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Effects of Dietary Gamnui (*Gnetum montanum*) Extract on Growth Performance, Immune Response, and Disease Resistance in Juvenile Striped Catfish (*Pangasianodon hypophthalmus*)

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Abstract

The present study investigated the effects of dietary supplementation with Gamnui (*Gnetum montanum*) extract on growth performance, immune response, and disease resistance in striped catfish (*Pangasianodon hypophthalmus*). Four experimental diets were formulated by adding Gamnui extract at 0 (G0), 2 (G2), 5 (G5), and 10 g kg^{-1} feed (G10), and administered to juvenile fish for six weeks. Growth parameters including final weight, weight gain, daily weight gain (DWG), specific growth rate (SGR), and feed conversion ratio (FCR) were recorded, along with relative internal organ indices. Post-feeding, fish were challenged with *Aeromonas veronii* to assess cumulative mortality and immune-related parameters. The results indicated no significant differences among dietary treatments in terms of final weight, weight gain, specific growth rate, or feed conversion ratio. Similarly, hepatosomatic (HSI), viscerosomatic (VSI), and gut indices (GSI) were unaffected. After bacterial challenge, the G10 group exhibited the lowest cumulative mortality, compared to the control (G0). Dietary Gamnui extract modulated several immune-related indicators: myeloperoxidase (MPO) activity increased consistently in fish receiving 10 g kg^{-1}, while glutathione (GSH) levels were elevated across all supplemented groups, indicating enhanced antioxidant capacity. Peroxidase activity exhibited a treatment-dependent response after infection, whereas lysozyme activity did not change.. In summary, dietary Gamnui extract did not influence fish growth performance but enhanced non-specific immune responses and disease resistance, particularly at 10 g kg^{-1}. These findings highlight the potential of this medicinal plant extract as a sustainable immunostimulant in aquafeed for striped catfish.

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Introduction

Aquaculture continues to play an increasingly important role in ensuring food security and promoting economic development in many countries, particularly in developing nations such as Vietnam. Among aquaculture species, striped catfish (*Pangasianodon hypophthalmus*) is considered a key species in the Mekong Delta, contributing substantially to the export value of aquatic products (Dao *et al.*, 2022). However, the rapid expansion of striped catfish farming has been accompanied by considerable challenges, notably disease outbreaks caused by bacterial pathogens such as *Edwardsiella ictaluri*, *Aeromonas hydrophila*, and *Flavobacterium columnare*, which have led to significant economic losses and have affected product quality (Tien *et al*., 2012; Pham Khanh Nguyen Huan *et al*., 2021; Bartie *et al*., 2023). Although antibiotics remain widely used in catfish farming, their routine application has become increasingly problematic. They contribute to antimicrobial resistance, lead to antibiotic residues in fish products, and pose risks to both public health and the environment (Andrieu *et al*., 2015). In response, there is growing interest in natural alternatives, particularly plant-derived products rich in bioactive compounds with immunostimulatory and disease-preventive properties, both in research and practical applications (Suraiya *et al*., 2022).

Among the native plant species of Vietnam, Gamnui, *Gnetum montanum*, a climbing vine belonging to the family Gnetaceae, is commonly found in mountainous regions of Northern and North Central Vietnam. In traditional medicine, Gamnui has been used to treat bone and joint disorders, inflammation, pain, and to support detoxification. Recent studies have revealed that this plant contains a variety of bioactive compounds, including flavonoids, saponins, alkaloids, and tannins, which exhibit antioxidant, antibacterial, and immunomodulatory properties (Vu Thi Lan Phuong *et al*., 2019; Nguyen Thu Hang *et al*., 2025). Several in vitro experiments have demonstrated that extracts from *Gnetum montanum* exhibit inhibitory effects against the growth of both Gram-negative and Gram-positive bacteria (Ong Binh Nguyen *et al.*, 2018). To date, no published study has assessed whether dietary *G. montanum* extract can modulate innate immunity or improve disease resistance in striped catfish (*Pangasianodon hypophthalmus*), a species highly susceptible to bacterial infections. The mechanisms through which its bioactive compounds may influence fish immune physiology also remain unclear.

Therefore, this study aimed to (i) evaluate the effects of different dietary levels of *G. montanum* extract on growth and feed utilization; (ii) examine changes in innate immune and antioxidant responses; and (iii) assess disease resistance against *Aeromonas veronii*. The findings are expected to provide the first scientific evidence supporting the potential use of *G. montanum* as a functional feed additive for sustainable antibiotic-free striped catfish production.

Materials and Methods

Experimental fish

Juvenile striped catfish (*Pangasianodon hypophthalmus*) (~4-5g) showing no signs of injury, deformity, or disease and exhibiting normal behavior were transported to the wet laboratory. Fish were acclimatized for one week prior to the experimental trial. During the acclimation period, fish were fed a commercial diet formulated for striped catfish at this developmental stage (Aquaxcel, Cargill, No. 7414, 40% crude protein), and rearing conditions were maintained similar to those used in the experimental system.

Diet preparation

The ethanolic extract of Gamnui used in this study was described in (Nguyen Thi Mai & Nguyen Thu Hang, 2025). Extract was dissolved in an equal volume of ethanol and uniformly sprayed onto a commercial feed (Aquaxcel,

Cargill, No. 7414, 40% crude protein) to achieve the following dietary treatments:

G0: Basal diet without *G. montanum* extract

G2: Diet supplemented with 2g extract per kg feed

G5: Diet supplemented with 5g extract per kg feed

G10: Diet supplemented with 10g extract per kg feed

After application, the diets were dried at 40°C for 8 hours and stored under refrigerated conditions. Inclusion levels were selected based on preliminary screening trials and previous findings on the use of guava and *Phyllanthus urinaria* extracts in striped catfish (Nhu *et al*., 2020).

Feeding trial

Striped catfish (*Pangasianodon hypophthalmus*) with an average body weight of 5.9 ± 0.1 g fish^{-1} were randomly distributed into 12 glass tanks (100L effective volume) at a stocking density of 30 fish per tank. The four dietary treatments (G0, G2, G5, G10) were arranged in triplicate. Continuous aeration was provided throughout the trial. Fish were hand-fed twice a day at a feeding rate of approximately 4-5% of their body weight, and daily feed intake was recorded.

Water quality parameters, namely temperature, pH, and dissolved oxygen (DO), were monitored daily, while nitrite (NO_2^-) and ammonia/ammonium (NH_3/NH_4^+) concentrations were measured weekly to ensure optimal rearing conditions. Temperature was measured using a mercury thermometer, DO with an automatic oxygen meter (Milwaukee MW600), and pH, NO_2^-, and NH_3/NH_4^+ using a Sera test kit (Germany).

Fish were weighed and measured biweekly to monitor growth and adjust feeding rates. At the end of the trial, fish were individually weighed, measured, and counted to determine the survival rate and feed conversion ratio (FCR). Data on visceral, liver, and intestine weights were collected to calculate the somatic indices.

The measured husbandry parameters were daily weight gain (DWG, g/fish/day), specific growth rate (SGR, % day^{-1}), weight gain (WG, %), feed conversion ratio (FCR), and survival rate (%). The somatic indices comprised the gastro-somatic index (GSI, %), viscerosomatic index (VSI, %), and hepatosomatic index (HSI, %). These were calculated based on the body weight, feed intake, intestinal weight and length, visceral weight, and liver weight. The growth and weight gains were calculated according to the formulas:

Daily weight gain (DWG, g fish^{-1} day^{-1}) = (FBW – IBW)/T,

Specific growth rate (SGR, % fish $^{-1}$ day^{-1}) = 100 × (Ln (FBW) – Ln (IBW))/T, and

Weight gain (WG, %) = 100 × (FBW – IBW)/IBW,

where FBW and IBW are the final and initial body weights, respectively, and T is the number of days. The other parameters were calculated with the formulas:

Feed conversion ratio (FCR) = Total consumed feed / total fish body weight gain,

Survival rate (%) = 100 × Final fish count/Initial fish count,

Gastro-somatic index (GSI, %) = 100 × Intestinal weight/fish body weight,

Visceral somatic index (VSI, %) = 100 × Visceral weight/fish body weight, and

Hepatosomatic index (HSI, %) = 100 × liver weight/fish body weight.

Bacterial challenge

Bacterial preparation

Aeromonas veronii bacteria were originally isolated from diseased striped catfish and preserved at the Laboratory of Aquatic Environment and Pathology, Faculty of Fisheries, Vietnam National University of Agriculture. The pathogenic bacteria were cultured on nutrient agar (NA) plates and incubated at 28°C for 24 hours. Colonies with characteristic morphology were selected and transferred into nutrient broth (NB) at 28°C for 24 hours. After incubation, the optical density (OD) of the bacterial suspension was measured at 610nm using a spectrophotometer, resulting in an OD of 1.686, which was considered equivalent to 10^9 CFU mL^{-1}. This stock solution

was then diluted with sterile physiological saline to obtain a working concentration of 5×10^6 CFU mL^{-1}, used for the experimental infection. All procedures were performed in a biosafety cabinet to prevent contamination. To verify the bacterial concentration, a 100,000-fold dilution of the stock was plated (20 µL per plate) on NA medium. After incubation at 28 °C for 24 hours, colony counts were used to calculate the actual bacterial concentration using the formula: Mi (CFU mL^{-1}) = Ai × Di V^{-1}, where Ai is the average colony count at a given dilution, Di is the dilution factor, and V is the volume (mL) of the suspension plated.

Bacterial infection

At the end of the feeding trial, fish from each treatment were intraperitoneally injected with *A. veronii* (from the Department of Environmental and Aquatic Diseases, Faculty of Fisheries, Vietnam National University of Agriculture) at a 50% lethal dose (LD_{50}; 5×10^6 CFU mL^{-1}; 0.1 mL $fish^{-1}$) as referenced in Vu Thi Thanh Huong *et al*. (2023). Following injection, fish were randomly distributed into 100L challenge tanks at a density of 10 fish per tank and monitored for mortality over a 14-day period. A negative control group consisting of fish from all the dietary treatments was injected with sterile physiological saline (0.1 mL $fish^{-1}$). Water temperature was recorded daily, and dead fish were counted and removed. Infected fish displayed typical disease symptoms, including hemorrhages on the scales and fin bases, as well as abdominal distension. The bacterial challenge experiment was conducted in a quarantine area completely isolated from other rearing systems to prevent the risk of cross-contamination.

Sample collection and analysis

At the end of the feeding trial and on day 2 of the post-challenge, samples of blood, liver, and kidney were collected from the experimental fish (three fish per tank) for analysis of immune parameters and antioxidant capacity. Blood samples were centrifuged at 4°C (7,500 × g for 10 minutes) to obtain serum for humoral immune assays. Lysozyme and peroxidase activities were determined according to the procedures described by Nguyen *et al*. (2024). Myeloperoxidase (MPO) and catalase (CAT) activities in kidney tissues, as well as glutathione (GSH) concentrations, were analyzed following the protocols of Nguyen *et al*. (2023). Each assay was conducted in triplicate.

Data analysis

Data were analyzed using one-way analysis of variance (ANOVA) in STATISTICA software version 10.0. Differences among treatment means were compared using the LSD (Least Significant Difference) test. For the growth performance and survival rate variables, replicate tanks were used as the statistical unit (n = 3), whereas for the other parameters, the number of fish sampled per treatment was used (n = 9). Statistical significance was considered at $P < 0.05$.

Results and Discussion

Growth performance, feed utilization, and survival rate

The results presented in **Table 1** show that the initial body weight (IBW) of fish did not differ significantly among the treatments at the start of the experiment ($P > 0.05$). After six weeks of feeding, no significant differences were observed in the final body weight (FBW), daily weight gain (DWG), specific growth rate (SGR), weight gain (WG), or feed conversion ratio (FCR) among the treatments ($P > 0.05$). These results indicate that dietary supplementation with Gamnui extract at the tested inclusion levels had no adverse effects on the growth performance or feed efficiency.

Fish more than doubled their initial weight by the end of the experiment, and survival rates were 100% across all the treatments, indicating that the experimental conditions were well suited for striped catfish at the fingerling stage.

Although herbal supplementation in aquaculture holds considerable potential, it is often accompanied by risks related to toxicity and the presence of anti-nutritional compounds such as tannins, saponins, and alkaloids. These substances may interfere with nutrient absorption, inhibit digestive enzymes, or suppress growth in fish (Tonsy *et al*., 2011; Zoral, 2023). Therefore, the finding that the

Table 1. Growth performance, feed conversion ratio, and survival of striped catfish fed diets supplemented with *Gnetum montanum* extract over a six-week feeding trial.

Parameters	Experimental diets			
	G0	G2	G5	G10
IBW (g fish^{-1})	5.9^{a} ± 0.1	5.9^{a} ± 0.1	5.9^{a} ± 0.1	5.9^{a} ± 0.1
FBW (g fish^{-1})	12.5^{a} ± 1.0	13.0^{a} ± 0.4	12.6^{a} ± 0.6	12.8^{a} ± 0.6
DWG (g fish^{-1} day^{-1})	0.16^{a} ± 0.02	0.18^{a} ± 0.01	0.17^{a} ± 0.02	0.17^{a} ± 0.01
SGR (% day^{-1})	1.77^{a} ± 0.13	1.85^{a} ± 0.06	1.81^{a} ± 0.15	1.83^{a} ± 0.10
WG (%)	110.9^{a} ± 11.5	117.8^{a} ± 5.5	113.8^{a} ± 13.5	116.0^{a} ± 9.2
FCR	1.53^{a} ± 0.16	1.52^{a} ± 0.10	1.49^{a} ± 0.15	1.46^{a} ± 0.11
Survival rate (%)	100	100	100	100

Note: G0, G2, G5, and G10 refer to dietary treatments supplemented with Gnetum montanum extract at 0, 2, 5, and 10 g kg^{-1} of feed, respectively. IBW and FBW: initial and final body weight; DWG: daily weight gain; SGR: specific growth rate; WG: weight gain; FCR: feed conversion ratio. Data are expressed as mean ± SD. Values within the same row sharing the same superscript letter are not significantly different (P >0.05).

dietary inclusion of Gamnui extracts at the tested levels did not negatively affect the growth performance or feed conversion in striped catfish is a positive outcome. This suggests that the extracts were standardized to biologically safe levels, exhibiting no signs of acute toxicity and no adverse effects on nutritional efficiency. The absence of significant growth improvement in fish fed *G. montanum* extract may be attributed to the functional profile of this plant. Its major bioactive compounds are primarily known for their antioxidant and immunomodulatory activities rather than growth-promoting effects. Comparable findings have been reported in studies using other immunostimulatory herbs, where immune parameters improved while growth remained unaffected (Yilmaz & Tan, 2023; Appuhami *et al*., 2025). These patterns suggest that herbs dominated by antioxidant and immune-boosting compounds often exert limited influence on nutrient assimilation, consistent with the responses observed in our study.

Previous studies have reported growth suppression when polyphenol-rich herbal extracts were administered at inappropriate dosages (Tonsy *et al*., 2011). Hence, the safety and stability of the Gamnui extract utilized in the present study provide a strong foundation for its future application as an immunostimulant, disease-preventive agent, or in combination with probiotics in antibiotic-free aquaculture strategies.

Somatic indices

The results of the somatic indices are presented in **Table 2**. No significant differences (P >0.05) were observed among the treatments for the hepatosomatic index (HSI, ranging from 2.50 to 2.86%), viscero-somatic index (VSI, 9.66 to 9.89%), gastro-somatic index (GaSI, 4.25 to 4.68%), or relative intestinal length (151.62 to 156.44%). Similar findings were reported by Bui Thi Bich Hang & Tran Thi Tuyet Hoa (2020), who observed no significant changes in internal organ indices when dietary pomegranate leaf extract was administered to striped catfish.

In the present study, the unaffected HSI values across the treatments suggest that the liver condition of the fish remained within a normal physiological range. This result is consistent with previous research in largemouth bass (*Micropterus salmoides*) fed fermented herbal additives for 56 days, in which no significant changes in VSI were reported (Jiang *et al*., 2023). These findings support the safety of the tested *Gnetum montanum* extract levels in terms of maintaining normal internal organ development.

Herbal products may contain bioactive compounds with potential toxicity that can negatively affect internal organs. Several studies have reported alterations in the hepatic condition and intestinal morphology in aquatic animals following exposure to certain herbal extracts (Yue *et al*., 2024). In the present study, the

Table 2. Somatic indices of striped catfish fed diets supplemented with *Gnetum montanum* extract after six weeks of feeding

Variables	Experimental diets			
	G0	G2	G0	G10
HSI (%)	2.50^{a} ± 0.44	2.86^{a} ± 0.26	2.62^{a} ± 0.42	2.54^{a} ± 0.41
VSI (%)	9.66^{a} ± 1.80	9.89^{a} ± 1.46	9.66^{a} ± 2.19	9.73^{a} ± 1.45
GaSI (%	4.31^{a} ± 1.09	4.68^{a} ± 0.86	4.40^{a} ± 1.68	4.25^{a} ± 0.40
Relative intestinal length (%)	156.44^{a} ± 29.65	151.62^{a} ± 43.82	156.24^{a} ± 31.33	152.33^{a} ± 40.62

Note: G0, G2, G5, and G10 correspond to diets supplemented with Gnetum montanum extract at 0, 2, 5, and 10 g kg^{-1} of feed, respectively. HSI: hepatosomatic index; VSI: viscerosomatic index; GaSI: Gastro-somatic index. Data are expressed as mean ± SD. Values within the same row sharing the same superscript letter are not significantly different (P >0.05).

absence of significant differences in the somatic indices between fish fed *Gnetum montanum* extract-supplemented diets and the control group suggests that the tested inclusion levels did not adversely affect the internal organ status in striped catfish. These findings provide preliminary evidence supporting the safe application of this plant extract in aquaculture.

Aeromonas veronii resistance

Temperature plays a critical role in bacterial development, making the close monitoring of temperature fluctuations during challenge trials essential. In the present study, water temperature in the infection system remained relatively stable, ranging from 25 to 29°C (**Figure 1**). These conditions were appropriate for the growth and maintenance of striped catfish.

A. veronii has a broad growth temperature range from 4 to 45°C, with optimal proliferation typically observed between 18 and 39°C. In this study, although the water temperature in the challenge tanks fell within the general growth range of *A. veronii*, it did not reach the optimal range for bacterial proliferation. This suggests that temperature may have had a limiting effect on the aggressiveness of the pathogen, while still allowing for a valid assessment of the fish's disease resistance under controlled conditions.

After 24 hours of incubation at 28°C, plating of the bacterial suspension diluted 100,000-fold resulted in 371 visible colonies. Based on the colony count and dilution factor, the actual bacterial concentration was calculated and found to be 1.85×10^{9} CFU mL^{-1}. Accordingly, the actual bacterial dose used for injection in the challenge experiment was 9.25×10^{6} CFU mL^{-1}.

Cumulative mortality of the experimental fish following the bacterial challenge is presented in **Figure 2**. After 14 days, the lowest cumulative mortality was observed in the G10 group (2.22 ± 3.85%), while the highest was recorded in the G2 group (7.14 ± 7.14%). Mortality in G2 was comparable to the control group G0 (6.67 ± 3.85%). These findings may be attributed to the presence of bioactive compounds in the Gamnui extract, which are known to exhibit immunostimulatory, antioxidant, and antimicrobial properties that could enhance disease resistance in fish.

The lowest mortality observed in the group fed 10 g kg^{-1} of extract-supplemented feed (G10) suggests that the higher concentration of active compounds contributed to improved protection against *A. veronii* infection. Mortality in G2 and G5 began within 24 hours post-infection, while the first deaths in G10 and G0 occurred on day 3 and day 4, respectively. No further mortalities were observed in G10 after day 4, indicating early and effective containment of the infection. In contrast, mortality in the G0 group accelerated rapidly after day 4 and ceased by day 6. The overall low cumulative mortality rates (< 50%) across the treatments may also be explained by suboptimal temperatures for *A. veronii* proliferation, as environmental conditions were not within the pathogen's ideal growth range. Modern pharmacological studies have confirmed multiple biological activities of Gamnui, including antioxidant, anti-inflammatory, and antibacterial effects (Ong Binh Nguyen *et al.*, 2018). These properties may act as natural antimicrobial agents under infection stress, which supports the notably low mortality rate in

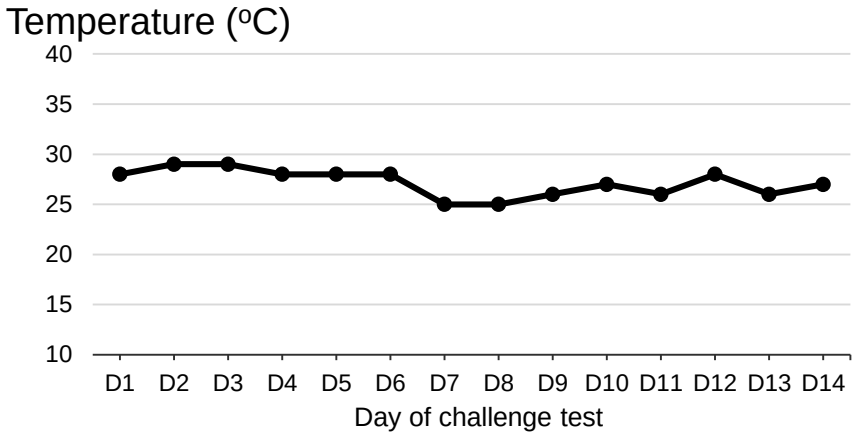


Figure 1. Mean water temperature during the bacterial challenge period

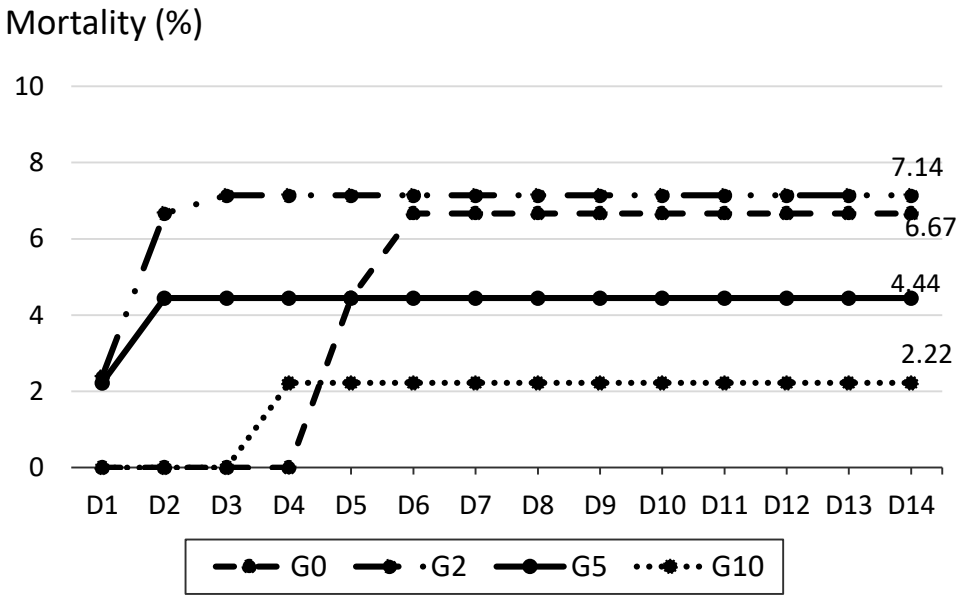


Figure 2. Cumulative mortality of experimental fish following challenge with *Aeromonas veronii*

the group receiving the highest inclusion level (10 g kg^{-1}) of the plant extract.

Aeromonas veronii has been identified as an important and emerging pathogen in freshwater fish species. It has been reported as the causative agent of disease outbreaks, septicemia, hemorrhagic symptoms, and gastrointestinal ulcerative syndromes in fish. Infected fish typically exhibit initial signs such as skin and fin hemorrhages, particularly around the fin bases, operculum, and anus as seen in **Figure 3**. Internal organs, including the liver and spleen, often appear pale and discolored, while the intestines may become swollen and hemorrhagic (**Figures 3 and 4**) (Hoai *et al*., 2019). These hemorrhagic manifestations are consistent with the clinical symptoms observed in infected fish in the present study.

Immune parameters

Peroxidase and lysozyme activities were analyzed in blood plasma collected at the end of the feeding trial and after the *A. veronii* challenge. The detailed results are presented in **Figure 5**. Lysozyme, also known as muramidase or N-acetylmuramide glycanhydrolase, is a bacteriolytic enzyme widely distributed throughout the body and plays a key role in the

Figure 3. External morphology and internal necropsy of striped catfish post-infection with *Aeromonas veronii*

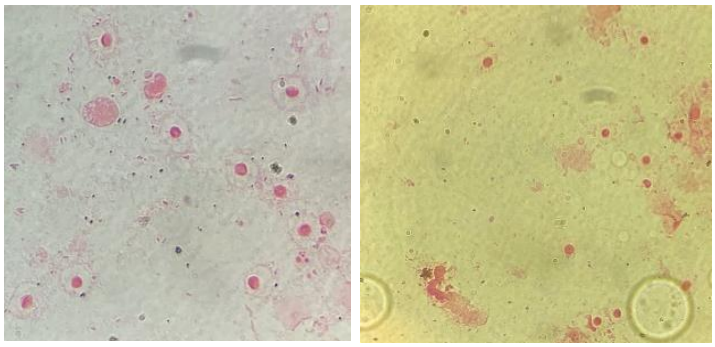


Figure 4. Fresh-stained liver samples of challenged fish (left) and control fish (right)

non-specific immune defense of most animal species (Saurabh & Sahoo, 2008). The results showed no significant differences ($P > 0.05$) in lysozyme activity among the treatments at either sampling point, indicating that dietary supplementation with Gamnui extract at the tested inclusion levels did not stimulate lysozyme activity. Lysozyme activity may have remained unchanged due to several factors, including the phytochemical profile of the extract, which may not strongly stimulate pathways associated with lysozyme production. In contrast with our results, Giri *et al.* (2015) reported a significant increase in lysozyme activity in *Labeo rohita* fed diets supplemented with 2% guava leaf extract for 60 days. Ethanol extracts of guava are known to contain compounds such as alkaloids, tannins, flavonoids, phenolic flavonoids, and ascorbic acid (Biswal *et al*., 2022), whereas Gamnui extract primarily contains stilbenes, flavonoids, alkaloids, and sterols. These differences in the phytochemical composition may account for the lack of variation in the lysozyme activity observed between the control and the treatments.

Another humoral immune parameter investigated was peroxidase activity, which refers to a broad class of enzymes involved in various biological processes. These enzymes are termed “peroxidases” due to their common function in breaking down peroxides. As shown in **Figure 5**, peroxidase activity in the fish blood plasma decreased significantly in the G10 group after the bacterial challenge compared to the control group G0 (P <0.05). However, a contrasting trend was observed in the myeloperoxidase (MPO) levels analyzed in kidney tissue, where fish from the G10 group exhibited higher MPO activity than the control. MPO is a well-known enzyme commonly found in the neutrophils of many fish species (Gan *et al*., 2023). It is believed to play more complex roles, including neutrophil activation and stimulation of other immune cells such as macrophages (Rizo-Téllez *et al*., 2022; Lin *et al*., 2024), thereby contributing to the overall inflammatory response. As presented in **Figure 6**, no significant differences in MPO activity were detected among the treatments at the end of the feeding period (T6; P >0.05). However, after the bacterial challenge, MPO activity was

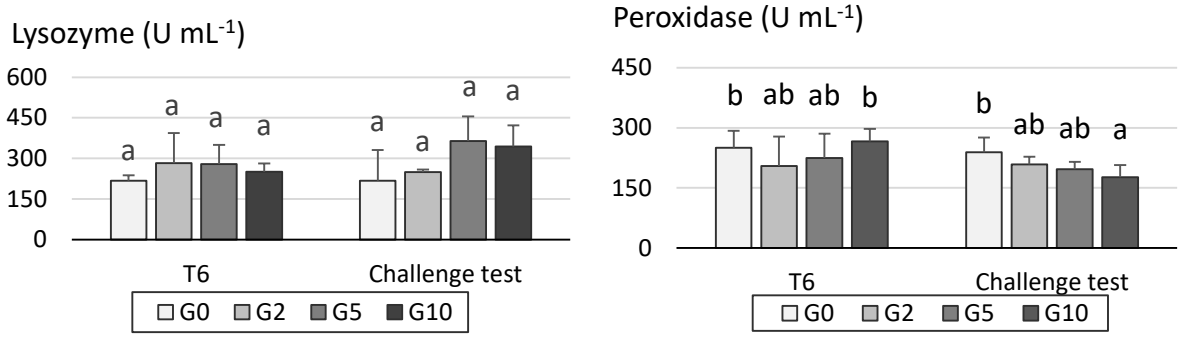
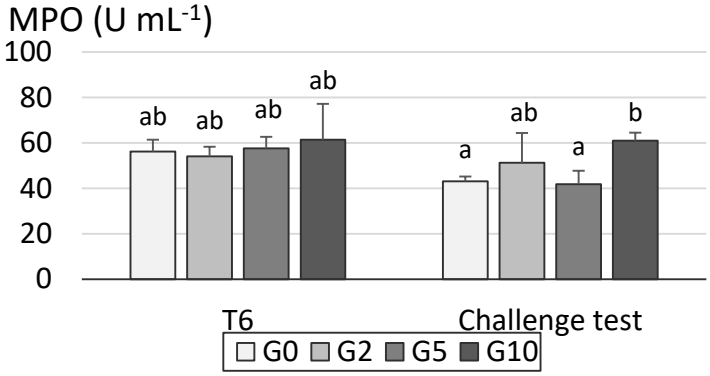


Figure 5. Lysozyme and peroxidase activities in blood plasma of striped catfish after six weeks of feeding and post-challenge with *Aeromonas veronii*

Note: G0, G2, G5, and G10 correspond to diets supplemented with Gnetum montanum extract at 0, 2, 5, and 10 g kg^{-1} of feed, respectively. Data are expressed as mean ± SD. Values within the same row sharing the same superscript letter are not significantly different (P >0.05).



Note: G0, G2, G5, and G10 correspond to diets supplemented with Gnetum montanum extract at 0, 2, 5, and 10 g kg^{-1} of feed, respectively. Data are expressed as mean ± SD. Values within the same row sharing the same superscript letter are not significantly different (P >0.05).

Figure 6. Myeloperoxidase (MPO) activity in the head kidney of striped catfish after six weeks of feeding and post-challenge with *Aeromonas veronii*

highest in the G10 group, suggesting that dietary supplementation of Gamnui extract at 10 g kg^{-1} promoted enhanced MPO activity in the kidneys.

These findings may reflect a regulatory balance within the humoral immune system, aimed at preventing host tissue damage during inflammatory responses. While MPO activity reached its peak in G10 after the challenge, serum peroxidase activity was simultaneously at its lowest in the same group. Since both enzymes contribute to oxidative activity and antimicrobial defense, excessive oxidation can adversely affect host recovery. Therefore, this opposing trend may represent a compensatory mechanism in which the activity of these oxidative enzymes is modulated to avoid excessive immune-mediated tissue damage (Nguyen *et al*., 2020).

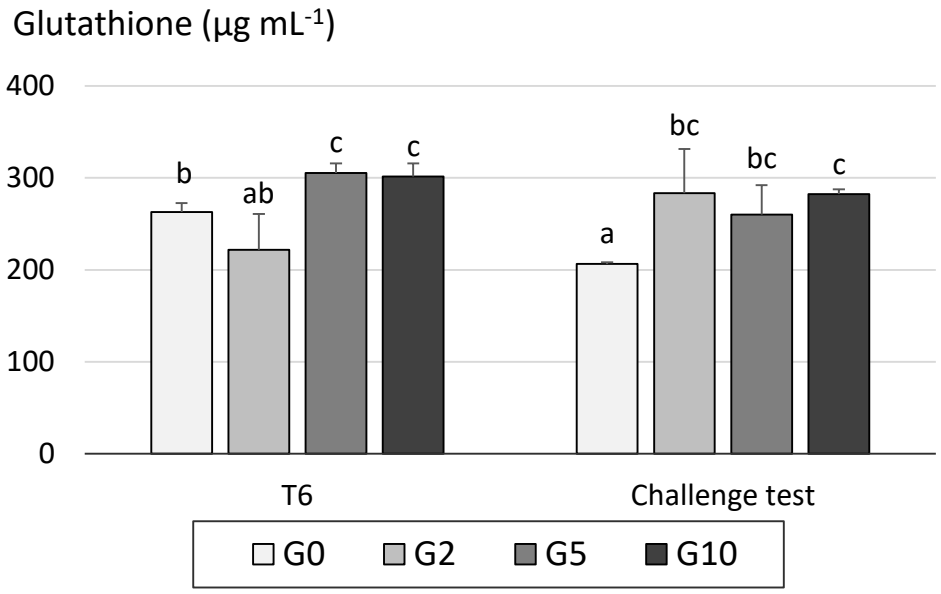
Antioxidant capacity of fish liver

Glutathione (GSH) is a key antioxidant compound found in plants, animals, fungi, and some bacteria. It plays a vital role in protecting cellular components from oxidative damage caused by reactive oxygen species (ROS), peroxides, and heavy metals (Silvagno *et al.*, 2020). At the end of the feeding trial (T6), hepatic GSH concentrations were highest in fish fed diets supplemented with Gamnui extract at 5 and 10 g kg^{-1} (G5 and G10). Following the bacterial challenge, GSH levels in all the extract-supplemented groups remained significantly higher than in the control group (P <0.05), with no significant differences observed among the Gamui extract-supplemented treatments (**Figure**

7). Flavonoids and stilbenes present in *G. montanum* are well-known for their antioxidant properties, particularly their ability to modulate intracellular redox systems. These compounds can enhance glutathione (GSH) levels by upregulating γ-glutamylcysteine ligase (the rate-limiting enzyme of GSH synthesis), promoting NADPH availability for GSH recycling, and directly scavenging reactive species that would otherwise deplete GSH. Because the MPO-peroxidase system contributes to ROS generation during inflammatory responses, increased GSH may help stabilize the MPO–peroxidase balance and prevent excessive oxidative activity. These mechanisms may explain the observed increase in GSH without a pronounced change in the MPO activity in the present study. Notably, the GSH concentrations in the control group declined after infection, whereas no such reduction was detected in the fish fed extract-supplemented diets, indicating that the extract conferred sustained antioxidant protection. During bacterial infection, various potent oxidants are generated as part of the host defense response to destroy bacterial membranes. However, excessive oxidative activity may also lead to host tissue damage. In the inflammatory cascade, alongside pro-inflammatory mediators such as eicosanoids that activate immune responses, the immune system also produces anti-inflammatory and antioxidant factors to mitigate oxidative stress and protect host tissues. These findings demonstrate that dietary supplementation with Gamnui extract effectively enhanced the antioxidant defense mechanisms in striped catfish, even at the lowest tested inclusion level (2 g kg^{-1}), offering protection against oxidative damage during infection.

Conclusions

Dietary inclusion of Gamnui extract at up to 10 g kg^{-1} did not impair the growth performance or feed conversion in striped catfish. After the *A. veronii* challenge, extract-fed fish showed reduced cumulative mortality, elevated MPO activity, and lower peroxidase levels. Hepatic GSH concentrations increased consistently across all the supplemented groups, suggesting improved antioxidant defense. Overall, these results indicate that Gamnui extract is a promising functional additive for antibiotic-free aquaculture.



Note: G0, G2, G5, and G10 correspond to diets supplemented with Gnetum montanum extract at 0, 2, 5, and 10 g kg^{-1} of feed, respectively. Data are expressed as mean ± SD. Values within the same row sharing the same superscript letter are not significantly different (P >0.05).

Figure 7. Glutathione (GSH) levels in striped catfish liver after six weeks of feeding and post-challenge with *Aeromonas veronii*

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