Genetics of Aquatic Organisms 8(3), GA832
https://doi.org/10.4194/GA832

RESEARCH PAPER

Expression Profiling of Innate Immune Genes in Nile Tilapia (*Oreochromis niloticus* Linnaeus, 1758) Upon Short Term Immunization with Bacterial Lipopolysaccharide (LPS) and Peptidoglycan (PGN)

Jannatul Ferdoush[1], Anwar Hossain[1,5], Md. Inja-Mamun Haque[1], Md. Ismail Hossen[2], Md. Abdullah Al-Mamun[3], Md. Mizanur Rahman[4], Md. Shafiujjaman[1,5], Shankar Chandra Mandal[1,*]

[1]Department of Fisheries, University of Dhaka, Dhaka 1000, Bangladesh.
[2]Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka 1000, Bangladesh.
[3]Department of Fisheries & Marine Science, Noakhali Science and Technology University, Noakhali-3814, Bangladesh.
[4]Department of Zoology, University of Dhaka, Dhaka 1000, Bangladesh.
[5]Center for Marine Environmental Studies, Ehime University, Bunkyo-Cho 3, Matsuyama, Ehime 790–8577, Japan.

How to Cite

Ferdoush, J., Hossain, A., Haque, Md. I-M., Hossen, Md.I., Al-Mamun, Md.A., Rahman, Md.M., Shafiujjaman, Md., Mandal, S.C. (2024). Expression Profiling of Innate Immune Genes in Nile Tilapia (*Oreochromis niloticus* Linnaeus, 1758) Upon Short Term Immunization with Bacterial Lipopolysaccharide (LPS) and Peptidoglycan (PGN). *Genetics of Aquatic Organisms, 8(3), GA832*. https://doi.org/10.4194/GA832

Article History
Received 21 November 2024
Accepted 26 December 2024
First Online 30 December 2024

Corresponding Author
E-mail: shankar@du.ac.bd

Keywords
Innate immunity
Nile tilapia
Lipopolysaccharide
Peptidoglycan

Abstract

Tilapia aquaculture is emerging throughout the world because of its fast growth and adaptability. However, disease is one of the impediments in emerging this sector. Boosting the immunity of tilapia by using immunostimulants can help in fighting the diseases. This study investigates how bacterial pathogen-associated molecular patterns (PAMPs), specifically lipopolysaccharide (LPS) and peptidoglycan (PGN), influence the regulation of expression of innate immune genes in Nile tilapia. In the current study, *E. coli* derived LPS and PGN were injected at 100 µg LPS/kg and 100 µg PGN/kg intraperitonially in treatment group and 100 µg PBS/kg in control group. Significant upregulation of *TLR2, TLR3, TLR8,* and *TLR9* was observed in the spleen, liver, gill, and brain at various time points post-treatment ($P<0.05$). Additionally, increased expression of *IRF3* and *TGF-β* was noted in multiple organs, along with elevated levels of pro-inflammatory cytokines *IL-1β* and *IL-10*, and antiviral protein viperin. These molecular responses suggest that *E. coli* derived LPS and PGN can effectively modulate inflammatory pathways, enhancing the immune defense mechanisms of tilapia. The findings highlight the potential of bacterial PAMPs to improve fish health, reduce mortality rates, and increase profitability in aquaculture by reducing the reliance on antibiotics.

Introduction

Tilapia, often called “aquatic chicken", is a popular species globally with valuable and affordable protein source (Maclean, 1984). Tilapia aquaculture has been popular because of its high growth and productivity in a short period. However, recent occurrences of bacterial and viral disease outbreaks present a significant threat to the global tilapia aquaculture industry (Abdel-Latif et al., 2020). Over the last several decades, many veterinary pharmaceuticals, especially antibiotics are being used to control disease outbreaks in tilapia aquaculture (Lupin, 2009). In aquaculture, immune stimulation is a helpful technique when vaccination or injectable therapy is challenging and time-consuming procedures, and when frequent chemotherapy increases the risk of drug-resistant pathogen strains emerging (Bondad-Reantaso et al., 2005; Maqsood et al., 2011). One of the first tasks the body must perform to defend against disease is the detection of pathogens. By identifying chemicals specific to bacteria that are not connected to human cells, is a critical function of innate immunity. Pathogen-associated molecular patterns, or PAMPs, are the term for these special molecules such as

lipopolysaccharide (LPS) and peptidoglycan (PGN). Lipopolysaccharide is a component of the cell walls of the majority of Gram-negative bacteria. On the contrary, PGN which makes up 40–90% of the dry mass of Gram-positive bacteria is its primary constituent (Inamura et al., 2001). According to Kumar et al. (2011), when PAMPs attach to defense cells' pattern-recognition receptors (PRRs), cytokines that cause inflammation and activate the complement system are released. This study also showed that tumor necrosis factor-alpha *(TNF-α)*, interleukin-1 beta *(IL-1β)* are examples of cytokines that are considered inflammatory because they induce inflammation, and this strengthens the body's protection by causing defense cells to produce a moderate number of cytokines. Pathogen associated molecular patterns (PAMPs) are used as immuno-stimulants and have been shown to boost resistance to a wide range of diseases (Magnadottir, 2010). Sevaraj et al. (2004) reported immunization of the *Cyprinus carpio* by LPS from *Aeromonas hydrophila* resulted in increased immunity and survivability. It has been demonstrated that PGN can increase the disease resistance of aquatic animals including fishes and shrimps as reported in *Litopenaeus japonicus* and *Pseudosciaena crocea* (Zhang et al., 2008).

Bony fish possess lymphoid organs, including the spleen, kidneys, and thymus, as they do not have bone marrow or lymph nodes like mammals (Biller-Takahashi & Urbinati, 2014). Among these, the spleen, a key peripheral immune organ (Rauta et al., 2012), actively releases antimicrobial peptides and cytokines during inflammatory responses (Hu et al., 2021). Beyond the roles in gas exchange, ion regulation, maintaining acid-base balance, osmoregulation, excretion of ammonia, hormone production, and immune defense, the fish gill also has other functions (Rombough, 2007). Notably, it is crucial for generating cells that produce antibodies following direct immersion vaccination (Dos Santos et al., 2001). The liver, which is key in bile secretion, detoxification, and metabolism of carbohydrates, proteins, and lipids, often has its role in immune functions overlooked. A study on immune gene expression post bacterial infection highlights the liver's involvement in immune responses (Martin et al., 2010). Although not an immune tissue, the fish brain can trigger a strong immune reaction characterized by the expression of cytokines (Novoa et al., 2010). One of the important immune organs in fish is head kidney. However, due to the small size of the fish used in the experiment, proper isolation of the head kidney was not feasible for all specimens. Consequently, it was excluded from this study.

Though several studies have shown the immunomodulatory effects of LPS and PGN in aquaculture species, very limited data are available on the immune responses of tilapia using bacterial PAMPs LPS and PGN as immunostimulants; and how these bacterial PAMPs induce the immunological response of Nile tilapia is not well studied. Moreover, the specific roles of key immune organs, such as spleen, gill, liver, and brain, in mediating immunological responses are poorly understood. These organs play critical roles in both systemic and localized immune responses, yet their involvement in tilapia immunity, particularly in response to bacterial PAMPs, warrants further investigation. Thus, the present study was conducted to investigate the effects of *E. coli* derived LPS and PGN on the immune response of Nile tilapia, focusing on the expression of particular innate immune genes, such as toll-like receptor 2 *(TLR2),* toll-like receptor 3 *(TLR3),* toll-like receptor 8 *(TLR8)*, toll-like receptor 9 *(TLR9)*, interferon 3 *(IRF3)*, transforming growth factor beta *(TGF-β)*, interleukin 1 beta *(IL-1β)*, interleukin 10 *(IL-10)*, and *viperin*.

Materials and Methods

Fish Collection, Experimental Set-Up and Fish Maintenance

In the present study 190 healthy male mono-sex Nile tilapia (20.87±5.58 g), showing no signs of disease such as bleeding, ascites, lethargy, or scale loss, were collected from a fish farm in Noakhali district, Bangladesh. The tilapia fish were housed in a 500-L capacity tank and conditioned with continuous aeration for seven days. Subsequently, they were randomly assigned to 80-L glass aquariums containing 60-L of water (15 fish per tank, two experimental groups, triplicated per group) and acclimatized for an additional week before starting the experiment. The experiment took place in an indoor facility at the University of Dhaka under uniform lighting to reduce potential confounders. Tanks of uniform size were utilized for fish rearing. Daily, water (30% of total volume) was replaced for maintaining water quality, keeping pH at 7.50±0.22, dissolved oxygen at 5.42±0.48 mg/L, and temperature at 26.46±0.92°C. Following acclimation, the fish remained in the tanks for 30 days of experiment.

LPS and PGN Stimulation and Sample Collection

To investigate the effects of lipopolysaccharide (LPS) and peptidoglycan (PGN) on immune function in Nile tilapia, the experiment was conducted where the fish (n=135) were allocated at random into two experimental groups and one control group each in triplicate. Each group consisted of 15 fish placed in an 80-L glass tank with 60-L of water. The fish were stimulated with LPS-B5 (lipopolysaccharide from *E. coli* 055: B5) and PGN-EK (peptidoglycan from *E. coli* K12), (InvivoGen, San Diego, USA). A previous study indicated that certain LPS may trigger immune reactions differently, with gene expression profiles varying by tissue and concentration (Boltaña et al., 2014). Thus, we utilized LPS and PGN from *E. coli* to offer a broad perspective on the immune system's role in defending against microorganisms. Fish from treatment groups

were treated with an intraperitoneal injection at a rate of 100 µg LPS and PGN/Kg fish and control group injected with 100 µg PBS/Kg fish. The dosage calculation for LPS and PGN was estimated based on previous study by Selvaraj et al. (2009). Fish were anesthetized using 0.1% of 2-phenoxyethanol before injection. Fish were provided feed to 10% of their body weight (BW) which was divided into two rations (5% of BW each time) at 9:00 and 16:00 h. At 24 h, 48 h, 72 h and on day 7, one fish from each replicate of each group was sacrificed to collect tissues including gill, brain, liver and spleen. Fish were anesthetized before sacrifice. The tissues were preserved in RNA later until used for the extraction of RNA.

Total RNA Extraction, cDNA Synthesis and Real-Time Quantitative PCR (qRT-PCR)

Total RNA was extracted from 50 mg of spleen, liver, gill and brain tissue using TRIzol™ Reagent (Thermo fisher Scientific) indicating the instructions of the manufacturer with some modifications. Both RNA amount and purity were determined using NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA). The RNA samples with ratio of 260/280 within 1.8-2.0 were used for downstream applications.

Following RNA extraction, cDNA synthesis was performed using PrimeScript™ 1st strand cDNA Synthesis Kit (Takara, Japan) based on the instruction of the manufacturer. Specific primers were used to amplify the selected genes with *β* -actin as housekeeping (internal control) gene. The expression of *TLR2, TLR3, TLR8, TLR9, IRF3, TGF-β, IL-1β, IL-10* and *viperin* in spleen, liver, gill and brain tissues of Nile tilapia was determined by qRT-PCR. The qPCR master mixture was prepared in a 20 µL volume following the manufacturer's recommendations. The volume contained 10 µL of TB Green® Premix Ex Taq™ (Tli RNaseH Plus) (Takara Bio, Japan), 0.5 µL each of forward and reverse primers (10 pM), 2 µL of cDNA (1:10 fold diluted) and 7 µL of nuclease free water. The qRT-PCR assay was carried out in a real time PCR machine (qTower3, Analytik Jena, Germany). The temperature profile for the assay was as follow: pre-treatment at 50°C for 2 minutes, followed by 40 cycles of denaturation at 95°C for 10 s, annealing at 56-60°C for 30 s, and extension at 72°C for 15 s; followed by melt curve analysis: 95°C for 10 s, 55°C for 5 s and 95°C for 30 seconds with cooling step at 40°C for 30 seconds to confirm single amplicon generation.

Statistical Analysis

The qRT-PCR data were first analyzed by Microsoft Excel (2013) for relative gene expression calculation in the *E. coli* derived LPS and PGN stimulated fish compared to those in the unstimulated control fish following $2^{-\Delta\Delta Ct}$ approach (Livak and Schmittgen 2001). Finally, one-way ANOVA and multiple comparisons were performed with GraphPad Prism version 9 (San Diego, CA, USA) to compare the expression of target genes between control and treatment groups.

Results

Expression of *TLRs* in Different Organs

A significantly higher level of *TLR2* expression was detected in spleen and gill at 24 h after treatment with PGN and LPS ($P<0.05$; Figure 1a & 1c). The expression of *TLR2, TLR3, TLR8* and *TLR9* was significantly increased in spleen of fish after 48 h of treatment with PGN and LPS ($P<0.05$; Figure 1a, 1e, 1i & 1m). Moreover, after 72 h, the expression of TLR9 was significantly upregulated in liver, gill, brain of LPS and PGN treated fish ($P<0.05$; Figure 1n, 1o & 1p). Furthermore, significantly higher levels of *TLR2, TLR3, TLR8* and *TLR9* expressions were detected in spleen ($P<0.05$; Figure 1a, 1e, 1i & 1m) and liver ($P<0.05$; Figure 1b, 1f, 1j & 1n) after 7 days of treatment with LPS and PGN.

Significant upregulation of *TLR2* expression was observed in the spleen and gill 24 hours after treatment with PGN and LPS ($P<0.05$; Figure 1a & 1c). At 48 hours post-treatment, the expression levels of *TLR2, TLR3, TLR8,* and *TLR9* were significantly increased in the spleen ($P<0.05$; Figure 1a, 1e, 1i & 1m). Furthermore, at 72 hours, *TLR9* expression was significantly elevated in the liver, gill, and brain of LPS- and PGN-treated fish ($P<0.05$; Figure 1n, 1o & 1p). After seven days of treatment with *E. coli* derived LPS and PGN, significantly higher expression levels of *TLR2, TLR3, TLR8,* and *TLR9* were detected in both the spleen ($P<0.05$; Figure 1a, 1e, 1i & 1m) and liver ($P<0.05$; Figure 1b, 1f, 1j & 1n).

Expression of *IRF3* and *TGF-β* in Different Organs

The expression of IRF3 was significantly upregulated in the spleen at 24 and 72 hours following treatment with PGN and LPS ($P<0.05$; Figure 2a). Similarly, the expression of *TGF-β* was significantly increased in the spleen on day 7 post-treatment ($P<0.05$; Figure 2e). At 48 hours, a significant increase in *IRF3* expression was observed in the gill and brain ($P<0.05$; Figure 2c & 2d), while *TGF-β* expression was significantly elevated in the brain at 48 hours in response to LPS ($P<0.05$; Figure 2h). Additionally, significant upregulation of both *IRF3* and *TGF-β* was detected in the liver and gill at 72 hours post-stimulation with *E. coli* derived LPS and PGN ($P<0.05$; Figure 2b, 2f, 2c & 2g).

Expression of *IL-1β, IL-10* and *Viperin* in Different Organs

The expressions of *IL-1β* and *IL-10* were significantly elevated in the gill at 24 hours post-treatment with LPS and PGN ($P<0.05$; Figure 3c & 3g). At

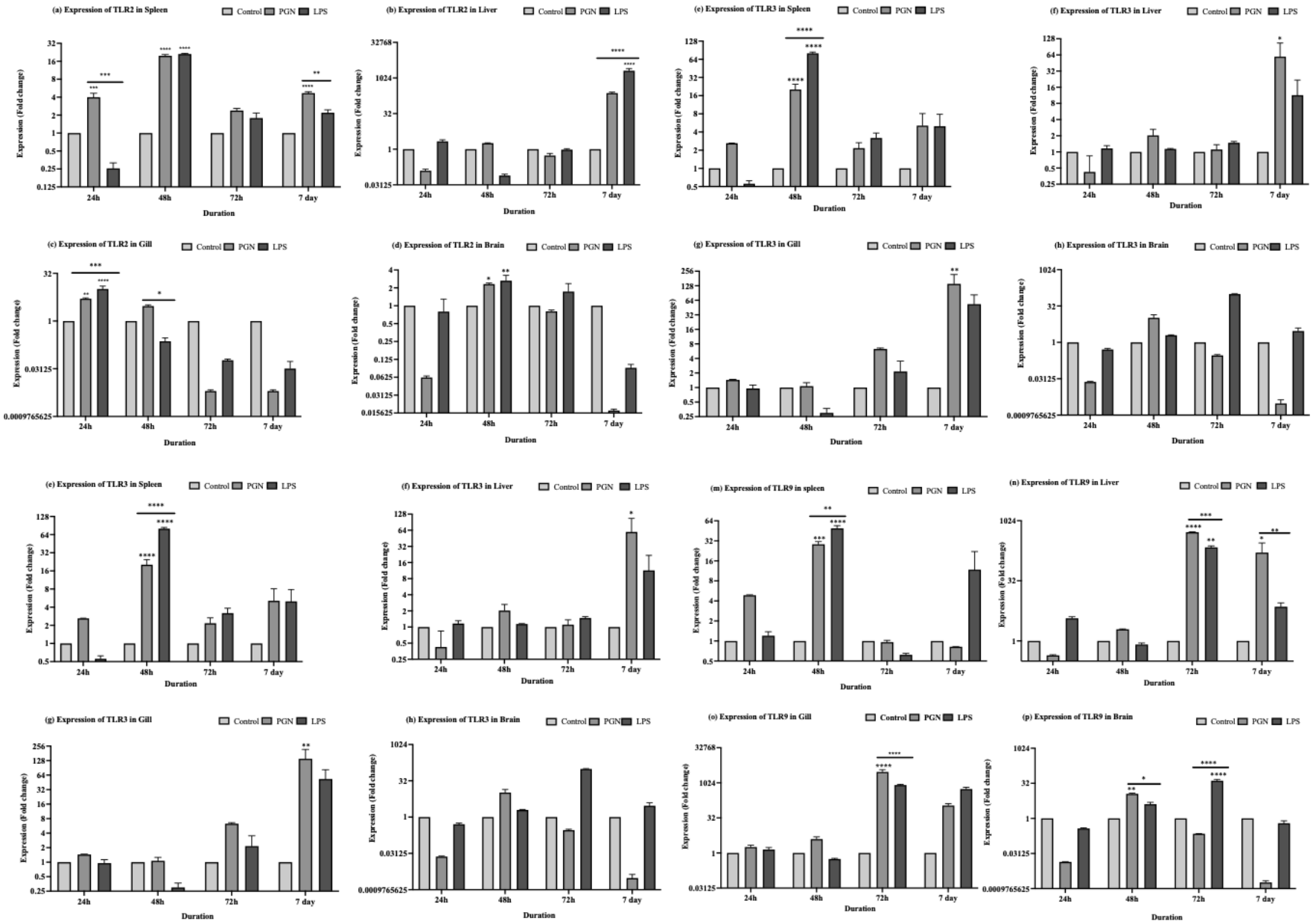

Figure 1. Expression of TLR2, TLR3, TLR8 and TLR9 in spleen (a, e, i and m), liver (b, f, j and n), gill (c, g, k and o) and brain (d, h, l and p) of control, *E. coli* derived LPS and PGN treated Nile tilapia. Data were presented as fold change compared to control. Bar with different shades and asterisk (*) are significantly different at P<0.05, P<0.01, P<0.001 and P<0.0001 which are represented as *, **, *** and ****, respectively. Asterisk upon horizontal line represents significantly different expression between PGN and LPS treated samples.

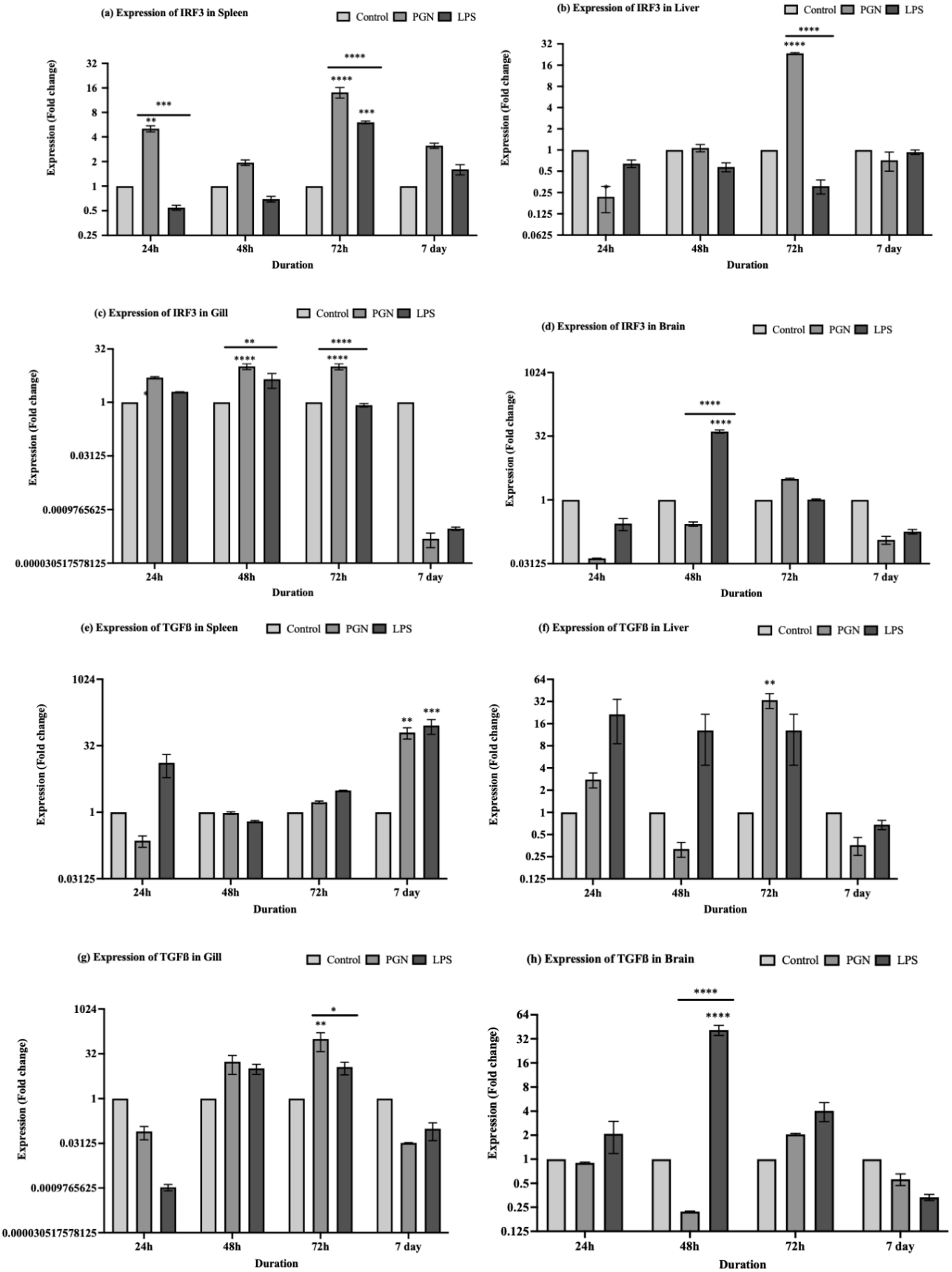

Figure 2. Expression of IRF3 and TGF-β in spleen (a and e), liver (b and f), gill (c and g) and brain (d and h) of control, *E. coli* derived LPS and PGN treated Nile tilapia. Data were presented as fold change compared to control. Bar with different shades and asterisk (*) are significantly different at P<0.05, P<0.01, P<0.001 and P<0.0001 which are represented as *, **, *** and ****, respectively. Asterisk upon horizontal line represents significantly different expression between PGN and LPS treated samples.

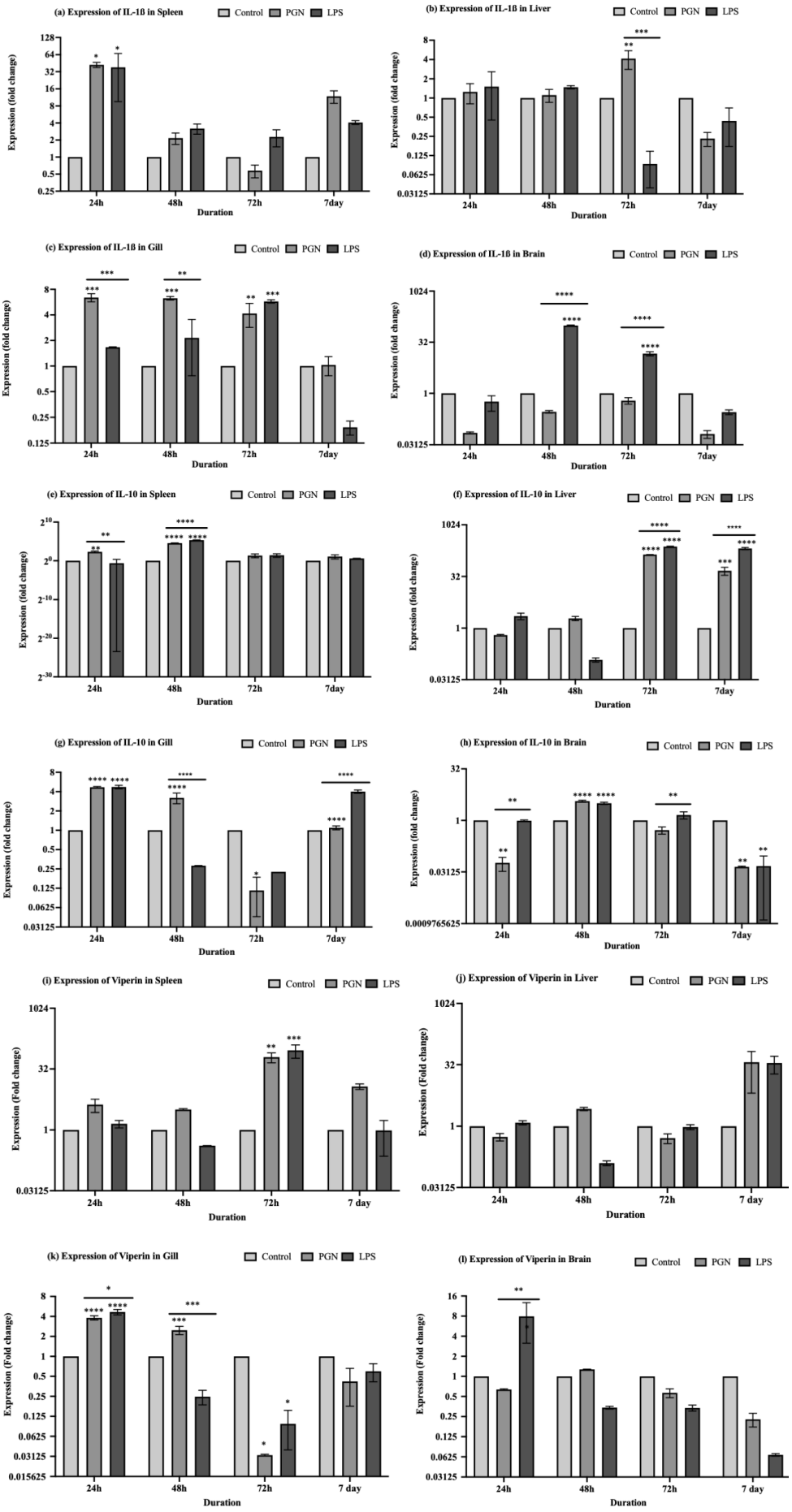

Figure 3. Expression of IL-1β, IL-10 and viperin in spleen (a, e and i), liver (b, f and j), gill (c, g and k) and brain (d, h and l) of control, *E. coli* derived LPS and PGN treated Nile tilapia. Data were presented as fold change compared to control. Bar with different shades and asterisk (*) are significantly different at P<0.05, P<0.01, P<0.001 and P<0.0001 which are represented as *, **, *** and ****, respectively. Asterisk upon horizontal line represents significantly different expression between PGN and LPS treated samples.

48 hours, significantly higher expression levels of *IL-1β* and *IL-10* were detected in the gill, spleen, and brain (P<0.05; Figure 3c, 3g, 3a, 3e, 3d & 3h). At 72 hours, significant upregulation of *IL-1β* and *IL-10* was observed in the liver and brain (P<0.05; Figure 3b, 3f, 3d & 3h). Interestingly, at day 7, *IL-10* expression was significantly increased in the liver and gill but decreased in the brain of LPS- and PGN-treated fish (P<0.05; Figure 3f, 3g & 3h). Viperin expression was significantly upregulated in the gill at 24 and 48 hours post-treatment with LPS and PGN (P<0.05; Figure 3k). Additionally, higher levels of viperin expression were detected in the spleen at 72 hours after LPS and PGN stimulation (P<0.05; Figure 3i).

Discussions

In the present study, the effects of bacterial PAMPs, including *E. coli* derived LPS and PGN, on the expression of innate immune molecules were investigated. The *TLR2* expression in fish spleen, liver, gill and brain tissues significantly increased at 24, 48, and 72 hours, as well as on day 7 following LPS and PGN treatment. Consistent with our findings, Zhang et al. (2016) reported increased *TLR2* expression in turbot fish (*Scophthalmus maximus*) after LPS and PGN stimulation, with upregulation observed in the spleen on days 1 and 7 and in the gills and muscle on days 4 and 7. The findings indicate that bacterial LPS and PGN can upregulate the TLR2 gene in both immune (e.g., liver, spleen) and non-immune tissues (e.g., brain, gill).

Additionally, TLR8 along with TLR9 expression patterns were similar in the spleen where significant upregulation was found at 48 hours after *E. coli* derived LPS and PGN injection while in liver and gill significant upregulation was detected on day 7 after LPS and PGN injection in Nile tilapia tissue. Therefore, TLR3, TLR8 and TLR9 may share immune functions to defend against microorganisms. Previous studies indicated that TLR3, TLR7, and TLR8 exhibited similar characteristics in regulating neuronal structure independently within the cell (Chen et al., 2017; Hung et al., 2018; Liu et al., 2013). This result demonstrated that *E. coli* derived LPS and PGN treatment significantly increased TLR3, TLR8 expression along with TLR9.

Moreover, *E. coli* derived LPS and PGN treatment also resulted in different expression of TLR8 and TLR9 gene expression, with TLR8 mRNA levels were found to be relatively high in the spleen at 24 and 72 hours and on day 7 post-injection with LPS and PGN. Elevated *TLR8* expression was also observed in the brain at 48 and 72 hours post-injection. For *TLR9*, significant increases in mRNA concentrations were observed throughout the brain at 72 hours and in the liver and gills at 72 hours after LPS and PGN injection. The different expression patterns of TLR8 and TLR9 might indicate their unique roles in Nile tilapia. Our results differed from previous studies by Le Manach et al. (2018) who found the highest TLR3 expression in the liver of silvery pomfret, and from studies on fish like carp, where TLR9 was TLR9 expressed less in tissues like the liver and spleen and most highly expressed in the gills and kidneys (Kongchum et al., 2011). However, it has been suggested that TLR gene expression comparisons should be made cautiously due to potential discrepancies arising from species differences, immune status, life stage, and genetic factors (Renshaw et al., 2002). These findings suggested that LPS and PGN have the potential to modulate TLR expression in fish, contributing to the regulation of innate immune responses.

Pro-inflammatory cytokines are essential for initiating and amplifying innate defense mechanism by recruiting immune cells to infection sites and promoting inflammation (Flavell, 2009). In this study, the pro-inflammatory cytokine *IL-1β* was significantly upregulated at 24, 48, and 72 hours in the spleen, gills, and brain of *E. coli* derived LPS and PGN-stimulated tilapia compared to unstimulated fish. These findings are aligned with a previous study conducted by Yoshimura et al. (1997), who demonstrated increased cytokine release, such as *TNF-α* and *IL-1β* from polymorphonuclear leukocytes (PMNs) of healthy donors following stimulation with lipopolysaccharide (LPS).

LPS binds to different TLRs, particularly *TLR4*, for generating pro-inflammatory cytokine, such as *IL-1β* to activate downstream signalling pathways (McCarthy et al., 2017; Chen et al., 2018). *TLR4* has not been reported in fish, thus, the *E. coli* derived LPS-induced immune response might be through receptor independent endocytosis. On the other hand, peptidoglycan (PGN) interacts with TLRs, notably *TLR2*, triggering downstream pathways like the *NF-κB* signalling cascade, which transcriptionally upregulates pro-inflammatory cytokines, including *IL-1β, IL-6,* and *TNF-α* (Lin et al., 2010). These mechanisms underline the critical role of LPS and PGN in modulating immune responses through cytokine production.

In this study, the expression of *TGF-β,* an anti-inflammatory cytokine was analysed, revealing significantly higher mRNA levels in *E. coli* derived LPS- and PGN-stimulated fish at various post-stimulation time duration. These findings align with earlier research, which showed that *E. coli* derived LPS stimulation increases *TGF-β* expression in the kidney of tilapia (Zhan et al., 2015). Similarly, *IL-10*, another anti-inflammatory cytokine was expressed at significantly higher level in the spleen, liver, gills, and brain of Nile tilapia at 24, 48, and 72 hours, as well as on day 7, following *E. coli* LPS and PGN stimulation compared to unstimulated fish. This observation is consistent with previous findings where LPS induces the expression of *IL-10* and *TGF-β* (Ferreira et al., 2021). When PGN activates specific pattern recognition receptors, such as NOD-like receptors (NLRs), it stimulates the production of anti-inflammatory cytokines, including *TGF-β* and *IL-10* (McLoughlin & Mills, 2011). PI3K/Akt and MAPK pathways are thought to regulate different anti-inflammatory cytokines expression (Zheng et al., 2013).

Anti-inflammatory cytokines, like *IL-10* and *TGF-β*, act as negative regulators of the immune response by mitigating excessive inflammation, promoting tissue repair, and facilitating the resolution of inflammation (Saraiva et al., 2020). These findings demonstrate that LPS and PGN play complex and dynamic roles in modulating immune responses to bacterial infections, as evidenced by their capacity to activate both pro-inflammatory and anti-inflammatory cytokine genes.

IRF-3 is also important in natural defence mechanism against different viruses (Abou-elmaatti et al., 2013). In the present study, *IRF-3* expression was analysed, revealing significant upregulation in various organs of Nile tilapia at different time points following stimulation with *E. coli* derived LPS and PGN. These findings align with previous research demonstrating that LPS stimulation increased *IRF-3* expression in the RTS-11 cell line (Holland et al., 2008). The current study also found increased expression of *viperin* in the spleen at 72 hours and in the gill at 24 and 48 hours post-stimulation with LPS and PGN. This observation is consistent with earlier findings showing that LPS significantly induced viperin expression in multiple tissues, including the kidney, liver, gill, fin, and spleen (Tharuka et al., 2019). Although viperin is primarily known for its antiviral properties, these results suggest that *E. coli* derived LPS and PGN may modulate viperin expression, potentially contributing to the production of inflammatory cytokines in tilapia. Despite these findings, the precise molecular mechanisms driving the differential expression of these genes remain unclear. The significant upregulation of TLRs and cytokines observed in this study suggests the activation of innate immune signalling pathways, including MyD88, NF-κB, PI3K/Akt, and MAPK. However, further investigation is necessary to unravel the specific interactions and regulatory mechanisms underlying the observed gene expression patterns.

The present study reveals that *E. coli* derived LPS and PGN significantly enhance the expression of immune genes in tilapia. These results imply that bacterial PAMPs may booster the immune system of tilapia, offering protection against pathogens. Enhanced fish health not only reduces mortality rates but also increases profitability for aquaculture operations. Furthermore, by relying less on antibiotics, producers can support global efforts to mitigate antibiotic resistance. These findings provide an eco-friendly strategy for sustaining fish health.

Conclusions

The findings of this study demonstrate that *E. coli* derived LPS and PGN significantly enhance the expression of *TLR2, TLR3, TLR8, TLR9, IRF3, TGF-β, IL-1β, IL-10, and viperin* in various organs of Nile tilapia. These molecular responses suggest that bacterial PAMPs may contribute in modulating inflammatory pathways thereby strengthening the immune defenses of tilapia. This enhanced immune response could lead to improved fish health, reduced mortality rates, and increased profitability in aquaculture operations. Moreover, utilizing bacterial PAMPs as an alternative to antibiotics supports global efforts to reduce antibiotic resistance, offering an environmentally sustainable approach to maintaining fish health. Further research should explore the specific molecular mechanisms and signalling pathways involved in these immune responses.

Ethical Statement

The experimental methodology was approved by the ethical review committee of the Faculty of Biological Sciences, University of Dhaka, Bangladesh (the ethics approval Ref. No. 210/Biol.Scs).

Funding Information

This research did not receive any specific grants from funding agencies in the public, commercial, or non-profit sectors.

Author Contribution

J.F.: Formal analysis, Investigation, Methodology, Writing – original draft. **A.H.:** Conceptualization, Formal analysis, Writing –review & editing. **M.I.M.H.:** Conceptualization, Data curation, Resources, Writing – review & editing. **M.I.H.** Formal analysis, Resources, Writing –review & editing. **A.A.M.:** Conceptualization, Resources, Writing –review & editing. **M.M.R:** Conceptualization, Formal analysis, Resources, Writing – review & editing. **M.S.:** Formal analysis, Investigation, Methodology, Writing –review & editing. **S.C.M.:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abdel-Latif, H. M., Dawood, M. A., Menanteau-Ledouble, S., & El-Matbouli, M. (2020). The nature and consequences of co-infections in tilapia: A review. *Journal of Fish Diseases*, *43*(6), 651–664.

Biller-Takahashi, J. D., & Urbinati, E. C. (2014). Fish Immunology. The modification and manipulation of the innate immune system: Brazilian studies. *Anais Da Academia Brasileira de Ciências*, *86*(3), 1484–1506. https://doi.org/10.1590/0001-3765201420130159.

Bondad-Reantaso, M. G., Subasinghe, R. P., Arthur, J. R., Ogawa, K., Chinabut, S., Adlard, R., Tan, Z., & Shariff, M. (2005). Disease and health management in Asian

aquaculture. *Veterinary Parasitology*, *132*(3–4), 249–272. https://doi.org/10.1016/j.vetpar.2005.07.005.

Chen, D., Sahin, A., Kam, W. R., Liu, Y., Darabad, R. R., & Sullivan, D. A. (2018). Influence of lipopolysaccharide on proinflammatory gene expression in human corneal, conjunctival and meibomian gland epithelial cells. *The Ocular Surface*, *16*(3), 382–389. https://doi.org/10.1016/j.jtos.2018.05.003.

Chen, C., Liu, H., & Hsueh, Y. (2017). TLR 3 downregulates expression of schizophrenia gene *Disc1* via MYD 88 to control neuronal morphology. *EMBO Reports*, *18*(1), 169–183. https://doi.org/10.15252/embr.201642586

Dos Santos, N. M., Taverne-Thiele, J., Barnes, A. C., van Muiswinkel, W. B., Ellis, A. E., & Rombout, J. H. (2001). The gill is a major organ for antibody secreting cell production following direct immersion of sea bass (*Dicentrarchus labrax*, L.) in a *Photobacterium damselae* ssp. *piscicida* bacterin: An ontogenetic study. *Fish & Shellfish Immunology*, *11*(1), 65–74.

Ferreira, L. F., Garcia Neto, P. G., Titon, S. C. M., Titon, B., Muxel, S. M., Gomes, F. R., & Assis, V. R. (2021). Lipopolysaccharide regulates pro- and anti-inflammatory cytokines, corticosterone, and melatonin in toads. *Integrative Organismal Biology*, *3*(1), obab025. https://doi.org/10.1093/iob/obab025.

Flavell, R. F. (2009). Cytokines in health and disease. *Hamdan Medical Journal*, *1*(1), 26–28.

Holland, J., Bird, S., Williamson, B., Woudstra, C., Mustafa, A., Wang, T., Zou, J., Blaney, S., Collet, B., & Secombes, C. (2008). Molecular characterization of IRF3 and IRF7 in rainbow trout, *Oncorhynchus mykiss*: Functional analysis and transcriptional modulation. *Molecular Immunology*, *46*(2), 269–285.

Hu, Y., Feng, L., Jiang, W., Wu, P., Liu, Y., Kuang, S., Tang, L., & Zhou, X. (2021). Lysine deficiency impaired growth performance and immune response and aggravated inflammatory response of the skin, spleen and head kidney in grown-up grass carp (*Ctenopharyngodon idella*). *Animal Nutrition*, *7*(2), 556–568. https://doi.org/10.1016/j.aninu.2020.07.009.

Hung, Y.-F., Chen, C.-Y., Shih, Y.-C., Liu, H.-Y., Huang, C.-M., & Hsueh, Y.-P. (2018). Endosomal TLR3, TLR7, and TLR8 control neuronal morphology through different transcriptional programs. *Journal of Cell Biology*, *217*(8), 2727–2742. https://doi.org/10.1083/jcb.201712113.

Inamura, S., Fukase, K., & Kusumoto, S. (2001). Synthetic study of peptidoglycan partial structures. Synthesis of tetrasaccharide and octasaccharide fragments. *Tetrahedron Letters*, *42*(43), 7613–7616.

Kongchum, P., Hallerman, E. M., Hulata, G., David, L., & Palti, Y. (2011). Molecular cloning, characterization and expression analysis of TLR9, MyD88 and TRAF6 genes in common carp (Cyprinus carpio). *Fish & Shellfish Immunology*, *30*(1), 361–371. https://doi.org/10.1016/j.fsi.2010.11.012.

Kumar, H., Kawai, T., & Akira, S. (2011). Pathogen Recognition by the Innate Immune System. *International Reviews of Immunology*, *30*(1), 16–34. https://doi.org/10.3109/08830185.2010.529976.

Le Manach, S., Sotton, B., Huet, H., Duval, C., Paris, A., Marie, A., Yépremian, C., Catherine, A., Mathéron, L., Vinh, J., Edery, M., & Marie, B. (2018). Physiological effects caused by microcystin-producing and non-microcystin producing Microcystis aeruginosa on medaka fish: A proteomic and metabolomic study on liver. *Environmental Pollution*, *234*, 523–537. https://doi.org/10.1016/j.envpol.2017.11.011

Lin, H.-Y., Tang, C.-H., Chen, Y.-H., Wei, I.-H., Chen, J.-H., Lai, C.-H., & Lu, D.-Y. (2010). Peptidoglycan enhances proinflammatory cytokine expression through the TLR2 receptor, Myd88, phosphatidylinositol 3-kinase/AKT and NF-kappaB pathways in BV-2 microglia. *International Immunopharmacology*, *10*(8), 883–891. https://doi.org/10.1016/j.intimp.2010.04.026.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta ct}$ method. *Methods*, *25*(4), 402–408. https://doi.org/10.1006/meth.2001.1262.

Liu, H.-Y., Hong, Y.-F., Huang, C.-M., Chen, C.-Y., Huang, T.-N., & Hsueh, Y.-P. (2013). TLR7 Negatively Regulates Dendrite Outgrowth through the Myd88-c-Fos-IL-6 Pathway. *Journal of Neuroscience*, *33*(28), 11479–11493. https://doi.org/10.1523/JNEUROSCI.5566-12.2013

Lupin, H. M. (2009). Human health aspects of drug and chemical use in aquaculture. *Fish Utilization and Marketing Service, FAO, Rome*, pages 95-103.

Maclean, J. L. (1984). Tilapia: The aquatic chicken. *ICLARM.*

Magnadottir, B. (2010). Immunological control of fish diseases. *Marine Biotechnology*, *12*(4), 361–379. https://doi.org/10.1007/s10126-010-9279-x.

Maqsood, S., Singh, P., Samoon, M. H., & Munir, K. (2011). Emerging role of immunostimulants in combating the disease outbreak in aquaculture. *International Aquatic Research (Islamic Azad University, Tonekabon Branch)*, *3*(3).

Martin, S. A., Douglas, A., Houlihan, D. F., & Secombes, C. J. (2010). Starvation alters the liver transcriptome of the innate immune response in Atlantic salmon (*Salmo salar*). *BMC Genomics*, *11*(1), 1–20.

McCarthy, G. M., Bridges, C. R., Blednov, Y. A., & Harris, R. A. (2017). CNS cell-type localization and LPS response of TLR signaling pathways. *F1000Research*, *6*, 1144. https://doi.org/10.12688/f1000research.12036.1.

McLoughlin, R. M., & Mills, K. H. G. (2011). Influence of gastrointestinal commensal bacteria on the immune responses that mediate allergy and asthma. *Journal of Allergy and Clinical Immunology*, *127*(5), 1097–1107. https://doi.org/10.1016/j.jaci.2011.02.012.

Nahid, M. A., Benso, L. M., Shin, J. D., Mehmet, H., Hicks, A., & Ramadas, R. A. (2016). TLR4, TLR7/8 agonist-induced miR-146a promotes macrophage tolerance to MyD88-dependent TLR agonists. *Journal of Leukocyte Biology*, *100*(2), 339–349. https://doi.org/10.1189/jlb.2A0515-197R.

Novoa, B., Mackenzie, S., & Figueras, A. (2010). Inflammation and Innate Immune Response Against Viral Infections in Marine Fish. *Current Pharmaceutical Design*, *16*(38), 4175–4184. https://doi.org/10.2174/138161210794519156.

Rauta, P. R., Nayak, B., & Das, S. (2012). Immune system and immune responses in fish and their role in comparative immunity study: A model for higher organisms. *Immunology Letters*, *148*(1), 23–33. https://doi.org/10.1016/j.imlet.2012.08.003

Renshaw, M., Rockwell, J., Engleman, C., Gewirtz, A., Katz, J., & Sambhara, S. (2002). Cutting Edge: Impaired Toll-Like Receptor Expression and Function in Aging. *The Journal of Immunology*, *169*(9), 4697–4701. https://doi.org/10.4049/jimmunol.169.9.4697

Rombough, P. (2007). The functional ontogeny of the teleost gill: Which comes first, gas or ion exchange? *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *148*(4), 732–742.

Saraiva, M., Vieira, P., & O'Garra, A. (2020). Biology and therapeutic potential of interleukin-10. *Journal of Experimental Medicine*, *217*(1), e20190418. https://doi.org/10.1084/jem.20190418.

Selvaraj, V., Sampath, K., & Sekar, V. (2009). Administration of lipopolysaccharide increases specific and non-specific immune parameters and survival in carp (*Cyprinus carpio*) infected with *Aeromonas hydrophila*. *Aquaculture*, *286*(3–4), 176–183.

Sevaraj, V., Sampath, K., & Sekar, V. (2004). Extraction and characterization of lipopolysaccharide from *Aeromonas hydrophila* and its effects on survival and hematology of the carp, *Cyprinus carpio*. *Asian Fisheries Science*, *17*(1/2), 163–173.

Tharuka, M. D. N., Priyathilaka, T. T., Yang, H., Pavithiran, A., & Lee, J. (2019). Molecular and transcriptional insights into viperin protein from Big-belly seahorse (*Hippocampus abdominalis*), and its potential antiviral role. *Fish & Shellfish Immunology*, *86*, 599–607. https://doi.org/10.1016/j.fsi.2018.12.006.

Yoshimura, A., Hara, Y., Kaneko, T., & Kato, I. (1997). Secretion of IL-1β, TNF-α, IL-8 and IL-1ra by human polymorphonuclear leukocytes in response to lipopolysaccharides from periodontopathic bacteria. *Journal of Periodontal Research*, *32*(3), 279–286. https://doi.org/10.1111/j.1600-0765.1997.tb00535.x.

Zhan, X., Ma, T., Wu, J., Yi, L., Wang, J., Gao, X., & Li, W. (2015). Cloning and primary immunological study of TGF-β1 and its receptors TβR I /TβR II in tilapia (*Oreochromis niloticus*). *Developmental & Comparative Immunology*, *51*(1), 134–140. https://doi.org/10.1016/j.dci.2015.03.008.

Zhang, C., Mai, K., Ai, Q., Duan, Q., Zhang, L., Li, H., & Wan, L. (2008). Effects of dietary peptidoglycan on growth and non‐specific defence of *Pseudosciaena crocea*. *Journal of Fisheries of China*, *32*(3), 411–416.

Zhang, H., Hu, G., Liu, Q., & Zhang, S. (2016). Cloning and expression study of a Toll-like receptor 2 (tlr2) gene from turbot, *Scophthalmus maximus*. *Fish & Shellfish Immunology*, *59*, 137–148. https://doi.org/10.1016/j.fsi.2016.10.001.

Zheng, Y., Yang, Y., Li, Y., Xu, L., Wang, Y., Guo, Z., Song, H., Yang, M., Luo, B., & Zheng, A. (2013). Ephedrine hydrochloride inhibits PGN-induced inflammatory responses by promoting IL-10 production and decreasing proinflammatory cytokine secretion via the PI3K/Akt/GSK3β pathway. *Cellular & Molecular Immunology*, *10*(4), 330–337.