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Sodium chloride treatment effects on rainbow trout suffering from proliferative kidney disease caused by *Tetracapsuloides bryosalmonae*

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ABSTRACT: The aim of the present study was to evaluate the effect of a long-term sodium chloride bath on rainbow trout *Oncorhynchus mykiss* naturally infected by *Tetracapsuloides bryosalmonae*. A total of 106 infected fish were divided into 2 groups. One group was left untreated and the other was treated with sodium chloride in increasing doses up a concentration of 0.8%. After 14 d, treatment was stopped and for a further 7 d the fish response to the sodium chloride bath was observed. Cumulative mortality was significantly lower in the treated group (19.2%) compared to the untreated group (31.5%) after 21 d. This corresponded to the lower but non-significant parasite intensity in kidney and spleen in the treated group after 14 d of treatment. However, lower prevalence of parasites in both tissues was recorded in the untreated group after 21 d of treatment, but a significant difference was observed only in spleen tissue. Furthermore, significant increases in leukocytes, hemoglobin, haematocrit, ferric reducing ability of plasma, and ceruloplasmin, and significant decreases in alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase activities were noticed in the treated group compared to the untreated group. In contrast, significant decreases in lysozyme concentration in the mucus and phagocyte oxidative burst in the blood were observed in the treated group. Histopathological examination revealed proliferative and reparative changes in parenchymatous tissues in the treated group. The 14- and 21-d salt bath used in rainbow trout with proliferative kidney disease was associated with a reduction in mortality and enhanced the reparative phase in the treated group.

KEY WORDS: PKD · Salt bath · Hematological indices · Biochemical indices · Immunological parameters · Oxidative stress parameters · Histopathology · Immunohistochemistry

INTRODUCTION

Proliferative kidney disease (PKD) is a parasitic disease in salmonid fish caused by *Tetracapsuloides bryosalmonae* (Myxozoa: Malacosporea), and it is associated with significant economic losses in both farmed and wild salmonid fish (Anderson et al. 1999, Canning et al. 2000). The life cycle of the parasite involves freshwater bryozoans as invertebrate hosts and salmonid fish as vertebrate hosts. Spores from

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infected bryozoans are released into the water, and infect the fish through the skin and gills (Feist et al. 2001). *Tetracapsuloides bryosalmonae* is recirculated via the blood and enters the kidney — the main target organ of the infection.

PKD development depends on water temperature and it occurs seasonally during the summer and autumn months. The disease progresses more rapidly and becomes more severe as temperatures increase. Clinical signs occur at water temperatures above 15°C (Clifton-Hadley et al. 1986b, Hedrick et al. 1993, Tops et al. 2006, Bettge et al. 2009), while the disease does not develop at low water temperature (<10°C) (Gay et al. 2001). The most frequent pathological signs are exophthalmus and abdominal distension. Other pathological changes include ascites, gill and liver pallor, darkened body, oedema, and spleen and renal enlargement (Clifton-Hadley et al. 1987). A heavily swollen and pale kidney tissue is often used as an indicator of PKD, but detection of *T. bryosalmonae* through histological examination remains the gold standard for diagnosis of the disease. In severe cases, PKD causes high mortality due to anaemia, stress, and secondary bacterial infections (Clifton-Hadley et al. 1986a). It is possible to avoid introduction of the parasite to a fish farm by using ground water rather than stream water in the farm, and occurrence of signs of this disease may be reduced through management practice (Ferguson 1981).

Treatment of PKD is problematic. Although fumagillin and its analogue TNP-470 are effective PKD treatments in salmonids, higher doses can cause anorexia or mortality due to depletion of splenic and renal hematopoietic tissue (Wishkovsky et al. 1990, le Gouvello et al. 1999, Morris et al. 2003, McGurk 2005), and neither pharmaceutical is currently approved for treating PKD in farmed fish destined for human consumption. Another possibility is treatment using NaCl, which is usually used for parasitic infections (Hedrick & Aronstien 1987, Noga 2010). NaCl has been recommended as a treatment for PKD at a concentration of 0.8–1.2% for 14 d. This treatment resulted in a reduction of mortality and a reduced occurrence of clinical and histopathological signs of infection (O'Hara 1985, Svobodová et al. 2007). Nevertheless, to our knowledge, there are still a limited number of studies providing us with a comprehensive evaluation of PKD treatment.

The aim of the present study was to evaluate the effect of NaCl baths in rainbow trout *Oncorhynchus mykiss* naturally infected by *T. bryosalmonae*. We focused on a comparison of alterations in hematological, biochemical, immunological and oxidative stress indices in both the treated and the untreated experimental groups. Histopathological examination of selected tissues and parasite prevalence and intensity of infection in kidney and spleen tissues was also performed.

MATERIALS AND METHODS

Fish and design of experiment

In the present study, 106 one-year-old rainbow trout *Oncorhynchus mykiss* were used, originating from the commercial farm BioFish s.r.o. (Highlands, near the city Kamenice nad Lipou, Czech Republic; 49.3194° N, 15.0946° E, 600 m above sea level). All fish used in our study exhibited a range of clinical and pathological changes typical for PKD, including enlarged body cavity, multiple petechial haemorrhages in skin, exophthalmus, haemorrhagic/non-haemorrhagic ascites, pale gills and liver, splenomegaly and markedly enlarged kidneys. The experiment was carried out in an experimental recirculation system at the Department of Ecology and Diseases of Game, Fish and Bees (University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic). Fish were divided into 4 tanks — 2 groups were treated with NaCl bath (26 fish in each tank) and the other 2 groups (27 fish in each tank) were left untreated. Each group was kept in laminate circular tank (1 m^3 in volume), with separate recirculating system. The treated group was linearly dosed with NaCl every day, reaching a final concentration of 0.8% on the 14th day of treatment. The therapy was stopped after 14 d, but fish responses were observed for a period of another 7 d after NaCl administration had been discontinued. The final concentration of NaCl at the end of the experiment in the treated group was 0.4%. Any debris from the bottom was removed and approximately 10% of the water volume was exchanged every day. During the test, the condition of the fish and the number of dead fish were recorded for each experimental group at 12 h intervals. Water quality was checked at 12 h intervals as well and no differences between the tanks were observed. The water temperatures ranged from 17.7 to 18.5°C, pH from 7.80 to 8.11 and saturation of oxygen from 84.8 to 98.2%. Both groups were fed commercial feed pellets (Biomar EFICO Alpha 717, 4.5 mm) at a total rate of 1% of body weight

twice a day. A photoperiod regime of 12 h light: 12 h dark was used.

After 14 d of treatment, 15 of the fish in the treated group and 15 of the fish in the untreated group were sampled. Additional sampling was carried out on Day 21 from the rest of the fish in both groups (27 fish from the treated group; 22 fish from the untreated group). Samples consisted of individual skin mucus, blood and selected tissues. Blood samples were taken by puncturing the caudal vein and were stabilized with sodium heparin (50 IU ml^{-1} of blood). Blood samples were used for establishing hematological, biochemical and immunological indices. After blood sampling, the fish were stunned with a blow to the head and killed by spinal transection, and other selected tissue samples were taken for further ana-lysis. On each sampling day, biometric data such as body length, body weight and liver weight were measured. The hepatosomatic index (HSI = [liver weight/body weight] × 100) and Fulton's coefficient (FCF = [body weight × 10^5/body $length^3$]) were also calculated. Experimental procedures were conducted in compliance with national legislation, i.e. Act 246/1992 Coll., on the protection of animals against cruelty, as amended and Decree 419/2012 Coll., on the Protection, Breeding, and Use of Experimental Animals, as amended.

Hematological and biochemical profile

One portion of heparinized blood was immediately used for establishing selected hematological indices such as red blood cell (RBC) count, hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC) count and differential leukocyte count (Svobodová et al. 2012). Another portion of heparinized blood was centrifuged (855 × *g*, 4°C, 10 min), and individual plasma samples were separated and stored at −85°C until further analysis was carried out. The analyzed biochemical indices included albumin, total protein, ammonia, glucose, triacylglycerols, lactate, cholesterol, calcium, phosphorus, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and creatine kinase (CK), and the indices were measured using a Konelab 20i biochemical analyzer (ThermoFisher Scientific) with commercial test kits (Biovendor).

Immunological profile

Immunological indices were analyzed in samples of heparinized blood (phagocyte oxidative burst), blood plasma (total immunoglobulin level [IgM], total complement activity) and skin mucus (lysozyme concentration).

Zinc sulphate precipitation was used for the determination of IgM in plasma samples according to McEwan et al. (1970). Quantification of IgM was based on the total protein level determined using a commercially available kit (DC Protein Kit, Bio-Rad Laboratories) before and after precipitation. The final IgM concentration was calculated as the difference between the total plasma protein and protein present in the supernatant after precipitation and centrifugation.

Lysozyme concentration was determined using radial diffusion in agarose gel containing *Micrococcus luteus* (CCM 169). Skin mucus was gently wiped off the fish using the blunt edge of an eye scalpel. Mucus samples of 15 µl were placed in a well cut into the agarose gel. The mean diffusion zone was measured following 24 h incubation at room temperature. Lysozyme concentration was converted to mg ml^{-1} according to a standard calibration curve (Poisot et al. 2009).

The bioluminescent strain of *Escherichia coli* K12 (pEGFPluxABCDEamp) was used to determine the total complement activity in the blood plasma (Atosuo et al. 2013). Bioluminescence was measured using an LM01-T luminometer (Immunotech). The results are expressed as the percentage loss of viability for bacterial cells killed by complement in the sample as compared to a positive control containing bacterial cells and phosphate buffer only. Percentage mortality was calculated from integrals (positive control taken as 100% viability) of the reaction curve area (Buchtíková et al. 2011).

Luminol-enhanced chemiluminescence was used to assess respiratory burst activity, taken as a measure of phagocyte activity. The reaction mixture contained 50× diluted blood in Hank's balanced salt solution (Sigma), 10^{-3} mol l^{-1} luminol (Molecular Probes) dissolved in borate buffer, and 0.25 mg ml^{-1} opsonized Zymosan A from *Saccharomyces cerevisiae* (Sigma) used as an activator. Zymosan A was opsonized by 30-min incubation with fish serum followed by 3-fold washing in sterile saline solution. The results are expressed as maximum respiratory burst intensity (reaction peak) and maximum respiratory burst intensity adjusted to 1000 phagocytic cells (Buchtíková et al. 2011).

Oxidative stress indices

Liver tissue and blood plasma samples were used for analysis of selected oxidative stress indices. All samples were stored at −85°C until analysis. After thawing, samples of liver tissue were homogenized using phosphate buffer (pH 7.2, 1:10 w/v). One part of the homogenate was used for determination of lipid peroxidation using thiobarbituric acid reactive substances (TBARS) according to Lushchak et al. (2005). Data were expressed as nmol of TBARS per gram of tissue wet weight. The second part of the homogenate was centrifuged for 20 min at 4°C and 10 500 × *g* and the supernatant was used for analysis of antioxidant enzyme activities (glutathione peroxidase, GPx; glutathione reductase, GR; and glutathione *S*-transferase, GST) and protein concentration. Acitivities of GPx, GR and GST were measured according to the methods of Flohe & Gunzler (1984), Carlberg & Mannervik (1975) and Habig et al. (1974), respectively. All enzyme activities were normalized and expressed per mg of protein concentration, which was determined using bicinchoninic acid (Smith et al. 1985). Ceruloplasmin activity in the plasma and the ferric-reducing ability of the plasma (FRAP) were measured using methods described by Sevcikova et al. (2016). All analyses were performed spectrophotometrically using a Varioskan Flash Spectral Scanning Multimode Reader. Only FRAP activity was determined using the Konelab 20i biochemical analyzer.

Histological and immunohistochemical examination

First, samples of selected tissues were fixed in 10% formalin for histopathological (liver, caudal kidney and spleen) and immunohistochemical (kidney and spleen) analyses. Formalin-fixed samples were dehydrated, embedded in paraffin wax, sectioned on a microtome at a thickness of 5 µm, stained with hematoxylin and eosin, and examined for histopathological changes. Sections for immunohistochemical examination were stained using a mouse monoclonal anti-*T. bryosalmonae* antibody (AquaMAb-P01, Aquatic Diagnostics), deparaffinized and washed, and endogenous peroxidase activity was blocked using 3% of hydrogen peroxide (Adams et al. 1992). Nonspecific binding sites were blocked with 10% goat serum (Sigma-Aldrich). Then, sections were incubated with mouse monoclonal anti-*T. bryosalmonae* antibody at a 1:100 ratio followed by biotin-conjugated goat anti-mouse IgG at a ratio of 1:1000. Parasites were visualized with the aid of streptavidin-HRP (Sigma-Aldrich) followed by AEC (3-amino-9-ethylcarbazole; Dako Chemicals) staining. Ten microscopic fields per slide (magnification ×200, total evaluated area 1.71 mm^2) were randomly selected and the mean number of parasites (intensity of infection) per field was determined in kidney and spleen tissues (Palikova et al. 2017).

Statistical analysis

Statistical evaluation was carried out using Unistat 5.6 for Excel. Data were first tested for normality using the Shapiro-Wilk test. For normally distributed data, an unpaired *t*-test was applied to test the differences between the treated and the untreated groups. In the case of non-normal distribution, data were subjected to the Mann-Whitney test. Statistical evaluation was carried out only between the treated and the untreated groups at the same time. For statistical evaluation, both duplicate groups were merged because we did not observed statistically significant differences between these replicates. The significance level was set at $p < 0.05$.

RESULTS AND DISCUSSION

The aim of our study was to evaluate the effect of an NaCl bath on rainbow trout *Oncorhynchus mykiss* naturally infected with *Tetracapsuloides bryosalmonae*. We focused on an assessment of alterations in selected biomarkers. The dose of NaCl was linearly increased for 14 d in the group of treated fish up to a maximum concentration of 0.8%, and fish responses were studied for a further period of 7 d. The design of our experiment was inspired by O'Hara (1985), who observed positive effects in Atlantic salmon *Salmo salar* when the water salinity was first adjusted to 0.8% and then increased to 1.2%. O'Hara (1985) found that mortality as well as clinical signs and symptoms of PKD were reduced during this treatment. Histopathology revealed a rapid healing process as regards melanisation, fibrosis, and destruction of agents. In view of their results, they suggested that the treatment reduced mortality and led to the destruction of the parasite.

In our study, on sampling Day 14, cumulative mortality was 15.4% (8 individuals) and 18.5% (10 individuals) in the treated and untreated groups, respectively. On sampling Day 21, mortality was 19.2%

(10 individuals) and 31.5% (17 individuals) in the treated and untreated groups, respectively (Fig. 1). A significant difference between the treated and untreated-groups was observed only on sampling Day 21. Biometric indices such as body length, body weight, liver weight, HSI and FCF did not differ between the treated and untreated groups (Table 1). During our experiment, both the treated and the untreated groups showed normal behavior. There were no signs of respiratory distress such as hyperventilation, fish floating on the water surface or increased rate of opercular movements.

Likewise, a non-significant decrease in the prevalence of parasites was detected in the kidney and spleen tissues after 14 d of treatment (Table 2). After 21 d of treatment, however, a lower prevalence of parasites in both tissues was recorded in the untreated group, but a significant difference was observed only in spleen tissue. Stimulation of non-specific defense mechanisms has already been described by several authors (Angelidis et al. 1987, Foott & Hedrick 1990, Lauriano et al. 2016, Palikova et al. 2017). Some authors (Burgos-Aceves et al. 2016, Palikova et al. 2017) concluded that the non-specific cellular response plays the most important role during the recovery period.

Immunological examination is important for the evaluation of fish health status during treatment of several diseases. The results of immunological examination in our study are shown in Fig. 2. In this study, the non-specific response represented by phagocytic oxidative burst decreased in the treated fish on Day 14, i.e. immediately after the treatment was finished. We suggest that this depletion is caused by the direct effect of NaCl application. In contrast, the results of phagocyte oxidative burst in the treated and untreated groups were comparable on Day 21, which is in accordance with the short half-life and rapid turnover of blood phagocytes. Lysozyme activity was significantly decreased in treated fish on both Day 14 and Day 21. Although the mechanism of the decrease in lysozyme activity is still unknown and further research is necessary, a direct effect of NaCl application could be supposed. Interestingly, Fast et al. (2002) described significantly higher lysozyme activity in Atlantic salmon reared in freshwater than in the same fish reared in salt water. Total immunoglobulin level appears to be stimulated by NaCl treatment; however, our study did not confirm any significant difference between the treated and the untreated groups.

Furthermore, histopathological examination revealed proliferative and reparative alterations in different tissues. In the treated group, a prevalence of proliferative and reparatory changes was found

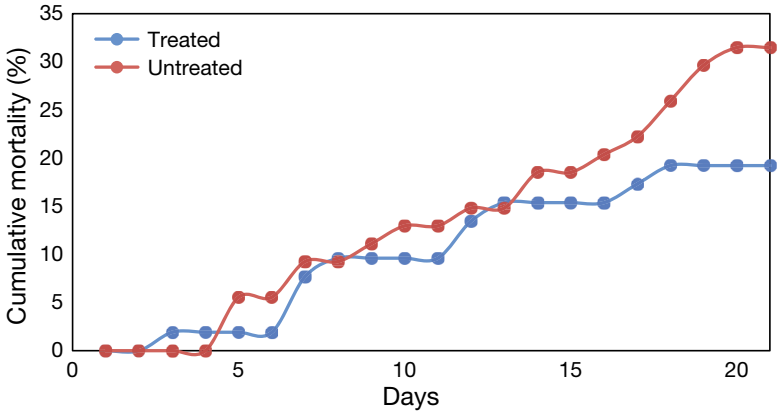

Fig. 1. Cumulative mortality of rainbow trout *Onchorhynchus mykiss* during NaCl treatment of proliferative kidney disease

Table 1. Biometric indices of rainbow trout *Onchorhynchus mykiss* during NaCl treatment of proliferative kidney disease. Data are means ± SEM. No significant differences between the treated and untreated groups were found at the same sampling point. his: hepatosomatic index; FCF: Fulton's condition factor

	Day 14		Day 21	
	Treated (n = 15)	Untreated (n = 15)	Treated (n = 27)	Untreated (n = 22)
Body length (mm)	203.9±2.7	198.9±5.8	204.7±7.4	191.3±4.3
Body weight (g)	133.1±5.7	130.7±10.3	124.5±9.6	108.0±8.3
Liver weight (g)	1.8±0.2	1.9±0.2	1.7±0.1	1.7±0.2
HSI	1.4±0.1	1.5±0.1	1.4±0.1	1.5±0.2
FCF	1.6±0.0	1.6±0.0	1.5±0.1	1.5±0.0

Table 2. Mean number of *Tetracapsuloides bryosalmonae* (mean from 10 fields, magnification ×200) and prevalence (%) in kidney and spleen of rainbow trout *Onchorhynchus mykiss*. Data are means ± SEM. Significant differences between the treated and untreated groups at the same sampling point are indicated in bold font (*$p < 0.05$)

	Day 14		Day 21	
	Treated (n = 15)	Untreated (n = 15)	Treated (n = 27)	Untreated (n = 22)
Kidney	1.1±0.7; 40%	2.0±1.0; 47%	2.0±1.1; 33%	3.0±1.9; 27%
Spleen	0.6±0.4; 27%	0.9±0.6; 40%	0.6±0.4; **33%***	0.6±0.7; **7%***

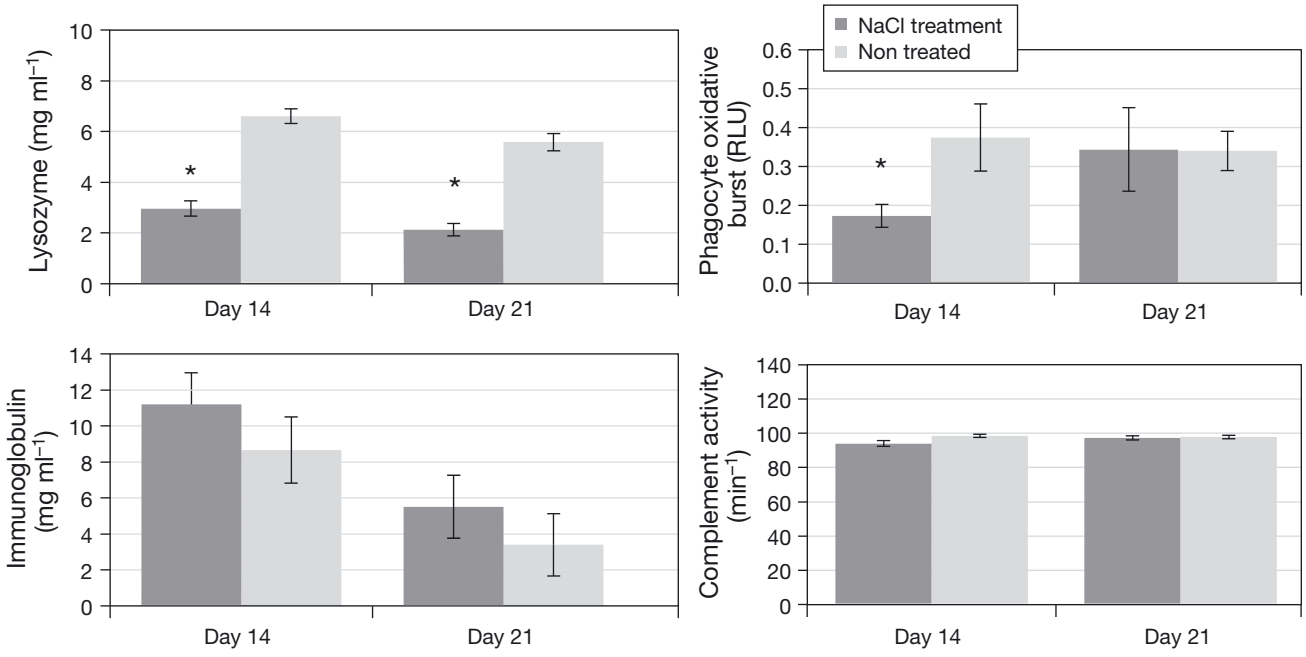

Fig. 2. Immunological indices in rainbow trout *Onchorhynchus mykiss* during NaCl treatment of proliferative kidney disease. Data are means ± SEM. Significant differences between the treated and untreated groups at the same sampling point are indicated with an asterisk (*$p < 0.05$). RLU: relative luminescence units (× 1000)

in the tissues evaluated, particularly in spleen and kidney parenchyme. In the kidney, a frequent occurrence of hyaline droplet degeneration of tubular lining was found (Fig. 3). There was mild parenchymatous degeneration of the liver. Proliferative changes were also found in the untreated group, with reactive changes being more readily observable than reparatory ones (Fig. 4). Damage to parenchymatous tissues was clearly greater in the untreated group than in the treated one. By contrast, the presence of parenchymatous degeneration of the liver was observed much less often. In addition, the hyaline droplet degeneration was only localized. Histopathological changes in the observed organs on Days 14 and 21 were similar both in the treated and the untreated groups. Our findings are in compliance with the results of other studies that also reported a decrease in erythropoiesis after kidney damage due to parasite infection (Clifton-Hadley et al. 1987, Palikova et al. 2017).

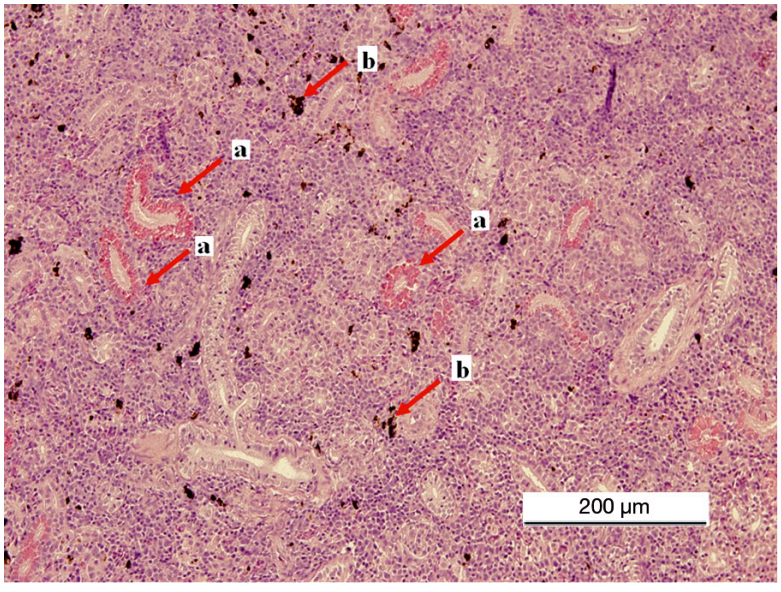

Fig. 3. Rainbow trout *Onchorhynchus mykiss* kidney (treated group). (a) Numerous areas of hyaline droplet degeneration of the tubular lining; and (b) small pigment deposits (H&E stain, magnification ×200)

Many alterations were observed in the hematological indices during the treatment as well (Table 3). A significant increase ($p < 0.05$) in Hct and Hb was found in the treated group compared to the untreated

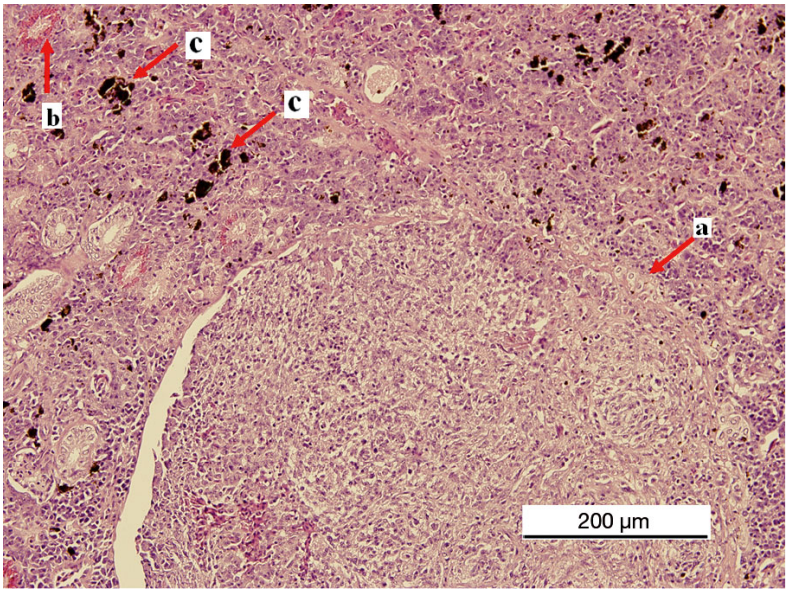

Fig. 4. Rainbow trout *Onchorhynchus mykiss* kidney (untreated group). (a) Nodular area of the fibro-granulation tissue; (b) areas of hyaline droplet degeneration; and (c) numerous pigment depositions (H&E stain, magnification ×200)

group after 21 d of treatment. In addition, the erythrocyte count increased in the treated group compared to the untreated group after 21 d, but this change was not significant. The remaining indices were comparable in the treated and the untreated group at the same sampling point. Decreases in Hct, Hb and erythrocyte counts are typical findings in the acute phase response to the disease, and they are restored during the regeneration phase (Hoffmann & Lommel 1984, Clifton-Hadley et al. 1987, Foott & Hedrick 1990, Palikova et al. 2017). An increase in the monitored hematological indices in the treated group indicates faster recovery of hematopoiesis and organism regeneration. Alterations of RBC indices relate to a decrease in the number of parasites in the kidney and spleen in the treated group and faster regeneration of parenchymatous organs as well. The reports concerning the WBC count and immune parameters during PKD are not uniform. Although a decrease in WBC count and neutrophilia were observed by Clifton-Hadley et al. (1987) and by Palikova et al. (2017), other studies (Angelidis et al. 1987, Foott & Hedrick 1990) observed increased leukocyte values and neutrophilia in rainbow trout with PKD. Palikova et al. (2017) described that during a period of 3 mo after PKD outbreak, fish were able to recover leukocyte counts to values seen in control, healthy fish. In our work, all WBC types were increased in the treated group on Day 14 compared to untreated fish. However, 1 wk later, WBC counts decreased again, to approximately the levels seen in the untreated group. We therefore assume that this effect was a part of an adaptive reaction to increasing salinity, rather than an indicator of faster recovery of renal tissue. Interestingly, Soltanian & Fereidouni (2017) tested an adaptive reaction of the marine species *Periophthalmus waltoni* to gradually decreasing salinity, and in their work, a similar trend was observed — an increase in WBC counts on day 17 followed by a decrease to close to control values. In the present study, significant changes in most plasma biochemical indices were observed during treatment (Table 4). Significant increases ($p < 0.05$) in the levels of ammonia, glucose, calcium and cholesterol were found in the treated group compared to the untreated group on both sampling days. In contrast, a significant decrease ($p < 0.05$) in LDH activity was observed in the treated group compared to the untreated group on both sampling days. In addition,

Table 3. Hematological indices in rainbow trout *Onchorhynchus mykiss* during NaCl treatment of proliferative kidney disease. Data are presented as means ± SEM. Significant differences between the treated and untreated groups at the same sampling point are indicated in **bold** (*$p < 0.05$). Hb: hemoglobin; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; MCV: mean corpuscular volume; Hct: hematocrit; RBC: red blood cell; WBC: white blood cell

	Day 14		Day 21	
	Treated	Untreated	Treated	Untreated
RBC (10^{12} l^{-1})	1.05±0.04	1.06±0.06	1.28±0.10	1.02±0.07
Hct (l l^{-1})	0.28±0.02	0.27±0.01	**0.30±0.01***	**0.25±0.02***
Hb (g l^{-1})	60.01±4.25	60.58±2.54	**64.95±2.50***	**52.70±4.07***
MCV (fl)	278.05±12.40	262.19±11.49	251.09±14.71	248.75±9.82
MCH (pg)	57.88±2.67	58.58±2.76	53.56±3.06	52.17±1.95
MCHC (g l^{-1})	0.21±0.01	0.23±0.01	0.21±0.00	0.21±0.01
WBC (10^9 l^{-1})	**38.27±4.57***	**18.00±3.67***	21.53±2.65	25.23±2.57
Lymphocytes (10^9 l^{-1})	**31.99±3.57***	**15.19±3.10***	17.56±2.32	21.33±2.67
Monocytes (10^9 l^{-1})	**2.91±0.73***	**1.18±0.20***	1.26±0.24	1.68±0.39
Neutrophil granulocytes (10^9 l^{-1})	**3.37±0.60***	**1.63±0.37***	2.77±0.72	2.52±0.37

Table 4. Plasma biochemical indices in rainbow trout *Onchorhynchus mykiss* during NaCl treatment of proliferative kidney disease (PKD). Data are means ± SEM. Significant differences between the treated and untreated groups at the same sampling point are indicated in **bold** (*p < 0.05). ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDH: lactate dehydrogenase; CK: creatine kinase

	Day 14		Day 21	
	Treated	Untreated	Treated	Untreated
Albumin (g l^{-1})	16.81±0.74	14.78±1.32	16.93±0.88	18.36±0.58
Total protein (g l^{-1})	42.84±2.02	37.61±2.87	42.13±2.30	45.75±1.11
Ammonia (µmol l^{-1})	**162.83±10.92***	**135.97±6.31***	**234.22±18.36***	**169.84±11.52***
Glucose (mmol l^{-1})	**3.78±0.28***	**3.10±0.15***	**5.37±0.28***	**3.57±0.28***
Triacylglycerols (mmol l^{-1})	**1.72±0.18***	**1.06±0.10***	2.23±0.25	1.88±0.21
Lactate (mmol l^{-1})	1.75±0.35	1.65±0.25	4.90±0.58	3.81±0.62
Calcium (mmol l^{-1})	**2.43±0.04***	**2.22±0.04***	**2.62±0.04***	**2.47±0.05***
Phosphorus (mmol l^{-1})	2.84±0.26	2.67±0.07	2.91±0.11	2.91±0.08
Cholesterol (mmol l^{-1})	**6.61±0.40***	**4.44±0.27***	**7.28±0.34***	**4.94±0.18***
ALP (µkat l^{-1})	1.09±0.18	0.84±0.12	**0.88±0.10***	**0.56±0.05***
ALT (µkat l^{-1})	**0.27±0.04***	**0.38±0.03***	0.28±0.02	0.33±0.02
AST (µkat l^{-1})	**6.35±0.48***	**8.22±0.54***	6.81±0.43	7.96±0.40
LDH (µkat l^{-1})	**15.73±3.35***	**24.38±2.03***	**15.80±1.53***	**19.95±1.43***
CK (µkat l^{-1})	34.44±5.62	51.70±10.27	54.77±8.97	51.46±12.48

significant raised levels ($p < 0.05$) of triacylglycerol content and ALP activity were observed in the treated group in comparison to the untreated group on sampling Days 14 and 21, respectively. Significant decreases ($p < 0.05$) in activities of ALT and AST were likewise observed in the treated group compared to the untreated group on sampling Day 14. The remaining indices monitored were found to be comparable in both groups at the same sampling point. A decrease in ALT, AST and LDH activities in the treated group compared to the untreated group indicated less damage to the parenchymatous organs due to lower parasite numbers and predominant proliferative-reparative processes in these tissues. In contrast, an increase in ammonia, glucose, triacylglycerols, calcium and cholesterol in the treated group compared to the untreated group was observed. We suggest that these changes could be caused by alterations in the osmotic rates in the organism as a result of the NaCl bath. As mentioned above, histopathological examination revealed hyaline droplet degeneration in the kidney tubular epithelium and mild parenchymatous degeneration of liver, which relate to a lower liver and kidney detoxification ability. We found that lipid and glycide metabolism was also affected, which corroborates results reported by Harikrishnan et al. (2003). The effect of saltwater treatment (1.05%) on energetic balance was also studied by Wang et al. (1997), who documented reduced feed conversion in the experimental group after 57 d of exposure. Significant increases in ammonia excretion, oxygen consumption and reduced body weight were observed as well.

Oxidative stress, incorporating both antioxidant defenses as well as oxidative damage, is a common effect in fish organisms exposed to different types of environmental factors such as changes in physico-chemical water quality indices, presence of pollutants or disease occurrence (Lushchak et al. 2005, Valavanidis et al. 2006, Faggio et al. 2015, Bartoskova et al. 2013, Faggio et al. 2016). The results of oxidative stress indices are given in Table 5. Significant increases in plasma oxidative indices such as FRAP and ceruloplasmin were discovered in the treated group after 14 d of the experiment. By contrast, a significant decrease in ceruloplasmin activity was detected in the treated group

Table 5. Oxidative stress indices in rainbow trout *Onchorhynchus mykiss* during NaCl treatment of proliferative kidney disease (PKD). Data are means ± SEM. Significant differences between the treated and untreated groups at the same sampling point are indicated in **bold** (*p < 0.05). Cp: ceruloplasmin; FRAP: ferric reducing ability of plasma; GR: glutathione reductase; GPx: glutathione peroxidase; GST: glutathione *S*-transferase; TBARS: lipid peroxidation

	Day 14		Day 21	
	Treated	Untreated	Treated	Untreated
Plasma				
FRAP (µmol l^{-1})	**580.06±35.00***	**479.05±32.42***	515.61±28.72	449.13±23.56
Cp (ΔA min^{-1} × 10 000)	**143.64±7.77***	**117.43±8.77***	**138.95±6.51***	**171.95±10.48***
Liver				
GR (nmol min^{-1} mg^{-1} protein)	4.35±0.39	4.37±0.52	**3.25±0.40***	**4.67±0.40***
GPx (nmol min^{-1} mg^{-1} protein)	56.18±4.36	55.10±2.93	51.81±6.85	51.00±2.82
GST (nmol min^{-1} mg^{-1} protein)	716.30±46.46	603.56±38.63	590.20±39.28	562.08±36.91
TBARS (nmol g^{-1} tissue)	12.35±1.19	12.17±0.64	10.25±0.59	9.47±0.70

after 21 d of treatment. FRAP is a non-specific test that measures the ferric to ferrous iron reduction and represents the antioxidant power of plasma. Ceruloplasmin has multiple functions including defense against oxidative stress (Haluzova et al. 2010) and it is an acute-phase reactant attendant in inflammatory responses (Bielli & Calabrese, 2002). An increase in ceruloplasmin activity after 14 d of treatment indicates an effective adaptation response of the organism to infection. However, no significant alterations between the treated and the untreated groups were observed in indices monitored in liver tissues. A significant decrease was observed only in GR activity in the treated group compared to the untreated group after 21 d of the experiment. This suggests that such changes may be a positive consequence of the salt bath.

CONCLUSIONS

The 21-day NaCl bath applied to rainbow trout *O. mykiss* with PKD caused a reduction in mortality and enhanced the reparative phase in the treated group, which corresponded to a lower intensity of parasitic infection in the kidney and spleen. A positive effect of the NaCl bath is also confirmed by a reduction in mortality, reparative changes in the parechymatous organs and an increase in hemoglobin as an indicator of faster hematopoiesis recovery. Similarly, a decrease in the activity of selected enzymes indicates a lower level of damage to tissues caused by parasites. On the basis of our results, we can recommend application of a long-term NaCl bath for effective treatment of PKD.

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