ASEAN
Journal of Scientific and Technological Reports
Online ISSN:2773-8752

Research article

Effect of Dietary Supplementation with *Durio zibethinus* Murr. cv. Monthong Rind on the Hematology and Innate Immune Response Against *Aeromonas hydrophila* in Red Tilapia (*Oreochromis niloticus x Oreochromis mossambicus*)

Duangjai Pisuttharachai[1*], Nataporn Sangkhonkhet[2], Warrapong Nalinanon[3], and Saichon Lerdsuwan[4]

[1] Fishery Science and Aquatic Resources, Disciplines of Technology Agriculture, King Mongkut's Institute of Technology Ladkrabang, Prince of Chumphon Campus, Chumphon, 86160, Thailand
[2] Scientist, King Mongkut's Institute of Technology Ladkrabang, Prince of Chumphon Campus, Chumphon, 86160, Thailand
[3] Fishery Science and Aquatic Resources, Disciplines of Technology Agriculture, King Mongkut's Institute of Technology Ladkrabang, Prince of Chumphon Campus, Chumphon, 86160, Thailand
[4] Animal Science, Disciplines of Technology Agriculture, King Mongkut's Institute of Technology Ladkrabang, Prince of Chumphon Campus, Chumphon, 86160, Thailand
* Correspondence: duangjai.pi@kmitl.ac.th

Citation:
Pisuttharachai, D.; Sangkhonkhet, N.; Nalinanon, W.; Lerdsuwan, S. Effect of dietary supplementation with *Durio zibethinus* murr. cv. monthong rind on the hematology and innate immune response against *Aeromonas hydrophila* in red tilapia (*Oreochromis niloticus x Oreochromis mossambicus*). *ASEAN J. Sci. Tech. Report*. **2025**, *28*(1), e255332. https://doi.org/10.55164/ajstr.v28i1.255332.

Article history:
Received: August 6, 2024
Revised: October 11, 2024
Accepted: October 17, 2024
Available online: December 14, 2024

Publisher's Note:
This article has been published and distributed under the terms of Thaksin University.

Abstract: *In vivo* activity study of durian rind (*Durio zibethinus* Murr. cv. Monthong) as a supplement in the tilapia diet was performed to evaluate its effect on the hematology and innate immune response against *Aeromonas hydrophila*. Red Tilapia (*Oreochromis niloticus* x *Oreochromis mossambicus*), with an initial average weight range of 40-45 g, were fed diets supplemented with durian rind at 0, 10, 15, and 20% for 140 days and were injected intraperitoneally with *A. hydrophila.* Blood was collected at 1 and 3 hours post-injection to determine the phagocytic activity and the durian rind efficiency in protecting fish red blood cells from hemolysin produced by *A. hydrophila*. Ensuing results showed that red blood cell counts in fish-fed durian rind supplemented diets, regardless of inclusion levels, did not decrease at 3 hours post bacterial infection and were significantly higher than control ($P<0.05$). Furthermore, we observed that the total white blood cells and phagocytic activity of all fish fed with diets supplemented with durian rind increased from 1-hour post-infection, indicating a heightened innate immune response. Taken together, durian rind supplementation of at least 10% in the tilapia diet can act as an immunostimulant and improve innate immune response and anti-hemolytic activity against *A. hydrophila* infection.

Keywords: *Durio zibethinus* Murr. cv. Monthong; *Oreochromis niloticus x Oreochromis mossambicus*; *Aeromonas hydrophila*; Hematological; Phagocytic activity

1. Introduction

Aeromonas hydrophila is one of the most concerning etiological agents in cultured finfish and is reportedly responsible for outbreaks in freshwater fish, including tilapia [1-4]. This bacterium produces several virulence factors, such as hemolysin, aerolysin, and cytotonic enterotoxins, which are harmful to its hosts [5, 6]. In fish, major symptoms include ulcerative lesions, hemorrhages, and necrosis of the visceral organs leading to death [1, 7]. Antibiotics often treat *A. hydrophila* infections [8-10]. Recently, consumers have become more concerned about residues in food, making it necessary to develop aquaculture

practices that deter disease outbreaks in cultured finfish and ensure that the products are safe for consumption. Among these, using herbal medicines and other natural products to replace antibiotics in treating fish diseases has been getting significant consideration.

Durian (*Durio zibethinus* Murr.), also referred to as the "king of fruits," is a tropical fruit grown in Southeast Asian countries, including Malaysia, Indonesia, the Philippines, and Thailand [11, 12]. Durian rind, which makes up about 75-80% of the whole fruit, is often discarded after durian fruit consumption or processing [13]. It was previously reported that durian rind is a good source of polysaccharides (PG) [14]. PG has been shown to exhibit anti-hemolytic [15, 16] and immunomodulating properties [17, 18]. As *A. hydrophila* can produce hemolysin that causes lysis of red blood cells, we investigated the effect of durian rind supplementation in fish diets on the innate immunological responses and its efficiency in preventing fish red blood cell hemolysis against this bacterium. Moreover, the valorization of durian waste as a potential dietary supplement relieves disposal problems and adheres to the country's drive for a circular economy.

2. Materials and Methods

2.1 Experimental design and feeding management

This study was approved by the Ethics Committee of King Mongkut's Institute of Technology Ladkrabang (Approval no. ACUC-KMITL-RES/2021/034). The experiment was carried out in a completely randomized design with 4 treatments and 4 replications. Four hundred red tilapia, acclimated before the feeding trials, with an average weight range of 40-45 g, were randomly distributed in 500-L plastic tanks with aeration at 25 fish/tank. The fish were fed diets supplemented with durian rind at 0 (control), 10, 15, and 20 % twice daily, at 8:30 a.m. and 4:00 p.m., at 3 % of their body weight for 140 days. The tanks were scrubbed daily, and water changes were done every two days at 50% water volume. Water quality parameters were monitored throughout the feeding trial to ensure optimal culture conditions.

2.2 Preparation of durian rind and experimental diets

The preparation of durian rind and the composition of the experimental diets were performed following the protocols described earlier [19]. The white inner rind from ripe durian was cleaned, cut into small pieces, dried at 60 ^{o}C for 48 hours, and then ground. The powdered rind was mixed with other feedstuff to make four experimental diets: Diet 1, control without powder rind; Diets 2-4, with powder rind at 10, 15, and 20 %, respectively. The pelleted experimental diets were stored in polyethylene bags at 4 °C until used.

2.3 Preparation of bacterial solution

The *Aeromonas hydrophila* strains were kindly provided by the Aquatic Animal Health Research and Development Division, Department of Fisheries, Bangkok Province, Thailand. The procedure was adapted from Julie et al. [20]. It was cultured in tryptic soy broth (DifcoTM, France) at 37 ^{o}C for 24 h. The pellet from the bacterial cultured broth was extracted by centrifuging at 5000 rpm for 10 min (Eppendorf 5920 R, Germany). The pellets were then suspended in sterile 0.85 % NaCl and adjusted to the optical density of 1.0 at 540 nm (Thermo Scientific-Evolution 201, United States of America), equivalent to a bacterial concentration of 4.1 x 10^7 CFU/ml.

2.4 Blood sampling for hematology and phagocytic activity

After the 140-day feeding trial, 4 fish were randomly collected from each tank. The fish were injected intraperitoneally with 0.1 ml bacterial solution containing 4.1 x 10^7 CFU/ml of *A. hydrophila*. This bacterial concentration was used based on an earlier LC50 analysis [19]. Blood was collected from two fish 1 hour after injection and the other 3 hours after injection. A total of 8 fish per treatment per period were evaluated. One milliliter of blood was taken from each fish using 3-mL syringes and 21-gauge needles [21] pre-rinsed with 0.5 M EDTA (Ajax-Finechem (Univar), Australia) as an anticoagulant. The total red blood cell count, white blood cell count, and phagocytic activity were determined.

2.5 Red blood cell and white blood cell counts

Following the protocol of Fazio et al. [22], blood samples were first attenuated at 1:250 using Dacie's fluid as a diluent to count the total red blood cells. To get the total white blood cell count, blood was initially

attenuated at 1:100 with Turk's fluid as diluent. After dilution, the cells were counted immediately in a Neubauer chamber.

2.6 Phagocytic activity

Leukocytes were separated from the blood by density gradient separation. Briefly, 1 ml of the lymphoprep (density 1.077) (Corning, United States of America) was distributed into corning centrifuge tubes. One milliliter of blood solution, composed of 0.5 ml of blood and 0.5 ml of RPMI medium (Sigma, United Kingdom), was meticulously layered on the top of the lymphoprep. The ready samples were centrifuged at 400 g for 30 min at 4 °C. After that, leukocytes between the lymphoprep and plasma layers were painstakingly removed and transferred into new corning tubes containing 1 ml RPMI medium and then centrifuged at 400 g for 10 min at 4 °C. Leukocytes were washed twice in RPMI medium and diluted to 1×10^6 cells/ml. Phagocytic activity was then evaluated. Two hundred microliters of leukocyte suspension were pipetted on top of a cover slide and incubated at 4 °C for 1 h. Subsequently, the cover slides were rinsed softly 3 times with RPMI medium. Then, 200 µL of latex beads (1×10^7 cells/ml) were dispensed onto the cover slide. The slides with latex beads were incubated at 4 °C for 1 h. After that, the cover slides were gently washed with RPMI medium and fixed with methanol 96 % (v/v). The dried cover slides were stained using Dip Quick staining. Randomly, two hundred leukocytes on each slide were observed for the number of ingested latex beads and phagocytizing cells under a microscope. The leukocyte phagocytic activity, phagocytic index, and average number of latex beads ingested per cell were calculated according to formulas described in Itami et al. [23] and Rengpipat et al. [24].

2.7 Data analysis

Statistical analyses were performed using ANOVA, and the mean difference in each experiment was compared using Duncan's new multiple range test with a statistical program at a 95 percent assurance level.

3. Results and Discussion

Earlier research on the effect of durian rind on the growth performance of red tilapia suggests that there was no significant difference in growth performance among fish fed different levels of durian rind in the diets, and the survival rate of these treatments was more than 90% [19[. Here, we wanted to know how tilapia, fed diets with durian rind, respond to bacterial infection. Our findings showed that the total red blood cell (RBC) counts of red tilapia fed a control diet decreased after 3 h post-infection with *A. hydrophila* (Fig. 1). Previous reports suggest that *A. hydrophila* infection could reduce the number of blood cells by advocating erythrocyte hemolysis [25, 26]. *A. hydrophila* has been shown to produce a variety of extracellular products which act as essential virulent factors, such as hemolysin which is associated with hemolysis [27-31]. On the other hand, fish fed with diets supplemented with durian rind at all inclusion levels showed no decrease in total RBCs at 3 h post-infection and were significantly higher than the control ($P<0.05$) (Fig. 1). This could be because of the polysaccharide (PG) contained in durian rind. Previous studies have demonstrated that polysaccharides from plant sources, for instance *Diaphragma juglandis fructus* (the dry wooden diaphragm inside walnuts), *Schinus terebinthifolius* (Brazilian pepper-tree) and *Schinus* mole (Peruvian pepper-tree, California pepper-tree) exhibit anti-hemolytic properties [32, 33]. Snega Priya et al. [17] demonstrated that zebrafish, given encapsulated *Artemia* with PG as food for 20 days and then challenged with *Vibrio anguillarum* by immersion, showed significantly higher total RBCs than control fish.

Moreover, previous reports showed that PG extracted from durians exhibited antibacterial activity against Gram-positive and Gram-negative bacteria, including *Streptococcus agalactiae*, *Vibrio harveyi*, *Staphylococcus aureus*, *Escherichia coli* [34-36]. Thunyakipisal et al. [37] showed scanning electron micrographs of bacteria incubated in PG. Irregular-shaped cells and various-sized blebs were found on the bacterial membrane incubated in PG. In contrast, bacteria incubated in the culture broth medium without PG appeared round-shaped (cocci) with a smooth surface, suggesting that the antibacterial activity of PG may affect bacterial membrane integrity. In addition to PG substances, flavonoid extract of durian rind also has antibacterial activity [38, 39].

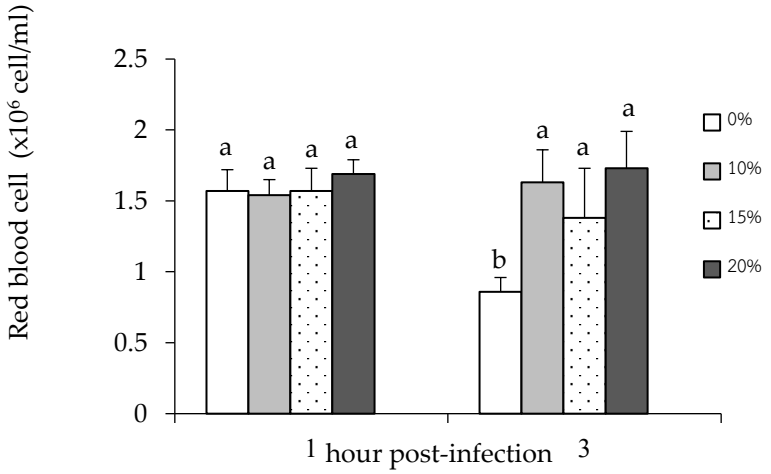

Figure 1. Red blood cell counts of red tilapia-fed diets supplemented with durian rind at varying inclusion levels and challenged with *A. hydrophila* at 1 and 3 hours post-infection. The data are represented as mean ± standard deviation (n = 8)

The innate immune system mediates the immune response to a bacterial infection almost instantaneously [40]. White blood cells (WBC), or leukocytes, are the key cells of vertebrate innate immunity in fish. Their major function is to combat infection by phagocytosing foreign organisms [41]. Therefore, the effect of durian rind on the innate immune response was examined through WBC counts and phagocytic activity. Our results indicate that a fish-fed diet supplemented with durian rind, regardless of inclusion levels, showed significantly increased WBC counts at 1 h post-infection with *A. hydrophila* compared to control ($P<0.05$). The increase in WBCs was directly proportional to the inclusion levels of durian rind in the diet (Fig. 2). Similarly, the increase in WBC count also increased phagocytic activity at 1 h post *A. hydrophila* infection. Fish fed a diet supplemented with 20% durian rind exhibited the highest phagocytic activity (Fig. 3A) and phagocytic index (Fig. 3B), both significantly different compared to control but not from 10% and 15% ($P<0.05$). The average number of the bead ingested per cell (ABPC) was not significant among treatment ($P>0.05$) (Fig. 3C). Our result is in line with Wang et al. [42] reported that crucian carp fed with plant PG exhibited enhanced innate immune response including serum bactericidal activity, lysozyme activity, total protein level, complement C3, superoxide dismutase activity as well as leukocyte phagocytosis activity. Hokputsa et al. [43] have isolated water-soluble polysaccharides from durian rinds (*Durio zibethinus*) and revealed that the principal components were pectic polysaccharides. Pectins are complex mixtures of polysaccharides and varieties of neutral sugars such as galactose, arabinose, glucose, mannose, and xylose [44]. Whole durian rind pectin yield is 73.67 % [45], of which white rind has 14.97% [46]. Pectin displays diverse biological activities, including immunomodulation [47]. The activity of pectin has been reported to increase the phagocytic activity of neutrophils, promote the formation of reactive oxygen species in these cells, and enhance NADPH oxidase activity, which plays a key role in the oxygen-dependent bactericidal activity of neutrophils [48]. Doan et al. [49] studied the effects of orange peel-derived pectin on the innate immune response of Nile tilapia (*Oreochromis niloticus*). Fish-fed orange peel-derived pectin showed significantly higher serum lysozyme and phagocytosis activity than the control.

The innate immunity of fish is propelled by pattern recognition receptors (PRRs), similar to immunological sensors, and protects the host from incursion of pathogens [50-52]. Several PRRs have been found and described in fish, including the Toll-like receptors (TLRs), retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs), and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) [52]. PRRs function by recognizing pathogen-associated molecular patterns (PAMPs) derived from various microbial pathogens such as lipopolysaccharide [53], Flagellin [54], peptidoglycans, and lipoteichoic acids [55]. The recognition of PAMPs by PRRs leads to the production of inflammatory responses [53] and antiviral or antibacterial responses [56, 57]. Other than PAMPs derived from microbial organisms, complex pectin from

plant cell walls has been gaining research interest as a source of novel innate immune modulators. Hyun et al. [58] investigated the interactions in pattern recognition for common glycostructures of pectic heteropolysaccharides (HPSs) by Toll-like receptors (TLRs). They showed that pectic HPSs were selectively clustered with TLR4 during endocytosis. In fish, TLR4s are presumed sensors of bacterial ligands [56, 57]. In the current study, the increase in total white blood cells, phagocytosis activity, and phagocytic index may be because of pectin in durian rind that can stimulate the fish's immune response and primes it to fight pathogens. Thus, when fish fed with durian rind supplemented feeds are infected with the bacteria, they can quickly respond and eliminate the pathogen better than the control group. Over time, the immune system of the control fish began to develop, which is why there was no difference in the total white blood cells, phagocytosis activity, and phagocytic index at 3 hours post-infection. These earlier reports and our current results, substantiate the potential of durian rind supplementation in fish diets.

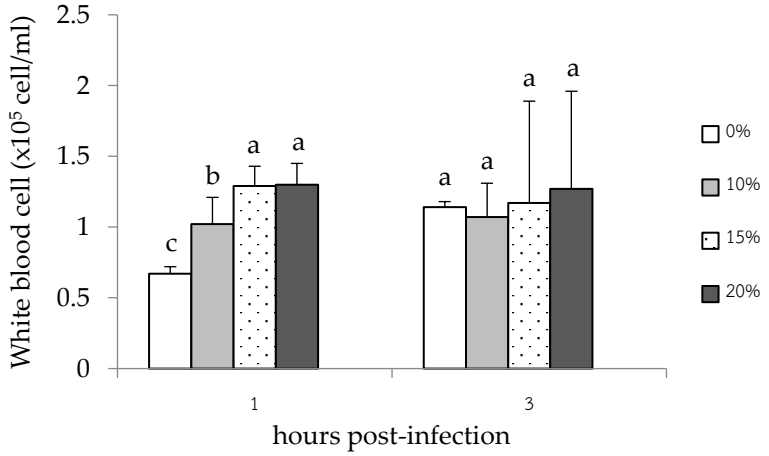

Figure 2. White blood cell counts of red tilapia-fed diets supplemented with durian rind at varying inclusion levels and challenged with *A. hydrophila* at 1 and 3 hours post-infection. The data are represented as mean ± standard deviation (n = 8)

4. Conclusions

Our results suggested that feed supplementation with durian rind can increase the level of white blood cells and phagocytic activity and prevent red blood cell lysis in red tilapia. Supplementing at least 10% durian rind can improve innate immune response and exhibit anti-hemolytic activity against *A. hydrophilla.* Furthermore, our results showed that discarded fruit wastes, such as durian peels, can be potentially used as feed additives/supplements in fish diets. We stress, however, that the current results are only limited to tilapia and that future studies can be conducted on its potential use as a feed additive for other cultured fish species. Further studies on other forms of durian rind supplemented in fish diet and combined with other feed additives to improve fish health are currently being undertaken.

5. Acknowledgements

The authors would like to thank Dr. Fernand F. Fagutao for his help in proofreading the manuscript.

Author Contributions: Conceptualization, D. P.; performed the experiments, D. P., N. S., W. N., and S. L.; writing-original draft preparation, D. P.; writing-review and editing, D. P.; supervision, D. P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

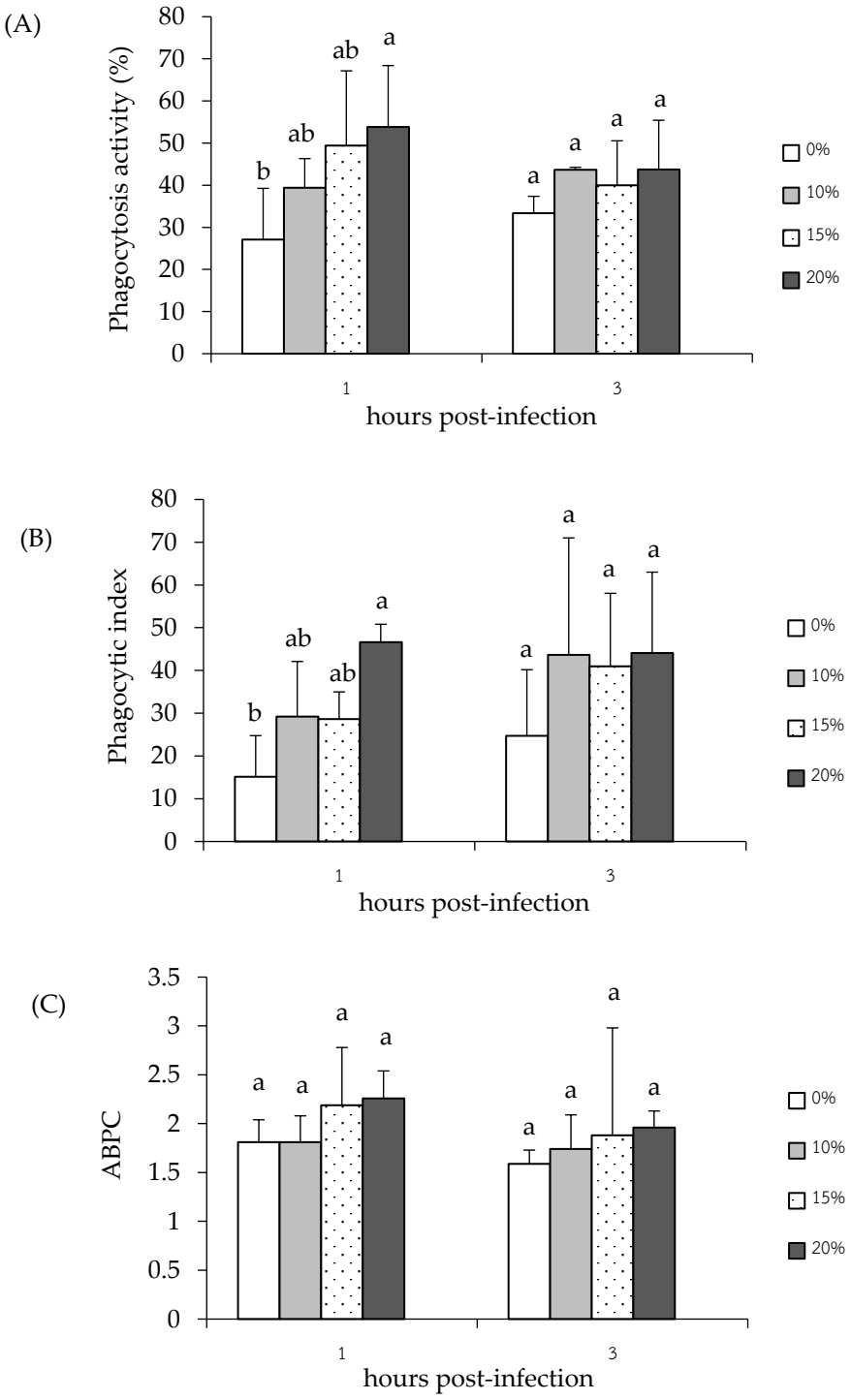

Figure 3. Phagocytosis activity (A), phagocytic index (B), and average number of beads ingested per cell : ABPC (C) of red tilapia-fed diets supplemented with durian rind at varying inclusion levels and challenged with *A. hydrophila* at 1 and 3 hours post-infection. The data are represented as mean ± standard deviation (n = 8)

References

[1] Yardimci, B.; Aydin, Y. Pathological findings of experimental *Aeromonas hydrophila* infection in Nile tilapia (*Oreochromis niloticus*). *Ankara Universitesi Veteriner Fakultesi Dergisi*. **2011**, *58*, 47-54.

[2] Pauzi, N. A.; Mohamad, N.; Azzam-Sayuti, M.; Yasin, I. S. M.; Saad, M. Z.; Nasruddin, N. S.; Azmai, M. N. A. Antibiotic susceptibility and pathogenicity of *Aeromonas hydrophila* isolated from red hybrid tilapia (*Oreochromis niloticus* × *Oreochromis mossambicus*) in Malaysia. *Veterinary World*. **2020**, *13*, 2166-2171.

[3] Saleh, A.; Elkenany, R.; Younis, G. Virulent and multiple antimicrobial resistance *Aeromonas hydrophila* isolated from diseased Nile tilapia fish (*Oreochromis niloticus*) in egypt with sequencing of some virulence-associated genes. *Biocontrol science*. **2021**, *26*, 167-176.

[4] Sukhavachana, S.; Ampolsak, K.; Poompuang, S. Positive genetic correlation between resistance to Aeromonasis and Streptococcosis in Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758). *Journal of Fisheries and Environment*. **2020**, *44*, 45–54.

[5] Sen, K.; Rodgers, M. Distribution of six virulence factors in Aeromonas species isolated from US drinking water utilities: a PCR identification. *Journal of applied microbiology*. **2004**, *97*, 1077-1086.

[6] Sherif, A. H.; AbuLeila, R. H. Prevalence of some pathogenic bacteria in caged-Nile Tilapia (*Oreochromis niloticus*) and their possible treatment. *Jordan Journal of Biological Sciences*. **2022**, *15*, 239-247.

[7] Swann, L.; White, M. R. Diagnosis and treatment of "*Aeromonas hydrophila*" infection of fish. *Aquaculture Extension Illinois- Indiana Sea Gran Program.* **1991**.

[8] Tipmongkolsilp, N.; del Castillo, C. S; Hikima, J. I; Jung, T. S; Kondo, H; Hirono, I; Aoki, T. Multiple drug-resistant strains of *Aeromonas hydrophila* isolated from tilapia farms in Thailand. *Fish Pathology*. **2012**, *47*(2), 56-63.

[9] Samal, S. K; Das, B. K; Pal, B. B. Isolation, biochemical characterization, antibiotic susceptibility study of *Aeromonas hydrophila* isolated from freshwater fish. *International Journal of Current Microbiology and Applied Sciences*. **2014**, *3*(12), 259-267.

[10] Ali, S.; Akhter, S.; Muhammad, A.; Khan, I.; Khan, W. A.; Iqbal, M. N.; Umar, S.; Ahmed, H.; Ali, Q. Identification, characterization and antibiotic sensitivity of *Aeromonas hydrophila* a causative agent of epizootic ulcerative syndrome in wild and farmed fish from Potohar, Pakistan. *Pakistan Journal of Zoology*. **2016**, *48*(3), 899-901.

[11] Rachtanapun, P.; Luangkamin, S.; Tanprasert, K.; Suriyatem, R. Carboxymethyl cellulose film from durian rind. *LWT - Food Science and Technology*. **2012**, *48*, 52-58.

[12] Foo, K. Y.; Hameed, B. H. Textural porosity, surface chemistry and adsorptive properties of durian shell derived activated carbon prepared by microwave assisted NaOH activation. *Chemical Engineering Journal*. **2012**, *187*, 53-62.

[13] Lubis, R.; Saragih, S. W.; Wirjosentono, B.; Eddyanto, E. (2018, July 18-19). *Characterization of durian rinds fiber (Durio zubinthinus, murr) from North Sumatera*. The 3rd international seminar on chemistry: green chemistry and its role for sustainability. Surabaya, Indonesia. https://doi.org/10.1063/1.5082474

[14] Pongsamart, S.; Panmuang, T. Isolation of polysaccharide from fruit-hulls of durian (*Durio zibethinus* L.). *Songklanakarin Journal of Science and Technology*. **1998**, *20*, 323-332.

[15] González-Vega, R.; Del-Toro-Sánchez, C. L.; Moreno-Corral, R.; López-Elías, J. A.; Reyes-Díaz, A.; García-Lagunas, N.; Carvajal-Millán, E.; Fimbres-Olivarría, D. Sulfated polysaccharide-rich extract from *Navicula incerta*: physicochemical characteristics, antioxidant activity, and anti-hemolytic property. *AIMS Bioengineering*. **2022**, *9*, 364-382.

[16] Yang, H.; Bai, J.; Ma, C.; Wang, L.; Li, X.; Zhang, Y.; Xu, Y.; Yang, Y. Degradation models, structure, rheological properties and protective effects on erythrocyte hemolysis of the polysaccharides from *Ribes nigrum* L. *International Journal of Biological Macromolecules*. **2020**, *165*, 738-746.

[17] Snega Priya, P.; Ashwitha, A.; Thamizharasan, K.; Harishkumar, M.; Dinesh, S.; Nithya, T. G.; Kamaraj, M. Synergistic effect of durian fruit rind polysaccharide gel encapsulated prebiotic and probiotic dietary supplements on growth performance, immune-related gene expression, and disease resistance in Zebrafish (*Danio rerio*). *Heliyon*. **2021**, 7, https://doi.org/10.1016/j.heliyon.2021.e06669

[18] Pholdaeng, K.; Pongsamart, S. Studies on the immunomodulatory effect of polysaccharide gel extracted from *Durio zibethinus* in *Penaeus monodon* shrimp against *Vibrio harveyi* and WSSV. *Fish and Shellfish Immunology*. **2010**, *28*, 555-561.

[19] Sangkhonkhet, N.; Phanchindawan, N.; Lerdsuwan, S.; Nalinanon, W.; Pisuttharachai, D. Effect of *Durio zibethinus* Murr. cv. Monthong rind as a dietary ingredient in feed on the growth performance and disease resistance against *Aeromonas hydrophila* in Red Tilapia (*Oreochromis niloticus x Oreochromis mossambicus*). *ASEAN Journal of Scientific and Technological Reports*. **2023**, *26*, 39-48.

[20] Julie, B.; Julio, C.G.; Ahmed, D. Effect of copper sulfate on *Aeromonas hydrophila* infection in channel catfish fingerlings. *North American Journal of Aquaculture*. **2012**, *74*, 494-498.

[21] Duran, U.; Çenesiz, S.; Şahin, B. Blood sampling techniques and preparing for analysis in rainbow trout (*Oncorhynchus mykiss*). *Black Sea Journal of Agriculture*. **2023**, *6*(1), 68-73.

[22] Fazio, F.; Marafioti, S.; Filiciotto, F.; Buscaino, G.; Panzera, M.; Faggio, C. Blood hemogram profiles of farmed onshore and offshore gilthead sea bream (*Sparus aurata*) from Sicily, Italy. *Turkish Journal of Fisheries and Aquatic Sciences*. **2013**, *13*, 415-422.

[23] Itami, T.; Takahashi, Y.; Tsuchihira, E.; Igusa, H.; Kondo, M. Enhancement of disease resistance of kuruma prawn *Penaeus japonicus* and increase in phagocytic activity of prawn hemocytes after oral administration of ß-1, 3-glucan (Schizophyllan). In L.M. Chou, A.D. Munro, T.J. Lam, T.W. Chen, L.K.K. Cheong, J.K. Ding, K.K. Hooi, H.W. Khoo, V.P.E. Phang, K.F. Shim & C.H. Tan (Eds.), *The 3rd Asian Fisheries Forum* (pp. 375–378). Asian Fisheries Society, Manila, Philippines. **1994**.

[24] Rengpipat, S.; Rukpratanporn, S.; Piyatiratitivorakul, S.; Menasaveta, P. Immunity enhancement in black tiger shrimp (*Penaeus monodon*) by a probiont bacterium (*Bacillus* S11). *Aquaculture*. **2000**, *191*, 271-288.

[25] Xiong, N.X.; Luo, S.W.; Fan, L.F.; Mao, Z.W.; Luo, K.K.; Liu, S.J.; Wu, C.; Hu, F.Z.; Wang, S.; Wen, M.; Liu, Q.F. Comparative analysis of erythrocyte hemolysis, plasma parameters and metabolic features in red crucian carp (*Carassius auratus* red var) and triploid hybrid fish following *Aeromonas hydrophila* challenge. *Fish and Shellfish Immunology*. **2021**, *118*, 369-384.

[26] Xiong, N. X.; Ou, J.; Fan, L. F.; Kuang, X. Y.; Fang, Z. X.; Luo, S. W.; Mao, Z. W.; Liu, S. J.; Wang, S.; Wen, M.; Luo, K. K.; Hu, F. Z.; Wu, C.; Liu, Q. F. Blood cell characterization and transcriptome analysis reveal distinct immune response and host resistance of different ploidy cyprinid fish following *Aeromonas hydrophila* infection. *Fish and Shellfish Immunology*. **2022**, *120*, 547-559.

[27] Allan, B. J.; Stevenson, R. M. Extracellular virulence factors of *Aeromonas hydrophila* in fish infection. *Canadian journal of microbiology*. **1981**, *27*, 1114-1122.

[28] Pansare, A. C.; Lewis, N. F.; Venugopal, V. Characterization of extracellular proteases of *Aeromonas hydrophila*. *Agricultural and Biological Chemistry*. **1986**, *50*, 1743-1749.

[29] Santos, Y.; Toranzo, A. E.; Barja, J. L.; Nieto, T. P.; Villa, T. G. Virulence properties and enterotoxin production of Aeromonas strains isolated from fish. *Infection and immunity*. **1988**, *56*, 3285-3293.

[30] Esteve, C.; Birbeck, T. H. Secretion of haemolysins and proteases by *Aeromonas hydrophila* EO63: separation and characterization of the serine protease (caseinase) and the metalloprotease (elastase). *Journal of applied microbiology*. **2004**, *96*. 994-1001.

[31] Subashkumar, R.; Thayumanavan, T.; Vivekanandhan, G.; Lakshmanaperumalsamy, P. Occurrence of *Aeromonas hydrophila* in acute gasteroenteritis among children. *The Indian journal of medical research*. **2006**, *123*, 61-66.

[32] Meng, Q.; Chen, F.; Xiao, T.; Zhang, L. Inhibitory effects of polysaccharide from *Diaphragma juglandis* fructus on α-amylase and α-d-glucosidase activity, streptozotocin-induced hyperglycemia model, advanced glycation end-products formation, and H_2O_2-induced oxidative damage. *International Journal of Biological Macromolecules*. **2019**, *124*, 1080-1089.

[33] Feriani, A.; Tir, M.; Hamed, M.; Sila, A.; Nahdi, S.; Alwasel, S.; Harrath, A. H.; Tlili, N. Multidirectional insights on polysaccharides from *Schinus terebinthifolius* and *Schinus molle* fruits: Physicochemical and functional profiles, *in vitro* antioxidant, anti-genotoxicity, antidiabetic, and antihemolytic capacities, and *in vivo* anti-inflammatory and anti-nociceptive properties. *International journal of biological macromolecules*. **2020**, *165*, 2576-2587.

[34] Maktrirat, R.; Pongsamart, S.; Ajariyakhajorn, K.; Chansiripornchai, P. Bactericidal effect of post-milking teat dip prepared from polysaccharide gel from durian rinds on streptococci causing clinical bovine mastitis. *Acta Horticulturae*. **2008**, *786*, 33-40.

[35] Pholdaeng, K.; Pongsamart, S. Studies on the immunomodulatory effect of polysaccharide gel extracted from *Durio zibethinus* in *Penaeus monodon* shrimp against *Vibrio harveyi* and WSSV. Fish and Shellfish Immunology. **2010**, *28*, 555-561.

[36] Lipipun, V.; Nantawanit, N.; Pongsamart, S. Antimicrobial activity (*in vitro*) of polysaccharide gel from durian fruit-hulls. *Songklanakarin Journal of Science and Technology*. **2002**, *24*, 31-38.

[37] Thunyakipisal, P.; Saladyanant, T.; Hongprasong, N.; Pongsamart, S.; Apinhasmit, W. Antibacterial activity of polysaccharide gel extract from fruit rinds of *Durio zibethinus* Murr. against oral pathogenic bacteria. *Journal of investigative and clinical dentistry*. **2010**, *1*, 120-125.

[38] Hong, J.; Du, H. X.; Hu, J. Y. Ultrasonic-assisted extraction of flavonoids from durian peel and their antioxidant and antimicrobial activities. *Journal of Henan Agricultural University*. **2014**, *48*, 653-657.

[39] Hong, J.; Hu, J. Y.; Zhang, X.; Ma, X. M.; Han, S. Antioxidant and antibacterial activities of flavonoids extracted from durian peel. *Guizhou Agricultural Sciences*. **2014**, *42*, 41-43.

[40] Janeway, C. A.; Travers, P.; Walport, M.; Shlomchik, M. J. *Immunobiology: The Immune System in Health and Disease.* New York: Garland Science. **2001**. https://www.ncbi.nlm.nih.gov/books/NBK27090/

[41] Peter, S. G.; Gakuya, D. W.; Maingi, N.; Mulei, C. M. Prevalence and risk factors associated with *Ehrlichia* infections in smallholder dairy cattle in Nairobi City County, Kenya. *Veterinary World*. **2019**, *12*, 1599–1607.

[42] Wang, E.; Chen, X.; Wang, K.; Wang, J.; Chen, D.; Geng, Y.; Lai, W.; Wei, X. Plant polysaccharides used as immunostimulants enhance innate immune response and disease resistance against *Aeromonas hydrophila* infection in fish. *Fish and Shellfish Immunology*. **2016**, *59*, 196-202.

[43] Hokputsa, S.; Gerddit, W.; Pongsamart, S.; Inngjerdingen, K.; Heinze, T.; Koschella, A.; Harding, S.; Paulsen, B. (2004). Water-soluble polysaccharides with pharmaceutical importance from Durian rinds (*Durio zibethinus* Murr.): Isolation, fractionation, characterisation and bioactivity. *Carbohydrate Polymers*. **2004**, *56*, 471-481.

[44] Garna, H.; Mabon, N.; Wathelet, B.; Paquot, M. New method for a two-step hydrolysis and chromatographic analysis of pectin neutral sugar chains. *Journal of Agricultural and Food Chemistry*. **2004**, *52*, 4652-4659.

[45] Hasem, N. H.; Fuzi, S. F.; Kormin, F.; Bakar, M. F.; Sabran, S. F. (2018, November 11-13). *Extraction and Partial Characterization of durian Rind Pectin* [Earth and Environmental Science 269]. International conference on biodiversity. Johor Darul Takzim, Malaysia. doi:10.1088/1755-1315/269/1/012019

[46] Wang, H. Y.; Liao, M. S.; Zhang, M.; Wen, B.; Li, P. S.; Huang, C. X.; Wang, X. B. Study on the extraction of pectin technology from durian shell. *Science and Technology of Food Industry*. **2012**, *33*, 246-250.

[47] Popov, S. V.; Ovodov, Y.S. Polypotency of the immunomodulatory effect of pectins. *Biochemistry (Mosc)*. **2013**, *78*, 823-835.

[48] Zaitseva, O. O.; Polezhaeva, T. V.; Khudyakov, A. N.; Solomina, O. N.; Laptev, D. S.; Svedentsov, E. P.; Utemov, S. V.; Kostyaev, A. A. Influence of pectins on NADPH oxidase and phagocytic activity of neutrophils during cryopreservation. *Cryo Letters*. **2013**, *34*, 544-548.

[49] Doan, H. V.; Hoseinifar, S. H.; Elumalai, P.; Tongsiri, S.; Chitmanat, C.; Jaturasitha, S.; Doolgindachbaporn, S. Effects of orange peels derived pectin on innate immune response, disease resistance and growth performance of Nile tilapia (*Oreochromis niloticus*) cultured under indoor biofloc system. *Fish and Shellfish Immunology*. **2018**, *80*, 56-62.

[50] Liao, Z.; Su, J. Progresses on three pattern recognition receptor families (TLRs, RLRs and NLRs) in teleost. *Developmental and comparative immunology*. **2021**, *122*, 104131, https://doi.org/10.1016/j.dci.2021.104131.

[51] Sahoo, B. R. Structure of fish Toll-like receptors (TLR) and NOD-like receptors (NLR). *International Journal of Biological Macromolecules*. **2020**, *161*, 1602-1617.

[52] Li, Y.; Li, Y.; Cao, X.; Jin, X.; Jin, T. Pattern recognition receptors in zebrafish provide functional and evolutionary insight into innate immune signaling pathways. *Cellular and molecular immunology*. **2017**, *14*, 80-89.

[53] Li, Y.; Xia, P.; Wu, J.; Huang, A.; Bu, G.; Meng, F.; Kong, F.; Cao, X.; Han, X.; Yu, G.; Pan, X.; Yang, S.; Zeng, X.; Du, X. The potential sensing molecules and signal cascades for protecting teleost fishes against lipopolysaccharide. *Fish and Shellfish Immunology*. **2020**, *97*, 235-247.

[54] Wangkahart, E.; Secombes, C. J.; Wang, T. Studies on the use of flagellin as an immunostimulant and vaccine adjuvant in fish aquaculture. *Frontiers in immunology*. **2019**, *9*, 3054. https://doi.org/10.3389/fimmu.2018.03054

[55] Ribeiro, C. M.; Hermsen, T.; Taverne-Thiele, A. J.; Savelkoul, H. F.; Wiegertjes, G. F. Evolution of recognition of ligands from gram-positive bacteria: similarities and differences in the TLR2-mediated response between mammalian vertebrates and teleost fish. *Journal of immunology*. **2010**, *184*, 2355-2368.

[56] Pietretti, D.; Wiegertjes, G. F. Ligand specificities of Toll-like receptors in fish: indications from infection studies. *Developmental and comparative immunology*. **2014**, *43*, 205-222.

[57] Zhang, J.; Kong, X.; Zhou, C.; Li, L.; Nie, G.; Li, X. Toll-like receptor recognition of bacteria in fish: ligand specificity and signal pathways. *Fish and Shellfish Immunology*. **2014**, *41*, 380-388.

[58] Hyun, G. H.; Cho, I. H.; Yang, Y. Y.; Jeong, D. H.; Kang, Y. P.; Kim, Y. S.; Lee, S. J., Kwon, S. W. Mechanisms of interactions in pattern-recognition of common glycostructures across pectin-derived heteropolysaccharides by Toll-like receptor 4. *Carbohydrate Polymers*. **2023**, *314*, 120921, https://doi.org/10.1016/j.carbpol.2023.120921