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Growth Performance and Immune Response of Striped Catfish as Affected by Dietary Pro-, Pre-, or Synbiotics Derived from *Lactobacillus* sp.

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Abstract

The present study aimed to investigate the effects of dietary supplementation of peptidoglycan, *Lactobacillus plantarum*, or their combination on the growth performance, disease resistance, and immune status of juvenile striped catfish. Fish were fed on a commercial feed supplemented with various concentrations of peptidoglycan (PG5.0: 5.0g per kg of feed), *Lactobacillus plantarum* (PL5.0: 5.0g per kg of feed), or the combination of the two (PP0.0: 0.0g + 0.0g per kg of feed; PP2.5: 2.5g + 2.5g per kg of feed); PP5.0: 5.0g + 5.0g per kg of feed). Fish were then fed at a rate of 3% of their body weight for six weeks. After the feeding trial, fish were infected with *Aeromonas veronii* at 50% the lethal dose (LD_{50}, 10^7 CFU mL^{-1}). Blood samples were collected after two, four, and six weeks of the experiment and on the second day after bacterial infection for hematological parameters and immune assays. The results demonstrated that supplementation with PP2.5 significantly improved the growth performance and feed utilization in fish, while the LP5.0-based diet reduced mortality in *A. veronii*-challenged fish, indicating enhanced disease resistance. The modifications in hematology and immune status were affected by the pro-, pre-, and synbiotics tested in this study. In conclusion, dietary supplementation with peptidoglycan and *L. plantarum* enhances growth, immunity, and disease resistance in striped catfish, supporting sustainable aquaculture practices for improved fish health and production efficiency, with the optimal ratio being 2.5g peptidoglycan + 2.5g *L. plantarum* or 5g *L. plantarum* per kg of diet in striped catfish.

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Keywords

Probiotics, prebiotics, bacterial infection, disease resistance

Introduction

The rapid expansion of aquaculture has made it one of the most significant contributors to global food security. Among aquaculture

species, the striped catfish (*Pangasianodon hypophthalmus*) holds a prominent position due to its high production yield, economic importance, and global market demand. Nevertheless, the intensification of aquaculture practices has introduced multiple challenges, such as suboptimal growth rates, reduced feed efficiency, and heightened vulnerability to diseases (Ahmad *et al*., 2021). These challenges highlight the necessity for innovative and sustainable approaches to enhance fish health, growth, and productivity while reducing environmental impacts.

In recent years, the incorporation of probiotics, prebiotics, and synbiotics into aquafeeds have attracted significant interest as promising strategies to improve fish health and performance. Probiotics, including *Lactobacillus* sp., are live microorganisms that provide health benefits to the host by regulating the gut microbiota, suppressing pathogenic bacteria, and boosting immune responses (Ringø *et al*., 2010). Prebiotics, in contrast, are non-digestible dietary components that selectively promote the proliferation of beneficial microorganisms within the intestinal tube (Mugwanya *et al.*, 2022; Wee *et al.*, 2024). When administered together, synbiotics foster a more favorable gut environment, thereby improving feed efficiency, promoting growth performance, and strengthening disease resistance in aquatic organisms. Synbiotics hold significant promise due to their synergistic effects in enhancing the growth and activity of beneficial gut microbiota, thereby optimizing nutrient absorption, strengthening immune function, and promoting overall health (Al-Habsi *et al.*, 2024; Amenyogbe *et al.*, 2024). Studies on the effects of synbiotics in aquaculture have shown promising results in various fish species, including crayfish (Alvanou *et al.*, 2023), Nile tilapia (Cavalcante *et al.*, 2020), common carp (Dehaghani *et al.*, 2015), *Labeo fimbriatus* (Pawar *et al.*, 2023), rainbow trout (Villumsen *et al.*, 2020), and Asian seabass (Susanto *et al.*, 2024). These studies have demonstrated improvements in growth performances, feed conversion ratios, and immune parameters, highlighting the potential of synbiotics as functional feed additives. However, research on the application of synbiotics in *P. hypophthalmus* remains limited. The study of Sutriana *et al*. (2021) demonstrated that a combined dietary probiotic and prebiotics significantly enhanced growth, intestinal morphology, and microbial balance in juvenile striped catfish. The use of *Lactobacillus* sp. as a probiotic component in synbiotics is of particular significance given its well-established probiotic characteristics, such as antimicrobial activity, modulation of gut microbiota, and immunostimulatory effects (Amenyogbe *et al.*, 2024). When combined with suitable prebiotics, such as oligosaccharides or inulin, *Lactobacillus*-based synbiotics may significantly enhance the growth performance, feed utilization, and immune responses of striped catfish. These improvements could reduce the reliance on antibiotics and other chemotherapeutics, contributing to more sustainable aquaculture practices (Dawood & Koshio, 2016).

This study aimed to assess the effects of dietary synbiotics derived from *Lactobacillus* sp. on the growth performance, feed utilization, and immune responses of striped catfish. Specifically, it investigated the impact of different synbiotic formulations on key growth parameters, feed conversion efficiency, and immunological responses. By elucidating the potential benefits of dietary synbiotics in striped catfish aquaculture, this research sought to provide valuable insights into their practical applications in aquafeeds and contributed to the advancement of sustainable and health-oriented aquaculture practices.

Materials and Methods

The protocols of the nutritional experiment and challenge test were approved by the Vietnam National University Animal Ethics Committee (I2-A-6670-1).

Diet preparation

Peptidoglycan compounds and *Lactobacillus plantarum* at a concentration of 5×10^8 CFU g^{-1} (Bio-floc Co. Ltd., Vietnam) were suspended in the same volumes of water (10mL

per 100g of feed) and mixed with commercial feed (Aquaxcel, Cargill, No. 7414) at different ratios as follows: PP0: commercial feed without supplementation; PG5: + 5.0g peptidoglycan per 1kg of commercial feed; LP5: + 5.0g *L. plantarum* per 1kg of commercial feed; PP2.5: (2.5g peptidoglycan + 2.5g *L. plantarum*) per 1kg of commercial feed; and PP5.0: (5.0g peptidoglycan + 5.0g *L. plantarum*) per 1kg of commercial feed. The experimental diets were prepared daily.

Diet preparation

Feeding trial

Healthy juvenile striped catfish (~13g/fish) were randomly allocated into composite tanks of 250L at densities of 30 fish per tank with three tanks per treatment. Fish were fed the experimental diets twice a day at 3% their body weight. Fish were weighed every two weeks to adjust the daily feed amount. The experiment was conducted for six weeks. The tank system was continuously aerated and the environmental parameters of temperature (from 25 to 27ºC), pH (7.0 to 7.5), oxygen (6.0 to 6.5 mg L^{-1}), NH_3/NH_4^+ (<0.1 mg L^{-1}), and NO_2 (<0.1 mg L^{-1}) were monitored twice a day. The feces were siphoned daily and approximate 30% of the water volume was renewed.

Bacterial infection

One day after the end of the feeding trial, fish from each treatment were mixed and allocated to an isolated tank system of 100L at a density of 10 fish per tank. Fish were then infected with *Aeromonas veronii* at 50% the lethal dose (LD_{50}, 10^7 CFU mL^{-1}, 0.1mL per fish). A batch of 10 fish from all the experimental groups (two fish per group) injected with physiological saline (0.1mL per fish) was used as a control without bacterial infection. The infected fish were then monitored for 14 days and the number of dead fish were recorded daily and removed out of the experimental system every two hours.

Sample collection and analysis

Blood plasma collection

On the 14th, 28th, and 42nd days (T2, T4, and T6) of the feeding trial, and the second day of the bacterial challenge, fish blood samples were collected (three fish per tank). Specifically, 1mL of blood from each fish was collected and put in a heparin tube (30µL of heparin in each tube) in which 0.1mL of fresh blood was used for the hematological analyses while a volume of 0.9mL of heparin blood was centrifuged to collect the plasma for the immune assays.

Hematological analysis

The hematological parameters, namely hematocrit (HCT), total white blood cell count (WBC), monocytes, neutrophils, lymphocytes, and red blood cell count (RBC), were analyzed following the manufacturer's protocols utilizing veterinary analyzers (URIT-3000 VETPLUS).

Lysozyme activity analysis

In a 96-well microplate, the lysozyme assay was started by mixing 10µL of plasma with 130µL of lyophilized *Micrococcus lysodeikticus* (0.6 mg mL^{-1} buffer, Sigma–Aldrich, MO, USA) suspended in phosphate buffer (pH 6.2). The absorbances at 450nm measured every 5 minutes during an interval of 30 minutes were used to calculate units of lysozyme activity. The lysozyme activity unit (U mL^{-1}) was quantified as the enzyme amount inducing a reduction in absorbance of 0.001 min^{-1} (Nhu *et al.*, 2019).

Peroxidase activity analysis

The plasma samples were added into a 96-well plate and each sample was replicated three times. Water wells were used as the blanks. Then, HBSS 1× (Thermo Fisher Scientific, USA) solution was added to each well for a total volume of 75µL. A volume of 25 µL of reaction solution (TMB, Thermo Fisher Scientific, USA) was supplemented into each well. The mixture was then incubated for 2min exactly. Finally, 25µL of 2M H_2SO_4 (Sigma, USA) was added after incubation. The value of the OD for each well was measured immediately by a spectrophotometer (Varioskan Lux, ThermoScientific) at 450nm. Peroxidase activity was determined by multiplying the difference between the OD of each sample and the blanks with Df (Df = 1000 (sample volume)$^{-1}$ used) and was represented by U mL^{-1} (Nguyen *et al.*, 2020).

Data collection, calculations, and analysis

After six weeks of the feeding experiment, the fish body weight and number of fish were calculated to determine the fish growth, protein efficiency ratio (PER), feed conversion ratio (FCR), and survival rate. Moreover, the intestinal indices, namely visceral somatic index (VSI), gastro-somatic index (GaSI), hepatosomatic index (HSI), and relative gut-length, were calculated based on the weight and length of the fish body, liver, and gut. The formulas to calculate these parameters are described as follows:

$$\text{Specific growth rate (SGR, \% day}^{-1}) = 100\ x\ \frac{(\text{Ln (FBW)} - \text{Ln (IBW)}}{\text{Days of experiment}}$$

$$\text{Daily weight gain } (DWG, g\ fish^{-1}\ day^{-1}) = \frac{\text{FBW} - \text{IBW}}{\text{Days of experiment}}$$

$$\text{Weight gain (WG, \%)} = 100\ x\ \frac{\text{FBW} - \text{IBW}}{\text{IBW}}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Weight of consumed feed}}{\text{Fish body weight gain}}$$

$$\text{Survival rate (\%)} = 100\ x\ \frac{\text{Final fish count}}{\text{Initial fish count}}$$

$$\text{Viscerosomatic index (VSI, \%)} = 100\ x\ \frac{\text{Viscera weight}}{\text{Fish body weight}}$$

$$\text{Intestinosomatic index (ISI, \%)} = 100\ x\ \frac{\text{Intestine weight}}{\text{Fish body weight}}$$

$$\text{Hepatosomatic index (HSI, \%)} = 100\ x\ \frac{\text{Liver weight}}{\text{Fish body weight}}$$

$$\text{Relative gutlength (\%)} = 100\ x\ \frac{\text{Gut length}}{\text{Fish body length}}$$

Data were subjected to one way ANOVA analyses with STATISTICA 10.0 software (Statsorf, Inc., East 14 Street, Tulsa, USA), followed by an LSD test using the diet replicate (n=3). Data were presented as means ± SD. Differences among groups were considered significant at *P-value* ≤0.05.

Results

Growth performance, feed utilization, and survival rate

The husbandry parameters were calculated based on the fish weight, fish number, and consumed feed, and the results are presented in **Table 1**. At the beginning, the fish body weights in the experimental groups were similar, around ~13 g fish^{-1} (**Table 1**, P >0.05). After the 6-week trial, significant differences were observed in the fish growth indices of FBW, WG, DWG, and SGR (P <0.05) indicating the supplementation of pro- or prebiotics induced modifications in the growth of the juvenile striped catfish. Specifically, the highest growth performances were recorded in PP2.5, namely the WG (84.8%), SGR (1.5% day^{-1}), and DWG (0.3g fish^{-1} day^{-1}) parameters (P <0.05). The growth variables

Table 1. Husbandry variables of fish fed on diets supplemented with pro-, pre-, or synbiotics after a six-week trial

	Experimental diets					
Parameters	PP0.0	PG5.0	LP5.0	PP2.5	PP5.0	*P-value*
IBW (g fish^{-1})	13.4^{a} ± 0.1	13.5^{a} ± 0.1	13.4^{a} ± 0.0	13.5^{a} ± 0.0	13.3^{a} ± 0.1	*0.152*
FBW (g fish^{-1})	22.0^{a} ± 0.5	23.9^{bc} ± 1.3	22.2^{ab} ± 1.4	24.9^{c} ± 1.6	22.4^{ab} ± 0.8	*0.025*
WG (%)	64.1^{a} ± 2.5	77.2^{bc} ± 8.9	65.6^{ab} ± 10.4	84.8^{c} ± 11.2	68.8^{ab} ± 4.6	*0.019*
SGR (% day^{-1})	1.2^{a} ± 0.0	1.4^{bc} ± 0.1	1.2^{ab} ± 0.2	1.5^{c} ± 0.1	1.2^{ab} ± 0.1	*0.022*
DWG (g fish^{-1} day^{-1})	0.2^{a} ± 0.0	0.2^{bc} ± 0.0	0.2^{ab} ± 0.0	0.3^{c} ± 0.0	0.2^{ab} ± 0.0	*0.018*
FCR	1.7^{c} ± 0.0	1.6^{b} ± 0.0	1.6^{b} ± 0.1	1.4^{a} ± 0.2	1.6^{bc} ± 0.1	*0.002*
Survival rate (%)	100	100	100	100	100	--

Note: PP0.0, PG5.0, LP5.0, PP2.5, and PP5.0: experimental treatments, IBW: initial body weight, FBW: final body weight, WG: weight gain, SGR: specific growth rate, DWG: daily weight gain, FCR: feed conversion ratio. Data are presented as means ± SD. The values with different letters in the same range denote significant differences (P <0.05).

observed in PG5.0 were similar to those in PP2.5 and also higher than the control diet (PP0.0), while the other LP5.0 and PP5.0 treatments did not differ from the control.

Similar to the growth performance parameters, the optimal values of the feed conversion ratio were also found in PP2.5 (P <0.05). The feed efficiency in both the PG5.0 and LP5.0 treatments were better than the control, while the ratio in PP5.0 was not different compared to the control.

Hepatosomatic, visceral, and intestinal indices

After six weeks of feeding, no significant differences were observed in the hepatosomatic index (HSI), viscerosomatic index (VSI), intestinosomatic index (ISI), or relative intestine length percentages of the juvenile striped catfish among the dietary treatments (**Table 2**, P >0.05). The HSI ranged from 1.7% to 2.2%, VSI from 6.7% to 7.6%, and ISI from 2.7% to 2.9%, while the relative intestine length varied from 134.0% to 164.2%. These results indicate that the dietary supplementation of pro-, pre-, or synbiotics derived from *Lactobacillus* sp. did not significantly affect the somatic indices or intestinal morphology of the juvenile striped catfish during the experimental period.

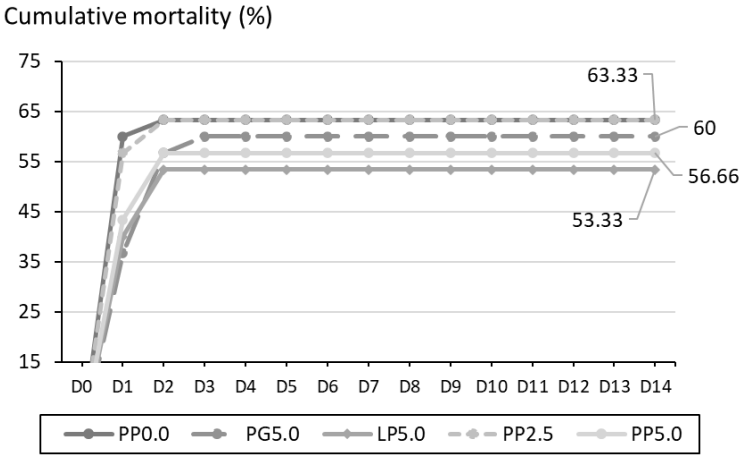
Fish mortality in the bacterial challenge

During the bacterial challenge test, the water temperature ranged from 25 to 26°C. The fish mortality was recorded daily and the cumulative mortality is displayed in **Figure 1**. After 14 days of the bacterial challenge, the lowest mortality was found in LP5.0 (53.3%), followed by PP5.0 (56.6%) and PG5.0 (60%), and the highest values were observed in PP0.0 and PP2.5 (63.3%). However, no significant differences were found between PG5.0 and PP0.0 (P >0.05). Dead fish

Table 2. Hepatosomatic, visceral, and intestinal indices of juvenile striped catfish fed on diets supplemented with pro-, pre-, or synbiotics after six weeks

Variables	*Experimental diets*					
	PP0.0	PG5.0	LP5.0	PP2.5	PP5.0	*P-value*
HSI (%)	$1.8^a \pm 0.1$	$1.9^a \pm 0.2$	$2.2^a \pm 0.4$	$2.0^a \pm 0.3$	$1.7^a \pm 0.5$	*0.131*
VSI (%)	$7.4^a \pm 0.6$	$7.6^a \pm 0.7$	$7.3^a \pm 0.9$	$7.2^a \pm 0.5$	$6.7^a \pm 0.5$	*0.184*
ISI (%)	$2.8^a \pm 0.5$	$2.9^a \pm 0.7$	$2.7^a \pm 0.9$	$2.9^a \pm 0.4$	$2.9^a \pm 0.5$	*0.966*
Relative intestine length (%)	$164.2^a \pm 25.4$	$148.0^a \pm 34.9$	$134.0^a \pm 33.4$	$137.9^a \pm 17.7$	$137.6^a \pm 39.2$	*0.460*

Note: PP0.0, PG5.0, LP5.0, PP2.5, and PP5.0: experimental treatments; HSI: Hepatosomatic index, VSI: Viscerosomatic index, ISI: Intestinosomatic index. Data are presented as means ± SD. The values with different letters in the same range denote significant differences (P <0.05).



Note: PP0.0, PG5.0, LP5.0, PP2.5, and PP5.0: experimental treatments.

Figure 1. Mortality (%) of fish infected with *Aeromonas veronii* after 14 days of bacterial challenge

were observed from the first day after bacterial injection, with the highest mortality recorded in PP0.0 and the lowest in PG5.0. From the third day to the 14th day of the challenge test, no mortalities were observed in any treatment.

Striped catfish infected with *A. veronii* exhibited various symptoms, including hemorrhages on their skin and fins, particularly at the fin base, operculum, and anus. Internal organs, including the liver and spleen, appeared pale, while the intestines were swollen and showed signs of hemorrhaging. Observations of the clinical signs in the infected fish are presented in **Figure 2**.

Immune parameters

Hematological parameters

Hematological parameters, namely leucocyte components, red blood cell (RBC) counts, and hematocrit (HCT), were analyzed in the experimental fish at weeks two, four, and six of the feeding trial and on day two post-challenge. The results are shown in **Figure 3**. After six weeks of feeding, white blood cell (WBC), monocyte, lymphocyte, and granulocyte counts showed an increasing trend, most notably in granulocytes. However, no significant differences were detected among the treatments for WBCs, monocytes, and lymphocytes at any sampling point. Granulocyte counts were highest in the PP5.0 group, with PP2.5 showing a comparable level and PP0.0 significantly lower (P <0.05). No significant differences were observed among PG5.0, LP5.0, and the control. Following bacterial infection, all the leucocyte components decreased except for granulocytes in the LP5.0 group.

Humoral immune indicators

Figure 4 illustrates the lysozyme activity across the different treatments and sampling points. At T2, the lysozyme activity was highest in LP5.0 (P <0.05), significantly differing from the PP0.0 and PP2.5 treatments, while the PP5.0 and PG5.0 treatments exhibited moderate activity. At T4, a decrease in the lysozyme activity was observed in PG5.0, LP5.0, and PP2.5 compared to T2 (P <0.05), while the PP0.0 and PP5.0 treatments maintained similar activity levels. At T6, the lysozyme activity remained low, similar to T4, with LP5.0 showing the lowest levels (P <0.05). In both the T4 and T6 samplings, the lysozyme activity in PP5.0 was higher than in LP5.0. Under bacterial infection, the lysozyme activity increased in PP0.0, LP5.0, and PG5.0, with PP2.5 showing the highest levels, while PP5.0 consistently displayed lower activity.

Regarding the results of the peroxidase activity, at T2, the peroxidase activity was elevated, with PP5.0 showing the highest values, which were statistically different from the other treatments (P <0.05). At T4, the highest activity was observed in the PP2.5 treatment, followed closely by PG5.0. At T6, a significant decrease in activity was noted across all treatments, with PP5.0 exhibiting the lowest value. Under bacterial infection, peroxidase

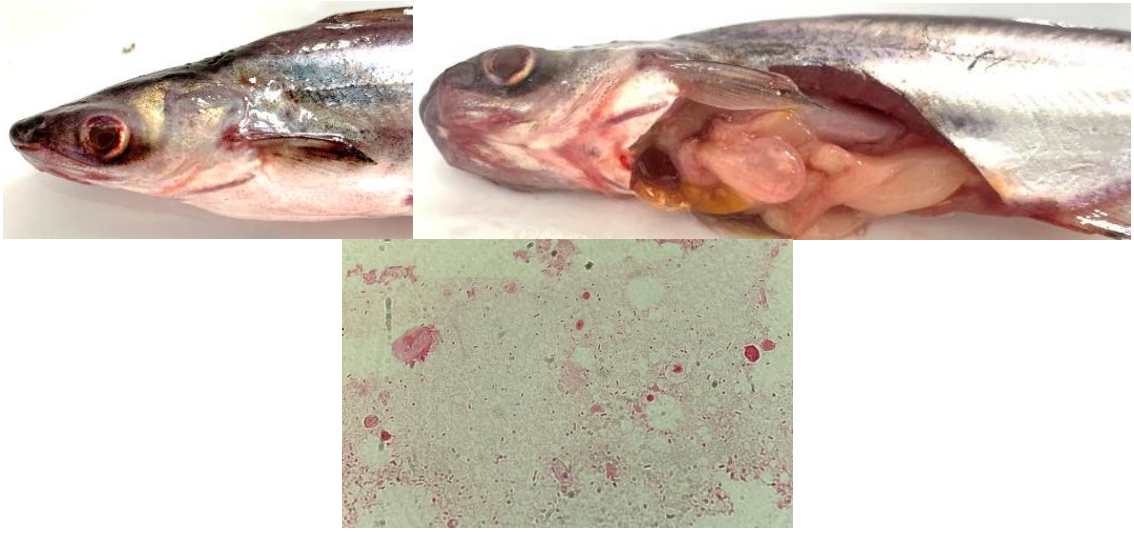
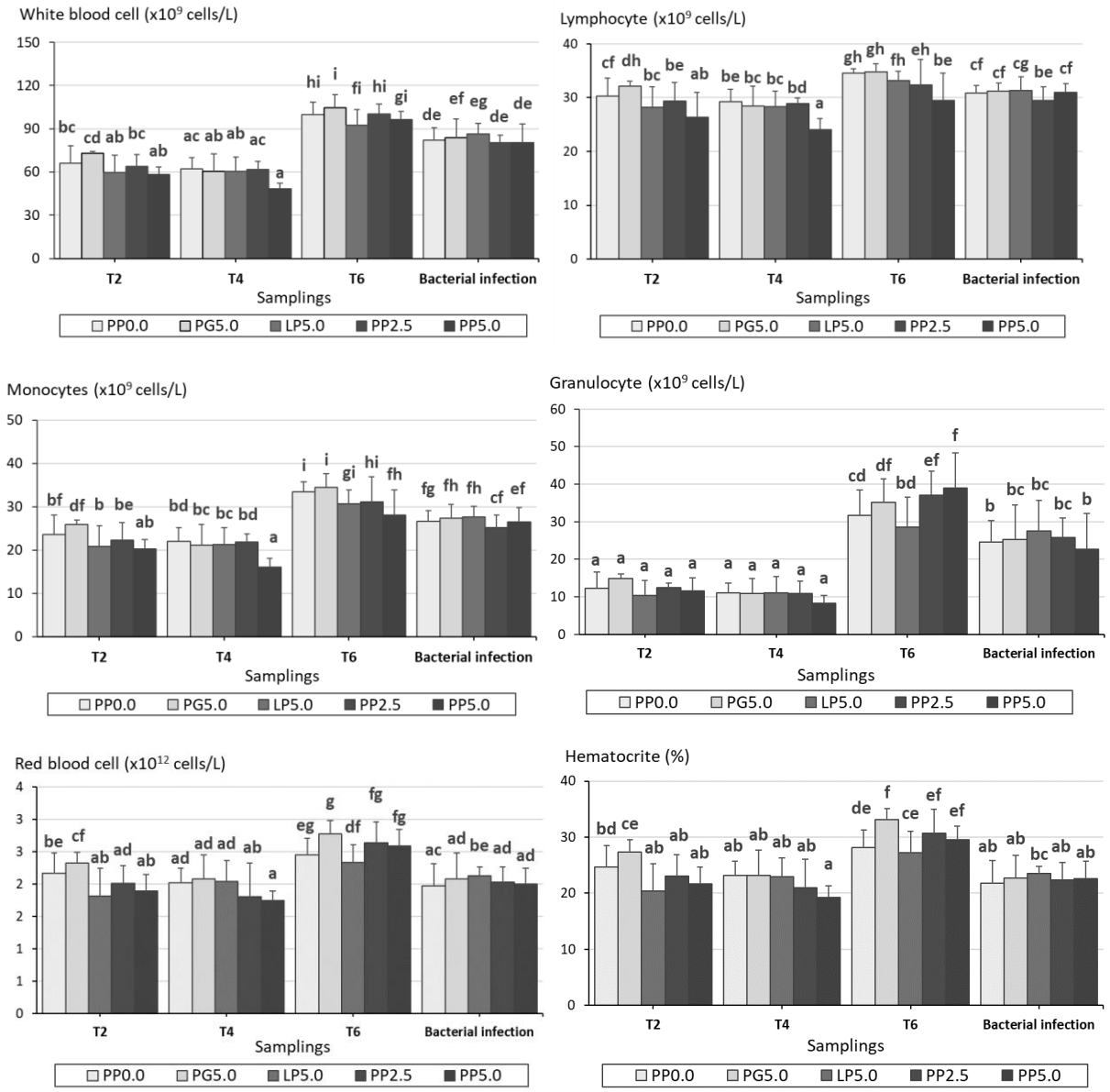


Figure 2. Clinical signs of external and internal lesions, as well as stained liver samples, were observed in infected fish



Note: PP0, PG5, LP5, PP2.5, and PP5.0: experimental treatments; T2, T4, and T6 are the samplings at the beginning and after 2, 4, and 6 weeks of the feeding trial; Ctrl is fish without bacterial infection. Data are presented as means ± SD. The values with different letters denote significant differences (P <0.05)

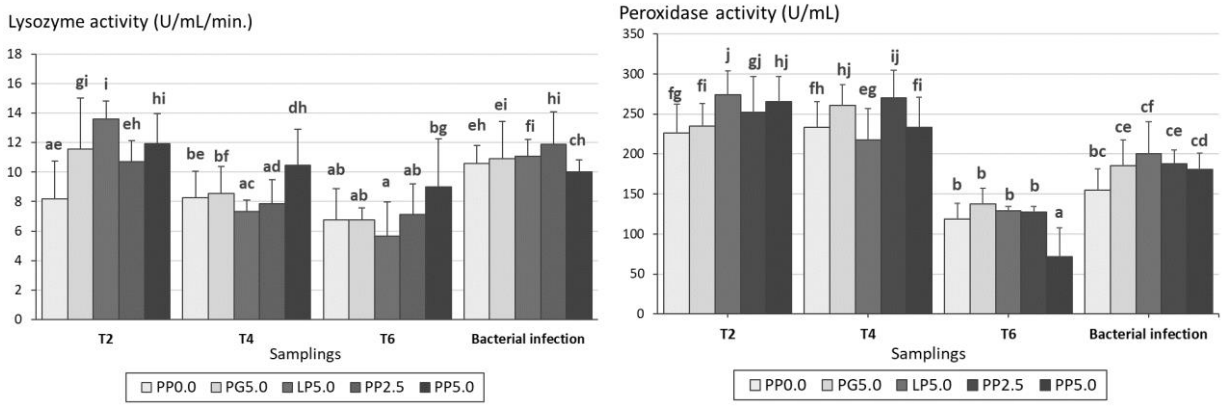
Figure 3. Hematological indices of striped catfish after 2, 4, and 6 week of feeding trial and bacterial challenge

activity increased compared to the T6 sampling, but no significant differences were found among the experimental groups.

Discussion

Influence of supplemental dietary pro-, pre-, and synbiotics on fish growth and feed utilization

At the start of the experiment, the initial body weights (IBW) showed no significant differences across treatments, indicating uniform starting conditions. However, after six weeks of the feeding trial, the studied parameters, namely final body weight (FBW), weight gain (WG), specific growth rate (SGR), and daily weight gain (DWG), demonstrated significant variations among the groups, with the highest values observed in the PP2.5 treatment, suggesting enhanced growth performance compared to the other treatments. Probiotics, prebiotics, and their combination as synbiotics have been reported to



Note: PP0, PG5, LP5, PP2.5, and PP5.0: experimental treatments; T2, T4, and T6 are the samplings at the beginning and after two, four, and six weeks of the feeding trial; Ctrl is fish without bacterial infection. Data are presented as means ± SD. The values with different letters denote significant differences (P <0.05)

Figure 4. Lysozyme and peroxidase activities in the blood plasma of striped catfish after two, four, and six weeks of feeding trials and the bacterial challenge

improve nutrient absorption and intestinal health, contributing to better growth metrics (Markowiak & Ślizewska, 2017; Okey *et al.*, 2018; Kaushik & Sharma, 2024). Interestingly, the growth performance of fish fed the PP2.5 diet was higher than those fed the PP5.0 and LP5.0 diets, suggesting that the combination of 2.5 g *L. plantarum* and 2.5 g peptidoglycan resulted in the best growth performance in the striped catfish. Similarly, the combination of probiotics and chitosan at doses of 3 g and 4 g per kg of diet, respectively, also improved the growth rate in Nile tilapia, as reported by Cavalcante *et al.* (2020). The PP2.5 treatment also exhibited the lowest feed conversion ratio (FCR) indicating superior feed efficiency. These results are consistent with previous studies showing that synbiotics improve feed digestibility and conversion efficiency (Sutriana *et al.*, 2021; Susanto *et al.*, 2024). Notably, the PP0.0 treatment had the highest FCR, reflecting suboptimal feed utilization in the absence of pro-, pre-, or synbiotics. In this study, the survival rate was consistently 100% across all the treatments, suggesting that the experimental diets, including the examined products, were safe and did not negatively affect fish health during the trial period. The PP2.5 diet demonstrated the best overall performance in terms of growth and feed utilization. While higher synbiotic levels (PP5.0) also improved growth performance compared to the control (PP0.0), the results suggest that an optimal dosage (2.5%) was more effective. Excessive synbiotics may not proportionally enhance growth and feed utilization, potentially due to microbiota imbalances or metabolic costs associated with higher supplementation levels (Mugwanya *et al.*, 2022; Wee *et al.*, 2024). These findings underscore the benefits of incorporating synbiotics into aquafeeds to improve growth, feed efficiency, and health. Such dietary interventions align with sustainable aquaculture practices by reducing feed wastage and enhancing productivity. However, further studies are needed to evaluate the long-term effects, economic feasibility, and the underlying mechanisms of synbiotic action in fish. Overall, the results highlight the positive impact of synbiotic supplementation on the growth performance and feed utilization of fish, with PP2.5 emerging as the optimal treatment. These findings contribute to the growing body of evidence supporting synbiotics as a promising dietary strategy for sustainable aquaculture.

Impacts of pro-, pre-, and synbiotics on immune status and disease resistance

The experimental results highlight the dietary modulation of hematological parameters in experimental fish. Significant differences were observed in lymphocyte counts, with a marked increase in the granulocyte count in fish fed the PP5.0 and PP2.5 diets compared to those fed

PP0.0 and LP5.0 after six weeks of the trial. Granulocytes, a type of white blood cell characterized by granules in their cytoplasm, play a key role in immune defense (Fischer *et al.*, 2006), particularly in secreting humoral immune molecules during immune responses. In our study, probiotics, prebiotics, and their combination may have acted as exogenous compounds, triggering an increase in innate immune responses, including leucocytes (Amenyogbe *et al.*, 2024), with synbiotics inducing the highest quantity of granulocytes. On the other hand, decreases in monocytes and lymphocytes were observed at T4 and T6 in the blood of fish fed the PP5.0 diet compared to PP0.0. Monocytes serve as precursors to macrophages, which are critical for pathogen clearance, while lymphocytes are essential for antigen recognition and the formation of immune memory (Secombes & Wang, 2012). These results can be explained by the fact that probiotics, prebiotics, and their combination activate non-specific immune responses (Hardy *et al.*, 2013; Mazziotta *et al.*, 2023), primarily humoral immunity, whereas lymphocytes are mainly involved in the production of specific immune factors. Consequently, blood cell components are regulated to maintain a balanced state. Therefore, when the granulocyte count increased in fish fed the PP5.0 diet, the monocyte and lymphocyte counts remained low. Interestingly, in the PP2.5 treatment, the leukocyte levels in the immune system were balanced and higher than in the control group.

The humoral immune indicators, including lysozyme and peroxidase activity, of striped catfish can be influenced by dietary supplementation with prebiotics (PG5.0), probiotics (LP5.0), and synbiotics (PP2.5, PP5.0). Lysozyme and peroxidase are crucial immune enzymes involved in the non-specific defense mechanisms of fish, playing critical roles in pathogen recognition and destruction (Mokhtar *et al.*, 2023). The lysozyme activity results indicated that supplementation with PP2.5 and PP5.0 enhanced lysozyme activity compared to the control group (PP0.0). At T2, LP5.0 exhibited the highest lysozyme activity, followed by PP5.0 and PG5.0, suggesting that early-stage dietary interventions can stimulate immune responses. At T4 and T6, activity slightly declined over time, likely due to physiological adaptation (Dawood & Koshio, 2016). The observed enhancement in lysozyme activity suggests that synbiotics provide a synergistic effect by modulating gut microbiota and stimulating immune cells (Ringø *et al.*, 2010). The temporal pattern of peroxidase activity was similar to that of lysozyme activity, with observed values decreasing at T6. These results indicate that synbiotic supplementation is a promising strategy for improving innate immune responses in striped catfish, potentially reducing the reliance on antibiotics in aquaculture.

With the support of an immune system stimulated by experimental bio-compounds, the lowest mortality rate was observed in fish supplemented with LP5.0, suggesting a beneficial effect of synbiotics in enhancing disease resistance. These findings are consistent with previous studies indicating that synbiotics improve gut health, enhance immune responses, and increase resistance to bacterial infections (Cavalcante *et al.*, 2020; Villumsen *et al.*, 2020; Pawar *et al.*, 2023). Prebiotics (PG5.0), probiotics (LP5.0), and synbiotics (PP5.0) demonstrated superior effects in reducing mortality, likely due to their role in modulating gut microbiota and stimulating non-specific immune mechanisms. Probiotics have been reported to enhance antimicrobial peptide production and competitively exclude pathogens, while prebiotics serve as substrates for beneficial bacteria, promoting intestinal health and immunity (Dawood & Koshio, 2016).

These findings in the current study have important implications for sustainable aquaculture. The use of synbiotics, particularly the combination of *L. plantarum* and peptidoglycan, offers a promising strategy to enhance fish health and performance without relying on antibiotics or synthetic chemicals. By improving growth, immunity, and disease resistance through bio-compounds, this approach can help reduce the negative effects of aquaculture and support more resilient and eco-friendly farming systems.

Conclusions

This study demonstrated that dietary supplementation with peptidoglycan, *Lactobacillus plantarum*, and their combination significantly enhances the growth performance, feed utilization, immune response, and disease resistance of juvenile striped catfish. The combination of peptidoglycan and *L. plantarum*, at 2.5 g kg^{-1} each, resulted in the best growth and feed efficiency, while the diet supplemented with 5 g *L. plantarum* kg^{-1} effectively reduced mortality in *Aeromonas veronii*-challenged fish, suggesting improved disease resistance. Overall, the findings support the use of synbiotics as a sustainable aquaculture strategy to improve fish health and production efficiency, thereby reducing the need for antibiotics in aquaculture systems.

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