The ability of recovery in common carp after nitrite poisoning

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ABSTRACT: The aim of the study was to assess the ability of recovery in common carp after nitrite poisoning and to distinguish the difference in nitrite poisoning of carp at two different chloride concentrations in water. Two groups of carp (group 1 and 2) were exposed to environmental nitrite concentration of 1.45 mmol/l NO_2^- at different chloride concentrations (group 1: 0.31 mmol/l Cl^- and group 2: 3.73 mmol/l Cl^-) for 48 hours. After that, they were transferred into nitrite-free water. In the group 1 mortality of 51% occurred during nitrite exposure and further 11% mortality was observed after 24 h in nitrite free water. No mortality occurred in group 2 and control. Nitrite and methaemoglobin concentrations (MetHb) markedly increased in group 1 (plasma: 10.5 ± 1.90 mmol/l NO_2^-, liver: 3.5 ± 1.15 mmol/kg NO_2^-, muscle: 1.5 ± 0.37 mmol/kg NO_2^-, and MetHb: 93 ± 6.1%) compared with control (plasma: 0.05 ± 0.04 mmol/l NO_2^-, liver: 0.02 ± 0.01 mmol/kg NO_2^-, muscle: 0.04 ± 0.01 mmol/kg NO_2^-, and MetHb: 3 ± 2.9%). After 24 h in nitrite-free water, the values mildly decreased but not significantly (plasma: 5.1 ± 1.49 mmol/l NO_2^-, liver: 1.8 ± 0.65 mmol/kg NO_2^-, muscle: 0.8 ± 0.23 mmol/kg NO_2^-, and MetHb: 84 ± 11.2%). After next 120 h the values decreased significantly and were compared with those found in the control group (traces of nitrite, MetHb: 3 ± 2.1%). Nitrite exposure caused also increase in plasma K^+ (3.8 ± 0.29 mmol/l), ammonia (230 ± 92 µmol/l), urea (1.7 ± 0.28 mmol/l) and uric acid concentration (66 ± 54 µmol/l) in group 1. On the other hand, values of haematocrit, erythrocyte count and haemoglobin concentration were markedly lower than control values. Most changes were corrected by the end of the recovery period, only plasma potassium concentration dropped bellow the control values. Nearly no changes were found in the group 2 compared with the control during the whole experiment duration. This shows the positive effect of chlorides on the fish resistance against nitrites.

Keywords: *Cyprinus carpio* L.; acute toxicity; haematology; ammonia; urea; uric acid; potassium

Nitrite is an intermediate product in bacterial nitrification and denitrification processes in the nitrogen cycle in ecosystems. The concentration of nitrite in natural waters is usually low (µmol/l). However, elevated concentrations of nitrite occur in water receiving nitrogenous wastes, in hypoxic environments or in effluents from industries producing metal, dyes, and celluloid (Pitter, 1999). In aquaculture, high levels of nitrite up to 2 mmol/l (Avnimelech et al., 1986) can be found causing sever health deterioration and even mass mortality of fish (Dvorak, 2004; Svobodova et al., 2005a). Nitrite problems are typically more likely in closed intensive culture systems due to insufficient, inefficient or malfunctioning filtration systems removing waste ammonia from water by means of nitrification.

Nitrite is actively taken up across the gills and accumulates in plasma, gills, liver, brain and muscle (Bath and Eddy, 1980; Margiocco et al., 1983; Gisbert et al., 2004). Nitrite subsequently enters tis-

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sues cells and within the red blood cells it oxidises haemoglobin to methaemoglobin, which is unable to transport oxygen (Cameron, 1971). Apart from methaemoglobinaemia, studies on fish and crustaceans have revealed that nitrite is a disrupter of a large variety of physiological functions, including ion regulatory, respiratory, endocrine and excretory processes (Jensen, 2003; Kroupova et al., 2005).

Nitrite toxicity to fish depends strongly on water quality (temperature; kation, anion and oxygen concentration) (Lewis and Morris, 1986). One of the most important factors influencing nitrite toxicity to fish is considered chloride concentration in water, i.e., nitrite toxicity decreases with increasing chloride concentration (Crawford and Allen, 1977; Lewis and Morris, 1986; Pistekova et al., 2005). The reason for such a positive effect of chlorides can be found in the mechanism of ion uptake in the gills of freshwater fish. Specifically, nitrite has an affinity for the branchial chloride uptake mechanism, presumably the Cl^-/HCO_3^- exchanger; thus, whenever nitrite is present in the ambient water, a part of the Cl^- uptake will be shifted to NO_2^- uptake (Williams and Eddy, 1986; Jensen, 2003). Similar behaviour of nitrite and chloride was explained by the similar hydrated size and hydration energy of chloride and nitrite ions. These findings indicate that lethal concentration of nitrite need to be expressed in relation to chloride content in water. European inland fisheries advisory commission (EIFAC) also recommended to monitor $Cl^-/N\text{-}NO_2^-$ ratio in aquaculture (EIFAC, 1984).

The aim of the study was to distinguish the difference in nitrite poisoning of common carp (*Cyprinus carpio* L.) at two different chloride concentrations in water. Specifically, to verify whether enhanced chloride concentration is sufficient to protect fish against high nitrite concentration represents 96hLC50 of nitrite and subsequently to study the ability of the fish to recover in nitrite-free water. The differences were assessed in terms of haematological and biochemical parameters of the blood and nitrite concentrations in selected tissues.

MATERIAL AND METHODS

Experimental animals

Two-year-old common carp (*Cyprinus carpio* L.; weight 280 ± 52 g, mean ± SD) were obtained from a local fish hatchery and maintained for 1 week in 300 l tanks with dechlorinated tap water. The fish were not fed during acclimation period and in the experiment.

Experimental protocol and fish sampling

The acute toxicity test was performed in a static assay for 48 h, the OECD Direction No. 203 was observed during the test. Fish were kept in tanks each containing 200 l of test solution. Basic chemical indices of diluting water (tap water) were as follows: $ANC_{4.5}$ (acid neutralisation capacity) 0.5 mmol/l; COD_{Mn} (chemical oxygen demand) 0.96 mg/l; total ammonia traces; NO_3^- 0.15 mmol/l; PO_4^{3-} traces; sum of $Ca^{2+} + Mg^{2+}$ 1.25 mmol/l, Cl^- 0.31 mmol/l. Oxygen saturation of water ranged between 68% to 96%, water temperatures in the test ranged from 20.1°C to 21.0°C and pH ranged from 7.08 to 7.51. At the beginning of the experiment, the fish were divided into three groups and exposed to different nitrite and chloride concentrations:

– group 1: 1.45 mmol/l NO_2^-, 0.31 mmol/l Cl^- (Cl^-/NO_2^- molar ratio: 0.21)
– group 2: 1.45 mmol/l NO_2^-, 3.73 mmol/l Cl^- (Cl^-/NO_2^- molar ratio: 2.57)
– control: traces NO_2^-, 0.31 mmol/l Cl^-

Each treatment had two replicates. Desired nitrite and chloride concentrations were obtained by addition of sodium nitrite ($NaNO_2$) and sodium chloride (NaCl) to the tap water.

After 48-hour exposure, each experimental group was separately transferred to nitrite-free water (tap water, 0.31 mmol/l Cl^-). Regeneration period lasted for 144 hours.

Fish were sampled immediately after 48-h nitrite exposition, after 24 h in nitrite-free water and after 144 h in nitrite-free water. Eight fish (four from each replicate tank) were randomly collected from each group at each sampling time to obtain samples of the blood, muscle and liver. The blood samples were taken from the heart with heparinised syringes (cardiac puncture) and the blood was stabilised by 40 IU of sodium heparin per 1 ml blood. Immediately after sampling, the erythrocyte count (RBC), haematocrit (PCV), haemoglobin (Hb), methaemoglobin (MetHb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and leukocyte count (WBC) were determined in the blood samples (Svobodova et al., 1991). Plasma was separated from cells by centrifu-

gation (10 min at 12 000 × g) at 4°C and plasma K^+, Cl^-, NO_2^-, ammonia (NH_3), urea nitrogen (UREA) and uric acid concentrations (URIC) were determined. After blood sampling, the fish were quickly stunned with a blow to the head, and approximately 1 g pieces of muscle and liver were taken. In these tissues, the nitrite concentration was also measured. The tissue samples were kept at –80°C until time of analysis to prevent nitrite oxidation.

Nitrite levels in the blood plasma and in selected tissues (muscle and liver) were determined spectrophotometrically using method of Shechter et al. (1972) based on diazotation and coupling reactions involving sulphanilic acid and Cleve's acid. An alkaline extraction using zinc sulphate and sodium hydroxide as deproteinizing agents was necessary due to the instability of nitrite in an acidic environment (Shechter et al., 1972). Plasma K^+ and Cl^- concentrations were measured by ion-selective electrodes (ADVIA 1650, Bayer). VETTEST 8008 Analyser (IDEXX Laboratories, Westbrook, ME, USA) was used for determination of plasma ammonia (NH_3), urea nitrogen (UREA) and uric acid (URIC) concentrations.

Statistical assessment of results was carried out by means of Statistica software 6.1 for Windows (StatSoft). Data were firstly tested for normality (Kolmogorov-Smirnov test) and homoskedasticity of variance (Bartlett's test). If those conditions were satisfied, two-way analysis of variance (ANOVA) was employed to determine whether there were any significant differences in measured variables between experimental groups. When a difference was detected ($P < 0.05$), Tukey's multiple comparison test was applied to identify which treatments were significantly different. If the conditions for ANOVA were not satisfied, the non-parametric Kruskal-Wallis's test was used (Zar, 1996).

RESULTS

Mortality

High fish mortality was noticed during nitrite intoxication (51%) and after 24 h in nitrite-free water (11%) in group 1. No mortality was observed in group 2 and control during the whole duration of the experiment.

Haematological parameters

After the acute nitrite exposure, the methaemoglobin content increased markedly ($P < 0.01$) in experimental group 1 compared with the experi-

Table 1. Haematological indices of common carp after 48-h nitrite exposure (E) and during recovery period (24-h R and 144-h R)

Indices	Group	Sample time (h)		
		48 (E) mean ± SD	24 (R) mean ± SD	144 (R) mean ± SD
Haemoglobin (g/l)	1	50.9 ± 10.86[b]	61.8 ± 7.04[a]	71.7 ± 8.21[a]
	2	83.0 ± 6.40[a]	78.6 ± 8.79[a]	77.7 ± 7.22[a]
	control	72.8 ± 14.61[a]	75.1 ± 7.09[a]	80.4 ± 5.19[a]
Erythrocyte count ($\times 10^{12}$/l)	1	1.05 ± 0.108[b]	1.28 ± 0.276[a]	1.50 ± 0.220[a]
	2	1.72 ± 0.227[a]	1.72 ± 0.198[a]	1.61 ± 0.200[a]
	control	1.54 ± 0.238[a]	1.51 ± 0.149[a]	1.47 ± 0.132[a]
Haematocrit (l/l)	1	0.20 ± 0.024[b]	0.21 ± 0.023[b]	0.28 ± 0.027[a]
	2	0.32 ± 0.035[a]	0.34 ± 0.023[a]	0.30 ± 0.025[a]
	control	0.30 ± 0.039[a]	0.31 ± 0.027[a]	0.30 ± 0.021[a]

E – group 1: 1.45 mmol/l NO_2^-, 0.31 mmol/l Cl^-; group 2: 1.45 mmol/l NO_2^-, 3.73 mmol/l Cl^-; control: nitrite-free tap water, 0.31 mmol/l Cl^-

R – nitrite-free tap water, 0.31 mmol/l Cl^-

Values are expressed as means ± SD. Significant differences ($P < 0.01$) between individual groups at each sample time are indicated by different letters (a, b)

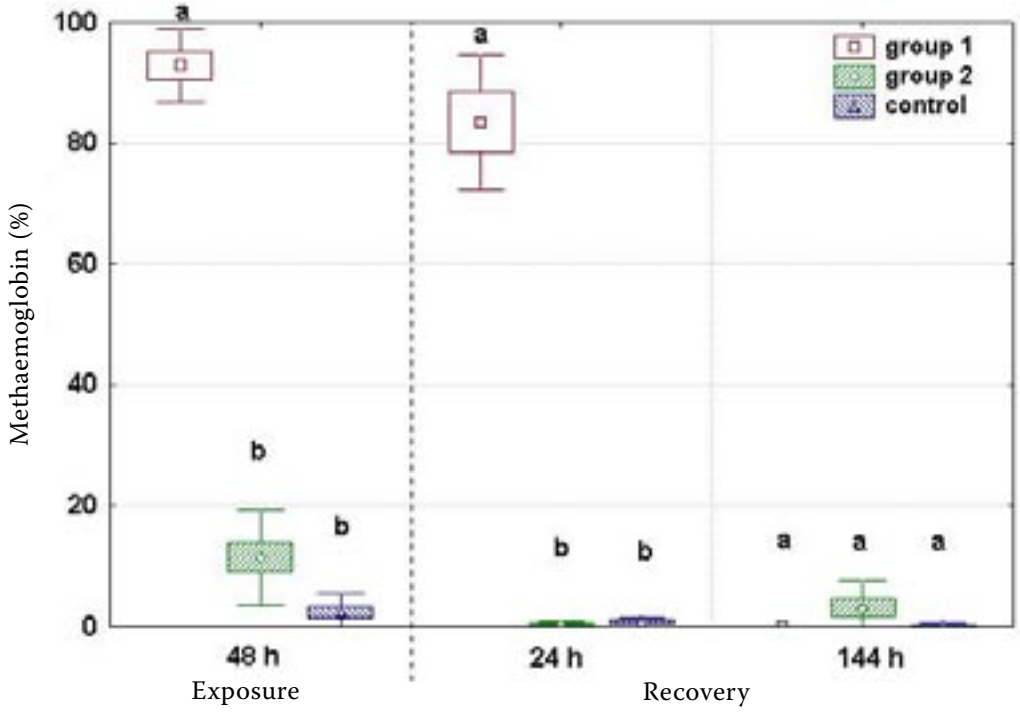

Figure 1. Methaemoglobin concentration in the blood of common carp after 48-h nitrite exposure and during recovery period (24-h and 144-h). Box: mean ± SE, whisker ± SD. Significant differences ($P < 0.01$) between individual groups at each sample time are indicated by different letters (a, b)

mental group 2 and control (Figure 1). On the other hand, haemoglobin concentrations, erythrocyte counts and haematocrit values were lower ($P < 0.01$) than in other groups of carp (Table 1).

Methaemoglobin concentration remained significantly higher in group 1 after 24 h in nitrite-free water, but significantly decreased and achieved control values by the end of the experiment (Figure 1). After 24 h in nitrite-free water, haemoglobin concentrations ($P < 0.01$) and erythrocyte counts ($P < 0.01$) significantly increased, reached control values and remained unchanged by the end of the experiment (Table 1). Haematocrit reached control values at the end of the recovery period. In group 2, haematological parameters did not differ significantly from control values during the whole duration of the experiment (Table 1).

Plasma parameters

Major changes were observed in plasma K^+ values (Table 2) in all of the test groups. Nitrite exposure caused elevation ($P < 0.01$) of potassium concentration in group 1 compared with other groups of carps. During recovery period, potassium levels were decreasing and at the end of the experiment dropped ($P < 0.01$) bellow the control values. On the other hand, K^+ values were significantly increasing during regeneration period in group 2 and control.

No statistically significant differences were observed in plasma Cl^- concentrations among all of the test groups (Table 2).

After nitrite poisoning, increased values of uric acid concentration ($P < 0.01$) were found in group 1 compared with other groups, where the values lay bellow the detection threshold (< 6 μmol/l) of the method used. However, in nitrite-free water, URIC levels dropped bellow the detection threshold even in group 1 (Table 2). Nitrite exposure also caused elevation of urea-N levels ($P < 0.01$) only in the blood plasma of the group 1. After 24 h in nitrite-free water, urea-N levels decreased and did not differ from control values (Table 2). Plasma ammonia concentrations were increased ($P < 0.01$) in group 1 and group 2 after nitrite exposure and even after 24 h in nitrite-free water. At the end of the experiment, ammonia levels dropped in these groups and were comparable with control values (Table 2).

Table 2. Blood plasma indices of common carp after 48-h nitrite exposure (E) and during recovery period (24-h R and 144-h R).

Indices	Group	Sample time (h)		
		48 (E) mean ± SD	24 (R) mean ± SD	144 (R) mean ± SD
K^+ (mmol/l)	1	3.8 ± 0.29[a]	3.0 ± 0.45[a]	1.7 ± 0.28[b]
	2	1.9 ± 0.48[b]	2.2 ± 0.40[b]	2.7 ± 0.43[a]
	control	1.8 ± 0.39[b]	1.5 ± 0.35[c]	2.7 ± 0.39[a]
Cl^- (mmol/l)	1	114 ± 8.0[a]	114 ± 4.9[a]	116 ± 2.2[a]
	2	118 ± 1.9[a]	109 ± 4.0[a]	117 ± 1.3[a]
	control	113 ± 2.8[a]	113 ± 3.0[a]	116 ± 3.5[a]
Ammonia (µmol/l)	1	230 ± 92[a]	245 ± 62[a]	93 ± 13[a]
	2	200 ± 45[a]	212 ± 42[a]	81 ± 19[a]
	control	90 ± 56[b]	137 ± 42[b]	84 ± 16[a]
Urea nitrogen (mmol/l)	1	1.7 ± 0.28[a]	1.1 ± 0.31[a]	1.1 ± 0.05[a]
	2	1.3 ± 0.24[b]	1.3 ± 0.12[a]	1.2 ± 0.18[a]
	control	1.2 ± 0.19[b]	1.3 ± 0.11[a]	1.2 ± 0.16[a]
Uric acid (µmol/l)	1	66 ± 54	11 ± 9	< 6
	2	< 6	< 6	< 6
	control	< 6	< 6	< 6

E – group 1: 1.45 mmol/l NO_2^-, 0.31 mmol/l Cl^-; group 2: 1.45 mmol/l NO_2^-, 3.73 mmol/l Cl^-; control: nitrite-free tap water, 0.31 mmol/l Cl^-

R – nitrite-free tap water, 0.31 mmol/l Cl^-

Values are expressed as means ± SD. Significant differences ($P < 0.01$) between individual groups at each sample time are indicated by different letters (a, b)

Table 3. Nitrite concentration in the liver and muscle of common carp after 48-h nitrite exposure (E) and during recovery period (24-h R and 144-h R)

Indices	Group	Sample time (h)		
		48 (E) mean ± SD	24 (R) mean ± SD	144 (R) mean ± SD
Liver NO_2^- (mmol/kg)	1	3.5 ± 1.15[a]	1.8 ± 0.65[a]	0.03 ± 0.011[a]
	2	0.02 ± 0.012[b]	0.01 ± 0.005[b]	0.01 ± 0.005[a]
	control	0.02 ± 0.010[b]	0.01 ± 0.005[b]	0.02 ± 0.005[a]
Muscle NO_2^- (mmol/kg)	1	1.5 ± 0.37[a]	0.8 ± 0.23[a]	0.04 ± 0.029[a]
	2	0.06 ± 0.018[b]	0.03 ± 0.006[b]	0.02 ± 0.005[a]
	control	0.04 ± 0.014[b]	0.02 ± 0.019[b]	0.02 ± 0.005[a]

E – group 1: 1.45 mmol/l NO_2^-, 0.31 mmol/l Cl^-; group 2: 1.45 mmol/l NO_2^-, 3.73 mmol/l Cl^-; control: nitrite-free tap water, 0.31 mmol/l Cl^-

R – nitrite-free tap water, 0.31 mmol/l Cl^-

Values are expressed as means ± SD. Significant differences ($P < 0.01$) between individual groups at each sample time are indicated by different letters (a, b)

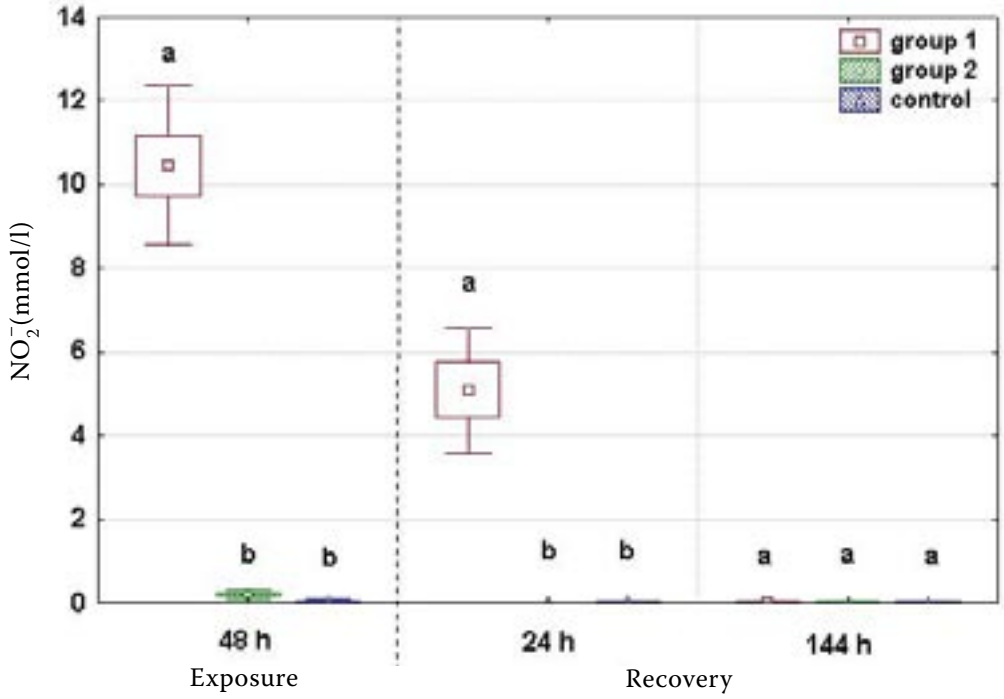

Figure 2. Nitrite concentration in the blood plasma of common carp after 48-h nitrite exposure and during recovery period (24-h and 144-h). Box: mean ± SE, whisker ± SD. Significant differences ($P < 0.01$) between individual groups at each sample time are indicated by different letters (a, b)

Nitrite concentration in plasma, liver and muscle

After 48-h nitrite exposure, carp of the group 1 accumulated the toxicant in the plasma, liver and muscle. The highest nitrite concentration was observed in the plasma, followed by the liver and muscle. After 24 h in nitrite-free water, there was mildly, but not significant, decrease in nitrite concentration in plasma, liver and muscle. A statistically significant decrease ($P < 0.01$) in nitrite levels wasn't observed until the end of the experiment (after 144 h in nitrite-free water), when it achieved control values (traces of nitrite) (Table 3, Figure 2).

In group 2, nitrite concentration did not differ significantly from control values during the whole duration of the trial (Table 3, Figure 2).

DISCUSSION

The exposure of fish to enhanced nitrite concentration (1.45 mmol/l) at low molar ratio of Cl^-/NO_2^- (0.21) caused sever health deterioration accompanied by high mortality of fish (51%) and resulted in increased blood plasma nitrite (10.5 mmol/l) represented a concentration 7 times higher than in the ambient water. Nitrite accumulation observed also Jensen et al. (1987) in common carp at similar conditions. Nitrite exposure of carp to 1 mmol/l NO_2^- at 0.3 mmol/l Cl^- (molar Cl^-/NO_2^- 0.3) for 48 h resulted in increased plasma levels of nitrite represented the concentration 5.4 higher than in surrounding water. Our results showed that nitrite also accumulate at lower extent in the liver (3.5 mmol/kg) and muscle (1.5 mmol/kg) of carp. However, only a few data are available for comparison on nitrite accumulation in tissues. Margiocco et al. (1983), who exposed rainbow trout to lower nitrite concentration (0.03 mmol/l NO_2^-) at 0.056 mmol/l Cl^- (molar Cl^-/NO_2^- 1.75) for 48 h, found also nitrite levels in the muscle (0.03 mmol/kg NO_2^-) similar to concentration in ambient water. In the liver, there were found nitrite concentration 9 times higher than the environmental one. Different way of nitrite accumulation observed Gisbert et al. (2004) in Siberian sturgeon exposed

to 9.3 mmol/l NO_2^- at 2.17 mmol/l (Cl^-/NO_2^- 0.23) for 18 hours. In this case, the highest nitrite concentrations were found in skeletal musculature (12.9 mmol/l NO_2^-), followed by the blood plasma (7.2 mmol/l NO_2^-) and liver (3.8 mmol/l NO_2^-). However, above-mentioned data are difficult to compare due to different experimental conditions (Cl^-/NO_2^- ratio in the first case and length of exposure in the second case) and different fish species used. As the rate of chloride (e.g. nitrite) uptake is known to be highly species dependent (Williams and Eddy, 1986).

High nitrite concentration in the blood plasma resulted in methaemoglobin formation and as a consequence extremely high methaemoglobin levels (93%) were detected. Nitrite accumulation in the blood was also accompanied by the low haematocrit value, haemoglobin concentration and erythrocyte count. One possibility might be that the decrease in above-mentioned haematological indices could be attributed to the oxidative haemolysis. Since formation of methaemoglobin is associated with formation of superoxide free radicals which denature critical proteins, including haemoglobin and components of the erythrocyte membrane. Alteration of haemoglobin leads to loss of haemoglobin solubility, resulting in structural damage to erythrocytes and it can subsequently cause rapid lysis of the erythrocytes (Bloom and Brandt, 2001; Everse and Hsia, 1997). Furthermore, increased potassium concentration was measured after nitrite poisoning. Since haemolysis results in elevated values of plasma potassium caused by the increased intraerythrocytic concentration of this ion relative to plasma (McDonald and Milligan, 1992). However, extracellular hyperkalemia can be also caused by a potassium efflux from skeletal musculature as described by Knudsen and Jensen (1997).

Our data also indicated some changes in nitrogen metabolism in fish exposed to nitrite at low molar ratio of Cl^-/NO_2^-; namely we detected elevated plasma ammonia, urea-N and uric acid concentration. Information on the interaction of nitrite with nitrogen metabolism is limited in fish. Jensen (2003) hypothesised that nitrite exposure could lead to depletion of adenylates in muscle and other tissues due to hypoxia and anaerobic energy production (anaerobic glycolysis) during nitrite poisoning. Conversion of AMP to NH_3 (and IMP) via AMP deaminase would then elevate the production of ammonia that could react with glutamate, forming glutamine, subsequently raising urea production. In addition, our previous study indicated that similar nitrite concentration caused some histological lesions in the gills of carp (Svobodova et al., 2005b). Whereas, elevated blood urea levels in teleost may serve as a clinical indication of respiratory and excretory compromise due to respiratory epithelial cell hypertropy and hyperplasia (Nelson et al., 1999). Elevated plasma levels of uric acid can be explained as a protection of fish against oxyhaemoglobin oxidation in the blood. Since Smith and Nunn (1984) reported that uric acid inhibited the oxidation of bovine oxyhaemoglobin by nitrite. Furthermore, Doblander and Lackner (1996) found out that oxidation (i.e., detoxification) of nitrite to nitrate by iron-containing proteins in trout hepatocytes was enhanced by the addition of uric acid. As uric acid is know to be an antioxidant protecting haem proteins from oxidation.

On the other hand, the same concentration of nitrite at higher molar ratio of Cl^-/NO_2^- (2.57) did not cause such health impairment as at lower ratio (0.21) and no mortality was observed. Also significantly lower nitrite levels were measured in the blood plasma and tissues and didn't differ significantly from the control values. This observation again verified the protective effect of enhanced chloride concentration on nitrite toxicity. Our results document that enhanced chloride concentrations protect fish from nitrite uptake and accumulation in the body. This fact contributes to the theory that nitrite has an affinity for the branchial chloride uptake mechanism in the fish gills (Williams and Eddy, 1986).

After 48-hour exposure, the fish were transferred into nitrite-free water and their ability of recovery from nitrite poisoning was monitoring. The health of fish in the first group was so affected by the nitrite action that fish continued to die even after 24 h in nitrite-free water. On the other hand, in the surviving fish, the recovery of erythrocyte counts and haemoglobin concentrations was observed. Methaemoglobin levels and nitrite concentrations in the blood plasma, liver and muscle mildly, but not significantly, decreased. However, it reached control values after 144 h under nitrite-free conditions. Haematocrit also reached normal values by the end of the recovery period. Normal plasma urea and uric acid concentrations were observed after 24 h post-nitrite exposition. Ammonia concentrations returned to normal levels by the end of the recovery period. Recovery process of potas-

sium levels evolved in different way. The values were increasing and dropped significantly bellow the control values at the end of the experiment. Knudsen and Jensen (1997) who observed similar K^+ deficit reported that the skeletal musculature might recover its normal K^+ levels at the expense of the extracellular K^+, thus reducing plasma potassium levels.

CONCLUSION

In conclusion, we can say that sensitivity of fish to nitrite is highly individual. Some fish died within 24 and 48 h of nitrite exposure, and even after 24 h under nitrite-free conditions but some fish survived and were able to recover nearly completely in the fresh water; thus in surviving fish, most of the nitrite-induced effects were reversible. Our results also shown marked positive effect of enhanced chloride content reducing both the nitrite uptake and toxicity even at extremely high concentrations of nitrite.

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