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EFFECTS OF DIETARY YEAST B-1.3/1.6-GLUCANS ON LIPID PEROXIDATION IN THE HEPATIC AND CARDIAC TISSUES OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WALBAUM), EUROPIAN WHITEFISH (*COREGONUS LAVARETUS* L.), AND GRAYLING (*THYMALLUS THYMALLUS* L.)

Tkachenko H., Doctor of Biological Sciences
https://orcid.org/0000-0003-3951-9005
Kurhaluk N., Doctor of Biological Sciences
https://orcid.org/0000-0002-4669-1092
Institute of Biology and Earth Sciences, Pomeranian University in Słupsk, Poland
Grudniewska J., Ph.D., https://orcid.org/0000-0002-4272-8337
Stanisław Sakowicz Inland Fisheries Institute, Rutki, Żukowo, Poland

Dietary β-glucans may be a useful tool to prime the host immune system and increase resistance against invading pathogens as the β-glucans influence the immune response. 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 cardiac function and the oxidative mechanisms underlying these effects. 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. Thirty healthy grayling weighing 34.9 ± 1.9 g, thirty healthy rainbow trout weighing 55.9 ± 2.1 g, and thirty healthy European whitefish weighing 43.3 ± 2.7 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 six groups. The groups were fed for 14 days as follows: the control groups comprising grayling (n = 15), rainbow trout (n = 15), and European whitefish (n = 15) received a control basal diet and the β-glucan groups were 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 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.

Keywords: **β-glucans, oxidative stress, lipid peroxidation, *Thymallus thymallus, Oncorhynchus mykiss, Coregonus lavaretus***

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. It forms the main components of the cell walls of some plants, fungi, bacteria, fungi, yeasts, and algae [17]. Antitumor, immunomodulatory, antimicrobial, antinociception, antiinflammatory, prebiotic, antioxidant, and antidiabetic are some of the different properties already described for β-glucans [8]. β-Glucans are a class of polysaccharides consisting of D-glucose units that are polymerized primarily *via* the β-1,3 glycosidic bonds, in addition to the β-1,4 and/or β-1,6 bonds [20]. Dietary β-glucans exert immunostimulatory and antitumor effects by acting on cells of the mucosal immune system *via* β-glucan receptors, such as dectin-1 [20]. The immunostimulatory activity of β-glucans occurs as a result of its attachment to specific receptors present on the immune cell surface [17].

In vitro, as well as *in vivo* animal and human studies demonstrate that especially β-glucans derived from fungi and yeast exhibit immunomodulatory properties [30]. Many studies have shown that β-glucan and mannan from yeast cell walls have the potential to replace antibiotics for the prevention and treatment of animal diseases, thereby reducing the development and spread of antibiotic-resistant bacterial pathogens [15]. β-Glucans appear to be effective at enhancing immune function and reducing susceptibility to infection and cancer [19].

In recent years, the effective immunomodulatory properties of β-Glucans derived from bacteria, fungi, algae, and plants have been extensively proved, not only in mammals but also in fish [30]. β-glucan naturally forms polysaccharides with glucose linked by β-glycosidic bonds [29] and can stimulate macrophages to actively fight against fish pathogens [6]. They can also enhance the activity of non-specific immune factors such as lysozyme and the complement system [11], alter immune cytokine-like gene expressions, such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) [17]. The β-glucan which forms a structural part of yeast, is able to generate immune activity in fish due to recognition by cellular receptors [16]. Non-digestible β-glucans may induce alterations in the composition of the gut microbiota and thereby indirectly influence the immune system and/or the bacterial community in the gut may help to digest non-digestible oligosaccharides such as β-glucans into short-chain fatty acids with a physiological effect of their own [21, 28].

Little is known about the biochemical changes in various tissues of salmonids after oral administration of β-glucan. The effects of dietary β-glucans on the general health status of three fish species (rainbow trout, European whitefish, grayling) 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 function and the oxidative mechanisms underlying these effects. 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.

Materials and methods. Fish and experimental design. Thirty healthy grayling (*Thymallus thymallus*) weighing 34.9 ± 1.9 g, thirty healthy rainbow trout (*Oncorhynchus mykiss*) weighing 55.9 ± 2.1 g, and thirty healthy European whitefish (*Coregonus lavaretus*) weighing 43.3 ± 2.7 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 six 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 groups comprising grayling (n = 15), rainbow trout (n = 15), and European whitefish (n = 15) received a control basal diet and the β-glucan groups were 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 [27].

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 [5].

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}·cm^{-1} [14].

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 [31].

Results and discussion. Oral administration of β-glucan resulted in better growth performance in rainbow trout fry compared to fish fed the other diets. As the

healthy and positive growth of fish that are in the early stages of development guarantees successful production in the aquaculture industry, the age of fish used in our study was chosen.

Lipid peroxidation can be described broadly as the process by which oxidizing agents, such as free radicals or non-radical species, attack lipids containing carbon-carbon double bonds, especially polyunsaturated fatty acids (PUFAs) that involve hydrogen abstraction from carbon, with oxygen insertion resulting in lipid peroxyl radicals and hydroperoxides [2]. Determining levels of MDA (malonaldehyde) by thiobarbituric acid reactive substances assay allows for measuring the levels of lipid peroxidation [13]. Levels of TBARS (nmol MDA·mg^{-1} protein) in the cardiac and hepatic tissues of rainbow trout *(O. mykiss)*, European whitefish (*C. lavaretus*), and grayling (*T. thymallus*) fed the β-glucan-supplemented diet were presented in Fig. 1.

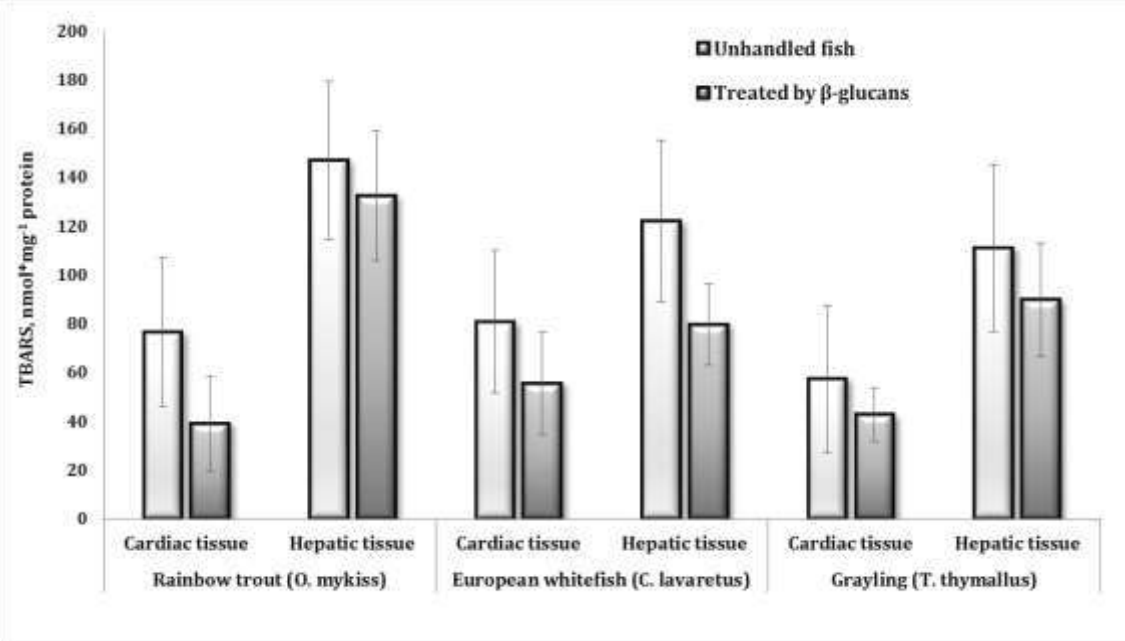

Fig. 1. Levels of TBARS (nmol MDA·mg^{-1} protein) in the cardiac and hepatic tissues of rainbow trout *(O. mykiss)*, European whitefish (*C. lavaretus*), and grayling (*T. thymallus*) fed the β-glucan-supplemented diet.

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.

In the current study, the levels of lipid peroxidation-related biomarkers (TBARS) were evaluated. Our results showed that feeding with low doses of β-glucans induced a decrease in TBARS level 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). In the hepatic tissue of European whitefish, the TBARS level was decreased to (79.59 ± 16.79 nmol·mg^{-1} protein) compared to the untreated fish (122.05 ± 33.14 nmol·mg^{-1} protein) (by 34.8%, p > 0.05) after 14 days of feeding with low doses of β-glucans. In the cardiac tissue, TBARS level was also decreased to (55.54 ± 20.76 nmol·mg^{-1} protein) compared to the untreated group (80.74 ± 29.13 nmol·mg^{-1} protein) (by 31.2%, p > 0.05). Similarly, 14 days of feeding graylings with low doses of β-glucans resulted in a decrease of the TBARS level in the hepatic tissue to (89.74 ± 23.44 nmol·mg^{-1} protein) compared to the untreated

fish (110.97 ± 34.34 nmol·mg^{-1} protein) (by 19.1%, p > 0.05). In the cardiac tissue, TBARS level was also decreased to (42.67 ± 11.22 nmol·mg^{-1} protein) compared to the untreated group (57.36 ± 30.23 nmol·mg^{-1} protein) (by 25.6%, p > 0.05) (Fig. 1).

Similar to our results, several authors have reported that β-glucans such as yeast glucan obtained from *Saccharomyces cerevisiae* incorporated in fish feed increase the growth rate of certain species [1, 7, 18]. The aim of an immunostimulant treatment is to improve immune response and disease resistance but it may also help to counteract the immunosuppressive effects of stress. The results of Dietrich-Muszalska and co-workers (2011) indicated that β-glucan seems to have distinctly protective effects against the impairment of plasma lipid molecules. These researchers showed that in the presence of β-glucan, lipid peroxidation in plasma samples treated with haloperidol was significantly decreased. Moreover, they did not observe the synergistic action of β-glucan and amisulpride on the inhibition of plasma lipid peroxidation. However, the β-d-glucan was found to be a more effective antioxidant, than the solution of pure resveratrol [9]. β-Glucan as a potentially safe and effective dietary supplement may be used for a prolonged time for systemic photoprotection of humans [24]. β-1,3-glucans can extend the lifespan, delay the onset of age-related biomarkers and exert an antioxidant action on the aged fish, *Nothobranchius guentheri*. It also implies that β-1,3-glucans may be potentially useful for health care in the elderly, including the extension of the lifespan [26].

As already suggested in previous studies [10], desensitization of the stress axis to the prolonged β-glucans stimulation probably occurred as a result of hormonal feedback regulation. However, this may also correspond to physiological exhaustion, resulting in the fish's inability to mount an adequate stress response. This may be particularly true when considering the administration of higher doses of β-glucans since it could constitute a more intense stimulation. This might be the case in healthy fish fed the low doses of β-glucans given the absence of a significant stress response either at 15 or 30 days of feeding while up-regulation of stress-related genes was observed for lower doses (at least after 15 days). Soltanian and co-workers (2014) reached a similar conclusion when feeding striped catfish (*Pangasianodon hypophthalmus*) with several doses of β-glucans (0.5, 1, and 2%) and evaluating the effects of the supplementation on resistance to a subsequent cold shock stressor. These authors reported lower post-stress mortality in fish fed low β-glucans doses while values increased the following feeding with high doses of β-glucans, thereby demonstrating the deleterious effects of a β-glucans overdose and the importance of appropriate dosage and duration of the treatment [25].

The effects of β-glucan on oxidative stress, inflammation, and copper transport in two intestinal regions of large yellow croaker under acute copper stress were investigated by Zeng and co-workers (2018). Fish were injected with β-glucan at a dose of 0 or 5 mg kg^{-1} body weight on 6, 4, and 2 days before being exposed to 0 and 368 μg Cu L^{-1} for 48 h. 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 MTs 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. But 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. β-glucan induced oxidative stress, inflammation, and copper transport varied between the anterior and mid intestines of fish under Cu stress [32].

The dietary pulse administration of a microalga (*Phaeodactylum tricornutum*) 37% enriched-β-glucans extract might be used as a counter-measure in the context of gut inflammation, due to its immune-tolerant and anti-oxidative effects. The effects of dietary supplementation with β-glucans extracted from yeast (*Saccharomyces cerevisiae*) and microalga (*P. tricornutum*) on gene expression, oxidative stress biomarkers, and plasma immune parameters in gilthead seabream (*Sparus aurata*) juveniles were evaluated by Reis and co-workers (2021). A practical commercial diet was used as the control (CTRL), and three others based on CTRL were further supplemented with different β-glucan extracts. One was derived from S. cerevisiae (diet MG) and two different extracts of 21% and 37% *P. tricornutum*-derived β-glucans (defined as Phaeo21 and Phaeo37), to give a final 0.06% β-glucan dietary concentration. Quadruplicate groups of 95 gilthead seabream (initial body weight: 4.1 ± 0.1 g) were fed to satiation three times a day for 8 weeks in a pulse-feeding regimen, with experimental diets intercalated with the CTRL dietary treatment every 2 weeks. After 8 weeks of feeding, all groups showed equal growth performance, and no changes were found in plasma innate immune status. Nonetheless, fish groups fed β-glucans supplemented diets showed an improved antioxidant status compared to those fed CTRL at both sampling points (i.e., 2 and 8 weeks). The intestinal gene expression analysis highlighted the immunomodulatory role of Phaeo37 diet after 8 weeks, inducing an immune tolerance effect in the gilthead seabream intestine, and a general down-regulation of immune-related gene expression [22].

Salah and co-workers (2017) evaluated the effect of different in-feed doses of β-1,3/1,6-glucans on the expression of antioxidant and stress-related genes (GST, HSP-70, Vtg), inflammation-related genes (Il-8, TNFα, CXC-chemokine, and CAS) and adaptive immune-related genes (MHC-IIβ, TLR-7, IgM-H, and Mx) of *Oreochromis niloticus* challenged and non-challenged with *Streptococcus iniae*. Six experimental groups were established: non-challenged control (non-supplemented diet), challenged control (non-supplemented diet), non-challenged supplemented with 0.1% β-glucan, challenged supplemented with 0.1% β-glucan, non-challenged supplemented with 0.2% β-glucan and challenged supplemented with 0.2% β-glucan. Fish were fed with β-glucan for 21 days prior challenge and then sampled after 1, 3, and 7 days post-challenge. In the non-challenged group, variable effects of the two doses of β-Glucans on the expression of the studied genes were observed; 0.1% induced higher expression of HSP70, CXC chemokine, MHC-IIβ, and MX genes. Meanwhile, 0.2% induced a better effect on the expression of Vtg, TNF-α, CAS, and IgM-H, and almost equal effects of both doses on GST and IL8. However, with the challenged group, 0.2% β-Glucans showed a better effect than 0.1% on day one post-challenge through significant up-regulation of GST, HSP, IL8, TNF-α, CXC, and MHC-IIβ, meanwhile, the effect of 0.1% was only on the expression of HSP70, MHC-IIβ, and TLR7 at day 3 post-challenge. No stimulatory role for both doses of β-Glucans on the expression of almost all genes at day 7 post-challenge. These researchers concluded that both doses of β-glucan can modulate the antioxidant, inflammation, stress, and immune-related genes in Nile tilapia, moreover, 0.2% β-Glucans showed a better protective effect with *Streptococcus iniae* challenge [23].

Glucans may be considered a potent protector against microwave radiation-induced cell damage. A significant decrease in the conjugated diene production, quanti-

fied as Klein oxidation index, was observed in the presence of a moderate amount of added glucan, as Babincová and co-workers (1999) demonstrated. The increase in the oxidation index was accompanied by enhanced carboxyfluorescein leakage as a result of liposome membrane destabilization. This process was markedly suppressed with glucan present in the liposome suspension [4]. Also, glucans as a potentially safe and effective dietary supplement may be used for a prolonged time for systemic photoprotection of humans [24]. B-glucans are antioxidants with the scavenging ability lying between that of alpha-tocopherol, which is known to be incorporated in the lipid bilayer, and the water-soluble antioxidant, mannitol [3]. β-D-glucan may serve as a source of bioactive compounds with effective antioxidant activity [12].

Conclusions. 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.

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ВПЛИВ ДІЄТИЧНИХ В-1,3/1,6-ГЛЮКАНІВ З ДРІЖДЖІВ НА ПЕРЕКИСНЕ ОКИСНЕННЯ ЛІПІДІВ У ПЕЧІНЦІ І СЕРЦІ РАЙДУЖНОЇ ФОРЕЛІ (ONCORHYNCHUS MYKISS WALBAUM), ЄВРОПЕЙСЬКОГО СИГА (COREGONUS LAVARETUS L.) І ХАРІУСА (THYMALLUS THYMALLUS L.)

Ткаченко Г., Курхалюк Н., Інститут біології наук про Землю, Поморська академія в Слупську, Польща.

Груднєвська Й., Інститут рибальства імені Станіслава Саковича, Руткі, Жуково, Польща

Дієтичні β-глюкани можуть бути корисним засобом для активізації імунної системи господаря та підвищення стійкості проти вторгнення патогенів. Це спонукало нас дослідити вплив β-1,3/1,6-D-глюканів з дріжджів, доданих до кормів протягом 14-денного періоду годування, на функціонування печінки та серця, а також на окиснювальні механізми, які лежать в основі ефектів β-глюканів. Ми оцінили перекисне окиснення ліпідів у печінці та серці райдужної форелі (Oncorhynchus mykiss), європейського сига (Coregonus lavaretus) і харіуса (Thymallus thymallus) після 14-денного періоду прийому β-глюканів. У дослідах використовували 30 здорових харіусів масою 34,9 ± 1,9 г, 30 здорових райдужної форелі вагою 55,9 ± 2,1 г і 30 здорових європейських сигів масою 43,3 ± 2,7 г. Рибу годували комерційною дієтою з розрахунку 1,5% маси тіла чотири рази на день. Після акліматизації рибу розділили на шість груп. Групи годували протягом 14 днів наступним чином: контрольні групи, що складалися з харіуса (n = 15), райдужної форелі (n = 15) і сига (n = 15), отримували контрольну основну дієту, а групи, які отримували β-глюкан, годували харчовим продуктом Yestimun® у дозі 1% основного корму (з 85% β-1,3/1,6-глюканів, Leiber GmbH, Bramsche, Germany). Основний корм доповнювали 1% порошком Yestimun® (доза: 1 кг на 99 кг маси риби). 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 зниження рівня маркерів перекисного окиснення ліпідів (TBARS) у тканинах печінки та серця райдужної форелі та європейського сига. Подібним чином, 14-денне годування харіусів низькими дозами β-глюканів призвело до зниження рівня TBARS як у печінці, так і у серці. Це дослідження підтверджує, що β-глюкани є корисними для сприяння росту та посилення антиоксидантної здатності пецінки і серця райдужної форелі, європейського сига та харіуса. Дійсно, ми припускаємо, що згодовування низьких доз β-глюканів може сприяти посиленню антиоксидантної функції, особливо за рахунок зниження біомаркерів перекисного окиснення ліпідів у печінці і серці цих видів риб.

Ключові слова: β-глюкани, окисуювальний стрес, перекисне окисуення ліпідів, Thymallus thymallus, Oncorhynchus mykiss, Coregonus lavaretus.