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Full Length Research Paper

Growth response and ionic regulation in common carp (C*yprinus carpio L*.) after chronic dietary copper exposure and recovery

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Effects of exposure of common carp juveniles (*Cyprinus carpio L*.) to dietary copper and its recovery rate were investigated with the aim of determining the growth and physiological impact. The fish were fed elevated copper diets (1000 and 2000 mgCukg^{-1} as diet 2 and diet 3 respectively) and control diet (5 mgCukg^{-}, as diet 1) for 42days and were then fed the control diet for a further 21days. After 42days of exposure to elevated copper diets, growth performance examined showed that there was significant increase at ($p<0.05$) in feed intake, %body weight, weight gain and condition factor by fish fed diet 2 compared to diet 1 and diet 3. There was, however, no difference in specific growth rate, feed conversion ratio, in all treatments ($p>0.05$). Hepatosomatic index increased significantly in fish fed both elevated diets compared to control diet ($p<0.05$). Recovery period on normal diet (control) showed no significant effect of copper recovery on fed intake, weight gain, %body weight, specific growth rate and feed conversion ratio in all treatments ($p>0.05$), but, fish fed diet 2 showed a significant reduction in condition factor compared to other diets ($p<0.05$). Tissue Na^{+}, Ca^{2+} K^{+} were disturbed throughout the experiment with sodium increasing from 257.82±2.50 to 388.14±1.32 μmol/g and calcium increasing from 499.54±6.81to 1025.94±9.16μmolg^{-1} reducing gill copper from 11.63±0.37to 0.00±0.00 mgCukg^{-1}. Intestinal copper decreased from 14.93 ±0.1 to 0.00±0.00 mgCukg^{-1} as a result of sodium increasing from 130.30±5.12 to 438.72±2.44 μmolg^{-1}. Increased gill copper of the 1000 mgCukg^{-1} diet exposed fish during exposure compared to the control was due to copper induced decrease in plasma ion regulatory sodium (Na ATPase activity), which protected fish from direct toxicity effect and could also suggest another pathway other than the common Na/Cu apical channel shared between sodium and copper through which copper binds to fish gill; diet 2 fish showing significant increase at ($p<0.05$) in haematocrit, red blood cell, white blood cell and neutrophil, and a significant reduction in lymphocyte and mean cell haemoglobin compared to diet 1 and diet 3, ($p<0.05$). This increase in blood indices is indicative of stress onset to which fish fed diet 2 is subjected. Fish fed diet 3 showed significant reduction in haematocrit, red blood cell, white blood cell and increased lymphocyte ($p<0.05$) and became anaemic with severe skin discoloration, indicative of a worsening effect of excess dietary copper exposure on the fish. There were no significant differences in moisture content of all tissues during and after copper exposure ($p<0.05$). Gills of fish fed diet 3 increased post-exposure, indicative of protection of the structural integrity of the gill to prevent hypoxia through oxygen supply from water.

Key words: Chronic, dietary copper, growth, ionic regulation, exposure, recovery, common carp.

INTRODUCTION

Copper is an essential trace element that plays a vital role in the physiology of animals for fetal growth and early post–natal development, for hemoglobin synthesis, connective tissue maturation especially in the cardiovascular system and in bones for proper nerve function and bone development, and inflammatory process. It is involved in different biochemical process of animal metabolism such as: enzyme – co enzyme catalytic reactions. It is associated with the function of a number of enzymes such as oxygenases including

cytochrome C oxidase and copper- zinc super oxide dismutase (Hinton and Lauren, 1990; Benneth et..al., 1995) and; ion transport for instance with ceruloplasmin (ferroxidase1), a putative copper transport protein required for the incorporation of iron into transferrin for it is transport in plasma (Linder, 1991).

It is an essential micronutrient for vertebrate animals especially fish, and has numerous functions, in addition to the ones stated above, in cellular biochemistry including vital roles in cellular respiration, and a cofactor for over 30 different enzymes (Linder, 1991). Copper deficiency leads to physiological disturbance. Symptoms include depression of growth, anaemia, bowing of legs, spontaneous fractures, ataxia of new borns, cardiac and vascular disorders and a depigmentation, decrease in some organs weight, depressed reproductive performance including egg production.Copper, though essential in fish diet, can be harmful when large single or daily intake occurs.

The dietary effect of copper varies from species to species (Atlantic Salmon), (Berntssen et al., 1999; Rainbow et al., 1985; Coho et al., 1972) and has severally been reported for most temperate fish such as Salmon (Berntssen et.al., 1999) and recently, Nile tilapia (Shaw and Handy, 2006), but little or no information on the dietary copper exposure and recovery in common carp has been reported. In Salmon, toxic effect of dietary copper includes reduced growth (Clearwater et al., 2002), severe lesions in the gut at high concentration (10 gKg^{-1} food; Handy, 1996), cell proliferation and metallothionein (Hinton and Lauren, 1990). Fatty change in the liver of salmon as well as altered haematology has been severally reported that copper when in excess, is stored in the liver and tend to increase after feed withdrawal leading to hepatic cell lysis and release of cell content in the liver. This is confirmed by (Shaw and handy, 2006). In their report in Nile tilapia, the recovery phase on normal diet without copper was characterized by a reduction in intestinal and branchial copper level after dietary cooper exposure was confirmed by elevated copper concentration in the intestine, liver and gills**.**

Knox et al. (1982) reported similar fatty changes in the liver of salmon, but there was altered haematology, contrary to report of Shaw and Handy (2006). Research on copper exposure and recovery in common carp would go a long way to add to sparse literature on dietary copper exposure and recovery in tropical fresh water and to know if difference in climate and region could account for differences in toxicity of dietary copper exposure at same level.

Dietary copper exposure in African catfish has also been reported severally (Handy et al., 2000), aqueous copper exposure and recovery on common carp (Karan et al., 1996); aqueous cadmium exposure in common carp (Witeska, 1998); aqueous zinc on common carp (Svobodova et al., 1994); dietary copper exposure and recovery in Nile tilapia (Shaw and Handy, 2006); as well as threshold for excess dietary copper toxicity on fresh water fish excluding carp (Clear water et. al., 2002; Lanno et al., 1985) have all been reported. But to my knowledge and literature review, no study on the dietary copper exposure and recovery in common carp (L) has been reported. This present study will not only examine chronic dietary copper toxicity on common carp, but it will also establish threshold for dietary copper toxicity by investigating growth and ionic response, which has not been reported for the fish.

The common carp, a benthic omnivore, is native to Asia and Eastern Europe (Taylor and Mahon, 1977). Reputed as a popular food fish and a highly cultivable species with year round breeding under tropical and subtropical conditions, the common carp also plays an important role in polyculture systems in seasonal reservoirs and ponds (Chakrabarty, 1982). It is the only exotic carp species that is known to breed naturally in lake, (Nathaniel and Edirisinghe, 1998). It has high fecundity and hatchability (Nathaniel and Edirisinghe, 2001).

It has been introduced into environments worldwide and can grow to a maximum length of 5 feet (1.5 m), a maximum weight of over 80lb (37.3 kg), and an oldest record age of at least 65 years (Panek, 1987). This age longevity of common carp makes it good for chronic toxicity test. Similarly, although they are very tolerant of most condition, the common carp prefer large bodies of show or standing water and soft, vegetable sediments. This makes it not unaffected by pollution from heavy metals since they eat anything near bank and bottom, thus ingesting contaminated food and water during feeding (Alabaster and Lloyd, 1980).

Fish unlike most terrestrial animal, can absorb some minerals, (inorganic elements) not only from their diet, but also from their external aquatic environment (Lall, 1989). Calcium (ca), sodium (Na), potassium (k), copper (Cu) and other essential minerals are generally derived from the water to satisfy part of the nutritional requirement of fish (Phillips et al., 1959). Inorganic elements, which are required for the normal life processes of fish, perform the following function: formation of skeletal muscles structures, electron transfer, regulation of acid-base balance/ equilibrium and osmoregulation; they are important component of hormones and enzymes and activate enzymes (Lall, 1989). Complex biochemical mechanisms control and regulate the uptake, storage, and excretion of various inorganic elements, allowing fish to live in a dynamic equilibrium with their aquatic medium. The electrolytes Na^{+}, Mg^{2+}, Ca^{2+}, Cl^{-} and HCO_3 play a major role in the osmotic and ionic regulation of extra – and intra cellular fluids in fish (Lall, 1989).

The exchange of ions from the surrounding water across the gill and skin of fish complicates the measurement of mineral requirement; although most

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essential elements known for terrestrial animals are also considered important for fish, quantitative requirement have been reported for only nine (9) minerals: calcium, phosphorus, magnesium, iron, copper, manganese, zinc, selenium, and iodine (Ogino and Taekeda, 1976; Watanabe et al., 1980; Lall and Hines, 1987; Gatlin and Wilson, 1986b, c; Bell and Coway, 1989; Lall and Hines, 1987) for selected fish species.

The aim of the research, which is the first of its kind, is to investigate and evaluate the chronic effects of varying dietary copper concentrations on the growth and physiology (tissue ions and moisture) of common carp (*Cyprinus carpio L) and* its recovery from exposure.

MATERIALS AND METHODS

Experimental design

Common carp were purchased from Oyo state Agricultural Development Programme (ADP) reputable fish farm in Ibadan, Oyo State. They were then placed under laboratory conditions in fish holding tanks with water temperature 27.4°±0.42°C and left unfed in the first 2 days to adapt to a change in environment before feeding them with normal diet.117 fish of average weight 19.43±14.09 g were then placed in 9 plastics of 52 L each in a water renewal method and were fed a control diet with no added copper to saturation for 14 days in order to acclimatize them to experimental conditions with 13 fish per tank. While fish in the first three containers remained on the control diet, fish in the second and last three containers were fed copper-loaded diets (1000 mg/kg dry weight feed) and (2,000 mg cupper/kg dry weight feed) respectively for 42 days. This was then was then followed by a 21 day recovery period with all containers fed the control diet (no added copper) diet. Throughout the experiment fish were fed to satiation twice a day in the morning and evening. Care was taken to ensure no uneaten food remained in the tanks during feeding and copper did not leach from the feed. To achieve these objectives, water was constantly and completely changed daily with fresh well water added and uneaten food removed after satiation was noted. Daily feed intake was calculated by subtracting weight of feed plus container after feeding from feed plus container before feeding. Copper concentrations in the different tank were measured in the analysis of water quality. Growth and nutritional performance in the different treatments were monitored throughout the experiment and the fish randomly sampled from each tank after 42 day of copper exposure for haematology, tissue ion analysis, and histology. Fish were not fed the day before sampling times in order to empty the gut and to facilitate dissection.

Diet

The control diet was purchased from a commercial animal feed dealer (Adom commercial Feeds, Ibadan, Oyo state, Nigeria) with a proximate composition from manufacturer's guidelines shown in Table 1.

Dietary preparations

The copper-supplemented diet was formulated by starch coating of the commercial feed with copper sulphate. In order to achieve a nominal copper concentration of 1000 mgCukg^{-1} feed, 1.1722 g of $CUSO_4$. $5H_20$ (AnalaR grade, BDH, poole, UK) was dissolved in 35 ml of deionised water with 1.2 g of starch to bind the copper to the food sticks. The starch solution was gradually sprayed onto 300 g of the commercial diet and mixed in a container to ensure even mixing of the food. The starch coat dried within minutes, and the copper diet was stored in airtight containers at – 20°C to prevent lipid oxidation. The other copper supplemented diet (2000 mgCu/kg) was also treated, but required higher $CuSO_4$. $5H_20$. In order to achieve a nominal copper concentration of 2000 mgCu/kg feed, 2.3503 g of $CuSO_4$ $5H_20$ was also dissolved in 35 ml of deionised water with 1.2 g of starch to bind the copper to the feed. It was then mixed thoroughly; the starch solution was sprayed onto 300 g of the pre-treated diet and stored at 20°C after moments of drying. The control diet was similarly treated except that no copper was added. The copper contents of the diets were confirmed by atomic absorption spectrophotometry (model 210 VGP) with the following specification for copper detection and analysis:

Growth and nutrition performance

Growth and nutritional performance was measured and described. Briefly, feed intake was calculated, daily for each tank by weighing feed containers before and after feeding. All fish were individually weighed at the start of the experiment and the end of 42 days of exposure. The individual fish weight was used because the periodic sacrifice of fish during the experiment prevented nutritional parameters being calculated from cumulative tank biomass as follows:

1) Specific growth rate (SGR (% day $^{-1}$) = $(\log_e W_2 - \log_e W_1)/(t_2 - t_1) \times 100$

where, t_1 = initial time point before exposure (days)
t_2 = final time point after exposure (days)
$W_{1\,(g)}$ = fish weights at t_1
$W_{2\,(g)}$ = fish weights at t_2

2) Feed conversion ratio (FCR) = feed intake (g) weight gain (g)
This was calculated from mean gain in body weight for each treatment for

a) The copper exposure phase (days 0 to 42),
b) Recovery phase (days 43 to 63)
c) Mean weight gain (g) =final weight—initial - weight
d) Condition factor (%) = weight (g) /length^3 (cm) × 100)
e) Hepato-somatic index for each fish (%) = liver weight (g)/body weight (g) × 100.

Tissue ion and moisture analysis

Tissues for trace metal analysis were oven dried to a constant weights, which were subtracted from the initial weight of each tissue before drying to get the moisture content and then expressed as percentage of initial weight; oven dried samples were then digested in nitric acid and then diluted to volume with distilled water. The samples were analyzed by atomic absorption spectrophotometry for copper (at 589.00 nm), K^+ (at 766.5 nm), Na^+ (at 589.00 nm), and Ca^{2+}(at 422.7 nm) using flame photometer (model: corning 410).

RESULTS

The physiological effects of dietary copper exposure and recovery on normal diet were studies throughout the entire length of the project. Growth performance, histology, hematology as well as tissue ion and moisture

Table 1. Proximate composition.

Crude protein	45%
Crude fat	12%
Crude fibre	1.5%
Phosphorus	1.2%
Preservatives	E280
Antioxidants	E321
Calcium	1.8%
Lysine	3.0%
Methionine	1.0%
Vitamin A	15,000 iu/kg
Vitamin D_3	2,000 iu/kg
Vitamin E	200 mg/kg
Vitamin C (stable)	150 mg/kg
$CUSO_4$	5 mg/kg

were investigated during the two phase of the experiment (exposure and recovery) water quality was monitored throughout the exposure phase and result showed that all parameters were within the range required and tolerated by common carp.

This study is a first report of chronic dietary copper exposure/toxicity in common carp; and overall, fish, in this research, accumulated excess copper in the liver and intestine. The fish were fed elevated copper diets (1000 and 2000 $mgCukg^{-1}$ as diet 2 and diet 3 respectively) and control diet (5 $mgCukg^{-1}$, as diet 1) for 42 days and were then fed the control diet for a further 21days. The results of the experiments showed that after 42 days of exposure to elevated copper diet there was significant increase at ($p<0.05$) in feed intake, %body weight, weight gain and condition factor by fish fed diet 2 compared to diet 1 and diet 3 (Table 6). There was, however, no difference in specific growth rate, feed conversion ratio, in all treatments ($p>0.05$). Hepatosomatic index increased significantly in fish fed both elevated diets compared to control diet ($p<0.05$). Recovery period on normal diet (control) showed no significant effect of copper recovery on fed intake, weight gain, %body weight, specific growth rate and feed conversion ratio in all treatments ($p>0.05$), but, fish fed diet 2 showed a significant reduction in condition factor compared to other diets ($p<0.05$) (Table 7). Tissue Na^+, Ca^{2+} K^+ were disturbed throughout the experiment with sodium increasing from to 388.14±1.32 μmol/g and calcium increasing from 499.54±6.81 to 1025.94±9.16 $\mu molg^{-1}$ reducing gill copper from 11.63±0.37 0.00±0.00 $mgCukg^{-1}$. Intestinal copper decreased from 14.93 ± 0.1 to 0.00±0.00 $mgCukg^{-1}$ as a result of sodium increasing from 130.30±5.12 to 438.72±2.44 $\mu molg^{-1}$ (Table 2). The reduction in intestinal calcium was sodium dependent as increasing sodium decreased calcium absorption. There was a significant increase in gill copper of the fish fed 1000 $mgCukg^{-1}$ diet compared to the control and 2000 $mgCukg^{-1}$ diet, which showed a significant accumulation of copper in the liver compared to 1000 $mgCukg^{-1}$ diet and control-fed fish (Table 2) and continued to increase post exposure on recovery diets compared to the other diets (Table 3). There was significant increase in haematocrit, red blood cell, white blood cell and neutrophil, and a significant reduction in lymphocyte and mean cell haemoglobin in fish fed the 1000 $mgCukg^{-1}$ compared to diet 1 and diet 3, ($p<0.05$). Fish fed diet 3 showed significant reduction in haematocrit, red blood cell, white blood cell and increased lymphocyte ($p<0.05$) and became anaemic with severe skin discoloration, indicative of a worsening effect of excess dietary copper exposure on the fish. There were not significant differences in moisture content of all tissues during and after copper exposure ($p<0.05$), although, gills of fish fed diet 2 showed reduction in moisture compared to diet 1 and diet 3-fed fish for both exposure and recovery phases, increasing from 75.3+3.20 to 79.5+6.44% after recovery for 21 days (Tables 4 and 5). Gills of fish fed diet 3 also increased post-exposure, indicative of protection of the structural integrity of the gill to prevent hypoxia through oxygen supply from water.

DISCUSSION

Copper accumulation

Copper accumulations in fish tissue have severally been reported (Playle et al., 1992; Shaw and handy, 2006; Pere and Pihan, 1991b; Lanno et al., 1985; Celechovska et al., 2007). In this study, copper accumulation in common carp also reflected the route of exposure (Kamunde et al., 2002), with large increase in copper content of the liver and intestine (Celechovska et al., 2007; Hinton and Lauren, 1990; Berntssen et al., 1999; Shaw and handy, 2006) and is consistent with previous studies on temperate species such as rainbow trout (Kamunde and wood, 2003); Atlantic Salmon (Berntssen et al., 1999; Lundebye et al., 1999). The gills showed increased copper accumulation post exposure in the 1000 mgCu/kg (diet 1) and 2000 mgCu/kg (diet 2). This cannot be explained by aqueous copper uptake, because gill morphology was normal (NVL) during and post exposure period (Shaw and Handy, 2006); rather, it was due to increased intestinal absorption of dietary copper.

Copper accumulation in fish does not depend on dietary level (Berntssen et al., 1999), but sodium dependent (Kjoss et al., 2005; Pyle et al., 2002; Erickson et al., 1996) as well as being calcium dependent (Mohson et al., 2007). In this research the time dependent reduction in the control was sodium and calcium dependent (Kjoss μmol/g and calcium increasing from 499.54±6.81 to 1025.94±9.16 $\mu molg^{-1}$ reducing gill copper from 11.63±0.37 to 0.00±0.00 $mgCukg^{-1}$. Intestinal copper decreased from 14.93±0.1 to 0.00±0.00 $mgCukg^{-1}$

Table 2. Sodium, calcium and potassium content in gills, liver and intestine of common carp fed recovery diet 1 (control), diet 2 and diet 3 after 42 days.

Content	Treatment	Gill	Liver	Intestine
Na	Initial	257.82±2.50^{a}	132.44±1.73^{a}	130.30 ±5.12^{a}
	Diet 1	388.14 ±1.32^{b}	407.47 ±0.29^{b}	438.72 ±2.44^{b}
	Diet 2	476.65 ±1.60^{c}	306.87 ±1.48^{c}	356.65 ±0.32^{c}
	Diet 3	427.57 ±0.71^{d}	356.78 ±0.47^{d}	597.74 ±3.89^{d}
K	Initial	226.20 ±6.12^{a}	87.07 ±2.25^{a}	86.48 ±0.87^{a}
	Diet 1	246.62 ±0.76^{b}	340.17 ±1.03^{b}	419.53 ±0.59^{b}
	Diet 2	320.40 ±1.17^{c}	300.25 ±1.03^{c}	420.29 ±1.38^{c}
	Diet 3	210.04 ±0.18^{d}	321.31 ±2.0^{d}	437.3 ±0.46^{d}
Ca	Initial	499.54 ±6.81^{a}	4.40 ±0.06^{a}	44.02 ±1.15^{a}
	Diet 1	1025.94 ±9.16^{b}	62.60 ±1.45^{b}	27.08 ±0.85^{b}
	Diet 2	644.17 ±0.10^{c}	3.27 ±0.03^{c}	3.21 ±0.02^{c}
	Diet 3	1420.94 ±3.53^{d}	26.48 ±0.75^{d}	20.84 ±0.09^{d}
*Cu	Diet 1	0.00±0.00^{ab}	34.67±1.00 ^{ab}	0.00±0.00^{b}
	Diet 2	15.67±0.33 ^{c}	152±1.15 ^{c}	267.67±1.76^{c}
	Diet 3	0.00+0.00^{b}	247.47±2.19^{d}	55.67±1.20^{d}

Data are means±S.E. (n = 3 per value), expressed in μmol/g of dry weight fish; letters with the same superscript in the same column are not significant (p>0.05); *Values of Cu are expressed in mgCu/kg of fish.

Table 3. Sodium, calcium and potassium content in gills, liver and intestine of common carp fed recovery diet for 21 days.

Content	Treatment	Gill	Liver	Intestine
Na	Diet 1	388.14±1.32^{a}	407.47±0.29^{a}	438.92±2.44^{a}
	Diet 2	300.13±7.53^{b}	281.28±7.67^{b}	260.99±7.72^{b}
	Diet 3	179.79±6.32^{c}	272.58±6.32^{c}	619.72±3.83^{c}
K	Diet 1	246.62±0.76 ^{a}	340.17±1.03^{a}	419.53±0.59^{a}
	Diet 2	171.35±5.91 ^{b}	220.79±3.7^{b}	284.73±3.71^{b}
	Diet 3	225.90±3.71^{c}	206.3±2.98^{c}	318.82±3.72^{c}
Ca	Diet 1	1025.94±9.16^{a}	62.60±1.45^{a}	27.08±0.85 ^{a}
	Diet 2	1036.32±4.4 ^{b}	23.29±2.20^{b}	44.91±2.88^{b}
	Diet 3	1585.24±3.63^{c}	23.29±2.20^{c}	10.81±2.20^{c}
*Cu	Diet 1	0.00±0.00^{a+}	34.6±1.00^{b}	0.00±0.00^{a}
	Diet 2	55.42±0.25^{b}	267.22±0.5^{b}	331.43±0.83^{b}
	Diet 3	364.49±5.22^{c}	290.99±0.70 ^{c}	78.02±0.87^{c}

Data are means±S.E (n=3/value), expressed as μmol/g letters with the same subscript in the same column are not significant (p>0.05). *****Values of Cu are expressed in mgCu/kg of fish.

as a result of sodium increasing from 130.30 ±5.12 to 438.72 ± 2.44 μmolg^{-1}. The reduction in intestinal calcium was sodium dependent as increasing sodium decreased calcium absorption (Flik and Verbost, 1993). Increased gill copper of the 1000 mgCukg^{-1} diet exposed fish during exposure compared to the control was due to copper induced decrease in plasma ion regulatory sodium (Na ATPase activity), which protected fish from direct toxicity effect (De Boeck et al., 2003) and could also suggest another pathway other than the common Na/Cu apical channel shared between sodium and copper through which copper binds to fish gill (Pyle et al., 2002). The later reason could be apt due to the fact that, although, inhibition of copper branchial /basolateral Na+/K+ ATPase cannot extrude intracellular Na+ into the blood (that is influx is inhibited), branchial influx of sodium was stimulated into the blood due to decreased plasma sodium from low intestinal uptake (Salman and Eddy,

Table 4. Tissue moisture during copper exposure for 42 days.

Treatment	Gill	Liver	Intestine
Initial	73.2±1.20^{a}	71.5±0.40^{a}	75.2±1.04^{a}
Diet 1	74.2±1.80^{a}	76.1±2.77^{b}	72.9±1.43^{a}
Diet 2	69.5±5.64^{a}	62.1±5.24^{a}	76.0±1.43^{a}
Diet 3	76.1±1.62^{a}	67.6±5.42^{a}	72.4±3.03^{a}

Data are means±S.E (n=3 per value), expressed as percentage (%) letters with the same subscript in the same column are not significant different (p>0.05).

Table 5. Tissue moisture during recovery phase on normal diet from 21 days.

Treatment	Gill	Liver	Intestine
Diet 1	77.5 ±1.81^{a}	78.0 3±1.82^{a}	74.3 ±4.46^{a}
Diet 2	75.3 ±3.20^{a}	78.63 ±3.19^{a}	75.9 ±2.00^{a}
Diet 3	79.5 ±6.44^{a}	76.3 ±7.13^{a}	73.6 ±18.30^{a}

Data are means±S.E (n=3 per value), expressed as percentage (%) letters with the same subscript in the same column are not significant different (p>0.05).

Table 6. Growth and nutritional performance of common carp fed control (diet 1), 1000 mgCuKg (diet 2) and 2000 mgCuKg^{-1} (diet 3) for 42 day (exposure phase).

Parameter	Treatment	Exposure
Weight gain (g)	D_1	8.50 ± 5.25^{a}
	D_2	16.2 ± 5.25^{b}
	D_3	-1.67 ± 4.37^{c}
Feed conversion ratio	D_1	0.46 ± 0.18^{a}
	D_2	0.18 ± 0.03^{a}
	D_3	0.51 ± 0.25^{a}
Specific growth rate (% day^{-1})	D_1	0.07 ± 0.05^{a}
	D_2	0.14 ± 0.02^{a}
	D_3	-0.09 ± 0.10^{a}
Condition factor (%)	D_1	1.45 ± 0.02^{a}
	D_2	1.60 ± 0.07^{b}
	D_3	1.41 ± 0.05^{a}
Hepatosomatic index (%)	D_1	0.99 ± 0.07^{a}
	D_2	1.47 ± 0.04^{b}
	D_3	1.74 ± 0.12^{b}
Mean ration size (% body weight)	D_1	0.84 ± 0.04^{a}
	D_2	1.08 ± 0.04^{b}
	D_3	1.06 ± 0.07^{b}

Data are means±S.E (n 3 per value), except for ration size where n= number of daily ration size. Letters with the same subscript in the same column are not significant (p>0.05).

Table 7. Growth and nutritional performance of common carp fed control diet for 21 days.

Parameter	Treatment	Recovery
Weight gain (g)	D_1	5.30 ± 0.60^{a}
	D_2	3.67 ± 2.97^{a}
	D_3	0.83 ± 1.87^{a}
Feed conversion ratio	D_1	0.26 ± 0.03^{a}
	D_2	1.60 ± 0.85^{a}
	D_3	0.28 ± 0.37^{a}
Specific growth rate (% day^{-1})	D_1	0.14 ± 0.03^{a}
	D_2	0.09 ± 0.02^{a}
	D_3	0.02 ± 0.05^{a}
Condition factor (%)	D_1	1.54 ± 0.02^{a}
	D_2	1.39 ± 0.01^{b}
	D_3	1.43 ± 0.02^{b}
Hepatosomatic index (%)	D_1	1.24 ± 0.02^{a}
	D_2	1.65 ± 0.02^{bc}
	D_3	1.84 ± 0.03^{d}
Mean ration size (% body weight)	D_1	0.90 ± 0.04^{a}
	D_2	0.90 ± 0.03^{a}
	D_3	0.89 ± 0.06^{a}

*Data are means ± S.E (n 3 per value), except for ration size where n= number of daily ration size. Letters with the same subscript in the same column are not significant (p>0.05).

1987). The copper accumulated in the gill of the 1000 mgCukg^{-1} exposed fish could, therefore, show that a second high-affinity mechanism for branchial copper uptake in the gills was independent of external sodium (Grossel and Wood, 2002). In the same vein, reduced gill copper of 2000 mgCu/kg (diet 3) exposed fish was due the fact that since sodium and copper shared similarly apical channel, increasing sodium and copper absorption from the intestine elevated plasma concentration of these two mineral/elements beyond the needs of the fish, branchial influx of sodium and copper were inhibited (Salman and Eddy, 1987).

The increased gill copper of the 1000 mgCu/kg diet (diet 2) compared to the control (diet 1) and the 2000 mgCu/kg (diet 3) is an indication of the beginning of stress to which fish is subjected and this stress response stimulated branchial sodium influx into the blood (Lin and Randall, 1995; Karnaky, 1997; Salman and Eddy, 1987) with resultants increase in haematocnt (PCV) (Vosyliene, 1999s). The increased coppers also showed or reflected systemic copper in the gill as a result of the absorption and distribution of dietary copper to the gill from the intestine (Shaw and Handy, 2006) whose copper was

significantly higher ($p<0.05$) compared to the control and the 2000 $mgCukg^{-1}$ diet fish. The high intestinal copper of the 1000 $mgCukg^{-1}$ exposed fish reflected the greater role-played by intestine in regulating absorption and metabolism of copper (Kamunde et al., (2002). This regulatory role by intestine explained the significant reduction in the intestinal copper of the 2000 mgCu/kg diet (diet 3) exposed fish compared to the 1000 mgCu/kg diet (diet 2) fish (Kamunde et al., 2002). In other words, homeostasis is primarily regulated at the intestinal level (Turnlund et al., 1998).The significant increase in liver copper of fish exposed to both elevated dietary copper (1000 and 2000 $mgCukg^{-1}$ reflected its role in copper metabolism and excretion (Berntssen et al., 1999; Hinton and Lauren, 1990). The continued copper accumulation in post exposure in all tissues indicated possible delayed toxic effects of dietary copper. This has also been reported for Nile tilapia (Shaw and Handy, 2006).

Tissue sodium, potassium and calcium

The presence of treatment-dependent changes in tissue ions (Na^{+}, K, Ca) suggest that dietary copper caused major osmotic disturbances which stimulated blood production (high red blood cell, haematocrit) of fish exposed to diet 2 (1000 $mgCukg^{-1}$). This has been reported by Vosyliene (1999) and Mazon et al. (2002). The time dependent increase in sodium and potassium indicated the ability of common carp to acclimatize to long time dietary copper exposure (Kamunde et al., 2002; McDonald and Wood, 1993) and could also reflect the excretion and detoxification capacity of common carp to copper (McDonald and Wood, 1993). The time dependent reduction in gill calcium is sodium dependent (Flik and Verbost, 1993) which explained the further reduction during elevated dietary exposure to 1000 mgCu/kg (diet 2) compared to the control as the gill sodium increased. The high level of gill calcium compared to other tissues in all treatments and the low level of intestinal calcium is due to the simple fact that the gill is the major and primary site of calcium absorption even though dietary calcium inclusion exceeded requirement 1-8% vs. 0.34% (Ogino and Takedal (1976) and uptake (Marshall et al., 1992; Flik and Verbost, 1993), while the intestinal contribution to calcium uptate comes to 30%. Similarly the presence of hypercalcaemic hormone, cortisol in the gill (Hanssen et al., 1989) has been reported to stimulate hypercalcaemia (high calcium), which is calcitrophic (Flik et al., 1989a; Flik and Perry, 1989) making fish capable of surviving extreme hypercalcaemia (up to 10 $mmol^{-1}$ total calcium, 4.5 $mmol^{-1}$ Ca^{2+}, Hanssen et al., 1989). Intestinal absorption of Calcium is inhibited by stanniocalcin (calcium reducing hormone) (Sundell et al., 1992), which reduces intestinal calcium absorption (Sundell and Bjornssen). The calcitropic action of cortisol (a stress hormone, De Boeck et al., 2003), independent of stanniocalcin (Verbost et al., 1993c) become noticeable only in the long term (Flik and Verbost, 1993). This could explain hypercalcaemia observed during the chronic dietary exposure of common carp to copper, which showed their stress response to elevated dietary copper (1000 $mgCukg^{-1}$ and 2000 $mgCukg^{-1}$). The post exposure phase was characterized by significant decrease in sodium and potassium in all tissue examined with increasing calcium after all fish were returned or fed normal diet. This reflected the continued calcitropic (hypercalcaemic) stress hormone, cortisol effect on common carp post exposure (Flik and Verbost, 1993), which initially protected fish against copper accumulation during the exposure phase, but now induced accumulation of the metal in all tissues post exposure (De Boeck et al., 2003). Increasing cortisol production reflects continued depletion of energy in common after exposure to elevated dietary copper. Similarly, the reduction in sodium accounted for the post exposure increase in all tissue examined of copper concentration post exposure (De Boeck et al., 2003). Tables 2 and 3 show ionic response to dietary copper in exposure and recovery phase, respectively.

Moisture

Although other parameters, including osmo regulatory ions, were significantly affected by dietary copper exposure and recovery, moisture remained unaffected and has been consistent with other research on copper toxicity (Mohsen et al., 2006; Shaw and Handy, 2006).The effects of dietary exposure and recovery of copper on the moisture content are presented in Tables 4 and 5.

Growth and nutritional performance

Fish exposed to diet (1000 $mgCukg^{-1}$) showed increase weight gain after 42 and this is reflected in the significant increase in condition factor, % body weight, increased feed intake, increased specific growth rate and increased hepatosomatic index, and reduced feed conversion ratio; although the weight gain, specific growth rate and feed conversion ratio were not statistically significant compared with other treatment. The increased weight gain of the diet 2 fish was an indication of the onset of stress-induced increase in haematologcal indices (haemoglobin, haematocrit and red blood cells) Vosyliene, (1999), and could be secondary response of fish to irritants (Folmar, 1993). Thus as a physiological mechanism of compensation (Vosyliene, 1999) hemoglobin increased to maintain oxygen supply to the fish which translated to increase in the rate of metabolism induced by Na/K ATPase action on blood (Garnong, 1999). Thus fish fed diet 2 tends to increase their feed

intake with consequent increase in weight gain observed after 42 days. However, fish fed diet 3 showed a significant decrease in weight gain which is reflected in decreased condition factor, specific growth rate, and an increased feed conversion ratio compared with other treatments. Mortality was recorded in the 2000 mgCukg-^{1} diet (diet 3) exposed treatment within 3 weeks. Several authors have noted reduction in growth rate during dietary copper exposure in fish (Clearwater et al., 2002). But others have not (Lanno et al., 1985; Handy et al., 1999; Kamunde et al., 2001). Reduced growth rate have been observed in Nile tilapia fed 2000 mgCukg^{-1} (Shaw and Handy, 2006) which is consistent with this research on common carp fed 2000 mgCukg^{-1} (diet 3). The recovery phase was characterized by decrease in feed intake by fish fed 1000 mgCukg^{-1} pre-exposure. This was reflected in reduced weight gain, specific growth rate, condition factor and increased feed conversion ratio post exposure. The 2000 mgCukg^{-1} diet (diet 3) fed fish also showed reduced weight, specific growth rate and a significantly reduced condition factor post exposure. The effects of dietary exposure and recovery on growth are shown in Tables 6 and 7.

In conclusion, copper, although essential in the diet of fish, involved in many physiological and developmental as well as growth, could be deleterious when dietary inclusions exceed that required for proper function of the body in view of its major effects in ionic imbalances in common carp (*Cyprinus carpio)*, which could trigger a whole lot of physiological and enzyme processes in the body, necessary for growth and development. Further research is necessary to determine dietary requirement of tropical fish species, including common carp (Cyprinus carpio).

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