УДК 639.09:615.2

The alterations in the oxidative stress biomarkers in the brain tissue of rainbow trout (*Oncorhynchus mykiss)* vaccinated against furunculosis

Halyna Tkachenko[1], Joanna Grudniewska[2]

[1] Department of Zoology and Animal Physiology, Institute of Biology and Environmental Protection, Pomeranian University in Slupsk, Arciszewski Str. 22B, 76–200 Slupsk, Poland;

[2] Department of Salmonid Research, Stanislaw Sakowicz Inland Fisheries Institute, 83–330 Żukowo, Poland

E-mail: tkachenko@apsl.edu.pl, biology.apsl@gmail.com

The use of vaccines for disease prevention in aquaculture has expanded both with regard to the number of fish species and number of microbial diseases. Despite the importance and success of vaccination, little is known about the mechanisms of oxidative stress and antioxidant defense in fish during vaccination against furunculosis. In the present study, we have determined the oxidative stress biomarkers and antioxidant defense in the brain tissue of rainbow trout (*Oncorhynchus mykiss*) vaccinated against furunculosis. The analysis of oxidative stress biomarkers has revealed significant alterations between vaccinated fish against furunculosis and unhandled controls. The brain tissue of vaccinated trout had lower level of aldehydic and ketonic derivatives of oxidatively modified proteins, as well as lipid peroxidation. The total antioxidant capacity becomes more susceptible to oxidative damage induced by vaccination against furunculosis. Glutathione-dependent enzymes and catalase activity were dependent to oxidative stress in vaccinated trout. Correlations between lipid peroxidation, carbonyl derivatives of protein damage, and antioxidant defense confirmed the assumption that oxidative stress could activate the antioxidant defenses for improve adaptive mechanisms during immunization. Understanding the role of biochemical changes in the tissues of vaccinated trout has important implications for understanding of the complex physiological alterations that occur in immunization, and also for improving aquaculture practices to maximize tissues growth and health of vaccinated trout.

Key words: furunculosis, rainbow trout *Oncorhynchus mykiss*, lipid peroxidation, oxidatively modified proteins, antioxidant defense, total antioxidant capacity

Introduction.

Motile aeromonads are often referred to as a complex of disease organisms that are associated with bacterial haemorrhagic septicaemias and other ulcerative conditions in fish [Cipriano, Austin, 2011]. Notwithstanding, motile aeromonads are ubiquitous in most freshwater environments and are common in the water column and in the upper layers of sediment [Hazen, 1979].

Motile aeromonads cause diverse pathological conditions that include acute, chronic and covert infections. Severity of disease is influenced by a number of interrelated factors, including bacterial virulence, the kind and degree of stress exerted on a population of fish, the physiological condition of the host and the degree of genetic resistance inherent within specific populations. Motile aeromonads differ interspecifically and intraspecifical-

ly in their relative pathogenicity or their ability to cause disease [Cipriano, Austin, 2011].

In the acute form of a motile aeromonad disease, a fatal septicaemia may occur so rapidly that fish die before they have time to develop anything but a few gross signs of disease. When clinical signs of infection are present, affected fish may show exophthalmia, reddening of the skin and an accumulation of fluid in the scale pockets. The abdomen may become distended as a result of an oedema and the scales may bristle out from the skin to give a 'washboard' appearance. The gills may haemorrhage and ulcers may develop on the dermis [Faktorovich, 1969; Cipriano, Austin, 2011].

Ogara and co-workers [1998] observed severe eye pathology and heavy mortality among yearling and older rainbow trout accompanying a severe outbreak of motile aeromonad septicaemia. Motile aeromonads were isolated from the eyes, liver and kidneys of affected fish. Histopathologically, fish may exhibit epithelial hyperplasia in the foregut, leptomeningeal congestion in the brain, as well as a thrombosis and inflammation in the perisclerotic region and corneal epithelium of the eye [Fuentes, Perez, 1998]. There may also be a severe branchitis, as indicated by leucocytic infiltration and dilation of the central venous sinus [Grizzle, Kiryu, 1993]. These authors also noted that catfish with septicaemic or latent infections had enlarged nuclei in the branchial epithelium and that there was a significant correlation between presences of these gill lesions and the severity of hepatic and pancreatic lesions.

The use of vaccines for disease prevention in aquaculture has expanded both with regard to the number of fish species and number of microbial diseases. Moreover, developed vaccines based on inactivated bacterial pathogens have proven to be very efficacious in fish [Sommerset et al., 2005]. Prophylactic measures against *Aeromonas salmonicida* subsp. *salmonicida*, the causative agent of furunculosis, have been an active field of research for decades. Villumsen and Raida [2013] reported that initial studies attempted vaccination via oral route and immersion. However, these vaccination methods proved insufficient when compared to intraperitoneally injected vaccines. The focus of vaccine research regarding *A. salmonicida* shifted towards the intraperitoneally injected vaccines during the 1980's and —90's, resulting in oil-adjuvanted vaccines providing high levels of protection over longer periods of time. The majority of this research has been conducted using salmon, while rainbow trout, which is also a commercially important species, has played a much less central role.

Håstein and co-workers [2005] submitted an update of the current situation worldwide about bacterial vaccines for fish. Vaccination is used in the commercial aquaculture of species like Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*), barramundi (*Lates calcarifer*), tilapia (*Tilapia* spp), turbot (*Scophthalmus maximus* L.), yellowtail (*Seriola quinqueradiata*), purplish and gold-striped amberjack (*Seriola dumereli*), striped jack (*Pseudocaranx dentex*) and channel catfish (*Ictalurus punctatus*). The range of bacterial infections for which vaccines are commercially available now comprises classical vibriosis (*Listonella anguillarum*, *Vibrio ordalii*), furunculosis (*Aeromonas salmonicida* subsp. *salmonicida*), cold-water vibriosis (*Vibrio salmonicida*), yersiniosis (*Yersinia ruckeri*), pasteurellosis (*Photobacterium damselae* supsp. *piscicida*), edwardsiellosis (*Edwardsiella ictaluri*), winter ulcer (*Moritella viscosa*), and streptococcosis/lactococcosis (*Streptococcus iniae*, *Lactococcus garviae*). Furthermore, experimental vaccines are used against diseases such as infection with *Vibrio harveyi* and *Photobacterium damsela* subsp. *damsela* in barramundi, piscirickettsiosis and bacterial kidney disease in salmonids, as well as infection with *Tenacibaculum maritimum* in turbot.

Despite the importance and success of vaccination, little is known about the mechanisms of oxidative stress and antioxidant defense in fish during vaccination against furunculosis. In the present study, we have determined the oxidative stress biomarkers and antioxidant defense in the brain tissue of rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)) vaccinated against furunculosis.

Materials and methods.

Experimental animals. Clinically healthy rainbow trout with a mean body mass of (135.5±1.5) g were used in the experiments. The study was carried out in a Department of Salmo-

nid Research, Stanislaw Sakowicz Inland Fisheries Institute (Żukowo, Poland). The experiments were conducted with the current laws in Poland, according to the Ethical Commission. Experiments were performed at a water temperature of 14.5±0.5 °C and the pH was 7.5. The dissolved oxygen level was about 12 ppm with additional oxygen supply with a water flow of 25 L/min, and a photoperiod of 7 hours per day. The fish were fed with commercial pelleted diet. 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 design. 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). Fish were vaccinated and grouped as follows: I) unhandled controls, II) vaccinated by vaccine against furunculosis. The vaccine against furunculosis is a vaccine containing an inactivated strain of *A. salmonicida* and *A. hydrofila* in concentration 1×10^{10} colony forming units (CFU). The vaccine was produce in Department of Epizootology, Faculty of Veterinary Medicine, University of Warmia and Mazury (Olsztyn, Poland). Immersion solution contained 1 liter of vaccine per 10 liters of water. It was prepared immediately prior to vaccination. Immersion lasted from 60 to 120 seconds. The fish were kept for 30 days at 14.5 °C after vaccination at a water temperature of 14.5±0.5 °C and the pH 7.5.

Sampling. The animals were quickly captured and killed on 31 days post vaccination (n=15 in each group). Brain tissue was removed *in situ*. Tissue sample was homogenized in ice-cold buffer (100 mM Tris-HCl, pH 7.2) using a glass homogenizer immersed in an ice water bath to a yield a 10% homogenate. Homogenates were centrifuged at 3,000g for 15 min at 4 °C. After centrifugation, the supernatant was collected and frozen at –20 °C until analyzed. Protein contents were determined using the method of Bradford [1976] with bovine serum albumin as a standard. Absorbance was recorded at 595 nm. All enzymatic assays were carried out at (22±0.5)°C using a Specol 11 spectrophotometer (Carl Zeiss Jena, Germany) in duplicate.

TBARS assay for lipid peroxidation. The level of lipid peroxidation was determined by quantifying the concentration of TBARS with the Kamyshnikov method (2004) for determining the malondialdehyde (MDA) concentration. This method is based on the reaction of the degradation of lipid peroxidation product, MDA, with TBA under high temperature and acidity to generate a colored adduct that is measured spectrophotometrically. The nmol of MDA per 1 mg of tissue protein was calculated by using $1.56\cdot10^5$ 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]. 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.

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 (1990) method. 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 [1988]. 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, EC1.6.4.2) activity in the tissue was measured according to the method described by Glatzle and co-workers [1974]. The GR activity was expressed as nmol NADPH per min per mg of tissue protein.

Glutathione peroxidase activity assay. Glutathione peroxidase (GPx, EC1.11.1.9) activity was determined by detecting the nonenzymat-

ic 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 (1986). 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 [Galaktionova et al., 1998]. 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 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 [Zar, 1999]. All statistical calculation was performed on separate data from each individual with STATISTICA 8.0.

Results.

Vaccination caused a significant decrease of the TBARS level in the brain tissue by 24.1% ($p=0.017$) (Fig. 1A). Aldehydic and ketonic derivatives of oxidatively modified proteins in the trout vaccinated against *Aeromonas* spp. were significantly reduced (by 27%, $p=0.008$ and by 24%, $p=0.008$, respectively) compared to the level in the controls (Fig. 1B).

SOD and GR activities were non-significantly inhibited, while CAT and GPx activity was increased in the brain tissue of vaccinated fish compared to control fish (Table 1). The TAC was sig-

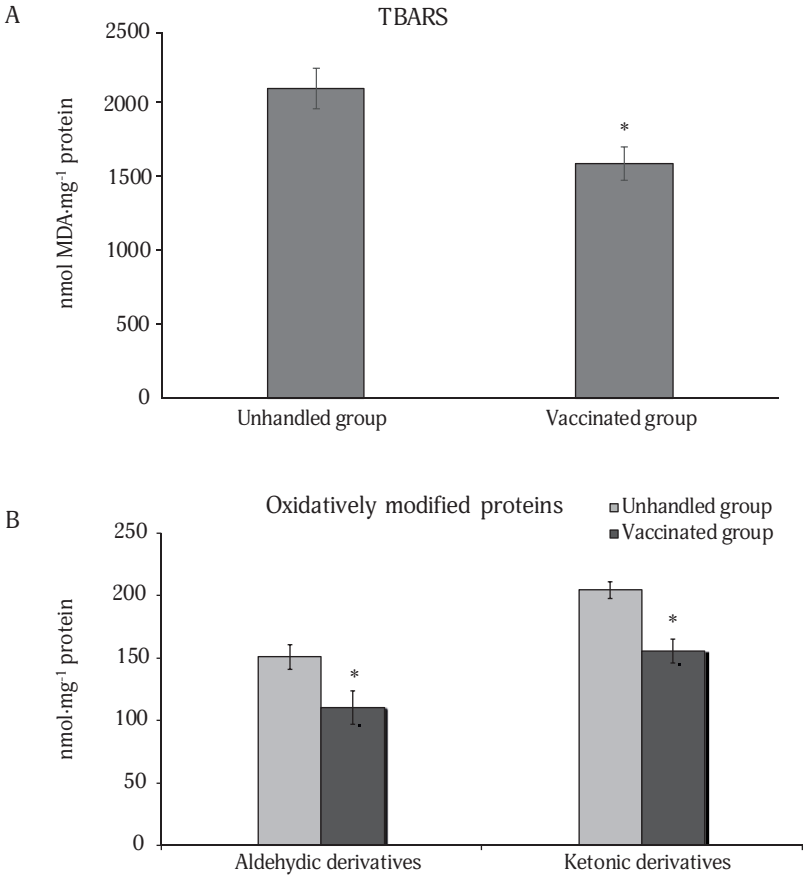


Fig. 1. TBARS level (A), aldehydic and ketonic derivatives of oxidatively modified proteins in the brain tissue of the trout vaccinated against *Aeromonas* spp. Data are represented as mean ± S.E.M.

* — the significant difference was shown as $p<0.05$ when compared vaccinated group and unhandled group values.

Table 1. Enzymatic antioxidant defenses in the brain tissue of the rainbow trout vaccinated against furunculosis

Antioxidant enzymes activity	Unhandled group (n=15)	Vaccinated group (n=15)
SOD, U·mg^{-1} protein	749.89±48.18	780.12±156.72
CAT, μmol·min^{-1}·mg^{-1} protein	119.67±10.21	95.74±6.91
GR, μmol·min^{-1}·mg^{-1} protein	1.747±0.199	1.956±0.208
GPx, μmol·min^{-1}·mg^{-1} protein	1104.15±183.13	945.65±149.80

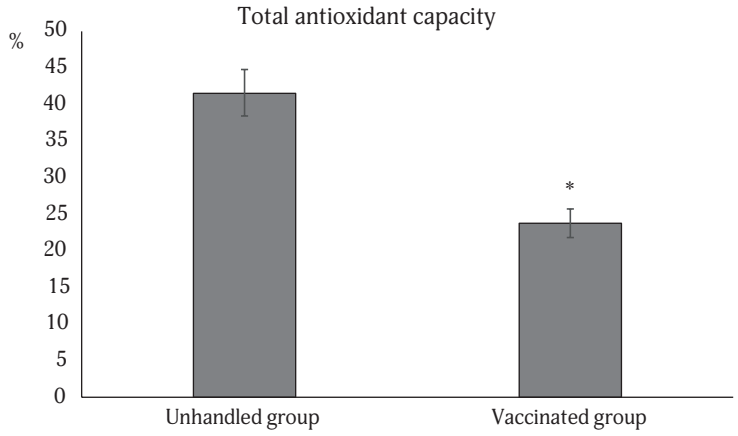


Fig. 2. TAC in the brain tissue of the trout vaccinated against *Aeromonas* spp. Data are represented as mean ± S.E.M.
* — the significant difference was shown as $p<0.05$ when compared vaccinated group and unhandled group values.

nificantly decreased in brain compared to those in the control (by 42.8%, p=0.000) (Fig. 2).·

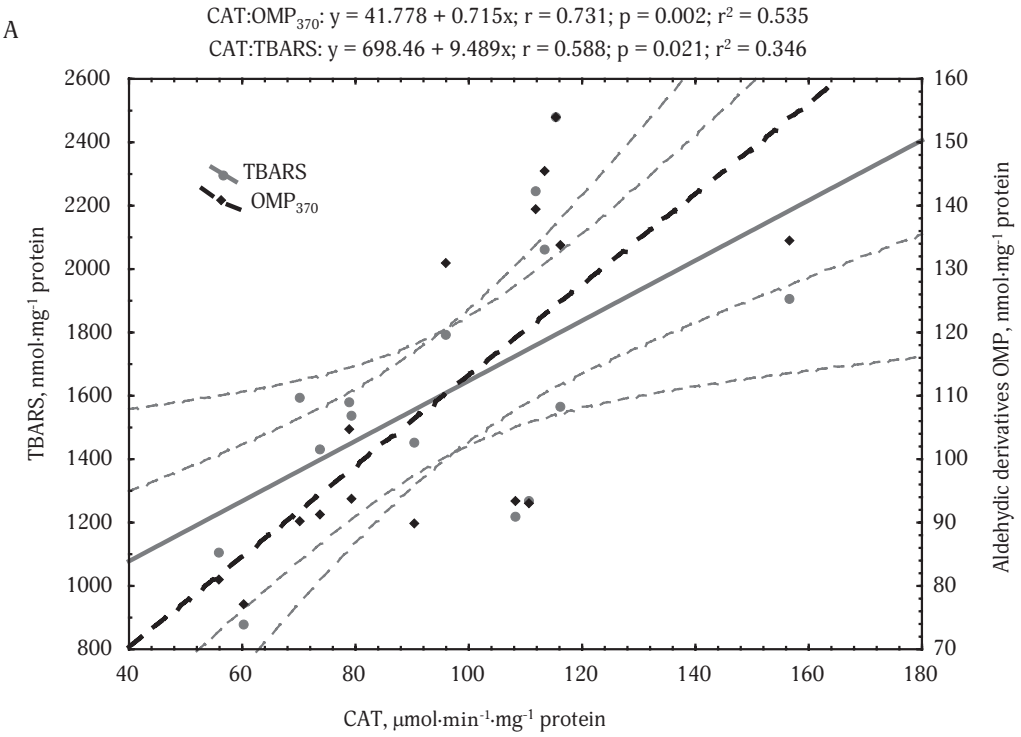
Several correlations between checked parameters were found (Fig. 3). In vaccinated group, CAT (r=0.731, p=0.002), GR (r=0.790, p=0.001), and GPx activity (r=0.709, p=0.003) correlated with aldehydic derivatives of OMP and TBARS level (r=0.588, p=0.021; r=0.673, p=0.006; r=0.732, p=0.002, respectively). Moreover, ketonic derivatives of OMP positively correlated with GR (r=0.802, p=0.000) and GPx activity (r=0.715, p=0.003) (Fig. 3).

Discussion.

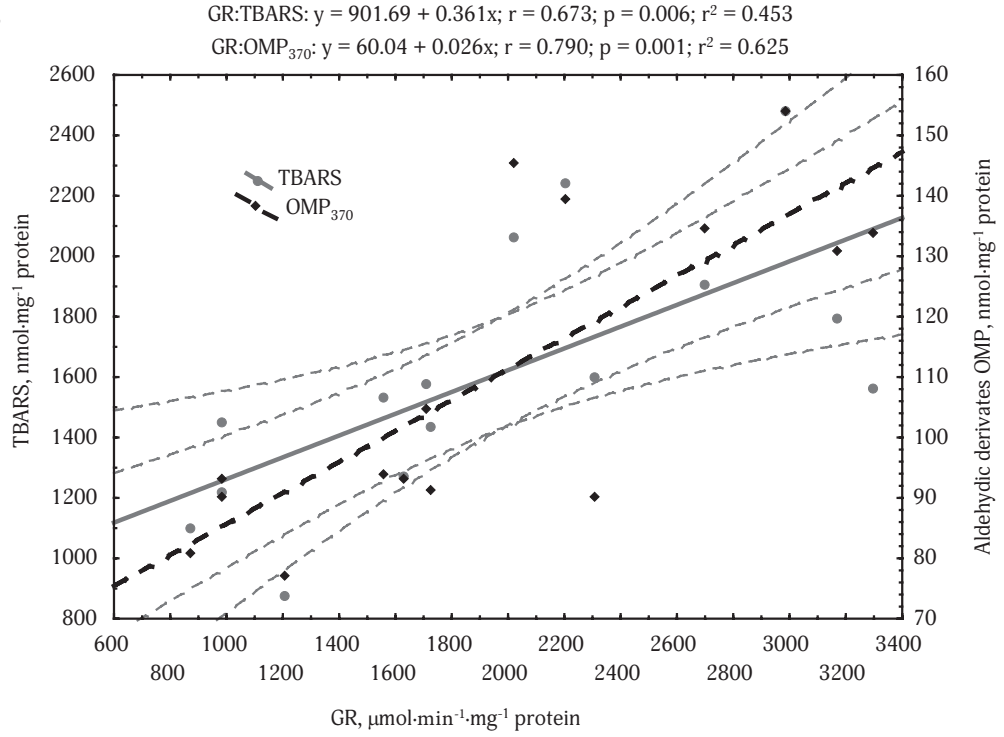
The study showed a post-treatment alterations in oxidative stress profile in the brain tissue of rainbow trout treated by vaccine against furunculosis. The decrease of lipid peroxidation (TBARS level), as well as aldehydic and ketonic derivatives of protein damage was observed (Fig. 1). However, the post-treatment TAC level was decreased after vaccination (Fig. 2). In our study, lower level of TBARS and proteins oxidation in the brain tissue of vaccinated fish apparently is caused by adaptation of brain metabolism to the vaccine against furunculosis during immunization.

In our previous study, we determined the influence of vaccination against furunculosis on the level of oxidative stress biomarkers (lipid peroxidation and oxidatively modified proteins) and biochemical enzymes activity (alanine and aspartate aminotransferases, lactate dehydrogenase) in the gills of brown trout, *Salmo trutta* m. *fario*. In contrary to our current study, the level of lipid peroxidation in the gills of brown trout treated by vaccine against furunculosis was significantly increased by 179% (p=0.000) compared to unhandled control. The content of aldehydic and ketonic derivatives of oxidatively modified proteins, as well as aminotransferases and lactate dehydrogenase (LDH) activities in the gills were significantly increased in the group vaccinated against furunculosis compared to unhandled group [Tkachenko, Grudniewska, 2015]. Interestingly, in the gills of rainbow trout, *O. mykiss*, the decrease of aldehydic and ketonic derivatives of protein damage was observed. However, the post-treatment level of

A


CAT:OMP370: y = 41.778 + 0.715x; r = 0.731; p = 0.002; r² = 0.535
CAT:TBARS: y = 698.46 + 9.489x; r = 0.588; p = 0.021; r² = 0.346
TBARS
OMP370
TBARS, nmol·mg-1 protein
Aldehydic derivatives OMP, nmol·mg-1 protein
CAT, µmol·min-1·mg-1 protein

B


GR:TBARS: y = 901.69 + 0.361x; r = 0.673; p = 0.006; r² = 0.453
GR:OMP370: y = 60.04 + 0.026x; r = 0.790; p = 0.001; r² = 0.625
TBARS
OMP370
TBARS, nmol·mg-1 protein
Aldehydic derivates OMP, nmol·mg-1 protein
GR, µmol·min-1·mg-1 protein

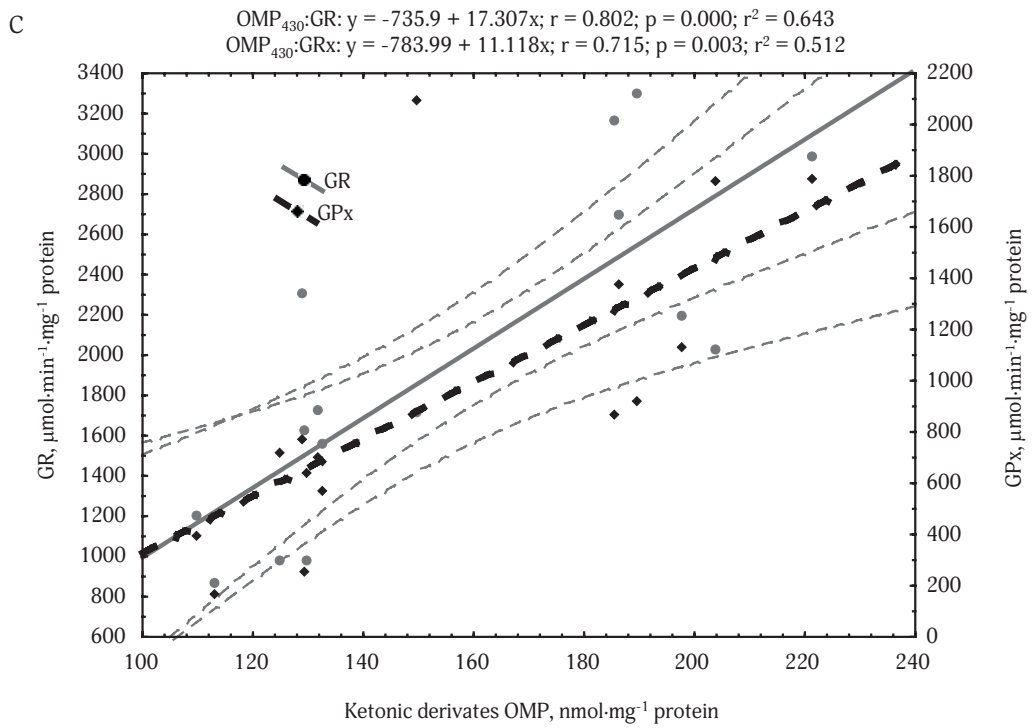


Fig. 3. Correlations between oxidative stress biomarkers in the brain tissue of the trout vaccinated against *Aeromonas* spp.

lipid peroxidation biomarker was increased. Higher level of lipid peroxidation in gills of vaccinated fish could be induced by respiratory burst through activation of phagocytic cells [Pietarinen-Runtti et al., 2000]. Our study reveals that vaccinated trout showed alterations in antioxidant defenses. Glutathione-dependent enzymes and catalase activity were decreased in vaccinated trout. Correlations between ketonic derivatives of protein damage, CAT and GPx activity confirm the assumption that carbonyl derivatives of oxidatively modified protein could inhibit the antioxidant defenses [Tkachenko et al., 2015].

Other researchers also presented different adverse and long-term protective effects following vaccination against *A. salmonicida* in rainbow trout, as well as results on different challenge methods used for infection of rainbow trout with *A. salmonicida*, a bacterial pathogen eliciting furunculosis. The various responses during vaccination against furunculosis are observed probably due to the different kind of vaccine, methods of trials and fish species. Numerous trials have been performed in order to optimize vaccination and to elucidate the specific antigens involved in protection. Results however, have been contradictory and certain antigens capable of producing agglutinating antibodies against *A. salmonicida* were not protective. Furthermore, several authors have suggested that the protection observed was, in part non-specific. In contrast, passive immunization of fish with immune sera directed against whole *A. salmonicida* antigens has shown that humoral factors were able to protect fish. The level of protection correlated with the level of specific antibodies in the serum [Bergh et al., 2013]. Villumsen and co-workers [2015] have noted adverse and long-term protective effects following oil-adjuvanted vaccination against *A. salmonicida* in rainbow trout. A commercial vaccine and an experimental auto vaccine, as well as their respective adjuvant formulations alone were used to evaluate their individual effects, both prior to and during an experimental waterborne infection challenge. Macro- and microscopic examination revealed signs of vaccine-induced adverse effects from 10 weeks to 14 months post vaccination. Both vaccines induced statistically significant protection during the experimental challenge ($P=0.018$ for both vaccines), as well as significantly elevated levels of specific circulat-

ing antibodies prior to and during the experimental challenge when compared to an unvaccinated control group. During the early, critical time points of the infection, both vaccines appeared to protect against pathological changes to the liver and spleen, which provides a probable explanation for the reduced mortality in the vaccinated groups. A significant correlation was found between levels of *A. salmonicida*-specific antibodies measured prior to challenge and the endpoint survival of each group after the experimental infection, and furthermore, the levels of these antibodies remained elevated for at least 14 months post vaccination.

Villumsen and Raida [2013] have examined the effect of a bath vaccination using an experimental *A. salmonicida* bacterin. Rainbow trout were vaccinated by a 5 min bath in a formalin-inactivated bacterin. Half of these fish was booster vaccinated using 50% of the initial vaccine dose 10 weeks post primary immunization. Along with an un-vaccinated control group, the fish were challenged by waterborne infection 24 weeks post primary immunization. Both vaccinated groups showed a significantly increased survival (>93% survival) compared to a 70% survival in the un-vaccinated control group. When comparing the survival of the single and dual immunization groups, there was no significant difference. ELISA showed no significant induction of specific circulating antibodies in either vaccinated group.

Rømer Villumsen and co-workers [2012] have examined the protection against infection with a Danish strain of *A. salmonicida* in both vaccinated and non-vaccinated rainbow trout. A commercial and an experimental auto-vaccine were tested. The protective effects of the vaccines were evaluated through an *A. salmonicida* challenge 18 weeks post vaccination. Both vaccines resulted in a significantly increased survival in the vaccinated fish during a 28 day challenge period relative to non-vaccinated fish. Throughout the entire experiment, the presence of specific antibodies in plasma was monitored using ELISA. A significant increase in specific antibody levels was seen in fish vaccinated with both vaccines during the 18 weeks between vaccination and challenge. Within 3 days post challenge, a significant decrease in specific antibodies occurred in vaccinated fish. A positive correlation was found between mean levels of specific antibodies pre challenge and overall survival. This correlation, along with the observed depletion of antibodies during the initial phase of infection, suggests that specific antibodies play an essential role in vaccine mediated protection against *A. salmonicida* in rainbow trout.

Vanya Ewart and co-workers [2008] have shown the early response of Atlantic salmon (*Salmo salar*) macrophages exposed *in vitro* to *A. salmonicida* cultured in broth and in fish. In their study, the early macrophage responses to *A. salmonicida* grown *in vivo* and *in vitro* were compared. Macrophage-enriched cell preparations from head kidney of Atlantic salmon were infected *in vitro* in 96-well microtitre dishes and changes in gene expression during the infection process were monitored using a custom Atlantic salmon cDNA microarray. *A. salmonicida* cultures grown in tryptic soy broth and in peritoneal implants were used to infect the macrophages. The macrophages were harvested at 0.5, 1.0 and 2.0 h after addition of the bacteria to the medium. Significant changes in gene expression were evident by microarray analysis at 2.0 h post-infection in macrophages infected with broth-grown and implant-grown bacteria; however, qPCR analysis revealed earlier up-regulation of JunB and TNF-α in macrophages exposed to the implant-grown bacteria. Up-regulation of those genes and others is consistent with the effects of extracellular products of aeromonad bacteria on macrophages and also suggests initiation of the innate immune response.

Interestingly, the vaccination against furunculosis in our study caused the decrease of oxidative modification of proteins in the brain tissue of vaccinated fish as compared with the control group. In our study, the activities of CAT, GR and GPx were significantly determined by alterations of oxidative stress biomarkers. CAT ($r=0.731$, $p=0.002$), GR ($r=0.790$, $p=0.001$), and GPx activity ($r=0.709$, $p=0.003$) correlated with aldehydic derivatives of OMP and TBARS level ($r=0.588$, $p=0.021$; $r=0.673$, $p=0.006$; $r=0.732$, $p=0.002$, respectively), while ketonic derivatives of OMP positively correlated with GR ($r=0.802$, $p=0.000$) and GPx activity ($r=0.715$, $p=0.003$) (Fig. 3). Correlative analysis confirmed that decrease of markers of protein damage and lipid peroxidation may cause to inhibition of CAT, GR and GPx activity (Fig. 3). The decrease in the antioxidant defenses observed

in the brain tissue could be due to the production of reactive oxygen species during vaccination.

Response of oxidative stress biomarkers in different tissues of fish is dependent of immune system activation and ROS generation due to respiratory burst in response to microbe recognition induced by vaccination. Paiva and Bozza [2014] described the mechanisms by which ROS directly kill microbes or interfere with the immune response, the role of ROS in pathogenic viral, bacterial, and protozoan infections. Phagocytes recognize microbes through many molecular patterns displayed by them and try to engulf them. Once a microbe is phagocytosed, the nature of the molecules recognized on microbe's surface dictates the treatment enacted within the phagosome. Respiratory burst, a process by which NADPH oxidase generates ROS in response to microbe recognition, is a possible outcome of this process and helps to get rid of many microbes. Once a pathogen is phagocytosed, it must subvert the respiratory burst, withstand its oxidative power, or escape the phagosome to survive. Microbe recognition sets the immune system in motion, and ROS are produced not only in the phagocyte respiratory burst but also in other cell compartments, such as mitochondria, as intermediaries in many signal transduction pathways, such as leukocyte pattern recognition receptor (PRR) signaling. The generation of ROS is a prerequisite to the formation of neutrophil extracellular traps (NETs); is actively involved in phagolysosomal formation and enzymatic degradation; autophagy; chemoattraction and inflammation; cell death of infection reservoirs; antigenic presentation, T-helper polarization, and lymphocyte proliferation; iron redistribution among tissues and cell compartment availability of iron. ROS are used by cells of the adaptive immune system as regulators of signal transduction by cell surface receptors [Williams, Kwon, 2004]. In our study, immunization against furunculosis was induced the decrease of lipid and protein oxidation in the brain tissue of rainbow trout (Fig. 1) in response, probably, to mobilization of antioxidant defense caused by immune system and respiratory burst activation.

Our results suggest that both the glutathione-mediated antioxidant defense system and endogenous CAT play a pivotal role in intracellular antioxidant defense against vaccinate-induced oxidative stress in fish. The impairment in the synthesis of enzymatic and nonenzymatic antioxidants of vaccinated fish may be the most important factor in reducing level of total antioxidant capacity (Fig. 2).

Conclusions.

The analysis of oxidative stress biomarkers has revealed a significant alterations between vaccinated fish against furunculosis compared to unhandled control. The brain tissue of vaccinated trout had lower level of aldehydic and ketonic derivatives of oxidatively modified proteins, as well as lipid peroxidation. The total antioxidant capacity become more susceptible to oxidative damage induced by vaccination against furunculosis. Glutathione-dependent enzymes and catalase activity were dependent to oxidative stress biomarkers in vaccinated trout. Correlations between TBARS level, carbonyl derivatives of protein damage, CAT, GR, and GPx activity confirmed the assumption that oxidative stress could activate the antioxidant defenses for improve adaptive mechanisms during immunization.

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

References

Bergh P.V., Burr S.E., Benedicenti O., von Siebenthal B., Frey J., Wahli T. 2013. Antigens of the type-three secretion system of *Aeromonas salmonicida* subsp. *salmonicida* prevent protective immunity in rainbow trout. Vaccine, 31(45): 5256–5261.

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.

Cipriano R., Austin B. 2011. Furunculosis and other Aeromonad Diseases // In Fish Diseases and Disorders. Vol. 3, Viral, Bacterial and Fungal Infections. Ed. by P.T.K. Woo and D.W.Bruno, 2011. P. 424–484.

Dubinina E.E., Burmistrov S.O., Khodov D.A., Porotov I.G. 1995. Oxidative modification of human serum proteins. A method of determining it // Vopr. Med. Khim., 41: 24–26 (In Russian).

Faktorovich K.A. 1969. Histological changes in the liver, kidneys, skin and brain of fish sick with red rot. Infectious Diseases of Fish and Their Control. Division of Fisheries Research, Bureau of Sport Fisheries

and Wildlife. Washington, DC. Translated from the Russian by R.M.Howland. Washington, DC, pp. 83–101.

Fuentes R.J.M., Perez H.J.A. 1998. Isolation of *Aeromonas hydrophila* in the rainbow trout (*Oncorhynchus mykiss*) // Veterinaria Mexico, 29: 117–119.

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).

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.

Grizzle J.M., Kiryu Y. 1993. Histopathology of gill, liver and pancreas and serum enzyme levels of channel catfish infected with *Aeromonas hydrophila* complex // J. of Aquatic Animal Health, 5: 36–50.

Håstein T., Gudding R., Evensen O. 2005. Bacterial vaccines for fish — an update of the current situation worldwide // Dev. Biol. (Basel), 121: 55–74.

Hazen T.C. 1979. Ecology of *Aeromonas hydrophila* in a South Carolina cooling reservoir // Microbial Ecology, 5: 179–195.

Kamyshnikov V.S. 2004. Reference book on clinic and biochemical researches and laboratory diagnostics, MEDpress-inform, Moscow, 463 p. (In Russian).

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).

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).

Levine R.L., Garland D., Oliver C.N., Amici A., Climent I., Lenz A. — G., Ahn B. — W., Shaltiel S., Stadtman E.R. 1990. Determination of carbonyl content in oxidatively modified proteins // Methods Enzymol., 186: 465–478.

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).

Ogara W.O., Mbuthia P.G., Kaburia H.F.A., Sorum H., Kagunya D.K., Nduthu D.I., Colquhoun D. 1998. Motile aeromonads associated with rainbow trout (*Oncorhynchus mykiss*) mortality in Kenya // Bulletin of the European Association of Fish Pathologists, 18: 7–9.

Paiva C.N., Bozza M.T. 2014. Are reactive oxygen species always detrimental to pathogens? // Antioxid. Redox Signal., 20(6): 1000–1037.

Pietarinen-Runtti P., Lakari E., Raivio K.O., Kinnula V.L. 2000. Expression of antioxidant enzymes in human inflammatory cells // Am.J.Physiol. Cell Physiol., 278(1): C118–125.

R mer Villumsen K., Dalsgaard I., Holten-Andersen L., Raida M.K. 2012. Potential role of specific antibodies as important vaccine induced protective mechanism against *Aeromonas salmonicida* in rainbow trout // PLoS One, 7(10): e46733.

Sommerset I., Krossøy B., Biering E., Frost P. 2005. Vaccines for fish in aquaculture // Expert. Rev. Vaccines, 4(1): 89–101.

Tkachenko G.M., Grudniewska J. 2015. Oxidative stress biomarkers in gills of brown trout (*Salmo trutta* m. *fario*) vaccinated against furunculosis // Scientific Medical Bulletin, 2(2): 83–89.

Tkachenko G.M., Grudniewska J., Kurhaluk N.N. 2015. Effects of vaccination against furunculosis on oxidative stress biomarkers and antioxidant defense in gills of rainbow trout (*Oncorhynchus mykiss*) // Proceedings of KSTU, 36: 1–12.

Vanya Ewart K., Williams J., Richards R.C., Gallant J.W., Melville K., Douglas S.E. 2008. The early response of Atlantic salmon (*Salmo salar*) macrophages exposed *in vitro* to *Aeromonas salmonicida* cultured in broth and in fish // Dev. Comp. Immunol., 32(4): 380–390.

Villumsen K.R., Koppang E.O., Raida M.K. 2015. Adverse and long-term protective effects following oil-adjuvanted vaccination against *Aeromonas salmonicida* in rainbow trout // Fish Shellfish Immunol., 42(1): 193–203.

Villumsen K.R., Raida M.K. 2013. Long-lasting protection induced by bath vaccination against *Aeromonas salmonicida* subsp. *salmonicida* in rainbow trout // Fish Shellfish Immunol., 35(5): 1649–1653.

Williams M.S., Kwon J. 2004. T cell receptor stimulation, reactive oxygen species, and cell signaling // Free Radic. Biol. Med., 37(8): 1144–1151.

Zar J.H. 1999. Biostatistical Analysis. 4th ed., Prentice-Hall Inc., Englewood Cliffs, New Jersey, 663 p.

Поступила в редакцию 16.04.16 *г.*
Принята после рецензии 25.07.16 *г.*

Содержание биомаркеров окислительного стресса в мозговой ткани радужной форели (*Oncorhynchus mykiss*) вакцинированной против фурункулеза

Ткаченко Г.М.[1], Грудневская Й.[2]

[1] Кафедра зоологии и физиологии животных, Институт биологии и охраны среды, Поморская Академия в Слупске, Польша

[2] Отдел исследований лососевых рыб, Институт пресноводного рыбного хозяйства им. С.Саковича, Польша

Использование вакцин для профилактики заболеваний в области аквакультуры расширилось как в отношении количества видов рыб, так и количества микробных заболеваний. Несмотря на важность и успешность вакцинации, мало известно о механизмах окислительного стресса и антиоксидантной защиты у рыб во время иммунизации. В настоящем исследовании мы определили содержание биомаркеров окислительного стресса и антиоксидантной защиты в мозговой ткани радужной форели (*Oncorhynchus mykiss*) вакцинированной против фурункулеза. Анализ содержания биомаркеров окислительного стресса показал значительные изменения между вакцинированными против фурункулеза и контрольной группами рыб. Выявлено более низкий уровень альдегидных и кетоновых производных окислительно модифицированных белков, а также процессов перекисного окисления липидов в мозговой ткани вакцинированной форели. Общая антиоксидантная активность была более чувствительной к окислительному повреждению, вызванному вакцинацией против фурункулеза. Активность глутатион-зависимых ферментов и каталазы коррелировали с содержанием маркеров окислительного стресса у вакцинированной форели. Корреляционный анализ подтвердил предположение, что окислительный стресс может активировать антиоксидантную систему для улучшения адаптационных механизмов во время иммунизации. Понимание роли биохимических изменений в тканях вакцинированных рыб имеет важное значение для понимания сложных физиологических изменений, которые происходят во время иммунизации, а также для улучшения методов аквакультуры для максимального роста тканей привитых рыб.

Ключевые слова: фурункулез, радужная форель *Oncorhynchus mykiss*, перекисное окисление липидов, окислительно модифицированные белки, антиоксидантная защита, общая антиокислительная активность