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ORIGINAL ARTICLE

Effects of ambient and dietary cadmium on haematological parametres in *Oreochromis mossambicus* acclimatised to low- and high-calcium water

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Abstract The haematological response to ambient (10μg Cd l^{-1}) or dietary (10μg Cd $fish^{-1}$ day^{-1}) cadmium for 2 and 14days was investigated in tilapia (*Oreochromis mossambicus*) acclimatised to low-calcium (0.2mM Ca^{2+}) and high-calcium water (0.8mMCa^{2+}). Significant reduction of erythrocytes (red blood cells), haemoglobin (Hb), packed cell volume (PCV), mean cell haemoglobin (MCH) and mean cell volume (MCV) occurred in fish exposed to ambient cadmium in low-calcium water. The anaemic response of significantly reduced Hb, PCV, MCH and MCV was also evident in fish exposed to dietary cadmium in low-calcium water. Except for increased mean cell volume of erythrocytes, both ambient and dietary cadmium had no effect on haematological indices in fish from high-calcium water. This study indicates that high-calcium water has a protective effect on cadmium toxicity.

Keywords Haematology · cadmium · *Oreochromis mossambicus*

Introduction

The increasing cadmium concentration in the lakes and rivers is mainly due to industrial processes and other anthropogenic activities. The toxic effects of cadmium on biological processes of aquatic organisms has been reviewed by Coombs (1979) and Wendelaar Bonga and Lock (1992). Fish blood has been widely used as an index of physiological and pathological changes caused by aquatic pollution or diseases (Blaxhall 1972). Haematological abnormalities after exposure to cadmium have been reported in *Pleuronectus flesus* (Johanssen and Larsson 1978), *Puntius conchonius* (Gill and Pant 1983), *Tilapia zillii* (Ghazaly 1992), *Cyprinus carpio* (Koyama and Ozaki 1984) and *Channa punctatus* (Karuppasamy et al. 2005). Most studies on cadmium toxicity in fish deal with acute lethal exposures, whereas information on the effects of low cadmium levels is limited. In natural freshwater bodies, the cadmium concentration is well below the LC_{50} values for most teleost species. Therefore, the LC_{50} studies are usually not relevant for evaluation of the impact of cadmium pollution on fish populations. Hence, studies on the effects of cadmium at water concentrations that are sublethal to fish need more attention.

In most studies reported earlier, fish have been exposed to ambient cadmium, while the information on the toxic effects of cadmium via the food is scarce. Cadmium can progressively concentrate through trophic transfers along the aquatic food chain and consequently form an important source of this metal (Dallinger et al. 1987). Dietary cadmium may therefore yield more representative information for field situations. In addition, water hardness (mainly Ca^{2+}) reduces the toxicity of cadmium in water or food (Pratap et al. 1989). Cadmium has been found to be less toxic to fish in hard water than soft water (Carroll et al. 1979; Pascoe et al. 1986). In this study, the effects of ambient and dietary cadmium on haematological parametres in *Oreochromis mossambicus* acclimatised to low- and high-calcium water were investigated.

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Materials and methods

Laboratory stock of male *O. mossambicus* ranging from 12 to 14cm in total length and 22.0 ± 4.2g in weight (mean ± SD) was used in the present study. The fish were maintained in 100-l aquaria (0.55mM Ca^{2+}) with circulating filtered water (Eheim pumps 1021) at 28°C on a daily12h photoperiod. The water pH ranged from 7.4 to 7.6.

Experimental design

Fish were divided into six groups, of which three were acclimatised to low-calcium water (0.2mM Ca^{2+}) and three to high-calcium water (0.8mM Ca^{2+}) for 2weeks. Artificial freshwater was prepared according to Flik et al. (1985). Acclimation of fish to low and high-calcium water, maintenance, preparation and administration of ambient cadmium (10μg Cd l^{-1}) or dietary cadmium (10μg Cd $fish^{-1}$ day^{-1}) were similar to those reported earlier (Pratap and Wendelaar Bonga 1993). Food was prepared as a mixture containing Tetramin tropical fish food (85%), gelatin (10%) and agar (5%), dissolved in warm distilled water. Cadmium was blended into this mixture. Samples of food were digested in HNO_3 followed by cadmium analysis in Atomic Absorption Spectrophotometre (Thermo Jarell Ash, MA, USA). The measured concentration did not deviate more than 2% of the calculated cadmium concentration. Groups 1, 2, 5 and 6 shown below were fed cadmium-free food. The water temperature, pH and photoperiod were maintained constant to ensure that changes in haematological parametres were not due to environmental variables.

The experimental set-up and cadmium exposure were as follows:

(a) group 1: low-Ca^{2+} water (0.2mM) containing 10μg Cd^{2+} l^{-1}
(b) group 2: high-Ca^{2+} water (0.8mM) containing 10μg Cd^{2+} l^{-1}
(c) group 3: low-Ca^{2+} water (0.2mM), 10μg Cd^{2+} $fish^{-1}$ day^{-1} via the food
(d) group 4: high-Ca^{2+} water (0.8mM) 10μg Cd^{2+} $fish^{-1}$ day^{-1} via the food
(e) group 5: low-Ca^{2+} water (0.2mM) control
(f) group 6: high-Ca^{2+} water (0.8mM) control

Sampling and analytical procedure

Fish from each tank were sampled after 2 and 14days. They were anaesthetised in 0.2% 2-phenoxyethanol. Blood was collected from the caudal vessel in heparinised micro-haematocrit capillaries. After centrifugation (Heraus-Christ Gmbh), the packed cell volume (PCV) was determined. Haemoglobin (Hb) content of the blood was determined spectrophotometrically according to Van Kampen and Jylstra (1961). The red blood cell (RBC) count was done by diluting blood with Hayem's fluid and counted using a Bucker Turk haemocytometre. The haematological indices: the mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and mean cell volume (MCV) were

Table 1 Effects of ambient (10 μg Cd^{2+} l^{-1}) and dietary (10 μg Cd^{2+} $fish^{-1}$ day^{-1}) cadmium on haematological parametres in *Oreochromis mossambicus* acclimatised to low-calcium (0.2 mM Ca^{2+}) and high-calcium (0.8 mM Ca^{2+}) water

	Days	Ambient cadmium		Dietary cadmium		Controls	
		Low-Ca^{2+} water	High-Ca^{2+} water	Low-Ca^{2+} water	High-Ca^{2+} water	Low-Ca^{2+} water	High-Ca^{2+} water
RBC × 10^6/μl	2	1.49±0.15	1.57±0.12	1.58±0.23	1.58±0.24	1.58±0.25	1.57±0.18
	14	1.42±0.12*	1.46±0.18	1.49±0.18	1.49±0.19	1.57±0.19	1.59±0.19
Hb, g/dl	2	7.62±0.65*	8.98±0.73	7.68±0.86*	9.18±0.96	8.89±0.64	8.84±0.63
	14	7.05±0.53*	8.75±0.59	8.04±1.32	8.18±0.38	8.21±0.81	8.58±0.42
PCV, %	2	21.5±3.81*	33.0±5.08	24.2±2.26*	32.8±3.05	30.1±3.85	31.4±4.12
	14	24.0±4.44*	33.8±6.32	32.5±4.16	33.2±4.60	32.6±3.98	31.9±5.02
MCH, %	2	50.14±4.31**	57.31±6.08	48.61±3.73*	58.64±4.01	56.26±4.56	56.30±3.51
	14	49.64±4.41*	46.55±7.37	53.95±7.33	54.89±4.22	53.92±4.26	53.96±4.66
MCHC, %	2	35.44±4.24**	27.21±4.43	31.73±3.30	27.98±3.14	29.53±4.75	28.15±4.52
	14	29.37±3.83	25.97±3.93	24.73±4.13	24.63±4.82	26.98±4.25	27.76±4.83
MCV, μ^3	2	144.29±15.40***	210.57±14.23	153.16±09.13***	207.23±12.71	198.51±13.40	200.10±12.28
	14	169.01±13.66***	233.10±17.93***	218.12±14.04**	222.81±15.11**	202.64±15.15	200.62±15.57

Values are expressed as mean ± SD of ten fish per group
*p<0.05; **p<0.01; ***p<0.001

calculated according to the formula described by Dacie and Lewis (1984). In normal circumstances, the physiology of fish can change with time, therefore Students' *t*-test was used to test each experimental group with their respective controls. Statistical analysis between groups was performed using analysis of variance (ANOVA) with significance level set at $p < 0.05$.

Results and discussion

In all the experimental groups, abnormal behaviour and morphological changes were not observed in fish exposed to ambient or dietary cadmium for 14days. Changes in fish blood parametres have been widely used as an index of the health status in polluted environments (Jezierska and Witeska 2001). The occurrence of cadmium at low concentrations in freshwater bodies can be toxic to fish due to bioaccumulation and may cause haematological crisis (Coombs 1979). In this study, the RBC count in fish after 2days exposure to ambient or dietary cadmium in low- and high-calcium water were not significantly different from their respective controls. On day14, a significant decrease of RBC occurred only in fish exposed to ambient cadmium in low-calcium water (Table 1). In high-calcium water, acclimatised tilapia exposed to ambient or dietary cadmium for 2 and 14 days, the RBC, Hb, PCV, MCH and MCHC were not different from their respective controls; however, a significant increase in MCV occurred in both the exposure routes after 14 days (Table 1). Reduced RBC, Hb and PCV were reported in flounders exposed to 5, 50 and 500 µg/l cadmium for 4 and 9 weeks (Johanssen and Larsson 1978), and in carp exposed to 5, 10 and 50 ppb cadmium for 30, 50, 70 and 90 days (Koyama and Ozaki 1984). Karupassamy et al. (2005) observed megaloblastic anaemia in air-breathing fish, *Channa punctatus*, exposed to 29 mg/l cadmium for 7, 15 and 30 days in which the RBC, Hb, PCV and MCHC had significantly declined while MCH and MCV were elevated.

On days 2 and 14, the Hb, PCV, MCH and MCV had reduced significantly while the MCHC on day 2 had significantly increased in fish exposed to ambient cadmium in low-calcium water. A similar significant decline of Hb, PCV, MCH and MCV occurred only on day 2 in fish exposed to dietary cadmium in low-calcium water. In tilapia exposed to ambient cadmium in low-calcium water, a transient decline of RBC count accompanied by reduced Hb, PCV and MCH is indicative of haemodilution due to disturbed osmo-ionic regulation and gill damage (Pratap et al. 1989; Pratap and Wendelaar Bonga 1993) that would elevate Cd^{2+} uptake and further suppress erythropoiesis (Houston and Keen 1984). Previous studies showed that when tilapia is exposed to ambient or dietary cadmium, the recovery of gill damage and altered homeostasis was more pronounced in fish from high-calcium water than low-calcium water. In this study, the most prominent effect of ambient and dietary cadmium on haematological parametres occurred in fish from low-calcium water.

Cd^{2+} is known to block Ca^{2+} channels and impedes normal membrane function of erythrocytes (Verbost et al. 1989). In vivo, Cd^{2+} poisoning caused loss of membrane permeability and resulted into anaemia due to increased erythrocyte haemolysis (Kunimoto et al. 1986). Various studies have shown that Cd^{2+} and Pb^{2+} causes haemolytic anaemia (Coombs 1979; Jezierska and Witeska 2001). Cd^{2+} probably affects iron metabolism (Houston and Keen 1984) while Pb^{2+} inhibits the δ-aminolevulinic acid dehydratase activity (Johanssen and Larsson 1978). In this study, the initial decline of RBC followed by reduced Hb, PCV and MCV in fish from low-calcium water suggests that premature cells were produced in response to Cd^{2+}-induced haemolysis and the oxygen-binding capacity could be affected. The significant increase of MCV of erythrocytes in tilapia exposed to ambient cadmium or fed cadmium food could mainly be attributed to loss of membrane integrity. Wu and Deng (2006) reported that although the numbers of circulating erythrocytes were normal after 12 h in tilapia-injected 3 mg/l cadmium, the size of RBCs and oxygen-binding capacity of haemoglobin was significantly reduced. During Cd^{2+}-induced stress, there is high energy demand and fish tries to maintain erythrocytes counts within the physiological limits using various mechanisms of compensation (Jezierska and Witeska 2001). The results of this study corroborates with the earlier findings that Ca^{2+} has a protective role against Cd^{2+} toxicity (Verbost et al. 1989; Pratap et al. 1989) because haematological indices in tilapia from low-calcium water were most affected.

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