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Systemic Pro-Inflammatory Signaling and Mucosal Protection Induced by Dietary β-Glucan in Fish

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Abstract

β-glucan stimulates systemic immunity, but its effects on mucosal immune regulation remain unclear, including whether it triggers non-specific activation that could disrupt beneficial microbiota. Therefore, this study aimed to investigate how β-glucan modulates mucosal immunity in fish, focusing on local molecular signaling and gene expression profiles in gut-associated lymphoid tissue (GALT) and gill-associated lymphoid tissue (GIALT), as well as its systemic effects. The experiment followed a fully randomized 2x2 factorial design (control diet vs. 0.1% β-glucan; sampling at 15 and 30 days). The expression of cytokine genes in the intestine and gills, serum immunological markers, were evaluated. β-glucan supplementation increased IL-8 and IL-10 expression in the intestine after 30 days, while reducing IL-6, IFN-γ, and HSP70 expression in the gills. These findings suggest that β-glucan is associated with a lower inflammatory/stress-related transcriptional profile in intestinal and gill mucosae, potentially contributing to the maintenance of tissue homeostasis and mucosal integrity. Additionally, in the bloodstream, β-glucan supplementation elevated lysozyme concentration and hemolytic activity of the alternative complement pathway, indicating an enhancement of systemic innate immune responses. These results demonstrate that β-glucan modulates both local and systemic immune functions in fish, enhancing humoral mechanisms against pathogens while supporting a more balanced mucosal immune profile. Future studies integrating histology, oxidative markers, and microbiome profiling should help clarify the underlying mechanisms.

INTRODUCTION

The mucosal surfaces of fish are continuously exposed to the aquatic environment and represent major portals of entry for microorganisms, including commensals, opportunistic microbes, and pathogens. To cope with this challenge while maintaining local equilibrium, teleosts rely on mucosa-associated lymphoid tissue (MALT), which integrates innate and adaptive immune components to support protective responses and immunological tolerance at mucosal interfaces (Gomez et al. 2013; Salinas and Magadán 2017). Mucosal immune activity is closely linked to epithelial function and to the host–microbe relationship that characterizes mucosal tissues (Lazado and Caipang 2014; Mai et al. 2025).

Among teleost mucosal sites, the intestine and gills are particularly relevant. The intestinal mucosa constitutes a key interface for nutrient absorption and immune surveillance, whereas the gills form a highly vascularized surface essential for gas exchange but also susceptible to environmental and infectious insults. In both tissues, pathogen- and microbe-associated molecular patterns are detected by pattern recognition receptors (PRRs), including Toll-like receptors (TLRs), which can initiate innate immune signaling and shape downstream cytokine responses (Cabillon and Lazado 2019; Su 2025). In this context, cytokine transcription profiles are widely used as indicators of local immune activation versus regulatory/anti-inflammatory tendencies in mucosal tissues (Johansson and Hansson 2016; Wang et al. 2024).

Infectious diseases remain a major constraint for aquaculture, compromising productivity and sustainability. Although antimicrobials can control outbreaks, their extensive use increases concerns regarding microbial resistance and environmental impacts (Biller and Urbinati 2014; Özcan and Arserim 2026). Therefore, dietary strategies that modulate host immunity, including immunostimulants, have been explored as complementary tools to improve fish health and reduce antimicrobial reliance (Muneeb et al. 2026).

β -glucan, a polysaccharide derived from yeast cell walls, is one of the most studied dietary immunostimulants in aquaculture due to its capacity to modulate innate immune functions, including humoral components such as lysozyme and complement activity, through PRR-mediated pathways (Brown and Gordon 2001; Choudhray et al. 2026; Reis et al. 2021; Zhao et al. 2025). However, how β -glucan supplementation shapes mucosal immune transcriptional responses in GALT and GIALT, and how these local profiles relate to systemic immune markers and disease outcomes, remains incompletely resolved.

Therefore, the present study evaluated the effects of dietary β -glucan on (i) cytokine gene transcription in the intestine and gills and (ii) systemic humoral immune markers in serum. By integrating mucosal transcriptional readouts with systemic immune indices, we aimed to clarify whether dietary β -glucan is associated with a predominantly pro-inflammatory or regulatory mucosal profile while enhancing systemic innate defenses.

MATERIALS AND METHODS

Installations, fish and experimental diet

The experiment was performed at the Aquaculture Laboratory of the College of Technology and Agricultural Sciences, São Paulo State University (FCAT/UNESP), Dracena, São Paulo, Brazil. A total of 408 Nile tilapia (*Oreochromis niloticus*, Linnaeus, 1758), averaging 25 g in weight and 10 cm in length, were sourced from farms in the Santa Fé do Sul region, São Paulo. These fish were allocated into twenty-four 130 L polyethylene tanks, each equipped with continuous water renewal and aeration systems utilizing air compressors and air stones. Following a 20-day acclimation period, during which fish were fed to apparent satiety twice daily with a commercial diet (~3% of body weight per day; Supra Acqua Line Juvenil, 42% crude protein, 2.5 mm pellet size; Alisul Alimentos, Maringá, São Paulo, Brazil), the fish were assigned into four treatment groups (18 fish per tank), with six replicate tanks per treatment. A 12 h light/dark photoperiod was maintained using artificial lighting controlled by timers. Water quality parameters were monitored daily, recording temperature at 26 ± 0.2 °C, dissolved oxygen at 7.10 ± 0.5 mg L^{-1}, and pH at 7.6 ± 0.1.

The experimental protocols received approval from the Ethics Commission for the Use of Animals (CEUA-FCAT/UNESP, number 17/2022). Two diets were formulated and prepared at the Fisheries Institute of São José do Rio Preto, São Paulo, in accordance with species-specific nutritional

requirements (Koch et al. 2021), a control diet devoid of β-glucan and an experimental diet supplemented with 0.1% β-glucan. Fish were fed to apparent satiety twice daily.

Table 1. Ingredients and calculated nutritional composition of the experimental diets.

Ingredients	Diets (%)	
	Control	**β-glucan**
Soybean meal	34.21	34.21
Viscera Flour	18.00	18.00
Corn	17.51	17.51
Wheat bran	9.00	9.00
Rice crackers	8.00	8.00
Meat and Bone Meal	6.68	6.68
Protenosis	3.44	3.44
Dicalcium phosphate	0.75	0.75
Soy oil	1.00	1.00
Premix	0.50	0.50
Antifungal	0.20	0.20
Salt	0.30	0.30
DL-Methionine	0.16	0.16
Antioxidant	0.05	0.05
Limestone	0.167	-
B-glucan	-	0.167
Proximate composition (%)		
Protein	34	34
Starch	27	27
Lipid	6.13	6.13
Ash	8.87	8.87
Crude Fiber	3.27	3.27

Enrichment per kilogram of feed: Vit.-A 12.000 UI; Vit.-D3 3.000 UI;Vit. E 150 mg; Vit. K3 150 mg; Vit. B1 20 mg; Vit. B2 20 mg; Vit. B6 17.5 mg; Vit. B12 40 mg; Vit. C 300 mg; Niacin 100 mg; Folic Acid 6 mg; Pantothenic Acid 50 mg; Biotin 1 mg; Antioxidant 25 mg; Copper 17.5 mg; Iron 100 mg; Iodine 0.80 mg; Manganese 50 mg; Zinc 120 mg; Selenium 0.50 mg; Inositol 125 mg; Choline 500 mg.

The product contains natural antioxidants designed to protect animal feed from oxidative deterioration and preserve its freshness. It includes tocopherol (INS 307), soy lecithin (INS 322), rosemary oil extract,

and sunflower oil. Allergens: contain soy derivatives; gluten-free.

 β-glucan MacroGard® Biorigin, São Paulo, Brazil.

Source: Prepared by Giovani Sampaio Gonçalves.

Experimental design and sampling trials

Fish were distributed into 24 tanks (17 fish per tank). The experiment was conducted in a completely randomized design into a 2 x 2 factorial arrangement, with 6 repetitions each and divided into 4 treatments, as: C15 (tilapias received a control diet (free of β-glucan) for 15 days and sampled at 15 days of experiment); β15 (experimental diet (1 kg^{-1} of β-glucan) for 15 days and sampled at 15 days of experiment); C30 (control diet (free of β-glucan) for 30 days and sampled on the 30th day of the experiment); β30 (experimental diet (1g kg^{-1} of β glucan) for 30 days and sampled at 30 days of experiment). For statistical purposes, individual fish were considered observational units, and “tank” was included as a random effect to account for the non-independence of fish within the same tank. In each sampling assay, two fish were removed from each tank (n = 12) and anesthetized with eugenol (0.1 g L^{-1} according to Ventura et al., 2020). In addition, whole blood samples were taken from the caudal blood vessels and allowed to clot and the serum was separated by centrifugation (3000×g for 5 min) and stored at −80 °C until further immunological analysis (lysozyme activity and hemolytic activity of alternative complement pathway (HAACP)). The intestine and gill were collected for gene expression.

RNA extraction, cDNA synthesis and real-time PCR (RT-qPCR)

The experimental procedures were conducted in accordance with the manufacturer's protocols for the iScript™ cDNA Synthesis Kit (Bio-Rad Laboratories, CA, USA) and SYBR® Green PCR Master Mix. Total RNA (1 µg) was reverse transcribed into complementary DNA (cDNA) using the iScript™ cDNA Synthesis Kit, following the recommended protocol: 5 minutes at 25 °C, 30 minutes at 42 °C, and 5 minutes at 85 °C. The resulting cDNA samples were stored at −20 °C until further analysis. Quantitative real-time PCR (qPCR) assays were prepared in 96-well plates, with each reaction (20 µL total volume) containing 10 µL of 2× SYBR® Green PCR Master Mix, 200 nM of each primer, and 2 µL of cDNA template. Reactions were performed in triplicate using the iCycler iQ™ Real-Time PCR Detection System (Bio-Rad Laboratories, CA, USA). The thermal cycling conditions were as follows: initial denaturation at 95 °C for 5 minutes, followed by 40 cycles of 95 °C for 30 seconds and 56 °C for 30 seconds. A melting curve analysis was conducted to confirm the specificity of the amplified products. Gene expression levels were quantified using the comparative CT method (2^−ΔΔCT), with elongation factor 1-alpha (ef1α) and β-actin serving as reference genes. The efficiency of the PCR reactions was validated to ensure accurate relative quantification.

Table 2. Sequences of cytokine-specific primers used for the amplification of transcribed RNAs extracted from gills and intestine.

Gene	Sequence Fw/Rv (5'-3')	Acess number
β-actin	E: GTGCCCATCTACGAGGGTTA E: CTCTTTGATGTCACGCACGA	KJ126772.1
IL-6	F: GCTGGAGGCCAATAAAATCA. R: CCTTCGTCAGTCTCCAGCTC	MN870642.1
IL-8	F:AGGAGACAGGACTGGAATGCACAA R:GGAGGCAGTATAGGTATCATCCTC	GQ355864.1
IL-10	F:CTTCCCCGTCAGGCTCAA R:CTGTCGGCAGAACCGTGTC	XM_013269189.3
IL-1β	F:TGCACTGTCACTGACAGCCAA R:ATGTTCAGGTGCATOTTGCGG	XM_019365844.1
TNF-α	F: TAGAAGGCAGCGACTCAA R: CCTGGCTGTAGACGAAGT:	NM_001279533.1
IFN-g	F: GGGTGGTGTTTTGGAGTCGT R: GTAGCGAGCCTGAGTTGTTGGTG	NM_001287402.1
Lys	F: AAGGGAAGCAGCAGCAGTTGTG R: CGTCCATGCCGTTAGCCTTGAG	XM_013265574.3
C3	F: CCTTGCCCTAGAAACACACAC R:TCCACGGACAGCAGCAGAATAAGG	XM_019347500.2
HSP70	F: AGCTGCCATTGCTTATGGGT R: TCCTCCCCACCAAGATGAGT	XM_003448890.5

Source: Prepared by the author.

IL-6 = Interleukin-6; IL-8 = Interleukin-8; IL-10 = Interleukin-10; TNF-α = Tumor Necrosis Factor-alpha; IFN-γ = Interferon-gamma; Lys = Lysozyme; C3 = Complement component 3; HSP70 = Heat Shock Protein 70.

Immunological assays

The lysozyme activity was measured using the turbidimetric assay described by Biller et al. (2021). The results were expressed in units of lysozyme per ml of serum. One unit is defined as the amount of sample required to reduce absorbance by 0.001/min at 450 nm compared to the control. The HAACP was measured according to Biller-Takahashi et al. (2012). The hemolysis was estimated by measuring

the optical density (OD) of the supernatant at 415 nm for 10 min. The value of 50% hemolysis was calculated from the hemolysis curves of diluted serum.

Statistical analysis

Prior to analysis, residuals were inspected for normality and homoscedasticity (Shapiro–Wilk test and Levene's test). Data were analyzed using linear mixed-effects models, with group (C15, β15, C30, β30) [or diet, time, and their interaction] as fixed effects and tank as a random effect to account for the non-independence of fish within tanks. When significant effects were detected, pairwise comparisons were performed using Tukey's post hoc test. Statistical significance was set at $P < 0.05$. The analysis was executed using SAS OnDemand for Academics (SAS Institute Inc., Cary, NC, USA).

RESULTS

Relative gene expression of genes in the intestine of tilapia supplemented with β-glucan, assessed by RT-qPCR.

The gene expression results in the intestine demonstrate the interaction between diet (control and β-glucan) and time (15 and 30 days), as well as other statistically significant interactions. No significant differences were observed in the expression of IL-6, TNF-α, Lys, C3 and HSP70 (Fig. 1) genes concerning diet and time interactions.

Notably, the expression of IL-8 and IL-10 (Fig.2) genes increased significantly in fish fed with β-glucan for 30 days compared to their respective controls ($P < 0.05$).

Conversely, IL-1β (Fig. 2) gene expression was lower in fish fed the β-glucan diet at both 15 and 30 days compared to those receiving the control diet at the corresponding times. Furthermore, a 30-day feeding period, with either control or β-glucan diets, resulted in elevated IL-1β expression compared to a 15-day feeding period ($P < 0.05$).

While IFN-γ (Fig. 2) gene expression did not show significant differences in diet and time interactions, a 15-day experimental duration independently led to a higher expression of IFN-γ compared to the 30-day period ($P < 0.05$).

Relative gene expression of genes in the gills of tilapia supplemented with β-glucan, assessed by RT-qPCR.

The gene expression results in gill tissues demonstrate the interaction between diet (control and β-glucan) and time (15 and 30 days), as well as other statistically significant interactions. No significant differences were observed in the expression of IL-6, IL-8, IL-10, IL-1β, TNF-α, IFN-γ, Lys, C3, and HSP70 (Fig. 3) genes concerning the diet-time interaction. However, independent of the time factor, β-glucan supplementation significantly reduced the expression of IL-6, IFN-γ, and HSP70 genes compared to the control group ($P<0.05$).

Immunological assays. Serum Immunological Markers

The data on serum markers demonstrate the interaction between diet (control and β-glucan) and time (15 and 30 days), as well as other statistically significant interactions. Lysozyme concentration increased in fish fed β-glucan for 30 days compared to their respective control group ($P<0.05$). Independent of time, β-glucan administration elevated lysozyme levels compared to the control diet ($P<0.05$) (Fig. 4). Hemolytic activity of alternative complement pathway increased in fish receiving the β-glucan diet at both 15 and 30 days compared to those on the control diet at the corresponding time points. Independent of time, β-glucan supplementation enhanced HAACP relative to the control diet ($P<0.05$) (Fig. 4).

DISCUSSION

Dietary β-glucan has been reported to stimulate systemic innate immunity in fish, including tilapia, with effects reflected in circulating biomarkers and in gene expression in internal immune-relevant organs (e.g., head kidney, spleen, liver) (Beneti et al. 2023; Choudhray et al. 2026; Douxfils et al. 2017; Duan et al. 2025; Fierro-Castro et al. 2023; Reis et al. 2021). In contrast, its impact on mucosal immune regulation is still less consistently characterized, particularly under non-infectious conditions. Because innate immune receptors detect conserved microbial-associated patterns present in both pathogenic and commensal microorganisms, β-glucan supplementation could influence immune tone at mucosal surfaces. The key question is whether this modulation strengthens protective surveillance without driving excessive local inflammation that could impair mucosal function.

Here, we report that β-glucan supplementation was associated with tissue-specific immunomodulation in mucosal sites, alongside evidence of systemic activation. In the intestine, β-glucan increased IL-8 expression while reducing IL-1β and TNF-α, whereas in the gills the overall profile was predominantly anti-inflammatory, with reduced IL-6, IFN-γ, and HSP70. Concurrently, increased serum lysozyme activity and alternative complement pathway hemolytic activity were consistent with an enhancement of systemic innate defense. These patterns suggest that β-glucan may support a more controlled inflammatory set-point at mucosal surfaces—particularly in the gills—while maintaining or enhancing systemic effector capacity. This tissue-dependent response highlights mucosal tissues as relevant targets for nutritional immunomodulation and supports future studies integrating barrier function and microbiota endpoints to clarify the functional consequences of these transcriptional changes.

In this study, we extend current evidence on β-glucan immunomodulation by focusing on mucosal tissues under non-challenged conditions. β-glucan supplementation was associated with a tissue-dependent transcriptional response in the intestine and gills, along with increased systemic innate effector activity. Rather than broadly amplifying inflammatory signaling at mucosal surfaces, β-glucan appeared to shift local cytokine expression toward a more controlled inflammatory set point while enhancing soluble defenses in the circulation.

In the intestine, β-glucan increased IL-8 expression but reduced key pro-inflammatory cytokines (IL-1β and TNF-α), suggesting an immune profile compatible with chemotactic signaling alongside restrained inflammatory amplification. In the gills, the response was comparatively less reactive and was characterized by reduced expression of IL-6, IFN-γ, and HSP70, consistent with dampened inflammatory and stress-related signaling in this tissue. At the systemic level, the higher serum lysozyme activity and alternative complement pathway hemolytic activity indicate enhanced innate humoral capacity. These results support the interpretation that β-glucan can differentially modulate mucosal versus systemic components of innate immunity, potentially favoring mucosal immune regulation while maintaining systemic preparedness against infection. Further studies integrating barrier-function and microbiota endpoints will be important to determine the functional consequences of these mucosal transcriptional shifts.

β-glucan supplementation did not increase IL-6 expression in the intestinal mucosa, suggesting that its local immunomodulatory effects in this tissue are not mediated by IL-6–dependent inflammatory signaling. By contrast, IL-6 expression in the gills decreased with β-glucan, a pattern consistent with reduced pro-inflammatory tone at this mucosal surface. Given the central role of IL-6 in innate immune regulation (Wang et al. 2020; Zhu et al. 2019; Yinjie et al. 2024), this downregulation may reflect a mechanism to limit unnecessary inflammation in a tissue continuously exposed to environmental microbes. In fish, IL-6 has been linked to antibacterial defenses—Wang et al. (2020) reported reduced bacterial loads after IL-6 administration in Siberian sturgeon, and Zhu et al. (2019) showed that IL-6 can promote other pro-inflammatory cytokines and enhance phagocytic/bactericidal activity in *Plecoglossus altivelis* macrophages. Therefore, the lack of intestinal IL-6 upregulation and the reduction of branchial IL-6 observed here support the interpretation that β-glucan does not simply amplify pro-inflammatory pathways at mucosal sites in the absence of infection but may instead favor a more controlled inflammatory set-point. Although we did not directly assess barrier integrity or microbiota composition, limiting IL-6–associated inflammation could be beneficial for preserving gill function (respiration and osmoregulation) and maintaining mucosal physiological stability.

β-glucan supplementation did not change TNF-α expression in the intestinal or gill mucosa, indicating that the mucosal responses observed here are unlikely to be driven by TNF-α–dependent inflammatory signaling. TNF-α is a key cytokine in host defense against bacteria, parasites, and viruses and has been associated with the regulation of B-cell responses, including aspects of lymphocyte development and antibody-related immunity (Yang et al. 2023). Together with IFN-γ, TNF-α can promote downstream inflammatory cascades, including the induction of other cytokines such as IL-1β and IL-6 (Ng et al. 2025). In line with this interpretation, we observed no upregulation of IL-6 in the intestine and a reduction of IL-6 in the gills, alongside reduced IL-1β in the intestine, supporting the interpretation that β-glucan did not elicit a broad pro-inflammatory mucosal response at baseline.

An important component of fish innate antibacterial defense, lysozyme is mainly produced in immune organs such as the head kidney and can be detected in blood serum, including in tilapia. (Li et al. 2021; Pan et al. 2026; Reis et al. 2021). Here, β-glucan supplementation did not significantly change lysozyme

(Lys) gene expression in the intestinal or gill mucosa, indicating no clear local mucosal upregulation at the transcriptional level. Thus, β-glucan–driven modulation of lysozyme may be more evident systemically (e.g., circulating activity/protein levels) and/or via mechanisms not captured by mucosal Lys mRNA. Our results reinforce the tissue- and context-dependent nature of β-glucan immunomodulation and suggest that baseline supplementation does not elicit a pronounced inflammatory response in mucosal tissues.

Complement components such as C3 have been reported to increase in different tissues and in serum after β-glucan administration (Choudhray et al. 2026; Reis et al. 2021). In Nile tilapia, Reis et al. (2021) showed that β-glucan supplementation combined with vaccination upregulated lysozyme, C3, and HSP70 mainly in the head kidney, with a more modest response in the spleen. β-glucan may also enhance alternative complement activity by promoting C3 cleavage (C3a/C3b) and downstream complement activation, contributing to pathogen control (Xie et al., 2020). In the present study, however, β-glucan did not increase C3 gene expression in intestinal or gill mucosa, indicating no evidence of a marked local complement-related activation at these mucosal sites at baseline.

The limited mucosal immunological responsiveness to compounds that are immunostimulatory in other tissues suggests that baseline protection at these sites may depend more on barrier functions and microbiota-associated mechanisms than on strong host effector upregulation. Indeed, commensal microorganisms can promote immune homeostasis, including the induction of anti-inflammatory cytokines, whereas pathogen exposure typically drives pro-inflammatory mediators such as IL-1β and TNF-α that are required for effective antimicrobial defense (Lamy-Besnier et al. 2026; Luan et al. 2023). In this context, we hypothesize that β-glucan may be sensed at mucosal surfaces as a commensal-like MAMP, rather than a pathogen-associated danger signal, thereby favoring a controlled, non-exuberant mucosal response.

Considering the anti-inflammatory profile observed in mucosal tissues, we next examined heat shock proteins (HSPs). HSPs are central mediators of cellular stress responses in fish and other organisms and contribute to cytoprotection by supporting protein quality-control and degradation pathways (Basu et al. 2002; Liu et al. 2026). In the intestine, β-glucan supplementation did not change HSP70 expression, whereas in the gills it decreased HSP70. Although β-glucan is widely described as an immunomodulator that can stimulate cell activation (e.g., phagocytosis) and biosynthetic demand—processes often associated with increased HSP70 expression (Reis et al. 2021; Salah et al. 2017)—our mucosal data suggest that, intestinal tissue did not experience sufficient stress or activation to trigger HSP70 upregulation. In the gills, the lower HSP70 expression may indicate reduced cellular stress and a lower requirement for protective stress responses, consistent with preserved epithelial integrity and local homeostasis. Given that epithelial stress pathways can shape host–microbe interactions at mucosal surfaces, the downregulation of HSP70 in the gills may be consistent with a less inflammatory milieu that favors microbiota stability and helps sustain key physiological functions (Gomez et al. 2013).

Furthermore, IL-8 and IL-10 expression increased in the intestinal mucosa of fish fed β-glucan for 30 days compared with the control group. In the gills, no significant changes were observed for either cytokine.

Monocytes and activated macrophages secrete interleukin-8 (IL-8), promoting the recruitment, chemotaxis, adhesion, and effector functions of neutrophils and other leukocytes to mucosal sites (Cao et al. 2025; Rossi and Zlotnik 2000;). Therefore, the upregulation of IL-8 suggests that β-glucan can improve immune cell recruitment to the intestine, reinforcing front-line defense mechanisms.

Interleukin-10 (IL-10) is a key anti-inflammatory cytokine that suppresses excessive activation of macrophages and other myeloid cells, modulates antigen-presenting cell maturation, and helps prevent tissue damage during immune responses (Kim et al. 2026; Ouyang and O'Garra 2019; Veenbergen and Samsom 2012). Thus, the increase in IL-10 indicates that β-glucan promoted regulatory signaling that can counterbalance chemokine-driven cell influx, contributing to immune homeostasis in the intestinal mucosa. The parallel induction of IL-8 (recruitment/alertness) and IL-10 (regulation) supports the interpretation that β-glucan enhances mucosal preparedness while limiting the risk of exacerbated inflammation, helping preserve epithelial integrity and potentially favoring a stable microbiota. This is relevant because uncontrolled intestinal inflammation can impair barrier function, increase permeability, and contribute to dysbiosis, which in turn facilitates microbial translocation and amplifies inflammatory activation (Wallaeys et al. 2023; Wang et al. 2024b). In contrast, the absence of significant modulation of these cytokines in the gills reinforces a tissue-specific response, consistent with distinct immune set-points and exposure dynamics at each mucosal site.

Interleukin-1β (IL-1β) is a central pro-inflammatory cytokine involved in the activation of innate and adaptive immune responses, including effects on lymphocyte activation and broader regulation of inflammatory and homeostatic processes (Eeckhout et al. 2020; Yang et al. 2022).

In rainbow trout (*Oncorhynchus mykiss*), IL-1β has also been associated with enhanced antiviral protection against infectious hematopoietic necrosis virus (IHNV) when incorporated into DNA-vaccine strategies (Cao et al. 2017). In the present study, however, IL-1β transcript levels were reduced in the intestine after 15 and 30 days of dietary β-glucan supplementation. This pattern is consistent with a dampening of intestinal inflammatory tone, which may help preserve epithelial homeostasis and support a more stable mucosal environment. Such stabilization could favor beneficial microbial communities and, consequently, contribute to improved digestive performance and resilience to opportunistic pathogens, as suggested in related feeding trials (Shi et al. 2021). Together, these findings reinforce that β-glucan effects can be tissue-specific, simultaneously supporting immune preparedness while preventing unnecessary inflammation at the intestinal mucosa.

Previous studies indicate that dietary β-glucan can modulate inflammatory signaling in a tissue- and context-dependent manner. Miest et al. (2016) evaluated turbot (*Scophthalmus maximus*) and reported an immunomodulatory profile after β-glucan feeding, with indicators consistent with reduced inflammatory stress and improved larval survival. In agreement with an anti-inflammatory tendency at

the intestinal mucosa, Huang et al. (2023) reported that dietary yeast cell wall significantly downregulated midgut IL-1β and TNF-α expression, indicating an attenuation of intestinal pro-inflammatory signaling and improved mucosal immune homeostasis. Similar conclusions were drawn by Falco et al. (2014), who reported reduced expression of pro-inflammatory cytokines in common carp after two weeks of β-glucan administration, suggesting attenuation of an acute inflammatory response, particularly in the intestine.

In the present study, IFN-γ gene expression decreased in the gills following β-glucan feeding. IFN-γ is involved in macrophage activation and antimicrobial effector pathways, including enhanced phagocytic activity and nitric oxide-related responses (Chen and Liu 2009; Grayfer et al. 2010; Zou et al. 2005). Although IFN-γ is typically associated with heightened cellular immunity, its downregulation at the gills may reflect a regulatory adjustment that helps limit excessive local inflammation at this highly exposed mucosal surface.

Selective responsiveness across immune organs after β-glucan administration has also been documented (Rawling et al. 2023). For example, Douxfils et al. (2017) reported marked responses in the head kidney and changes in serum immune parameters in rainbow trout (*Oncorhynchus mykiss*), supporting the idea that β-glucan effects vary across compartments. Collectively, these findings reinforce that β-glucan can shape mucosal immune tone rather than uniformly amplifying inflammation, and that local factors (including resident microbiota and baseline tissue immune set-points) may contribute to the observed tissue-specific outcomes.

Although mucus production was not directly evaluated, β-glucan supplementation has been reported to influence mucosal secretions and barrier-related traits, which may contribute to a luminal environment that favors commensal microbiota (Cigarroa-Ruiz et al., 2023; Gomez et al. 2013; Marel et al. 2012; Wang et al. 2024a). The bidirectional relationship between intestinal microbiota and host health is well established, with impacts on nutrient processing, barrier function, and immune homeostasis (Hooper 2009; Sekirov et al. 2010; Shi et al. 2021). In this context, the anti-inflammatory profile observed after β-glucan administration may reflect a compartmentalized modulation of mucosal immunity that differs from systemic responses. Accordingly, shifts in cytokine transcription within gut-associated immune compartments (e.g., GALT/GIALT) may represent early molecular events that precede broader changes in cellular recruitment and contribute to the maintenance of tissue homeostasis.

Blood biomarkers were also evaluated, as circulating immune parameters provide valuable insight into fish defense status and the effects of nutritional or environmental interventions on the immune system (Li et al. 2021; Rauta et al. 2012). Thirty days of β-glucan administration resulted in a significant increase in lysozyme activity, whereas hemolytic complement activity increased after 15 and 30 days of supplementation. Lysozyme is an antimicrobial enzyme present in mucus, eggs, and blood and is frequently used as a biomarker of innate immunocompetence and responses to environmental and nutritional modulation in fish (Biller and Urbinati 2014; Manchanayake et al. 2025). The complement system is also a central effector of innate defense, participating in opsonization, phagocytosis,

chemotaxis, inflammation, and lysis of foreign cells (Bavia et al. 2022). Therefore, the increased lysozyme and complement activity observed here support an immunomodulatory effect of β-glucan and suggest an enhanced capacity for early innate responses.

Previous studies support these observations. Koch et al. (2021) reported increased lysozyme activity in the liver and intestine of tilapia fed β-glucan. In pacu, β-glucan supplementation has been associated with immunomodulatory effects following stressful handling and bacterial challenge (Lopes et al. 2022), and with increased lysozyme levels and hemolytic activity of the alternative complement pathway after transport handling (El-Murr et al. 2019). Complement-related responses have also been described under challenge conditions. Sixty-day oligosaccharide and β-glucan supplementation improved growth, antioxidant/innate immunity, and survival after *Streptococcus iniae* challenge in Nile tilapia (Eissa et al. 2024). Moreover, Choudhray et al. (2026) showed that dietary β-glucan increased lysozyme and complement activities and improved growth performance and feed efficiency, which were accompanied by higher digestive enzyme activities, suggesting enhanced nutrient utilization.

CONCLUSION

This study provides evidence that β-glucan modulates fish innate immunity in a compartment-specific manner. Overall, β-glucan supplementation was associated with a systemic pro-inflammatory profile, whereas the intestinal and branchial mucosae exhibited a comparatively anti-inflammatory pattern, consistent with improved local inflammatory control. Accordingly, future studies should investigate whether β-glucan reshapes intestinal microbial communities and their metabolites, and how these changes relate to mucosal immune function and disease resistance.

Systemic innate immunity represents a major first line of defense in fish, and its activation by β-glucan may increase the availability of early effector mechanisms that contribute to host protection. In parallel, the apparent reduction in intestinal and branchial inflammation may help preserve epithelial barrier integrity and limit oxidative and structural damage—processes that are critical for digestion, nutrient absorption, and gas exchange. Our results suggest that β-glucan can support mucosal homeostasis by modulating local inflammation while maintaining systemic innate immune responsiveness.

Declarations

AUTHOR CONTRIBUTIONS

Conceptualization, Ingrid Camargo dos Reis, Basia Schlichting Moromizato, Camino Fierro-Castro, Jaqueline Dalbello Biller; **Funding acquisition,** Jaqueline Dalbello Biller; **Investigation, analysis and interpretation of data**, Ingrid Camargo dos Reis, Isabella Amigo Cordeiro Vaz, Basia Schlichting Moromizato, Camino Fierro-Castro, Giovanni Sampaio Gonçalves, Jaqueline Dalbello Biller; **Methodology**, Ingrid Camargo dos Reis, Helio José Montassier; **Writing-original draft**, Ingrid Camargo dos Reis; **Writing-review & editing**, Camino Fierro-Castro, Helio José Montassier, Jaqueline Dalbello Biller; Giovanni Sampaio Gonçalves; **Supervision**, Jaqueline Dalbello Biller.

DECLARATION OF COMPETING INTEREST

The authors declare no conflicts of interest.

CLINICAL TRIAL NUMBER

Clinical trial number: not applicable.

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Figures

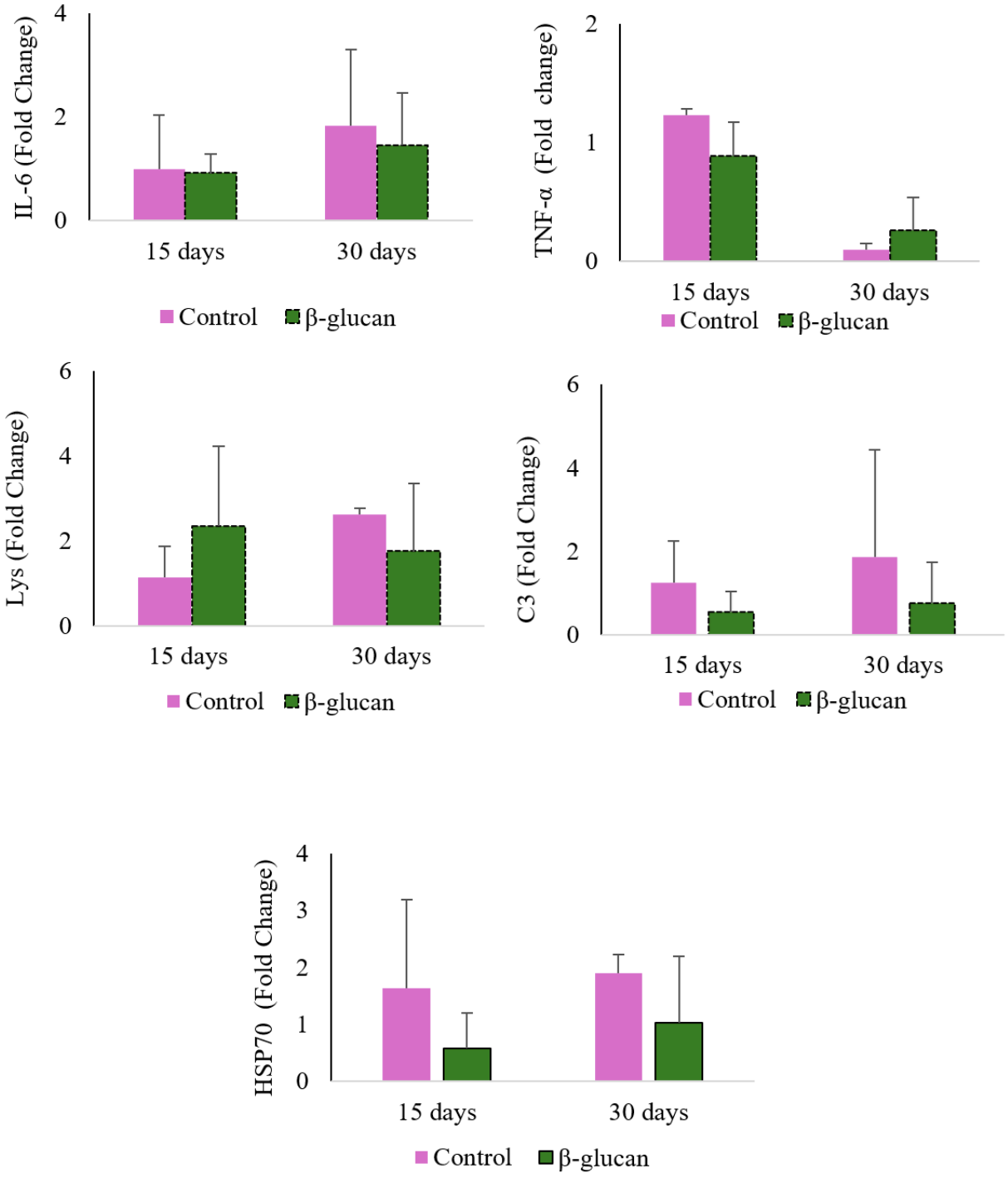


Figure 1

Relative expressions of IL-6, TNF-α, Lys, C3 and HSP70 genes in the intestine of tilapia supplemented with β-glucan, using qPCR-RT. Different letters indicate significant differences. P <0.05. C. Values are means ± standard error in duplicate.

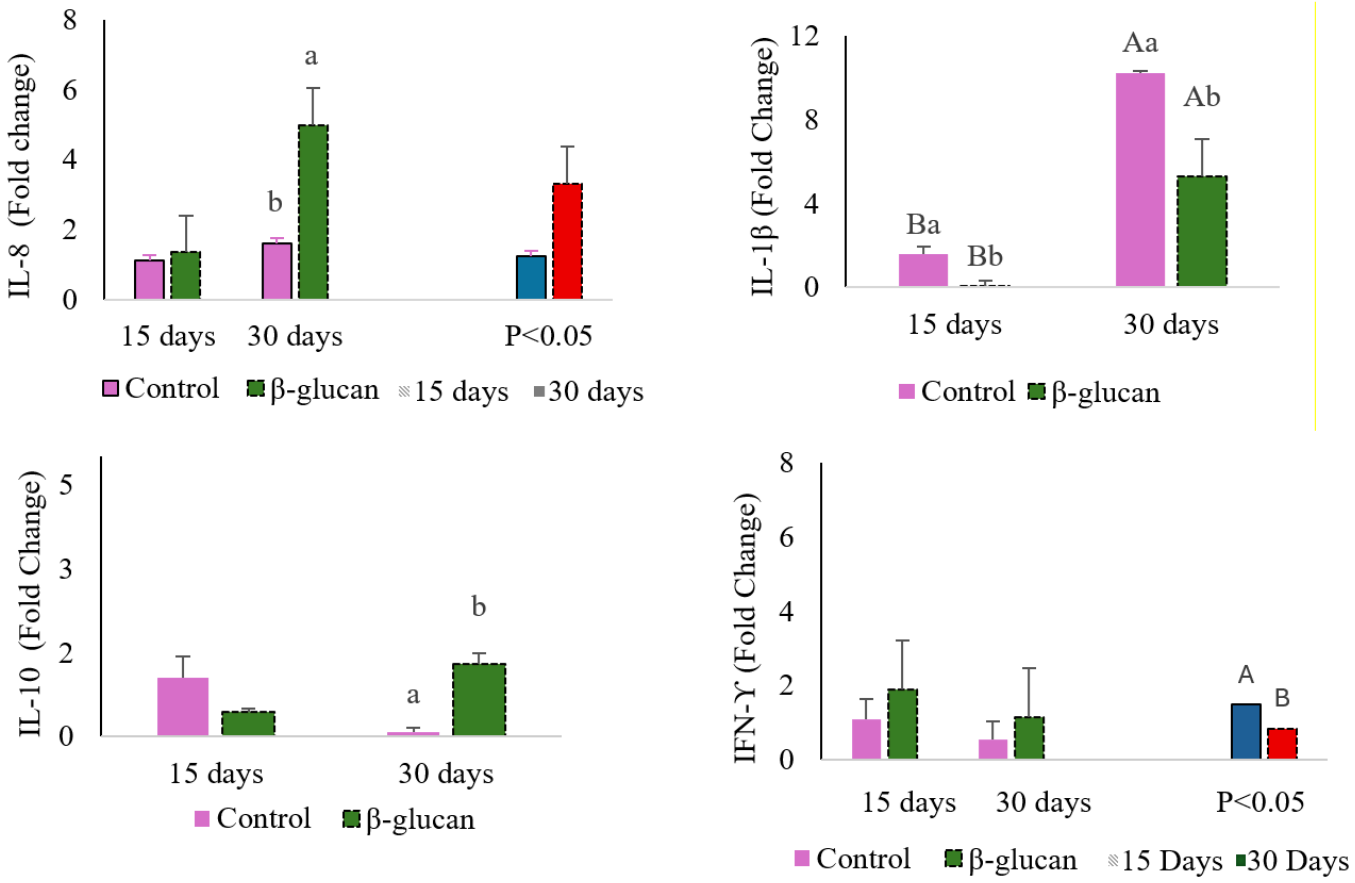


Figure 2

Relative expressions of IL-8, IL-10, IL-1β and IFN-γ genes in the intestine of tilapia supplemented with β-glucan, using qPCR-RT. Different letters indicate significant differences. P <0.05. C. Values are means ± standard error in duplicate.

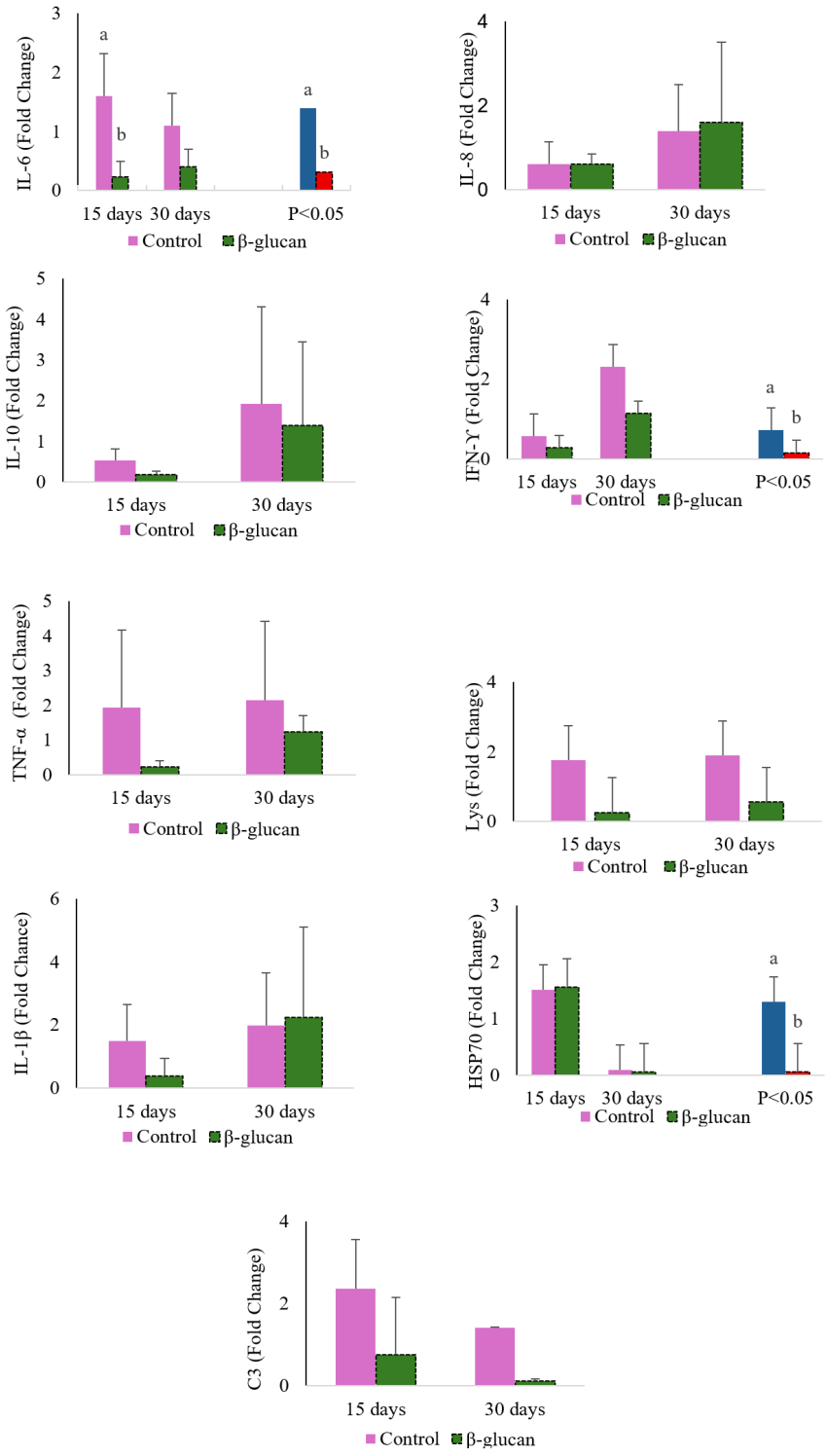


Figure 3

Relative expression of IL-6, IL-8, IL-10, IL-1β, TNF-α, IFN-γ, Lys, C3, and HSP70 in the gills of tilapia supplemented with β-glucan, using qPCR-RT. Different letters indicate significant differences. P <0.05. Values are means ± standard error in duplicate.

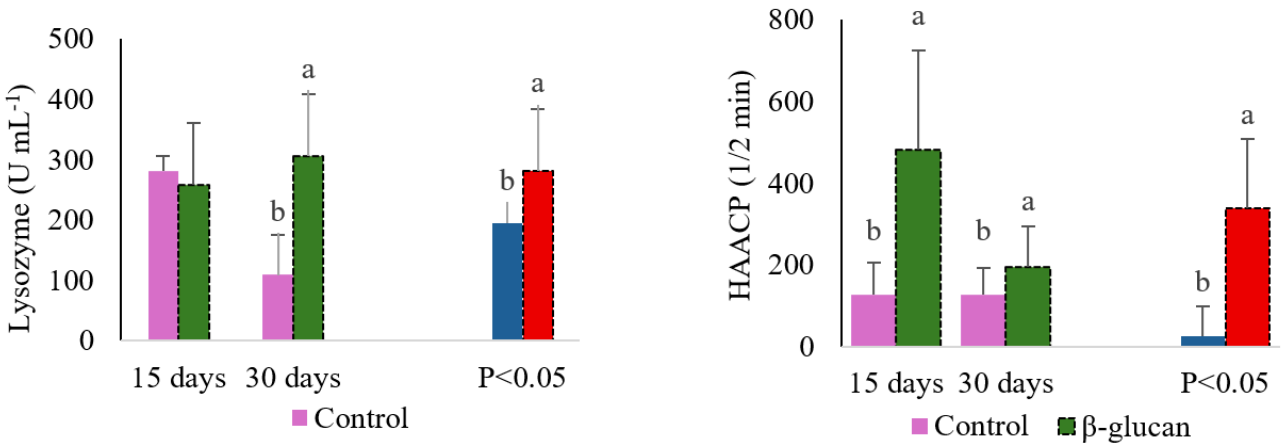


Figure 4

Lysozyme and Hemolytic activity of alternative complement pathway (HAACP) of tilapia supplemented with β-glucan. Different letters indicate significant differences. P <0.05. Values are means ± standard error in duplicate.