УДК 57.044:577.3:639.3:612.062

# Biochemical profile in the muscle tissue of rainbow trout (*Oncorhynchus mykiss*) exposed to Disinfectant "CIP" formulated with peracetic acid and hydrogen peroxide

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Recent research on substances such as acidic agents, e. g. disinfectants based on peracetic acid (PAA) and hydrogen peroxide show prophylactic effects against bacterial, virus, fungal infections. Such disinfectants, however, are important and effective as chemiotherapeutant agents but may have damaging effects due to the generation of reactive oxygen species (ROS) and formation of oxidation products. The aim of this study was to evaluate the impact of disinfection procedure with Disinfectant "CIP" based on 15% PAA and 20% $H_2O_2$ on the oxidative stress biomarkers (levels of 2-thiobarbituric acid reactive substances, aldehydic and ketonic derivatives of oxidatively modified proteins) and biochemical indices [alanine- (ALT) and aspartate aminotransferases (AST), lactate dehydrogenase (LDH), lactate, pyruvate] in the muscle tissue of juvenile rainbow trout *Oncorhynchus mykiss*. In the disinfectantive exposure, fish were bathed with Disinfectant "CIP" in final concentration 16 mL per $m^3$. Fish were bathed with Disinfectant "CIP" for 20 min and repeated three times every 3 days. Significantly decrease in oxidative stress biomarkers was observed in the fish exposed to Disinfectant "CIP", which may be due to excitation of defense mechanism of the fish to counter the stress induced by disinfection. In this study, the activities of ALT and AST were significantly lower in fish after treated by Disinfectant "CIP" than that of fish in control group. The rate of amino acid transformation *via* transamination is slowed down by Disinfectant "CIP". The observation that lactate level was decreased in muscle samples with decreased of LDH activity in fish treated by Disinfectant "CIP", supports the hypothesis that lactate is not transferred from white muscle *via* the blood stream before it accumulated in liver. Results suggested that the given concentration of Disinfectant "CIP" could be applied as disinfective agent in salmonid aquaculture; however, further studies are required for safe use.

**Key words:** disinfection, rainbow trout *Oncorhynchus mykiss*, muscle tissue, lipid peroxidation, oxidatively modified proteins, aminotransferases, lactate dehydrogenase, lactate, pyruvate.

## Introduction

Peracetic acid (PAA) is a strong disinfectant with a wide spectrum of antimicrobial activity. The use of PAA as a disinfectant has been drawing more attention in recent years due to its bactericidal, virucidal, fungicidal, and sporicidal effectiveness as demonstrated in various industries [Kitis, 2004]. The results of Koivunen and Heinonen-Tanski (2005) demonstrated also that PAA could be a good alternative disinfection method for elimination of enteric microbes from different wastewaters.

PAA is a therapeutic agent used for disinfection in aquaculture as well [Meinelt et al., 2015]. The practical application of the products with high molecular PAA: $H_2O_2$ ratios should be prioritized if *Aeromonas salmonicida* and *Yersinia ruckeri* are diagnosed [Meinelt et al., 2015]. The disinfection efficiency of PAA towards microorganisms can be ranked as following on a general basis: bacteria>viruses>bacterial spores>protozoan cysts [Liberti, Notarnicola, 1999; Rudd, Hopkinson, 1989; Kitis, 2004].

The combination of PAA and hydrogen peroxide was found to be synergistic [Alasri et al., 1992, 1993]. Baldry (1983) was compared the antimicrobial properties of aqueous solutions of PAA and hydrogen peroxide ($H_2O_2$) against vegetative bacteria, bacterial spores and yeasts. $H_2O_2$ is more effective as a sporicide than as a bactericide [Baldry, 1983]. $H_2O_2$ is also effective for treating certain external bacterial infections and flagellate infestations in some species of ornamental fish (serpae tetra *Hyphessobrycon eques*, tiger barb *Puntius tetrazona*, blue gourami *Trichogaster trichopterus*, suckermouth catfish *Hypostomus plecostomus*, and green swordtail *Xiphophorus hellerii*) [Russo et al., 2007]. The effects of treatment with $H_2O_2$ against a progressive infection with flatworm parasite *Zeuxapta seriolae* were determined by Mansell and co-workers (2005). McAndrew and colleagues (1998) also demonstrated the effects of $H_2O_2$ treatment on different life-cycle stages of the salmon louse, *Lepeophtheirus salmonis*. The mobile adult and pre-adult stages of *L. salmonis* readily reattached to Atlantic salmon after $H_2O_2$ treatment. Adult female lice reattached in significantly lower numbers than untreated controls [McAndrew et al., 1998]. Meinelt and co-workers (2015) compared the effectiveness of 6 commercial PAA products with different molecular PAA: $H_2O_2$ ratios to reduce bacterial growth of *Aeromonas salmonicida* and *Yersinia ruckeri* and to determine effective concentrations and exposure times. All products reduced colony-forming units (CFUs) of *A. salmonicida* and *Y. ruckeri*. $H_2O_2$ is not the driving force in the reduction of *A. salmonicida* and *Y. ruckeri* growth by PAA *in vitro*, because products with higher molecular PAA: $H_2O_2$ ratios inhibited growth better than products with lower molecular PAA: $H_2O_2$ ratios at the same PAA concentration [Meinelt et al., 2015].

A successful therapy of *Ichthyophthirius multifiliis*, one of the most dangerous diseases in aquaculture and ornamental fish breeding worldwide, with PAA is only possible while fighting the free living stages theronts and tomonts [Meinelt et al., 2007]. The toxicity of Wofasteril E400 (40% PAA) to free-living forms of *I. multifiliis* was determined shortly after tomonts were physically removed from the surface of the fish and at 2.5 and 24 h after removal. Results indicate that 0.6 to 0.9 mg $L^{-1}$ PAA killed 39 to 82% of the newly released tomonts within 48 h when treated immediately [Meinelt et al., 2009]. Results suggest that disinfectants based on PAA could be used for additional disease treatments in freshwater aquaculture; however, more information is needed to evaluate its usefulness on various diseases, species and culture settings [Straus et al., 2012a, b].

In mammals, acute toxicity of PAA after oral administration consisted from erythrocytic infiltration and exudation with morphological changes in the whole of the lungs, inflammatory to necrotic changes in the mucosa of the gastrointestinal tract, hyperaemia of the liver and spleen and damage in the renal tubules in the rats, while repeated use of bactericidal concentrations of PAA or formulations containing this substance could cause irritative changes in the respiratory tract and skin, mainly inflammatory and necrotic changes of the target organs, lung and mucosa [Busch, 1973]. The predominant systemic effects seen after exposure of animals by inhalation or dermal contact are reduced body weight gain, blood count changes and inflammatory reactions in the liver.

PAA is a strong oxidizing disinfectant, which is currently sold in several commercial compounds. Strong PAA solutions have to be handled with care but the PAA ($CH_3CO_3H$) itself breaks down in water into acetic acid ($C_2H_4O_2$) and hydrogen peroxide ($H_2O_2$) and further into water and oxygen, which all are rather harmless compounds [Jussila et al., 2011]. The mechanism of the toxicity might be that PAA and $H_2O_2$ are exogenous sources of reactive oxygen species (ROS). They can pass the cell membrane and increase the endogenous ROS concentration [Liu et al., 2016]. Hydrogen peroxide can transport across membranes by free diffusion and is scavenged by antioxidant enzymes, i. e. catalase and glutathione peroxidase under normal conditions

[Bienert et al., 2006]. Excess hydrogen peroxide can react with free Cu and Fe in the cytosol and form hydroxyl radicals leading to damage in macromolecules [Cavaletto et al., 2002].

Therefore, the aim of this study was to evaluate the impact of disinfection procedure with Disinfectant "CIP" based on 15% PAA and 20% $H_2O_2$ on the oxidative stress biomarkers (levels of 2-thiobarbituric acid reactive substances (TBARS), aldehydic and ketonic derivatives of oxidatively modified proteins) and biochemical indices (alanine- and aspartate aminotransferases, lactate dehydrogenase, lactate, pyruvate) in the muscle tissue of juvenile rainbow trout (*Oncorhynchus mykiss* Walbaum).

## Materials and methods

**Experimental Fish.** Juveniles of rainbow trout at the age of 0+ (in the first year of life) came from spawning in Department of Salmonid Research, Inland Fisheries Institute (Rutki, Zukowo, Poland). Fish were fed daily of feed with using of tape feeders. Feed daily dose was calculated according to the applicable feed table, depending on the water temperature. Fish were starved one day prior to experiment. Forty four clinically healthy rainbow trout with a mean body mass of 45.8±1.2 g were used in the experiments. Experiments were performed at a water temperature of 16±2 °C and the pH was 7.5—7.6. The dissolved oxygen level was about 12 ppm with additional oxygen supply. Every morning were measured temperature and the oxygen content in the water, using thermometer and oxygen meter, respectively. All enzymatic assays were carried out at Department of Zoology and Animal Physiology, Institute of Biology and Environmental Protection, Pomeranian University in Slupsk (Poland).

**Experimental Procedure.** The fish were divided into two groups and held in 250-L square tanks (70 fish per tank). As disinfecting agent was used Disinfectant "CIP" solution based on 15% PAA and 20% $H_2O_2$ (firm "Biochem-Art", Gdansk, Poland). It was admitted to market by the license No. 0508/04 of Minister of Health in Poland (27.01.2004) as biocidal product named Disinfectant "CIP" [Grudniewska et al., 2008]. In the disinfectant exposure, fish were bathed with Disinfectant "CIP" solution in final concentration 16 mL per $m^3$ (Group II). Fish were bathed for 20 min and repeated three times every 3 days. Bathing with Disinfectant "CIP" were taken in the morning before fish feeding. Control fish (Group I) were handled at same manner as Group II but without Disinfectant "CIP" treatment. Two days after the last bathing, twenty two individuals from each group were sampled. Fish were not anesthetized before tissue sampling.

**Tissue isolation.** Muscle tissue was removed from trout after decapitation. One trout was used for each homogenate preparation. Briefly, muscle tissue was excised, weighted and washed in ice-cold buffer. The minced tissue was rinsed clear of blood with cold isolation buffer and homogenized in a glass Potter-Elvehjem homogenising vessel with a motor-driven pestle on ice. The isolation buffer contained 100 mM tris-HCl; pH of 7.2 was adjusted with HCl.

**Analytical methods.** Thiobarbituric acid (TBA), oxidized and reduced glutathione (GSSG and GSH), NADPH, 5,5-dithio-bis-2-nitrobenzoic acid (DTNB), ethylenediaminetetraacetic acid (EDTA), thrichloroacetic acid (TCA), quercetin, hydrogen peroxide, ammonium molybdate, sodium aside, t-butylhydroperoxide, Tween 80, urea acid, 2,4-dinitrophenyl hydrazine (DNFH) were obtained from Fluka (Buchs, Switzerland). All other chemicals were of analytical grade. All enzymatic assays were carried out at 25±0.5 °C using a Specol 11 spectrophotometer (Carl Zeiss Jena, Germany). The enzymatic reactions were started by adding the homogenate suspension. The specific assay conditions are presented subsequently. Each sample was analyzed in triplicate. The protein concentration in each sample was determined according to Bradford (1976) using bovine serum albumin as a standard.

*TBARS assay for lipid peroxidation*. The level of lipid peroxidation was determined by quantifying the concentration of TBARS according to Kamyshnikov (2004) for determining the malondialdehyde (MDA) concentration. The nmol of MDA per 1 mg of tissue protein was calculated by using $1.56 \cdot 10^5$ $mM^{-1}$ $cm^{-1}$ as extinction coefficient.

*The carbonyl derivatives content of protein oxidative modification (OMP) assay.* The rate of protein oxidative destruction was estimated from the reaction of the resultant carbonyl derivatives

of amino acid reaction with 2,4-Dinitrophenylhydrazine (DNFH) as described by Levine and co-workers (1990) and as modified by Dubinina and co-workers (1995). DNPH was used for determining carbonyl content in soluble and insoluble proteins. Carbonyl groups were determined spectrofotometrically from the difference in absorbance at 370 nm (aldehydic derivatives, $OMP_{370}$) and 430 nm (ketonic derivatives, $OMP_{430}$) and expressed in nmol per mg of tissue protein.

*Assays of ALT (E.C. 2.6.1.2) and AST (E.C. 2.6.1.1) activities*. ALT and AST activity was analyzed spectrophotometrically by standard enzymatic method [Reitman, Frankel, 1957]. The ketoacids produced by the enzyme action reacts with 2,4-dinitrophenylhydrazine producing hydrazone complex measured calorimetrically at 530 nm. ALT and AST activities were expressed as μmol pyruvate per h per mg of protein.

*Assay of LDH (E.C. 1.1.1.27) activity*. The colorimetric method of Sevela and Tovarek (1959) was used for the determination of LDH activity LDH activity was expressed as μmol pyruvate per h per mg of protein.

*Assays of lactate and pyruvate concentrations*. Lactate and pyruvate concentration was measured according to the procedure described by Herasimov and Plaksina (2000). One mL of tissue homogenate sample was added to 6 mL distilled water and 1 mL metaphosphoric acid (10%). The mixture was centrifuged at 800g for 5 min to separate the supernatant. 1 mL $CuSO_4$ (25%) and 0.5 g $Ca(OH)_2$ were added to the supernatant, which was then mixed for 30 min. The mixture was centrifuged at 1,000g for 10 min. For lactate concentration assay the resulting supernatant was resuspended in 3 mL ρ-dimethylamino benzaldehyde and 1 mL NaOH (25%). Solutions were heated in a water bath at 37°C for 45 min, which was then centrifuged at 1000g for 10 min. The absorbance was measured at 420 nm. Solution with ρ-dimethylamino benzaldehyde and NaOH (25%) was used as blank. For pyruvate concentration assay the resulting supernatant was resuspended in 0.1 mL $CuSO_4$ (10%), 4 mL $H_2SO_4$, and 0.1 mL hydroquinone, which was then heated in a water bath at 100°C for 15 min. The absorbance was measured at 430 nm. Calibration curve of lactate (0.1—5 mM) and pyruvate (0.1—5 mM) was used, and results were expressed in nmol per mg protein.

**Statistical analysis.** The mean ± S.E.M. values was calculated for each group to determine the significance of inter group difference. All variables were tested for normal distribution using the Kolmogorov-Smirnov and Lilliefors test ($p>0.05$). Significance of differences between the oxidative stress biomarkers and biochemical indices (significance level, $p<0.05$) was examined using Mann-Whitney *U* test. Correlations between parameters at the set significance level were evaluated using Spearman's correlation analysis [Zar, 1999]. All statistical calculation was performed on separate data from each individual with STATISTICA 8.0 (StatSoft, Krakow, Poland).

## Results

TBARS as biomarkers of lipid peroxidation as well as aldehydic and ketonic derivatives as biomarkers of protein oxidation in the muscle tissue of rainbow trout disinfected by Disinfectant "CIP" are presented in Table 1. TBARS and ketonic derivatives of oxidatively modified proteins showed significant decrease by 24% ($p=0.047$) and by 31% ($p=0.003$), respectively between controls and Disinfectant "CIP"-treated group (Table 1).

Biochemical profile in the muscle tissue of rainbow trout treated by Disinfectant "CIP" are shown in Fig. 1. The ALT, AST, and LDH ac-

**Table 1.** Oxidative stress biomarkers in the muscle tissue of the trout treated by CIP-Disinfectant.

| Oxidative stress biomarkers | Control group (n=22) | Disinfectant "CIP"-treated group (n=22) |
|---|---|---|
| TBARS, nmol·mg$^{-1}$ protein | 534.30±43.96 | 403.71±30.70* |
| Aldehydic derivatives of OMP, nmol·mg$^{-1}$ protein | 18.66±1.60 | 15.93±2.78 |
| Ketonic derivatives of OMP, nmol·mg$^{-1}$ protein | 13.52±1.20 | 9.38±1.77* |

Data are represented as mean ± S.E.M.
* — the significant difference was shown as $p<0.05$ when compared disinfected group and control group values.

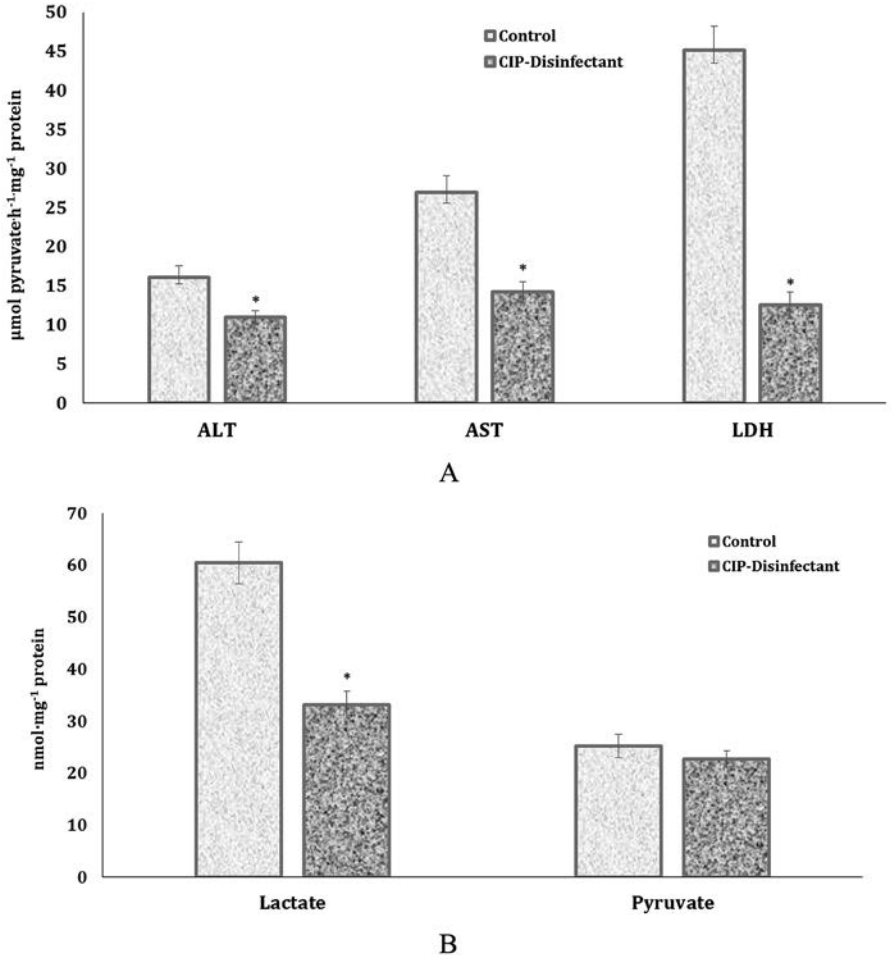


**Fig.** 1. Activity of alanine- and aspartate aminotransferases, lactate dehydrogenase (A), lactate and pyruvate level (B) in the muscle tissue of the trout treated by Disinfectant "CIP".

Data are represented as mean ± S.E.M. * — the significant difference was shown as $p<0.05$ when compared disinfected group and controls values.

tivity was lower by 32% ($p=0.009$), by 47% ($p=0.000$), and by 72% ($p=0.000$), respectively after Disinfectant "CIP" traetment. The lactate level in the muscle tissue of disinfected trout was significantly decreased by 45% ($p=0.000$) compared to the controls (Fig. 1).

Several correlations between checked parameters were found (Figs 2 and 3). In control group, ALT activity correlated positively with LDH activity ($r=0.833$, $p=0.000$) and pyruvate level ($r=0.691$, $p=0.000$), as well as AST activity correlated positively with LDH activity ($r=0.790$, $p=0.000$) and pyruvate level ($r=0.798$, $p=0.000$). LDH activity correlated positively with TBARS ($r=0.497$, $p=0.019$) and ketonic derivatives of OMP ($r=0.440$, $p=0.040$). Pyruvate level correlated positively with LDH ($r=0.539$, $p=0.001$) and lactate level ($r=0.678$, $p=0.001$) (Fig. 2).

In group treated by Disinfectant "CIP", ALT correlated positively with LDH activity ($r=0.662$, $p=0.002$) and lactate level ($r=0.978$, $p=0.000$), as well as LDH activity ($r=0.899$, $p=0.000$) and lactate level ($r=0.681$, $p=0.005$). AST activity correlated positively with ALT ($r=0.633$, $p=0.003$) and LDH activity ($r=0.499$, $p=0.025$), while LDH activity correlated positively with aldehydic ($r=0.510$, $p=0.022$) and ketonic derivatives of OMP ($r=0.457$, $p=0.043$) (Fig. 3).

## Discussion

Our study revealed significantly decrease of oxidative stress biomarkers in the muscle tissue,

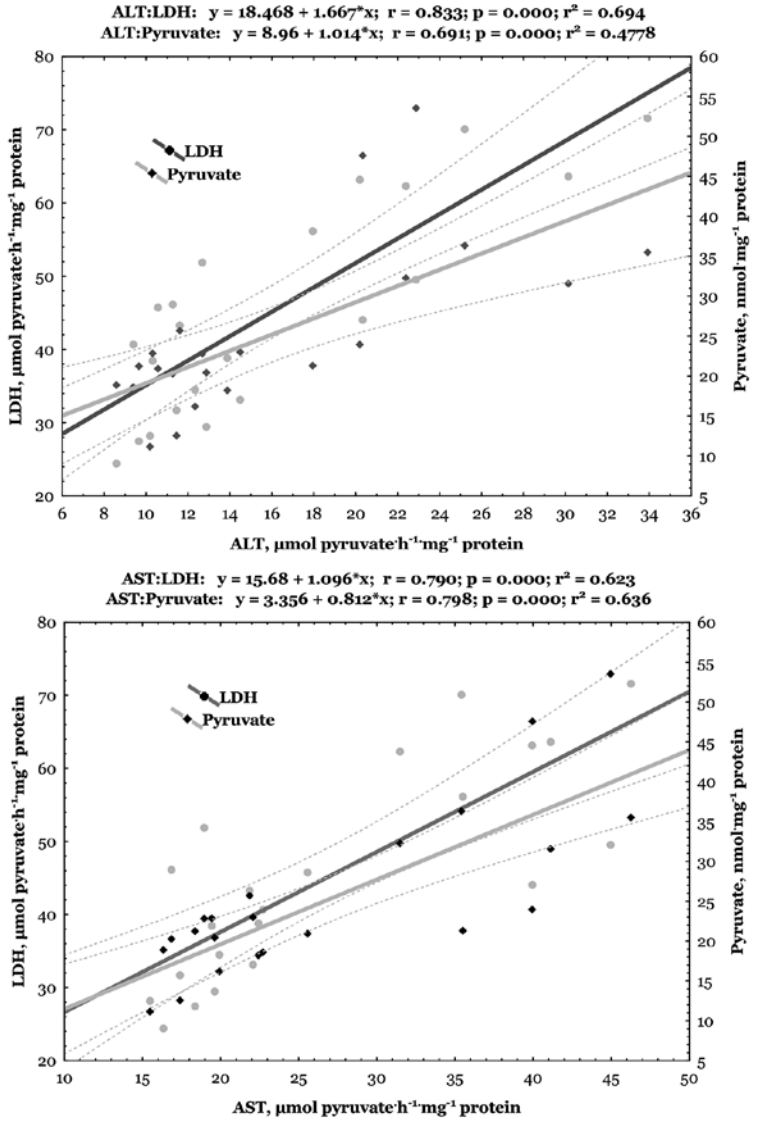


**Fig.** 2.

a clear oxidative stress inhibition. Lipid peroxidation, a complex process resulting from free radical reactions in biological membranes forms lipid hydroperoxides which decompose double bonds of unsaturated fatty acids and destructs membrane lipids [Carvalho Cdos et al., 2012]. The exposure of Disinfectant "CIP" to rainbow trout also caused a significant decrease in ketonic derivatives of oxidatively modified proteins (Fig. 1B). Similar significantly decrease in lipid peroxidation (TBARS as indirect marker of lipids peroxidation) was observed in the fish treated by Disinfectant "CIP" (Table 1), which may be due to excitation of defense mechanism of the fish to counter the stress of toxicant.

ALT and AST are the most important aminotransferases in fish tissues [Fynn-Aikins et al., 1995]. Moreover, the activity of AST or ALT is closely related to amino acid metabolism in fish and the transaminase activity enhanced with the increase of amino acid metabolism [Cai et al., 2015]. Activities of serum ALT, AST, and LDH have been commonly used in the diagnosis of fish diseases as well as in the detection of tissue damage caused by environmental pollution. An increase of these enzyme activities in the extracellular fluid or serum is a sensitive indicator of

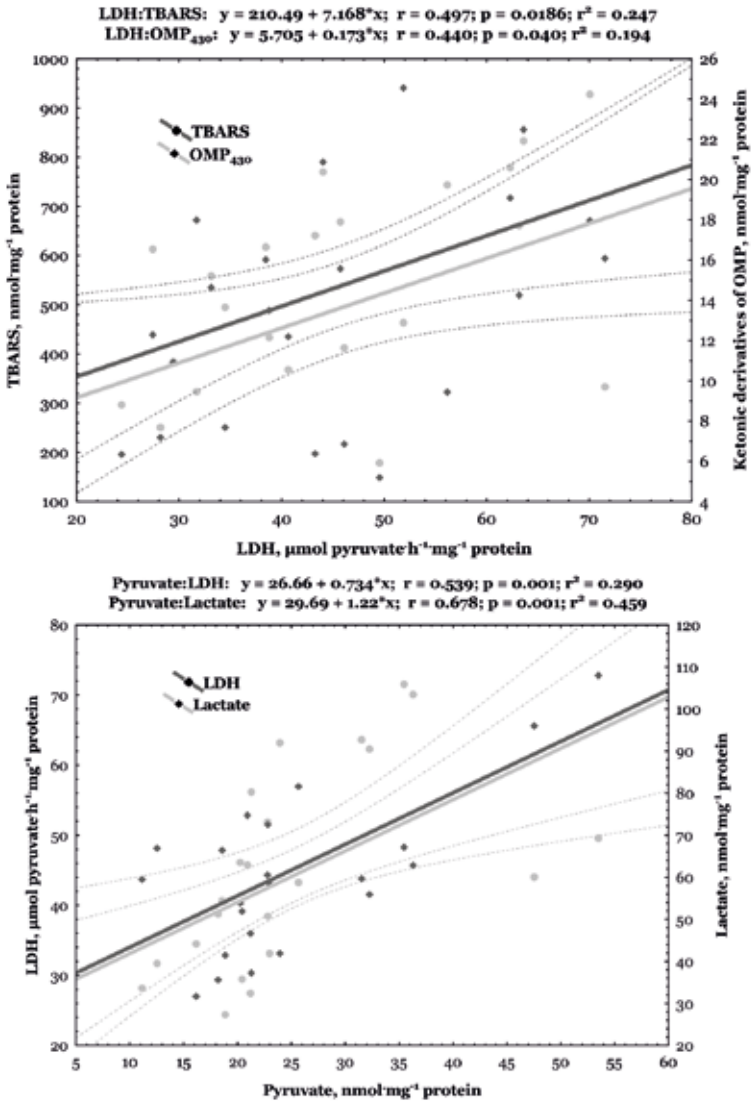


**Fig.** 2. Correlations between oxidative stress biomarkers and biochemical indices in the muscle tissue of rainbow trout from unhandled controls.

even minor cellular damage and indicates stress-based tissue impairment [Firat et al., 2011]. In this study, the activities of ALT and AST were significantly lower in fish treated by Disinfectant "CIP" than that of fish in control group (Fig. 1A). This suggests that combination PAA with hydrogen peroxide could decrease the amino acid metabolism of muscle tissue in rainbow trout. This indicates that the rate of amino acid transformation *via* transamination is slowed down by Disinfectant "CIP" treatment.

LDH (EC1.1.1.27) plays a crucial role in maintaining aerobic metabolism by converting lactate to pyruvic acid. The cytosolic enzyme LDH is commonly used as a cell lysis marker in medical and toxicology domains [Morcillo et al., 2016; Diop et al., 2016]. The observation that lactate level was decreased in muscle samples with decreased of LDH activities in fish treated by Disinfectant "CIP" (Figs 1 and 2), supports the hypothesis that lactate is not transferred from white muscle *via* the blood stream before it accumulated in liver. Muscle lactate level in disinfected trout was considerably lower with constant level of pyruvate level (Fig. 1). This was probably caused by an inhibition of gluconeogenesis to maintain levels

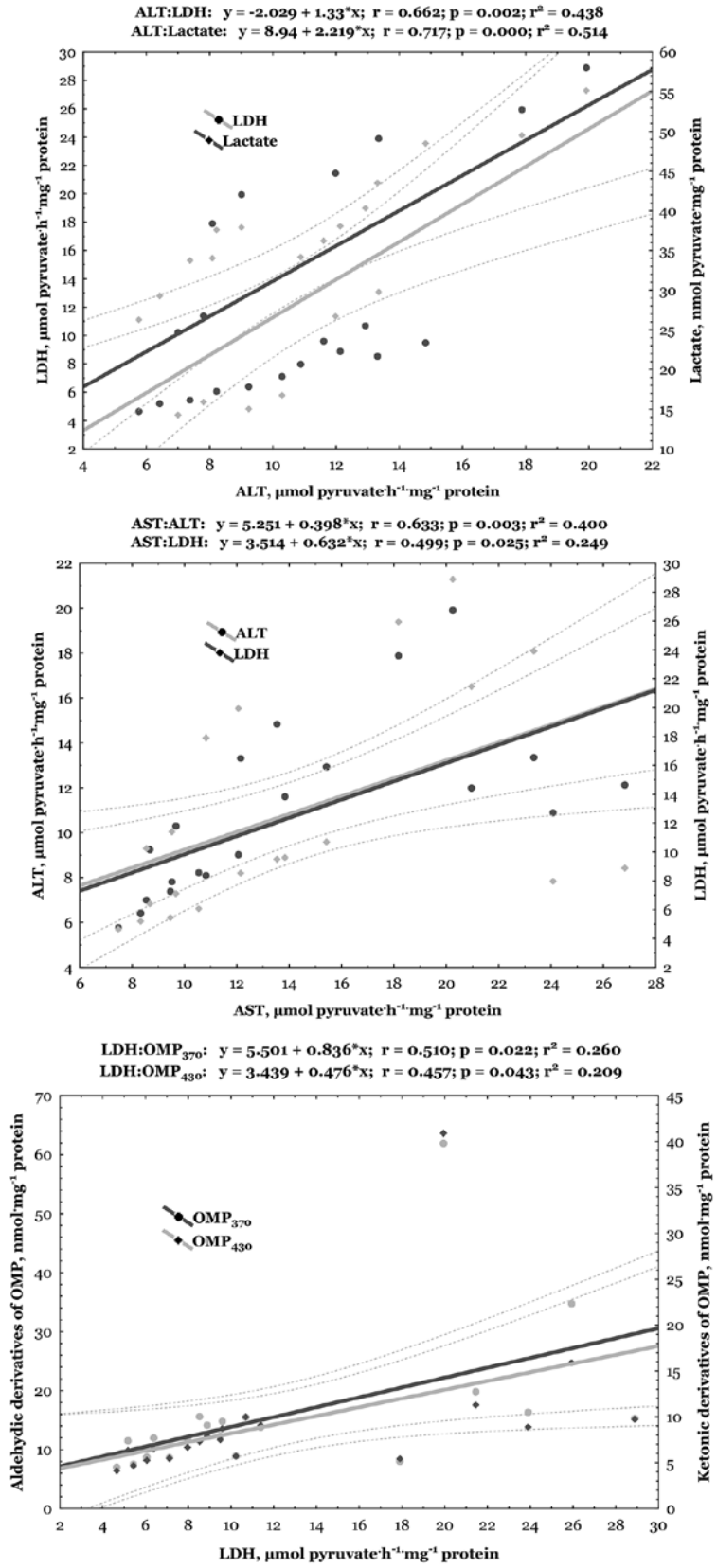


**Fig.** 3. Correlations between oxidative stress biomarkers and biochemical indices in the muscle tissue of the trout treated by Disinfectant "CIP".

of circulation glucose. Consequently, decreased serum glucose levels can be caused. Thus, it can be assumed that Disinfectant "CIP" affected anaerobic metabolism in trout muscle.

Correlations between oxidative stress biomarkers, amino acid metabolism and aerobic/anaerobic metabolism suggested about a significant role of these indices in assessment of different disinfectant influence and seems to be a sensitive indicators of the fish responses to disinfected procedures (Figs 2 and 3). Our results showed a significant correlation of ALT and AST with the LDH activity and pyruvate level in the muscle tissue of control group indicating about link between amino acid metabolism and aerobic metabolism (Fig. 3). TBARS and ketonic derivatives of OMP are correlated with LDH activity, which suggested that oxidative stress is linked with anaerobic metabolism both in Disinfectant "CIP"-treated and control groups (Figs 3 and 4). Thus, the activity of muscle ALT, AST and LDH together with lactate and pyruvate concentrations as well as oxidative stress biomarkers seems to be a sensitive indicators of the fish responses to treatment by Disinfectant "CIP".

In our previous study [Tkachenko et al., 2014], the effects of Disinfectant "CIP" on the oxidative stress biomarkers and antioxidant defenses [superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx), total antioxidant capacity] in muscle, gill, hepatic and cardiac tissues of rainbow trout were determined. Lipid peroxidation and carbonyl contents were shown to change according to the tissue type. Exposure to Disinfectant "CIP" led to a significant decrease of lipid peroxidation in the muscle tissue and carbonyl content in the muscle and gill tissues. The inhibition of SOD and CAT activity in muscle, hepatic and cardiac tissues was observed probably due to increased oxidative stress during disinfection; however, hepatic and cardiac GPx activity was increased, in an attempt to counteract oxidative stress. We suggest that oxidative stress during the oxidation of PAA and hydrogen peroxide may be counteracted by the antioxidant system in various tissues of rainbow trout. Correlative analysis between oxidative stress biomarkers and antioxidant defense confirm pivotal role of SOD and CAT against oxidative stress caused by Disinfectant "CIP" [Tkachenko et al., 2014].

The present finding is also in agreement with the results of other researchers according useful effects of PAA application in aquaculture [Meinelt et al., 2007; Hébert et al., 2008; Meinelt et al., 2009; Straus, Meinelt, 2009; Picón-Camacho et al., 2012; Straus et al., 2012a, b; Pedersen et al., 2013; Chupani et al., 2016]. Aquaculture-related research with PAA products includes *in vitro* assessments, where promising disinfection action has been documented [Meinelt et al., 2009; Straus, Meinelt, 2009; Picón-Camacho et al., 2012]. The demonstrated *in vitro* efficacy of the PAA-based product suggests its great potential especially to control *I. multifiliis* infections in commercial aquacultural systems [Picón-Camacho et al., 2012]. PAA concentrations of 0.3 ppm were able to kill all theronts of *I. multifiliis* in 120 min. Straus and co-workers (2012b) determined the effectiveness of PAA for fungus control on channel catfish (*Ictalurus punctatus*, Rafinesque) eggs.

The disinfection strategy with PAA can modify the immune system in fish at the level of T lymphocyte proliferation. The evaluation the impacts of various wastewater disinfection processes on the immune system of juvenile rainbow trout was assessed by Hébert and co-workers (2008). The trout were exposed to a primary-treated effluent for 28 days before and after one of each of the following treatments: ultraviolet radiation, ozonation and PAA. Immune function was characterized in leucocytes from the anterior head kidney by the following three parameters: phagocytosis activity, natural cytotoxic cells function and lymphocyte (B and T) proliferation assays. The results of Hébert and co-workers (2008) showed that the fish mass to length ratio was significantly decreased for the primary-treated and all three disinfection processes. Exposure to the primary-treated effluent led to a significant increase in macrophage-related phagocytosis; the addition of a disinfection step was effective in removing this effect. Both unstimulated and mitogen-stimulated T lymphocyte proliferation in fish decreased dramatically in fish exposed to the ozonated effluent compared to fish exposed to either the primary-treated effluent or to aquarium water. Stimulation of T lymphocytes proliferation was observed with the PAA treatment group [Hébert et al., 2008].

The effects of PAA on crayfish affected by zoospores of *Aphanomyces astaci*, the agent of

crayfish plague, were investigated through assessment of histological changes and oxidative damage by Chupani and co-workers (2016). The gill was the most affected organ, infiltrated by granular hemocytes and displaying malformations of lamella tips and disorganization of epithelial cells. The extent and frequency of histological alterations were more pronounced in animals exposed to 10 $mg \cdot L^{-1}$ [Chupani et al., 2016].

Liu and co-workers (2015) have evaluated whether repeated applications of low concentration PAA could induce continuous stress to the carp (*Cyprinus carpio*). The stress response was estimated by the increase of water cortisol released from the carp. The results showed that the increase of water cortisol became less significant and occurred earlier along repeated applications of low concentration PAA. It indicates faster but reducing stress response of the carp. They conclude that PAA at low concentration is an adaptable stressor to carp, and regular applications should cause no chronic stress. Moreover, low concentration of PAA is suitable to be applied regularly in recirculating aquaculture systems [Liu et al., 2015]. The study of Straus and co-workers (2015) determined the acute toxicity of PAA to 12 fish species in well water. The experiments were designed to provide the 24 h $LC_{50}$, LOEC (Lowest Observed Effect Concentration) and NOEC (No Observed Effect Concentration) values for each species at ~23 °C. Black fathead minnows (*Pimephales promelas*) and blue tilapia (*Oreochromis aureus*) were most and least sensitive, respectively. The mean LOEC value for all species tested was 3.7 mg/L PAA with the range of 1.9 mg/L to 5.8 mg/L [Straus et al., 2015].

Therefore, PAA has the potential to control infections in aquaculture. Low doses of PAA require planning and surveillance to adjust to situations where exposure to organic matter is inevitable, since PAA half-lives can be in the order of a few min [Pedersen et al., 2013].

## Conclusions

Significantly decrease in oxidative stress (TBARS as indirect marker of lipids peroxidation and ketonic derivatives of oxidatively modified proteins) was observed in the fish treated by Disinfectant "CIP", which may be due to excitation of defense mechanism of the fish to counter the stress of disinfection. In this study, the activities of ALT and AST were significantly lower in fish treated by Disinfectant "CIP" than that in control group. The rate of amino acid transformation *via* transamination is slowed down by Disinfectant "CIP" treatment. The observation that lactate level was decreased in muscle samples with decreased of LDH activity in fish treated by Disinfectant "CIP", supports the hypothesis that lactate is not transferred from white muscle *via* the blood stream before it accumulated in hepatic tissue.

Biochemical profiles in the muscle tissue can provide important information about the internal environment of the organism, as the unfavorable changes are the first ones to earliest affect the tissues. The present study showed that the changes in the enzymes activities, metabolites, and oxidative stress biomarkers in fish treated by Disinfectant "CIP" were regarded as the biochemical manifestation of the toxic actions of PAA and hydrogen peroxide. We concluded that the alterations in biochemical parameters can be thus used as rapid and sensitive indicators of monitoring toward the impact of various disinfectants on aquatic organisms.

*This work was supported by grant of the Pomeranian University for Young Scientists.*

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*Поступила в редакцию 15.03.2017 г.*
*Принята после рецензии 20.04.2017 г.*

# Биохимический профиль в мышечной ткани радужной форели (*Oncorhynchus mykiss*) после обработки дезинфицирующим средством „CIP" на основании перуксусной кислоты и перекиси водорода

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Недавние исследования таких веществ, как кислые дезинфицирующие средства, особенно на основе перуксусной кислоты и пероксида водорода демонстрируют эффективное профилактическое действие против бактериальных, вирусных и грибковых инфекций в аквакультуре. Однако такие дезинфектанты могут иметь разрушительные последствия, связанные с образованием активных форм кислорода (АФК) и образованием продуктов окисления. Цель этого исследования состояла в том, чтобы оценить воздействие дезинфицирующего средства CIP на основе 15% перуксусной кислоты и 20% $H_2O_2$ на биомаркеры окислительного стресса [уровни ТБК-активных веществ, альдегидные и кетоновые производные окислительно-модифицированных белков] и биохимические показатели [аланин- (АЛТ) и аспартатаминотрансферазы (АСТ), лактатдегидрогеназа (ЛДГ), лактат, пируват] в мышечной ткани радужной форели *Oncorhynchus mykiss*. Рыбы подвергались воздействию дезинфицирующего средства CIP в конечной концентрации 16 мл/м$^3$ в течение 20 мин, процедуру повторяли три раза каждые 3 дня. Значительное снижение содержания маркеров окислительного стресса наблюдалось у рыб, подвергшихся воздействию дезинфицирующего средства CIP, что может быть связано с активацией защитных механизмов рыб для противодействия стресс-индуцированной дезинфекции. В этом исследовании активность АЛТ и АСТ была значительно ниже у рыб в группе дезинфицированных рыб. Скорость трансформации аминокислот через трансаминацию замедляется в мышечной ткани дезинфицированных рыб. Наблюдение за снижением уровня лактата в образцах мышц с уменьшением активности ЛДГ подтверждает гипотезу о том, что лактат не переносится из мышечной ткани через поток крови для его накопления в печени. Наши результаты показывают, что данная концентрация средства CIP может применяться как дезинфицирующий агент в аквакультуре лососевых. Однако для безопасного использования необходимы дальнейшие исследования его токсичности для рыб.

**Ключевые слова:** дезинфекция, радужная форель *Oncorhynchus mykiss*, мышечная ткань, пероксидация липидов, окислительно-модифицированные белки, аминотрансферазы, лактатдегидрогеназа, лактат, пируват.