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Effects of Cadmium, Naphthalene, and DDVP on Gut Carbohydrases Activity in Bream (*Abramis brama* L.) and Mozambique Tilapia (*Oreochromis mossambicus* Peters)

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Previous research has shown that sublethal concentrations of cadmium, naphthalene and dichlorvos (DDVP) decreased growth rates in bream and Mozambique tilapia (Gerassimov et al. 1989; Pavlov et al. 1991). One of the factors known to affect fish growth is the activity of gut digestive enzymes such as of lipases, proteases, carbohydrases (Fange and Grove 1979). We assumed that toxicant-induced inhibition of the digestive enzyme activity and, consequently, the impaired digestion of food may contribute to the reduction of growth in fish exposed to toxicants. However, the influence of toxicants on digestive enzyme activities is poorly studied. The contribution of toxicant-induced changes of digestive enzymes activity to growth rate retardation in exposed fish remains unknown. The goal of this study was to examine the influence of an organophosphorus insecticide DDVP, a polyaromatic hydrocarbon naphthalene, and a metal cadmium on fish gut carbohydrase (CH) activity.

MATERIALS AND METHODS

Adult and young-of-the-year bream (Abramis brama L.) (average weight 700 g and 4.5 g, respectively) and 6-mon old Mozambique tilapias (Oreochromis mossambicus Peters) (average weight 6.2 g) were used in this study. Adult bream were caught in July in the Rybinsk reservoir (Russia, 57°N, 36°E) and were analyzed just after they were caught. Bream young-of-the-year were hatched from eggs of parent fish caught in the wild and spawned in the experimental ponds. During the first 6

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mon these fish were kept in the ponds and then transferred to laboratory aquaria with flowing well water for the next 6 mon before the experiments were started. Tilapias were obtained from the stock population raised in laboratory for several generations. Both young-of-the-year bream and tilapia were fed ad libitum once daily artificial trout chow containing 46 g crude protein, 11 g fat, 18 g carbohydrates, and 16 g ash per 100 g.

Technical grade DDVP (O,O-dimethyl-O-(2,2-dichlorovinyl) phosphate; 80 % of active ingredient), technical grade naphthalene (96% pure) and cadmium sulfate (chemical grade) were tested. DDVP was purchased from Volgograd Pesticide Producing Factory, Volgograd, Russia, naphthalene was purchased from Tcherepovets Coal Processing Factory, Tcherepovets, Russia, and cadmium sulfate was purchased from Red Chemist Co., St. Petersburg, Russia. The DDVP concentration was calculated according to active ingredient and cadmium concentration according to total cadmium. Cadmium sulfate was predissolved in distilled water, and the organic compounds were predissolved in acetone and then dissolved in distilled water. After that the toxicants were introduced to diluter toxicant chambers in the in vivo experiments or to experimental media in the in vitro experiments. In the in vivo study final acetone concentration in the experimental aquaria did not exceed 0.005 % (v/v). In the in vitro study final acetone concentration did not exceed 0.001 % either in control or test media. Special experiments carried out previously revealed that these concentrations of acetone did not affect enzyme activity and no acetone controls were carried out in the in vivo study.

To examine the effects of toxicants in vivo we exposed Mozambique tilapia to sublethal concentrations of toxicants during 60 d. Two groups of fish (12 specimens each) were exposed to each toxicant. The fish were placed into 30-L Plexiglas aquaria and toxicants were delivered using the diluter (Vinogradov and Tagunov 1989) so that 90 % water replacement was achieved in every 4 hr. Well water with average temperature 22°C (21°–23° C), pH 7.7 (7.7-7.9), Ca^{2+} content about 40 mg/L, and oxygen content 7.0 mg/L (6.5 – 7.5 mg/L) was

used for the experiment. Before the experiment the fish were kept 14 d in the aquaria to acclimate to the experimental conditions. The tested toxicants concentrations were 0.46 mg/L for DDVP, 1.5 mg /L for naphthalene and 5.0 mg/L for cadmium, that was about 1/15 LC50 according to original data (Frumin et al. 1992). The feeding regimen during the exposure was equal to the preexposure period.

The fish were sampled 15, 30, 45, and 60 d after the beginning of exposure. For analysis 3 fish from each group (6 fish/assay) were decapitated, the guts were removed and weighed. In the case of adult fish the CH activity was analyzed only in gut mucosa. In the case of younger fish the CH activity was analyzed in whole gut. The homogenates were prepared in Ringer's solution (103 mM NaCl, 1.9 mM KCl, 0.45 mM $CaCl_2$, 1.5 mM NaH_2PO_2, 0.17 mM KH_2PO_4, 1.37 mM $MgSO_4$). All preparation procedures were performed at 0 - 4°C. In the cadmium study phosphate ions were excluded from Ringer's solution to prevent precipitation of cadmium. To determine total CH activity 1 mL of homogenate was mixed with 1 mL of 1.8 g/L solution of soluble potato starch (chemical grade, Shostka Chemical Reagents, Shostka, Ukraine), that served as substrate. The mixture was incubated for 30 min at 20°C. After the incubation the total hexoses concentration produced during the hydrolisis of starch was determined using colorimetric procedure by Nelson (1944) as modified by Ugolev and Iezuitova (1969). D-glucose (2mM, chemical grade, Shostka Chemical Reagents, Shostka, Ukraine) was used as a reference standard for hexoses determination. Total CH activity is primarily the sum of activities of α -amylase (EC 3.2.1.1), γ -amylase (EC 3.2.1.3), and maltases (EC 3.2.1.20) (Ugolev and Iezuitova 1969) and was expressed as µmol hexoses/g wet tissue/min. The conditions for CH activity determination are optimal for freshwater teleosts (Kuz'mina and Golovanova 1980).

In the <u>in vitro</u> experiments before the incubation with starch, the homogenates (from 8 fish per each concentration) were incubated with toxicant solutions for 60 min. The homogenates from the same fish served as controls and were incubated for the same time in Ringer's solution without the toxicants. The nominal

toxicant concentrations in the in vitro study were 0.05, 0.1, 0.5, 2.5, 5, 10, 25, and 50 mg/L for total cadmium, 0.3, 1.5, 3, and 15 mg/L for naphthalene, and 0.2, 2, 20, and 100 mg/L for DDVP. The results of the in vivo study were analyzed statistically using Student's t-test (Lakin 1969). Results of the in vitro assay were statistically analyzed using one-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference test for difference of means (Steel and Torrie 1980), at a 5% level of significance.

RESULTS AND DISCUSSION

The results of the in vivo study are given in Table 1. Gut CH activities in tilapia exposed to DDVP were higher 30 d and lower 60 d after exposure compared with control. Exposure to naphthalene did not alter fish gut CH activity significantly. CH activity in cadmium-exposed fish were lower than in control (ranging from 35.1 % to 82.5 % of control) during all exposure period except day 30 when the differences were not statistically significant.

In all tested concentrations of both naphthalene and DDVP in vitro did not affect gut CH activity either in bream or tilapia (Tabl.2). Mean CH activities fluctuated between 2.8 and 3.6,103.5 and 116.7, 51.2 and 60.3 µmol/g tissue /hr in adult and young-of-the-year bream and in tilapia, respectively. One-way ANOVA showed that CH activities were significantly influenced by cadmium in vitro in adult ($F=2.87$, $P=0.0342$) and young-of-the-year bream ($F=6.208$, $P=0.0005$) and in tilapia ($F=35.49$, $P<0.0001$). Incubation of gut homogenates with cadmium concentrations 50 mg/L and 25 mg/L caused significant inhibition of CH activities in both species (Table 2). In tilapia this inhibition was more pronounced. Cadmium concentration 5 mg/L caused significant inhibition of CH activity only in tilapia. Lower cadmium concentrations did not affect gut CH activity in both species. The study revealed that cadmium effect upon gut CH activity in young-of-the-year bream and in tilapia was concentration-dependent and higher concentrations resulted in more pronounced enzyme inhibition. In adult bream the effect of cadmium was not concentration-dependent.

Table 1. *In vivo* effects of DDVP, naphthalene, and cadmium on total gut CH activity (μmol/g tissue/hr) in Mozambique tilapia.

Duration of exposure, d	Control	DDVP	Naphthalene	Cadmium
15	86.6±2.2	89.46.6	85.3±3.2	59.6±5.6*
30	69.3±5.0	84.1±1.4*	90.3±10.2	57.2±7.4
45	82.8±8.0	81.5±5.2	75.3±6.0	47.1±4.7*
60	84.1±4.0	69.4±2.9*	82.5±9.8	29.5±4.6*

Means±standard error are presented.
* – significantly different from control ($P<0.05$)

Control gut CH activity in adult bream was one to two orders lower then in younger bream and in tilapia. Our previous research has shown that gut CH activity (in younger fish CH activity was determined in whole gut, in adult fish in gut mucosa) in bream caught in wild in general did not depend on fish age (Golovanova and Kuz'mina 1984). The values of this activity in wild bream were close to that in adult bream determined in present study. It is known that total CH activity may be increased by carbohydrate-rich food (Karasov and Diamond 1983). We assume that higher values revealed in young-of-the-year bream in this study are due to induction of enzyme activity by the carbohydrate-rich artificial food rather than due to age-dependent changes. The fact that natural food of wild bream contains 2-5% of carbohydrates (Kuz'mina et al. 1979) while the artificial food of young-of-the-year bream in our study contains greater amount of carbohydrates (18%) supports this assumption. As for tilapia this species is normally phytoplanctivorous fish. It was found earlier that total CH activities in herbivorous fish are higher than in carnivorous (Fange and Grove 1979). This seems to be a more likely reason for higher gut CH activity in tilapia compared with zoobenthivorous wild bream.

The data presented here demonstrates that the gut CH activity in tilapia is more sensitive to cadmium

Table 2. *In vitro* cadmium effect on total gut CH activity (μmol/ g tissue /hr) in bream and Mozambique tilapia

Cadmium concentration, mg/L	Bream Adult	Bream Young-of-the-year	Tilapia
Control	2.66±0.44^{A}	112.05±1.84^{A}	52.56±0.98^{A}
0.5	2.42±0.19^{B}	109.70±5.75^{A}	51.62±1.75^{A}
5	2.62±0.92^{A}	107.48±3.72^{A,B}	43.73±2.10^{B}
25	2.40±0.32^{B}	100.47±3.52^{B}	36.43±2.28^{B}
50	2.45±0.18^{B}	91.68±4.26^{C}	30.28±1.64^{B}

Means±standard error are presented. In control N=24 fish/assay, in other variants N=8 fish/assay. Means in the same column with the same letter superscript are not significantly ($P<0.05$) different (Tukey's HSD test)

in vitro than in bream. Consequently, the digestion process in phytoplanctivorous tilapia may be more sensitive to negative influence of cadmium compared with bream. However, cadmium inhibited CH activity in bream as well. This inhibition was evident both in adult bream that consumed natural food in wild and in young-of-the-year bream fed artificial food. As we noted *in vitro* cadmium effect upon adult bream CH activity was not concentration-dependent as opposite to young-of-the-year bream and tilapia. The possible explanation of this fact is that in adult bream we determined CH activity and evaluated cadmium effect in gut mucosa while in younger bream and in tilapia we studied whole gut CH activity.

Change in gut CH activity revealed in the *in vivo* study may be either due to presence or absence of food within the gut or due to direct impact of the toxicants. In the case of DDVP the results have shown that *in vitro* this toxicant did not influence enzyme activity, but altered it *in vivo*. We assume that change of gut CH activities

revealed in DDVP-exposed fish was not the result of direct toxicant action upon enzyme and more likely was due to changing food content in gut. Food content may depend on motivation to feed (appetite) and on ability of fish to find, to capture and to process food. The alteration of fish feeding behavior resulting in decreased food consumption was often observed in fish exposed to toxicants (Sandheinrich and Atchison 1990). Comparison of the data on in vivo and in vitro naphthalene and cadmium effects on gut amilolitic enzyme activity shows that the results of both studies are consistent. Naphthalene did not affect gut CH activity while cadmium decreased it both in vivo and in vitro. The inhibition of CH activity by cadmium in vitro revealed that the decrease of enzyme activity in the gut of cadmium-exposed fish may be due to direct toxicant effect on the enzymes. Thus the reduction of growth rate in naphthalene and DDVP exposed fish is not the result of toxicant-induced impairment of food digestion by the inhibition of digestive enzymes and has another reason, such as impaired feeding behavior. The inhibition of gut CH activity by cadmium may negatively influence digestion and may contribute to growth retardation in cadmium-exposed fish.

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