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ORAL VACCINATION AGAINST *YERSINIA RUCKERI*: BIOMARKERS OF PROTEIN OXIDATION IN THE HEPATIC TISSUE OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WALBAUM)

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The aim of this study was to evaluate the one-month effect of oral vaccination against Yersinia ruckeri based on oxidative stress biomarkers in the hepatic tissue of rainbow trout (Oncorhynchus mykiss Walbaum). The vaccine consisted of three strains of Y. ruckeri (serotype O1) obtained from rainbow trout from different farms where fish showed clinical signs of enteric redmouth disease. The vaccine was produced at the Department of Fish Diseases, National Veterinary Research Institute in Pulawy (Poland) according to the process covered by patent no. P.428259. The concentrated vaccine was added to the fish feed. Treatment was administered three times at one-day intervals. Livers were sampled one month after immunisation. Our results show that immunisation with the anti-Yersinia vaccine did not alter the liver tissue of rainbow trout. aldehydic and ketonic derivatives of oxidatively modified proteins (OMP) were not significantly altered in the hepatic tissue of vaccinated fish prior to immunisation. One month after immunisation, the levels of ketonic derivatives of OMP in the hepatic tissue of untreated trout were reduced compared to the untreated group before immunisation. Similar to the untreated groups, the levels of ketonic derivatives of OMP in the hepatic tissue of the vaccinated group one month after immunisation were reduced compared to the vaccinated group before immunisation. A statistically non-significant decrease in the total antioxidant capacity (TAC) levels was observed between the values obtained in the untreated and vaccinated groups before vaccination and one month after vaccination. Understanding the role of oxidative stress in the tissues of vaccinated trout has important implications for understanding the complex physiological changes that occur during vaccination, and also for improving aquaculture practices to maximise tissue growth and health of vaccinated trout.

Key words: *rainbow trout Oncorhynchus mykiss, Yersinia ruckeri, immunization, oxidative stress, carbonyl derivatives, liver.*

ПЕРОРАЛЬНА ВАКЦИНАЦІЯ ЩОДО *YERSINIA RUCKERI*: БІОМАРКЕРИ ОКИСНЕННЯ БІЛКІВ У ТКАНИНІ ПЕЧІНКИ РАЙДУЖНОЇ ФОРЕЛІ (*ONCORHYNCHUS MYKISS* WALBAUM)

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Метою даного дослідження була оцінка одномісячного ефекту пероральної вакцинації щодо Yersinia ruckeri на основі біомаркерів окиснювального стресу в тканині печінки райдужної форелі (Oncorhynchus mykiss Walbaum). Вакцина складалася з трьох штамів Y. ruckeri (серотип O1), отриманих від райдужної форелі з різних ферм, де у риб спостерігали клінічні ознаки кишкової хвороби. Вакцину було виготовлено у Відділі хвороб риб Національного ветеринарного науково-дослідного інституту в Пулавах (Польща) згідно технології, описаної в патенті P.428259. Концентровану вакцину додавали в корм для риб. Годування цим препаратом проводилося тричі з інтервалом в один день. Обробку проводили тричі з інтервалом в один день. Зразки печінки брали через місяць після імунізації. Наші результати показують, що імунізація вакциною проти ієрсиніозу не спричинила змін в тканині печінки райдужної форелі. Вміст альдегідних та кетонових похідних окиснювально модифікованих білків (ОМБ) був істотно не змінений в тканині печінки вакцинованих риб до імунізації. Через місяць після імунізації рівні кетонових похідних ОМБ у печінковій тканині форелі контрольної групи були знижені порівняно з контрольною групою до імунізації. Подібно до контрольних груп, рівні кетонових похідних ОМБ у тканині печінки вакцинованої групи через місяць після імунізації були знижені порівняно з вакцинованою групою до імунізації. Статистично неістотне зниження рівнів загальної антиоксидантної активності (ЗАА) спостерігалося між значеннями, отриманими в контрольних і вакцинованих групах до вакцинації та через місяць після вакцинації. Розуміння ролі окиснювального стресу в тканинах вакцинованої форелі має важливе значення для розуміння складних фізіологічних змін, які відбуваються під час вакцинації, а також для вдосконалення практики аквакультури для максимального росту тканин і збереження здоров'я вакцинованої форелі.

Ключові слова: *райдужна форель Oncorhynchus mykiss, Yersinia ruckeri, імунізація, окиснювальний стрес, карбонільні похідні, печінка*

Introduction. *Yersinia ruckeri,* a Gram-negative strain of the family *Enterobacteriaceae,* is the causative agent of enteric redmouth disease (ERM) and yersiniosis in salmonids (Tobback E. et al., 2007). It is now a ubiquitous pathogen that has been isolated from fish populations around the world, as well as from other taxa and environmental samples. *Y. ruckeri* is known to cause disease in several farmed and wild species, including other salmonid species such as Atlantic salmon (*Salmo salar* L.) (Ross A. J. et al., 1966; Wheeler R. W. et al., 2009; Bastardo A. et al., 2012; Ghosh B. et al., 2016). It is now a ubiquitous pathogen that has been isolated from fish populations around the world, as well as from other taxa and environmental samples (Kumar G. et al., 2015; Ghosh B. et al., 2016). It can be transmitted vertically from parent

to offspring and horizontally in the water column from both clinically infected fish and asymptomatic carriers, and is therefore capable of infecting fish at early stages of development (Ghosh B. et al., 2016).

The disease is so named because of the subcutaneous bleeding that can occur at the corners of the mouth, in the gums, and on the tongue. Other clinical signs include exophthalmia, darkening of the skin, splenomegaly, and inflammation of the lower intestine with accumulation of thick yellow fluid. The bacterium enters the fish through the secondary gill lamellae, from where it spreads to the blood and internal organs (Kumar G. et al., 2015). Infected fish and asymptomatic carriers are the main sources of infection, spreading bacteria in their faeces. The gills are thought to be the entry point for *Y. ruckeri* rods, but the likelihood of disease depends on the virulence of the particular strain. Characteristic clinical signs of yersiniosis, such as bleeding around the mouth, are caused by extracellular products (ECPs) of *Y. ruckeri* (Pękala A. & Antychowicz J., 2010).

Vaccination plays an important role in the protection of salmonids against the bacterial pathogen *Yersinia ruckeri* (Ormsby M. J. et al., 2016). ERM has been successfully controlled for several decades using bacterin vaccines administered by immersion (Welch T. J. & LaPatra S., 2016). The immune mechanisms responsible for protection may include both cellular and humoral elements (Raida M. K. et al., 2011). Oral administration is the "ideal method" for delivering vaccines to fish, as the vaccine is incorporated into the fish feed. It is less labour intensive than injection and immersion and is suitable for vaccinating large numbers of fish of all sizes. It avoids the handling stress experienced by the fish in the other two methods. The main disadvantage of this route of administration is that lower levels of protection are achieved and the duration of protection induced is shorter (Thompson K.D. & Adams A., 2004).

The study by Fajardo C. and co-workers (2022) aimed to evaluate the short-term innate immune response of rainbow trout to *Y. ruckeri* infection. A number of factors related to the innate immune response were evaluated, including the determination of haematological parameters, oxidative stress biomarkers and analysis of the expression of immune-related genes. The results showed a significant decrease in several haematological parameters (white blood cell count, haematocrit, neutrophils, monocytes, lymphocytes and platelets) and oxidative stress indicators (SOD) between the control and infected groups. There were also significant differences in gene expression levels between the infected and control groups. Most of these genes (*il-1β, il-8, il-10, tnf-α1, tnf-α2, socs3, mmp-9, cath, hsp-70, saa, fer, pcb*) were upregulated within the first 24 h after infection (Fajardo C. et al., 2022).

Several factors can increase oxidative stress (e.g. intensive production, heat stress, polyunsaturated fatty acids, and impaired fat quality), uncontrolled inflammatory responses (e.g. high n-6 to n-3 ratio in cell membranes), and impaired immune development (e.g. micronutrient deficiency) (Lauridsen C., 2019). Free radical-induced oxidation plays a crucial role in normal physiology and biochemistry. It is also a universal non-specific link in the physiological mechanisms of the development of various pathological syndromes (Dröge W., 2002; Schieber M. & Chandel N. S., 2014). Increased oxidative stress leads to changes in proteins. One possibility for oxidatively modified proteins is the formation of protein aggregates due to the appearance of intermolecular bonds, more often disulfide bonds. The second possibility is the fragmentation of proteins into low-molecular-weight substances. In both cases, proteins become more susceptible to proteolytic degradation and conformational rearrangement. This process produces ketonic dinitrophenylhydrazones, which are markers of aggregation of protein molecules and a more pronounced stage of oxidative stress, and aldehydic dinitro-

phenylhydrazones, which are markers of fragmentation and milder stages of oxidative stress development (Stadtman E. R., 2001).

Therefore, it would be valuable to investigate the effects of vaccination against *Y. ruckeri* on the health status of trout in general and on the levels of oxidative stress biomarkers in different tissues. The present study aims to elucidate the effects of vaccination against *Y. ruckeri* on liver function and the oxidative mechanism underlying these effects by determining relevant biomarkers of protein oxidation and total antioxidant capacity (TAC) one month after oral vaccination against *Y. ruckeri*.

Materials and methods.

Experimental animals. Rainbow trout (*Oncorhynchus mykiss* Walbaum) weighing 105-135 g were used in the experiments. The study was conducted at the Department of Salmonid Research, Stanislaw Sakowicz Inland Fisheries Institute in Olsztyn (Poland). The experiments were conducted at a water temperature of 14.5 ± 0.5 °C and a pH of 7.5. Dissolved oxygen levels were approximately 12 ppm, with supplemental oxygen provided by a water flow of 25 litres per minute and a photoperiod of 12 hours per day. Fish were fed a commercial pelleted diet at optimum levels using 12-hour fish belt feeders. Daily dietary allowances were calculated in accordance with current dietary guidelines. All biochemical assays were performed at the Department of Zoology and Department of Animal Physiology, Institute of Biology, Pomeranian University in Słupsk (Poland).

Experimental design. The fish were divided into two groups: I) untreated control and II) vaccinated against *Y. ruckeri*. The fish were kept in 1000 L square tanks (150 fish per tank) under the same environmental conditions. The vaccine was produced in the Department of Fish Diseases, National Veterinary Research Institute in Pulawy (Poland) according to the procedure covered by patent no. P.428259. The prepared vaccine at a concentration of $1 \cdot 10^9$ cells per ml was used for inoculation of fish *per os*. The vaccine concentrate was added to the fish feed and administered three times at one-day intervals.

Fish were maintained at 14.5 ± 0.5°C and pH 7.5 for 30 days after vaccination. In the current study, 15 rainbow trout from the untreated control and 15 vaccinated trout were used before vaccination and one month after vaccination. Liver samples were taken from the rainbow trout one month after vaccination.

Sampling. Animals were captured and decapitated 31 days after vaccination. The liver was excised in situ. The organs were perfused with cold isolation buffer and homogenised using a glass H500 homogeniser with a motor-driven pestle immersed in an ice-water bath to obtain a 1:9 (weight/volume) homogenate. The isolation buffer contained 100 mM Tris-HCl; the pH was adjusted to 7.2 with HCl. The homogenates were centrifuged at 3,000 rpm for 15 min at 4°C. After centrifugation, the supernatant was collected and frozen at -25°C until analysis. Protein content was determined by the method of Bradford M. (1976) using bovine serum albumin as standard (Bradford M. M., 1976). Absorbance was recorded at 595 nm. All assays were performed in duplicate at 22 ± 0.5°C. Biochemical reactions were initiated by the addition of tissue supernatant. The specific assay conditions were as follows.

Assay for carbonyl groups of oxidatively modified proteins. Carbonyl groups were measured as an indication of oxidative damage to proteins according to the method of Levine R. L. and co-workers (1990) as modified by Dubinina E. E. and co-workers (1995). Samples were incubated with 10 mM 2,4-dinitrophenylhydrazine (DNTP) in 2 M HCl for 1 h at room temperature. Blanks were run without DNTP. The proteins were then precipitated with TCA and centrifuged at 3,000 g for 20 min. The protein pellet was washed three times with ethanol : ethyl acetate (1:1) and incubated at 37 °C until

complete resuspension. Carbonyl content was measured spectrophotometrically at 370 nm (aldehydic derivatives, OMP_{370}) and 430 nm (ketonic derivatives, OMP_{430}) (molar extinction coefficient 22,000 $M^{-1} \cdot cm^{-1}$) and expressed as nmol per mg of protein (Levine R. L. et al., 1990; Dubinina E. E. et al., 1995).

Total antioxidant capacity (TAC) assay. TAC was estimated spectrophotometrically at 532 nm according to the Tween 80 oxidation method (Galaktionova L. P. et al., 1998). TAC levels were expressed as %.

Statistical analysis. Results are expressed as mean ± S.D. Statistical analysis was performed using the STATISTICA 13.3 package (TIBCO Software Inc., USA). Significant differences between means were measured using a multiple-range test at a minimum of $p < 0.05$. Non-normally distributed data were log-transformed. Statistical tests with 95% confidence intervals ($\alpha = 0.05$) were used to determine the significance of differences between the parameters studied (Stanisz A., 2006, 2007). Data were tested for homogeneity of variance using the Levene test and for normality using the Kolmogorov-Smirnov test. For data with a normal distribution, a t-Student test was used for paired comparisons. An ANOVA test was used for comparative analysis between the values obtained in the untreated and vaccinated groups in the first month after vaccination.

Research results. The levels of aldehydic and ketonic derivatives of oxidatively modified proteins in the liver tissue of trout treated orally with the *Y. ruckeri* vaccine during the first month after immunisation are shown in Figure 1.

An increase in the levels of aldehydic derivatives of OMP (by 12.1%, $p > 0.05$) in the hepatic tissue of vaccinated trout compared to untreated trout was observed before immunisation, while a decrease was observed one month after immunisation (by 24%, $p > 0.05$) compared to untreated trout. An increase in the aldehydic derivatives of OMP in the hepatic tissue of the untreated group was observed one month after immunisation (by 22.4%, $p < 0.05$) compared to the group before immunisation. On the other hand, a decrease of aldehydic derivatives of OMP was observed in the liver tissue of the vaccinated group one month after immunisation compared to the values obtained in the liver tissue of the vaccinated group before immunisation (by 17%, $p < 0.05$) (Fig. 1A).

One month after immunisation, the levels of ketonic derivatives of OMP in the hepatic tissue of untreated trout were reduced (by 27.6%, $p < 0.05$) compared to the untreated group before immunisation. Similar to the untreated groups, the levels of ketonic derivatives of OMP in the hepatic tissue of the vaccinated group one month after immunisation were reduced (by 52%, $p < 0.05$) compared to the vaccinated group before immunisation. The decrease in ketonic derivatives of OMP in the hepatic tissue of the vaccinated group was observed before vaccination (by 2%, $p > 0.05$) and one month after vaccination (by 34.9%, $p < 0.05$) compared to the untreated groups (Fig. 2B).

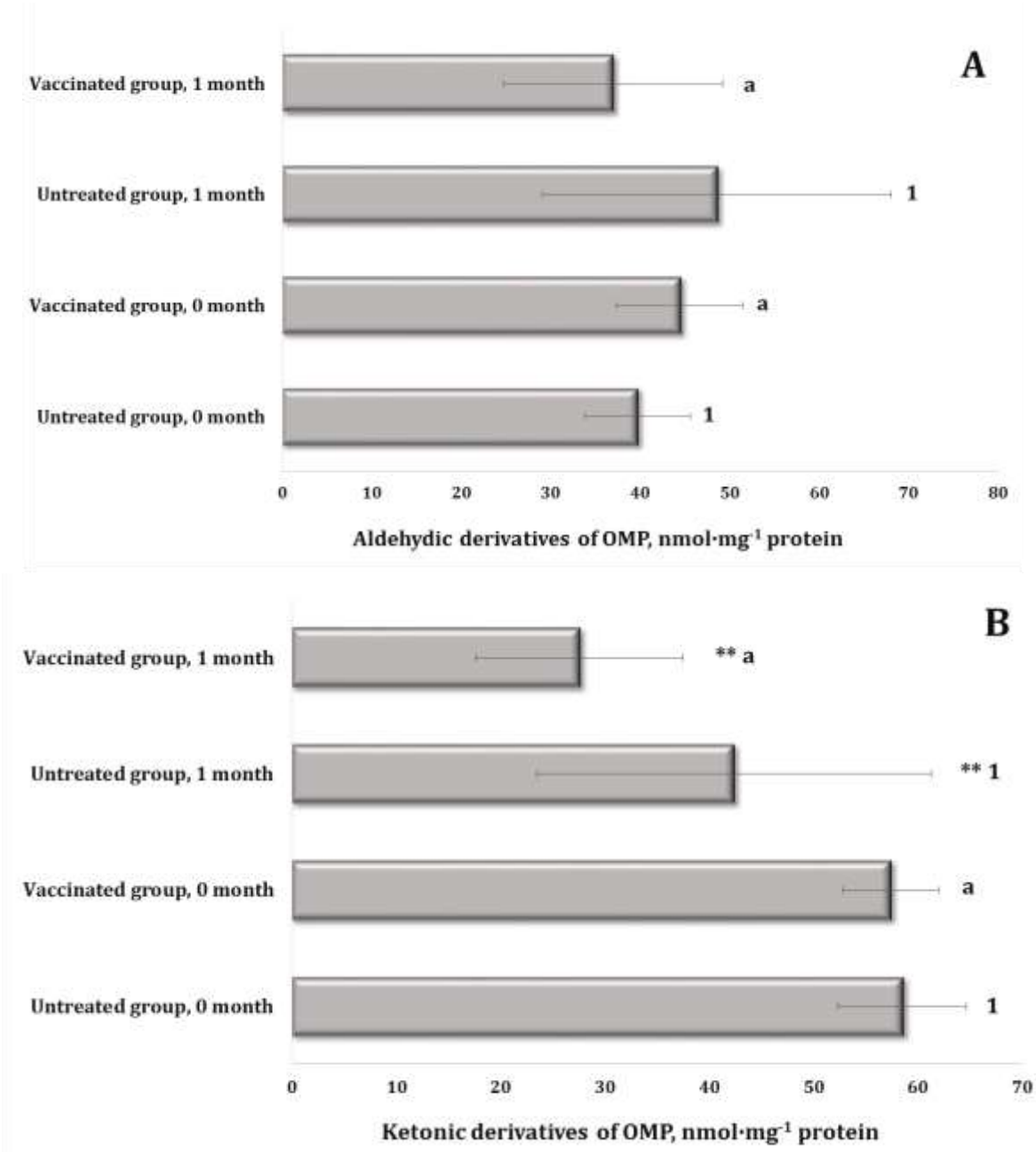

Fig. 1. **Levels of aldehydic and ketonic derivatives of oxidatively modified proteins (nmol·mg^{-1} protein) in the hepatic tissue of trout treated orally with the *Y. ruckeri* vaccine one month after immunisation. Data are expressed as mean ± S.D. (n = 15).**

^{1} – statistically significant changes ($p < 0.05$) between values obtained in the control group, 0 months vs. 1 month;

^{a} – statistically significant changes ($p < 0.05$) between values obtained in the vaccinated group, 0 months vs. 1 month;

*** – statistically significant changes ($p < 0.05$) between values obtained in the control group vs. vaccinated group 1 month after vaccination.*

TAC levels (%) in hepatic tissue of trout treated with *Y. ruckeri* vaccine one month after immunisation are shown in Figure 2.

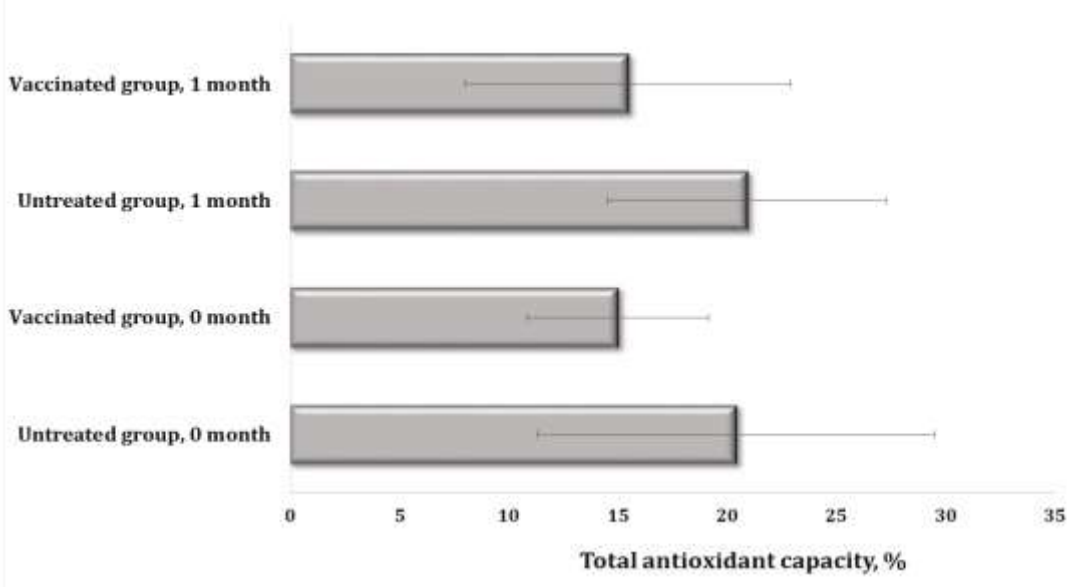

Fig. 2. **TAC levels (%) in hepatic tissue of trout treated with the *Y. ruckeri* vaccine at one month after immunisation.**

Data are presented as mean ± S.D. (n = 15).

One month after vaccination (20.91 ± 6.37 %), the same value of TAC was obtained in the untreated group as before vaccination (20.40 ± 9.08 %). Similar to the untreated group, the vaccinated groups achieved the same level of TAC after one month (15.45 ± 7.44%) as before vaccination (15.0 ± 4.17%). A statistically non-significant decrease in TAC level was observed between the values obtained in the untreated and vaccinated groups before vaccination (by 26.5%, $p > 0.05$) and one month after vaccination (by 26.1%, $p > 0.05$) (Fig. 2).

Discussion. The current study aimed to elucidate the effects of vaccination against *Y. ruckeri* on liver function and the oxidative mechanism underlying these effects by determining relevant biomarkers of protein oxidation and total antioxidant capacity (TAC) one month after oral vaccination against *Y. ruckeri*. In this study, our results clearly demonstrate that immunisation with the anti-Yersinia vaccine did not alter the hepatic tissue of rainbow trout. Aldehydic and ketonic derivatives of OMP were not significantly altered ($p > 0.05$) in the hepatic tissue of vaccinated fish before immunization. One month after immunisation, the levels of ketonic derivatives of OMP in the hepatic tissue of untreated trout were reduced (by 27.6%, $p < 0.05$) compared to the untreated group before immunisation. Similar to the untreated groups, the levels of ketonic derivatives of OMP in the hepatic tissue of the vaccinated group one month after immunisation were reduced (by 52%, $p < 0.05$) compared to the vaccinated group before immunisation (Fig. 1). A statistically non-significant decrease in TAC level was observed between the values obtained in the untreated and vaccinated groups before vaccination (by 26.5%, $p > 0.05$) and one month after vaccination (by 26.1%, $p > 0.05$) (Fig. 2).

In our previous study, we investigated the influence of vaccination against enteric redmouth disease on oxidative stress biomarkers and antioxidant defences in muscle and gill tissue of rainbow trout vaccinated against *Y. ruckeri* in the first and second month after vaccination (Tkachenko et al., 2016a, b, 2022). No significant difference in lipid peroxidation levels was observed in either the first or second month after vaccination, while aldehydic and ketonic derivatives of OMB were significantly lower in the vaccinated group in the second month compared to the first month after vaccination

($p < 0.05$). The content of ketonic derivatives of OMB in the muscles was higher in the first month after vaccination than in the untreated group. All this led to a decrease in glutathione peroxidase (GPx) activity and a low level of TAC. Correlations between catalase activity and lipid peroxidation and TAC confirmed the key role of catalase in the antioxidant defence during immunisation. From a broader perspective, it is suggested that immunisation of fish with the *Yersinia* vaccine is associated with induced free radical formation and oxidative stress. Free radicals would therefore be responsible, at least in part, for the induction of both humoral and cellular elements of immunity and increased protective immunity against *Y. ruckeri* infection (Tkachenko et al., 2016a, b).

In addition, a statistically significant reduction in lipid peroxidation between the mean values in the immunised groups at the first and second month after vaccination indicated effective adaptive antioxidant defence mechanisms in the fish for immunity against *Y. ruckeri*. A similar reduction in lipid peroxidation was observed between the mean values in the control group of fish after the first and second months of the study. The reduction in aldehydic and ketonic derivatives of oxidatively modified proteins in the liver of vaccinated trout two months after immunisation was caused by a high antioxidant capacity of the liver. Activation of proteolytic degradation of modified amino acid residues may be one reason for the reduction of oxidatively modified derivatives resulting from adaptation to immunisation. A high level of total antioxidant capacity in the liver of individuals from the control and immunised groups in the second month after vaccination indicated the powerful adaptability of the liver to help defend against oxidative stress induced by immunisation. The increased aspartate aminotransferase activity in the liver of individuals from the control and immunised groups in the second month was noted. Activation of aminotransferases indicates the metabolic conversion of proteins and carbohydrates. The significant decrease in lactate concentration and lactate dehydrogenase activity in liver tissue reflects the dynamic changes in aerobic-anaerobic and aerobic metabolism as well as total energy supply. A significant decrease in the level of lactate in liver tissue in the second month of the study in both the control and immunised groups indicates the proper functioning of the mechanisms of metabolic activity in the long-term effects of vaccination. The decrease in the levels of pyruvate and lactate in liver tissue in both the control and immunised groups in the second month after vaccination confirms the high adaptive capacity of the liver in compensating for the metabolic changes that occur as a result of immunisation. The correlative dependence between the levels of oxidative stress markers and metabolites in the liver of rainbow trout vaccinated against *Y. ruckeri* in the first and second month after vaccination confirms the important role of metabolites and energy conversion enzymes in the liver as a response to oxidative stress induced by immunisation against *Y. ruckeri*. Our results confirm that the vaccine against *Y. ruckeri* has no adverse effect on the condition and metabolism in the liver of fish. The metabolic changes recorded in our study are evidence that the vaccine against *Y. ruckeri* has no negative effects (Tkachenko et al., 2015).

To determine the effects of vaccination against *Y. ruckeri* on the health status of rainbow trout in general and on oxidative stress biomarkers and metabolic parameters in particular, and to identify mechanisms underlying the susceptibility of fish to vaccination, we compared liver and heart function and the oxidative mechanism underlying these effects by detecting relevant biomarkers of lipid peroxidation and protein oxidation as well as aerobic-anaerobic metabolism in trout immunised against *Y. ruckeri* 30 days after vaccination and in healthy individuals. In our study, hepatic aminotransferase activities were positively associated with biomarkers of oxidative stress in trout vaccinated against *Y. ruckeri*. Similar associations were also observed in the heart tissue of

vaccinated trout. Decreased aldehyde and ketone derivatives of oxidatively modified proteins and reduced aminotransferase and lactate dehydrogenase activities were sensitive to vaccination of trout against *Y. ruckeri* and can be used as biomarkers to evaluate vaccine effects in the liver of rainbow trout. Understanding the role of biochemical changes in tissues of vaccinated trout has important implications not only for understanding the complex physiological changes that occur during immunisation, but also for improving aquaculture practices to maximise tissue growth and health of vaccinated trout (Tkachenko et al., 2016c). Time-dependent changes in oxidative stress biomarkers and activities of lysosomal and antioxidant enzymes in liver tissue of rainbow trout following vaccination against *Y. ruckeri* were evaluated in our previous study (Tkaczenko et al., 2023).

The immune pathways stimulated following injection vaccination of salmonids against enteric redmouth disease have been studied by many researchers. For example, Wangkahart E. and co-workers (2019) analysed the expression of a large set of genes encoding cytokines, acute phase proteins (APPs) and antimicrobial peptides (AMPs) in the spleen and gills in response to ERM vaccination in rainbow trout (*Oncorhynchus mykiss*). Many immune genes in teleost fishes are known to have multiple paralogs that can show differential responses to ERM vaccination, highlighting the need to determine whether all genes present respond in a similar manner. ERM vaccination immediately activated a balanced inflammatory response with correlated expression of both pro- and anti-inflammatory cytokines (e.g. IL-1β1-2, TNF-α1-3, IL-6, IL-8 and IL-10A, etc.) in the spleen. The increase in pro-inflammatory cytokines may explain the systemic up-regulation of APPs (e.g. serum amyloid A protein and serum amyloid protein P) and AMPs (e.g., cathelicidins and hepcidin) seen in both spleen and gills. These researchers also observed an upregulation of all α-chains but only one β-chain (p40B2) of the IL-12 family of cytokines, suggesting that specific IL-12 and IL-23 isoforms with different functions may be produced in the spleen of vaccinated fish. Notably, the expression of Th1 cytokines (IFN-γ1-2) and a Th17 cytokine (IL-17A/F1a) was also upregulated and correlated with increased expression of the IL-12 family α-chains and most pro- and anti-inflammatory cytokines, APPs and AMPs. These expression profiles suggest that ERM vaccination activates host innate immunity and the expression of specific IL-12 and IL-23 isoforms, leading to a Th1 and Th17 biased immune response. A late induction of Th2 cytokines (IL-4/13B1-2) was also observed, which may have a homeostatic role and/or involvement in antibody production (Wangkahart E. et al., 2019).

In the study by Zuo and co-workers (2020), SNP analyses indicated that ERM resistance in rainbow trout is a multi-locus trait. Gene expression in surviving fish suggested that several immune genes are associated with the resistance-conferring trait. Immune genes encoding inflammatory cytokines (IL-1β, IL-2A, IL-6A, IL-8, IL-10A, IL-12, IL-17A/F2A, IL-17C1, IL-17C2, IL-22, IFNγ, TNFα), acute phase reactants (SAA, C3, cathelicidins, lysozyme) were differentially expressed in moribund trout with clinical signs at 7 dpc (CS) and exposed fish without clinical signs at the same sampling time (NCS). Correlations (negative or positive) between gene expression and bacterial load suggested the involvement of immune genes in protection. Down-regulation of adaptive immune genes including *IgDm*, *IgDs*, *IgT* and *TCR-β* was observed mainly in CS and NCS fish, whereas survivors showed up-regulation of effector molecule genes such as cathelicidins, complement and lysozyme, suggesting their role in clearance of infection (Zuo S. et al., 2020).

The protective effects of autogenous and commercial ERM immersion vaccines (bacterins based on *Yersinia ruckeri*, serotype O1, biotypes 1 and 2) for rainbow trout (*Oncorhynchus mykiss*) were compared in the study by Yang H. and co-workers (2021)

to evaluate whether the use of local pathogen strains for immunisation could improve protection. The expression of genes encoding immune factors (IL-1β, IL-6, IL-8, IL-10, IFN-γ, MHCI, MHCII, CD4, CD8, TCRβ, IgM, IgT, IgD, cathelicidins 1 and 2, SAA and C3) and the density of immune cells in organs were recorded. Both vaccines were protective, as judged by the reduced bacterial load in exposed fish. Innate immune genes were upregulated in all groups following bacterial challenge, but significantly more in unvaccinated naive fish, in which the density of SAA-positive immune cells increased. Immunoglobulin genes were upregulated on day 5 post-challenge, and fish vaccinated with the high commercial dose of bacterin showed increased IgM levels by ELISA on day 14 post-challenge, indicating that vaccine dose correlated with protection. Both vaccine types provided protection to rainbow trout when exposed to live *Y. ruckeri* and no significant difference was observed between commercial and autogenous vaccines (Yang H. et al., 2021).

The response of oxidative stress biomarkers in different fish tissues depends on the activation of the immune system and the generation of reactive oxygen species (ROS) due to the 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 (Paiva C. N. & Bozza M. T., 2014). Phagocytes recognise microbes by the many molecular patterns they display and attempt to engulf them. Once a microbe is phagocytosed, the nature of the molecules recognised on the surface of the microbe determines the treatment that takes place inside the phagosome. Respiratory bursting, a process in which NADPH oxidase generates ROS in response to microbe recognition, is a possible outcome of this process and helps to eliminate many microbes (Paiva C. N. & Bozza M. T., 2014). Once a pathogen is phagocytosed, it must subvert the respiratory burst, withstand its oxidative power or escape the phagosome to survive (Paiva C. N. & Bozza M. T., 2014). Microbe recognition triggers the immune system, and ROS are generated not only in the phagocyte respiratory burst, but also in other cell compartments, such as mitochondria, as intermediaries in many signalling pathways, such as leukocyte pattern recognition receptor (PRR) signalling. ROS generation is a prerequisite for the formation of neutrophil extracellular traps (NETs); is actively involved in phagolysosome formation and enzymatic degradation; autophagy; chemoattraction and inflammation; cell death of infection reservoirs; antigen presentation, T helper polarisation and lymphocyte proliferation; iron redistribution between tissues; and iron availability in cellular compartments (Paiva C. N. & Bozza M. T., 2014). ROS generated by NADPH oxidase play an important role in antimicrobial host defence and inflammation. The release of high concentrations of ROS aids in the clearance of invading bacteria. ROS can cross the membranes of bacterial pathogens and damage their nucleic acids, proteins and cell membranes. Some pathogens are able to directly prevent the oxidative burst of phagocytes by secreting effector proteins or toxins that interfere with the translocation of the NADPH oxidase complex or signalling pathways required for its activation (Nguyen G. T. et al., 2017).

Conclusions. The current study aimed to elucidate the effects of vaccination against *Y. ruckeri* on liver function and the oxidative mechanism underlying these effects by determining relevant biomarkers of protein oxidation and total antioxidant capacity (TAC) one month after oral vaccination against *Y. ruckeri*. In this study, our results clearly demonstrate that immunisation with the anti-*Yersinia* vaccine did not alter the hepatic tissue of rainbow trout. Aldehydic and ketonic derivatives of OMP were not significantly altered in the hepatic tissue of vaccinated fish before immunization. One month after immunisation, the levels of ketonic derivatives of OMP in the hepatic tissue

of untreated trout were reduced compared to the untreated group before immunisation. Similar to the untreated groups, the levels of ketonic derivatives of OMP in the hepatic tissue of the vaccinated group one month after immunisation were reduced compared to the vaccinated group before immunisation. A statistically non-significant decrease in TAC level was observed between the values obtained in the untreated and vaccinated groups before vaccination and one month after vaccination. Understanding the role of oxidative stress in the tissues of vaccinated trout has important implications for understanding the complex physiological changes that occur during vaccination, and also for improving aquaculture practices to maximise tissue growth and health of vaccinated trout.

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