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Sodium chloride bath – A cheap and safe tool for antiparasitic treatment of fish

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Abstract: Sodium chloride is widely used in aquaculture due to its antiparasitic effects and its ability to reduce stress during fish transport and manipulation. The aim of this study was to assess the safety of short-term exposure to sodium chloride for the common carp (*Cyprinus carpio*). In our experiment, fish were placed into a sodium chloride bath (c = 30 g l^{-1}; T = 30 min; t = 20 °C) and the effects of the treatment were assessed immediately after the bath (T0) and 24, 48 and 240 h later (T24, T48 and T240, respectively), with non-treated fish serving as control groups. Though significant differences compared to the controls were observed in the treated fish sampled at T0, T24 and T48, these effects were only temporary and all the affected parameters (i.e., haemoglobin, haematocrit, plasmatic lactate, cholesterol, alanine aminotransferase, albumin, phosphorus and ceruloplasmin) had completely recovered within 10 days of exposure, suggesting that the treatment of carp with a sodium chloride bath represents a safe approach suitable for therapy of parasitic infections.

Keywords: aquaculture; fish parasite; salt water treatment

The occurrence of pathogens represents a serious threat to aquacultural fish farms as it can lead to a range of diseases causing significant economic losses. While larval fish stages are particularly sensitive toward parasitic diseases (Dulski et al. 2020), all fish infected by parasites tend not to thrive and may even die (Barber 2007; Garcia-Magana et al. 2019). Fish parasitic infections can be treated with various chemotherapeutics, which are usually administered in the form of therapeutic baths. Using this approach, medicinal substances dissolved in water act externally on the gills or skin, or may be absorbed through the skin and gills into the fish's body (Palikova et al. 2019). While a number of alternatives for treating parasitic infections exist in aquaculture, almost all have limitations. Optimally, a treatment

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should comply with several requirements, i.e., it should be effective against parasites at concentrations that are not toxic to the fish themselves and the compound used should be cheap and easily soluble in water. Furthermore, where the flesh of the fish is intended for human consumption, there should be no antiparasitic agent residue retained in the fish's tissue over the long-term (Lieke et al. 2019).

Common disinfectants and antiparasitic agents presently used in aquaculture include formaldehyde, peracetic acid and potassium permanganate (Svobodova et al. 2007; Valeta et al. 2016). In many countries, malachite green has been extensively used in aquaculture; however, highly stable and potentially toxic residues of the parent compound, as well as its metabolite leucomalachite green, have been detected in fish tissues after absorption, representing a health risk to both the fish and humans, as the final consumers of fish meat (Hashimoto et al. 2011; EFSA CONTAM Panel 2016; Valeta et al. 2016). For this reason, the use of malachite green is not authorised in EU fisheries and the possible presence of residue in fish meat intended for the EU market is carefully monitored (European Commission 2002; European Commission 2019). Formaldehyde, another antiparasitic agent, is usually applied as a 36% to 38% solution known as formalin. The first recorded use of formalin was in the beginning of the 20th century, specifically in 1909. However, while it is extremely effective against protozoan and monogenean parasites of both farmed and marine fish (Alderman and Michel 1992; Tavares-Dias 2021), it is potentially carcinogenic. A further disadvantage of formalin is that it can remove oxygen from the water (Svobodova et al. 2007; Palikova et al. 2019). Potassium permanganate, as a strong oxidiser, can act as an effective agent against fish diseases; however, its action is non-selective and it will oxidise any organic matter, including fish mucus and gill tissue (Svobodova et al. 2007; Valeta et al. 2016). Moreover, therapeutic concentrations of the compound are very close to those lethal for fish, while high potassium permanganate concentrations in water can cause undesirable changes in the water colour (Svobodova et al. 2007; Valeta et al. 2016).

A number of more environmentally friendly disinfectant/antiparasitic methods exist, such as treatment by hydrogen peroxide or peracetic acid. These substances are highly reactive and degrade easily into harmless by-products; nevertheless, their low stability can pose a problem in that an effective concentration of the active compounds can decrease very rapidly in water (Svobodova et al. 2007; Pedersen et al. 2009; Valeta et al. 2016).

A further alternative for fish antiparasitic treatment is the use of sodium chloride, or common table salt (Dulski et al. 2020). Sodium chloride is an easily available and cost-effective compound that has often been used in practice; indeed, it is sometimes referred to as the "aspirin of aquaculture" (Bolivar et al. 2004). It is known that common salt has effectively been used in fish treatment since the 1950s (Sellose and Rowland 1990). In addition to the antiparasitic therapy, it has also been used as an agent for reducing the effects of nitrite poisoning, for stimulating mucus production and for reducing stress from manipulation or transportation (Bolivar et al. 2004; Maciel et al. 2018). Previous studies have suggested that immersion in sodium chloride baths helps reduce the occurrence of important protozoans, helminths and fungal pathogens involving ectoparasitic genera such as *Cryptobia*, *Ichthyobodo*, *Chilodonella*, *Trichodina* and *Trichodinella* (Noga 1995; Svobodova et al. 2007; Palikova et al. 2019). Nevertheless, therapeutic bath conditions, especially the sodium chloride concentration and duration and temperature of the bath, need to be carefully considered in relation to the species, developmental stage and the health of the fish being treated. The most common conditions applied include preparation of a solution of 10 g to 30 g of sodium chloride per 1 litre of water, with the application lasting 15 min to 30 min (maximum one hour). The bath temperature should be kept above 5 °C at all times, as once it drops below that temperature, the treatment efficiency may be substantially reduced (Noga 1995; Svobodova et al. 2007; Palikova et al. 2019). Unlike some of the above-mentioned antiparasitics, sodium chloride is relatively harmless to humans (Svobodova et al. 2007) and, if necessary, the bath can be repeated several times (Svobodova et al. 2007).

The aim of this study was to assess the safety of short-term sodium chloride baths for the common carp (*Cyprinus carpio*), one of the most commonly produced and consumed fish in Central Europe (Nebesky et al. 2016), by examining the fish histology and a range of haematological, biochemical and oxidative stress indices. The bath conditions (c = 30 g l^{-1}; T = 30 min; t = 20 °C) were chosen so that they matched the actual therapeutic concentrations used in aquaculture for treating parasitic infections.

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MATERIAL AND METHODS

Experimental design

In this experiment, we used eighty two-year-old common carp from the Mendel University fishery farm (a breeder of experimental animals approved by the Ministry of Agriculture of the Czech Republic). At the time of testing, all the fish were of good health status with no ecto- or endoparasites. Before the experiment started, the 80 carp were randomly divided into eight groups, which were then placed into glass tanks (i.e., 10 fish per tank) filled with 180 l^{-1} of dechlorinated water (t = 20 ± 1 °C) and left to acclimatise for fourteen days. Subsequently, four groups were treated in 99.8% sodium chloride baths [sodium chloride concentration (c) = 30 g l^{-1}; duration of exposure (T) = 30 min, temperature (t) = 20 °C)], while the other four groups were left untreated to serve as controls. Immediately after exposure (T0), the fish from one of the treated groups and an untreated control were sampled for further analysis (see below), while the remaining three groups of treated and untreated fish were transferred into tanks filled with fresh dechlorinated water. One treatment group and one untreated control were then sampled at 24, 48 and 240 h after exposure (T24, T48 and T240, respectively). The water temperature, oxygen concentration and pH were recorded in all the tanks twice per day, and half of the water volume was changed every 24 hours. Over the course of the experiment, the water temperature ranged from 19 °C to 21 °C, the pH values ranged from 7.9 and 8.4 and the dissolved oxygen levels never fell below 60% saturation. The concentration of ammonia, chlorides and nitrites ranged from 0.05 mg l^{-1} to 1.14 mg l^{-1}, 78 mg l^{-1} to 110 mg l^{-1} and 0.2 mg l^{-1} to 1.94 mg l^{-1}, respectively. The fish mortality, feed intake and behaviour were also monitored throughout the experiment.

Upon sampling, blood was taken from the tail vein for the haematological and biochemical examination and determination of the oxidative stress. The fish were then sacrificed for the histological examination of the tissues (see below) by first stunning the fish with a blow to the head and then killing it through spinal transection. The sacrifice procedure was conducted in compliance with national Czech legislation on the protection of animals against cruelty (Act No. 246/1992 Coll.) and the relevant laws on the protection, breeding and use of experimental animals (Decree No. 419/2012 Coll.).

Haematological profile

The blood for the haematological and biochemical examination was sampled from the tail vein of each fish and transferred into tubes containing sodium heparin (10 µl of anticoagulant and 1 ml of blood) and chilled. The total red blood cell count (RBC; erythrocytes) was determined microscopically in a Burker chamber, with Natt-Herrick's solution used to stain the blood cells. The blood for the determination of packed cell volume (PCV; haematocrit) was sampled with a haematocrit capillary and centrifuged (18 928 *g*, 3 minutes). The haemoglobin (Hb) concentration was determined spectrophotometrically using the cyanohaemoglobin method. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were also examined. The MCV was calculated as the PCV multiplied by 1 000 and divided by the RBC; the MCH was calculated as the Hb concentration divided by the RBC and the MCHC was calculated as the Hb divided by the PCV. The differential leucocyte counts were monitored by optical microscopy after staining blood smears on glass slides with a Pappenheim solution, according to the methodology of Svobodova et al. (2012), with six different cell populations distinguished, i.e., lymphocytes, monocytes, myelocytes, metamyelocytes, neutrophil granulocytes (bands) and neutrophil granulocytes (segments).

Biochemical indices

The blood for the biochemical analysis was centrifuged for 10 min at 4 °C and a relative centrifugal force of 800 *g*. The plasma, thus obtained, was stored at −80 °C for further analysis. The concentrations of the albumin, ammonia, total protein, cholesterol, glucose, triacylglycerols, lactate, calcium and phosphorus were determined using a Konelab 20i biochemical analyser (Thermo Fischer Scientific, Waltham, USA).

The same instrument, together with commercial test kits (Biovendor, Brno, Czech Republic), were used for the determination of the plasmatic enzyme activity, specifically alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH).

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Oxidative stress indices

The blood plasma samples were also used for the determination of two oxidative stress indices, specifically ceruloplasmin and FRAP (ferric reducing ability of plasma).

The FRAP was measured by reducing the ferric ions to ferrous ions at a low pH using a Konelab 20i biochemical analyser (Thermo Fischer Scientific, Waltham, USA), while the ceruloplasmin activity was determined using *p*-phenylenediamine, the concentration being expressed as the absorbance increase per minute × 10 000, as described in detail in Haluzova et al. (2010).

Histopathology

A histological examination was carried out on a total of 48 fish (i.e., six fish per group), with six different tissue samples taken from each fish, i.e., liver, spleen, anterior and posterior kidneys, gills and skin. Each sample was fixed in 10% formalin, dehydrated and then embedded into paraffin blocks. These were then processed using a microtome to obtain ultrathin 4 µm slices. The slices were then installed onto microscopic slides and stained with haematoxylin and eosin (H&E).

Finally, the microscopic slides were examined under an optical microscope in order to detect any pathologies.

Statistical analysis

The data obtained were analysed using Unistat for Excel v6.5 statistical software, with the results presented as the mean ± SD (n = 10 in each group). First, the data were tested for normality using the Shapiro-Wilks test. An unpaired t-test was used for the normally distributed data. In the case of non-normal distribution, the data were subjected to a non-parametric Mann-Whitney test. The statistical evaluations were carried out only between the control group and treated group at the same time point. The differences between the groups were considered as significant at $P < 0.05$ and $P < 0.01$.

RESULTS AND DISCUSSION

Haematological profile

Sodium chloride baths have often been used in aquaculture to reduce the occurrence of some parasitic diseases (Bolivar et al. 2004); nevertheless, a careful assessment of the possible toxic effects is still required in order to confirm the safety of this therapeutic approach.

In this study, we only observed significant differences for the PCV and Hb in the T24 treatment group ($P < 0.05$), with no differences observed in the other haematological indices for any other time period (Table 1). Regarding the leucocyte dif-

Table 1. Haematological indices for the common carp (*Cyprinus carpio*) after treatment in a sodium chloride bath ($\bar{x}$ ± SD), n = 10

Index	T0		T24		T48		T240	
	treated	control	treated	control	treated	control	treated	control
RBC (T l^{-1})	2.02 ± 0.58	2.14 ± 0.41	1.68 ± 0.45	1.89 ± 0.64	1.64 ± 0.32	1.76 ± 0.56	2.08 ± 0.34	2.28 ± 0.57
Hb (g l^{-1})	61.75 ± 2.96	63.48 ± 4.17	**58.10 ± 3.99***	**54.19 ± 6.44***	62.20 ± 7.47	61.34 ± 5.01	63.30 ± 6.32	59.45 ± 4.35
PCV (l l^{-1})	0.32 ± 0.02	0.32 ± 0.01	**0.33 ± 0.03***	**0.29 ± 0.03***	0.32 ± 0.04	0.32 ± 0.03	0.35 ± 0.03	0.33 ± 0.03
MCV (fl)	158.6 ± 34.13	149.2 ± 39.65	195.4 ± 52.24	155.1 ± 49.19	197.9 ± 24.10	183.0 ± 64.94	168.4 ± 38.35	145.2 ± 32.79
MCH (pg)	30.60 ± 6.99	29.77 ± 8.09	34.56 ± 8.31	28.74 ± 7.78	37.92 ± 6.79	34.85 ± 12.02	30.49 ± 6.67	26.04 ± 7.06
MCHC (l l^{-1})	0.19 ± 0.01	0.20 ± 0.01	0.18 ± 0.01	0.19 ± 0.02	0.19 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.18 ± 0.02

Hb = haemoglobin; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; MCV = mean corpuscular volume; PCV = packed cell volume (haematocrit); RBC = red blood cell count; SD = standard deviation

Asterisk and bold font indicate a significant difference between the control and treated groups at the same time point; *$P < 0.05$

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ferential counts, we observed a significant increase for the lymphocytes ($P < 0.05$) and a significant decrease ($P < 0.05$) in the neutrophil myelocytes and metamyelocytesin the T0 treated group (Table 2), as well as a significant decrease in the percentage of monocytes in the T48 group ($P < 0.01$).

As the T240 group showed no significant impacts in regards to any of the haematological indices investigated, this would suggest that a period of 10 days is sufficient to allow the complete recovery from any effects of the sodium chloride therapeutic bath.

The haematological indices in fish usually cover a relatively wide range due to exogenous (e.g., water temperature, oxygen content) and endogenous (e.g., age, sex) factors (Svobodova et al. 2012). Nevertheless, our haematological profile results are consistent with those of previous studies. For example, Enevova et al. (2018), who studied the effect of salt baths (8 g l^{-1}, 14 and 21 days) on parasitic kidney disease caused by *Tetracapsuloides bryosalmonae* in rainbow trout (*Oncorhynchus mykiss*), described a significant increase ($P < 0.05$) in the PCV and Hb at 21 days after immersion, suggesting that the increase represented a compensatory reaction to the stress that contributed to their gradual recovery. Similarly, Demska-Zakes et al. (2021), who tested the effects of a 30 min sodium chloride bath on pikeperch (*Sander lucioperca*) at T0 and T24, observed that the haemoglobin levels, haematocrit, and red blood cell count were significantly increased in fish treated with either a 10 g l^{-1} or 20 g l^{-1} sodium chloride concentration, with the changes in the haematological profile persisting after 24 hours. Brown et al. (2001), in testing the osmoregulatory ability of pikeperch by transferring the fish from freshwater to saline water (c = 16 g l^{-1} sodium chloride), recorded an increase in both the blood PCV and Hb within 24 h; however, both haematological indices were restored to control values over the following five days while remaining in the saline water. According to these authors, the initial rise in the PCV was probably the result of an adrenergic stress response, with the osmotic water loss also contributing to the observed effect (Brown et al. 2001). According to Svobodova et al. (2012), salinity stress can also trigger changes in the differential leucocyte count, which would explain the significant increase in the percentage of lymphocytes observed in our study in the T0 treated fish.

Biochemical indices

Changes in the plasmatic biochemical profile are considered a highly sensitive indicator of the fish's condition (Svobodova et al. 2012). Probably the most sensitive biochemical index investigated in our study was the plasmatic lactate. Surprisingly, while a significant increase ($P < 0.01$) in the lactate concentration was detected in the T0 treated fish, significantly lower lactate concentrations were

Table 2. Differential leucocyte counts for the common carp (*Cyprinus carpio*) after treatment in a sodium chloride bath ($\bar{x}$ ± SD), $n = 10$

Cell types (%)	T0		T24		T48		T240	
	treated	control	treated	control	treated	control	treated	control
Lymphocytes	**94.80 ± 2.20***	**91.30 ± 3.53***	93.50 ± 4.14	95.15 ± 1.80	88.55 ± 6.07	86.05 ± 7.49	92.30 ± 2.21	94.00 ± 2.79
Neutrophilic myelocytes	**2.25 ± 1.13***	**2.95 ± 0.99***	3.13 ± 1.95	2.07 ± 0.89	4.05 ± 2.78	4.05 ± 2.86	2.00 ± 2.16	1.60 ± 1.78
Neutrophilic metamyelocytes	**2.65 ± 1.25***	**4.15 ± 0.44***	2.11 ± 1.08	1.95 ± 0.60	3.90 ± 2.09	4.20 ± 2.23	2.80 ± 0.92	1.70 ± 1.89
Neutrophilic bands	1.25 ± 0.35	1.29 ± 0.39	1.00 ± 0.00	1.00 ± 0.00	2.19 ± 0.66	2.00 ± 1.49	1.30 ± 1.57	0.80 ± 0.63
Segmented neutrophils	1.14 ± 0.38	1.39 ± 0.70	1.29 ± 0.76	1.10 ± 0.22	1.39 ± 0.60	2.39 ± 1.24	0.70 ± 0.67	1.30 ± 1.25
Monocytes	1.33 ± 0.41	1.20 ± 0.40	2.00 ± 0.85	1.44 ± 0.46	**1.56 ± 0.60****	**2.80 ± 0.92****	0.90 ± 0.74	0.60 ± 0.52

SD = standard deviation

Asterisk and bold font indicate significant difference between control and treated group at the same time point; *$P < 0.05$, **$P < 0.01$

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observed in the T24 ($P < 0.05$) and T48 ($P < 0.01$) treated fish (Table 3). In the T0 group, the cholesterol was significantly lower ($P < 0.05$) and the phosphorus concentration significantly higher ($P < 0.05$) than the controls. Finally, we recorded a significantly lower ($P < 0.05$) ALT activity in the T24 group, and significantly lower ($P < 0.01$) albumin concentrations in the T48 group. No significant differences were observed in any of the biochemical indices for the T240 treated fish (Table 3).

Our results are comparable with those reported by Demska-Zakes et al. (2021), who placed pikeperch in sodium chloride baths at concentrations of either 10 or 20 g l^{-1} for 30 or 60 minutes. They reported increased lactate and glucose levels in the fish sampled immediately after the bath, and no significant differences against the control after 24 hours. The increase in the lactate levels, also observed in the T0 fish in our experiment, may have occurred due to activation of a stress re-

Table 3. Plasma biochemical indices for the common carp (*Cyprinus carpio*) after treatment in a sodium chloride bath ($\bar{x}$ ± SD), $n = 10$

Index	T0		T24		T48		T240	
	treated	control	treated	control	treated	control	treated	control
Albumin (g l^{-1})	10.97 ± 1.60	11.84 ± 1.88	10.93 ± 2.44	10.37 ± 2.14	**10.9 ± 2.16****	**13.95 ± 1.90****	10.58 ± 2.55	9.66 ± 2.55
ALP (µkat l^{-1})	0.29 ± 0.22	0.42 ± 0.29	0.36 ± 0.27	0.44 ± 0.28	0.51 ± 0.42	0.37 ± 0.24	0.4 ± 0.24	0.39 ± 0.23
ALT (µkat l^{-1})	0.42 ± 0.09	0.59 ± 0.36	**0.41 ± 0.13***	**0.55 ± 0.16***	0.40 ± 0.11	0.57 ± 0.48	0.17 ± 0.15	0.18 ± 0.04
AST (µkat l^{-1})	3.42 ± 2.05	2.62 ± 1.49	3.21 ± 2.20	3.81 ± 2.42	3.83 ± 1.96	2.36 ± 1.16	1.44 ± 0.67	1.08 ± 0.23
Ammonia (µmol l^{-1})	174.9 ± 56.58	165.0 ± 52.93	233.0 ± 56.98	201.0 ± 82.34	202.1 ± 45.49	209.7 ± 48.89	262.5 ± 85.25	223.1 ± 69.59
Total protein (g l^{-1})	28.06 ± 3.23	31.33 ± 3.88	27.96 ± 3.87	27.23 ± 2.06	28.12 ± 2.62	30.61 ± 3.38	26.90 ± 2.52	24.99 ± 1.71
Cholesterol (mmol l^{-1})	**4.92 ± 0.63***	**5.93 ± 0.90***	5.47 ± 0.80	5.61 ± 0.57	5.56 ± 1.11	6.16 ± 1.34	5.90 ± 0.93	5.59 ± 1.00
Phosphorus (mmol l^{-1})	**2.39 ± 0.58***	**1.70 ± 0.47***	3.01 ± 0.45	2.94 ± 0.48	2.59 ± 0.34	3.17 ± 0.76	3.73 ± 0.78	3.17 ± 0.98
Glucose (mmol l^{-1})	6.58 ± 1.40	7.46 ± 2.15	5.70 ± 2.24	7.24 ± 2.61	7.93 ± 2.44	8.10 ± 2.07	5.30 ± 1.29	5.21 ± 0.66
LDH (µkat l^{-1})	20.43 ± 25.59	7.68 ± 8.17	10.71 ± 9.77	20.06 ± 24.45	10.19 ± 10.80	6.83 ± 11.00	2.87 ± 3.02	2.30 ± 2.00
Lactate (mmol l^{-1})	**3.23 ± 1.06****	**1.90 ± 0.73****	**3.73 ± 1.14***	**4.84 ± 0.96***	**3.83 ± 0.75****	**5.23 ± 1.14****	6.51 ± 1.78	6.79 ± 2.32
TAG (mmol l^{-1})	2.91 ± 0.52	3.26 ± 0.50	3.02 ± 0.50	3.01 ± 0.79	3.49 ± 0.67	3.36 ± 0.64	2.49 ± 0.33	2.29 ± 0.33
Calcium (mmol l^{-1})	2.65 ± 0.51	2.69 ± 0.32	2.91 ± 0.28	2.79 ± 0.31	2.62 ± 0.55	3.14 ± 0.53	2.95 ± 0.48	3.17 ± 0.46

ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; LDH = lactate dehydrogenase; SD = standard deviation; TAG = triacylglycerols

Asterisk and bold font indicate a significant difference between the control and treated groups at the same time point; *$P < 0.05$, **$P < 0.01$

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sponse through adrenergic stimulation (Galkova 2015). Lactate, which is easily transported between organs, plays a crucial role in cellular metabolism, where it serves as the main energy source. This is of particular importance in stressful situations. Thus, a decrease in the plasma lactate at T24 and T48 may suggest an accelerated metabolic transformation within the fish or stress adaptation (Bakker et al. 2013). The increase in the phosphorus concentration after the treatment is explained by the metabolic acidosis caused by the increase in the lactate concentration, while the decrease in the plasmatic concentrations of the cholesterol (T0) and albumin (T48) may be the result of the reduced synthesis (Doubek et al. 2014).

Oxidative stress indices

A range of water pollutants are able to trigger the generation of reactive oxygen species (ROS) in living organisms, thereby inducing oxidative stress. Antioxidant defence systems (either enzymatic or non-enzymatic), the main role of which is to protect organisms from ROS, can be used to evaluate the toxic effects of various chemicals, especially those causing oxidative damage (Valavanidis et al. 2006). The main role of non-enzymatic antioxidants (e.g., glutathione, vitamin E, ascorbate, FRAP, ceruloplasmin, urate) is to scavenge and bind free radicals (Birben et al. 2012). In our study, while the ceruloplasmin was significantly lower ($P < 0.01$) in the T0 treated fish, there were no differences in either the ceruloplasmin or FRAP in the fish sampled at T24, T48 or T240 (Table 4).

Unlike our study, Enevova et al. (2018) described a significant increase in the plasma oxidative indices, including the FRAP and ceruloplasmin, an acute-phase reactant (Haluzova et al. 2010), in the treated group 14 days after immersion, indicating an effective adaptation response to stress. Our results suggest that fish adapt relatively quickly to sodium chloride baths and that, with the used water temperatures and exposure times, they represent a safe therapeutic treatment for fish (Svobodova et al. 2007; Palikova et al. 2019).

Histopathology

In our study, we observed no histopathological changes in the liver, spleen, anterior and posterior kidneys, gills or skin in all the treated and control groups (T0, T24, T48 and T240). Similarly, Enevova et al. (2018) observed no histopathological changes in the groups treated with an 8 g l^{-1} sodium chloride solution and the untreated groups after 14 and 21 days. In comparison, formalin and peracetic acid baths, which are also used as antiparasitic agents, cause strong histopathological effects, with extensive changes observed on the gills and skin in common carp immediately and after 24 h, 48 h and 10 days, and numerous mucinous elements on the skin, following exposure to a formalin treatment bath (Chmelova et al. 2016). Moreover, structural damage to the lamella and numerous mucinous cells have been observed on the gills of common carp (Chmelova et al. 2016), whereas Chupani et al. (2014) observed pathological alterations in the secondary and primary la-

Table 4. Plasma oxidative stress indices for the common carp (*Cyprinus carpio*) after treatment in a sodium chloride bath ($\bar{x}$ ± SD), $n = 10$

Index	T0		T24		T48		T240	
	treated	control	treated	control	treated	control	treated	control
FRAP (μmol l^{-1})	642.8 ± 126.79	672.2 ± 101.97	631.8 ± 101.06	653.0 ± 117.23	551.9 ± 96.73	588.8 ± 89.95	617.3 ± 77.89	602.4 ± 129.24
Cp (ΔA min^{-1} × 10 000)	**18.96 ± 2.96****	**25.44 ± 5.64****	22.63 ± 5.23	25.20 ± 6.11	25.73 ± 4.21	33.27 ± 14.23	25.73 ± 4.21	23.30 ± 3.38

Cp = ceruloplasmin; FRAP = ferric reducing ability of plasma; SD = standard deviation

Asterisk and bold font indicate a significant difference between the control and treated groups at the same time point; **$P < 0.01$

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mellae, including haemorrhaging, fusions and focal degeneration of the secondary lamellae in grass carp (*Ctenopharyngodon idella*) after immersion in a peracetic acid treatment bath.

The prevention of fish parasitic infections is an important issue in aquaculture as it can lead to economic losses. While numerous methods exist for treating parasitic infections, most have serious disadvantages that limit their use. Previous use sodium chloride baths have suggested that they are a promising alternative to other, potentially more harmful, alternatives, not least as, in particular, it is inexpensive, the compound is easily soluble in water and the method is usually very effective against a wide range of (ecto)parasites. Moreover, sodium chloride does not leave toxic residue in the fish meat. Our study, which was designed to evaluate the safety of such therapeutic sodium chloride baths, used a sodium chloride concentration, bath temperature and immersion duration ($c = 30$ g l^{-1}, $T = 20$ °C, $t = 30$ min) that was as similar to the actual field use as possible.

Our results suggest that while the treatment can induce mild, short-term stress levels in fish, the physiological functions completely recover within 10 days of treatment, with no long-term histopathological, haematological, biochemical or oxidative stress impacts. Based on these results, therapeutic sodium chloride baths represent one of the safest options for treating (ecto)parasitosis in aquaculture.

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Conflict of interest

The authors declare no conflict of interest.

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