

# Induction of oxidative stress and antioxidant defenses in the livers of sea trout, *Salmo trutta* L., with ulcerative dermal necrosis

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**Abstract.** The aim of this study was to investigate the lipid peroxidation (thiobarbituric acid reactive substance content) and oxidative modified protein levels (stable 2,4-dinitrophenyl hydrazine derivates of the carbonyl groups), and antioxidant defense system (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase activity, and total antioxidative activity) in livers of males and females from a control group (healthy specimens) and a group of sea trout, *Salmo trutta* L., affected by ulcerative dermal necrosis (UDN). Ulcerative dermal necrosis tends to induce protein oxidative destruction and negatively affects the livers of infected trout by initiating oxidative stress. UDN-affected males are characterized by higher expressed oxidative-modified protein levels in their livers compared to healthy specimens. Our results indicate there is a connection between the level of oxidative stress and antioxidant defenses in the livers of trout with UDN. Increased levels of protein oxidation and modified activities of antioxidant defenses caused declines in total antioxidant activity in the livers of UDN-affected females. The glutathione-mediated antioxidant defense system and endogenous catalase play critical roles in the intracellular antioxidant defenses under UDN-induced oxidative stress.

**Keywords:** sea trout, ulcerative dermal necrosis, liver, oxidative stress, oxidative destruction of proteins, antioxidant defenses

## Introduction

Sea trout, *Salmo trutta* L., is a commercially important species, with landings in Poland exceeding 600 tons a year (Was and Wenne 2002). During 2007-2009 a distinct increase of moribund wild salmonids (brown and rainbow trout) with ulcerative dermal necrosis (UDN) was recorded in the north Pomeranian region. UDN is a historical condition affecting salmonids that has come and gone for over a century, and a definitive diagnosis has yet to be made. Substantial work has been performed on UDN in fish (Roberts 1993, Kane et al. 2000, Law 2001, Vogelbein et al. 2001, Bruno et al. 2007, Harikrishnan et al. 2009, 2010).

The primary causative agent of the ulcerative syndrome appears to be one or more members of the fungal genus *Aphanomyces*, which causes necrosis of muscle tissue as it invades the body; in some cases the hyphae extend into the visceral organs (Vogelbein et al. 2001). Isolates from infected fish in Asia, Africa, and the USA have recently been described as *Aphanomyces invaderis* (Vandersea et al. 2006, Sosa et al. 2007, Andrew et al. 2008, Saylor et al. 2010). *A. piscicida* was described as the primary cause of mycotic granulomatosis (Vogelbein et al. 2001). Infected fish also usually suffer from severe bacterial septicaemia involving a range of opportunistic pathogens. A variety of parasites have also been reported in diseased fish, but their presence is inconsistent (Kane et al. 2000, Law 2001).

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Associated viral infections also occur frequently (Frerichs 1995, John et al. 2001). The fungus, by itself, cannot normally invade fish, and it is postulated that some co-factor, such as epidermal damage (which may be initiated by an array of agents), severe environmental stress, or viral infection, is required to initiate this complex and exceedingly serious condition (Kane et al. 2000, Law 2001). Recent studies have shown that the opportunistic pathogens *Aeromonas hydrophila* and *A. sobria* might also be involved in the pathogenesis of the ulcerative syndrome (Candan 1990, McGarey et al. 1991, Lilley et al. 1998, Rahman et al. 2002, Majumdar et al. 2007, Harikrishnan et al. 2009, 2010).

The clinical symptoms of ulcerative syndrome include clinical dropsy, ulceration, and widespread tissue necrosis (Roberts 1993). The disease manifests as ulcerative skin lesions with an extensive superficial reddening of the dermis, often associated with necrosis of the fins (Aoki and Hirono 1991, Kane et al. 2000, Law 2001, Vogelbein et al. 2001, Bruno et al. 2007, Harikrishnan et al. 2009, 2010).

The effects of reactive oxygen species (ROS) generation have been postulated to be a major contributor of different diseases (Yagi 1993). It has been shown that oxidative damage implies the development of ROS, which in turn modify the structure of proteins, lipids, and nucleic acids by interacting with them (Patrick 2003). The oxidative modification of proteins by ROS is implicated in the etiology or progression of disorders and diseases (Dalle-Donne et al. 2003, Foster et al. 2003). Oxidative modified proteins might be, more efficiently than other oxidative stress biomarkers, employed to monitor disease progression and outcome (Di Rosa et al. 2009).

The depletion of antioxidant defenses and the changes in the activities of various antioxidant enzymes indicative of lipid and protein oxidation have been implicated in oxidative tissue damage. ROS are known to convert amino groups of proteins and thereby alter protein structure and function. An increase in the number of modified carbonyl groups correlates with protein damage caused by oxidative stress (Halliwell and Gutteridge 1999; Hermes-Lima 2004; Lushchak and Bagnyukova 2006). UDN

seems to be quite capable of causing oxidative stress in the livers, muscle tissues, hearts, and spawn of brown trout (Kurhalyuk et al. 2009, 2010).

The objective of the current study was to examine the responses of biomarkers for oxidative stress in livers of males and females from control (healthy specimens) and UDN-affected trout from the Słupia River (central Pomerania, northern Poland). The activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPX), and total antioxidative activity (TAA) were measured.

## Materials and Methods

### Fish

Adult sea trout, 3-5 years of age, were collected during migration from a site on the Słupia River in Słupsk, northern Poland. The fish were caught in co-operation with personnel from the Słupia Valley Landscape Park and the Polish Angling Association branch in Słupsk. The sea trout were sampled in November and December from 2007 to 2009.

### Sampling

Samples for analysis were collected immediately after catch from 61 and 70 males and females (control group) as well as 81 males and 65 females of sea trout affected by UDN (study group). The fish were caught quickly and sacrificed after being anesthetized. Individuals from both groups were transported to the Department of Animal Physiology, Institute of Biology and Environmental Protection, Pomeranian University (Słupsk, Poland). After the fish were caught microbiological tests were carried out (Szewczyk 2005). The cultivation of samples on *Aeromonas* Isolation Agar for detecting *Aeromonas* spp. suggested that *Aeromonas hydrophila* complex caused ulcerative dermal necrosis. The pathogen was isolated from the infected sea trout.

The specimens from each group were dissected, and one fish was used for each preparation. The livers from the trout were homogenized in cold 0.1 M tris-HCl buffer (pH 7.4). The protein content of the samples was determined according to Bradford (1976) using bovine serum albumin as the standard.

## Analytical methods

Thiobarbituric acid (TBA), oxidized and reduced glutathione (GSSG and GSH), NADPH, and 5,5-dithiobis-2-nitrobenzoic acid, ethylenediamine-tetraacetic acid (EDTA), trichloroacetic 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  $25 \pm 0.5^\circ\text{C}$  using a Specol 10 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 level of lipid peroxidation was determined by quantifying the concentration of 2-thiobarbituric acid reactive substrates (TBARS) with the Kamyshnikov (2004) method for determining the malondialdehyde (MDA) concentration. This method is based on the reaction of the degradation product of lipid peroxidation, MDA, with TBA under conditions of high temperature and acidity to generate a colored adduct that is measured spectrophotometrically. A 2.1 mL sub-sample of liver homogenate was mixed with 1 mL of TCA and 1 mL of TBA reagent. The mixture was heated in a boiling water bath for 10 minutes. After cooling, the mixture was centrifuged at 3,000 g for 10 minutes. The MDA level was expressed in  $\mu\text{mol}$  MDA per mg protein by using  $1.56 \times 10^5 \text{ mM}^{-1} \text{ cm}^{-1}$  as the molar extinction coefficient.

The rate of protein oxidative destruction was estimated from the reaction of the resultant carbonyl derivatives of the amino acid reaction with DNFH as

described by Levine et al. (1990) and modified by Dubinina et al. (1995). DNPH was used for determining the carbonyl content in soluble and insoluble proteins. A quantity of 1 mL 0.1M DNPH (dissolved in 2M HCl) was added to 0.1 mL of the tissue samples after the protein was denatured. After adding the DNPH solution (or 2M HCl to the blanks), the tubes were incubated for 1 h at  $37^\circ\text{C}$ . The tubes were centrifuged for 20 min at 3,000 g. After centrifugation, the supernatant was decanted, and 1 mL of an ethanol-ethylacetate solution was added to each tube. Following the mechanical disruption of the pellet, the tubes were allowed to stand for 10 min and then centrifuged again (20 min at 3,000 g). The supernatant was decanted and the pellet was rinsed with ethanol-ethylacetate two times. After the final rinse, the protein was solubilized in 2.5 mL of 8M urea solution. To speed up the solubilization process, the samples were incubated in a  $90^\circ\text{C}$  water bath for 10 min. The final solution was centrifuged to remove any insoluble material. The carbonyl content was calculated from the absorbance measurement at 370 nm and 430 nm and an absorption coefficient  $22,000 \text{ M}^{-1} \text{ cm}^{-1}$ . Carbonyl groups were determined spectrophotometrically from the difference in absorbance at 370 nm (aldehyde derivatives,  $\text{OMP}_{370}$ ) and 430 nm (ketonic derivatives,  $\text{OMP}_{430}$ ) and expressed in E per mg of tissue protein.

Superoxide dismutase (SOD, E.C. 1.15.1.1) activity was measured with the method by Kostiuk et al. (1990). SOD activity was assessed by its ability to dismutate superoxide produced during quercetin auto-oxidation in an alkaline medium (pH = 10.0). Briefly, 1.0 mL of C reagent was mixed with 0.1 mL liver homogenate (1:1000). C reagent was made *ex tempore* (mixture of equal volumes of 0.1 M K<sub>2</sub>Na-phosphate buffer, pH = 7.8 and 0.08M EDTA), pH of C reagent was adjusted to 10.0 by adding tetramethylenediamine. Distilled water (0.1 mL) was added to blank vials instead of liver homogenate. 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  $\mu\text{M}$  in dimethyl sulfoxide). Absorbance at 406 nm was measured

immediately and after 20 min. Activity is expressed in units of SOD per mg of tissue protein.

Catalase (CAT, E.C. 1.11.1.6) activity was determined by measuring the decrease of  $\text{H}_2\text{O}_2$  in the reaction mixture using a spectrophotometer at a wavelength of 410 nm with the method by Koroliuk et al. (1988). The reaction was initialized by adding 0.1 mL of liver homogenate into the incubation medium (2 mL of 0.03% solution of  $\text{H}_2\text{O}_2$ ). The duration of this reaction was 10 min at room temperature. The reaction was terminated by rapidly adding 1.0 mL of 4% ammonium molybdate solution to 12.5 mM  $\text{H}_2\text{SO}_4$  and 1 mL 125 mM  $\text{H}_2\text{SO}_4$ . The blank assay included 0.1 mL of distilled water instead of liver homogenate. All samples were centrifuged at 3,000 g for 5 min. The absorbance of the solution obtained was measured at 410 nm and was compared with that of the blank. One unit of catalase activity is defined as the amount of enzyme required for the decomposition of 1  $\mu\text{mol}$   $\text{H}_2\text{O}_2$  per min per mg of protein.

Glutathione reductase (GR, E.C. 1.6.4.2) activity in liver homogenate was measured according to the method described by Glatzle et al. (1974). 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 liver homogenate. The rate of NADPH oxidation was followed spectrophotometrically at 340 nm. A control without NADPH was used, and the GR activity was expressed as nmol NADPH per min per mg tissue protein.

Glutathione peroxidase (GPX, E.C. 1.11.1.9) activity in liver homogenate was measured spectrophotometrically as described by Moin (1986). The assay mixture contained 0.8 mL of 0.1 M Tris-HCl with 6mM EDTA and 12mM sodium aside (pH 8.9), 0.1 mL of 4.8 mM GSH, 0.2 mL of liver homogenate, 1 mL of 20 mM t-butylhydroperoxide, and 0.1 mL of 0.01M 5,5-dithiobis-2-nitrobenzoic acid. The rate of GSH reduction was followed spectrophotometrically at 412 nm. GPX activity is expressed as  $\mu\text{mol}$  GSH per min per mg tissue protein.

The TAA level in the liver homogenate was estimated spectrophotometrically at 532 nm following the method with Tween 80 oxidation (Halaktionova et al. 1998). Briefly, 0.2 mL of liver homogenate was

added to 2 mL 1% Tween 80. The blank assay included 0.1 mL of distilled water instead of sample. The mixture was heated in a boiling water bath for 48 hours at 37°C. After cooling, 1 mL of TCA was added and the mixture was centrifuged at 3,000 g for 10 min. After centrifugation, 2 mL of supernatant and 2 mL of TBA reagent was mixed. The mixture was heated in a boiling water bath at 95°C for 15 minutes. The absorbance of the obtained solution was measured at 532 nm and was compared with that of the blank. TAA was expressed in %.

### Statistical analysis

Results are expressed as mean  $\pm$  SEM. Significance of differences in enzyme activity in the livers of sea trout (significance level,  $P < 0.05$ ) was examined using one-way ANOVA followed by multiple-range test (significance level,  $P < 0.05$ ), analysis of variance (test F), Levene's and Tukey HSD test (test of reasonably important difference for bumpy numerical force of attempt). Correlation analysis between levels of TBARS and protein oxidative destruction and enzyme activities in livers at the set significance level were determined with the regression method (Zar 1999). The Pearson correlation was used to examine linear correlation among data. All statistical calculations were performed on separate data from each specimen with Statistica version 8.0.

### Results

The lipid peroxidation level in the livers of males and females from the control (healthy specimens) and UDN-affected trout are presented in Fig. 1. Basal values of TBARS levels in the livers from control males were 15.8% ( $P < 0.05$ ) higher than in females. In response to UDN infection, males and females showed a gradual increase in lipid peroxidation level ( $F = 10.60$ ,  $P < 0.001$ ) compared to the control fish by 10.1% ( $P > 0.05$ ) and 11.6% ( $P > 0.05$ ), respectively, but the induction of lipid peroxidation in the livers of UDN-affected trout was not significant.



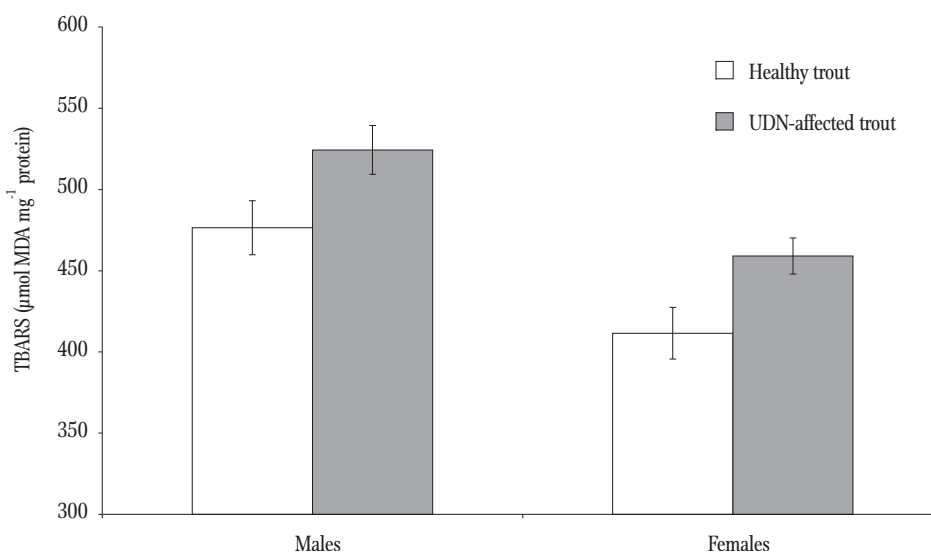


Figure 1. Lipid peroxidation, measured by TBARS level ( $\mu\text{mol MDA mg}^{-1}$  protein) in liver from males and females of control (healthy specimens) and UDN-affected sea trout. Each value represents the mean  $\pm$  SEM.

The effects of UDN infection on rates of protein oxidative destruction, measured as carbonyl oxidation levels, in the livers are shown in Fig. 2. UDN infection induced an increase of aldehyde derivatives in the livers from males by 45.4% ( $P < 0.001$ ) and by 43% ( $P < 0.001$ ) from UDN-affected females (Fig. 2a). The level of ketonic derivatives in the livers of females affected by UDN was significantly higher by 31% ( $P < 0.001$ ) than in the values from control trout (Fig. 2b).

No significant changes in liver SOD activity were found as a consequence of UDN infection either in males or females (Tables 1 and 2). Regarding CAT, its activity ( $F = 63.54$ ,  $P < 0.001$ ) was significantly decreased as a consequence of UDN infection in males (by 36.4%,  $P < 0.001$ ) and females (by 40.1%,  $P < 0.001$ ). GR activity ( $F = 144.85$ ,  $P < 0.001$ ) was decreased by 55% ( $P < 0.001$ ) from UDN-affected females as compared to controls, but no differences in GR activity between healthy and UDN-affected males

**Table 1**

Antioxidant enzyme activities in the livers of UDN-affected males of sea trout from the Słupia River

Antioxidant enzymes activities	Healthy trout	UDN-affected trout	P
SOD ( $\text{U mg}^{-1}$ protein)	$182.79 \pm 8.58$	$186.67 \pm 5.79$	0.984
CAT ( $\mu\text{mol H}_2\text{O}_2 \text{ min}^{-1} \text{ mg}^{-1}$ protein)	$49.24 \pm 1.92$	$31.30 \pm 1.05$	0.000
GR ( $\text{nmol NADPH}_2 \text{ min}^{-1} \text{ mg}^{-1}$ protein)	$48.92 \pm 2.33$	$46.87 \pm 1.87$	0.964
GPX ( $\mu\text{mol GSH min}^{-1} \text{ mg}^{-1}$ protein)	$98.53 \pm 3.88$	$59.57 \pm 2.53$	0.000

**Table 2**

Antioxidant enzyme activities in the livers of UDN-affected females of sea trout from the Słupia River

Antioxidant enzymes activities	Healthy trout	UDN-affected trout	P
SOD ( $\text{U mg}^{-1}$ protein)	$173.06 \pm 6.01$	$174.12 \pm 8.68$	0.999
CAT ( $\mu\text{mol H}_2\text{O}_2 \text{ min}^{-1} \text{ mg}^{-1}$ protein)	$47.41 \pm 1.13$	$28.41 \pm 1.28$	0.000
GR ( $\text{nmol NADPH}_2 \text{ min}^{-1} \text{ mg}^{-1}$ protein)	$117.83 \pm 4.38$	$53.03 \pm 2.17$	0.000
GPX ( $\mu\text{mol GSH min}^{-1} \text{ mg}^{-1}$ protein)	$123.49 \pm 4.02$	$42.07 \pm 2.12$	0.000

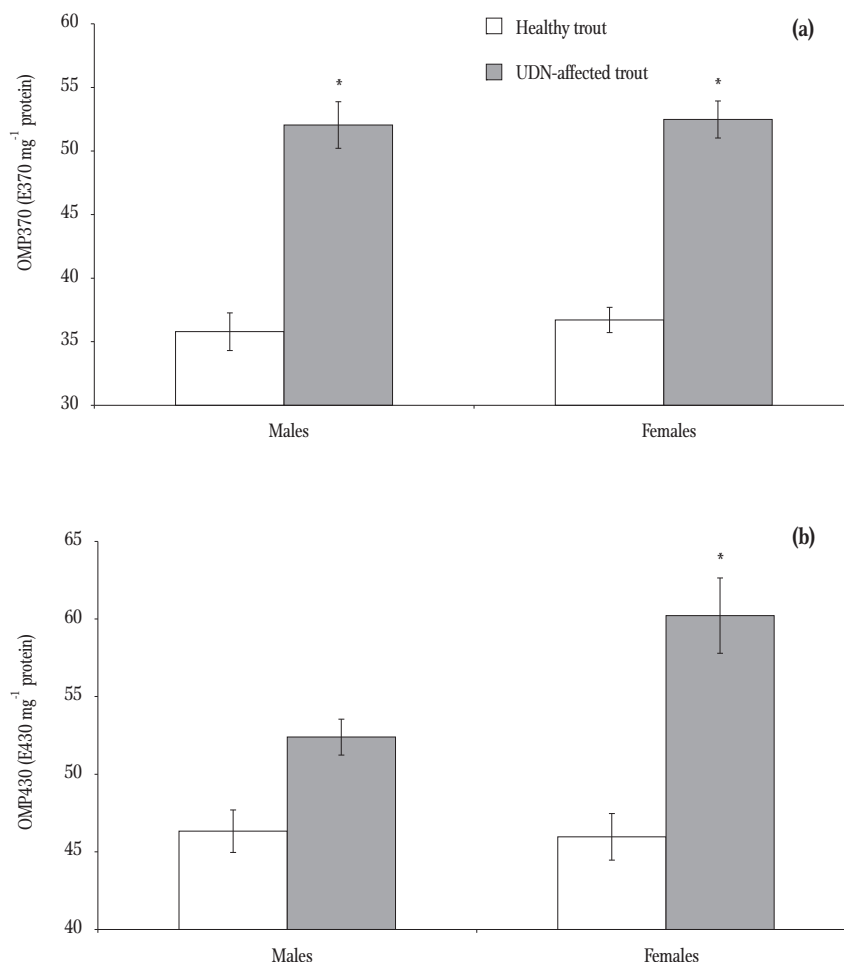


Figure 2. Oxidatively modified protein (OMP) content ( $\text{E mg}^{-1}$  protein) measured by the quantity of carbonyl oxidation (aldehyde derivatives, A; ketonic derivatives, B) in the livers of males and females from control (healthy specimens) and UDN-affected sea trout. Each value represents the mean  $\pm$  SEM. \*The significant change was shown as  $P < 0.05$  as compared to control group values.

**Table 3**

Correlation analysis among lipid peroxidation, oxidative modified protein levels, and antioxidant defense parameters in the livers from males and females of sea trout affected by ulcerative dermal necrosis (UDN)

Relation	Correlation coefficient, r	P
<b>Males</b>		
TBARS-OMP430, control	0.285	0.026
OMP370-OMP430, control	0.266	0.039
OMP370-GPX, UDN	0.300	0.007
OMP430-SOD, UDN	-0.268	0.016
OMP430-GR, UDN	0.310	0.005
SOD-GR, UDN	0.318	0.004
<b>Females</b>		
OMP370-OMP430, control	0.480	0.000
TBARS-OMP370, UDN	0.282	0.023

were found. UDN infection significantly affected GPX activity ( $F = 132.35$ ,  $P < 0.001$ ), which was inhibited by 39.5% ( $P < 0.001$ ) and 65.9% ( $P < 0.001$ ) in the livers of males and females, respectively.

Regarding the total antioxidative activity (Fig. 3), the UDN infection significantly decreased TAA by 34.4% ( $P < 0.01$ ) in UDN-affected females, but no differences in TAA between healthy and UDN-affected males were found.

Ketonic derivatives of carbonyl oxidation from healthy males correlated with TBARS levels ( $r = 0.285$ ,  $P < 0.05$ ) and aldehyde derivatives ( $r = 0.266$ ,  $P < 0.05$ ) (Table 3). The relationship between liver GPX activity and OMP<sub>370</sub> levels from UDN-affected males was positive ( $r = 0.300$ ,  $P = 0.007$ ), while between liver SOD activity and OMP<sub>430</sub> levels it was

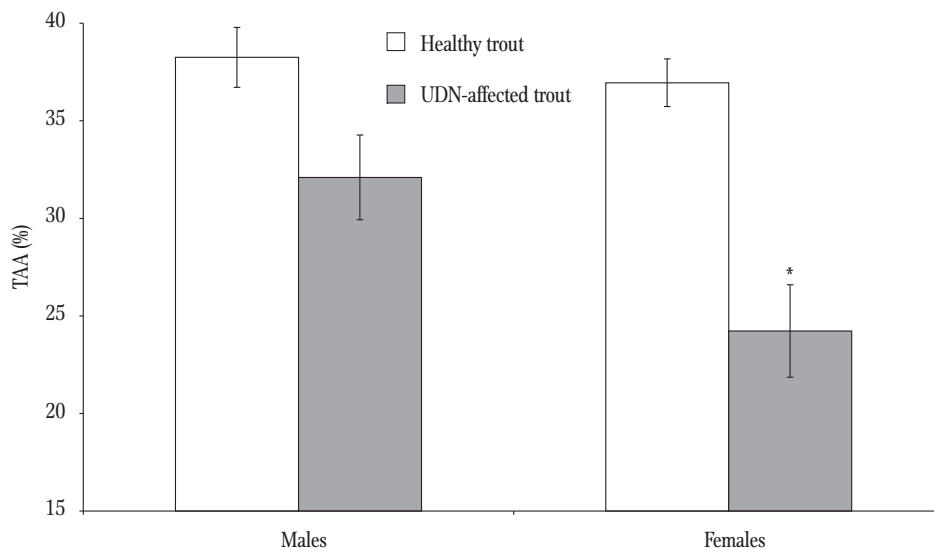


Figure 3. Total antioxidative activity (%) in the livers of males and females from control (healthy specimens) and UDN-affected sea trout. Data are means  $\pm$  SEM. \*The significant change was shown as  $P < 0.05$  as compared to control values.

inverse ( $r = -0.268$ ,  $P < 0.05$ ). Liver GR activity from UDN-affected males correlated positively with SOD activity ( $r = 0.318$ ,  $P < 0.01$ ) and ketonic derivates of carbonyl oxidation ( $r = 0.310$ ,  $P < 0.01$ ). The relationship between OMP<sub>370</sub> and TBARS levels in the livers of UDN-affected females was positive ( $r = 0.282$ ,  $P < 0.05$ ).

## Discussion

The present study investigates the physiological response of the antioxidant defense system in the livers of sea trout affected by UDN. Specifically, we addressed the question of whether oxidative stress occurs during UDN infection and whether the antioxidant defense system is induced. The interplay between these defense systems is supposed to be significant for the passive survival of oxidative stress caused by UDN. Enzymatic antioxidant defenses are key components of biochemical machinery that permit species survival during oxidative stress (Halliwell and Gutteridge 1999, Hermes-Lima 2004, Lushchak and Bagnyukova 2006).

The necessity of conducting the research presented herein stems from the fact that external factors such as pathogens, infectious agents, toxins,

heavy metal, and radiation can lead to increased free radicals and other ROS. Although low levels of ROS are essential in many biochemical processes, accumulations of ROS can damage biological macromolecules such as lipids, proteins, carbohydrates, and DNA (Yagi 1993). Oxidative damage can be minimized by antioxidant defense mechanisms that protect cells against cellular oxidants and repair systems thus preventing the accumulation of oxidative damaged molecules. Antioxidant enzymes (SOD, CAT, GR, GPX, reduced and oxidized glutathione, etc.) play vital roles in protecting cellular damage from the harmful effects of ROS (Halliwell and Gutteridge 1999, Hermes-Lima 2004, Lushchak and Bagnyukova 2006).

The etiology of UDN outbreaks remains unknown. There has been no conclusive evidence of the involvement of any particular organism as the primary pathogen (Roberts 1993). Harikrishnan et al. (2009) examined histopathological and recovery changes in lateral muscles, gills, livers, and hearts in *A. hydrophila* – infected goldfish, *Carassius auratus* (L.). The results of these studies provide evidence that *A. hydrophila* induces in *A. hydrophila* – infected fish the appearance of a muscular hemorrhagic protuberance, which progressed into an extensive ulcerative dermatitis associated with focal

hemorrhage, edema, and dermal necrosis exposing the underlying muscle. The progression of the disease affected muscle, gills, livers, and, finally, hearts. Histopathological changes in *A. hydrophila* – infected livers were characterized by developed granulomatous inflammation and necrosis of hepatocytes (Harikrishnan et al. 2009). By day 18 after infection, multiple fibromas and macrophage granulomas were observed; by day 24 after infection, the livers of the infected fish showed deep vacuolization and granulation in the cytoplasm, pyknosis of the hepatocyte nuclei, necrosis, granulomatous inflammation, and large numbers of macrophages and fibroblasts. The livers exhibited focal necrosis of the hepatocytes with tubular degeneration of the intestinal microvilli and hepato-cellular necrosis by day 30 after infection. By day 36 after infection, liver tissues appeared edematous and were congested with necrotic foci showing fibrin deposition or slight haemorrhage in the pulp, inflammation, free pre-granulomatous tissue, and mature granuloma (Harikrishnan et al. 2009).

Law (2001) suggests that skin ulcers in fish can have many different etiologies, including infectious agents, toxins, physical causes, immunologic causes, and nutritional and metabolic perturbations. Ulcerative lesions are likely to be initiated by a series of factors that lead ultimately to a breach of the normal barrier function of the skin. Kane et al. (2000) reported that lesions in fish are associated with a variety of organisms including parasites and bacterial, viral, and fungal infectious agents. Trauma, suboptimal water quality, and other abiotic stress factors can result in the loss of homeostasis. Certain conditions (such as disease, exposure to toxins, aging, exercise, etc.) can increase the rate of oxidative damage, a condition called oxidative stress (Pryor 1986). Oxidative stress has been defined as a disturbance in the balance between the production of ROS, or free radicals and antioxidant defenses, which can lead to a series of biochemical and physiological changes, thus, altering normal body homeostasis and tissue injury (Halliwell and Gutteridge 1999).

Our previous study indicated that the pathophysiological mechanism of UDN impact is

connected with decreases of the most important antioxidant enzymes (SOD, CAT, GR, and GPX) and TAA in muscle, livers, hearts, and the spawn of the infected trout. It has been suggested that increases of oxidative stress markers and the intensity of lipid peroxidation levels is caused by UDN (Kurhalyuk et al. 2009, 2010). Our present study indicates that the livers of sea trout affected by UDN undergo protein oxidation due to the oxidizing effect of ROS. The content of aldehyde and ketonic derivatives, as end-products of protein oxidative destruction, increased in most cases. It appears that UDN caused the accumulation of that end-product of protein oxidation in the livers of UDN-affected trout. Decreased cell antioxidant defenses is followed by the production of lipid and protein peroxidation products (Yagi 1993, Halliwell and Gutteridge 1999). The correlative relationships between prooxidative parameters and activities of antioxidant enzymes also agree, as was expected if the peroxidation process is subsequent to the consumption of intracellular antioxidants. The content of aldehyde and ketonic derivatives confirmed that the protein oxidation starts rapidly in UDN-affected females. Thus, our findings indicate that UDN in sea trout is associated with oxidative stress and is characterized by marked changes in protein structure and function.

Several diseases and many common environmental pollutants recognized as toxic were found to have harmful effects on the lipids, proteins, and carbohydrates of cells (Beal 2002, Cesaratto et al. 2006). For example, protein carbonyl formation can occur as result of oxidative stress and has been shown to play an important role in a number of diseases (Floyd et al. 2001, Foster et al. 2003). ROS generated during many diseases are known to convert amino groups of proteins and thereby alter protein structure or function. An increase in the number of modified carbonyl groups correlates with protein damage caused by oxidative stress (Beal 2002, Foster et al. 2003, Dalle-Donne et al. 2003).

The oxidative modification of proteins by ROS is implicated in the etiology or progression of different disorders and diseases (Floyd et al. 2001, Beal 2002). These reactive species form through a large number of



physiological and non-physiological reactions. An increase in the rate of their production or a decrease in their rate of scavenging increases the oxidative modification of cellular molecules, including proteins (Nguyen and Donaldson 2005). Oxidative modified proteins are not repaired and must be removed by proteolytic degradation, and a decrease in the efficiency of proteolysis causes an increase in the cellular content of oxidative modified proteins (Ercal et al. 2001, Beal 2002). Protein oxidation, as well as final products of lipid peroxidation and changes in levels of endogenous antioxidants, has been successfully utilized as biological markers of oxidative stress conditions (Dalle-Donne et al. 2003, Foster et al. 2003).

In order to protect against oxidative stress, organisms have developed antioxidant systems consisting of low-molecular weight compounds (glutathione, ascorbic and uric acid, tocopherols, etc.) and proteins including antioxidant enzymes (Lushchak and Bagnyukova 2006). No significant differences were observed for SOD activity in the livers of UDN-affected trout. SOD catalyzes the conversion of superoxide radicals into molecular oxygen and hydrogen peroxide that can in turn be destroyed by CAT or GPX reactions (Yagi 1993). The depression in SOD activity can result in cellular injury by superoxide radicals and the inactivation of enzymes by interaction with oxygen radicals (Hunt et al. 1998). This situation can reflect the correlative link between ketonic derivatives of protein oxidative destruction and SOD activity in the livers of UDN-affected males ( $r = 0.393$ ,  $P < 0.001$ ). CAT is an antioxidant enzyme that catalyzes the decomposition of  $H_2O_2$  to form water and molecular oxygen (Yagi 1993, Hunt et al. 1998). The decreased CAT activity in the livers of UDN-affected trout indicates a reduced capacity to scavenge  $H_2O_2$  produced in response to oxidative stress induced by UDN.

Multiple data indicate that glutathione-dependent enzymes are important to the resistance of oxidative stress in fish (Lushchak and Bagnyukova 2006). In animals, GPX detoxifies both  $H_2O_2$  and organic hydroperoxides, glutathione-S-transferase detoxifies various compounds by conjugating them with glutathione, GR reduces oxidized glutathione using

NADPH (Halliwell and Gutteridge 1999, Hermes-Lima 2004). Decreases of the glutathione-mediated antioxidant defense system results in oxidative stress and increased cytotoxicity, whereas the elevation of intracellular GSH levels is recognized as an adaptive response to oxidative stress (Sagara et al. 1998).

In our study the GR and GPX activities were significantly decreased in the livers of UDN-affected fish with respect to the control group. These results suggest that both the glutathione-mediated antioxidant defense system and endogenous catalase play critical roles in intracellular antioxidant defenses under UDN-induced oxidative stress. The importance of the glutathione-mediated antioxidant defense system in protection against oxidative stress was also demonstrated in rainbow trout adrenocortical cells (Dorval and Hontela 2003). Decreases of glutathione-dependent enzyme activity is associated with the decline of the total antioxidative activity under UDN-induced oxidative stress in the livers of infected trout.

In conclusion, our results indicate changes in oxidative stress intensity in the livers of trout affected by ulcerative dermal necrosis. In summation, increased protein oxidative destruction modifies antioxidant defenses and causes changes in CAT, GR, and GPX activity. It is important to note the predominance of UDN participation in decreased total antioxidative activity and antioxidant defenses in the livers of infected females.

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## Streszczenie

### Występowanie stresu oksydacyjnego i obrona antyoksydacyjna w wątrobie troci wędrownej *Salmo trutta* L. z wrzodziejącą martwicą skóry

Celem niniejszej pracy była analiza występowania stresu oksydacyjnego i zmian w mechanizmach obrony antyoksydacyjnej, określonych za pomocą zawartości produktów, reagujących z kwasem 2-tiobarbiturowym (TBARS-produktów), poziomu oksydacyjnej modyfikacji białek (aldehadowe i ketonowe pochodne, OMB), aktywności najważniejszych enzymów antyoksydacyjnych (dysmutazy nadadtlenkowej, katalazy, glutationowej reduktazy i peroksydazy) oraz całkowitej

zdolności antyoksydacyjnej w tkance wątroby samców i samic troci wędrownej *Salmo trutta* L. z wrzodziejącą martwicą skóry (WMS) w okresie tarła z lat 2007-2009. Na podstawie przeprowadzonych badań stwierdzono, że występowanie WMS u osobników różnej płci powoduje wzrost procesów oksydacyjnej modyfikacji białek (zarówno aldehydowych jak i ketonowych pochodnych) z jednoczesnym obniżeniem aktywności podstawowych enzymów obrony antyoksydacyjnej.

W największym stopniu te negatywne zmiany dotyczyły aktywności katalazy i enzymów antyutleniania systemu glutatio-  
nu. Zaobserwowano także, że u samic w porównaniu do grupy  
chorych na WMS samców występuje istotne statystycznie ob-  
niżenie poziomu całkowitej zdolności antyoksydacyjnej. Ana-  
liza współczynników korelacyjnych zależności wykazała, że  
w grupie chorych samców otrzymano większą liczbę powiązań  
pomiędzy intensyfikacją procesów lipoperoksydacji,

oksydacyjnej modyfikacji białek i parametrami funkcjonowa-  
nia systemu pro- i antyutleniania w tkance wątroby osobni-  
ków troci wędrownej. Wyniki badań sugerują, że występowanie  
WMS u troci wędrownej w analizowanej tkance  
negatywnie modyfikuje mechanizmy obrony antyoksydacyj-  
nej, co w posumowaniu niekorzystnie wpływa na przeżywal-  
ność chorych osobników i obniża wydajność procesów  
tańlowych.