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ACTIVITY OF ANTIOXIDANT ENZYMES IN THE CARDIAC AND HEPATIC TISSUES OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WALBAUM) FED A DIET SUPPLEMENTED WITH B-GLUCANS

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This study investigates the effects of dietary β-glucans on the activity of key antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPx)] in the hepatic and cardiac tissues of rainbow trout (Oncorhynchus mykiss Walbaum). Supplementation with β-glucans significantly modulated catalase (CAT) and glutathione peroxidase (GPx) activities in both tissues, suggesting enhanced antioxidant defence mechanisms. In the hepatic tissue, a 264.6% increase in CAT activity ($p < 0.05$) and a 311% increase in GPx activity ($p < 0.05$) was observed, indicating enhanced hydrogen peroxide detoxification and oxidative stress resistance. A similar enhancement of antioxidant capacity was observed in the heart, with an increase in cardiac CAT activity of 135.7% ($p < 0.05$). Although changes in superoxide dismutase (SOD) activity were not statistically significant, a trend of decreased hepatic SOD activity and increased cardiac SOD activity was observed, potentially reflecting tissue-specific oxidative defense strategies. Glutathione reductase (GR) activity decreased in both tissues, albeit not significantly, suggesting possible adaptations in the glutathione cycle. The findings of this study emphasise the tissue-specific modulation of antioxidant pathways by dietary β-glucans and their potential role in enhancing oxidative stress resistance in aquaculture species. The data suggest that dietary β-glucans induce tissue-specific modulations of antioxidant enzyme activities in rainbow trout, potentially enhancing oxidative resilience in liver and heart tissues. These effects are likely to arise from the bioactive properties of β-glucans, which are known to influence immune and oxidative defence pathways. It is recommended that future studies focus on long-term feeding trials and the inclusion of additional biomarkers to facilitate a comprehensive understanding of the physiological effects of β-glucan supplementation.

Keywords: *Rainbow trout, Oncorhynchus mykiss, β-glucans, antioxidant enzymes, catalase, glutathione peroxidase, superoxide dismutase, oxidative stress, hepatic tissue, cardiac tissue.*

АКТИВНІСТЬ АНТИОКСИДАНТНИХ ФЕРМЕНТІВ У СЕРЦЕВІЙ ТА ПЕЧІНКОВІЙ ТКАНИНАХ РАЙДУЖНОЇ ФОРЕЛІ (*ONCORHYNCHUS MYKISS* WALBAUM), ЯКУ ГОДУВАЛИ ДІЄТОЮ, ДОПОВНЕНОЮ В-ГЛЮКАНАМИ

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У цьому дослідженні ми вивчали вплив дієтичних β-глюканів на активність ключових антиоксидантних ферментів [супероксиддисмутази (SOD), каталази (CAT), глутатіонредуктази (GR) і глутатіонпероксидази (GPx)] у печінці та серцевій тканині райдужної форелі (Oncorhynchus mykiss Walbaum) після 14-денного періоду перорального прийому добавок з β-глюканами. Добавка β-глюканів значно змінила активність каталази (CAT) і глутатіонпероксидази (GPx) в обох тканинах, що свідчить про покращення антиоксидантних механізмів захисту. У печінці було зафіксовано збільшення активності CAT на 264,6% ($p < 0,05$) та GPx на 311% ($p < 0,05$), що вказує на покращену детоксикацію перекису водню та підвищену стійкість до окиснювального стресу. Подібне посилення антиоксидантної активності спостерігалося і в серці, з підвищенням активності CAT в серцевій тканині на 135,7% ($p < 0,05$). Хоча зміни в активності супероксиддисмутази не були статистично значущими, спостерігалася тенденція до зменшення активності SOD у печінці та збільшення її активності у серці, що потенційно відображає тканинно-специфічні стратегії окиснювального захисту. Активність глутатіонредуктази знижувалася в обох тканинах, хоча й незначно, що може вказувати на можливі адаптації в циклі глутатіону. Результати цього дослідження підкреслюють тканинно-специфічну модуляцію антиоксидантних шляхів дієтичними β-глюканами та їх потенційну роль у підвищенні стійкості до окиснювального стресу. Дані свідчать, що β-глюкани у складі раціону райдужної форелі спричиняють тканинно-специфічну модуляцію активності антиоксидантних ферментів, що потенційно підвищує окиснювальну стійкість печінки та серцевої тканини у цього виду риб. Ці ефекти, ймовірно, зумовлені біоактивними властивостями β-глюканів, відомих своєю здатністю впливати на імунні та окиснювальні захисні шляхи. Майбутні дослідження зосередимо на довгострокових випробуваннях β-глюканів в годівлі лососевих риб та включенні додаткових біомаркерів для повного розуміння фізіологічних ефектів добавок з β-глюканами.

Ключові слова: *Райдужна форель, Oncorhynchus mykiss, β-глюкани, антиоксидантні ферменти, каталаза, глутатіонпероксидаза, супероксиддисмутаза, окиснювальний стрес, печінка, серцева тканина*

Introduction. The supplementation of aquaculture diets with bioactive compounds has emerged as a key strategy to improve the health and growth performance of farmed fish (Torres-Maravilla E. et al., 2024). Among these compounds, β-glucans – naturally occurring polysaccharides found in the cell walls of fungi, yeasts and cereals – have gained considerable attention due to their immunostimulatory and antioxidant

properties (Singla A. et al., 2024). The incorporation of β-glucans in aquafeeds has been extensively studied for their role in boosting the immune system and improving resistance to pathogenic challenges (Rodrigues M. V. et al., 2020). However, their influence on the activity of antioxidant enzymes in vital organs, such as the heart and liver tissues of fish, remains underexplored.

Rainbow trout (*Oncorhynchus mykiss* Walbaum), an economically important species in aquaculture, serves as a valuable model for studying the effects of dietary supplementation on fish physiology. As ectothermic organisms, fish are particularly susceptible to oxidative stress caused by environmental and metabolic factors, which can disrupt cellular homeostasis and affect overall health (Uiuiu P. et al., 2021; Banaee M. et al., 2023). The liver, as the primary organ responsible for metabolism and detoxification, and the heart, which is critical for circulatory function, are particularly vulnerable to oxidative damage (Cichoż-Lach H. and Michalak A., 2014; Allameh A. et al., 2023). Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) play a pivotal role in mitigating the deleterious effects of reactive oxygen species (ROS), thereby maintaining cellular integrity (Jomova K. et al., 2023).

It has been hypothesised that dietary β-glucans may modulate oxidative stress responses by increasing the activity of antioxidant enzymes. This modulation is hypothesised to be facilitated by the activation of signalling pathways that regulate cellular redox balance (Yu C. et al., 2021). Despite the encouraging evidence from studies in other animal models, the specific effects of β-glucans on the enzymatic antioxidant defence systems in fish tissues are not yet fully understood. Investigation of these effects is crucial for optimising aquafeed formulations and improving the sustainability of aquaculture practices.

The present study aims to evaluate the activity of key antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPx)] in the cardiac and hepatic tissues of rainbow trout fed a diet supplemented with β-glucans. By elucidating the biochemical responses to β-glucan supplementation, this research aims to provide insights into the potential health benefits and mechanisms of action of β-glucans. The findings will contribute to the development of functional aquafeeds designed to improve the resilience and performance of farmed fish under varying environmental conditions.

Materials and methods.

Fish and experimental design. Thirty healthy rainbow trout (*Oncorhynchus mykiss*), with an average weight of 55.9 ± 2.1 g, were selected for the experiments. The fish were housed in an indoor system equipped with a freshwater supply, adequate aeration and an internal power filter. Water quality parameters were maintained at a temperature of 16 ± 2 °C, dissolved oxygen levels of 12 ± 0.5 ppm and pH between 7.4 and 7.6. During a 14-day acclimation period, the trout were fed a commercial basal diet at 1.5% of body weight (BW) four times a day.

Subsequent to the acclimation period, the fish were randomly divided into two groups and housed in aerated 250 L square tanks containing dechlorinated tap water, with each group occupying one tank (15 fish per tank). Throughout the feeding trial, the photoperiod conditions remained naturally occurring. The study was conducted at the Department of Salmonid Research, National Inland Fisheries Research Institute (Rutki, Poland).

The feeding trial lasted for a period of 14 days, during which the control group (n = 15) received a basal diet, while the β-glucan group (n = 15) received a diet supplemented with the Yestimun® powder at 1% of the basal diet (containing 85% β-1,3/1,6-glucans,

Leiber GmbH, Bramsche, Germany). The basal diet was supplemented with 1% Yestimun® powder (1 kg per 99 kg, w/w). This insoluble, highly purified preparation contains natural polysaccharides, including β-1,3/1,6-D-glucans, derived from brewer's yeast (*Saccharomyces cerevisiae*), which typically contains approximately 30% β-glucans on a dry weight basis.

Throughout the feeding trial, survival and weight gain were meticulously monitored. At the conclusion of the 14-day period, the fish were euthanised by decapitation, and the liver and heart tissues were collected for further analysis. It is noteworthy that the experiments were performed in duplicate.

Hepatic and cardiac tissue isolation. Tissue samples were collected from the fish post-decapitation, with one fish utilised for each homogenate preparation. The liver and heart were then excised, weighed and rinsed in ice-cold buffer. The tissues were then minced and washed with ice-cold 100 mM Tris-HCl isolation buffer in order to remove any residual blood. The tissues were then homogenised in 10 volumes (v/w) of isolation buffer and subsequently subjected to a centrifugation process at 3,000 g at 4°C for a duration of 10 minutes. The resulting clarified upper layer was stored in a -22°C freezer for subsequent analyses of enzymatic activity and oxidative stress biomarkers. The isolation buffer comprised 100 mM Tris-HCl, adjusted to pH 7.2 with HCl.

Biochemical assays. All enzymatic assays were performed at 23 ± 1°C using a Specol 11 spectrophotometer (Carl Zeiss Jena, Germany). The initiation of the enzymatic reactions was facilitated by the addition of the homogenate suspension. The detailed assay conditions are described below, and each sample was analysed in duplicate. Protein concentrations in the samples were determined by the Bradford M. M. method (1976) using bovine serum albumin as the standard.

Superoxide dismutase activity. Superoxide dismutase (SOD, E.C. 1.15.1.1) activity was evaluated by its ability to dismutate superoxide radicals generated during the auto-oxidation of quercetin in an alkaline environment (pH 10.0), according to the method described by Kostiuk V. A. et al. (1990). The reaction was initiated by adding 0.1 mL of quercetin (1.4 μM dissolved in dimethylsulfoxide) to the reaction mixture. Absorbance at 406 nm was recorded immediately following the addition of quercetin and again after a period of 20 minutes. SOD activity was expressed in units per milligram of tissue protein.

Catalase activity. Catalase (CAT, E.C. 1.11.1.6) activity was determined by monitoring the reduction of H_2O_2 levels in the reaction mixture, measured spectrophotometrically at 410 nm, as described by Koroliuk M. A. et al. (1988). The reaction was initiated by adding 0.1 mL of the homogenate sample to the incubation medium consisting of 2 mL of 0.03% H_2O_2 solution and 1.0 mL of 4% ammonium molybdate dissolved in 12.5 mM H_2SO_4 solution (used as a blank). The absorbance of the reaction mixture was compared with the blank. One unit of catalase activity was defined as the amount of enzyme required to decompose 1 μmol H_2O_2 per minute per milligram tissue protein.

Glutathione reductase activity. Glutathione reductase (GR, E.C. 1.6.4.2) activity was measured by the method described by Glatzle D. et al. (1974). The assay quantifies NADPH consumption spectrophotometrically in the presence of oxidised glutathione (GSSG). GR catalyses the reduction of GSSG while oxidising NADPH, resulting in a decrease in absorbance at 340 nm. The reaction mixture contained 2.4 mL of 67 mM sodium phosphate buffer (pH 6.6), 0.2 mL of 7.5 mM GSSG and 0.1 mL of the homogenate sample. The oxidation rate of NADPH was monitored at 340 nm and quantified on the basis of the molar extinction coefficient of 6.22 $mM^{-1} \cdot cm^{-1}$. GR activity was expressed as nmol NADPH oxidised per minute per milligram tissue protein.

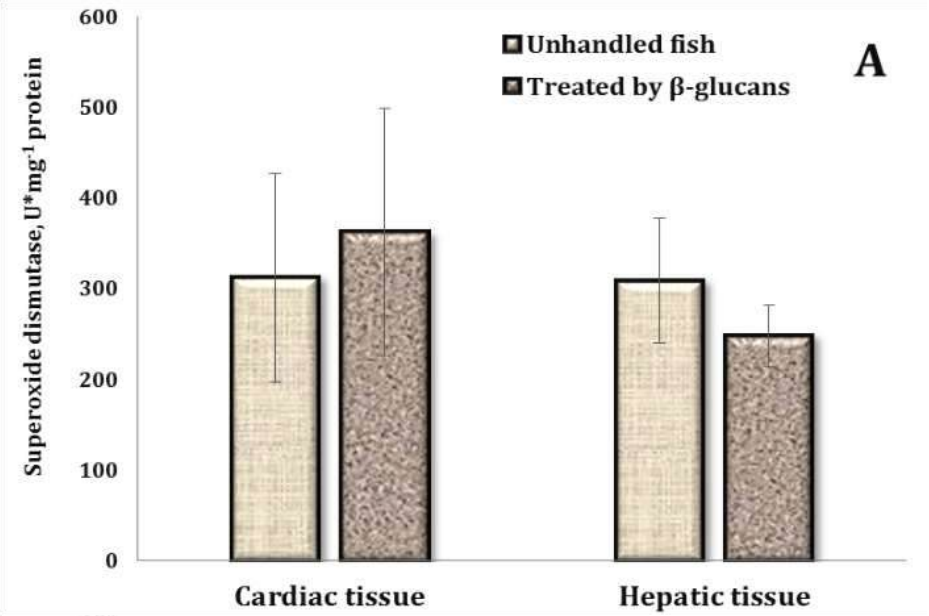
Glutathione peroxidase activity. Glutathione peroxidase (GPx, E.C. 1.11.1.9) activity was determined by measuring the non-enzymatic consumption of reduced glutathione (GSH) in a reaction mixture containing 5,5-dithiobis-2-nitrobenzoic acid (DTNB), as described by Moin V. M. (1986). The test mixture consisted of 0.8 mL 0.1 M Tris-HCl buffer containing 6 mM EDTA and 12 mM sodium azide (pH 8.9), 0.1 mL 4.8 mM GSH, 0.2 mL homogenate sample, 1 mL 20 mM t-butyl hydroperoxide and 0.1 mL 0.01 M DTNB. The rate of GSH oxidation was monitored spectrophotometrically at 412 nm. GPx activity was expressed as nmol of GSH oxidised per minute per milligram tissue protein.

Statistical analysis. Basic statistical analyses were performed using the Statistica 13.3 software package (TIBCO Software Inc., USA). Homogeneity of variance was assessed using Levene's test for equality of error variances, while normality of data was assessed using the Kolmogorov-Smirnov test. The results obtained are presented as the mean ± standard deviation (S.D.). The determination of significant differences between means was conducted through the implementation of a multiple range test, with a minimum significance level of $P < 0.05$. Comparisons between the control and experimental groups were made using multivariate analysis of variance (MANOVA) followed by Bonferroni's post-hoc test. Statistical differences were considered significant at $P < 0.05$. For data sets that did not follow a normal distribution, logarithmic transformation was applied according to Zar J. H. (1999).

Results. The activity of key antioxidant enzymes in the cardiac and hepatic tissues of rainbow trout fed a diet supplemented with β-glucans is shown in Figure 1.

The results of the present study demonstrated that the administration of low doses of β-glucans induced a decrease in SOD activity in the hepatic tissue of rainbow trout to (247.83 ± 33.90 U·mg^{-1} protein), in comparison to the untreated trout (309.17 ± 68.99 U·mg^{-1} protein) (by 19.8%, $p > 0.05$). In the cardiac tissue, an increase in SOD activity was observed, reaching (362.84 ± 136.13 U·mg^{-1} protein) in the experimental group compared to (312.56 ± 115.05 U·mg^{-1} protein) in the untreated trout (by 16.1%, $p > 0.05$) (Fig. 1A). The administration of low doses of β-glucans resulted in a statistically significant increase in CAT activity (Fig. 1B). In the hepatic tissue of rainbow trout, CAT activity was increased to (148.88 ± 48 μmol·min^{-1}·mg^{-1} protein) in comparison to the untreated trout (62.83 ± 15.3 μmol·min^{-1}·mg^{-1} protein) (by 264.6%, $p < 0.05$). In the cardiac tissue, CAT activity increased to (43.75 ± 16.08 μmol·min^{-1}·mg^{-1} protein) compared to the untreated trout (12.0 ± 4.89 μmol·min^{-1}·mg^{-1} protein) (by 135.7%, $p < 0.05$) (Fig. 1B).

The results obtained demonstrated that the administration of low doses of β-glucans induced a decrease in GR activity in the cardiac tissue of rainbow trout to (6.73 ± 1.3 nmol·min^{-1}·mg^{-1} protein), in comparison to the untreated trout (9.88 ± 6.73 nmol·min^{-1}·mg^{-1} protein) (by 31.9%, $p > 0.05$). In the hepatic tissue, GR activity was decreased to (27.2 ± 7.1 nmol·min^{-1}·mg^{-1} protein) compared to the untreated trout (52.31 ± 20.7 nmol·min^{-1}·mg^{-1} protein) (by 48%, $p > 0.05$) (Fig. 1C). The administration of low doses of β-glucans resulted in a modification in GPx activity (Fig. 1D). In the hepatic tissue of rainbow trout, GPx activity was increased to (738.45 ± 189 nmol·min^{-1}·mg^{-1} protein) in comparison to the untreated trout (179.66 ± 60.52 nmol·min^{-1}·mg^{-1} protein) (by 311%, $p < 0.05$). In the cardiac tissue, GPx activity was reduced to (352.32 ± 177.43 nmol·min^{-1}·mg^{-1} protein) in comparison to the untreated trout (406.24 ± 133.88 nmol·min^{-1}·mg^{-1} protein) (by 13.3%, $p > 0.05$) (Fig. 1D).


Superoxide dismutase, U*mg-1 protein
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500
400
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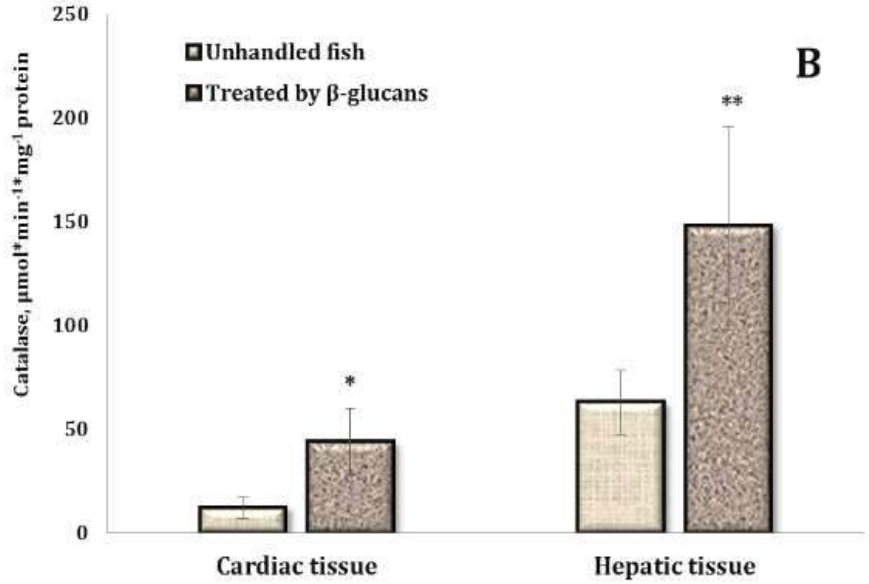
Unhandled fish
Treated by β-glucans
A
Cardiac tissue
Hepatic tissue


Catalase, µmol*min-1*mg-1 protein
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200
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Unhandled fish
Treated by β-glucans
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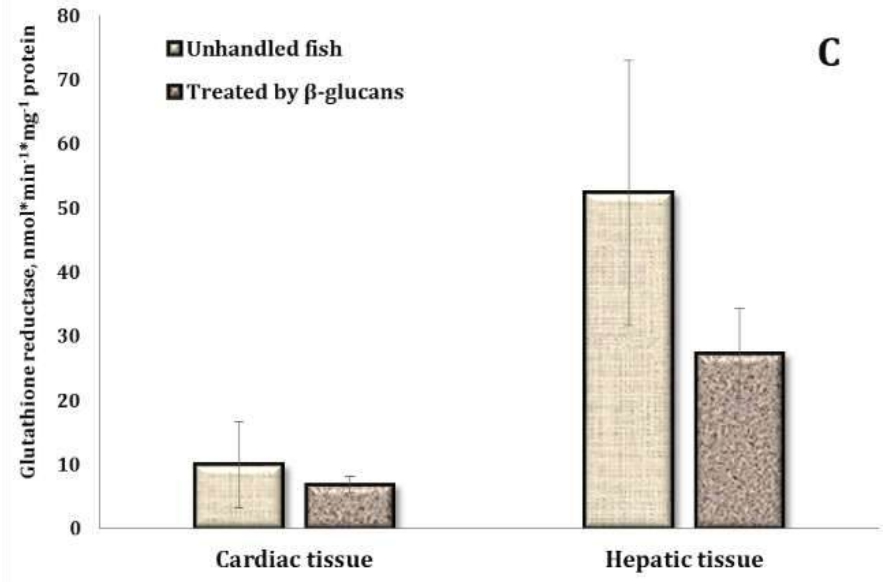
Cardiac tissue
Hepatic tissue


Glutathione reductase, nmol*min-1*mg-1 protein
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70
60
50
40
30
20
10
0
Unhandled fish
Treated by β-glucans
C
Cardiac tissue
Hepatic tissue

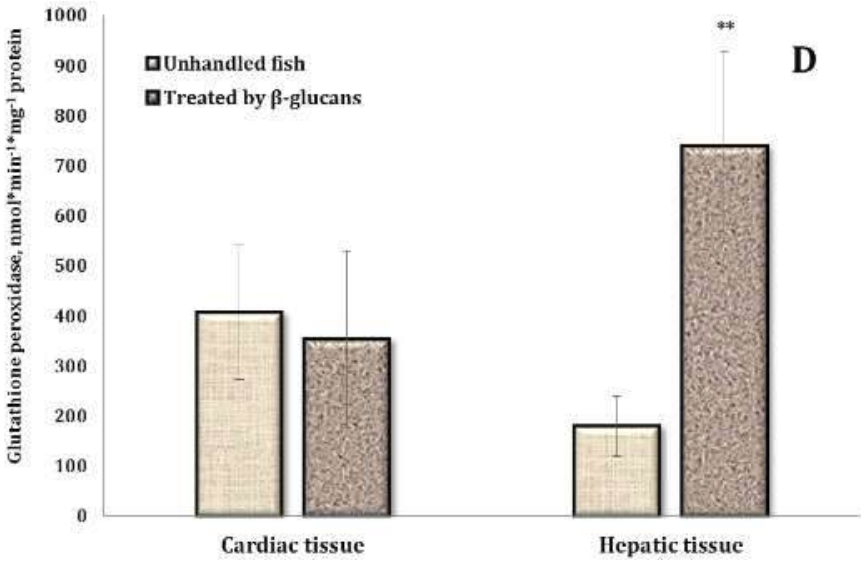

Fig. 1. **The activity of key antioxidant enzymes [superoxide dismutase (SOD, A), catalase (CAT, B), glutathione reductase (GR, C), and glutathione peroxidase (GPx, D)] in the cardiac and hepatic tissues of rainbow trout fed a diet supplemented with β-glucans.**

The results are expressed as mean ± S.D.

** and ** – Differences between the control and experimental groups were analyzed with MANOVA and Bonferroni's post-hoc test. Differences were considered significant at P < 0.05.*

Discussion. The results of this study offer insight into the modulatory effects of dietary β-glucans on antioxidant enzyme activities in the hepatic and cardiac tissues of rainbow trout. A notable finding was the differential response of superoxide dismutase (SOD) activity between hepatic and cardiac tissues. The decline in hepatic SOD activity in trout treated with β-glucans suggests a potential reduction in oxidative stress levels or a shift in the oxidative defence strategy within the liver. Conversely, the observed increase in cardiac SOD activity suggests enhanced superoxide detoxification, which could be a compensatory mechanism to counteract oxidative stress in cardiac tissue. While the changes in SOD activity did not reach statistical significance, the observed trends merit further investigation to elucidate tissue-specific responses to β-glucan supplementation.

The marked increase in catalase (CAT) activity in both hepatic and cardiac tissues following β-glucan supplementation underscores the pivotal function of this enzyme in mitigating oxidative stress by degrading hydrogen peroxide. The marked increase in hepatic CAT activity (by 264.6%, $p < 0.05$) compared to the control group underscores the pivotal role of the liver in detoxification processes. A similar increase in cardiac CAT activity (135.7%, $p < 0.05$) suggests a robust increase in antioxidant capacity, which may protect against oxidative damage in cardiac tissue. The findings suggest that β-glucans stimulate hydrogen peroxide detoxification pathways, thereby enhancing tissue resistance to oxidative stress.

Conversely, a decrease in glutathione reductase (GR) activity was observed in both liver and heart tissues, though these changes did not attain statistical significance. This decline could be indicative of alterations in the glutathione cycle, potentially reflecting an adaptive response to dietary β-glucans. The pronounced decrease in hepatic GR activity (by 48%) suggests that the hepatic glutathione pool may be differentially

modulated by β-glucan supplementation. However, further studies are required to clarify the implications of this finding for cellular redox balance.

Glutathione peroxidase (GPx) activity exhibited divergent responses in liver and heart tissues. The marked increase in hepatic GPx activity (by 311%, $p < 0.05$) indicates an increased use of reduced glutathione for peroxide detoxification, thus highlighting the central role of the liver in oxidative defence. Conversely, the modest decrease in cardiac GPx activity suggests that other antioxidant systems may compensate for peroxide detoxification in the heart. These observations underscore the tissue-specific modulation of the glutathione system by β-glucans.

In our previous study (Tkachenko H. et al., 2023), we explored the impact of dietary β-glucans on lipid peroxidation in the hepatic and cardiac tissues of three fish species: rainbow trout (*Oncorhynchus mykiss*), European whitefish (*Coregonus lavaretus*), and graylings (*Thymallus thymallus*), following a 14-day supplementation period. The results indicated a significant reduction in 2-thiobarbituric acid reactive substances (TBARS) levels, a marker of lipid peroxidation, in both tissues for rainbow trout and European whitefish. In a similar manner, graylings that were fed low doses of β-glucans for a period of 14 days exhibited decreased TBARS levels in both hepatic and cardiac tissues. These findings suggest that dietary β-glucans can effectively enhance the antioxidant defence systems in these fish species, mitigating oxidative stress by reducing lipid peroxidation. This antioxidative effect appears consistent across the examined species and tissues, demonstrating the potential of β-glucans as a beneficial dietary supplement in aquaculture. The hypothesis that low-dose β-glucan supplementation enhances antioxidant capacity in fish is supported by the observed reduction in TBARS levels in key tissues such as the liver and heart. These results are consistent with the broader understanding of β-glucans as functional feed additives with both health-promoting and growth-enhancing properties in aquaculture species (Tkachenko H. et al., 2023).

It is well established that dietary β-glucans possess immunostimulatory and antitumour properties, primarily through the activation of mucosal immune system cells via specific β-glucan receptors (Nakashima A. et al., 2018; Zhong X. et al., 2023). The receptors that have been identified include dendritic cell-associated C-type lectin-1 (Dectin-1), complement receptor 3 (CR3), differentiation cluster 11b (CD11b)/CD18, αMβ2-integrin, macrophage differentiation antigen-1 (Mac-1), lactosylceramide (LacCer), and scavenger receptors (SRs) (Zhong X. et al., 2023). β-glucans and mannans derived from yeast cell walls have been identified as alternatives to antibiotics for the prevention and treatment of animal diseases, as well as the mitigation of the emergence and spread of antibiotic-resistant bacterial pathogens (Liu Y. et al., 2021; Bar-Dagan H. et al., 2023).

When administered orally, β-glucans are absorbed through the gastrointestinal tract, taken up by tissue macrophages, and fragmented. These fragments are then transported to the bone marrow and reticuloendothelial system, where they are released and subsequently taken up by other immune cells, triggering various immunological responses (Barton C. et al., 2016; Singh R. P. and Bhardwaj A., 2023). These characteristics have prompted research into the utilisation of β-glucan particles as vaccine platforms against invasive fungal diseases (Vetvicka V. et al., 2020). Additionally, β-glucan particles are being explored as oral vaccine delivery systems, functioning both as carriers and adjuvants (Mirza Z. et al., 2017; Wu Y. et al., 2023).

As a group of polysaccharides with inherent immunostimulatory characteristics, β-glucans present promising opportunities for developing novel vaccine adjuvants. Their compatibility, safety, and tolerability render carbohydrate structures such as

polygalactans, fructans, β-D-glucans, α-D-glucans, D-galactose, and D-glucose attractive candidates for use as vaccine adjuvants and immunomodulatory (Colaço M. et al., 2022; Liang X. et al., 2024). Key factors influencing the toxicological and adjuvant properties of β-glucan-based formulations include the particle size and the method of antigen encapsulation or surface adsorption (Colaço M. et al., 2022; Jesus S. et al., 2024).

Experimental research has illuminated the mechanisms of immune activation by β-D-glucans, particularly the roles of dectin-1 and C3-iCR3 receptors. In order to optimise the therapeutic application of β-glucans, it is essential to thoroughly define biologically active molecules and to comprehensively characterise glucans from various sources chemically and biologically (Tsoni S. V. and Brown G. D., 2008; Mata-Martínez P. et al., 2022).

It is noteworthy that the physicochemical properties of β-glucans include antioxidant activity, enabling them to scavenge reactive oxygen species (ROS). In addition to their role in disease prevention, β-glucans have a significant role in the human diet as a source of fibre, which has been demonstrated to reduce cholesterol absorption, enhance digestive processes, and stimulate the production of short-chain fatty acids in the intestines (Kofuji K. et al., 2012; Nakashima A. et al., 2018).

In order to evaluate the capacity of β-glucans as natural antioxidants, an investigative approach was undertaken by Song H. S. and Moon K. Y. (2006) that entailed the examination of their antioxidant properties through the utilisation of five distinct *in vitro* methods. These methodologies encompassed the assessment of lipid peroxidation value (POV), nitric oxide (NO) scavenging, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, reducing power, and β-carotene diffusion assay. The β-glucans evaluated encompassed extracts from *Saccharomyces cerevisiae* KCTC 7911, along with both water-soluble and water-insoluble forms derived from the yeast mutant *S. cerevisiae* IS2. In the POV test, the antioxidant activities of all β-glucans were either comparable to or exceeded that of vitamin C, a well-known antioxidant. It is noteworthy that both β-glucan and water-insoluble mutant β-glucan exhibited 2.5 times the potency of vitamin C at a dosage of 2 mg. Conversely, in the NO and DPPH tests, which evaluate radical scavenging capacity, vitamin C exhibited approximately 100 times greater activity than the β-glucans. Notably, the β-glucans exhibited higher radical scavenging activity than N-acetyl-L-cysteine (NAC), a recognised radical scavenger, in the DPPH test. Furthermore, the water-insoluble mutant β-glucan demonstrated 2.6-fold and 5-fold greater activity than the water-soluble β-glucan in the NO and DPPH tests, respectively, suggesting that all β-glucans possess the capacity to effectively scavenge radicals such as NO and DPPH. In the reducing power and β-carotene diffusion assays, β-glucans exhibited lower antioxidant profiles in comparison to vitamin C. Nevertheless, β-glucan and the water-insoluble mutant β-glucan displayed marginal reducing power and significant β-carotene diffusion activity. These findings underscore the differential antioxidant potentials of the various β-glucan forms. The results obtained demonstrate that β-glucans, particularly the water-insoluble mutant form, possess notable antioxidant properties and could serve as effective natural antioxidants. The activity of β-glucans across a range of assays indicates their potential for application in health and nutrition as a viable alternative to synthetic antioxidants (Song H. S. and Moon K. Y., 2006).

The collective evidence suggests that dietary β-glucans induce tissue-specific modulations of antioxidant enzyme activities in rainbow trout, potentially enhancing oxidative resilience in liver and heart tissues. These effects are likely to arise from the bioactive properties of β-glucans, which are known to influence immune and oxidative defence pathways.Future studies should focus on long-term feeding trials and the

inclusion of additional biomarkers to fully understand the physiological effects of β-glucan supplementation.

Conclusions. The present study demonstrates that dietary supplementation with low doses of β-glucans significantly influences antioxidant enzyme activities in the hepatic and cardiac tissues of rainbow trout. The marked increases in CAT and GPx activities in the liver and heart highlight the potential of β-glucans to enhance oxidative defence mechanisms. Conversely, the observed decreases in SOD and GR activities reflect a complex, tissue-specific response to β-glucan supplementation. The findings of this study provide valuable insights into the role of dietary β-glucans in modulating oxidative stress and support their use as a functional feed additive to improve fish health and resilience.

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