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TEMPORAL MODULATION OF OXIDATIVE STRESS AND ANTIOXIDANT CAPACITY IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WALBAUM) FOLLOWING *YERSINIA RUCKERI* VACCINATION

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This study investigated the temporal effects of Yersinia ruckeri vaccination on markers of oxidative stress and antioxidant capacity in the livers of rainbow trout (Oncorhynchus mykiss Walbaum). Oxidative stress was assessed using 2-thiobarbituric acid-reactive substances (TBARS), oxidatively modified protein (OMP) derivatives and total antioxidant capacity (TAC) at three time points: baseline and one and two months post-vaccination. TBARS levels exhibited a transient peak one month after vaccination, suggesting a short-term increase in lipid peroxidation associated with immune activation. In contrast, TAC increased progressively over the experimental period, reaching significantly higher levels in vaccinated fish after two months, which is indicative of enhanced systemic antioxidant defences. OMP derivatives exhibited moderate variability: aldehydic forms peaked in the control group at one month, while ketonic forms were highest at baseline. However, no significant vaccination effect was observed. Correlation analysis revealed a strong negative association between TBARS and TAC (r = −0.58), supporting the role of antioxidant capacity in mitigating oxidative damage. Effect size and variability analyses confirmed substantial time- and treatment-related changes, particularly with regard to TBARS reduction and TAC enhancement in vaccinated fish. Overall, the results demonstrate a biphasic oxidative response to vaccination characterised by an initial oxidative challenge followed by adaptive antioxidant upregulation, highlighting the importance of modulation of redox balance in immune protection and aquaculture health management.

Keywords: liver, rainbow trout, *Yersinia ruckeri*, vaccination, 2-thiobarbituric acid-reactive substances (TBARS), oxidatively modified protein (OMP) derivatives, total antioxidant capacity (TAC)

ЧАСОВА МОДУЛЯЦІЯ ОКСИДАТИВНОГО СТРЕСУ ТА АНТИОКСИДАНТНОЇ АКТИВНОСТІ У РАЙДУЖНОЇ ФОРЕЛІ (*ONCORHYNCHUS MYKISS* WALBAUM) ПІСЛЯ ВАКЦИНАЦІЇ ПРОТИ *YERSINIA RUCKERI*

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У цьому дослідженні вивчали часовий вплив вакцинації щодо Yersinia ruckeri на показники оксидативного стресу та антиоксидантної активності у печінці райдужної форелі (Oncorhynchus mykiss Walbaum). Оксидативний стрес оцінювали за допомогою продуктів, які реагують з 2-тіобарбітуровою кислотою (TBARS), похідних окиснювально модифікованих білків (OMP) та загальної антиоксидантної активності (TAC) у трьох часових точках: на початковому етапі, через один та два місяці після вакцинації. Рівні TBARS демонстрували тимчасовий пік через місяць після вакцинації, що свідчить про короткочасне підвищення пероксидації ліпідів, пов'язане з активацією імунної відповіді. Натомість TAC поступово зростала протягом усього експерименту, досягаючи значно вищих рівнів у вакцинованих риб через два місяці, що вказує на посилення системного антиоксидантного захисту. Похідні OMP проявляли помірну варіабельність: альдегідні форми досягали максимуму у контрольній групі через місяць, тоді як кетонові форми були найвищими на початковому етапі. Проте значного впливу вакцинації на рівні похідних OMP не виявлено. Кореляційний аналіз показав істотну негативну кореляцію між TBARS і TAC (r = −0,58), що підтверджує роль антиоксидантної активності у зменшенні оксидативного ушкодження. Аналіз ефекту та варіабельності підтвердив суттєві зміни, пов'язані з часом і впливом обробки, особливо щодо зниження TBARS та підвищення TAC у вакцинованих риб. Загалом результати демонструють біфазну оксидативну реакцію на вакцинацію, що характеризується початковим оксидативним стресом, за яким слідує адаптивне підвищення антиоксидантного захисту, підкреслюючи важливість модуляції редокс-балансу для імунного захисту та роль терапевтичних і профілактичних процедур в аквакультурі.

Ключові слова: печінка, райдужна форель, *Yersinia ruckeri*, вакцинація, продукти, які реагують з 2-тіобарбітуровою кислотою (TBARS), похідні окиснювально модифікованих білків (OMP), загальна антиоксидантна активність (TAC)

Introduction. Vaccination is a key part of preventing disease in aquaculture, as it reduces the morbidity and mortality caused by infectious pathogens (Mondal H. and Thomas J., 2022; Kumar A. et al., 2024). *Yersinia ruckeri*, the causative agent of enteric redmouth disease (ERM), is a significant bacterial pathogen affecting salmonids worldwide and causing substantial economic losses in freshwater aquaculture (Kumar G.

et al., 2015). Although effective vaccines against *Y. ruckeri* have been developed and are widely used, the physiological consequences of vaccination beyond immune protection are not well understood.

One area of emerging interest is the interaction between vaccination and oxidative stress. Activation of the immune system often triggers the production of reactive oxygen species (ROS) as part of the host's defence mechanisms (Andrés C. M. C. et al., 2022; Afzal S. et al., 2023). While ROS play a crucial role in clearing pathogens, excessive or prolonged ROS generation can damage cellular macromolecules, including lipids, proteins and nucleic acids (Schieber M. and Chandel N. S., 2014; Juan C. A. et al., 2021). An organism's ability to maintain redox homeostasis depends on balancing pro-oxidant processes with antioxidant defences, which can be either enzymatic or non-enzymatic (Rahal A. et al., 2014).

In fish, markers of oxidative stress such as 2-thiobarbituric acid reactive substances (TBARS) and oxidatively modified proteins (OMPs), alongside measures of total antioxidant capacity (TAC), have been used to evaluate the physiological response to environmental, nutritional and immunological challenges (Schumann S. et al., 2023; Oliveira J. et al., 2024; Grădinariu L. et al., 2025). However, few studies have investigated how these redox biomarkers change over time following vaccination (Tkachenko H. et al., 2014; Kurhaluk N. et al., 2024; Grădinariu L. et al., 2025), particularly in economically important species such as the rainbow trout (*Oncorhynchus mykiss* Walbaum) (D'Agaro E. et al., 2022).

Furthermore, given its central role in metabolic regulation and detoxification, the liver is a critical organ for assessing systemic oxidative stress (Li S. et al., 2015; Allameh A. et al., 2023). Due to its high metabolic activity and exposure to circulating immune mediators, hepatic tissue is particularly sensitive to fluctuations in redox balance (Smith R. L. et al., 2018). By focusing on liver-specific responses, this study provides an insight into how vaccination may influence oxidative processes at an organ level.

The timing of sample collection after vaccination is crucial for capturing the dynamic nature of redox changes. Acute responses may differ significantly from longer-term adaptations, and distinguishing between these phases can help to establish whether the oxidative stress is transient or sustained (Pickering A. M. et al., 2013). In this study, fish were sampled at multiple time points following immunisation to capture both immediate and delayed effects on oxidative biomarkers.

This study aimed to evaluate the temporal dynamics of oxidative stress markers and antioxidant capacity in the livers of rainbow trout following vaccination against *Yersinia ruckeri*. Through the integration of biochemical analyses and statistical modelling, we sought to clarify the relationships between lipid peroxidation, protein oxidation and antioxidant status, as well as determining the extent to which vaccination influences these parameters. Understanding these interactions could provide valuable insights into fish physiology and contribute to the development of health management strategies in aquaculture.

Ultimately, integrating oxidative stress profiling into vaccine evaluation protocols could provide a more comprehensive understanding of fish health. If vaccination induces measurable oxidative shifts, these could serve as early indicators of physiological strain or adaptation. Such insights could inform the formulation of vaccines, dosing schedules and post-vaccination monitoring practices, thereby contributing to more sustainable and welfare-conscious aquaculture systems.

Materials and methods.

Fish. The experiments used rainbow trout (*Oncorhynchus mykiss* Walbaum) weighing 105-135 g. Prior to the commencement of the experiment, all individuals were acclimated to laboratory conditions for 14 days, with the same water quality parameters being employed as during the trial.

The study was conducted at the Department of Salmonid Research at the Stanisław Sakowicz Inland Fisheries Institute in Olsztyn, Poland. Experiments were conducted at a water temperature of 14.5 ± 0.5 °C and a pH of 7.5. Dissolved oxygen levels were maintained at approximately 12 ppm with supplemental oxygen provided by a continuous water flow of 25 litres per minute and a photoperiod of 12 hours of light followed by 12 hours of darkness. The fish were fed a commercial pelleted diet at optimum levels using 12-hour automatic belt feeders. Daily dietary allowances were calculated in accordance with current nutritional guidelines for salmonids.

All biochemical assays were performed at the Department of Zoology and the Department of Animal Physiology at the Institute of Biology, Pomeranian University in Słupsk, Poland. All procedures involving animals were conducted in accordance with national and EU regulations for the protection of animals used for scientific purposes. The experimental protocol was approved by the Local Ethical Committee for Animal Experiments in Olsztyn.

Experimental design. The rainbow trout were acclimated to laboratory conditions for 14 days prior to the experiment. The fish were randomly assigned to two groups: (1) an untreated control group and (2) a group vaccinated against *Yersinia ruckeri*. Each group was held in a separate 1,000-litre square tank (150 fish per tank) under identical environmental conditions.

The vaccine was produced at the Department of Fish Diseases at the National Veterinary Research Institute in Puławy, Poland, according to a procedure covered by patent no. P.428259. The prepared vaccine, at a concentration of 1×10^9 cells ml^{-1}, was administered orally. It was mixed with commercial fish feed and given to the fish three times at one-day intervals. Following vaccination, the fish were kept at a temperature of 14.5 ± 0.5 °C, a pH level of 7.5, a dissolved oxygen level of ~12 ppm, and a photoperiod of 12 hours of light followed by 12 hours of darkness. In the present study, 15 untreated control trout and 15 vaccinated trout were sampled at three time points: baseline (0), one month and two months after vaccination.

Tissue collection and sample preparation. Thirty-one and sixty-one days after vaccination, the rainbow trout were humanely euthanised by decapitation. Immediately afterwards, the liver was excised in situ. The organs were perfused via the hepatic portal vein with an ice-cold isolation buffer to remove any remaining blood. This buffer solution consisted of 100 mM Tris-HCl with a pH of 7.2.

The liver tissue was then homogenised using a glass H500 homogeniser with a motor-driven pestle and immersed in an ice–water bath to obtain a 1:9 (weight/volume) homogenate. The homogenates were then centrifuged at 3,000 rpm (approximately 1,000×g) for 15 minutes at 4°C. After centrifugation, the supernatant was collected and stored at −25 °C until analysis.

The protein content of the supernatant was determined using the Bradford method (Bradford, M.M., 1976) with bovine serum albumin (BSA) as the standard. Absorbance was measured at 595 nm using a UV–Vis spectrophotometer (Spekol 11, Carl Zeiss, Jena, Germany). All assays were performed in duplicate at 22 ± 0.5 °C and biochemical reactions were initiated by adding tissue supernatant to the reaction mixture.

Specific assay conditions for each parameter are described in the following subsections.

2-Thiobarbituric acid reactive substances (TBARS) assay. Lipid peroxidation levels were assessed by measuring the concentration of 2-thiobarbituric acid reactive substances (TBARS) according to the method of Buege J.A. and Aust S.D. (1978), with minor modifications. This assay is based on the reaction between 2-thiobarbituric acid (TBA; Sigma-Aldrich, St. Louis, MO, USA) and malondialdehyde (MDA) or similar aldehydic products of lipid peroxidation. Under high temperature and acidic conditions, TBA reacts with MDA to form a pink chromogen with a maximal absorbance at 532 nm.

Briefly, 0.5 ml of supernatant was mixed with 2.5 ml of TBA reagent (0.375% TBA, 15% trichloroacetic acid, and 0.25 N HCl) and heated in a boiling water bath for 15 min. After cooling on ice, samples were centrifuged at 3,000 × g for 10 min to remove precipitated proteins. The absorbance of the supernatant was measured at 532 nm against reagent blanks using a UV–Vis spectrophotometer (Spekol 11, Carl Zeiss, Jena, Germany).

The TBARS concentration was calculated using the molar extinction coefficient for the MDA–TBA complex ($\varepsilon = 1.56 \times 10^5\ M^{-1}\cdot cm^{-1}$) and expressed as nanomoles of MDA equivalents per milligram of protein.

Assay for carbonyl groups in oxidatively modified proteins. Carbonyl groups were determined as a marker of protein oxidation using the method of Levine R. L. et al. (1990) with some modifications. Briefly, liver homogenate supernatant aliquots were incubated with 10 mM 2,4-dinitrophenylhydrazine (DNPH; Sigma-Aldrich, St. Louis, Missouri, USA) in 2 M hydrochloric acid (HCl) for one hour at room temperature and in the dark. Blank samples were prepared under identical conditions, but without DNPH.

The proteins were then precipitated using 20% trichloroacetic acid (TCA), after which the mixture was centrifuged at 3,000 g for 20 minutes at 4 °C. The protein pellet was washed three times with a mixture of ethanol and ethyl acetate (1:1, v/v) to remove any remaining reagents or lipids. The pellet was then incubated at 37 °C in an 8 M urea solution until completely resuspended. The carbonyl content was measured spectrophotometrically at 370 nm for aldehydic derivatives (OMP_{370}) and at 430 nm for ketonic derivatives (OMP_{430}), using a molar extinction coefficient of 22,000 $M^{-1}\cdot cm^{-1}$. The results were expressed as nmol of carbonyl groups per mg of protein (Levine et al., 1990).

Total antioxidant capacity (TAC) assay. TAC was determined spectrophotometrically using the Tween 80 oxidation method (Galaktionova L. P. et al., 1998). Absorbance was measured at 532 nm and TAC values were expressed as a percentage relative to the control oxidation value.

Statistical analysis. Statistical analyses were performed to evaluate the effects of anty-*Yersinia* vaccination and sampling time on oxidative stress markers and total antioxidant capacity (TAC) in liver tissue. Six experimental groups were included: three control groups that were not treated and three groups that received the anti-*Yersinia* vaccine at 0, 1 and 2 months after vaccination. Four biochemical parameters were assessed: 2-thiobarbituric acid reactive substances (TBARS; nmol per mg of protein) as a marker of lipid peroxidation, and aldehydic and ketonic derivatives of oxidatively modified proteins (nmol per mg of protein), as well as TAC (%).

Data were analysed using two-way ANOVA to assess the main effects of time and vaccination status, as well as their interaction. When significant effects were detected, post hoc pairwise comparisons were performed using the Tukey–Kramer honest significance test (HSD). Effect sizes were calculated using Cohen's d and partial eta squared (η^2). Pearson's correlation coefficients (r) were determined to assess the associations between the oxidative stress markers and TAC. Multiple linear regression analysis was used to identify the strongest predictors of TBARS levels, including TAC, vaccination status and time. Variability in the data was expressed as the coefficient of variation (CV%) (Stanisz A., 2006, 2007).

Normality and homogeneity of variances were verified using Shapiro–Wilk and

Levene's tests, respectively. All tests were two-tailed and differences were considered statistically significant at $p < 0.05$. The statistical analyses were conducted using Statistica 13.3 (TIBCO Software Inc., Palo Alto, USA).

Results. Figure 1 shows the levels of TBARS (a key indicator of lipid peroxidation), aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity in the hepatic tissues of rainbow trout that were orally immunised against *Y. ruckeri* at 0, 1 and 2 months post-vaccination.

A statistical analysis was performed to evaluate the effects of anti-*Yersinia* vaccination and time on oxidative stress markers and antioxidant capacity in liver tissue. The study included six groups: three control groups that received no treatment and three groups that received the anti-*Yersinia* vaccine (at 0, 1 and 2 months after vaccination). Four biochemical parameters were assessed: TBARS as a marker of lipid peroxidation (nmol·mg^{-1} protein); aldehydic and ketonic derivatives of oxidatively modified proteins (nmol·mg^{-1} protein); and total antioxidant capacity (TAC, %).

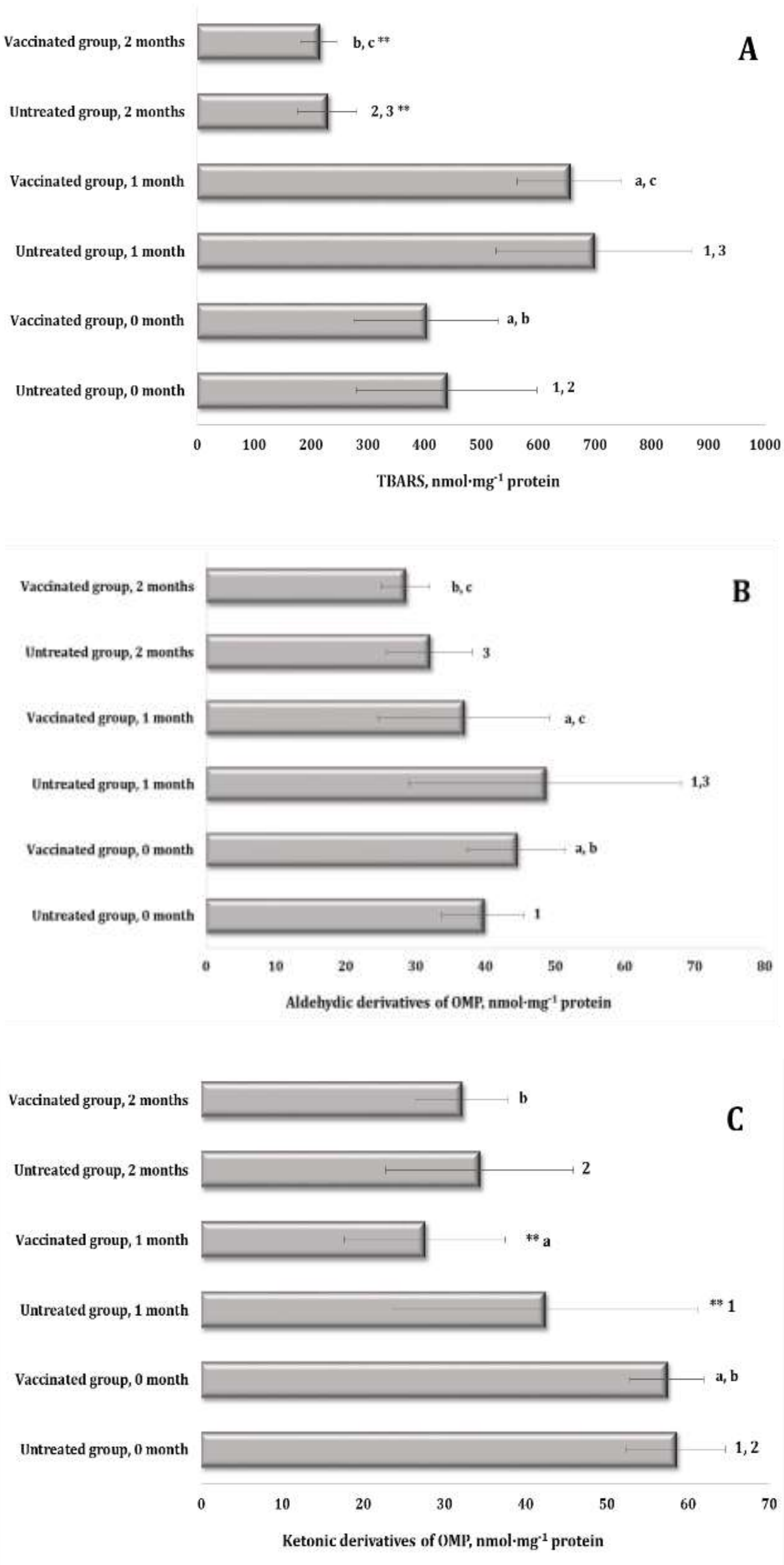
TBARS levels varied significantly across groups and time points. One-way ANOVA revealed significant main effects of time ($F_{2.78} = 14.62$, $p < 0.001$) and group ($F_{1.78} = 6.87$, $p = 0.011$), as well as a significant interaction effect ($F_{2.78} = 4.23$, $p = 0.018$). Post hoc Tukey HSD tests showed that TBARS levels peaked at one month in both the control group (698.30 ± 172.43 nmol·mg^{-1} protein) and the vaccinated group (655.67 ± 91.95 nmol·mg^{-1} protein), followed by a significant decrease at two months (control group: 228.64 ± 51.89 nmol·mg^{-1} protein; vaccinated group: 214.18 ± 32.20 nmol·mg^{-1} protein; $p < 0.01$) (Fig. 1A).

Levels of aldehydic and ketonic derivatives of oxidatively modified proteins exhibited moderate variability (Fig. 1B, 1C). Aldehydic derivative levels peaked at 1 month in the control group (48.57 ± 19.50 nmol·mg^{-1} protein) compared to the vaccinated group (36.91 ± 12.25 nmol·mg^{-1} protein) (Fig. 1B), while ketonic derivative levels peaked at 0 months in the control group (58.49 ± 6.13 nmol·mg^{-1} protein) compared to the vaccinated group (57.34 ± 4.59 nmol·mg^{-1} protein) (Fig. 1C). ANOVA revealed a significant time effect for ketonic derivatives ($F_{2.78} = 4.76$, $p = 0.012$), but no significant group effect.

TAC values increased over time, reaching their highest levels after two months. A two-way ANOVA revealed significant effects of both time ($F_{2.78} = 21.34$, $p < 0.001$) and group ($F_{1.78} = 9.45$, $p = 0.003$). At 2 months, the vaccinated group showed significantly higher TAC levels (54.62 ± 12.84%) than the control group (49.41 ± 13.45%) ($p < 0.01$) (Fig. 1D).

Pearson's correlation analysis revealed a moderate positive correlation between TBARS and aldehydic derivatives of oxidatively modified proteins ($r = 0.42$, $p < 0.01$), a weaker correlation with ketonic derivatives of oxidatively modified proteins ($r = 0.35$, $p = 0.03$) and a strong negative correlation between TBARS and TAC ($r = -0.58$, $p < 0.001$). Multiple linear regression identified TAC as the strongest predictor of TBARS levels ($\beta = -5.2$, $p < 0.001$). Vaccination status was also a significant factor ($\beta = -45.6$, $p = 0.017$), indicating reduced oxidative stress in vaccinated animals. Time had a non-linear effect, with the highest TBARS values occurring at one month.

For TBARS changes between 1 and 2 months, Cohen’s d ranged from 1.12 (large) in the control group to 1.35 (very large) in the vaccinated group, indicating a substantial reduction in oxidative stress over time. For TAC, Cohen's d between 0 and 2 months was 1.48 in the vaccinated group, reflecting a significant increase in antioxidant capacity after immunisation.


A
Vaccinated group, 2 months
Untreated group, 2 months
Vaccinated group, 1 month
Untreated group, 1 month
Vaccinated group, 0 month
Untreated group, 0 month
b, c **
2, 3 **
a, c
1, 3
a, b
1, 2
0 100 200 300 400 500 600 700 800 900 1000
TBARS, nmol·mg-1 protein
B
Vaccinated group, 2 months
Untreated group, 2 months
Vaccinated group, 1 month
Untreated group, 1 month
Vaccinated group, 0 month
Untreated group, 0 month
b, c
3
a, c
1,3
a, b
1
0 10 20 30 40 50 60 70 80
Aldehydic derivatives of OMP, nmol·mg-1 protein
C
Vaccinated group, 2 months
Untreated group, 2 months
Vaccinated group, 1 month
Untreated group, 1 month
Vaccinated group, 0 month
Untreated group, 0 month
b
2
** a
** 1
a, b
1, 2
0 10 20 30 40 50 60 70
Ketonic derivatives of OMP, nmol·mg-1 protein

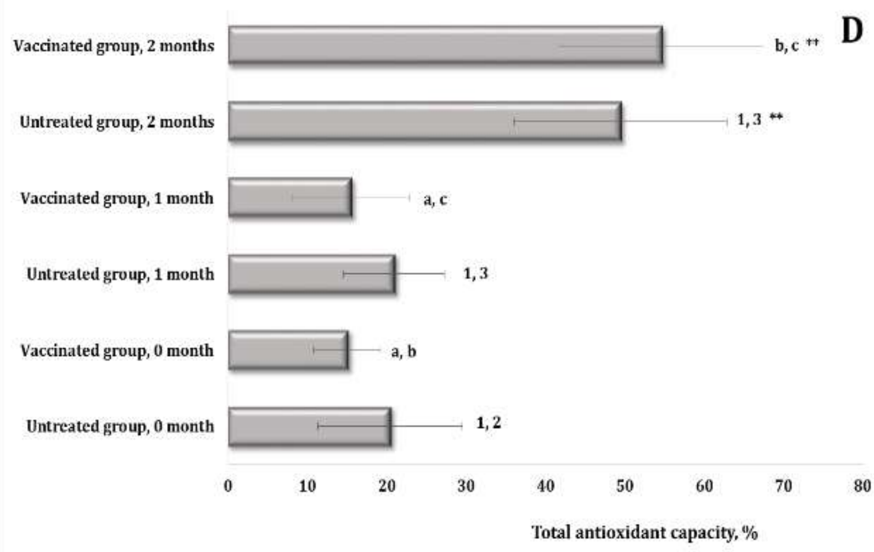


Fig. 1. The levels of TBARS levels, a key indicator of lipid peroxidation (A), aldehydic and ketonic derivatives of oxidatively modified proteins (B, C), and total antioxidant capacity (D) in the hepatic tissues of rainbow trout that were orally immunised against *Y. ruckeri* at 0, 1, and 2 months after vaccination.

Data are presented as means ± S.D. (n = 10).

[**] Significant differences (p < 0.05) between the untreated control group and the group vaccinated against Y. ruckeri;

[1] Significant differences (p < 0.05) between the untreated control group at 0 and 1 months after vaccination against Y. ruckeri;

[2] Significant differences (p < 0.05) between the untreated control group at 0 and 2 months after vaccination against Y. ruckeri;

[3] Significant differences (p < 0.05) between the untreated control group at 1 and 2 months after vaccination against Y. ruckeri;

[a] Significant differences (p < 0.05) between the group vaccinated against Y. ruckeri at 0 and 1 months after vaccination;

[b] Significant differences (p < 0.05) between the group vaccinated against Y. ruckeri at 0 and 2 months after vaccination;

[c] Significant differences (p < 0.05) between the group vaccinated against Y. ruckeri at 1 and 2 months after vaccination.

Two-way ANOVA revealed that time accounted for 41% of the variance in TBARS levels (partial $\eta^2 = 0.41$), while vaccination status accounted for 17% (partial $\eta^2 = 0.17$). For TAC, time accounted for 38% of the variance and group differences accounted for 22%, suggesting a meaningful physiological impact of the vaccine. The coefficient of variation (CV%) was highest for TBARS at 1 month (24.6%), which is consistent with elevated oxidative stress and variability in the response to Yersinia exposure between individuals. In contrast, TAC showed the lowest CV% (12.3%) at 2 months in the vaccinated group, indicating a more uniform antioxidant response post-vaccination.

Overall, these findings emphasise the robust protective effect of anti-*Yersinia* vaccination, characterised by reduced lipid peroxidation and enhanced antioxidant defences over time.

Discussion. This study investigated the temporal and immunological effects of *Yersinia ruckeri* vaccination on oxidative stress markers and antioxidant capacity in the livers of rainbow trout. The results showed clear time-dependent patterns of biochemical modulation, indicating that vaccination measurably affects the redox balance in fish.

TBARS levels, a key indicator of lipid peroxidation, varied significantly between time points and treatment groups. The peak observed one month after vaccination suggests a transient increase in oxidative stress, which is likely to be linked to immune activation and metabolic adaptation during the post-vaccination phase (Fig. 1A). Similar transient increases in TBARS have been reported in teleosts following immunostimulation or pathogen exposure, reflecting the increased production of reactive oxygen species (ROS) during the innate immune response (Torrealba D. et al., 2019; Yu Y. et al., 2020; Usman S. et al., 2021; Hissen K. L. et al., 2023). The subsequent decline in TBARS levels at two months, which was particularly pronounced in vaccinated fish, indicates the activation of compensatory antioxidant mechanisms, which may involve the upregulation of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase (Tkachenko H. et al., 2014; Shastak Y. and Pelletier W., 2023). The reduction in TBARS in vaccinated fish (214.18 ± 32.20 nmol·mg^{-1} protein) compared to controls (228.64 ± 51.89 nmol·mg^{-1} protein) suggests that immunisation protects against sustained lipid oxidative damage.

The variability of oxidatively modified protein (OMP) derivatives was moderate. Aldehydic derivatives peaked at one month in the control group, while ketonic derivatives were at their highest level at baseline (Fig. 1B, 1C). This pattern suggests that the kinetics of protein oxidation may differ from those of lipid peroxidation: aldehydic forms may reflect ongoing damage, while ketonic forms may represent earlier oxidative events (Pizzimenti S. et al., 2013; Frijhoff J. et al., 2015). Although ANOVA indicated a significant time effect for ketonic derivatives, no significant group effect was observed. This suggests that vaccination has a stronger impact on lipid oxidation and antioxidant capacity than on protein oxidation. Previous studies in fish have also found that markers of protein oxidation are generally less responsive than TBARS to immunological or environmental stressors (Dinardo F. R. et al., 2021).

Total antioxidant capacity (TAC) increased progressively over the experimental period, reaching its highest levels at two months post-vaccination. This increase was statistically significant for both time and group, with TAC values being higher in vaccinated fish (54.62% ± 12.84%) than in controls (49.41% ± 13.45%) (Fig. 1D). This aligns with the hypothesis that immunisation triggers systemic antioxidant defences, possibly via the transcriptional activation of antioxidant genes in response to ROS signalling (Birben E. et al., 2012; Hong Y. et al., 2024). The enhancement of TAC in vaccinated animals may represent an adaptive, protective mechanism aimed at restoring redox homeostasis after the initial oxidative challenge (Kurutas E. B., 2016; Liu S. et al., 2025).

Correlation analysis revealed moderate correlation between TBARS and aldehydic OMP ($r = 0.42$), weak correlation between TBARS and ketonic OMP ($r = 0.35$), and strong negative correlation between TBARS and TAC ($r = -0.58$). These findings emphasise the interconnected nature of oxidative stress pathways and suggest that higher antioxidant capacity is associated with lower lipid peroxidation. Multiple regression analysis identified TAC as the strongest predictor of TBARS, emphasising the central role of antioxidant defences in controlling oxidative damage.

Effect size analysis confirmed significant time- and treatment-related changes, particularly for TBARS and TAC shifts, with large Cohen's d values. For TBARS, the decrease between one and two months corresponded to a large-to-very-large effect size in both groups, with vaccinated fish demonstrating greater improvement. Changes in TAC between 0 and 2 months in vaccinated fish yielded a Cohen’s d of 1.48, indicating a substantial enhancement of systemic antioxidant defences. Coefficient of variation (CV%) analysis revealed the greatest variability in TBARS at one month (24.6%), consistent with inter-individual differences in oxidative responses, and the lowest CV% for TAC in vaccinated fish at two months (12.3%). This suggests a more uniform antioxidant adaptation post-vaccination.

Importantly, these redox-related changes are consistent with the immunological observations reported by Raida M. K. et al. (2011). They demonstrated that immersion vaccination of rainbow trout against *Y. ruckeri* significantly increased plasma IgM titres and provided complete protection at 8 and 26 weeks post-vaccination. Antibody levels correlated with reduced bacteraemia and enhanced bactericidal activity of plasma *in vitro*. Integrating these findings with our data suggests that the biphasic oxidative response – initial oxidative stress followed by enhanced antioxidant capacity – may support the development of robust humoral immunity by limiting oxidative damage to immune cells and plasma proteins involved in pathogen neutralisation.

The proposed link between redox modulation and humoral immunity is further supported by the work of Rømer Villumsen K. et al. (2012), who investigated protection against *Aeromonas salmonicida* induced by vaccination in rainbow trout. They found that both commercial and experimental vaccines significantly improved survival following bacterial challenge, inducing sustained increases in specific antibody levels during the 18-week post-vaccination period. Notably, antibody titres prior to infection were positively correlated with survival, while a rapid decline in antibodies was observed within three days post-challenge. This indicates the active utilisation of these immune effectors in defending against pathogens. These findings reinforce the idea that the efficacy of vaccination in salmonids depends not only on the generation of strong antibody responses, but also on preserving the functional capacity of these antibodies during infection. In this context, the enhanced antioxidant capacity observed in our study could play a critical role in maintaining antibody stability and effectiveness, thereby supporting long-term protection in aquaculture settings.

Taken together, these findings suggest that vaccination against *Y. ruckeri* induces a biphasic oxidative stress response in rainbow trout. This is characterised by an initial increase in lipid peroxidation, followed by a significant increase in antioxidant capacity. These adaptive changes may represent an important physiological mechanism that supports the development of effective immune protection while limiting oxidative damage. These results are consistent with reports in other fish species, in which vaccination or immune stimulation has been shown to modulate redox balance (Zhang C. et al., 2021; Nadarajapillai K. et al., 2023; Dagoudo M. et al., 2023; Khansari A. R. et al., 2025), and could inform future strategies aimed at optimising fish health and vaccine efficacy in aquaculture.

In summary, the data demonstrate that anti-*Yersinia* vaccination influences oxidative stress dynamics in a time-dependent manner. The transient increase in TBARS followed by enhanced TAC indicates a coordinated physiological response to an immunological challenge. These findings provide insight into the redox-modulatory effects of vaccination and highlight the usefulness of TBARS, protein oxidation markers and TAC as sensitive indicators of hepatic oxidative status.

Conclusions. This study provides evidence that vaccination against Yersinia ruckeri modulates oxidative stress and antioxidant capacity in the livers of rainbow trout over time. The observed biphasic response – an initial, transient increase in lipid peroxidation, followed by a pronounced rise in total antioxidant capacity – suggests a coordinated physiological adjustment to the immunological challenge. Lipid peroxidation markers (TBARS) were found to be more sensitive to the effects of vaccination than indicators of protein oxidation, while antioxidant capacity was found to be a strong negative predictor of oxidative damage. These findings emphasise fish's ability to activate compensatory antioxidant mechanisms following vaccination and highlight the importance of oxidative stress biomarkers as tools for monitoring fish health and vaccine efficacy in aquaculture systems.

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