tissue is in direct contact with water and osmoregulation, gill easily disposed of Cu can. The gills also, shown a small increase in Cu content during exposure in this experiment. Fish exposed to dietary metal shown concurrent increases in gill metal concentrations and elevated metallothionein synthesis in the epithelial cells of gill (Dang *et al.*, 2001). Furthermore, dietary metal can enter the gill epithelium from the blood, which cause substantial damage to the gill and decrease its osmoregulatory capacity (Pratap and Bonga, 1993; Kim *et al.*, 2006). As this research, many authors have emphasized metals accumulation in liver, digestive system and gill and the least was shown in muscle (Lorentzen *et al.*, 1998; Kamunde *et al.*, 2002; Hoyle *et al.*, 2007). Because muscle tissue is less than the capacity of the heavy metals bind to and the other by low metabolic activity of Cu accumulation has observed.

Growth and nutritional performance: In this study, dietary Cu exposure resulted in reduction of rainbow trout growth, SGR and feed intake (Table 2). Reduced feed intake, which was found in fish fed the diet containing higher dietary Cu, could be one of the reasons for lower weight gain of fish since lower feed intake would reduce the amount of nutrients availability for growth. Furthermore, toxicants that interfere with energy-yielding reactions indirectly inhibit the syntheses of RNA, DNA and protein. Moreover, the physiological changes permitting metal detoxification and homeostasis cost energy and reduced growth caused by exposure to metal has been attributed to metabolic costs associated with metal detoxification (Kim *et al.*, 2006). Several research have noted reductions in growth during dietary Cu exposure in rainbow trout (Kamunde *et al.*, 2002; Lanno *et al.*, 1985), but others have not (Handy *et al.*, 1999; Kamunde *et al.*, 2001; Kamude and Wood, 2003). Clearwater *et al.* (2002) argues that the threshold for dietary Cu toxicity on growth rate is about 664-730 mg Cu kg^{-1} DW feed, equating to a dosage of 35-45 mg Cu kg^{-1} BW day^{-1}. In the present experiment, growth, SGR and FI were significantly reduced at a dietary Cu concentration of 424 mg Cu kg^{-1} (daily Cu doses of 8.48 mg Cu kg/bw/day, ration of 2.1% BW day^{-1}) compared to D1 group. The difference can probably be explained by differences in water temperature and food regurgitation by the fish.

Secondary stress responses such as depletion of glycogen tissue reserves, lipolysis, inhibition of protein synthesis and catabolism of muscle protein occur typically in sublethal exposed fish (Jobling, 1994). Lett *et al.* (1976) and DeBoeck *et al.* (1997) and Berntsen *et al.* (1999) reported that lipid content decreased with increment of Cu concentration in diets. Conversely, in this study, the muscle lipid content increased with increment of Cu concentration in diets.

Also, the highest lipid intake and lipid accumulation were observed from 123 and 424 mg Cu kg^{-1} group. The determination of the lowest weight gain in these groups again shows that the fish stores energy in the muscular tissue as fat instead of using it to grow (Table 4).

After 60 days of exposure, the muscle protein content of the 123 mg and 424 mg Cu kg^{-1} group was significantly lower than the D1 and D2. Furthermore the highest N intake and the lowest N accumulation were observed from 424 mg Cu kg^{-1} group (Table 3). DeBoeck *et al.* (1997) suggest that first glycogen stores are depleted as glucose source followed by protein breakdown and subsequent gluconeogenesis from amino acids to maintain glucose levels. Berntssen *et al.* (1999) reported, protein catabolism appears to be the primary source for release of stored energy in dietary Cu stressed fish. Moreover, in this study, the increase in fat storage in the muscular tissue following the decrease in weight may be related to the degeneration of protease enzymes.

Although, it's known that copper causes recession in growth, lower food utilization rates and changes in body compositions in fishes two results that differ from other studies were obtained in the study. The first of those results is the recession of growth, which was detected in lower Cu values such as 123 and 424 mg Cu kg^{-1}. The second one is the increase in fat content of the fish muscular tissue contrary to the other studies. The parameters that were acquired indicate that the increase in contamination of the aquatic ecosystem and diversification of contaminants day by day leads to a decrease in the tolerances of species living in this environment against to the contaminants.

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