*дсотка гемолізованих еритроцитів у коней до і після тренінгу. Відсоток гемолізу протягом перших 30 с, а також максимальний відсоток гемолізованих еритроцитів були значно вищими у літній сезон як до, так і після тренінгу. Максимальний гемоліз відзначали у весняно-літній період до і після тренінгу (5,5 та 6,5 хв. відповідно). Як до, так і після тренінгу спостерігали збільшення часу гемолізу у коней навесні. Найменш стійкі до соляної кислоти еритроцити спостерігали влітку, більш високу їх резистентність – восени і взимку. Можна припустити, що сезонні зміни можуть виникати з-за більшої м'язової активності коней, а також більш високої температури навколишнього середовища, особливо в літній період. Результати показали, що тренування навесні і восени може зробити благотворний вплив за рахунок підвищення стійкості еритроцитів до коней, що використовується для рекреаційної верхової їзди.*

*Ключові слова: рекреаційна верхова їзда, Поморське воєводство (Польща), висота, кислотно-індукований гемоліз, резистентність еритроцитів.*

УДК 57.044:577.3:639.3:612.062

# ANTIOXIDANT DEFENSES IN THE HEPATIC TISSUE OF GRAYLING (*THYMALLUS THYMALLUS* LINCK) AFTER CHLORAMINE-T DISINFECTION (OVERVIEW)

**Tkachenko H.,** PhD, assistant Professor
Department of Zoology and Animal Physiology, Institute of Biology and Environmental Protection, Pomeranian University in Slupsk, Poland
**Grudniewska J.,** PhD, assistant Professor
Department of Salmonid Research, Stanislaw Sakowicz Inland Fisheries Institute, Poland

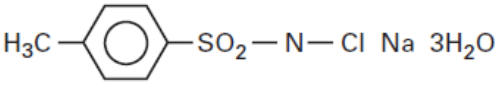
*The aim of the current study was to examine the effects of disinfection by Chloramine-T on the hepatic tissue of grayling (Thymallus thymallus Linck) using antioxidant defense (superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, total antioxidant capacity) to observe the its toxic effects. The endpoints obtained from this study will be useful to monitor the effects of disinfective procedure with Chloramine-T for this species of fish. Our results showed that the catalase activity was increased after Chloramine-T influence. In contrary, the glutathione peroxidase activity in the hepatic tissue of grayling disinfected by Chloramine-T was decreased compared to the controls. Non-significant decrease of total antioxidant capacity level in the hepatic tissue of grayling as a consequence of disinfection with Chloramine-T was found. The antioxidant enzymatic defenses are considered an important control point for the homeostatic adjustments to disinfection-induced stress. The biomarkers of antioxidant defense in this study will therefore be suitable for the monitoring in safety of disinfected procedures. Preliminary results are highly promising and practical implications for a more robust and suitable evaluation of disinfected procedures are possible using these biomarkers.*

Keywords: **Chloramine-T, disinfection, grayling *Thymallus thymallus*, hepatic tissue, antioxidant defense, total antioxidant capacity.**

Chloramine-T (n-chloro-para-toluene sulfonamide sodium salt), as an antimicrobial agent, has had widespread use in a broad range of practices, including medical, dental, veterinary, food processing, and agricultural, as well as a disinfectant for disinfection surfaces and instruments. In agricultural practices, it has been approved as a broadspectrum biocide for foot-and-mouth disease, swine vesicular disease, diseases of poultry, and tuberculosis, while in medicine in the treatment of burns, in whirlpools for the treatment of wounds, and as an oral mouthwash (*Chloramine-T…*).

In aquaculture, it has traditionally been used to treat external bacterial infections in salmonid aquaculture [3]. It has also been reported to be effective for the treatment of monogenean trematodes (skin and gill flukes). Chloramine-T functions by slowly breaking down to hypochlorous acid, with the release of oxygen and chlorine. The active ingredient is p-toluene sulfonamide [1]. Toxicity, as well as effective dose, is dependent upon water hardness, pH and temperature [23]. Bacterial gill disease is caused by a variety of Gram-negative bacteria (myxobacteria, aeromonads, and pseudomonads) (*Chloramine-T…*). The disease is highly contagious among cultured salmonids and can lead to substantial fish losses. An approved therapeutant to control bacterial gill disease is needed to enable the production of salmonids for restoration of fish stocks and for sport and commercial fisheries (*Chloramine-T…*). As a therapeutic agent, it is used as an effective treatment of bacterial gill disease in freshwater or marine aquaria, garden ponds, or other aquatic systems at concentrations ranging from 6.5 to 10.0 mg/L [23.1 to 35.5 μM] [3, 21] and as a preventative, prophylactic, and disinfectant treatment in many fresh water hatcheries (*Chloramine-T…*) [21, 28].

Organic chlorine compounds (N-chloro compounds), which contain the =N–Cl group, show microbicidal activity. Examples of such compounds, are Chloramine-T, diChloramine-T, halazone, halane, dichloroisocyanuric acid, sodium and potassium dichloroisocyanurates and trichloroisocyanuric acid. All appear to hydrolyze in water to produce an imino (=NH) group [22, 27].




Chloramine T
(sodium-*p*-toluene-sulphonchloramide)

Their action is claimed to be slower than that of the hypochlorites, although this can be increased under acidic conditions [14]. Experiments where equal weights of disinfectants were used suggested that the greater penetrating power of monochloramine compensated for its limited disinfection activity. Studies of LeChevallier and coworkers [14] showed that monochloramine was as effective as free chlorine for inactivation of biofilm bacteria. In fish studies, chloramine was poorly absorbed from water, and that which was absorbed was rapidly metabolized to the residue marker, p-Toluenesulfonamide. A second, as of yet unidentified, metabolite may also exist (*Chloramine-T…*).

However, despite the wide use of Chloramine-T, only few studies have examined its toxicity to fish [21, 25]. Accumulating evidence of other researchers has shown that Chloramine-T causes oxidative stress by inducing the generation of reactive oxygen species (ROS) [24, 26, 27]. The data suggest that HOCl and monochloramine can increase endothelial permeability by causing very rapid cytoskeletal shortening and cell retraction, possibly as a result of the oxidation of intracellular sulfhydryls [24]. Therefore, the aim of the current study was to examine the effects of disinfection by Chloramine-T on the hepatic tissue of grayling (*Thymallus thymallus* Linck) using antioxidant

defense (superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, total antioxidant capacity).

**Materials and methods. Fish.** Twenty clinically healthy grayling (*Thymallus thymallus*) were used in the experiments. The study was carried out in a Department of Salmonid Research, Inland Fisheries Institute (Rutki, Poland). Experiments were performed at a water temperature of 16±2 °C and the pH was 7.5. The dissolved oxygen level was about 12 ppm with additional oxygen supply. All biochemical assays were carried out at Department of Zoology and Animal Physiology, Institute of Biology and Environmental Protection, Pomeranian University in Słupsk (Poland).

The fish were divided into two groups and held in 250-L square tanks (70 fish per tank) supplied with the same water as during the acclimation period (2 days). On alternate days, the water supply to each tank was stopped. In the disinfectant exposure, grayling (n=10) were exposed to Chloramine-T in final concentration 9 mg per L. Control group of grayling (n=10) were handled in the same way as Chloramine-T exposed groups. Fish were bathed for 20 min and repeated three times every 3 days. Two days after the last bathing fish were sampled. Fish were not anesthetized before tissue sampling.

**Hepatic tissue isolation.** Hepatic tissue samples were removed from grayling after decapitation. One grayling was used for each homogenate preparation. Briefly, hepatic tissue were excised, weighted and washed in ice-cold buffer. The minced tissue was rinsed clear of blood with cold isolation buffer and homogenized in a homogenizer H500 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.** 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 [2] using bovine serum albumin as a standard (Bradford 1976).

***Superoxide dismutase activity assay*.** Superoxide dismutase (SOD, E. C. 1.15.1.1) activity was assessed by its ability to dismutate superoxide produced during quercetin auto-oxidation in an alkaline medium (pH 10.0) by Kostiuk and co-workers [13] method. Briefly, 1.0 mL of C reagent was mixed with 0.1 mL of sample. C reagent was made *ex tempore* (mixture of equal volumes of 0.1M K,Na-phosphate buffer, pH 7.8 and 0.08M EDTA solution); pH of C reagent was adjusted to 10.0 by adding TEMED. Distilled water (0.1 mL) was added to blank vials instead of blood sample. The total volume of all samples was brought up to 2.4 mL using distilled water. The reaction was initiated by adding 0.1 mL of quercetin (1.4 μM dissolved in dimethyl sulphoxide). Absorbance at 406 nm was measured immediately and after 20 min addition of quercetin solution. Activity is expressed in units of SOD per mg of tissue protein.

***Catalase activity assay*.** Catalase (CAT, E.C. 1.11.1.6) activity was determined by measuring the decrease of $H_2O_2$ in the reaction mixture using a spectrophotometer at the wavelength of 410 nm by the method of Koroliuk and co-workers [12]. The reaction was initialized by adding 0.1 mL of sample into the incubation medium (2 mL of 0.03 % $H_2O_2$ solution) and to 1.0 mL of 4 % ammonium molybdate dissolved in 12.5 mM $H_2SO_4$ solution (blank sample). The duration of reaction was 10 min at room temperature. The reaction was terminated by rapid adding 1.0 mL of 4 % ammonium molybdate dissolved in 12.5 mM $H_2SO_4$ solution to incubation medium and 1 mL of 125 mM $H_2SO_4$ to all samples. All samples were centrifuged at 3,000 g for 5 min. The ab-

sorbance of the obtained solution was measured at 410 nm and compared with that of the blank. One unit of catalase activity is defined as the amount of enzyme required for decomposition of 1 μmol $H_2O_2$ per min per mg of tissue protein.

***Glutathione reductase activity assay.*** Glutathione reductase (GR, EC 1.6.4.2) activity in the tissue was measured according to the method described by Glatzle and co-workers [10]. The enzymatic activity was assayed spectrophotometrically by measuring $NADPH_2$ consumption. In the presence of GSSG and NADPH, GR reduces GSSG and oxidizes $NADPH_2$, resulting in a decrease in the absorbance at 340 nm. The enzyme assay mixture contained 2.4 mL of 67 mM sodium phosphate buffer (pH 6.6), 0.2 mL of 7.5 mM oxidized glutathione, and 0.1 mL of sample. The rate of NADPH oxidation was followed spectrophotometrically at 340 nm. Quantification was performed based on a molar extinction coefficient of 6.22 $mM^{-1}$ $cm^{-1}$ of NADPH. The GR activity was expressed as nmol NADPH per min per mg of tissue protein.

***Glutathione peroxidase activity assay.*** Glutathione peroxidase (GPx, EC 1.11.1.9) activity was determined by detecting the nonenzymatic utilization of GSH (the reacting substrate) at an absorbance of 412 nm after incubation with 5,5-dithiobis-2-nitrobenzoic acid (DTNB) according by the method of Moin [17]. The assay mixture contained 0.8 mL of 0.1 M Tris-HCl buffer with 6 mM EDTA and 12 mM sodium azide (pH 8.9), 0.1 mL of 4.8 mM GSH, 0.2 mL of sample, 1 mL of 20 mM t-butylhydroperoxide, and 0.1 mL of 0.01 M 5,5-dithiobis-2-nitrobenzoic acid. The rate of GSH reduction was followed spectrophotometrically at 412 nm. GPx activity is expressed as μmol GSH per min per mg of tissue protein.

***Total antioxidant capacity assay*.** The TAC level in the sample was estimated by measuring the TBARS level following Tween 80 oxidation. This level was determined spectrophotometrically at 532 nm by Galaktionova and co-workers [9]. Plasma inhibits the $Fe^{2+}$/ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level. Briefly, 0.1 mL of sample were added to 2 mL of 1 % Tween 80 reagent, 0.2 mL of 1 mM $FeSO_4$, and 0.2 mM of 10 mM ascorbic acid. In the blank assay, 0.1 mL of distilled water were used instead of the sample. The mixture was heated in a boiling water bath for 48 h at 37 $^oC$. After cooling, 1 mL of 20 % TCA was added. The mixture was centrifuged at 3,000·g for 10 min. After centrifugation, 2 mL of supernatant and 2 mL of 0.25 % of TBA reagent were mixed. The mixture was heated in a boiling water bath at 95$^oC$ for 15 min. The absorbance of the obtained solution was measured at 532 nm. The absorbance of the blank was defined as 100 %. The level of TAC in the sample (%) was calculated with respect to the absorbance of the blank.

**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 level (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 [39]. All statistical calculation was performed on separate data from each individual with STATISTICA 8.0.

**Results.** The main enzymatic antioxidants include superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). Each of these enzymes is responsible for the reduction of different reactive oxygen species (ROS), and they are located in different cellular compartments [18]. Antioxidant defense in the hepatic tissue of grayling disinfected by Chloramine-T are shown in Fig. 1.

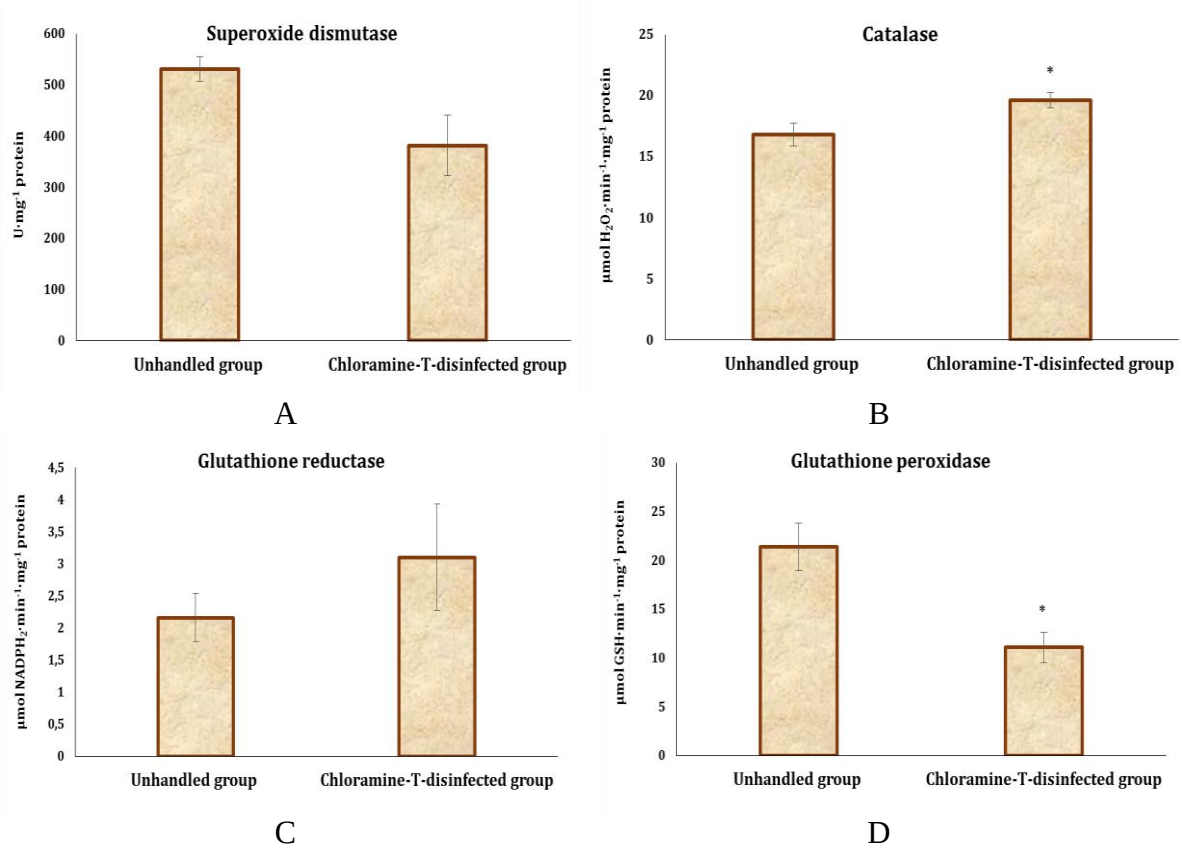



**Fig. 1. Antioxidant enzymes' activities in the hepatic tissue of grayling disinfected by Chloramine-T. Data are represented as mean ± S.E.M.**

* *the significant change was shown as $p<0.05$ when compared values of unhandled (n=10) and disinfected groups (n=10).*

The CAT activity was increased by 16.7 % (p=0.019) after Chloramine-T influence. In contrary, the GPx activity in the hepatic tissue of grayling disinfected by Chloramine-T was decreased by 48 % (p=0.010) compared to the controls. SOD acts on superoxide radicals to form oxygen and the lesser reactive non-radical species, hydrogen peroxide, while GR can regenerate oxidized glutathione (GSSG, glutathione disulfide) to reduced glutathione (GSH). In our study, the activity of these enzymes were not changed in the hepatic tissue of grayling disinfected by Chloramine-T compared to the controls. The CAT is located mainly in peroxisomes and mitochondria and also removes $H_2O_2$. CAT requires iron as a cofactor, and similar to GPx and SOD, its activity is highest in highly oxidative muscle fibers [11].

Non-significant decrease of TAC level in the hepatic tissue of grayling as a consequence of disinfection with Chloramine-T was found (Fig. 2).

Given the central role of liver in many homeostatic processes, this organ was targeted for the study of antioxidant defenses. Our results showed that Chloramine-T disinfection markedly decrease GPx activity and increase CAT activity with non-significant alterations of total antioxidant capacity (Figs 1 and 2).

The extensive studies on enzyme activity of GPx have been carried out in respect to acute stress in fish. Glutathione peroxidase (GPxs) is the largest and the most studied selenoprotein family. Cytosolic glutathione peroxidase (cGPx, GPx1) and phospholipid hydroperoxide glutathione peroxidase (PHGPx, GPx4) are widely distributed throughout tissues, and play a pivotal role in regulating the oxidative status in the cell [19]. Castro and co-workers [4] demonstrated that GPx enzyme activity was in-

creased in response to heat shock in fish fed with a diet containing 55 % protein and decreased in fish fed with 45 % protein. Pacitti and co-workers [19] have concluded that trout GPx1 transcripts expression level may represent a sensitive biomarker for selenium intake, helping to evaluate if selenium concentration and chemical speciation impact on cell homeostasis. Interestingly, GPx1a was the most sensitive to selenium availability in non-stressful conditions, whereas GPx1b1 and GPx1b2 were highly induced by exposure to selenium levels that had some toxic effects on the cells. Although the different concentrations tested of the two selenocompounds modulate GPx1 transcript expression to various degrees, no significant change of GPx1 enzymatic activity was detectable [19]. Previous studies have shown that liver GPx activity varies greatly (orders of magnitude) amongst different fish species even when they are fed the same diet [37].

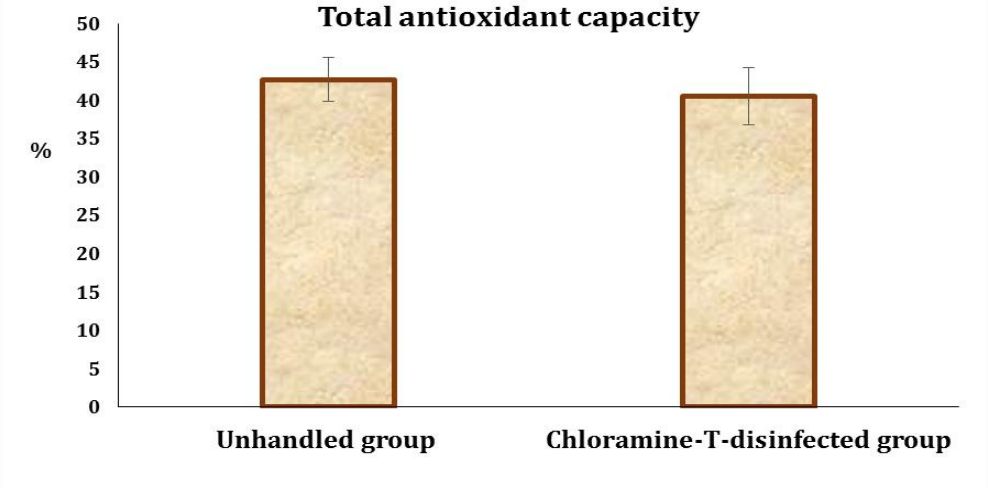



**Fig. 2. Influence of Chloramine-T on total antioxidant capacity in the hepatic tissue of grayling (*Thymallus thymallus*). Data are represented as mean ± S.E.M.**

* *the significant change was shown as $p<0.05$ when compared values of unhandled ($n=10$) and disinfected groups ($n=10$).*

In the present study, we found that liver GPx enzyme activity was significantly lower in the disinfected group. This suggested that the Chloramine-T-disinfected fish were experiencing greater oxidative stress. Our several previous studies have sought to link GPx antioxidant enzyme activity with oxidative stress in various tissue of disinfected grayling [29, 30]. Our results showed that Chloramine-T disinfection markedly increase aldehydic and ketonic derivatives of oxidative protein in hepatic tissue, while significantly decrease of carbonyl derivatives in cardiac tissue of grayling was observed [31, 32]. In the muscle tissue of grayling, Chloramine-T bathing markedly decrease lipid peroxidation with non-significant decrease of aldehydic and ketonic derivatives of oxidative proteins. However, reduced lipid peroxidation results in decrease of total antioxidant capacity. Moreover, decreased lipid peroxidation level causes decrease of aldehydic and ketonic derivatives of oxidatively modified proteins [33]. Our results also showed that Chloramine-T non-significantly decrease lipid peroxidation as well as aldehydic and ketonic derivatives of oxidative proteins in the gills of grayling. No statistically significant alterations in the activities of antioxidant defenses instead catalase and superoxide dismutase activity in the gill tissue of grayling disinfected by Chloramine-T were noted [34].

The effects of disinfection by Chloramine-T on the muscle tissue of grayling using oxidative stress biomarkers [levels of 2-thiobarbituric acid reactive substances (TBARS) and oxidative modified protein (OMP) derivatives] and antioxidant defense

(superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, total antioxidant capacity) was studied in our previous study [35]. Our results showed that Chloramine-T bathing markedly decrease lipid peroxidation with non-significant decrease of aldehydic and ketonic derivatives of oxidatively modified proteins. However, reduced lipid peroxidation results in decrease of total antioxidant capacity. Moreover, decreased lipid peroxidation level causes decrease of aldehydic ($r = 0.854$, $p = 0.002$) and ketonic derivatives of oxidatively modified proteins ($r = 0.852$, $p = 0.002$). Fish developed tissue-specific enzyme responses, such as decrease in superoxide dismutase and catalase activity as well as total antioxidant capacity in muscle tissue with decrease of lipid peroxidation as response to the Chloramine-T disinfection. Correlative analysis has revealed positive correlations between oxidative stress biomarkers (aldehydic and ketonic derivatives of oxidatively modified proteins, TBARS as marker of lipid peroxidation) and antioxidant defenses (Tkachenko and Grudniewska 2016f).

In our previous study [29, 34, 36], we assessed the influence of Chloramine-T on oxidative stress biomarkers and metabolic alterations in various tissues of grayling and rainbow trout (*Oncorhynchus mykiss* Walbaum). Chloramine-T bathing markedly decrease aldehydic and ketonic derivatives of oxidative protein, and aminotransferases activity only in rainbow trout liver, and their elevation is a compensatory mechanism to impaired metabolism. No significant changes were found in oxidative stress biomarkers between control and Chloramine-Treated brown trout. For grayling, Chloramine-T exposure caused significantly elevation in the levels of severe oxidative stress biomarkers in the liver. Increased aldehydic and ketonic derivatives of oxidative protein could modify lactate and pyruvate levels, aminotransferases and lactate dehydrogenase activities, principally causing increased enzymes activity due to oxidative stress in the liver of chloramine-exposed fish [36].

The effects of disinfection by Chloramine-T using oxidative stress biomarkers (levels of 2-thiobarbituric acid reactive substances and derivatives of oxidatively modified proteins) and biochemical enzymes' activity [alanine- and aspartate aminotransferases (ALT and AST), lactate dehydrogenase (LDH)] were assessed in the muscle tissue of rainbow trout [30]. Our results showed that Chloramine-T bathing caused the decrease of the lipid peroxidation as well as ALT and AST activity and significant decrease of LDH activity (by 339 %, $p = 0.017$) compared to controls. Chloramine-T markedly affected on lactate and pyruvate metabolism and resulted to decrease of LDH activity. Correlative analysis revealed that the lipid peroxidation level is correlated with ALT and AST activity in the muscle tissue of unhandled control group. In the muscle tissue of trout disinfected by Chloramine-T, LDH activity is correlated positively with ALT and AST activity. Thus, the skeletal muscles of fish play an important role in the processing of lactate through the gluconeogenic and glycogenic pathways including a greater potential for biosynthesis [30, 34].

Cellular oxidative stress occurs when pro-oxidant forces overwhelm antioxidant defenses. These antioxidant defenses comprise enzymatic and non-enzymatic mechanisms [38]. SOD is important in the disproportionation of superoxide anions into hydrogen peroxide ($H_2O_2$) and dioxygen [8]. The SOD-CAT system provides the first line of defense against oxygen toxicity and is usually used as a biomarker of ROS production [15]. Results of the present investigation indicated that Chloramine-T significantly increase the CAT activity. The alteration of CAT activity revealed that hepatic tissue might suffer from oxidative stress. CAT is mainly located in the peroxisomes and, along with glutathione peroxidase, is responsible for the reduction of $H_2O_2$ produced from the metabolism of long chain fatty acids in peroxisomes [8]. CAT has one of the highest turnover rates of all enzymes: one molecule of CAT can convert

millions of molecules of hydrogen peroxide to water and oxygen per second [6]. In the current study, hepatic CAT activity in grayling was significantly increased by Chloramine-T disinfection (Fig. 1).

These enzymes can be induced by reactive oxygen species and they may be useful indicators of oxidative stress. The induction of antioxidants can provide sensitive early warning signals of incipient oxidative stress. Of the antioxidant enzymes, SOD and CAT are considered as the first-line defenses against oxidative stress. They have related functions and are essential for the conversion of ROS to harmless metabolites. To be specific, SOD catalyzes dismutation of superoxide radical anion to $H_2O$ and $H_2O_2$, and CAT reduces $H_2O_2$ to less toxic $H_2O$ and $O_2$. In the present study, CAT activity was significantly increased in Chloramine-T-treated groups. The increased response of the enzyme possibly suggested an activation of the antioxidant system in keeping the antioxidant defense balance, which could be due to the excessive $H_2O_2$ production under Chloramine-T exposure, resulting in the accumulation of the oxidative substances in the cells [15].

**Conclusions.**

1. In the present study we analyzed the antioxidant response in the hepatic tissue of grayling after disinfection by Chloramine-T, and the results highlight responses which lead to the attainment of a new steady state, and emphasize the complexity of the adaptive response of antioxidant defenses.

2. The antioxidant enzymatic defenses are considered an important control point for the homeostatic adjustments to disinfection-induced stress.

3. The biomarkers of antioxidant defense in this study will therefore be suitable for the monitoring in safety of disinfected procedures. Preliminary results are highly promising and practical implications for a more robust and suitable evaluation of disinfected procedures are possible using these biomarkers.

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

**References**

1. Booth, N. H., McDonald, L. E. (1988). *Veterinary Pharmacology and Therapeutics* (6th ed.). Iowa State Press, Ames Iowa, pp. 774–777.

2. Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.,* 72, 248–254.

3. Bullock, G. L., Herman, R. L., Waggy, C. (1991). Hatchery efficacy trials with Chloramine-T for control of bacterial gill disease. *J. Aquat. Animal Health*, 3, 48–50.

4. Castro, C., Pérez-Jiménez, A., Guerreiro, I., Peres, H., Castro-Cunha, M., Oliva-Teles, A. (2012). Effects of temperature and dietary protein level on hepatic oxidative status of Senegalese sole juveniles (*Solea senegalensis*). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.,* 163 (3–4), 372–378.

5. Chloramine-T [127-65-1] and Metabolite p-Toluenesulfonamide [70-55-3]. Review of Toxicological Literature. Prepared for Scott Masten, Ph.D., National Institute of Environmental Health Sciences, Submitted by Karen E. Haneke, M.S. Integrated Laboratory Systems, 2002.

6. El-Gendy, K. S., Radwan, M. A., Gad, A. F. (2009). *In vivo* evaluation of oxidative stress biomarkers in the land snail, the a pisana exposed to copper-based pesticides. *Chemosphere*, 77 (3), 339–344.

7. Ferreira, D., da Motta, A. C., Kreutz, L. C., Toni, C., Loro, V. L., Barcellos, L. J. (2010). Assessment of oxidative stress in *Rhamdia quelen* exposed to agrichemicals. Chemosphere, 79 (9), 914–921.

8. Filipak, Neto, F., Zanata, S. M., Silva de Assis, H. C., Nakao, L. S., Randi, M. A., Oliveira Ribeiro, C. A. (2008). Toxic effects of DDT and methyl mercury on the hepatocytes from *Hoplias malabaricus*. *Toxicol. In Vitro*, 22 (7), 1705–1713.

9. Galaktionova, L. P., Molchanov, A. V., Elchaninova, S. A., Varshavskiy, B. Y. (1998). The lipid peroxidation processes in patients with ulcerous illness of stomach and duodenum. *Clin. Lab. Diagnostics*, 6, 10–14 (In Russian).

10. Glatzle, D., Vuilleumier, J. P., Weber, F., Decker, K. (1974). Glutathione reductase test with whole blood, a convenient procedure for the assessment of the riboflavin status in human. *Experientia*, 30, 665–667.

11. Gomes, E. C., Silva, A. N., Rubino de Olivera, M. (2012). Oxidants, antioxidants and beneficial role of exercise-induced production of reactive species. Oxidative Medicine and Cellular Longevity, 2012, 1–12.

12. Koroliuk, M. A., Ivanova, L. I., Maĭorova, I. G., Tokarev, V. E. (1988). A method of determining catalase activity. *Laborotornoe Delo*, 1, 16–19 (in Russian).

13. Kostiuk, V. A., Potapovich, A. I., Kovaleva, Zh. V. 1990. A simple and sensitive method of determination of superoxide dismutase activity based on the reaction of quercetin oxidation. Voprosy Meditsinskoĭ Khimii, 36, 88–91 (in Russian, Abstract in English).

14. LeChevallier, M. W., Cawthon, C. D., Lee, R. G. (1988). Inactivation of biofilm bacteria. *Appl. Environ. Microbiol*., 54 (10), 2492–2499.

15. Li, C., Qin, L., Qu, R., Sun, P., Wang, Z. (2016). Responses of antioxidant defense system to polyfluorinated dibenzo-p-dioxins (PFDDs) exposure in liver of freshwater fish *Carassius auratus*. *Ecotoxicol. Environ. Saf*., 126, 170–176.

16. Li, Z. H., Zlabek, V., Velisek, J., Grabic, R., Machova, J., Kolarova, J., Li, P., Randak, T. (2011). Acute toxicity of carbamazepine to juvenile rainbow trout (*Oncorhynchus mykiss*): effects on antioxidant responses, hematological parameters and hepatic EROD. *Ecotoxicol. Environ. Saf.*, 74 (3), 319–327.

17. Moin, V. M. (1986). A simple and specific method for determining glutathione peroxidase activity in erythrocytes. *Labaratornoe Delo*, 12, 724–727 (in Russian, Abstract in English).

18. Nunes-Silva, A., Freitas-Lima, L. G. (2015). The association between physical exercise and reactive oxygen species (ROS) production. *J. Sports Med. Doping Stud*., 5, 1.

19. Pacitti, D., Wang, T., Page, M. M., Martin, S. A., Sweetman, J., Feldmann, J., Secombes, C. J. (2013). Characterization of cytosolic glutathione peroxidase and phospholipid-hydroperoxide glutathione peroxidase genes in rainbow trout (*Oncorhynchus mykiss*) and their modulation by in vitro selenium exposure. *Aquat. Toxicol*., 130–131, 97–111.

20. Powell, M., Harris, J. (2004). Influence of oxygen on the toxicity of Chloramine-T to Atlantic salmon smolts in freshwater and seawater. *J. of Aquatic Animal Health*, 16 (2), 83–92.

21. Powell, M. D., Perry, S. F. (1996). Respiratory and acid-base disturbances in rainbow trout (*Oncorhynchus mykiss*) blood during exposure to chloramine T, paratoluenesulfonamide, and hypochlorite. *Can. J. Fish Aquat. Sci*., 53, 701–708.

22. *Principles and practice of disinfection, preservation, and sterilization*. – 5th ed. / edited by Adam P. Fraise, Jean-Yves Maillard, Syed A. Sattar. Rev. ed. of Rus-

sell, Hugo & Ayliffe's principles and practice of disinfection, preservation & sterilization, 2004.

23. Rach, J. J., Bills, T. B., Marking, L. L. (1988). Effects of physical and chemical factors on the toxicity of Chloramine-T. *United States fish and Wildlife Service Information Bulletin*, 69–88.

24. Sakuma, S., Miyoshi, E., Sadatoku, N., Fujita, J., Negoro, M., Arakawa, Y., Fujimoto, Y. (2009). Monochloramine produces reactive oxygen species in liver by converting xanthine dehydrogenase into xanthine oxidase. *Toxicol. Appl. Pharmacol*., 239 (3), 268–272.

25. Sanchez, J. G., Speare, D. J., Macnair, N., Johnson, G. R., Aquaculture Assoc. of Canada, S. A., N. B. [C.], (1997). Effect of prophylactic Chloramine-T treatment on growth performance and condition indices of rainbow trout (*Oncorhynchus mykiss*). In: M. D. B. Burt & S. L. Waddy (Eds.), Aquaculture Association of Canada Special Publications, no. 2 (pp. 51-57). Department Pathology & Microbiology, Atlantic Veterinary College, 550 University Ave., Charlottetown, P.E. C1A 4P3, Canada: Aquaculture Association of Canada.

26. Stanley, N. R., Pattison, D. I., Hawkins, C. L. (2010). Ability of hypochlorous acid and N-chloramines to chlorinate DNA and its constituents. *Chem. Res. Toxicol.,* 23 (7), 1293–1302.

27. Tatsumi, T., Fliss, H. (1994). Hypochlorous acid and chloramines increase endothelial permeability: possible involvement of cellular zinc. *Am. J. Physiol*., 267 (4 Pt 2), 1597–1607.

28. Thorburn, M. A., Moccia, R. D. (1993). Use of chemotherapeutics on trout farms in Ontario. *J. Aquat. Anim. Health*, 5, 85–91.

29. Tkachenko, G. M., Grudniewska, J. (2015). Tissue-specific response of protein oxidation in the grayling (*Thymallus thymallus* L.) disinfected by Chloramine-T. *Scientific Medical Bulletin*, 1 (1), 76–82.

30. Tkachenk,o H., Grudniewska, J. (2016). Biochemical changes in the muscle tissue of rainbow trout (*Oncorhynchus mykiss* Walbaum) disinfected by Chloramine-T. Baltic Coastal Zone. *J. of Ecology and Protection of the Coastline*, 20, 101–116.

31. Tkachenko, H., Grudniewska, J. (2016). Influence of Chloramine-T on oxidative stress biomarkers in the muscle tissue of grayling (*Thymallus thymallus*). *Scientific journal «Kaliningrad State Technical University News*», 42, 49–58.

32. Tkachenko, H., Grudniewska, J. (2016). Influence of Chloramine-T on oxidative stress biomarkers in the cardiac tissue of grayling (*Thymallus thymallus* Linn.). *Globalisation and regional environment protection. Technique, technology, ecology*. Scientific editors Tadeusz Noch, Wioleta Mikołajczewska, Alicja Wesołowska. Gdańsk, Gdańsk High School Publ., 2016. – P. 213-234.

33. Tkachenko, H., Grudniewska, J. (2016). Lipid and protein oxidation in the muscle tissue of grayling (*Thymallus thymallus* Linn.) after Chloramine-T disinfection. *Materials of the International Forum "The Current State and Prospects for the Development of Aquaculture in the Caspian Region",* dedicated to the 85th anniversary of Dagestan State University and the 75th anniversary of Professor F. Magomayev. Ed. F. Magomayev, S. Chalayeva, S. Kurbanova, A. Shakhnazova (Makhachkala, 17–19 October, 2016). Makhachkala, Printing house IPE RD, 168–175.

34. Tkachenko, H., Grudniewska, J. (2016). Effect of Chloramine-T disinfection on oxidative stress biomarkers in the gill tissue of grayling (*Thymallus thymallus*). *Trudy VNIRO (Труды ВНИРО*), 162, 150–160.

35. Tkachenko, H., Grudniewska, J. (2016). Oxidative stress biomarkers in the muscle tissue of grayling (*Thymallus thymallus* Linck) after Chloramine-T disinfection. *Słupskie Prace Biologiczne*, 13, 231–252.

36. Tkachenko, H., Kurhaluk, N., Grudniewska, J. (2012). Effects of Chloramine-T exposure on oxidative stress biomarkers and liver biochemistry of rainbow trout, *Oncorhynchus mykiss* (Walbaum), brown trout, *Salmo trutta* (L.), and grayling, *Thymallus thymallus*. *Arch. Pol. Fish.*, 21, 41–51.

37. Tocher, D. R., Mourente, G., van der Eecken, A., Evjemo, J. O., Diaz E., Bell, J. G., Geurden, I., Lavens, P., Olsen, Y. (2002). Effects of dietary vitamin E on antioxidant defence mechanisms of juvenile turbot (*Scophthalmus maximus* L.), halibut (*Hippoglossus hippoglossus* L.) and sea bream (*Sparus aurata* L.). Aquac. Nutr., 8, 195–207.

38. Xia, J., Zhao, H. Z., Lu, G. H. (2013). Effects of selected metal oxide nanoparticles on multiple biomarkers in *Carassius auratus*. Biomed. Environ. Sci., 26(9), 742-749.

39. Zar, J. H. (1999). Biostatistical Analysis. 4th ed., Prentice-Hall Inc., Englewood Cliffs, New Jersey.

*АНТИОКСИДАНТНАЯ ЗАЩИТА В ТКАНИ ПЕЧЕНИ ХАРИУСА (ХАРИУС THYMALLUS LINCK) ПОСЛЕ ДЕЗИНФЕКЦИИ ХЛОРАМИНОМ-Т (ОБЗОРНАЯ)*

*Ткаченко Г., Институт биологии и охраны среды Поморского университета (Слупск, Польша)*

*Грудниевска Ж., Институт внутреннего рыболовства им. Станислава Саковича (Жуково, Польша)*

*Целью исследования являлось изучение влияния дезинфекции хлорамином-Т на печеночную ткань хариуса (Thymallus thymallus Linck) с использованием антиоксидантной защиты (супероксиддисмутаза, каталаза, глутатионредуктаза, глутатионпероксидаза, общая антиоксидантная способность) для наблюдения его токсического воздействия. Конечные точки, полученные в результате этого исследования, будут полезны для мониторинга влияния дезинфекционной процедуры с хлорамином-Т на этот вид рыб. Полученные результаты показали, что активность каталазы повышена после воздействия хлорамина-Т. Напротив, активность глутатионпероксидазы в ткани печени хариуса обеззараживаемой Хлорамином-Т, была снижена по сравнению с контролем. Установлено несущественное снижение общего уровня антиоксидантной способности печеночной ткани хариуса в результате дезинфекции хлорамином-Т. Антиоксидантная ферментативная защита считается важной контрольной точкой для гомеостатической адаптации к дезинфекционно-индуцированному стрессу. Таким образом, биомаркеры антиоксидантной защиты в данном исследовании можно применять для контроля безопасности процедуры дезинфекции. Предварительные результаты являются весьма многообещающими для более надежной и подходящей оценки процедур дезинфекции с использованием изученных биомаркеров.*

*Keywords: Хлорамин-Т, дезинфекция, хариус Thymallus thymallus, печеночная ткань, антиоксидантная защита, полная антиоксидантная емкость.*

*АНТИОКСИДАНТНИЙ ЗАХИСТ У ТКАНИНІ ПЕЧІНКИ ХАРІУСА (ХАРІУС THYMALLUS LINCK) ПІСЛЯ ДЕЗИНФЕКЦІЇ ХЛОРАМІНОМ-Т (ОГЛЯДОВА)*

*Ткаченко Г., Інститут біології та охорони середовища Поморского университета (Слупск, Польща)*

*Груднієвська Ж., Інститут внутрішнього риболовства ім. Станислава Саковича (Жуково, Польща)*

*Метою дослідження було вивчення впливу дезінфекції хлораміном-Т на печінкову тканину харіуса (Thymallus thymallus Linck) з використанням антиоксидантного захисту (супероксиддисмутаза, каталаза, глутатионредуктаза, глутатіонпероксидаза, загальна антиоксидантна здатність) для спостереження його токсичного впливу. Кінцеві точки, отримані в результаті цього дослідження будуть корисні для моніторингу впливу дезінфекційної процедури з хлораміном-Т на цей вид риб. Отримані результати показали, що активність каталази підвищена після дії хлораміну-Т. Навпаки, активність глутатіонпероксидази в тканинах печінки харіуса знезаражуємо Хлораміном-Т, була знижена порівняно з контролем. Встановлено несуттєве зниження загального рівня антиоксидантної здатності печінкової тканини харіуса в результаті дезінфекції хлораміном-Т. Ферментативний антиоксидантний захист вважається важливою контрольною точкою для гомеостатичної адаптації до дезінфекційно-індукованого стресу. Таким чином, біомаркери антиоксидантного захисту в даному дослідженні можна застосовувати для контролю безпеки процедури дезінфекції. Попередні результати є досить перспективними для більш надійної і відповідної оцінки процедур дезінфекції з використанням вивчених біомаркерів.*

*Ключові слова: Хлорамін-Т, дезинфекція, харіус Thymallus thymallus, печінкова тканина, антиоксидантний захист, повна антиоксидантна ємність.*

УДК 636.92.033.084.1

# ФОРМУВАННЯ ВАГОВИХ, ЛІНІЙНИХ ТА М'ЯСНИХ ПОКАЗНИКІВ У КРОЛІВ М'ЯСО–ШКУРКОВОГО НАПРЯМУ ЗА ВИКОРИСТАННЯ КОМБІНОВАНОГО ТИПУ ГОДІВЛІ

**Аксьонов Є. О.,** асп.[1]
Інститут тваринництва НААН

*Викладено результати досліджень особливостей росту, розвитку (ріст внутрішніх органів – серце, легені, печінка, нирки, селезінка), формування м'ясної продуктивності (маса м'язової, кісткової, жирової тканин) та її якості у кролів м'ясо-шкуркового напряму продуктивності за застосування комбінованого типу годівлі у віці 1–30–45–60–90–120–150 діб. Визначено забійний вихід. Встановлена інтенсивність росту та розвитку кроленят з урахуванням абсолютного, середньодобового та відносного приростів. Визначено екстер'єрні особливості розвитку молодняку за допомогою лінійних промірів.*

*У зв'язку з цим зроблено наголос на тому, що отримання продукції вищої споживчої якості забезпечується на комбінованому типі годівлі.*

Ключові слова: **абсолютний та відносний приріст, комбінований тип годівлі, кролівництво, м'ясна продуктивність, середньодобовий приріст.**

[1] *Науковий керівник – д. с.-г. н., професор, Помітун І. А.*