DOI 10.32900/2312-8402-2023-130-231-243
UDC 639.2: 577.115.4

OXIDATIVE STRESS BIOMARKERS IN THE CARDIAC AND HEPATIC TISSUES OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WALBAUM) FED THE B-GLUCAN-SUPPLEMENTED DIET

Tkaczenko Halina, Doctor of Biological Sciences
https://orcid.org/0000-0003-3951-9005
Kurhaluk Natalia, Doctor of Biological Sciences
https://orcid.org/0000-0002-4669-1092
Institute of Biology, Pomeranian University in Słupsk, Poland
Grudniewska Joanna, Ph.D., https://orcid.org/0000-0002-4272-8337
Department of Salmonid Research, Stanisław Sakowicz Inland Fisheries Institute, Rutki, Żukowo, Poland

Treatment with β-glucans has been found to stimulate various aspects of immune responses such as resistance to infections and resistance to environmental stress. The effects of dietary β-glucans on the general health status of rainbow trout, as well as oxidative stress biomarkers in different tissues specifically should be explored. This prompted us to investigate the effects of dietary yeast β-1,3/1,6-D-glucans supplemented for a 14-day feeding period on liver and heart function and the oxidative mechanisms underlying these effects. We assessed the levels of lipid peroxidation, derivatives of the oxidatively modified proteins (OMP), and the total antioxidant capacity (TAC) in the hepatic and cardiac tissue of rainbow trout (Oncorhynchus mykiss Walbaum) after a 14-day period of oral supplementation with β-glucans. Thirty healthy rainbow trout weighing 55.9 ± 2.1 g were used in the experiments. The fish were fed with a commercial basal diet at a rate of 1.5% body weight four times a day. After acclimation, the fish were randomly divided into two groups. The groups were fed for 14 days as follows: the control group comprising rainbow trout (n = 15) received a control basal diet and the β-glucan group (n = 15) was fed with the Yestimun® food product at a dose of 1% of the basal feed (with 85% of β-1.3/1.6-glucans, Leiber GmbH, Bramsche, Germany). The basal feed was supplemented with 1% of Yestimun® powder (dose: 1 kg per 99 kg, w/w). This insoluble and highly purified preparation contains natural polysaccharides, e.g. β-1,3/1,6-D-glucans derived from Spent Brewers' Yeast (Saccharomyces cerevisiae). Yeast cell walls typically contain approximately 30% of β-glucans of dry weight. Our results showed that feeding with low doses of β-glucans induced a statistically non-significant decrease in TBARS levels in the hepatic and cardiac tissues of rainbow trout. The feeding with low doses of β-glucans induced non-significant changes in the TAC levels both in the hepatic and cardiac tissues of rainbow trout. Levels of aldehydic and ketonic derivatives of OMP in the cardiac and hepatic tissues of rainbow trout fed the β-glucan-supplemented diet were at the same levels as in the untreated controls. In conclusion, our results unambiguously showed that β-glucan did not induce oxidative stress in the hepatic and cardiac tissues of rainbow trout.

Keywords: β-glucans, lipid peroxidation, oxidatively modified proteins, total antioxidant capacity, liver, heart, Oncorhynchus mykiss.

Біомаркери окисного стресу в серцевій і печінковій тканинах райдужної форелі (*Oncorhynchus mykiss* Walbaum), яку годували дієтою з добавками β-глюкану

Ткаченко Г., д. б. н., https://orcid.org/0000-0003-3951-9005
Кургалюк Н., д. б. н., https://orcid.org/0000-0002-4669-1092
Інститут біології, Поморський університет у Слупську, Польща
Груднєвська Й., Ph.D., https://orcid.org/0000-0002-4272-8337
Інститут рибальства імені Станіслава Саковича, Руткі, Жуково, Польща

Було встановлено, що лікування β-глюканами стимулює різні аспекти імунної відповіді, такі як стійкість до інфекцій і стійкість до стресу навколишнього середовища. Вплив β-глюканів на загальний стан здоров'я райдужної форелі, а також на біомаркери окисного стресу в різних тканинах слід вивчити окремо. Це спонукало нас дослідити вплив β-1,3/1,6-D-глюканів дріжджів, доданих протягом 14-денного періоду годування, на функцію печінки та серця та окиснювальні механізми, що лежать в основі цих ефектів. Ми оцінювали рівні перекисного окиснення ліпідів, похідних окиснювально модифікованих білків (ОМБ) і загальну антиоксидантну активність (ТАС) у тканинах печінки та серця райдужної форелі (Oncorhynchus mykiss Walbaum) після 14-денного періоду перорального прийому добавок з β-глюканами. В дослідах використовували 30 здорових особин райдужної форелі вагою 55,9 ± 2,1 г. Рибу годували комерційною дієтою з розрахунку 1,5% маси тіла чотири рази на день. Після акліматизації рибу випадково розділили на дві групи. Групи годували протягом 14 днів наступним чином: контрольна група райдужної форелі (n = 15) отримувала контрольну основну дієту, а група β-глюканів (n = 15) отримувала харчовий продукт Yestimun® у дозі 1% основного корму (складається з 85% β-1,3/1,6-глюканів, Leiber GmbH, Bramsche, Німеччина). Базальний корм доповнювали 1% порошком Yestimun® (доза: 1 кг на 99 кг маси). Цей нерозчинний і високоочищений препарат містить природні полісахариди, β-1,3/1,6-D-глюкани, отримані з відпрацьованих пивних дріжджів (Saccharomyces cerevisiae). Клітинні стінки дріжджів зазвичай містять приблизно 30% β-глюканів. Наші результати показали, що годування низькими дозами β-глюканів викликало статистично незначуще зниження рівня TBARS у тканинах печінки та серця райдужної форелі. Годування низькими дозами β-глюканів викликало незначні зміни рівнів ТАС як у печінковій, так і серцевій тканинах. Рівні альдегідних і кетонових похідних ОМБ у серцевій і печінковій тканинах райдужної форелі, яку годували дієтою з добавками β-глюканів, були на тому ж рівні, що й у контрольних групах. На закінчення, наші результати однозначно показали, що β-глюкан не викликає окисного стресу в печінкових і серцевих тканинах райдужної форелі.

Ключові слова: β-глюкани, перекисне окиснення ліпідів, окиснювально модифіковані білки, загальна антиоксидантна активність, печінка, серце, Oncorhynchus mykiss

Aquaculture is a rapidly growing part of agriculture worldwide, accounting for about 44 percent of the world's total fish production. Today, preventive and management measures in aquaculture are central to dealing with outbreaks of various diseases (Assefa, A., & Abunna, F., 2018). Immunostimulators are considered an effective means of increasing the immune status of cultivated organisms. Among the various immunostimulants used in aquaculture practice, one of the promising immunostimulants

is β-glucan, which is a homopolysaccharide of a glucose molecule linked by a glycosidic bond (Meena at al., 2013). β-glucans are a group of β-D-glucose polysaccharides that form the major constituents of the cell wall of some plants, fungi, bacteria, mushrooms, yeast, and seaweeds (Singh, R. P., & Bhardwaj, A. 2023). β-glucans from different sources differ in their structure, conformation, physical properties, receptor binding affinity, and therefore biological functions (Han, at al., 2020). They are known for their metabolic and immunomodulatory properties, including anticancer, immunomodulatory, antimicrobial, antiviral, antinociception, antiinflammatory, prebiotic, antioxidant, antidiabetic, etc. (Dalonso, at al., 2015); Mirończuk-Chodakowska, I., at al., 2021). The immunostimulatory activity of β-glucan results from its attachment to specific receptors present on the surface of immune cells (Ciecierska, A. at al.,2019). In addition, β-D-glucans are also suitable for use in nanomedicine to prepare natural nanocarriers for the delivery of drugs or biological molecules [Lehtovaara, B. C., & Gu, F. X. 2011; Soto, E. R. at al., 2012; Vannucci, L., at al., 2013)

Dietary β-glucans also exert immunostimulatory and antitumor effects by activating mucosal immune system cells through β-glucan receptors (Nakashima, A., at al., 2018). Currently, the following β-glucan receptors have been identified: dendritic cell (DC)-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., at al., 2023). β-glucan and mannan from yeast cell walls can replace antibiotics for the prevention and treatment of animal diseases, and reduce the development and spread of antibiotic-resistant bacterial pathogens (Liu, Y., at al.,2021).

Orally administered β-glucans are absorbed through the gastrointestinal tract and taken up by tissue macrophages (Barton, C., at al., 2016). Here they are fragmented, transported to the bone marrow and the reticuloendothelial system, and eventually released and taken up by other immune cells, leading to various immunological effects (Chan, G. C., at al., 2009). Based on these properties, β-glucan particles have been evaluated as potential vaccines against invasive fungal diseases (Edwards J. E. 2012), and beta-glucan particles have been proposed as a delivery system for oral vaccines, acting both as a carrier and as an adjuvant (Huang, H., at al., 2013). Beta-glucans are a group of polysaccharides with inherent immunostimulatory properties, which makes it very promising to create new adjuvants for vaccines based on β-glucans (Colaço, M. at al., 2022). Carbohydrate structures – polygalactans, fructans, β-D-glucans, α-D-glucans, D-galactose, and D-glucose – are desirable candidates for vaccine adjuvants and immunomodulators because they perform important functions in nature and are often biocompatible, safe, and well tolerated (Abbasi, A. at al., 2022). The size of the particles and the loading method of the antigen encapsulated in the particles or adsorbed on their surface will influence the toxicological and adjuvant properties of the adjuvant powder (Colaço, M., at al., 2022).

Experimental studies have largely elucidated the underlying mechanisms involved in immune stimulation induced by β-D-glucans, especially with regard to the involvement of dectin-1 and C3-iCR3. A clear definition of biologically active molecules and a more detailed chemical and biological characterization of glucans from various sources seem necessary in order to better define the rationale for their use in the therapy of relevant pathologies (Vannucci, L., at al., 2013). The main physicochemical properties of β-glucans include their antioxidant properties, which are responsible for scavenging reactive oxygen species (ROS), as well as their role as a dietary fiber to prevent cholesterol absorption, improve digestion, and produce short-chain fatty acids

in the intestines (Nakashima, A., at al., 2018).

Little is known about the biochemical changes in various tissues of salmonids after oral administration of β-glucans. The effects of dietary β-glucans on the general health status of rainbow trout, as well as oxidative stress biomarkers in different tissues specifically should be explored. This prompted us to investigate the effects of dietary yeast β-1,3/1,6-D-glucans supplemented for a 14-day feeding period on liver and heart function and the oxidative mechanisms underlying these effects. We assessed the levels of lipid peroxidation, derivatives of the oxidatively modified proteins (OMP), and the total antioxidant capacity (TAC) in the hepatic and cardiac tissue of rainbow trout (*Oncorhynchus mykiss* Walbaum) after a 14-day period of oral supplementation with β-glucans.

Materials and methods.

Fish and experimental design. Thirty healthy rainbow trout (*Oncorhynchus mykiss*) weighing 55.9 ± 2.1 g were used in the experiments. The fish were kept in an indoor system with a supply of freshwater with adequate aeration and internal power filter. The water quality parameters were as follows: a temperature of 16 ± 2 °C, 12 ± 0.5 ppm of dissolved oxygen, and a pH value of 7.4–7.6. During the acclimation period (14 days), the fish were fed with a commercial basal diet at a rate of 1.5% body weight (BW) four times a day. After acclimation, the fish were randomly divided into two groups kept in aerated 250-L square tanks containing dechlorinated tap water (70 fish per tank). One tank comprised one group. Natural photoperiod conditions were maintained throughout the feeding trial. The experimental part of the study was carried out in the Department of Salmonid Research, Stanislaw Sakowicz Inland Fisheries Institute (Rutki, Poland).

The groups were fed for 14 days as follows: the control group comprising rainbow trout (n = 15) received a control basal diet and the β-glucan group (n = 15) was fed with the Yestimun® food product at a dose of 1% of the basal feed (with 85% of β-1.3/1.6-glucans, Leiber GmbH, Bramsche, Germany). The basal feed was supplemented with 1% of Yestimun® powder (dose: 1 kg per 99 kg, w/w). This insoluble and highly purified preparation contains natural polysaccharides, e.g. β-1,3/1,6-D-glucans derived from Spent Brewers' Yeast (*Saccharomyces cerevisiae*). Yeast cell walls typically contain approximately 30% of β-glucans of dry weight (Stier, H., at al., (2014).

The survival rate of fish in the different treatment groups was recorded during the feeding trial. An increase in fish weight was observed as well. At the end of the 14-day feeding period, the fish were decapitated, and the liver and heart were dissected. Blood was sampled with plastic syringes from the caudal vein. The experiments were performed in duplicate.

Hepatic and cardiac tissue isolation. Tissue samples were removed from fish after decapitation. One fish was used for each homogenate preparation. Briefly, the liver and heart were excised, weighed, and washed in the ice-cold buffer. The minced tissue was rinsed clear of blood with ice-cold 100 mM Tris-HCl isolation buffer, homogenized in 10 vol. (v/w) in isolation buffer, and centrifuged at 3,000 × g at 4 °C for 10 min. The resulting supernatant was stored in a refrigerator at −22 °C and used for analyses of enzyme activities and biomarkers of oxidative stress. The isolation buffer contained 100 mM Tris-HCl; the pH value was adjusted to 7.2 with HCl.

Biochemical assays. All enzymatic assays were carried out at 24 ± 0.5°C with the use of a Specol 11 spectrophotometer (Carl Zeiss Jena, Germany). The homogenate suspension was added to start the enzymatic reactions. The specific assay conditions are presented below. Each sample was analyzed in duplicate. The protein concentration in each sample was determined as in Bradford (1976) using bovine serum albumin as a

standard.

2-Thiobarbituric acid reactive substances (TBARS). Lipid peroxidation was determined in aliquots of 10% hepatic and cardiac tissue homogenates from the treated and control groups with the procedure developed by Kamyshnikov (2004). The absorbance of each aliquot was measured at 540 nm, and the lipid peroxidation level was expressed as nanomoles of TBARS formed per milligram of protein (nmol MDA·mg^{-1} protein) using a molar extinction coefficient of $1.56 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$ (Kamyshnikov, V.S. (2004).

The carbonyl derivatives of oxidatively modified proteins (OMP) assay. The rate of protein oxidative destruction was estimated from the reaction of the resultant carbonyl derivatives of amino acid reaction with 2,4-dinitrophenylhydrazine (DNFH) as described by Levine et al. (1990) and as modified by Dubinina et al. (1995) (Dubinina, E. at al., 1995; Levine, R. L.,at al., 1990). DNFH was used for determining carbonyl content in soluble and insoluble proteins. Carbonyl groups were determined spectrophotometrically from the difference in absorbance at 370 nm (aldehydic derivatives, OMP_{370}) and 430 nm (ketonic derivatives, OMP_{430}).

Measurement of total antioxidant capacity (TAC). The TAC level in samples was estimated by measuring the 2-thiobarbituric acid reactive substances (TBARS) level after Tween 80 oxidation. This level was determined spectrophotometrically at 532 nm (Galaktionova, L. P., at al.,1998). The sample inhibits the Fe^{2+}/ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level. The level of TAC in the sample (%) was calculated with respect to the absorbance of the blank sample.

Statistical analysis. The basic statistical analysis was performed using the Statistica 13.3 package (TIBCO Software Inc.). The data were tested for homogeneity of variance using Levene's test of equality of error variances. Normality was checked with the Kolmogorov-Smirnov test. The results are expressed as mean ± S.D. Significant differences among the means were measured with the use of a multiple-range test at min. $P < 0.05$. Differences between the control and experimental groups were analyzed with MANOVA and Bonferroni's post-hoc test. Differences were considered significant at $P < 0.05$. Non-normally distributed data were log-transformed (Zar, J.H.,1999).

Results.

Levels of TBARS (nmol MDA·mg^{-1} protein) and TAC (%) in the cardiac and hepatic tissues of rainbow trout *(O. mykiss)* fed the β-glucan-supplemented diet were presented in Fig. 1.

Our results showed that feeding with low doses of β-glucans induced a decrease in TBARS levels in the hepatic tissue of rainbow trout to (132.41 ± 26.65 nmol·mg^{-1} protein) compared to the untreated trout (147.03 ± 32.33 nmol·mg^{-1} protein) (by 9.9%, $p > 0.05$). In the cardiac tissue, TBARS level was also decreased to (39 ± 19.55 nmol·mg^{-1} protein) compared to the untreated trout (76.51 ± 30.29 nmol·mg^{-1} protein) (by 49%, $p > 0.05$) (Fig. 1A). The feeding with low doses of β-glucans induced non-significant changes in the TAC levels (Fig. 1B). In the hepatic tissue of rainbow trout, TAC level was at the same level (25.88 ± 2.35 %) compared to the untreated trout (25.90 ± 2.52 %). In the cardiac tissue, the TAC level was decreased to (27.01 ± 3.68 %) compared to the untreated trout (28.69 ± 3.91 %) (by 5.9%, $p > 0.05$) (Fig. 1A).

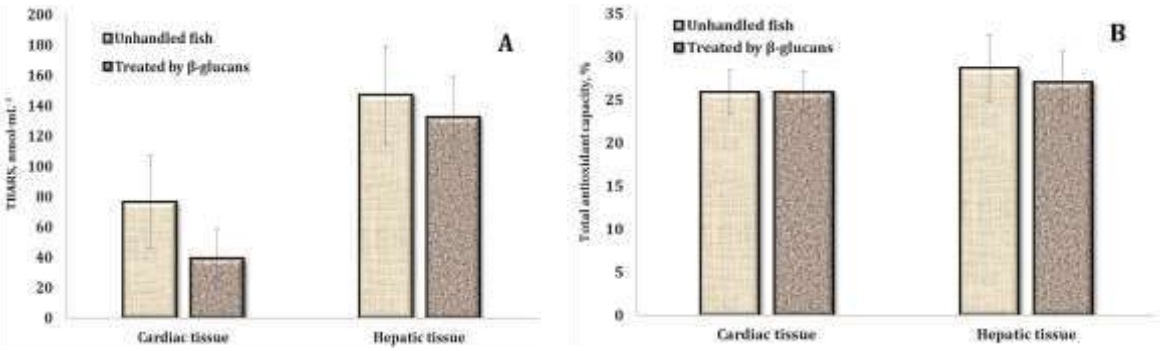

Fig. 1. **Levels of TBARS (nmol MDA·mg^{-1} protein) and TAC (%) in the cardiac and hepatic tissues of rainbow trout *(O. mykiss)* fed the β-glucan-supplemented diet.**

The results are expressed as mean ± S.D. 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.

Levels of aldehydic and ketonic derivatives of OMP (nmol·mg^{-1} protein) in the cardiac and hepatic tissues of rainbow trout *(O. mykiss)* fed the β-glucan-supplemented diet were presented in Fig. 2.

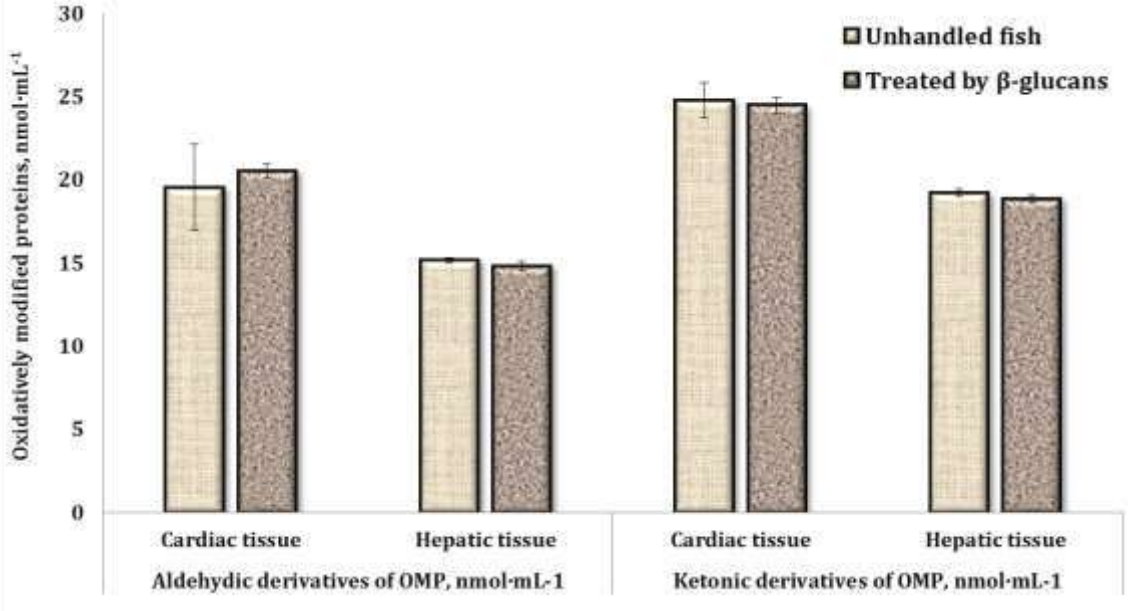

Fig. 2. **Levels of aldehydic and ketonic derivatives of OMP (nmol·mg^{-1} protein) in the cardiac and hepatic tissues of rainbow trout *(O. mykiss)* fed the β-glucan-supplemented diet.**

The results are expressed as mean ± S.D. 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.

Our results revealed that feeding with low doses of β-glucans induced an increase in aldehydic derivatives of OMP level in the hepatic tissue of rainbow trout to (20.52 ± 0.44 nmol·mg^{-1} protein) compared to the untreated trout (19.54 ± 2.60 nmol·mg^{-1} protein) (by 5%, p > 0.05). In the cardiac tissue, level of aldehydic

derivatives of OMP was at the same level (14.82 ± 0.27 nmol·mg^{-1} protein) compared to the untreated trout (15.17 ± 0.15 nmol·mg^{-1} protein) (Fig. 2). The feeding with low doses of β-glucans induced non-significant changes in the levels of ketonic derivatives of OMP (Fig. 2). In the hepatic tissue of rainbow trout, level of ketonic derivatives of OMP was at the same level (24.49 ± 0.50 nmol·mg^{-1} protein) compared to the untreated trout (24.78 ± 1.08 nmol·mg^{-1} protein). In the cardiac tissue, the level of ketonic derivatives of OMP was decreased to (18.86 ± 0.24 nmol·mg^{-1} protein) compared to the untreated trout (19.23 ± 0.22 nmol·mg^{-1} protein) (by 1.9%, $p > 0.05$) (Fig. 2).

Discussion. Our results showed that feeding with low doses of β-glucans induced a statistically non-significant decrease in TBARS levels in the hepatic and cardiac tissues of rainbow trout. The feeding with low doses of β-glucans induced non-significant changes in the TAC levels both in the hepatic and cardiac tissues of rainbow trout (Fig. 1). Membrane destruction as a pathological phenomenon is primarily due to the involvement of cell membrane lipids in the processes of lipid peroxidation (LPO), which leads to a change in lipid-protein bonds, the stability of the enzyme complexes and other membrane macromolecules, damage to nucleic acids, and disruption of cellular metabolism (Gaschler, M. M., & Stockwell, B. R., 2017; van Ginkel, G., & Sevanian, A., 1994). The classic biochemical indicators of the state of animals, which are used in assessing the effect of various medicals and toxicants on the body, include products of lipid peroxidation (LPO), which can be assessed by the accumulation of primary LPO products as diene conjugates (DC) and diene ketones (DK), as well as one of end metabolites of lipid peroxidation – malonic dialdehyde (MDA) (Gaweł, S., at al., 2004, Lefèvre, G., at al., 1998). Total antioxidant capacity (TAC) is a measure of the ability of a food or substance to reduce oxidative stress in the body. Determination of antioxidant activity plays an important role in assessing the body's defense against oxidative stress (Rubio, C. P., at al., 2016).

Levels of aldehydic and ketonic derivatives of OMP in the cardiac and hepatic tissues of rainbow trout fed the β-glucan-supplemented diet were at the same levels as in the untreated controls (Fig. 2). To date, the concept of oxidative degradation of proteins as the earliest and most reliable indicator of oxidative stress has already been clearly and voluminously formed. Since proteins are present in all tissues and organs, it is their modification that can be a reliable indicator of pathological processes both at the local and general body levels. Since proteins perform specific functions (often having a clearly visible and easily fixed manifestation), the assessment of the qualitative and quantitative aspects of oxidative modification of proteins has a number of advantages in the diagnosis of pathological conditions Hawkins, C. L., & Davies, M. J. 2019; Squier T. C. 2001); Stadtman E. R., 2001).

It has been shown that in a wide range of pathologies of the most diverse etiologies, it is an oxidative modification specifically proteins (and not lipids or nucleic acids) that is one of the earliest and most reliable markers of their presence and even just their occurrence. In addition, it has been established that oxidatively modified proteins can exist in living organisms for a long time (hours, days, and even years; for example, lipofuscin), while the primary intermediates of oxidative stress (free radicals, lipid peroxidation products) exist in a free state for much shorter (usually a few minutes, maximum a few hours). This circumstance also makes it possible to consider the phenomenon of oxidatively modified proteins in living organisms as relatively stable diagnostic parameters of their structural and functional state, which is of great importance in clinical practice (Kehm, R., at al., 2021).; Squier, T. C., & Bigelow, D. J., 2000).

Similar to our results, several authors have reported that β-glucans incorporated

in fish feed increase the non-specific resistance mechanisms and raise the counts of intestinal immune cells [Akhtar, M. S.,at al.,2021); Cornet, V.,at al., 2021; Kazuń, B., at al., 2020; Koch, J. F. A., at al., 2021). For example, Kazuń and co-workers (2020) compared the effect of dietary supplementation of β-1,3/1,6-glucan, Lactobacillus plantarum bacteria or their mixture on the growth performance, selected parameters of the immune system, as well as the liver and intestinal histology of roach. Fish were fed for 14 days with four different diets, each treatment was carried out in triplicate. In the control group, fish were fed dry commercial starter feed Aller Performa 2 (Aller Aqua, Denmark). The other experimental fish groups received the same commercial starter feed supplemented with 1% β-1,3/1,6-glucan (Leiber® Beta-S) in group G; 10^8 CFU *L. plantarum* g^{-1} in group L; 1% β-1,3/1,6-glucan + 10^8 CFU *L. plantarum* g^{-1} in group G + L. The stimulating effect of the tested preparations was evaluated once the feeding with commercial feed exclusively was resumed and 2 weeks afterward. No effect on the survivability and growth performance of the fish was observed in any of the groups. Supplementation of feed with β-1,3/1,6-glucan improved selected parameters of innate humoral immunity and the pinocytotic activity of phagocytes. Increased respiratory burst activity of head kidney phagocytes (RBA) was observed in groups L and G + L, and the effect persisted for 2 weeks after the commercial feed regime was resumed. An analogous tendency was determined for the killing activity of phagocytes (PKA) of the head kidney with respect to *Aeromonas hydrophila*, although this effect appeared only during the feed supplementation period. Supplying roach with β-1,3/1,6-glucan, singly or with L. plantarum, had no effect on the proliferation of mitogen-activated lymphocytes. However, an increase in the number of CD3-positive cells and goblet cells was noticed in the digestive system of the L-group fish (Kazuń, B., at al.,2020).

An eight-week feeding trial was performed by Akhtar and co-workers (2021) to assess the effect of different dietary levels (0, 0.5, 1.0, and 1.5%) of β-glucan (sourced from *Saccharomyces cerevisiae*) on growth, survival, immunological parameters (immune gene expression, lysozyme, and antiprotease), total antioxidant status, thermal tolerance, and disease resistance of *Tor putitora* fry (Akhtar, M. S., at al.,2021). Feeding of moderate doses (0.5 and 1.0%) of β-glucan significantly improved survival but not weight gain percentage as compared to that received unsupplemented control and the highest dose (1.5%) of glucan. Supplementation of β-glucan in diets differentially influenced the mRNA expression of cytokine and other immune genes. On the other hand, the dietary inclusion of β-glucan markedly improved total antioxidant levels and extended the thermal tolerance limits at both ends. After feeding β-glucan for eight weeks, the fish were bath-challenged with a bacterial pathogen, *Aeromonas salmonicida*. The challenge study results revealed that β-glucan intake improved most of the studied immune parameters, resulting in lower mortality. Overall, dietary inclusion of β-glucan (0.5-1.0%) was efficient in improving the immune responses, thermal tolerance, and disease resistance of *T. putitora* fry (Akhtar, M. S., at al.,2021).

In the study conducted by Valérie Cornet and co-workers (2021), the immunostimulant effects of two β-glucan types extracted from wild-type baker's yeast (*S. cerevisiae*) and its null-mutant Gas1 were investigated. Gas1 has a β-1,3-glucanosyltransferase activity necessary for cell wall assembly. Using a positive (commercial product MacroGard®) and negative control (a diet without glucans), these researchers evaluated the immune responses and disease resistance of rainbow trout juveniles (mean weight, ~44 g) fed control, low (0.2%) and high (0.5%) doses of Macrogard®, Gas1, and Wild type-β-glucan after a short-term (15 days, D15) or mid-term (36 days, D36) feeding periods (Cornet, V., at al., 2021). These researchers found that β-glucan supplemented diets did not affect growth performance, mortality, splenic

index, or leukocyte respiratory burst activity on D15 nor D36. Each β-glucan triggered different immune effectors, depending on the doses or length of exposure compared to others and the negative control. Indeed, a high dose of MacroGard® significantly increased lysozyme activities at D15 compared with the control and other diets. At D36, MacroGard β-glucan enhanced the production of lymphocytes in comparison with the control diet. Regarding WT β-glucan, at D36, WT-β-glucan, especially the high dose, provided the highest enzymatic activities (lysozyme and ACH50) and Ig level. On D36, Gas1 also increased lysozyme activity, Ig proportion, and some immune genes (mcsfra, hepcidin) compared with MacroGard®. Besides, both doses of Gas1-β-glucans increased the resistance of juveniles to bacterial infection highlighted by a higher survival rate at 14 days post-challenge compared with the control and other types and doses of β-glucans (Cornet, V., at al., 2021).

Dietary β-glucan (MacroGard®) improves innate immune responses and disease resistance in Nile tilapia regardless of the administration period. Koch and co-workers (2021) demonstrated that β-glucan improved the innate immune responses and the tilapia's resistance to disease, and this protection could be observed up to 10 days post-feeding trial, adding *in vivo* evidence that β-glucan may contribute to a trained innate immunity. Additionally, these researchers showed that a longer period of administration did not cause immunosuppression as previously hypothesized but promoted further growth and immune performance (Kehm, R., at al., 2021). Sabioni and co-workers (2020) demonstrated that β-glucan enhances the respiratory activity of leukocytes suppressed by stress and modulates blood glucose levels in pacu (*Piaractus mesopotamicus*). These findings confirm the immunomodulatory action of glucan and add evidence showing that glucan can have a role in stress response (Sabioni, R. E., at al., 2020). . Also, Do Huu and co-workers (2016) revealed that dietary β-glucan improved growth performance, *Vibrio* counts, hematological parameters, and stress resistance of pompano fish, *Trachinotus ovatus* L. (Do Huu, H., at al., 2016).

The dietary intake of 0.75% β-glucan improved resistance to ammonia stress to a certain degree by activating the anti-oxidative system and reducing brachial ammonia uptake as shown by Ciji and co-workers (2023). The study investigated the effects of dietary administration of β-glucan on aquaporins and antioxidative & immune gene expression in endangered golden mahseer, *Tor putitora* juveniles, exposed to ammonia stress. For that, fish were fed experimental diets having 0 (control/basal), 0.25, 0.5, and 0.75% β-d-glucan for five weeks and then exposed to ammonia (10 mgL-1 total ammonia nitrogen) for 96 h. Administration of β-glucan differentially influenced the mRNA expression of aquaporins, anti-oxidative, and immune genes in ammonia-exposed fish. For instance, the transcript abundance of catalase and glutathione-s-transferase in gill varied significantly among the treatment groups, with the lowest levels in 0.75% β-glucan fed groups. At the same time, their hepatic mRNA expression was similar (Ciji, A., AT AL., 2023).

Zeng and co-workers (2018) evaluated and investigated the effects of β-glucan on oxidative stress, inflammation, and copper transport in two intestinal regions of a large yellow croaker (*Larimichthys crocea*) under acute copper stress. Biochemical indicators (MDA, Cu content, MTs protein levels, Cu,Zn-SOD, CAT and iNOS activities), gene expressions of oxidative stresses (Cu,Zn-SOD, CAT, Nrf2, MTs and MTF-1), inflammatory responses (NF-κB, iNOS, IL-1β, IL-6 and TNF-α) and Cu transporters (ATP7A, ATP7B and CTR1) were determined. In the anterior intestine, β-glucan increased MT levels, activities of Cu,Zn-SOD, CAT, and iNOS, mRNA levels of MTs, CAT, iNOS, ATP7A, and ATP7B, and reduced Cu content and CTR1 gene expression to inhibit Cu-induced MDA. However, β-glucan had no effect on

inflammatory gene expressions. In the mid intestine, β-glucan increased activities of Cu,Zn-SOD and iNOS, mRNA levels of Cu,Zn-SOD, CAT, and iNOS to maintain MDA content. However, unlike the anterior intestine, β-glucan had no effect on Cu transporter gene expressions. Furthermore, transcription factors (Nrf2, NF-κB, and MTF-1) paralleled with their target genes in the mid-intestine, but no correlation was observed between NF-κB and IL-1β and TNF-α gene expressions in the anterior intestine (Zeng, L.,at al., 2018)..

In our previous study (Tkachenko H., at al., 2023), we assessed relevant lipid peroxidation in the hepatic and cardiac tissue of rainbow trout (*Oncorhynchus mykiss*), European whitefish (*Coregonus lavaretus*), and graylings (*Thymallus thymallus*) after a 14-day period of supplementation with β-glucans. Our results showed that feeding with low doses of β-glucans induced a decrease in TBARS levels in the hepatic and cardiac tissues of rainbow trout, and European whitefish. Similarly, 14 days of feeding graylings with low doses of β-glucans resulted in a decrease in the TBARS levels both in the hepatic and cardiac tissues. This study confirms that dietary β-glucan is beneficial for promoting growth and enhancing antioxidant capacity against oxidative stress in rainbow trout, European whitefish, and graylings. Indeed, we cautiously hypothesized that feeding low β-glucans doses may help to boost antioxidant function, especially by the decrease of biomarkers of lipid peroxidation in the hepatic and cardiac tissues of these fish.

Conclusions. The effects of dietary β-glucans on the oxidative stress biomarkers in different tissues specifically were explored. We investigated the effects of dietary yeast β-1,3/1,6-D-glucans supplemented for a 14-day feeding period on liver and heart function and the oxidative mechanisms underlying these effects. We assessed the levels of lipid peroxidation, derivatives of the oxidatively modified proteins (OMP), and the total antioxidant capacity (TAC) in the hepatic and cardiac tissue of rainbow trout after a 14-day period of oral supplementation with β-glucans. Our results showed that feeding with low doses of β-glucans induced a statistically non-significant decrease in TBARS levels in the hepatic and cardiac tissues of rainbow trout. The feeding with low doses of β-glucans induced non-significant changes in the TAC levels both in the hepatic and cardiac tissues of rainbow trout. Levels of aldehydic and ketonic derivatives of OMP in the cardiac and hepatic tissues of rainbow trout fed the β-glucan-supplemented diet were at the same levels as in the untreated controls. In conclusion, our results unambiguously showed that β-glucan did not induce oxidative stress in the hepatic and cardiac tissues of rainbow trout.

References

Abbasi, A., Rahbar Saadat, T., & Rahbar Saadat, Y. (2022). Microbial exopolysaccharides-β-glucans-as promising postbiotic candidates in vaccine adjuvants. *International journal of biological macromolecules*, 223(Pt A), 346–361. https://doi.org/10.1016/j.ijbiomac.2022.11.003.

Akhtar, M. S., Tripathi, P. H., Pandey, A., & Ciji, A. (2021). β-glucan modulates non-specific immune gene expression, thermal tolerance and elicits disease resistance in endangered *Tor putitora* fry challenged with *Aeromonas salmonicida*. *Fish & shellfish immunology*, 119, 154–162. https://doi.org/10.1016/j.fsi.2021.09.038.

Assefa, A., & Abunna, F. (2018). Maintenance of Fish Health in Aquaculture: Review of Epidemiological Approaches for Prevention and Control of Infectious Disease of Fish. *Veterinary medicine international*, 2018, 5432497. https://doi.org/10.1155/2018/5432497.

Barton, C., Vigor, K., Scott, R., Jones, P., Lentfer, H., Bax, H. J., Josephs, D. H., Karagiannis, S. N., & Spicer, J. F. (2016). Beta-glucan contamination of

pharmaceutical products: How much should we accept?. *Cancer immunology, immunotherapy: CII*, 65(11), 1289–1301. https://doi.org/10.1007/s00262-016-1875-9.

Chan, G. C., Chan, W. K., & Sze, D. M. (2009). The effects of beta-glucan on human immune and cancer cells. *Journal of hematology & oncology*, 2, 25. https://doi.org/10.1186/1756-8722-2-25.

Ciecierska, A., Drywień, M. E., Hamulka, J., & Sadkowski, T. (2019). Nutraceutical functions of beta-glucans in human nutrition. *Roczniki Panstwowego Zakladu Higieny*, 70(4), 315–324. https://doi.org/10.32394/rpzh.2019.0082.

Ciji, A., Tripathi, P. H., Pandey, A., & Akhtar, M. S. (2023). Expression of genes encoding non-specific immunity, anti-oxidative status and aquaporins in β-glucan-fed golden mahseer (*Tor putitora*) juveniles under ammonia stress. *Fish and shellfish immunology reports*, 4, 100100. https://doi.org/10.1016/j.fsirep.2023.100100.

Colaço, M., Panão Costa, J., & Borges, O. (2022). Glucan Particles: Choosing the Appropriate Size to Use as a Vaccine Adjuvant. *Methods in molecular biology (Clifton, N.J.)*, 2412, 269–280. https://doi.org/10.1007/978-1-0716-1892-9_13.

Cornet, V., Khuyen, T. D., Mandiki, S. N. M., Betoulle, S., Bossier, P., Reyes-López, F. E., Tort, L., & Kestemont, P. (2021). GAS1: A New β-Glucan Immunostimulant Candidate to Increase Rainbow Trout (*Oncorhynchus mykiss*) Resistance to Bacterial Infections With *Aeromonas salmonicida achromogenes*. *Frontiers in immunology*, 12, 693613. https://doi.org/10.3389/fimmu.2021.693613.

Dalonso, N., Goldman, G. H., & Gern, R. M. (2015). β-(1→3),(1→6)-Glucans: medicinal activities, characterization, biosynthesis and new horizons. *Applied microbiology and biotechnology*, 99(19), 7893–7906. https://doi.org/10.1007/s00253-015-6849-x.

Do Huu, H., Sang, H. M., & Thanh Thuy, N. T. (2016). Dietary β-glucan improved growth performance, *Vibrio* counts, haematological parameters and stress resistance of pompano fish, *Trachinotus ovatus* Linnaeus, 1758. *Fish & shellfish immunology*, 54, 402–410. https://doi.org/10.1016/j.fsi.2016.03.161.

Dubinina, E. E., Burmistrov, S. O., Khodov, D. A., & Porotov, I. G. (1995). Okislitel'naia modifikatsiia belkov syvorotki krovi cheloveka, metod ee opredeleniia [Oxidative modification of human serum proteins. A method of determining it]. *Voprosy meditsinskoi khimii*, 41(1), 24–26.

Edwards J. E. (2012). Fungal cell wall vaccines: an update. *Journal of medical microbiology*, 61(Pt 7), 895–903. https://doi.org/10.1099/jmm.0.041665-0.

Galaktionova, L. P., Molchanov, A. V., El'chaninova, S. A., & Varshavskiĭ, B. I.a (1998). Sostoianie perekisnogo okisleniia u bol'nykh s iazvennoĭ bolezn'iu zheludka i dvenadtsatiperstnoĭ kishki [Lipid peroxidation in patients with gastric and duodenal peptic ulcers]. Klinicheskaia laboratornaia diagnostika, (6), 10–14.

Gaschler, M. M., & Stockwell, B. R. (2017). Lipid peroxidation in cell death. *Biochemical and biophysical research communications*, 482(3), 419–425. https://doi.org/10.1016/j.bbrc.2016.10.086.

Gaweł, S., Wardas, M., Niedworok, E., & Wardas, P. (2004). Dialdehyd malonowy (MDA) jako wskaźnik procesów peroksydacji lipidów w organizmie [Malondialdehyde (MDA) as a lipid peroxidation marker]. *Wiadomosci lekarskie (Warsaw, Poland: 1960)*, 57(9-10), 453–455.

Han, B., Baruah, K., Cox, E., Vanrompay, D., & Bossier, P. (2020). Structure-Functional Activity Relationship of β-Glucans From the Perspective of Immunomodulation: A Mini-Review. *Frontiers in immunology*, 11, 658.

https://doi.org/10.3389/fimmu.2020.00658.

Hawkins, C. L., & Davies, M. J. (2019). Detection, identification, and quantification of oxidative protein modifications. *The Journal of biological chemistry*, 294(51), 19683–19708. https://doi.org/10.1074/jbc.REV119.006217.

Huang, H., Ostroff, G. R., Lee, C. K., Specht, C. A., & Levitz, S. M. (2013). Characterization and optimization of the glucan particle-based vaccine platform. *Clinical and vaccine immunology: CVI*, 20(10), 1585–1591. https://doi.org/10.1128/CVI.00463-13.

Kamyshnikov, V.S. (2004). *A reference book on the clinic and biochemical research and laboratory diagnostics*. MEDpress-inform, Moscow.

Kazuń, B., Małaczewska, J., Kazuń, K., Kamiński, R., Adamek-Urbańska, D., & Żylińska-Urban, J. (2020). Dietary administration of β-1,3/1,6-glucan and *Lactobacillus plantarum* improves innate immune response and increases the number of intestine immune cells in roach (*Rutilus rutilus*). *BMC veterinary research*, 16(1), 216. https://doi.org/10.1186/s12917-020-02432-1

Kehm, R., Baldensperger, T., Raupbach, J., & Höhn, A. (2021). Protein oxidation - Formation mechanisms, detection and relevance as biomarkers in human diseases. *Redox biology*, 42, 101901. https://doi.org/10.1016/j.redox.2021.101901

Koch, J. F. A., de Oliveira, C. A. F., & Zanuzzo, F. S. (2021). Dietary β-glucan (MacroGard®) improves innate immune responses and disease resistance in Nile tilapia regardless of the administration period. *Fish & shellfish immunology*, 112, 56–63. https://doi.org/10.1016/j.fsi.2021.02.014

Lefèvre, G., Beljean-Leymarie, M., Beyerle, F., Bonnefont-Rousselot, D., Cristol, J. P., Thérond, P., & Torreilles, J. (1998). Evaluation de la peroxydation lipidique par le dosage des substances réagissant avec l'acide thiobarbiturique [Evaluation of lipid peroxidation by measuring thiobarbituric acid reactive substances]. *Annales de biologie clinique*, 56(3), 305–319.

Lehtovaara, B. C., & Gu, F. X. (2011). Pharmacological, structural, and drug delivery properties and applications of 1,3-β-glucans. *Journal of agricultural and food chemistry*, 59(13), 6813–6828. https://doi.org/10.1021/jf200964u

Levine, R. L., Garland, D., Oliver, C. N., Amici, A., Climent, I., Lenz, A. G., Ahn, B. W., Shaltiel, S., & Stadtman, E. R. (1990). Determination of carbonyl content in oxidatively modified proteins. *Methods in enzymology*, 186, 464–478. https://doi.org/10.1016/0076-6879(90)86141-h.

Liu, Y., Wu, Q., Wu, X., Algharib, S. A., Gong, F., Hu, J., Luo, W., Zhou, M., Pan, Y., Yan, Y., & Wang, Y. (2021). Structure, preparation, modification, and bioactivities of β-glucan and mannan from yeast cell wall: A review. *International journal of biological macromolecules*, 173, 445–456. https://doi.org/10.1016/j.ijbiomac.2021.01.125.

Meena, D. K., Das, P., Kumar, S., Mandal, S. C., Prusty, A. K., Singh, S. K., Akhtar, M. S., Behera, B. K., Kumar, K., Pal, A. K., & Mukherjee, S. C. (2013). Beta-glucan: an ideal immunostimulant in aquaculture (a review). *Fish physiology and biochemistry*, 39(3), 431–457. https://doi.org/10.1007/s10695-012-9710-5.

Mirończuk-Chodakowska, I., Kujawowicz, K., & Witkowska, A. M. (2021). Beta-Glucans from Fungi: Biological and Health-Promoting Potential in the COVID-19 Pandemic Era. *Nutrients*, 13(11), 3960. https://doi.org/10.3390/nu13113960.

Nakashima, A., Yamada, K., Iwata, O., Sugimoto, R., Atsuji, K., Ogawa, T., Ishibashi-Ohgo, N., & Suzuki, K. (2018). β-Glucan in Foods and Its Physiological Functions. *Journal of nutritional science and vitaminology*, 64(1), 8–17. https://doi.org/10.3177/jnsv.64.8.

Rubio, C. P., Hernández-Ruiz, J., Martinez-Subiela, S., Tvarijonaviciute, A., & Ceron, J. J. (2016). Spectrophotometric assays for total antioxidant capacity (TAC) in dog serum: an update. *BMC veterinary research*, 12(1), 166. https://doi.org/10.1186/s12917-016-0792-7.

Sabioni, R. E., Zanuzzo, F. S., Gimbo, R. Y., & Urbinati, E. C. (2020). β-Glucan enhances respiratory activity of leukocytes suppressed by stress and modulates blood glucose levels in pacu (*Piaractus mesopotamicus*). *Fish physiology and biochemistry*, 46(2), 629–640. https://doi.org/10.1007/s10695-019-00739-x.

Singh, R. P., & Bhardwaj, A. (2023). β-glucans: a potential source for maintaining gut microbiota and the immune system. *Frontiers in nutrition*, 10, 1143682. https://doi.org/10.3389/fnut.2023.1143682.

Soto, E. R., Caras, A. C., Kut, L. C., Castle, M. K., & Ostroff, G. R. (2012). Glucan particles for macrophage targeted delivery of nanoparticles. *Journal of drug delivery*, 2012, 143524. https://doi.org/10.1155/2012/143524.

Squier T. C. (2001). Oxidative stress and protein aggregation during biological aging. *Experimental gerontology*, 36(9), 1539–1550. https://doi.org/10.1016/s0531-5565(01)00139-5.

Squier, T. C., & Bigelow, D. J. (2000). Protein oxidation and age-dependent alterations in calcium homeostasis. *Frontiers in bioscience: a journal and virtual library*, 5, D504–D526. https://doi.org/10.2741/squire.

Stadtman E. R. (2001). Protein oxidation in aging and age-related diseases. *Annals of the New York Academy of Sciences*, 928, 22–38. https://doi.org/10.1111/j.1749-6632.2001.tb05632.x.

Stier, H., Ebbeskotte, V., & Gruenwald, J. (2014). Immune-modulatory effects of dietary Yeast Beta-1,3/1,6-D-glucan. *Nutrition journal*, 13, 38. https://doi.org/10.1186/1475-2891-13-38.

Tkachenko H., Kurhaluk N., Grudniewska J. 2023. Effects of dietary yeast β-1.3/1.6-glucans on lipid peroxidation in the hepatic and cardiac tissues of rainbow trout (*Oncorhynchus mykiss* Walbaum), European whitefish (*Coregonus lavaretus* L.), and grayling (*Thymallus thymallus* L.). *Scientific and Technical Bulletin of the Institute of Animal Science of the National Academy of Agrarian Science of Ukraine*, 129, 16-25. https://doi.org/10.32900/2312-8402-2023-129-16-25.

van Ginkel, G., & Sevanian, A. (1994). Lipid peroxidation-induced membrane structural alterations. *Methods in enzymology*, 233, 273–288. https://doi.org/10.1016/s0076-6879(94)33031-x.

Vannucci, L., Krizan, J., Sima, P., Stakheev, D., Caja, F., Rajsiglova, L., Horak, V., & Saieh, M. (2013). Immunostimulatory properties and antitumor activities of glucans (Review). *International journal of oncology*, 43(2), 357–364. https://doi.org/10.3892/ijo.2013.1974.

Zar, J.H. (1999). *Biostatistical Analysis.* 4th ed., Prentice-Hall Inc., Englewood Cliffs, New Jersey.

Zeng, L., Wang, Y. H., Ai, C. X., & Zhang, J. S. (2018). Differential effects of β-glucan on oxidative stress, inflammation and copper transport in two intestinal regions of large yellow croaker *Larimichthys crocea* under acute copper stress. *Ecotoxicology and environmental safety*, 165, 78–87. https://doi.org/10.1016/j.ecoenv.2018.08.098.

Zhong, X., Wang, G., Li, F., Fang, S., Zhou, S., Ishiwata, A., Tonevitsky, A. G., Shkurnikov, M., Cai, H., & Ding, F. (2023). Immunomodulatory Effect and Biological Significance of β-Glucans. *Pharmaceutics*, 15(6), 1615. https://doi.org/10.3390/pharmaceutics15061615.