BIOMARKERS OF LIPID AND PROTEIN OXIDATION IN DIFFERENT TISSUES OF FURUNCULOSIS-AFFECTED SEA TROUT (*SALMO TRUTTA* M. *TRUTTA* L.) COLLECTED FROM THE BALTIC SEA

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Furunculosis, caused by the bacterium *Aeromonas salmonicida* and *A. hydrophila*, is an important infectious disease affecting several salmonid species, including sea trout (*Salmo trutta* m. *trutta* L.), in aquaculture and wild populations worldwide (O'Brien et al., 1994; Hill, 1996; Cipriano and Austin, 2011). The severity of disease is influenced by a number of interrelated factors, including bacterial virulence, the type and level of stress applied to a fish population, the physiological state of the host, and the level of genetic resistance present in specific populations. Motile aeromonads differ interspecifically and intraspecifically in their relative pathogenicity or ability to cause disease. Pathological conditions attributed to members of the motile aeromonad complex may include dermal ulceration, tail or fin rot, ocular ulceration, erythrodermatitis, haemorrhagic rot disease and scale protrusion disease (Cipriano and Austin, 2011; Menanteau-Ledouble et al., 2016). This disease poses significant economic and environmental challenges due to its impact on fish health, survival and productivity. While efforts have been made to control furunculosis through vaccination and antibiotic treatment, the disease remains a persistent threat, particularly in regions such as the Baltic Sea.

One of the key aspects of furunculosis pathogenesis is the induction of oxidative stress in the host organism, leading to the oxidation of lipids and proteins in various tissues (Tkachenko et al., 2014). Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and antioxidant defence mechanisms, resulting in cellular damage and dysfunction. Understanding the oxidative stress response in furunculosis-affected sea trout is essential to elucidate the mechanisms of disease progression and to develop effective therapeutic interventions (Juan et al., 2021). In recent years, biomarkers of lipid and protein oxidation have emerged as valuable tools for assessing oxidative stress and its consequences in aquatic organisms (Valavanidis et al., 2006; Regoli and Giuliani, 2014). These biomarkers, which include lipid peroxidation products such as malonic dialdehyde (MDA) and protein carbonyls, provide quantitative measures of oxidative damage in different tissues and offer insights into the severity and progression of oxidative stress-related diseases (Margaritelis et al., 2016; Dennis et al., 2019).

Despite the importance of oxidative stress in the pathogenesis of furunculosis, there is limited research investigating biomarkers of lipid and protein oxidation in sea trout affected by the disease, particularly in the Baltic Sea region. Therefore, this study aims to fill this knowledge gap by investigating the levels of MDA and protein carbonyls in different tissues of furunculosis-affected sea trout collected from the Baltic Sea. The aim of the current study was to investigate the responses of oxidative stress biomarkers in different tissues (muscle, gills, liver, heart, milt/spawn) of healthy sea trout (*Salmo trutta* m. *trutta* L.) and naturally furunculosis-affected trout sampled from the Słupia River, part of the Baltic Sea basin where adult trout spawn (northern Poland, Central Pomeranian region). Biomarkers of oxidative stress [2-thiobarbituric acid reactive substances (TBARS) as lipid peroxidation biomarkers, aldehydic and ketonic derivatives of oxidatively modified proteins (OMP) and total antioxidant capacity (TAC)] were measured in different tissues of healthy and furunculosis-affected trout.

Adult sea trout (*Salmo trutta* m. *trutta* L.), 3-5 years old, were collected from sites in the Słupia River (Słupsk, northern Poland). Fish were caught in close cooperation with the Słupia Valley Landscape Park and the Polish Angling Association in Słupsk. Samples for analysis from healthy males and females (control group) and females of sea trout affected by furunculosis (study group) were taken immediately after fishing. Microbiological tests were carried out after capture.

These tests indicated that the *Aeromonas hydrophila* complex caused furunculosis. The pathogen was isolated from the infected sea trout. Samples from each group were prepared. One fish was used per preparation. Each sample was homogenised in cold Tris-HCl buffer (100 mM, pH 7.2) to obtain a 10 % (w/v) tissue homogenate. The protein content of each sample was determined by the Bradford method (1976) using bovine serum albumin as the standard.

The level of lipid peroxidation was determined by quantifying the concentration of 2-thiobarbituric acid reactive substances (TBARS) according to Kamyshnikov (2004). The rate of protein oxidative damage was estimated from the reaction of the resulting carbonyl derivatives of the amino acid reaction with 2,4-dinitrophenylhydrazine (DNPH) as described by Levine et al. (1990) and modified by Dubinina et al. (1995). DNPH was used to determine the carbonyl content of soluble and insoluble proteins. The TAC content in the sample was estimated spectrophotometrically at 532 nm according to the Tween 80 oxidation method (Galaktionova et al., 1998).

The mean ± S.E.M. values were calculated for each group to determine the significance of the differences between the groups. The Kruskal-Wallis one-way analysis of variance with ranks test was used to assess the differences between the groups studied (significance level, $p < 0.05$). Correlations between parameters at the set significance level ($p < 0.05$) were determined by the regression method. Interactions were determined by Spearman's rank (Zar, 1999). All statistical calculations were performed on separate data from each individual using STATISTICA version 13.3 (TIBCO Inc., USA).

An imbalance between the production of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, hypochlorous acid, hydroxyl, alkoxyl and peroxyl radicals, and the antioxidant defence against them produces oxidative stress, which increases tissue damage by releasing pro-oxidant forms of reactive iron, which can drive Fenton chemistry and lipid peroxidation, and by depleting protective sacrificial antioxidants (Gutteridge, 1995). TBARS levels were significantly higher in muscle tissue (by 8.87 %, $p = 0.001$), gills (by 37.72 %, $p = 0.01$) and liver (by 139.15 %, $p = 0.000$) of males with furunculosis compared to controls (healthy samples). A decreased TBARS level in milt to (282.2 ± 41.37) nmol/mg protein was found in males with furunculosis compared to (756.31 ± 85.67) nmol/mg protein in healthy trout. Decreased TBARS levels in gills (by 45.5 %, $p = 0.005$) and increased TBARS levels in spawn (by 179 %, $p = 0.031$) were found in infected females compared to healthy females.

Oxidative modification of proteins by reactive species has been implicated in the aetiology or progression of a variety of disorders and diseases (Levine, 2002). Furunculosis induces an increase in aldehydic derivatives of OMP in muscle tissue (by 60.45 %, $p = 0.005$), liver (by 54.82 %, $p = 0.002$) and heart (by 99.4 %, $p = 0.040$) in infected males and their increase in muscle tissue (by 126 %, $p = 0.000$), liver (by 59.4%, $p = 0.000$) and heart (by 65.43 %, $p = 0.000$) in infected females compared to healthy samples. In addition, significantly higher levels of aldehydic derivatives of OMP were found in gills (by 93.8 %, $p = 0.006$) and lower levels in milt (by 67.6 %, $p = 0.040$) of infected males compared to females. The ketonic derivatives of protein oxidation in muscle, gills and liver of males with furunculosis were significantly higher by 62.67 % ($p = 0.000$), 90.1 % ($p = 0.005$) and 48.05 % ($p = 0.000$), respectively, than in healthy males. Similar increases in ketonic derivatives of protein oxidation were found in muscle (by 78.4 %, $p = 0.000$), liver (by 24.6 %, $p = 0.002$) and heart (by 48.7 %, $p = 0.001$) of infected females compared to healthy females. A significantly higher level of aldehydic derivatives of OMP was found in the liver (by 24.5 %, $p = 0.002$) of furunculosis-affected males compared to females. A significantly higher level of ketonic derivatives (by 14.5 %, $p = 0.007$) was found in the heart tissue of infected females compared to males. With regard to total antioxidant capacity, furunculosis significantly decreased TAC levels by 30 % ($p = 0.001$) in the liver, by 47 % ($p = 0.000$) in the heart and by 39 % ($p = 0.006$) in the milt of furunculosis-affected males and by 20 % ($p = 0.040$) in the liver of furunculosis-affected females compared to healthy samples. The TAC level was significantly higher (by 64 %, $p = 0.006$) in the heart of the furunculosis-affected females compared to the males.

Thus, in both males and females, biomarkers of lipid peroxidation and protein damage in the different tissues of furunculosis-affected trout showed higher values compared to healthy trout. Increased lipid peroxidation was observed in muscle tissue, gills, liver tissue and milt of furunculosis-affected males. Aldehydic and ketonic derivatives of oxidatively modified proteins were higher in muscle, heart and liver tissues, milt and spawn of furunculosis-affected males and females. Total antioxidant capacity was decreased, especially in liver and heart tissues of furunculosis-affected males and females. This study encourages efforts to increase the knowledge of oxidative stress biomarkers for the identification of *Aeromonas*-induced disorders and specific fish responses typical of furunculosis in salmonids.

In conclusion, this study provides valuable insights into the oxidative stress response in furunculosis-affected sea trout collected from the Baltic Sea and sheds light on the systemic effects of the disease on lipid and protein oxidation in different tissues. By quantifying biomarkers such as TBARS and protein carbonyls, we have demonstrated the presence of oxidative damage in furunculosis-affected sea trout, highlighting the role of oxidative stress in the pathophysiology of the disease. Our findings reveal tissue-specific variations in the levels of lipid and protein oxidation, suggesting differential susceptibility to oxidative damage in different organs. The observed changes in oxidative stress biomarkers provide evidence for the systemic effects of furunculosis on sea trout physiology, with implications for overall health and disease progression.

The identification of biomarkers of lipid and protein oxidation in furunculosis-affected sea trout represents an important step towards understanding the mechanisms underlying disease pathogenesis and progression in this species. By elucidating the oxidative stress response in sea trout exposed to furunculosis, our study contributes to a broader understanding of host-pathogen interactions and immune responses in aquatic organisms. Furthermore, our findings have implications for the development of targeted therapeutic interventions and biomonitoring strategies for the management of furunculosis in both aquaculture and wild sea trout populations. By identifying tissue-specific biomarkers of oxidative stress, we provide potential targets for therapeutic intervention to mitigate the detrimental effects of furunculosis on the health and survival of sea trout.

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МОДЕЛЮВАННЯ ПРОЦЕСІВ ОЧИЩЕННЯ ВОДИ АКВАКУЛЬТУРНИХ ПІДПРИЄМСТВ ВІД РОЗЧИННИХ ФОРМ НІТРОГЕНУ З ВИКОРИСТАННЯМ БАЗАЛЬТОВИХ ТУФІВ

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Нітроген в рибоводних системах представлений у формі аміаку (NH_3), іонів амонію (NH_4^+), нітритів (NO^{2-}) та нітратів (NO^{3-}). Їх накопичення у водному середовищі зумовлено життєдіяльністю самих об'єктів аквакультури, оскільки основним продуктом білкового метаболізму у риб є амоній. Окрім того, вирощування риби за інтенсивних технологій відбувається при використанні кормів з високим вмістом протеїнів, які є додатковими джерелами нітрогену у водному середовищі. Отже, розробка методів ефективного вилучення розчинних форм нітрогену з води рециркуляційних рибоводних систем є нагальною проблемою промислової аквакультури.

Перспективним методом очистки води є використання адсорбційних матеріалів, які здатні активно поглинати розчинені форми азоту з води. Прикладом таких адсорбентів є