Neuroendocrinology Letters Volume 37 Suppl. 1 2016
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

Effects of therapeutic formalin bath on selected indices in fish

Livia Chmelova [1], Pavla Sehonova [1], Veronika Doubkova [1], Lucie Plhalova [1], Jana Blahova [1], Frantisek Tichy [2], Helena Modra [3], Zdenka Svobodova [1]

1 Department of Animal Protection, Welfare and Behaviour, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic
2 Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic
3 Department of Territorial Studies, Faculty of Regional Development and International Studies, Mendel University in Brno, Czech Republic

Correspondence to: Lívia Chmelová, MSc.
Department of Animal Protection, Welfare and Behaviour
University of Veterinary and Pharmaceutical Sciences Brno
Palackeho tr. 1946/1, 612 42 Brno, Czech Republic.
TEL: +420 541 562 788; FAX: +420 541 562 790; E-MAIL: H14394@vfu.cz

Submitted: 2016-06-24 Accepted: 2016-10-30 Published online: 2016-12-18

Key words: **haematological profile; biochemical profile; histopathological profile; *Cyprinus carpio***

Neuroendocrinol Lett 2016; **37**(Suppl. 1):67–77 **PMID:** 28263533 NEL370916A09

Abstract

OBJECTIVES: The aim of this study is to assess the effects of a treatment bath in a formalin solution on fish, focusing on haematological, biochemical and histopathological profiles.

METHODS: A total of 96 common carps (*Cyprinus carpio*) were randomly assigned to eight groups. Four experimental groups were placed in the test solution for 60 minutes. The concentration of the formalin bath was 0.17 ml/l (38% formaldehyde), with the water temperature of 20 °C. The effects of the bath were monitored immediately (E0) and 24 hours (E24h), 48 hours (E48h) and 10 days (E10d) after the bath. There was a control group (C0, C24h, C48h, C10d) for each of the experimental groups.

RESULTS: Histopathological indices were strongly affected. Extensive changes were found on gill and skin immediately and also after 24 h, 48 h and 10 d of the formalin treatment bath. A plurality of mucinous elements was observed on the skin. Moreover, structural devastation of lamellas and numerous mucinous cells were observed on the gill. Changes were also found in haematological and biochemical indices.

CONCLUSIONS: It can be concluded that after applying the treatment bath in the formalin solution, the monitored profiles were affected immediately after the bath and also after 10 days (histopathological changes of skin and gill). Such significant impact of therapeutic formalin bath should be considered in treatment of fish.

ORIGINAL ARTICLE

Abbreviations:

ALB - albumin
ALP - alkaline phosphatase
ALT - alanine aminotransferase
AST - aspartate aminotrasferase
C_0 - control group tested immediatelly after formalin bath
C_{24h} - control group tested 24 hours after formalin bath
C_{48h} - control group tested 48 hours after formalin bath
C_{10d} - control group tested 10 days after formalin bath
CHOL - cholesterol
E_0 - experimental group tested immediatelly after formalin bath
E_{24h} - experimental group tested 24 hours after formalin bath
E_{48h} - experimental group tested 48 hours after formalin bath
E_{10d} - experimental group tested 10 days after formalin bath
GLU - glucose
Hb - haemoglobin concentration
HSI - hepatosomatic index
LACT - lactate
LDH - lactate dehydrogenase
MCH - mean erythrocyte haemoglobin
MCHC - mean corpuscular haemoglobin concentration
MCV - mean erythrocyte volume
PCV - haematocrit
PHOS - inorganic phosphorus
RBC - red blood cells
TG - triacylglycerols
TP - total protein
WBC - white blood cells

INTRODUCTION

Formalin (36–38% formaldehyde aqueous solution) is one of the most effective and widely used compounds applied in fish culture for the therapeutic and prophylactic treatment of fungal infections and for the removal of external parasites of fish and fish eggs. It is effective against most protozoans and also against some of the larger parasites, such as monogenetic trematodes. Formalin effectively kills parasites on gill, skin and fins (Noga 2000). It is used at a concentration from 0.17 to 0.25 ml/l, depending on the water temperature. In case of finding species of genus *Cryptobia*, *Ichthyobodo*, *Chilodonella*, *Trichodina*, *Trichodinella*, *Dactylogyrus*, *Gyrodactylus*, *Thaparocleidus* and surface mycoses the bathing time is between 30–60 minutes (Svobodová *et al.* 2007).

The toxicity of formalin in fish in waters of different quality was studied by Phelps (1975). His study found that formalin was more toxic to channel catfish (*Ictalurus punctatus*) in soft water than in hard water. Bills *et al.* (1977) observed that water hardness did not affect the toxicity of formalin in fish. However, in soft water with pH 9.5 formalin was more toxic than at pH 6.5 and 8.5. Chinabut *et al.* (1988) found no significant differences in the effects of formalin in water of different hardness and pH on the *Cyprinus carpio*. Similar results were reported by Marking *et al.* (1972) and Piper and Smith (1973). Temperature can influence the toxicity of formalin as well. Noga (2000) and Svobodová *et al.* (2007) both suggest to regulate the concentration of formalin treatment bath according to the temperature. It means that higher formalin concentration (0.25 ml/l) should be used when the temperature is lower (up to 10 °C) and lower concentration (0.17 ml/l) should be used when the temperature is higher (more than 15 °C). Low temperature could significantly prolong recovery period which follows various treatments used in aquaculture (Kristan *et al.* 2014).

The tolerance for chemicals can vary depending on species and treatment conditions (Hoffman & Meyer 1974; Kabata 1985). The toxicity of a chemical to fish should be determined before applying any chemical therapy. This is particularly important as the recommended concentrations and treatment times for some therapeutics are near the lethal level for certain cultured species. The therapeutic index represents the range between the therapeutic and lethal concentration and it is often expressed as a ratio of lethal concentration (LC50) and effective therapeutic concentration (EC50). In general, suggested value should be no less than 2–3 (Schäperclaus 1992). For *Poecilia reticulata* the therapeutic index of formaldehyde is 4.9 and for *Danio rerio* it is 5.24 (Dolezelova *et al.* 2009).

Formaldehyde has been classified as a carcinogen. The effect of aldehyde dehydrogenase is being used to produce formic acid (Gupta 2012). This acid then oxidizes very slowly to carbon dioxide and water and it accumulates significantly in tissues. It causes metabolic acidosis, accumulates in the retina, optic nerve and basal ganglia of the brain, resulting in permanent visual impairment and extrapyramidal symptoms. In mammals, formaldehyde permeates into the body by inhalation, through skin and the eye. Tests in animals have shown potential neurotoxicity (Pitten *et al.* 2000). Smith and Piper (1972) observed haematological and histopathological changes in rainbow trout after 1 hour of treatment at a concentration of 167 and 250 mg/l of formalin. The toxic effect of formalin at concentrations of 0, 25, 50 and 75 mg/l in an 8 week test on common carp reduced the growth of common carp fry (Chinabut *et al.* 1988).

The aim of this study was to assess the effects of formalin treatment bath on haematological, biochemical and histopathological profiles of fish. The experiment was carried out with *C. carpio* because this fish is considered to be an appropriate model organism when conducting drug safety studies.

MATERIALS AND METHODS

The experiment was carried out on two-year old common carp (*C. carpio*) obtained from a commercial fish farm (Rybnikářství Pohořelice, a.s., Czech Republic). Fish were allowed to acclimate to the conditions of the experiment for 14 days in semi-static system tanks with dechlorinated tap water which was changed every 24 hours. Water in the tanks was individually aerated, with inspections of the pH, temperature and oxygen concen-

tration carried out every 12 hours. During the acclimatization fish were fed with commercial pellets with spirulina (protein 37%, fat 6%) *ad libitum*. The feeding was stopped 24 hours before the exposure.

After the acclimation period a total of 96 carps were randomly assigned to 8 groups (each group consisting of 12 fish). Bath was performed in water with the addition of formalin (38% formaldehyde) at a concentration of 0.17 ml/l and lasted for 60 minutes. The temperature of the water was 20 °C.

The first group was tested immediately after the bath (E_0), other groups were tested 24 hours (E_{24h}), 48 hours (E_{48h}) and 10 days (E_{10d}) after the bath. A control group without exposure to the tested substance (C_0, C_{24h}, C_{48h}, C_{10d}) was assigned to each of the experimental groups. Behavior of the fish was observed during the bath. After the bath the fish (except E_0 and C_0) were placed in the semi-static system tanks with dechlorinated tap water which was changed every 24 hours. During the recovery period fish were fed with the same commercial pellets *ad libitum*.

Water parameters

The water temperature during the recovery period ranged from 19 to 22 °C; the pH was between 7.9 and 8.4 and the dissolved oxygen did not fall below 60% saturation.

The basic physical and chemical parameters of the tap water used in the tests were acid neutralization capacity ($ANC_{4.5}$) 1.0–1.2 mmol/l; chemical oxygen demand (COD_{Mn}) 0.9–1.2 mg/l; total ammonia below the limit of determination (<0.03 mg/l); nitrates 11.2–13.1 mg/l; nitrites below the limit of determination (<0.02 mg/l); Cl^- 17.8–20.1 mg/l; Σ Ca+Mg 3.01 mg/l.

Biometric indices

The fish were stunned with a blow to the head and killed by spinal transection. The biometrical indices (body and liver weight), from which somatic parameters such as the hepatosomatic index were derived and calculated, were defined afterwards. The hepatosomatic index (HSI) was calculated using the following formula:

$$HSI = [liver\ weight \times 100] / [body\ weight]$$

Health status examination

During the bath and recovery period the behavior of the fish, clinical and pathological changes were observed. The parasitological examination was conducted primarily by focusing on the gill and skin before the bath for 12 carps and after the bath (immediately, 24 h, 48 h and 10 d) for 12 subjects of the control and experimental groups. The prevalence (%) and the intensity of infection were evaluated. The intensity of infection was expressed as either rare for one individual parasite in the three fields of view or midrange for 2–3 parasites in the three fields of view (magnification 60×).

Haematological profile

Blood samples were taken from each of the fish by cardiac puncture and stabilized with sodium heparin (50 IU per 1ml of blood). Heparinized blood samples were used for evaluation of haematological indicators, including erythrocyte count (RBC), haemoglobin concentration (Hb), haematocrit (PCV), mean erythrocyte volume (MCV), mean erythrocyte haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), leukocyte count (WBC) and leukogram (Svobodova *et al.* 2012).

Biochemical profile

Blood samples were centrifuged (855 g, 10 min, 4 °C) and plasma samples were used for the analysis of selected biochemical indices, including albumin (ALB), total protein (TP), glucose (GLU), ammonia (NH_3), triacylglycerols (TG), lactate (LACT), cholesterol (CHOL), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), calcium, and inorganic phosphorus (PHOS). Plasma biochemical indices were measured using a biochemical automatic analyzer Konelab 20i (ThermoScientific, Czech Republic) and commercial test kits (BioVendor, Czech Republic).

Histopathogical profile

Samples of gill, liver, cranial, and caudal kidney of six fish from each group were immediately fixed in buffered 10% neutral formalin. The samples were later dehydrated, embedded in paraffin wax, sectioned on a microtome at a thickness of 4 μm and stained with haematoxylin and eosin.

Statistical analysis

Statistical assessment was carried out using statistical program R. Data did not meet the assumption of normality, therefore logarithmic transformation was used for the analysis. Results were tested for statistical significance using methods which allow multiple comparisons of mean values – analysis of variance. The statistical evaluation was always carried out between the experimental and control group at the same time. Differences were considered statistically significant when $p<0.05$. The original values were used in the figures.

RESULTS

Biometric indices

The fish body weight varied between 169 and 483 g and it was constant during the recovery period. There were no significant differences in body weight and HSI between the control and experimental groups (Figure 1).

Health status

Before the bath some rare occurrences of *Trichodina epizootica* on the skin and some rare occurrences of

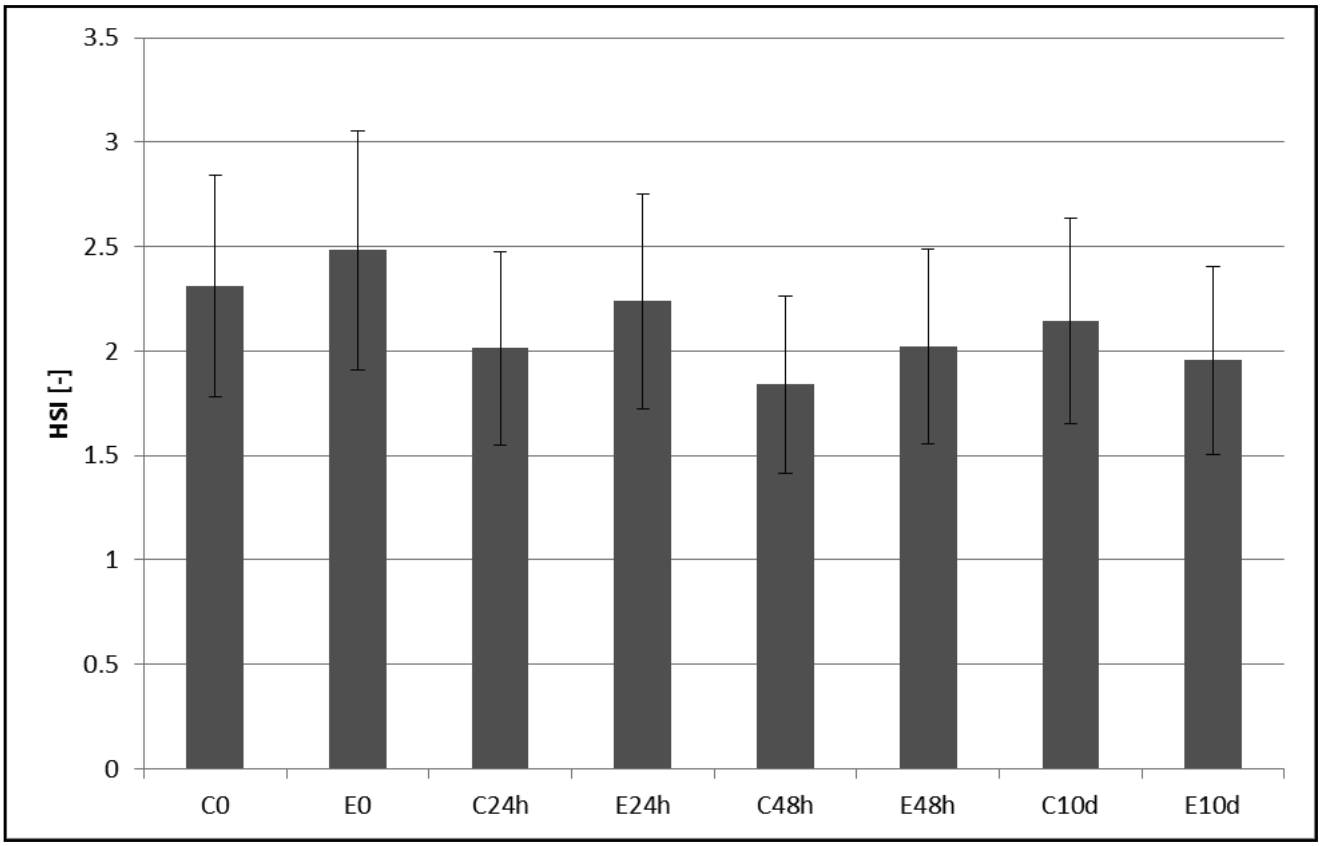

Fig. 1. Hepatosomatic index (mean ± SEM). Comparison of control and experimental group at each time.

Tab. 1. Prevalence (%) and intensity of parasite infection (rarely for one individual parasite in the three fields of view, middle for 2–3 parasites in the three fields of view).

Time	Parasites	Control (prevalence %)				Experiment (prevalence %)			
		Skin		Gill		Skin		Gill	
		Rarely	Middle	Rarely	Middle	Rarely	Middle	Rarely	Middle
0	Trichodina epizootica	100	0	0	0	0	0	0	0
	Gyrodactylus sprostonae	0	0	100	0	0	0	0	0
24h	Trichodina epizootica	58	42	17	0	33	0	17	0
	Gyrodactylus sprostonae	0	0	17	0	0	0	0	0
48h	Trichodina epizootica	17	83	17	0	33	0	0	0
	Gyrodactylus sprostonae	0	0	8	0	0	0	0	0
10d	Trichodina epizootica	75	25	17	0	17	0	0	0
	Gyrodactylus sprostonae	0	0	75	0	0	0	0	0

Gyrodactylus sprostonae on the gill were found. After the bath the experimental groups only had rare occurrences of parasites, but in the control groups we noted an upward trend of parasitic manifestation (Table 1).

Fish behavior

During the experiment both the control and experimental groups showed normal behavior. There were no signs of respiratory distress such as rapid ventilation, increased rate of gill opercular movements or fish floating on the water surface.

Haematological profile

Our results show no significant differences in haematological indices between control and experimental groups. These differences only started to appear 10 days after the treatment. There was a statistically significant increase in the value of RBC ($p<0.01$) and values of Hb and PCV ($p<0.05$) 10 days after the therapeutic formalin bath (E_{10d}) (Table 2). Significantly decreased values of basophils were found in white blood cells 10 days after the bath (Table 3).

Biochemical profile

The results showed a significant reduction ($p<0.001$) in ALT activity (Figure 2) and increasing ($p<0.01$) ammonia (Figure 3) in the experimental group E_{48h}, glucose ($p<0.001$) (Figure 4) and calcium ($p<0.001$) (Figure 6) increased in the experimental group E_{10d} and there was also a trend to an increase in lactate levels (Figure 5) for the experimental group E_{10d} ($p=0.07$). Other monitored indices (ALP, AST, total protein, cholesterol, phosphorus, LDH, triacylglycerols) showed no statistically significant differences between the experimental and control groups. These data are not presented.

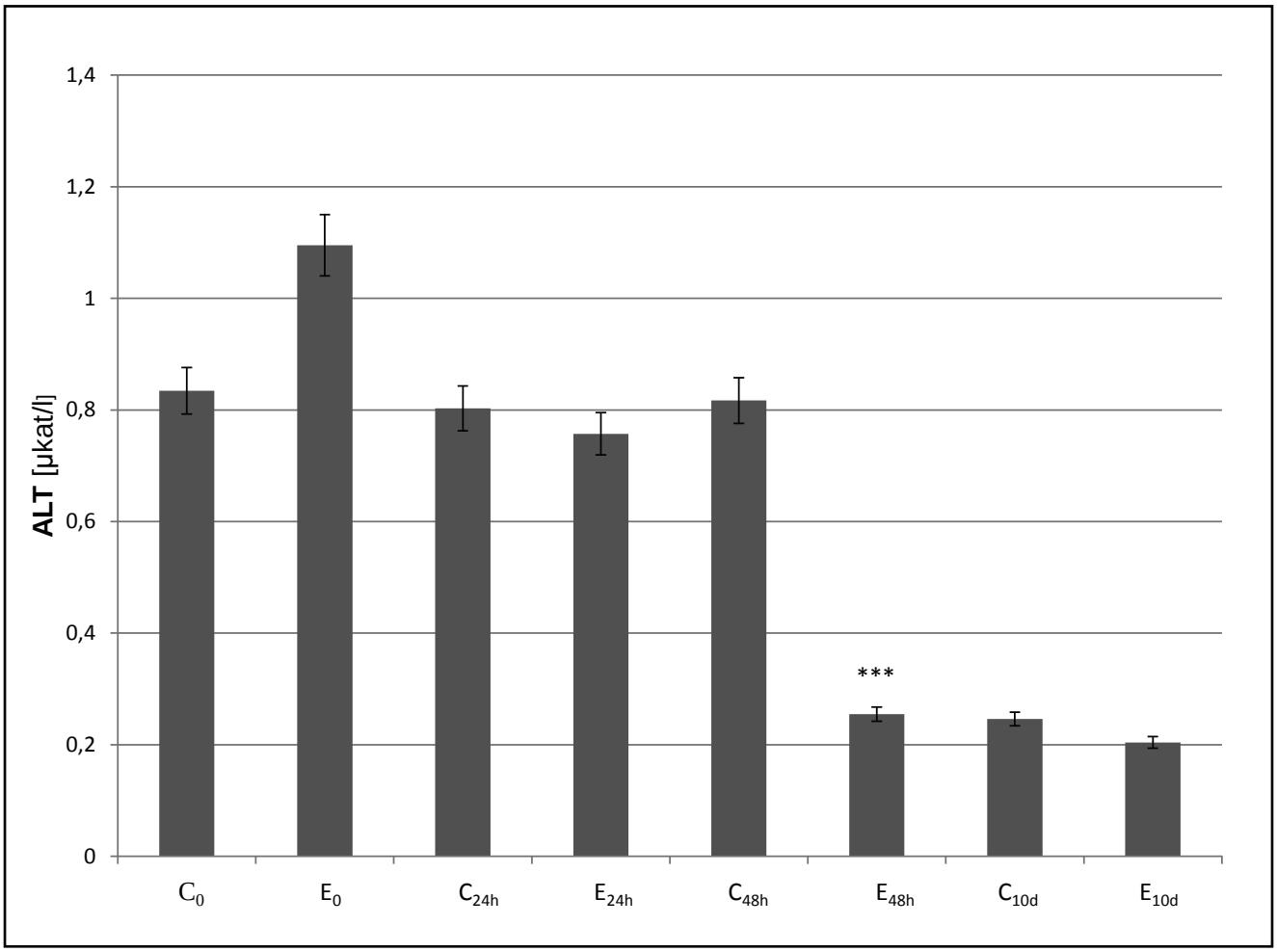

Fig. 2. Results of ALT activity after formalin bath – comparison of control and experimental group at each time. Data are expresed as mean +/- SEM (***$p<0.001$)

Tab. 2. Results of red blood cell profile – comparison of control and experimental group at each time.

	RBC (T/l)	Hb (g/l)	PCV (l/l)	MCV (fl)	MCH (pg)	MCHC (l/l)
C_0	1.68±0.08	64.5±2.96	0.32±0.01	196±9.34	38.8±1.39	0.20±0.01
E_0	1.60±0.12	62.0±3.71	0.30±0.01	200±18.59	39.7±1.64	0.21±0.01
C_{24h}	1.61±0.11	66.3±2.41	0.29±0.01	188±11.33	43.7±3.22	0.23±0.01
E_{24h}	1.64±0.10	66.9±7.08	0.27±0.01	170±10.53	41.1±3.93	0.24±0.02
C_{48h}	1.50±0.06	63.9±2.57	0.30±0.01	204±7.93	43.0±1.68	0.21±0.01
E_{48h}	1.61±0.07	65.4±2.96	0.31±0.01	197±8.54	41.6±2.69	0.21±0.01
C_{10d}	1.35±0.07	60.6±3.42	0.26±0.01	197±5.57	45.1±1.66	0.23±0.00
E_{10d}	1.71±0.08**	70.9±2.96*	0.30±0.01*	180±6.93	42.0±1.88	0.23±0.01

Data are expresed as mean ± SEM (*$p<0.05$, **$p<0.01$)

Tab. 3. Results of white blood cell profile – comparison of control and experimental group at each time.

				Neutrofile granulocytes			
	WBC (G/l)	**Lymfocytes (G/l)**	**Monocytes (G/l)**	**Myelocytes (G/l)**	**Metamyel. (G/l)**	**Eosinophils (G/l)**	**Basophils (G/l)**
C_0	39.92±4.87	33.15±3.96	1.14±0.27	3.42±0.63	1.79±0.75	0.79±0.19	0.54±0.23
E_0	34.13±4.39	27.30±3.40	0.60±0.16	2.46±0.85	2.01±0.74	1.07±0.33	0.80±0.26
C_{24h}	69.71±8.01	60.77±6.69	1.51±0.56	2.89±0.81	2.15±0.51	1.46±0.33	0.92±0.29
E_{24h}	63.33±6.64	56.18±6.12	1.43±0.35	1.90±0.29	1.78±0.31	1.32±0.42	0.74±0.30
C_{48h}	32.64±3.40	29.38±4.78	1.00±0.24	2.91±0.54	1.65±0.36	1.05±0.21	0.89±0.25
E_{48h}	45.21±5.84	34.33±4.99	0.82±0.20	4.19±1.17	2.29±0.73	1.36±0.27	2.23±1.07
C_{10d}	62.13±4.30	53.94±3.99	0.25±0.09	9.83±5.13	1.00±0.27	0.78±0.17	1.30±0.30
E_{10d}	75.14±7.08	63.29±8.22	0.31±0.20	3.35±0.68	1.04±0.38	0.56±0.18	0.33±0.17*

Data are expressed as mean ± SEM (*p<0.05)

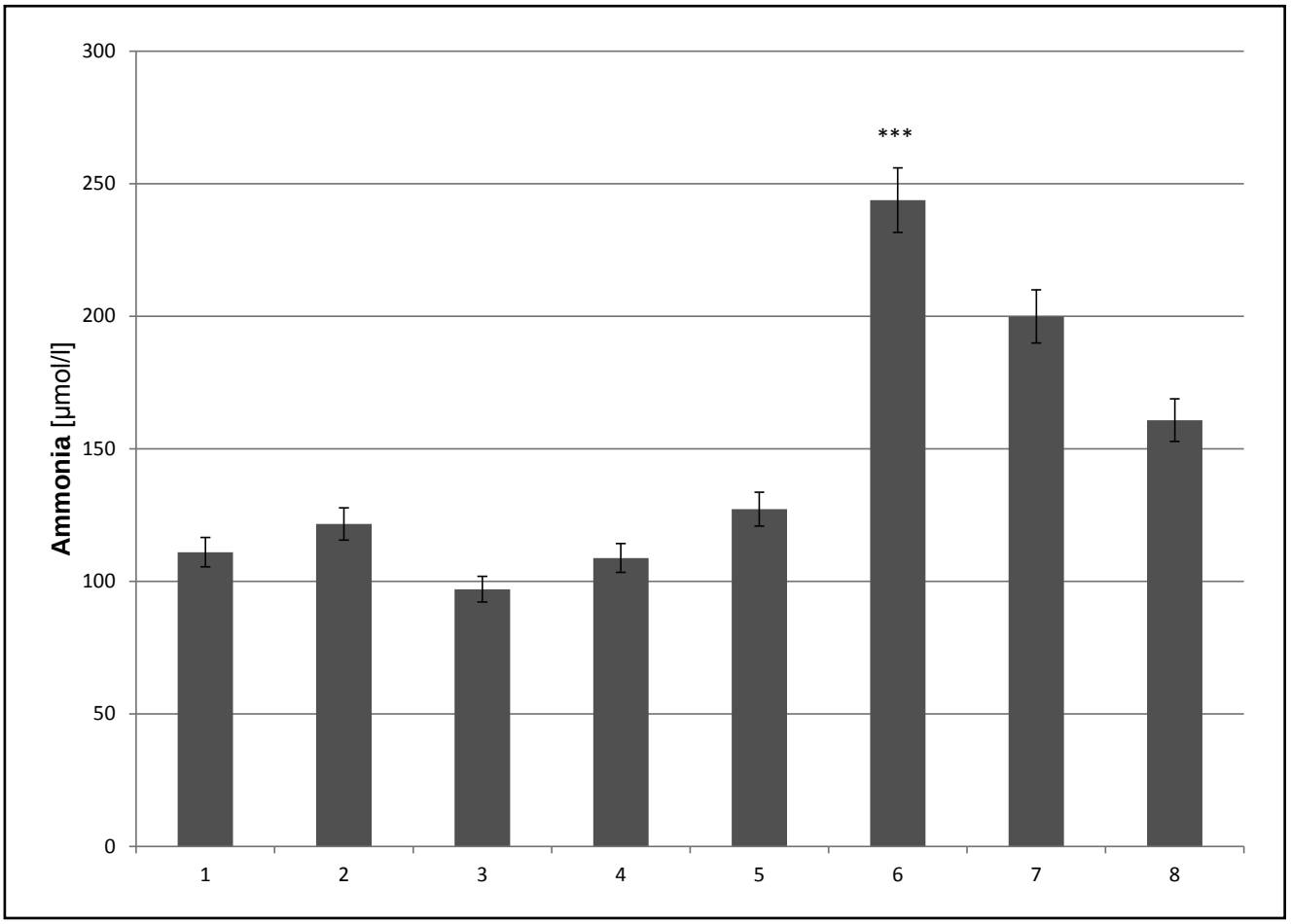

Fig. 3. Results of ammonia concentration after formalin bath – comparison of control and experimental group at each time. Data are expresed as mean ± SEM (**p<0.01)

Histopathological profile

There were extensive changes on gill and skin discovered within the scope of histopathological examination.

All experimental groups had a plurality of mucinous elements on the skin (Figure 7).

Devastation of the lamellas and inflammatory necrotic masses on the gill occurred only in one sample from the experimental group E_0. In surviving districts the lamellas were deformed, with a separated surface layer and numerous mucinous cells. An amount of

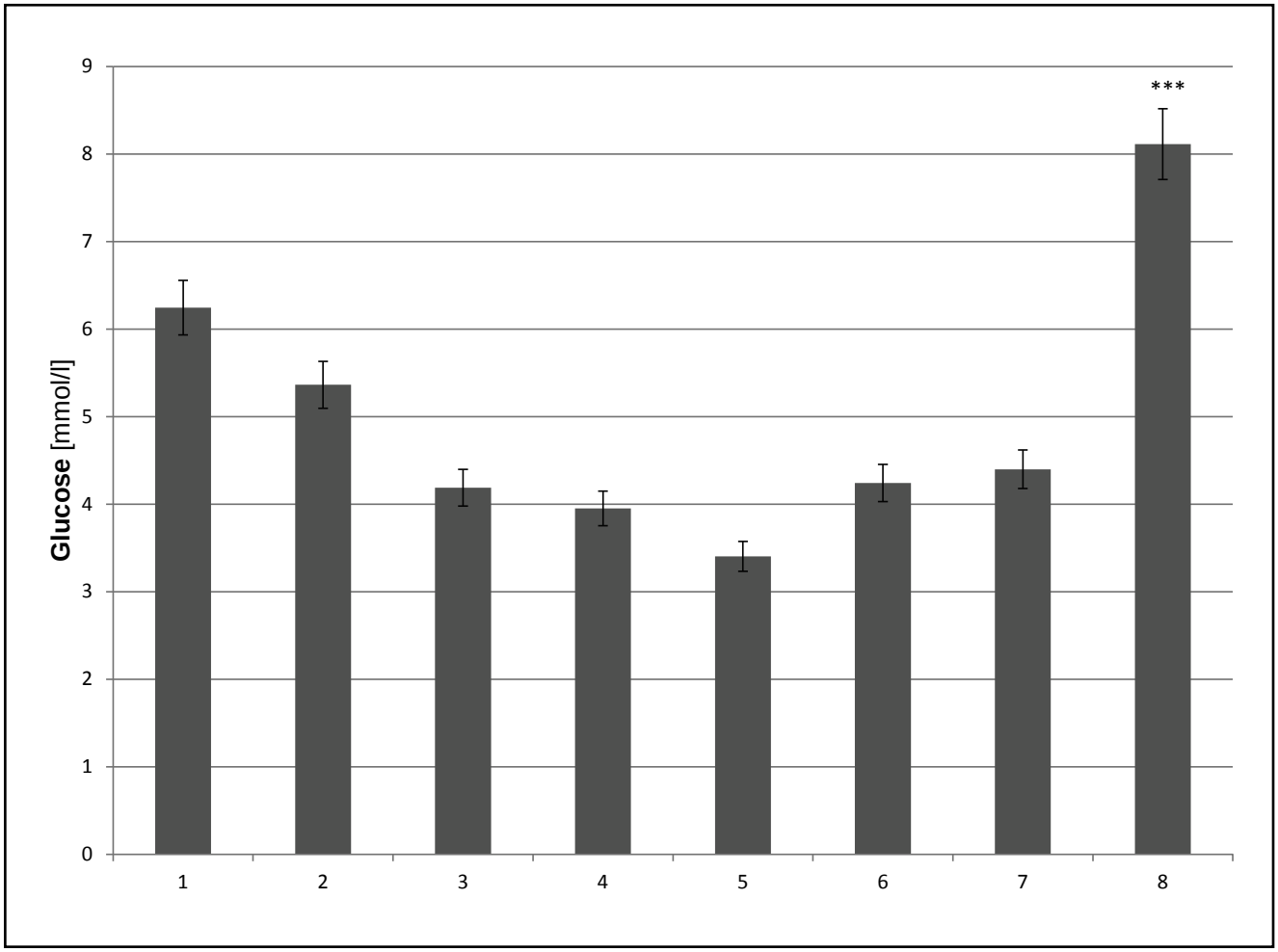

Fig. 4. Results of glucose concentration after formalin bath – comparison of control and experimental group at each time. Data are expresed as mean ± SEM (***p<0.001)

mononuclear cellulisation was observed among the bases of the lamellas for tested fish in E_0 (Figure 8). Similar changes were observed also on the gill at E_{24h}.

Structural devastation of lamellas with numerous mucinous cells were found in groups E_{48h} (Figure 9) and E_{10d}. Hyperemia was observed in groups C_0, C_{24h} and C_{48h}. In group C_{10d} captured spherical structures (susp. parasite) were observed among the lamellas.

In the cranial kidney we observed congestion, otherwise there was a normal appearance of the haematopoietic population, perivascular precipitated pigment and present spherical formation of homogeneous eosinophilic substance for all experimental and control groups.

The caudal kidney of both experimental and control groups showed congestion and small siderin deposits. There were no differences between the tubular system and glomeruli of both control and experimental groups. For E_{24h}, E_{48h}, E_{10d} eosinophilic spherical formations were present sporadically.

Congestive parenchyma with dissemination of siderin deposits and moderate glycogen content were found in the liver of all experimental and control groups.

Congestion and siderin deposits were observed in the spleen of all the experimental and control groups.

DISCUSSION

Results of this study show that the formalin bath affected the organism of the *C. carpio* not only immediately but also 10 days later, mostly in case of histopathological changes. However, there were no differences between the control and experimental groups in case of HSI, which is considered to be an index of energy status (Chellappa *et al.* 2006).

A lower occurrence of *Trichodina epizootica* and *Gyrodactylus sprostonae* was observed after the formalin bath. Effectiveness of the formalin treatment was demonstrated previously on *Trichodina* (Singhal *et al.* 1986). The disinfection success of a formalin treatment was also proven for *Gyrodactylus spp.* (Rahn *et al.* 2015).

All the fish in the control and experimental groups in our study showed normal behaviour after 60 min-

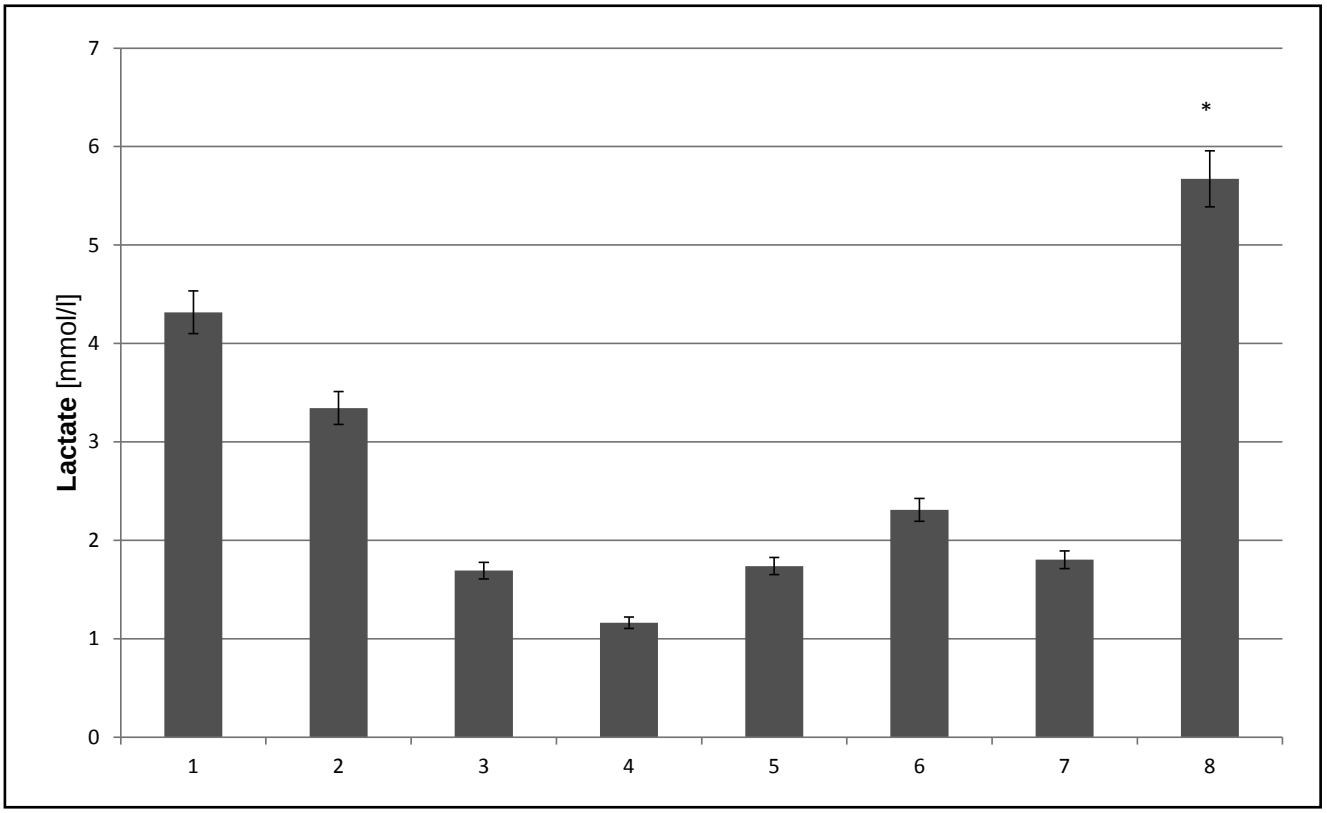

Fig. 5. Results of lactate concentration after formalin bath – comparison of control and experimental group at each time. Data are expresed as mean ± SEM (*p=0.07).

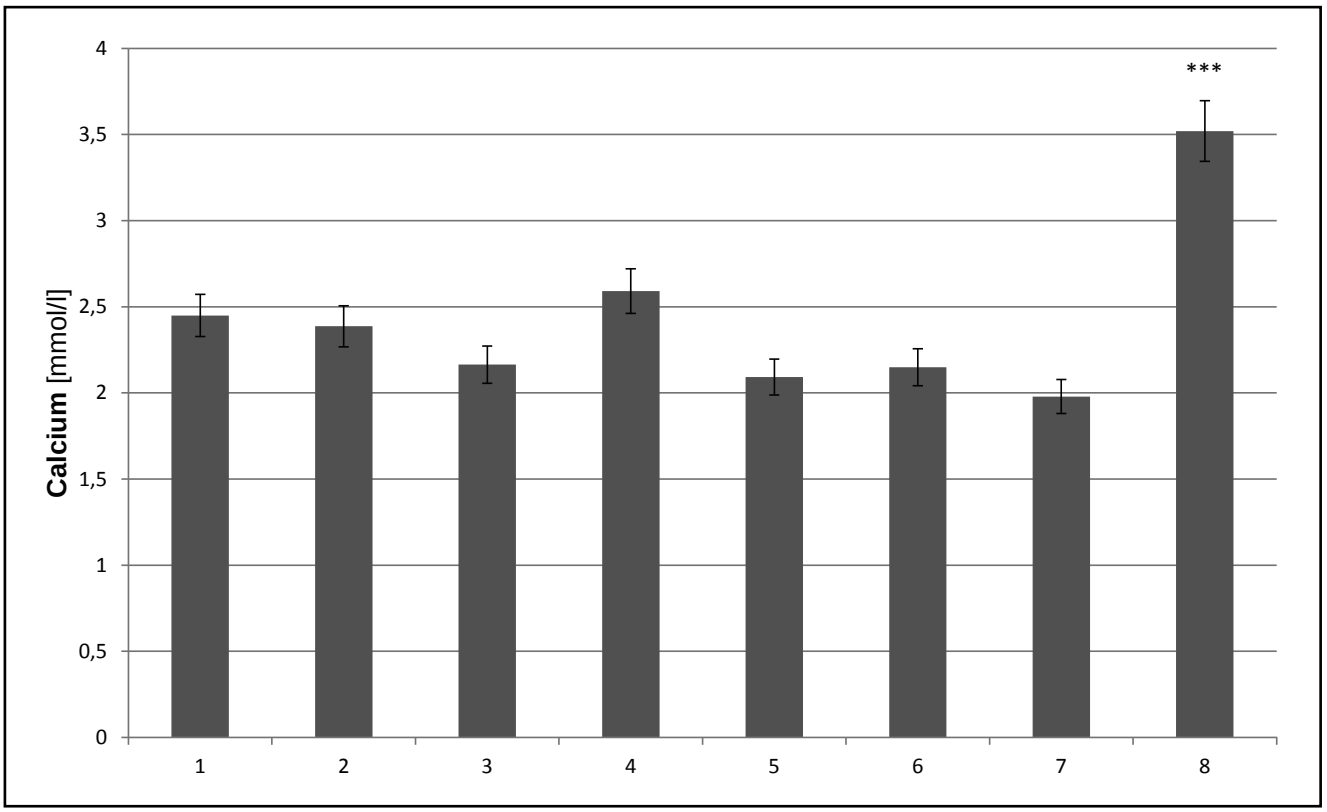

Fig. 6. Results of calcium concentration after formalin bath – comparison of control and experimental group at each time. Data. are expresed as mean ± SEM (***p<0.001).

utes of exposure. In contrast, Mohammed *et al.* (2012) observed increased swimming movements and decreased rate of opercular movements after 96 hours of exposure to formalin at a concentration of 40 mg/l in *Danio* rerio. These differences in observations can be explained by different species of fish and also by different duration of exposure.

Haematological parameters could be used as parameters of physiological stress by toxic chemicals in fish. Jung *et al.* (2003) observed a methaemoglobin formation in the blood of an olive flounder (*Paralichthys olivaceus*) at concentrations of formalin above 250 mg/l after a 3-hour bath, but there was no haemolysis at any concentration. Immediately after the exposure to a treatment in a formalin bath of 100 to 300 mg/l for 1 hour no significant changes in haematological and haemochemical values in the fish were found. Wedemeyer and Yasutake (1974) and Kakuta *et al.* (1991) reported that after the formalin exposure of *C. carpio, Oncorhynchus mykiss* and *Oncorhynchus tshawytscha* the value of Hb did not change significantly. This is consistent with our results for experimental groups (E_0, E_{24h}, E_{48h}) which were tested immediately, after 24 hours and after 48 hours.

Our results show a statistically significant increase in the value of RBC, Hb and PCV 10 days after the therapeutic formalin bath (E_{10d}). An explanation for an increased red blood picture could be the result of a long-term damage of gill. Smith and Piper (1972) reported that rainbow trout treated with formalin at concentrations 167 and 250 mg/l for 1 hour had an elevation of haematocrit and immature erythrocytes. Nieminen *et al.* (1983) observed increased Hb values measured in the fish exposed twice to 250 mg/l formalin or four times to 1 250 mg/l formalin for 30 min during a 24-hour interval. 10 days after the bath significantly decreased values of basophils were found in white blood cells. This could be a sign of an allergic reaction to the formalin treatment as the basophils are essential for many biological responses, such as allergic conditions and inflammatory disorders (Huang *et al.* 2016).

Wedemeyer (1971) found a reduction in chloride and calcium ions in rainbow trout exposed to formalin, but for steelhead trout Wedemeyer and Yasutake (1974) reported a reduction in chloride ions, increased calcium and hypercholesterolaemia. The experimental group E_{10d} (10 days after exposure) showed increased amount of calcium. This could be due to the differences in sensitivity of various species.

Kakuta *et al.* (1991) observed increased plasma glucose in formalin treated carp. In our test the level of glucose increased only in the group E_{10d} (10 days after a formalin bath), probably as a response of the organism to the metabolism changes in E_{10d}. There was also a trend to an increase in lactate levels (p=0.07) in this group.

Our study has shown a statistically significant reduction in ALT and an increase in ammonia for the experimental group E_{48h}. Changes in the enzymatic activities of aquatic organisms are widely used to demonstrate

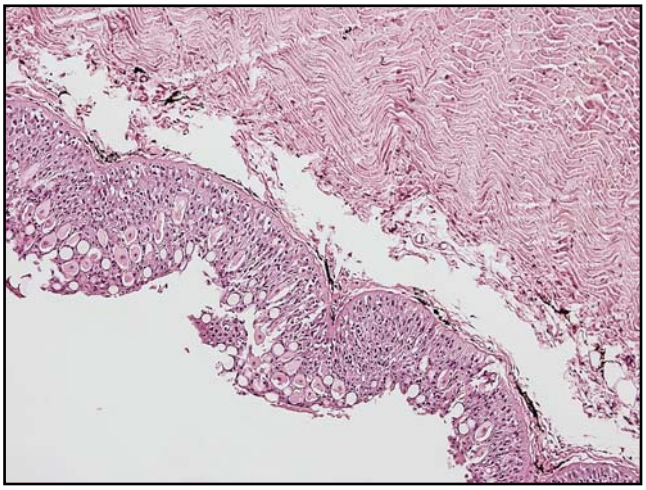

Fig. 7. Histopathological changes in skin of experimental group 10 days after formalin bath (plurality of mucinous elements), (H+E), magnification 200×.

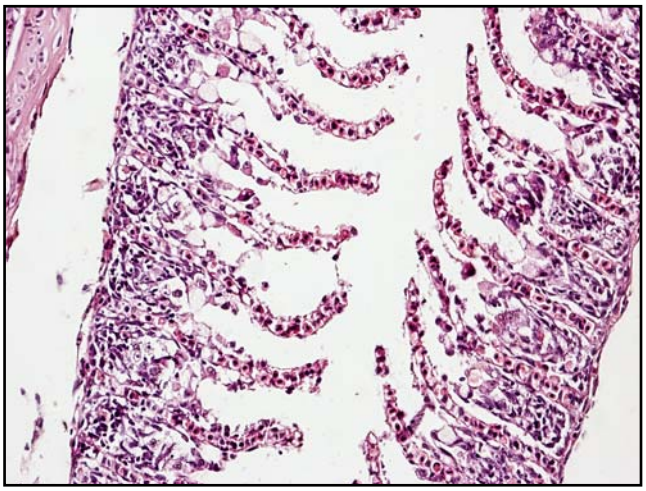

Fig. 8. Histopathological changes in gill of the experimental group immediately after a formalin bath (deformed lamellas with the separated surface layer, numerous of mucinous cells, massive mononuclear cellulisation between the bases of lamellas), (H+E), magnification 400×.

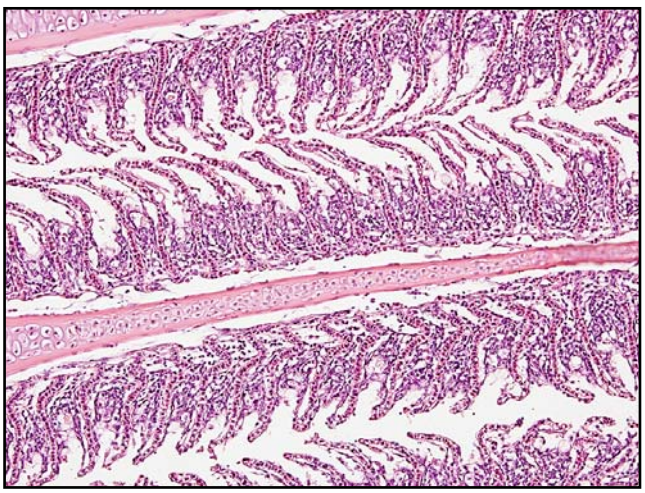

Fig. 9. Histopathological changes in gill of the experimental group 48hours after a formalin bath (deformed leaves of gill with occasional absence of surface layer, numerous mucinous cells,), (H+E), magnification 200×.

tissue damage and also to diagnose fish diseases (Nemcsok & Boross 1982; Pacheco & Santos 2002). In human organisms with chronic hepatocellular injury ALT is elevated, however, as fibrosis progresses ALT activities typically decline (Kim *et al.* 2008). We could suppose that a similar process could have caused a reduction in the ALT in fish.

During prophylactic bath with formaldehyde the fish can be affected in the following ways: damage such as gill hyperplasia, disorganization of liver arrangement, kidney necrosis (Santos *et al.* 2012) and massive vacoulations of the skin (Adeyemo *et al.* 2012). Pahor-Filho *et al.* (2015) described histological observation in the gill of juvenile mullet (*Mugil liza)* in all groups exposed to formaldehyde in 1h-profylactic bath at concentrations 67.5, 135, 270, 405 and 540 mg/l, where hyperplasia and detachment of the respiratory epithelium were observed. This corresponds to our observation of histopathological changes mainly on skin and gill. All experimental groups showed a devastation of the lamellas in the gill, which were deformed in the surviving areas with the separated surface layer. This kind of damage was also noticed in treatment with peracetic acid (Chupani *et al.* 2014).

Histopatological examination of the skin revealed fragments of skin with mucinous elements which is in accordance with the findings of Buchmann *et al.* (2004). In their previous studies they demonstrated extensive damage of the epithelial structure of fins and changes in skin composition due to formalin exposure. There were no differences between the experimental and control groups in histopathology of liver, caudal and cranial kidney and spleen.

CONCLUSIONS

Our results demonstrated that after therapeutic bath in formalin some monitored profiles were affected both immediately after the bath and also 10 days after the bath. It is thus necessary to consider the type and use of therapeutic bath based on the health status of the fish. This indicates that it is necessary to be aware of the impact of formalin treatment baths on histopathological markers.

ACKNOWLEDGEMENTS

This work was funded by the project IGA VFU Brno 225/2015/FVHE and by the project NAZV QJ1210013. Authors would like to thank Mr. Charles du Parc for English proofreading.

REFERENCES

1 Adeyemo OK, Akano OF, Emikpe BO (2012). Effects of formalin on spawning success and organ histology in *Clarias gariepinus*. Research Journal of Environmental Toxicology **6:** 42–50.

2 Bills TD, Marking LL, Chandler JH Jr (1977). Formalin: Its toxicity to nontarget aquatic organisms, persistence, and counteraction. In: U.S. Fish and Wildlife Service Investigation in Fish Control **73**: 1–7.

3 Buchmann K, Bresciani J, Jappe C (2004). Effects of formalin treatment on epithelial structure and mucous cell densities in rainbow trout, *Oncorhynchus mykiss* (Walbaum), skin. Journal of Fish Diseases **27**: 99–104.

4 Chellappa S, Huntingford FA, Strang RHC, Thomson RY (2006). Condition factor and hepatosomatic index as estimates of energy status in male three-spined stickleback. Journal of Fish Biology **47**: 775–787.

5 Chinabut SC, Limsuwan C, Tonguthai K and Pungkachonboon T (1988). Toxic and sublethal effect of formalin on freshwater fishes. NACA/WP/88/73, network of Aquaculture Centres in Asia-Pacific (NACA), Bangkok, Thailand, 200 p.

6 Chupani L, Stara A, Velisek J, Zuskova E (2014). Evaluation of the toxic effect of peracetic acid on grass carp (*Ctenopharyngodon idella*) juveniles. Neuroendocrinology Letters **35**: 86–92

7 Dolezelova P, Macova S, Plhalova L, Pistekova V, Svobodova Z, Bedanova I, *et al.* (2009). Comparison of the sensitivity of different species to medical substances. Neuroendocrinology Letters **30**: 248–252.

8 Gupta RC (2012). Veterinary Toxicology: Basic and Clinical Principles. 2nd ed. Boston: Academic Press, 1438 p.

9 Hoffman GL and Meyer FP (1974). Parasites of freshwater fishes: a review of their control and treatment. T.F.H. Publications, 224 p.

10 Huang L, Jiang P, Jianlin W, Hua Z, Jiye C, Ting L, *et al.* (2016): A rapid and sensitive assay based on particle analysis for cell degranulation detection in basophils and mast cells. Parmacological Research **111**, 374–383.

11 Jung SH, Sim DS, Park MS, Jo Q, Kim Y (2003). Effects of formalin on haematological and blood chemistry in olive flounder, *Paralichtys olivaceus* (Temminck et Schlegel). Aquaculture Research **34**: 1269–1275.

12 Kabata Z (1985). Parasites and diseases of fish cultured in the tropics. Taylor and Francis Ltd., 318 p.

13 Kakuta L, Namba K, Uematsu K, Murachi S (1991). Physiological response of the formalin exposure – I. Effects of formalin on urine flow, heart rate, respiration. Comparative Biochemistry and Physiology **100C**: 405–411.

14 Kim WR, Flamm SL, Di Bisceglie AM (2008). Serum aktivity of alanine aminotransferase (ALT) as an indicator of health and disease. Hepatology **47**: 1363–1370.

15 Kristan J, Stara A, Polgesek M, Drasovean A, Kolarova J, Priborsky J, *et al.* (2014). Efficacy of different anaesthetics for pikeperch (*Sander lucioperca L.*) in relation to ater temperature. Neuroendocrinology Letters **35**: 81–85.

16 Marking LL, King EL Tr, Chandler JH Jr (1972). Section of toxicology. Qarterly report of progress for July-September 1972. Fish control laboratory, La Crosse, Wisconcin, pp. 3–8.

17 Mohammed VN, Sheriff A, Mohideen S, Azmathullah NPG and Research Department of Zoology, The new College, Chennai (2012). Toxicity of formalin on behaviour and respiration in *Danio rerio*. International Journal of Environmental Sciences **2**: 1904–1908.

18 Nemcsok J, Boross L (1982). Comparative studies on the sensitivity of different fish species to metal pollution. Acta biologica Academiae Scientiarum Hungaricae **33**: 23–27.

19 Nieminen M, Pasanen P, Laitinen M (1983). Effects of formalin treatment on the blood composition of salmon (*Salmo salar*) and rainbow trout (*Salmo gairdneri*). Comparative Biochemistry and Physiology C **76**: 265–269.

20 Noga EJ (2000). Fish Disease Diagnosis and Treatment, Iowa State University Press, 563p.

21 Pacheco M, Santos MA (2002). Biotransformation, genotoxic, and histopathological effects of environmental contaminants in European eel (*Anguilla anguilla L.*). Ecotoxicology and Environmental Safety **53**: 331–347.

22 Pahor-Filho E, Kleber Campos Filho M, Klosterhoff M, Romano LA, Percira J Jr. (2015). Histopathological and behaviour effects of formaldehyde treatment in juvenile mullet, *Mugil liza* (Valenciennes). Aquaculture Research **46**: 3040–3045.

23 Phelps RP (1975). Toxicity and efficacy of five chemotherapeutics used in aquaculture when applied to waters of different quality. Ph.D. thesis, Auburn University, Auburn, Alabama. 103p.
24 Piper RG and Smith CE (1973). Factors influencing formalin toxicity in trout. Progressive Fish-Culturist **352**: 78–81.
25 Pitten FA, Kramer A, Herrmann K, Bremer J, Koch S (2000). Formaldehyde neurotoxicity in animal experiments. Pathology, Research and Practice **196**: 193–198.
26 Rahn AK, Hammer DA, Bakker TCM (2015). Experimental infection with the directly transmitted parasite *Gyrodactylus* influences shoaling behaviour in sticklebacks. Animal Behaviour **107**: 253–261.
27 Santos RF, Dias HM, Fujimoto RY (2012). Acute toxicity and histopathology in ornamental fish amazon bluespotted corydora (*Corydoras melanistius*) exposed to formalin. Anais da Academia Brasileira de Ciencias **84**: 1001–1007.
28 Schäperclaus W (1992). Fish diseases. CRC Press, 748p.
29 Singhal RN, Swam j, Davies RW (1986). Chemotherapy of six ectoparasitic diseases of cultured fish. Aquaculture **54**: 165–171.
30 Smith CE, Piper RG (1972). Pathological effect in formalin-treated rainbow trout (*Salmo gairdneri*). Journal of the Fisheries Research Board of Canada **29**: 328–329.
31 Svobodová Z, Kolárová J, Navrátil S, Veselý T, Chloupek P, Tesarčík J, *et al.* (2007). Nemoci sladkovodních a akvarijních ryb. Informatorium, 264 p (in Czech).
32 Svobodova Z, Pravda D, Modra H (2012). Unified methods of haematological examination of fish (in Czech). Faculty of Fisheries and Protection of Waters, Vodnany, Methods No. 122, pp. 38.
33 Wedemeyer G (1971). The stress of formalin treatments in rainbow trout (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*). Journal of the Fisheries Research Board of Canada **28**: 1899–1904.
34 Wedemeyer G, Yasutake WT (1974). Stress of formalin treatment in juvenile spring chinook salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*). Journal Fisheries Research Board of Canada **31**: 179–184.