ACTA VET. BRNO 2026, 95: 165–176; https://doi.org/10.2754/avb202695020165

Dietary β-glucans modulate the internal environment and gut microbiome of rainbow trout (*Oncorhynchus mykiss*)

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Received April 29, 2026
Accepted June 15, 2026

Abstract

Rainbow trout (*Oncorhynchus mykiss*) is one of the most important freshwater fish species in intensive aquaculture. The aim of this study was to evaluate the effects of dietary supplementation with yeast-derived β-1,3/1,6-glucans at different concentrations on the health status of rainbow trout. An eight-week feeding trial was conducted with four groups of fish: a control group without supplementation and three experimental groups receiving β-glucans at concentrations of 0.2%, 0.5%, and 1.0% of total feed weight. Fish health was comprehensively assessed using production parameters, haematological, biochemical, and immunological indices, as well as analysis of the intestinal microbiome. The results demonstrated that higher β-glucan doses (0.5% and 1.0%) led to a significant increase in the hepatosomatic index and albumin, calcium, glucose and lipid metabolites in blood plasma. Plasma protein contents and white blood cell count increased only at the 0.5% dose. Overall diversity of the gut microbiome was not significantly affected by supplementation; however, correlation network analysis revealed pronounced reorganization of microbial interactions and dose-dependent changes in the abundance of selected bacterial genera. This study confirms that β-1,3/1,6-glucans can modulate physiological and structural organization of the gut microbiome in a dose-dependent manner, representing a promising tool to support fish health under intensive aquaculture conditions.

Prebiotics, fish health, haematology, aquaculture additives

Rainbow trout (*Oncorhynchus mykiss*) is one of the most widely farmed freshwater fish species worldwide, primarily due to its rapid growth, efficient feed conversion and high adaptability to intensive aquaculture systems (D'Agaro et al. 2022). Advances in selective breeding, nutrition, and management have substantially improved production efficiency in recent decades. However, intensification of aquaculture is associated with increased exposure to stressors such as high stocking densities, handling and environmental fluctuations, which may negatively affect physiological balance and immune function (Murray and Peeler 2005).

Under intensive rearing conditions, chronic stress can impair immune responsiveness and compromise fish health, reducing welfare and production performance (Murray and Peeler 2005). Maintaining immune homeostasis and physiological stability is therefore essential for sustainable aquaculture. Preventive strategies, including water quality management, vaccination and biosecurity, play a key role in reducing

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disease incidence, particularly under high-density conditions. A comprehensive approach integrating nutrition, environmental control and routine health monitoring is recommended to support physiological balance and immune competence in salmonid fish (Palíková et al. 2015). Consequently, nutritional strategies using functional feed additives have gained attention as tools to support fish health without excessive immune stimulation.

Prebiotics represent an important group of functional feed additives and are defined as non-digestible dietary components that selectively stimulate beneficial intestinal microorganisms, thereby improving gut health and systemic immune function. In fish, intestinal microbiota contribute not only to digestion and nutrient absorption but also to immune regulation and maintenance of mucosal barrier integrity (Seong et al. 2014).

Among the prebiotics used in aquaculture, β-glucans are the most extensively studied immunomodulatory compounds. These structurally diverse polysaccharides occur naturally in yeast, fungi and cereals, with β-1,3/1,6-glucans derived from *Saccharomyces cerevisiae* being most commonly used in fish nutrition. Their activity is associated with interactions with pattern recognition receptors on immune cells, leading to modulation of innate immune mechanisms (Tokunaka et al. 2000; Seong et al. 2014). Dietary β-glucans have been shown to influence phagocytic activity, respiratory burst, lysozyme activity and cytokine production in teleost fish, enhancing non-specific immune responses (Soltys and Quinn 1999; Seong et al. 2014; Minářová et al. 2021).

A growing body of evidence suggests that dietary β-glucans may also modulate the gut microbiome of fish, thereby contributing to their systemic physiological effects. The intestinal microbiota play a central role in digestion, nutrient assimilation, and mucosal immunity, and their composition is sensitive to dietary inputs, including prebiotics (Nayak 2010; Ghanbari et al. 2015). Several studies have demonstrated that supplementation with yeast-derived β-1,3/1,6-glucans can alter microbial community structure in salmonids, often increasing the relative abundance of beneficial taxa such as lactic acid bacteria and butyrate-producing groups associated with improved barrier function and anti-inflammatory activity (Merrifield et al. 2011; Menanteau-Ledouble et al. 2022). These shifts in microbial composition may enhance short-chain fatty acid production and support the gut–immune axis, contributing to improved immune tone and resilience under intensive aquaculture conditions (Nayak 2010; Ghanbari et al. 2015). Therefore, evaluating the impact of β-glucans on the intestinal microbiome is essential for understanding their holistic effects on rainbow trout health and overall physiological balance (Dawood et al. 2015).

Immunomodulatory effects of β-glucans have also been reported in salmonids, including rainbow trout, where supplementation has been associated with changes in immune-related and physiological indices (Lauridsen and Buchmann 2010; Ji et al. 2017). However, these effects depend on factors such as species, developmental stage, dosage and duration of administration. While moderate supplementation may support immune competence and stress resilience, excessive or prolonged stimulation may disrupt immune homeostasis (Lauridsen and Buchmann 2010).

These findings highlight the importance of evaluating β-glucans not only in terms of disease resistance but also regarding their impact on overall health and physiological balance. Therefore, the present study aimed to investigate the effects of dietary supplementation with varying concentrations of yeast-derived β-1,3/1,6-glucans on the health status of rainbow trout. Fish health was comprehensively assessed using production parameters, haematological, plasma biochemical, and immunological indices to elucidate dose-dependent systemic responses under standard rearing conditions, alongside gut microbiome analysis.

Materials and Methods

Fish rearing conditions

The experiment was carried out in compliance with the Czech law on protection of animals against cruelty, as approved by the Czech Ministry of Education, Youth and Sports (Permit No. MSMT-2380/2023-5).

The study was performed on rainbow trout (*Oncorhynchus mykiss*) aged 8 months. The fish originated from the Biely Potok – Slovryb a.s. SK 9-3, farm in Slovakia. When stocked, the fish had a mean body weight of 49.92 ± 7.65 g and a mean total length of 151.87 ± 7.85 mm. Each experimental group comprised 68 fish, which were distributed into four 80-l tanks (4 replicates, 17 fish per tank). The photoperiod was set at 14 h of light and 10 h of dark. After stocking, a 14-day adaptation period followed, during which all groups were fed with a standard commercial rainbow trout food Efico Enviro 921, 3 mm (BioMar, Brande, Denmark). The fish were fed three times a day with a diet without added β-glucans, corresponding to the control diet used throughout the experiment.

Preparation of β-glucans-enriched pellets

Commercial Biomar EFICO Enviro 921 pellets (3 mm) were used to prepare the experimental feed. These pellets contain: 47.00% protein; 26.10% fat; 7.00% ash; 1.00% TM fibre; phosphorus 0.91%; calcium 0.88%; sodium 0.39%; ingredients: fish meal, rapeseed oil, horse beans, blood meal, wheat gluten, soya concentrate, wheat, fish oil, guar protein, monoammonium phosphate. The pellets served as the basic carrier for the application of the additive-β-glucans. The amount of β-glucans was determined according to the individual experimental variants (0.2%, 0.5%, 1% of total feed weight). The β-glucans (*Saccharomyces cerevisiae*, Zhuhai Frong Tech Co., LTD., Zhuhai, China) were added using a dry coating method, in which the powdered supplement was poured onto the surface of the pellets and the mixture was intensively stirred by shaking in a closed container until the pellets completely absorbed the powder and their surface was evenly coated. The pellets prepared in this way were then used for the experiment.

Statistical analysis of non-microbiome data

Statistical analysis was carried out using statistical software Unistat for Excel 6.5. At first, all indices were tested for normality and homogeneity of variance using Shapiro-Wilk test and Levene's test, respectively. If normality of data was achieved, data were evaluated using one-way ANOVA and *post hoc* Tukey Honestly Significant Difference test to determine differences among groups. Kruskal-Wallis ANOVA followed by a multi-sample median test was subjected to non-normally distributed data. Evaluation was performed only among groups at the same time point. The differences were considered significant or highly significant at $P < 0.05$ or $P < 0.01$, respectively. All results were reported as mean ± standard deviation (SD).

Experimental design

The feeding trial was conducted over an 8-week period. During the experiment, the control group (group C) was fed with Effico Enviro 921, 3 mm pellets. The experimental groups (P1–P3) received the same basal diet supplemented with β-1,3-1,6-glucan at the following concentrations: 0.2% for group P1; 0.5% for group P2; and 1.0% for group P3. The fish were fed at a rate of 1.5% of the fish stock weight, divided into three feedings per day. Throughout the feeding trial, mortality, behavioural changes, and feed intake were regularly monitored and recorded. Control weighing of the fish was performed three times and feeding rations were adjusted according to the obtained results. The water temperature (15.73 ± 0.76 °C), dissolved oxygen content (9.56 ± 0.30 mg/l), and pH (7.12 ± 0.20) were measured daily in each tank and in the filter. Every second day, a water analysis was performed in the filter to determine the concentrations of nitrites (0.50 ± 0.20 mg/l), ammonia (0.047 ± 0.037 mg/l), and chlorides (161.86 ± 53.57 mg/l).

Sampling procedure

At the end of the 8-week feeding trial, 40 fish from each experimental group (10 specimens per replicate) were sampled and blood samples of 20 fish from each group (5 specimens per replicate) were collected by puncture of caudal vessels using heparinised syringes (50 IU of heparin sodium salt per 1 ml of blood; Heparin Léčiva, Zentiva, Prague, Czech Republic) immediately. The fish were subsequently euthanized by a blow to the head followed by cutting the branchial vessels. The collected blood was used for haematological examination, plasma biochemistry, and immunological analyses.

Production parameters

Subsequently, fitness and production parameters were determined: total length (TL), standard length (SL), body weight (W), eviscerated weight (EW), liver weight (LW), spleen weight (SW), Fulton's condition factor (Fc), Clark's condition factor (Cc), hepatosomatic index (HSI), lienosomatic index (LSI), weight gain (WG; WG = 100 × [final weight - initial weight]/initial weight), feed conversion ratio (FCR; FCR = feed consumed [g, dry weight]/[weight gain, g]) and specific growth rate (SGR; SGR = 100 × ln [final weight/initial weight]/days of experiment) (Khajepour et al. 2012).

Haematological, biochemical, and immune indices

Red blood cell count (RBC), white blood cell count (WBC), haematocrit (Ht), haemoglobin (Hb), mean cell corpuscular volume (MCV), mean cell corpuscular haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and ratio of lymphocytes and phagocytes were assessed according to Svobodová et al. (2012).

Respiratory burst activity (RBA) (as a measure of phagocyte activity) was measured using chemiluminescence enhanced by luminol (Sigma-Aldrich Merck KGaA, Darmstadt, Germany) as described by Papežíková et al. (2016). Chemiluminescence kinetics were measured for 90 min using a Cytation 3M reader (BioTekInstruments, Inc., Winooski, VT, USA). The results were expressed as time of a maximum respiratory burst intensity (peak time), peak of chemiluminescence, total intensity of respiratory burst defined as the integral of the reaction curve area and integral per 1 000 phagocytes. The remaining heparinized blood was centrifuged (800 *g*, 10 min, 4 °C) to obtain plasma. Plasma separated from samples was used to determine biochemical properties. Measurements were performed photometrically with a Konelab 20i biochemical analyser and commercial kits (Biovendor, Brno, Czech Republic). The indices measured included total protein, albumin, triglycerides, cholesterol, creatinine, calcium, phosphorus, magnesium, glucose, aspartate aminotransferase, alkaline phosphatase, alanine aminotransferase and urea.

Microbiome

Six individuals from each experimental group were used to assess microbial community composition of the gut contents. As part of the necropsy, the caudal part of the intestine was removed, ligated at both ends and immediately frozen at −80 °C and stored until further processing. For DNA isolation, the intestine was opened in a flow box, 100 µg of intestinal contents collected from the terminal part of the intestine. Isolation was performed using the commercially available EZNA soil kit (Omega Bio-tek, Norcross, GA, USA), according to the manufacturer's instructions, the isolated DNA being stored at −20 °C until amplification. Upon thawing, the commercially available 16S Barcoding kit (Oxford Nanopore Ltd., Oxford, UK) was used to amplify the entire 16S gene and to subsequently prepare the amplicon for sequencing. Twenty-five amplification cycles were performed with primers and barcodes on a Bio-Rad Mini cycler (Bio-Rad, Hercules, CA, USA). Amplicons were purified using AMPure XP Beads (Beckmann-Coulter, Brea, CA, USA), according to the kit manufacturer's instructions. The amount of DNA was quantified fluorometrically using the DeNovix High Sensitivity Assay kit on a DeNovix QFX fluorometer (DeNovix, Wilmington, DE, USA). Based on the resulting amount of DNA, individual libraries were then pooled for sequencing on a third-generation Minion nanopore sequencer (Oxford Nanopore Ltd., Oxford, UK) using the reagents provided with the 16S Barcoding kit and according to the manufacturer's instructions. Reads were filtered and those with a Phred score < 8 were removed from the analysis. Barcode-sorted fastq files were concatenated and used for further analysis. After low sequencing depth samples (one 0.2%, one 0.5% and one 1.0% β-glucans sample) were removed, gut content samples sequencing data analysis revealed 223,883 total reads, with 10,661 average reads per sample and a minimum count of 1,779 reads. After removing singletons, 102 OTUs (Operation Taxonomic Unit) remained.

For statistical analysis, 68 low abundance OTUs (with a maximum of 4 occurrences in 20% of samples) were excluded from the analysis, 68 low abundance OTUs (minimum count four in 20% of samples) were excluded from the analysis of the gut content microbial community, and 4 features were excluded based on interquartile range. A bioinformatic analysis was performed using a pipeline described previously (Mann et al. 2021), while taxonomic classification of individual reads was performed using the RDP16S_v18 database (Cole et al. 2014). The OTU table obtained and the auxiliary mapping file describing individual samples were then uploaded to the Microbiome Analyst online tool (https://www.microbiomeanalyst.ca). Owing to differences in sample read counts, the data were first rarefied on normalised by total sum scaling. Subsequent analysis of alpha diversity (Shannon index, Chao1, Kruskall-Wallis ANOVA), beta diversity (principal coordinate analysis PCoA) based on Bray-Curtis distance and PERMANOVA), correlation network and Microbiome Dysbiosis Index Analysis (MD) at the genus level were performed.

Results

Production parameters

Production parameters are presented in Table 1. While group P1 showed statistically significant differences only when compared to groups P2 and P3 in SL, TL, BW, and EW, fish from groups P2 and P3 were found to have significantly higher liver weight compared to the control group, which was also reflected in a significant increase in HSI. No significant differences were observed for the remaining production parameters (Table 1).

Plasma biochemical indices

Plasma biochemical indices are presented in Table 2. While group P1 showed statistically significant differences in cholesterol and glucose (compared to groups P2 and P3), albumin, Mg, TAG and UREA (compared to P2) and in Pi (compared to P3), fish from groups P2 and P3 exhibited significantly higher contents of albumin, Ca, glucose and TAG than the control group. Group P2 was also found to have significantly higher Mg and urea compared to all other groups, while TP was significantly increased compared to control and P1. No significant differences were observed for the remaining plasma biochemical indices (Table 2).

Table 1. Production parameters of rainbow trout after the 56-day trial (mean ± SD; n = 40).

Indicator	C	P1	P2	P3
Standard length (mm)	182.53 ± 9.99^{ab}	178.62 ± 11.51^{b}	186.18 ± 14.13^{a}	182.98 ± 15.96^{a}
Total length (mm)	207.73 ± 10.74^{ab}	203.82 ± 12.36^{b}	213.68 ± 13.11^{a}	207.90 ± 16.65^{a}
Body weight (g)	120.61 ± 17.35^{ab}	113.16 ± 20.73^{b}	128.94 ± 18.50^{a}	121.84 ± 22.36^{a}
Eviscerated weight (g)	103.20 ± 15.49^{ab}	96.48 ± 18.40^{b}	108.54 ± 15.85^{a}	103.41 ± 19.18^{a}
Hepatosomatic index	1.37 ± 0.30^{b}	1.43 ± 0.42^{bc}	1.57 ± 0.22^{ac}	1.61 ± 0.23^{a}
Lienosomatic index	0.18 ± 0.09	0.20 ± 0.07	0.19 ± 0.08	0.17 ± 0.05
Liver weight (g)	1.40 ± 0.31^{b}	1.37 ± 0.42^{b}	1.70 ± 0.33^{a}	1.64 ± 0.32^{a}
Spleen weight (g)	0.19 ± 0.10	0.19 ± 0.07	0.20 ± 0.08	0.17 ± 0.06
Fulton's condition factor	1.98 ± 0.16	1.96 ± 0.13	2.02 ± 0.35	1.98 ± 0.23
Clark's condition factor	1.69 ± 0.13	1.67 ± 0.10	1.69 ± 0.26	1.68 ± 0.19
Weight gain	239.25 ± 12.61	243.25 ± 13.07	189.75 ± 112.53	233.75 ± 9.95
Specific growth rate	1.62 ± 0.06	1.61 ± 0.08	1.58 ± 0.10	1.58 ± 0.05
Feed conversion ratio	0.81 ± 0.34	0.82 ± 0.04	0.80 ± 0.06	0.83 ± 0.02

C – control group; P1 – β-glucans 0.2%; P2 – β-glucans 0.5%; P3 – β-glucans 1%

Significant differences between groups are marked with different superscripts. ^{a,b} Different superscripts within a row denote significance ($P < 0.05$)

Table 2. Plasma biochemical indices (mean ± SD; n = 20) of rainbow trout after the 56-day trial.

Parameter	C	P1	P2	P3
Albumin (g/l)	11.37 ± 2.53^{b}	12.81 ± 2.81^{bc}	15.44 ± 2.87^{a}	14.51 ± 2.98^{ac}
TP (g/l)	38.56 ± 4.18^{bc}	37.98 ± 5.41^{b}	43.06 ± 3.63^{a}	41.71 ± 3.72^{ac}
AST (µkat/l)	6.86 ± 2.94	7.72 ± 2.78	8.16 ± 2.26	8.13 ± 2.26
ALP (µkat/l)	2.44 ± 1.15	2.15 ± 0.90	2.82 ± 1.38	2.64 ± 0.65
Ca (mmol/l)	2.31 ± 0.21^{b}	2.40 ± 0.32^{ab}	2.58 ± 0.20^{a}	2.54 ± 0.21^{a}
Glucose (mmol/l)	3.52 ± 0.49^{b}	3.74 ± 0.47^{b}	5.15 ± 1.12^{a}	4.87 ± 0.73^{a}
Cholesterol (mmol/l)	6.97 ± 1.25^{ab}	6.64 ± 1.89^{b}	7.96 ± 1.38^{a}	7.88 ± 1.11^{a}
Creatinine (µmol/l)	23.77 ± 10.29	20.26 ± 3.89	22.90 ± 6.14	20.86 ± 4.13
Pi (mmol/l)	2.89 ± 0.44^{ab}	2.97 ± 0.42^{a}	3.08 ± 0.46^{a}	2.59 ± 0.24^{b}
Mg (mmol/l)	0.89 ± 0.14^{b}	0.93 ± 0.14^{b}	1.09 ± 0.08^{a}	0.98 ± 0.07^{b}
TAG (mmol/l)	2.15 ± 0.69^{b}	2.30 ± 0.97^{bc}	3.25 ± 0.84^{a}	2.98 ± 1.00^{ac}
Urea (mmol/l)	0.55 ± 0.23^{b}	0.52 ± 0.19^{b}	0.76 ± 0.23^{a}	0.50 ± 0.14^{b}

C - control group, P1 - β-glucans 0.2% , P2 - β-glucans 0.5%, P3 – β-glucans 1%; ALT – alanine aminotransferase; AST – aspartate aminotransferase; ALP – alkaline phosphatase; Ca – calcium; Pi – inorganic phosphorus; Mg – magnesium; TP – total protein; TAG – triacylglycerides

Significant differences between groups are marked with different supercripts. ^{a,b} Different superscripts within a row denote significance ($P < 0.05$)

Haematological indices and phagocytic activity

Haematological indices and phagocytic activity are presented in Table 3. The kinetic curves of phagocytic activity are shown in Fig. 1.

While group P1 showed statistically significant differences only when compared to group P3 in MCV and MCHC, fish from group P2 were found to have significantly higher WBC compared to the control group. No significant differences were observed for the remaining haematological indices (Table 3).

Group P1 showed the highest values of oxidative burst of phagocytes, but it was significant only when compared to groups P2 and P3 in peak value and to P2 in integral of chemiluminescence, respectively. The peak time was significantly decreased when compared to P3 and control group. On the other hand, the lowest values of oxidative burst of phagocytes were found in fish from group P2 where the peak value was significantly decreased compared to the control group. No significant differences were observed for the integral re-calculated to 1000 phagocytes in the sample (Table 3).

Table 3. Haematological indices and phagocytic activity (mean ± SD; n = 20) of rainbow trout after the 56-day trial.

Indicator	C	P1	P2	P3
RBC (10^{12}/l)	1.25 ± 0.28	1.19 ± 0.18	1.19 ± 0.23	1.09 ± 0.16
Hb (g/l)	76.67 ± 9.00	77.62 ± 10.27	78.73 ± 8.09	76.53 ± 6.75
MCV (fl)	315.59 ± 63.11^{ab}	327.48 ± 75.61^{b}	358.27 ± 114.48^{ab}	370.14 ± 47.79^{a}
MCH (pg)	63.52 ± 11.00	66.87 ± 14.15	69.30 ± 18.66	71.20 ± 9.24
MCHC (g/l)	202.95 ± 17.11^{ab}	205.40 ± 15.94^{a}	195.56 ± 16.09^{ab}	192.93 ± 15.92^{b}
WBC (G/l)	35.30 ± 9.10^{b}	37.25 ± 10.09^{ab}	43.25 ± 14.33^{a}	37.05 ± 6.40^{ab}
Phagocytes (G/l)	1.69 ± 0.81	1.71 ± 0.96	2.25 ± 1.74	1.96 ± 1.18
Lymphocytes (G/l)	33.61 ± 8.72	35.54 ± 9.95	41.00 ± 13.23	35.06 ± 6.23
ICL (RLU/min)	126 676 ± 63 459^{ab}	146 458 ± 53 187^{a}	82 678 ± 44 884^{b}	102 664 ± 60 063^{ab}
P-T (min)	39.90 ± 7.09^{a}	36.75 ± 6.22^{b}	40.50 ± 6.45^{ab}	43.20 ± 8.67^{a}
ICL RC (RLU/min)	1 539 ± 879	1 808 ± 813	1 682 ± 2 731	1 612 ± 1 733

C – control group; P1 – β-glucans 0.2%; P2 – β-glucans 0.5%; P3 – β-glucans 1%; RBC – red blood cell count; Hb – haemoglobin; Ht – haematocrit; MCV – mean cell corpuscular volume; MCH – mean cell corpuscular haemoglobin; MCHC – mean cell corpuscular haemoglobin concentration; WBC – white blood cells count; RLU – relative light unit; ICL – integral of chemiluminescence; P-T – peak time; ICL RC – integral of chemiluminescence re-calculated to 1 000 phagocytes

Significant differences between groups are marked with different supercripts. ^{a,b} Different superscripts within a row denote significance ($P < 0.05$)

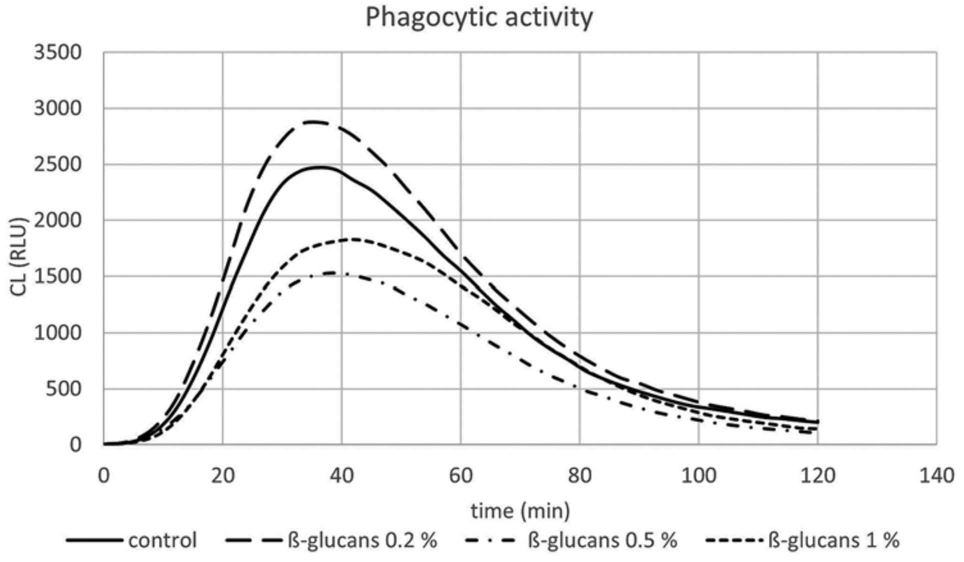

Fig. 1. Phagocyte activity kinetics. The curves represent the intensity of respiratory burst over time (in min). Chemiluminescence (CL) signal intensity is expressed as relative light units (RLU).

Microbiome

After low sequencing depth samples (one in P1, one in P2, one in P3) were removed, gut content samples sequencing data analysis revealed 223,883 total reads, with 10,661 average reads per sample and a minimum count of 1,779 reads. After filtering, 30 OTUs remained for analysis of gut content microbial community composition. Owing to differences in the number of reads between libraries, rarefaction to the minimum library size (1,752 reads) and normalisation with total sum scaling was performed. The normalised data were then visualised using a rarefaction curve. Initially, a rapid increase in the number of OTUs was observed; however, these curves gradually decreased and then flattened, indicating that the sequencing depth was sufficient to capture diversity in the samples, allowing for reliable analysis.

At the phylum level, Firmicutes (87.9%, 72.0%, 99.0% and 65.6%), Fusobacteria (11.2%, 27.4%, 0.1% and 33.5%) and Proteobacteria (0.1%, 0.5%, 0.0% and 0.0%) were found in intestinal content samples from the caudal part of the intestine in C, P1, P2, and P3, respectively.

At the genus level, analysis of intestinal content samples revealed the presence of dominant genera *Streptococcus*, *Peptostreptococcus, Clostridium sensu stricto* and *Cetobacterium* (except for P2), less abundantly *Peptoniphilus* and *Lactococcus*. Low abundance genera in the intestinal content were *Leuconostoc*, *Lactobacillus*, *Enterococcus*, *Vagococcus*, *Hespelia*, *Romboutsia*, *Paucilactobacillus*, *Eubacterium* and *Staphylococcus*.

Comparison of both alpha diversity indexes (Shannon, Chao1, ANOVA) and beta diversity (PERMANOVA) showed non-significant differences between control and β-glucan groups ($P > 0.05$). Community composition remained broadly similar across diets, but SparCC networks ($|P| \geq 0.5$) indicated that strong co-occurrence relationships among dominant genera were reorganized, suggesting changes in community organization (Plates XII–XIII, Figs 2–5). The combined SparCC network (all groups, Fig. 2) revealed two major microbial interaction clusters. First, a densely connected fermentation guild consisting of *Streptococcus*, *Peptoniphilus*, *Peptostreptococcus*, *Enterococcus* and *Lactococcus* and second, *Clostridium sensu stricto* negatively correlated with several taxa including *Vagococcus, Hespellia, Paucilactobacillus* and *Romboutsia*. In the low β-glucan group (P1), correlation network shows three weakly connected smaller clusters with interactions mildly differing from control network (Fig. 3; MD = 1.2786); in the medium β-glucan group (P2), most of the taxa form one large cluster with stronger interconnection and strongly restructured microbial interactions compared to control network (Fig. 5; MD = −4.5339); and the high β-glucan group (P3) shows strongly restructured microbial interactions compared to the control group, but also differing from those visible in the medium group P2 (Fig. 5; MD = 4.8742). Two-group comparison revealed significant difference in *Cetobacterium* abundance (Plate XIV, Fig. 6; EdgeR, adjusted $P = 0.006$) and significant difference in *Clostridium sensu stricto* abundance between medium (P2) and high β-glucan group (P3) (Plate XIV, Fig. 7; EdgeR, adjusted $P = 0.047$).

Discussion

Production parameters

Beta-glucans are widely reported as functional feed additives that can stimulate growth performance and survival in cultured fish through nutrition and its effect on the immune system (Ji et al. 2017). However, in our experiment, the effects on production parameters were generally not very pronounced and differences were found especially among β-glucan supplemented groups, rather than in comparison with the control group.

The most sensitive indices examined were liver weight and hepatosomatic index, which significantly increased in fish administered 0.5% and 1.0% β-glucans. The increase reflects enhanced metabolic activity rather than pathological liver enlargement, as no concomitant elevation of hepatic enzyme activities was detected. The liver plays a central role in nutrient metabolism, acute-phase protein synthesis, and immune regulation in fish, and its enlargement is frequently associated with improved nutritional status and anabolic processes (Causey et al. 2018; Cornet et al. 2021).

Growth-promoting effects of β-glucans and other functional feed additives have been reported in salmonids, where moderate supplementation improved feed utilization efficiency and growth performance (Kühlwein et al. 2014; Ji et al. 2017). Other sources confirm that β-glucans can improve growth and biochemical profiles when included in fish diets in appropriate amounts and also play a key role in gluconeogenesis and glycogen storage, which may be associated with increased liver weight (Kühlwein et al. 2014; Marandel et al. 2015; Ji et al. 2017; Satiro et al. 2024).

From a broader perspective, functional feeds may improve growth indirectly by enhancing gut functionality, nutrient absorption, and immune–metabolic interactions (Merrifield et al. 2011; Wang et al. 2019). These authors reported that immunostimulatory feed additives can support growth when immune activation remains balanced and does not impose excessive energetic costs. The studies by Dawood et al. (2015), Ji et al. (2017) and Khanjani et al. (2021) further highlight that immunostimulants such as β-glucans promote growth performance and immune responses across aquatic species when optimally dosed.

Plasma biochemical indices

Plasma biochemical indices revealed substantial metabolic modulation in β-glucan-supplemented groups, particularly at inclusion levels of 0.5% and 1.0%. Elevated concentrations of total protein and albumin suggest enhanced hepatic synthetic activity and improved nutritional status, which are frequently associated with improved immune competence in fish (Hrubec et al. 2000; Pashay et al. 2025). These findings correspond with the observed increase in liver weight and hepatosomatic index. Such biochemical responses are commonly reported in fish receiving functional feeds enriched with immunostimulants, including β-glucans (Dobšíková et al. 2012; Menanteau-Ledouble et al. 2022).

Increased glucose and triacylglyceride contents in higher-dose groups likely reflect intensified energy metabolism and anabolic processes. According to Dobšíková et al. (2012), plasma biochemical profiles are highly sensitive indicators of nutritional and physiological status in fish, and moderate increases in these parameters are commonly observed in animals receiving functional or immunostimulant-enriched diets.

Changes in mineral concentrations, particularly increase in calcium (P2, P3) and magnesium (P2), further indicate improved metabolic balance, as these ions are essential for enzymatic activity, immune signalling, and cellular homeostasis (Flik et al. 2009). Similar biochemical adaptations were reported by Minářová et al. (2021) and Menanteau-Ledouble et al. (2022) in rainbow trout under optimized nutritional regimes, even though Menanteau-Ledouble et al. (2022) tested 10-g individuals, while our experiment utilized 8-month-old trout weighing approximately 50 g.

Importantly, no significant elevation of hepatic enzymes (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase) was observed, indicating that increased metabolic activity did not result in liver damage or physiological stress (Dobšíková et al. 2012). This observation aligns with conclusions by Ji et al. (2017) and Cornet et al. (2021), who emphasized that β-glucans act as metabolic and immunological modulators rather than stress-inducing agents when properly formulated in aquafeeds.

Haematological indices and phagocytic activity

Haematological indices were only moderately affected by β-glucan supplementation, indicating physiological modulation rather than pathological alteration of the haematopoietic system. Alterations in erythrocyte indices (MCV, MCHC) were dose-dependent but remained within physiological reference ranges (Dobšíková et al. 2012). Such changes are generally interpreted as adaptive responses linked to metabolic demands rather than indicators of anaemia or erythropoietic dysfunction (Hrubec et al. 2000). The absence of marked changes in the red blood cell count, haemoglobin concentration, or haematocrit further supports the conclusion that β-glucan supplementation did not negatively affect haematological stability. Similar findings have been reported in rainbow trout and carp fed immunostimulant-enriched diets, where immune activation occurred without disruption of basic haematological homeostasis (Selvaraj 2005; Kühlwein et al. 2014; Minářová et al. 2021). According to Dawood et al. (2015), β-glucans modulate immune parameters while preserving haematological homeostasis when used at appropriate dietary levels. The dependence of the immune response on the concentration of β-glucans in the feed and the length of administration is also described in the study of Douxfils et al. (2017), where β-glucans (0.1%, 0.2%, and 0.5%) were administered for only 15 and 30 days.

A significant increase in white blood cell count was observed in fish receiving 0.5% β-glucans compared to the control group, suggesting proliferation and mobilization of leukocytes. This is a common response to immunostimulatory compounds, reflecting enhanced immune readiness (Tokunaka et al. 2000; Cornet et al. 2021). According to Cornet at al. (2021), leukocyte activity and immune responsiveness are impacted following β-glucan supplementation in fish diets. The absence of significant differences in phagocyte and lymphocyte counts supports the interpretation that β-glucans primarily affect immune cell functionality rather than cell abundance. Such qualitative modulation is considered advantageous in aquaculture, as it enhances immune preparedness with minimal energetic costs and without evidence of inducing chronic inflammation (Dobšíková et al. 2012; Cornet et al. 2021). Phagocytic activity demonstrated dose-dependent modulation by β-glucan supplementation.

Enhanced respiratory burst activity observed at lower inclusion levels, followed by reduced activity at higher doses, indicates a biphasic immunomodulatory response. This phenomenon is characteristic of immunostimulants, where moderate stimulation enhances innate immune function, whereas excessive or prolonged exposure may activate regulatory feedback mechanisms (Sakai 1999; Bricknell and Dalmo 2005). Beta-glucans are recognized by pattern recognition receptors on phagocytic cells, leading to enhanced respiratory burst, cytokine production, and microbial killing capacity (Selvaraj et al. 2005; Dobšíková et al. 2012). The enhancement of immune mechanisms is further supported by Khanjani et al. (2021), who reported that dietary β-glucans significantly stimulate the immune response in rainbow trout. However, sustained stimulation can lead to functional adaptation or immune tolerance-like effects, as described by Whyte (2007) in teleost innate immunity.

Microbiome analysis

At the phylum level, the gut microbiome of rainbow trout was dominated by Firmicutes and Fusobacteria. Fusobacteria, represented primarily by the genus *Cetobacterium*, were nearly absent in the 0.5% β-glucan group but remained abundant in the 0.2% and 1.0% β-glucan groups, indicating a non-linear dose-dependent response of the microbiome to dietary β-glucan exposure. Dose–response relationships are often non-linear, and higher intake levels do not necessarily provide greater benefits (Bechthold et al. 2019; Zhao et al. 2026). On the other hand, medium β-glucan group showed increased abundance of *Clostridium sensu stricto*. It corresponds with previously described effect of yeast-derived β-glucans increasing the abundance of beneficial gut bacteria, specifically *Clostridium sensu stricto* (Shi et al. 2025).

Alpha diversity indices (Shannon, Chao1; ANOVA, $P > 0.05$) comparison and principal coordinate analysis based on Bray–Curtis dissimilarities (PERMANOVA, $P > 0.05$) did not reveal significant differences in overall microbial community composition among treatments. Network analysis further suggested substantial restructuring of microbial associations despite the absence of significant differences in global community composition.

The combined SparCC network revealed two major microbial interaction clusters. A densely connected fermentation guild consisting of genera *Streptococcus*, *Peptoniphilus*, *Peptostreptococcus*, *Enterococcus* and *Lactococcus* exhibited strong positive associations, suggesting shared metabolic functions related to substrate use. In contrast, *Clostridium sensu stricto* formed negative correlations with several taxa including *Vagococcus* and *Romboutsia*, indicating potential niche differentiation or competitive interactions. Several taxa, particularly *Streptococcus* and *Peptostreptococcus*, occupied central positions within the network and may represent putative keystone members of the trout gut microbiome. While community composition stays similar, ecological interactions reorganize. Ecological relationships among microbes are represented by cooperation within the same guild and competition or cooperation between different guilds. These interactions play a crucial role in dynamic relationships of the microbiome that influence host fitness (Wu et al. 2021).

In conclusion, the presented results demonstrate that dietary β-glucans act as dose-dependent functional feed additives in rainbow trout metabolic activity, and innate immune function without inducing physiological stress. Higher β-glucans levels (0.5–1.0%) primarily supported hepatic metabolism, and favourable plasma biochemical profiles, whereas lower supplementation (0.2%) was associated with enhanced phagocytic responsiveness. Importantly, β-glucan-induced changes across production, haematological, and biochemical indices remained within physiological limits, indicating balanced immunometabolic modulation rather than overstimulation. These findings highlight the necessity of dose optimisation when incorporating β-glucans into practical aquafeed formulations, as different β-glucans levels may be strategically applied depending on production goals, whether targeting growth efficiency or immune preparedness. From an applied aquaculture perspective, β-glucans represent a promising nutritional tool for improving fish performance and health management under intensive rearing conditions.

The moderate β-glucan dose was related to the strongest microbiome relationship shift. This can be probably explained by concept, that moderate doses sometimes trigger the largest ecological reorganization before the system stabilizes again at higher doses. The medium β-glucan diet (0.5%) resulted in the strongest restructuring of microbial interaction networks and a marked reduction in *Cetobacterium* dominance together with a shift in *Clostridium sensu stricto* abundance. These results suggest that improved growth performance may be associated with a functional reorganization of the gut microbial community, although causal relationships require further investigation.

Acknowledgements

This study was supported through project IGA 216/2023/FVHE (University of Veterinary Sciences Brno, Czech Republic) and European Regional Development Fund in the Operational Program Research, Development and Education and The Czech Ministry of Education, Youth and Sports: PROFISH grant (CZ.02.1.01/0.0/0.0/16_019/0000869). We are grateful to Mgr. Petr Linhart, Pd.D. for help with statistical analysis. Computational resources were provided by the e-INFRA CZ project (ID: 90254), supported by the Ministry of Education, Youth and Sports of the Czech Republic.

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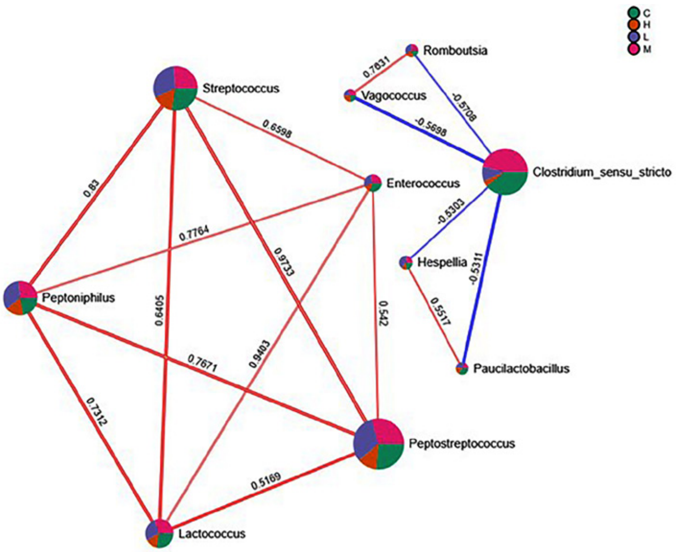

Fig. 2. Correlation network in all groups: C – control, L – low (0.2%) β-glucan P1, M – medium (0.5%) β-glucan P2, H – high (1.0%) β-glucan P3; node – genus, edge – correlation between connected nodes with correlation coefficient (SparCC, 200 permutations, |*P*| ≥ 0.5)

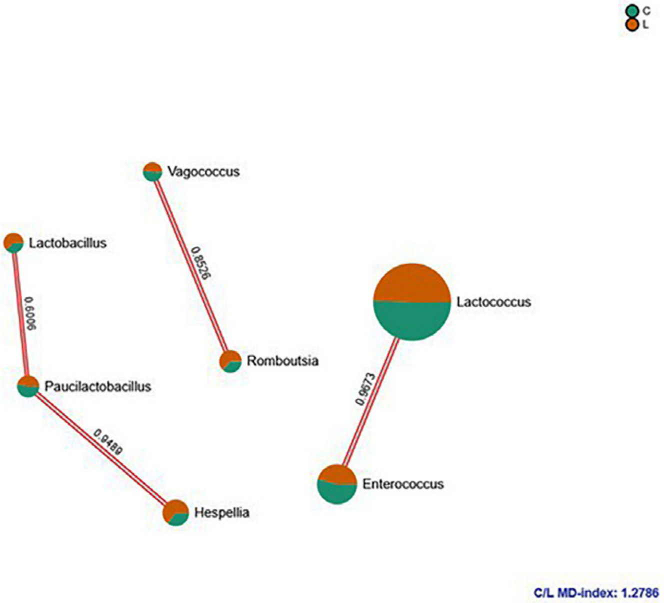

Fig. 3. Correlation network – control and low β-glucan group (C – control, L – low (0.2%) β-glucan P1; node – genus, edge – correlation between connected nodes with correlation coefficient (SparCC, 200 permutations, |*P*| ≥ 0.5); C/L Microbiome Dysbiosis Index (MD) = 1.2786

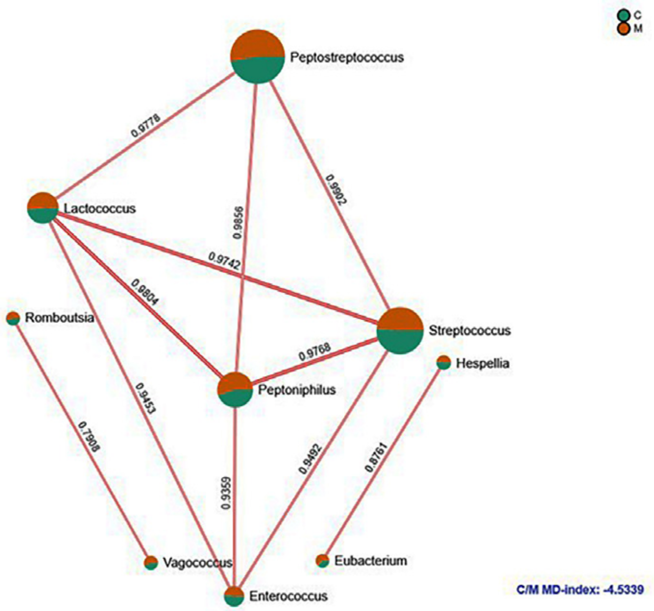

Fig. 4. Correlation network – control and medium β-glucan group (C – control, M 0.5% β-glucan P1; node – genus, edge correlation with correlation coefficient (SparCC, 200 permutations, |*P*| ≥ 0.5); C/M Microbiome Dysbiosis Index (MD) = −4.5339

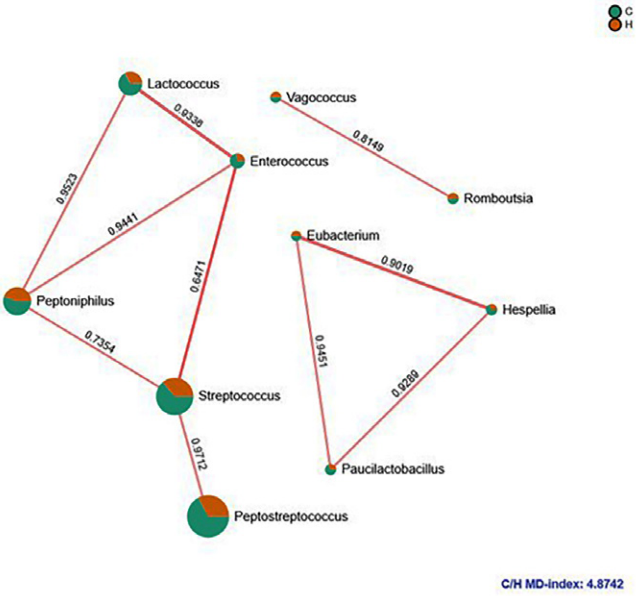

Fig. 5. Correlation network – control and high β-glucan group (C – control, H – 1.0% β-glucan P3; node – genus, edge correlation with correlation coefficient (SparCC, 200 permutations, |*P*| ≥ 0.5); C/H Microbiome Dysbiosis Index (MD) = 4.8742

Plate XIV

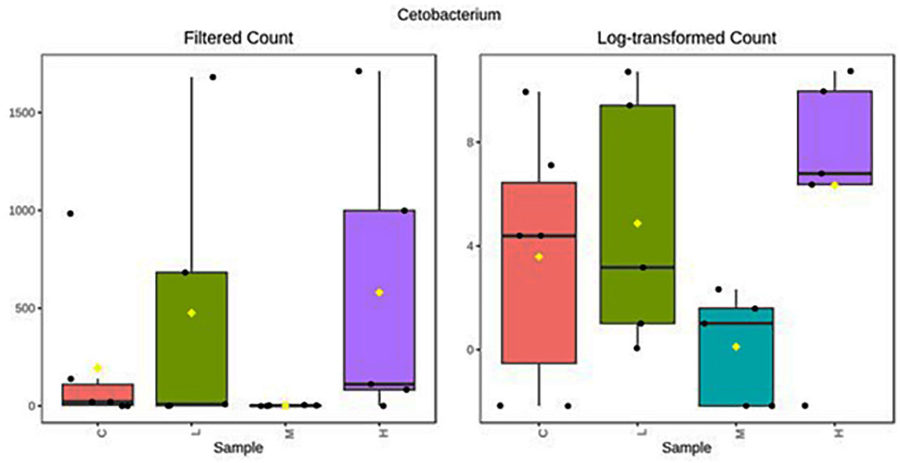

Fig. 6. Difference in abundance of genus *Cetobacterium* between control (C) and β-glucan groups (L – low (0.2%) P1, M – medium (0.5%) P2, H – high (1.0%) P3; difference in *Cetobacterium* abundance between medium (P2) and high (P3) β-glucan group; EdgeR, FDR = 0.006)

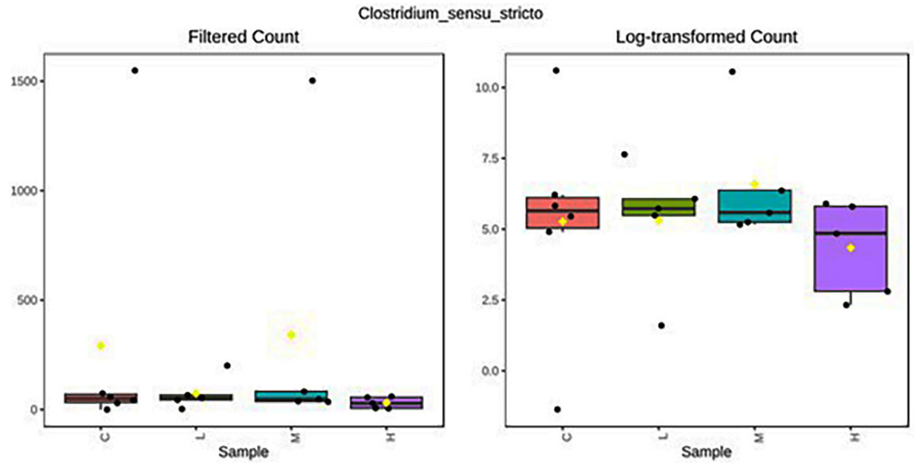

Fig. 7. Difference in abundance of genus *Clostridium sensu stricto* between control (C) and β-glucan groups (L – low (0.2%) P1, M – medium (0.5%) P2, H – high (1.0%) P3; difference in *Clostridium sensu stricto* abundance between medium (P2) and high (P3) β-glucan group; EdgeR, FDR = 0.047)