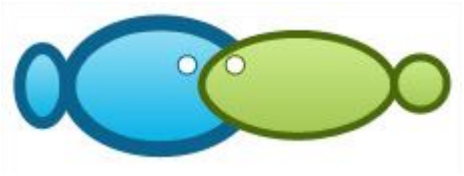

# Effect of *Lactobacillus plantarum* on growth performance, immune responses, and disease resistance of striped catfish (*Pangasianodon hypophthalmus*)

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**Abstract**. A 8 weeks long feeding trial was carried out to investigate the effect of a potential probiotic bacterial strain, *Lactobacillus plantarum*, supplemented in diets on growth performance and immune responses of striped catfish (*Pangasianodon hypophthalmus*). The feeding trial had three treatments supplemented with $10^6$, $10^7$, and $10^8$ CFU *L. plantarum* $g^{-1}$ of feed, and one control group without probiotic supplementation. Fish fed with *L. plantarum* diets showed significantly higher growth performance compared to those supplemented with the control diets ($p < 0.05$) after feeding trial. The weight gain, daily weight gain, and specific growth rate of the treatment supplemented with $10^7$ CFU $g^{-1}$ was the highest. The survival rates were not significantly different between the treatments. In terms of immunity, fish blood was sampled for hematological parameters, lysozyme, and complement activity. Results also showed that hematological parameters (total white blood cells, monocytes, lymphocytes, neutrophils, and thrombocytes were significantly higher ($p < 0.05$) in the probiotic supplemented groups compared to those of the control group. Total lysozyme activity and complement activity was significantly higher in the probiotic supplemented groups than in the control group ($p < 0.05$), indicating higher immunity in the probiotic supplemented group. After completion of feeding trial, the fish were challenged with *Edwardsiella ictaluri* for evaluating the protection against injected bacteria. Accumulated mortalities after bacterial challenge were higher in the control group compared to the probiotic-fed group. The results suggest that *L. plantarum* has potential for improving the growth performance and modulating the immune response of the striped catfish larvae.
**Key Words**: hematological parameters, *Edwardsiella ictaluri*, immune system, probiotics, striped catfish.

**Introduction**. Over the last two decades, the Mekong Delta of Vietnam has become the home to the explosive farming sector of the native catfish (*Pangasianodon hypophthalmus*), locally known as striped or tra catfish. This fish has become an important fish species worldwide, and Vietnam is the largest producing country with a total of 1.553 million tons in 2020 (MARD 2021). One of the critical factors for its farming success is the seed production and associated development of the hatchery techniques (Nguyen et al 2013). Under the large-scale business, it might be stressful for the fish as they are exposed to traumatic conditions, various diseases, and deterioration of environmental conditions. As the striped catfish is cultured in an intensive system at high densities, infectious disease agents are likely to be transmitted easily between individuals. According to a survey conducted by Bui et al (2010), the most common diseases in nursery farms appear during the rainy season. They are listed as Bacillary necrosis of pangasius (BNP) (25%), parasites (20%), hemorrhagic disease (9%), and less common diseases include white gill, stinking tail, redhead disease, red spot disease, and red-mouth disease. Bacillary necrosis pangasius (Edwardsiellosis or BNP) was identified as the most common disease of juveniles on nursery farms (Bui et al 2010). Commercial methods, such as anti-microbial agents and vaccination have been used to combat fish diseases, but they have had limited success in preventing and treating aquatic diseases. The use of antibiotics and other chemotherapeutics to treat fish and shellfish diseases has resulted in drug-resistant pathogens (Le et al 2005) and residues

of fish accumulation (Rico et al 2013). In aquaculture management, finding less toxic and more environmentally sustainable therapies has become a top priority.

Probiotics are the microbial assistants that can prevent pathogens from proliferating in the gastrointestinal tract of cultured species, which can also aid in digestion, improving water quality, and stimulating the immune response (Verschuere et al 2000). Probiotics have been used in aquaculture to protect the cultured species against bacteria, viruses, parasites, and fungi (Balcázar et al 2006). Probiotics can also be used potentially as a vital disease control strategy (Austin et al 1995). The use of probiotics in feed has proven to stimulate growth, increase survival, and enhance the fish's immune system in various experiments. Probiotics do not have any residues in fish muscle, and many of them have been tested for various species to date in aquaculture. Many of them have also proved beneficial (Irianto & Austin 2002; Thy et al 2017; Akter et al 2019). Striped catfish fed with probiotics showed a significant increase in serum bactericidal activity and phagocytic activity (Biswas et al 2016). Previous studies have confirmed that dietary supplementation of *L. plantarum* has increased growth and survival as well as enhanced immune response including phagocytosis, complement activity, and lysozyme activity in various freshwater and marine species (Butprom et al 2013; Giri et al 2013; Akter et al 2019). This study was conducted to find the effects of *L. plantarum* on the growth performance and immune response of the striped catfish through oral administration.

## Material and Method

***Lactobacillus plantarum and feed preparation***. The commercial probiotics *L. plantarum* (containing $1.1 \times 10^{10}$ CFU $g^{-1}$) used in this study was produced by Khanh Hoa company, Viet Nam. The commercial pellet (32% crude protein, Grobest, Viet Nam) was used as the control feed. This basal feed was supplemented with three levels of *L. plantarum* ($10^6$, $10^7$, $10^8$ CFU $g^{-1}$ diet) by using the method of Butprom et al (2013). The *L. plantarum* was homogenized with sufficient water, sprayed on feed, and mixed well slowly part by part. Also, the same amount of water was added to the control feed. The feed was dried at room temperature for 4 hrs and coated with 1% of squid liver oil, then dried for 4 hrs. The feeds were transferred to the plastic bags and stored at 4°C during the experiment.

***Fish and experimental design***. This study was conducted from April to August 2021. The striped catfish with an initial body weight of 2-3 g were selected from a local hatchery (Can Tho city, Viet Nam) and transferred to the wet laboratory of the College of Aquaculture and Fisheries, Can Tho University. Before the experiment, the fish was acclimatized in the composite tanks (2 $m^3$) with continuous aeration for two weeks. Fish were fed to satiation 2 times a day with chosen commercial feed. The tanks were siphoned every two days to remove uneaten feed and dead fish, and 20% of water was also replaced with new water.

The experiment was conducted using a completely randomized design with four treatments, including treatment 1 (T1): $10^6$ CFU *L. plantarum* per gram feed, treatment 2 (T2): $10^7$ CFU *L. plantarum* per gram feed, treatment 3 (T3): $10^8$ CFU *L. plantarum* per gram feed, and treatment 4 (T4): control (without *L. plantarum* supplementation) with three replicates. The experiment was conducted in circular plastic tanks (500-L each) with continuous aeration. Each tank contained 70 fish. The fish were fed two times a day at the feeding rate of 3% of body weight. Uneaten feed was removed daily by bottom siphoning. The experiment lasted for 8 weeks. The fish were counted and weighed at the end of the $8^{th}$ week (W8) for growth parameters and survival rate. Blood samples were collected at the $4^{th}$ week (W4) and $8^{th}$ week (W8) of the experiment for hematological and immunological assays. At the end of the experiment, the fish were also used in a bacterial challenge experiment.

***Sample collection***. Blood samples were collected through a caudal vein from 4 fish per tank (12 fish per treatment) using a 1 mL syringe at the end of the W4 and W8 for

hematological parameters, lysozyme, and complement assay. Afterward, 10 µL of whole blood was utilized to enumerate the number of red blood cells, and a small drop of whole blood was used for white blood cells count. The rest sample was kept in the Eppendorf tube and centrifuged to separate and collect serum for immunological assay, i.e., lysozyme and complement activity.

***Growth performance analysis***. Fish were gently captured, weighed, and counted from each experimental tank before the commencement ($W_i$) and the end ($W_f$) of the experiment to calculate the growth parameters and survival rate following the formulae:

Weight gain (WG, g) = Final weight ($W_f$) – Initial weight ($W_i$);

Daily weight gain (DWG, g day$^{-1}$) = ($W_f$ – $W_i$)/experimental time (days);

Specific growth rate (SGR, % day$^{-1}$) = 100 × [($l_n$($W_f$) – $l_n$($W_i$)] / experimental time (days);

Survival rate (SR, %) = (Final No. of fish / Initial No. of fish) × 100

***Immune parameters analysis***. Total red blood cells (RBCs) were counted in duplicate for each sample by Neubauer hemocytometer using Natt-Herrick solution as a diluent stain (Natt & Herick 1952). White blood cells (WBC) and differential WBC count were determined following Supranee et al (1991). Briefly, a drop of whole blood was smeared on the lame. The smear was quickly dried and then fixed with methanol (95%) and stained with Wright's and Giemsa (Rowly 1996). The results of each blood cell were calculated according to Hrubec et al (2000):

RBC (cells mm$^{-3}$) = C × 10 × 5 × 200

where: C = total red blood cells in 5 cells of counting area, 10 = distance between lamellae to counting chamber is 0.1 mm, 5 = square of 5 cells of counting area is 0.2 mm$^3$, 200 = dilution factor.

Each WBC = (No. of each type of WBC × Total WBC)/200

Lysozyme activity was determined by measuring the lysis of *Micrococcus lysodeikticus* according to the method described by Ellis (1990). In microplates with 96 wells, the lysozyme activity assay was started by mixing 10 µL plasma with 130 µL lyophilized *M. lysodeikticus* (Sigma) suspension at a concentration of 0.6 mg mL$^{-1}$ in phosphate buffer, pH 6.2. Between 0 and 30 minutes, the difference in absorption at 450 nm was measured and used to calculate lysozyme activity in units (Hang et al 2016). One unit represents the amount of lysozyme that caused a 0.001 decrease in absorbance.

Following Sunyer & Tort (1995) and Milla et al (2010), the alternative complement pathway was investigated using rabbit red blood cells (RRBC, Biomerieux, Craponne, France) as targets. RRBC suspension (3%) diluted in veronal buffer (Biomerieux) was combined with serial dilutions of plasma or spleen lysate in 10 µL (60 mL of total volume). The samples were centrifuged at 2,000 g for 10 minutes at room temperature after being incubated for 100 minutes at 28°C. By mixing 60 mL of veronal buffer with 10 mL of RRBC, spontaneous hemolysis was obtained. By adding 60 mL of distilled water to RRBC, complete lysis was obtained. At 405 nm, the absorbance was measured. Calculations that were accurate were used to estimate complement activity (Sunyer & Tort 1995).

### *Bacterial challenge test*

*Bacterial preparation*. Bacteria strain (*Edwardsiella ictaluri*) was obtained from the bacterial collection of the Department of Aquatic Animal Pathology, the College of Aquaculture and Fisheries, Can Tho University, and prepared accordingly to Hang et al (2013). Then, the bacteria were diluted until reaching the required concentration (1.48 × 10$^5$ CFU mL$^{-1}$) and used for the challenge experiment.

*Challenge experiment*. After 8 weeks of the supplemented *L. plantarum* experiment, the fish were selected and challenged with *E. ictaluri*. The experiment was designed with 5 treatments (10 fish per tank) including 4 treatments: T1 (10$^6$), T2 (10$^7$), and T3 (10$^8$) from *L. plantarum* supplementation. The control treatment of the experiment was divided into two more treatments in this experiment, T4 (positive control) and T5 (negative control). The fish in T1, T2, T3, and T4 were injected with 0.1 mL of *E. ictaluri* (1.48 ×

$10^5$ CFU $mL^{-1}$) which can cause 50% mortality ($LD_{50}$) while the fish in the negative control treatment (T5) were injected with 0.1 mL of saline buffer (0.85% NaCl). Ten fish will be stocked in each circular plastic tank (100-L) with continuous aeration. Fish were fed the chosen commercial feed at satiation level during the experiment. The clinical signs and mortality of fish were observed and recorded twice daily for 14 days. The head kidney of moribund fish was collected for bacterial confirmation.

The mortality rate was calculated as the percentage of the number of deaths in a specific period in a population during that period. Mortality rate (%) = (No. of dead fish/total No. fish) × 100.

### *Bacterial confirmation*

*DNA extraction*. Total nucleic acid was extracted from 50 to 100 mg kidney of experimentally infected fish and followed the method of Taggart et al (1992). The DNA pellet was dissolved in 50 µL TE buffer (10 mM Tris, 1 mM EDTA, pH 7.0) and stored at -20°C.

*Polymerase Chain Reaction (PCR)*. *E. ictaluri* was detected by the PCR method, following the protocol of Panangala et al (2007). The PCR reaction contained 1X buffer 10X; 1.5 mM $MgCl_2$; 200 µM dNTPs; 2.5 U Taq DNA polymerase; 0.4 µM f-primer (EiFd-1); 0.4 µM r-primer (EiRs) and 20 ng DNA of sample. The cycling parameters consisted of an initial denaturation at 95°C for 4 min, followed by 30 cycles of denaturation 95°C for 30 s, annealing at 55°C for 45 s, extension at 72°C for the 30 s, and a final extension at 72°C for 10 min. A negative control (no template DNA) and positive controls (purified DNA from bacteria) were included in each batch of reactions. Amplification was performed in a T-gradient thermocycler and PCR products were analyzed by electrophoresis on 1% agarose gels. Gels were stained in the dark with Ethidium bromide and scanned and documented with a Gel Doc XR System (Bio-Rad).

***Statistical analysis***. All data were processed using Excel software, and the statistical analysis was done using the SPSS version 23. One-way ANOVA was performed for statistical analysis, and significant differences between means were tested at the 5% probability level using Duncan multiple range test.

## Results

***Survival rate and growth parameters***. Survival rate (SR) and growth performance of the striped catfish (mean initial weight 3.2 g) were evaluated and compared at the end of the 8th week of the experiment. Results showed that the SR of fish in all treatments was high (91-97%) but was not significantly different among treatments (Table 1). The highest SR was recorded to be 96.7±2.65% in the treatment fed with $10^8$ CFU $g^{-1}$, while the lowest was seen on $10^6$ CFU $g^{-1}$ (91.4±5.15%) compared to the control group (92.4±4.18%).

Table 1

Survival rate and growth performance of the striped catfish at the end of 8th weeks of the experiment

| *Treatment* | *SR (%)* | *WG (g)* | *DWG (g day$^{-1}$)* | *SGR (% day$^{-1}$)* |
|---|---|---|---|---|
| $10^6$ CFU $g^{-1}$ | 91.4±5.15 | 17.6±3.41$^{ab}$ | 0.29±0.06$^{ab}$ | 3.10±0.26$^{ab}$ |
| $10^7$ CFU $g^{-1}$ | 95.7±3.77 | 19.4±1.66$^{b}$ | 0.32±0.03$^{b}$ | 3.25±0.11$^{b}$ |
| $10^8$ CFU $g^{-1}$ | 96.7±1.65 | 17.8±2.89$^{ab}$ | 0.30±0.05$^{ab}$ | 3.12±0.22$^{ab}$ |
| Control (0) | 92.4±2.18 | 16.1±1.78$^{a}$ | 0.27±0.03$^{a}$ | 2.99±0.15$^{a}$ |

Values are means±SD of different treatments (n = 12). Within the columns, values with the same letters are not significantly different ($p > 0.05$).

The growth performance of fish indicated that the *L. plantarum* fed treatments had significantly higher WG (g) ($10^6$, $10^7$, and $10^8$ CFU $g^{-1}$) than the control group. It was found that $10^7$ CFU $g^{-1}$ has a highly significant weight gain (WG) (19.4±1.66 g) than the control treatment (16.1±1.78 g). Similarly, the DWG (g $day^{-1}$) and SGR (% $day^{-1}$) were significantly higher in *L. plantarum* fed groups than in the control group. The treatment

with $10^7$ CFU $g^{-1}$ showed the highest value of WG, DWG, and SGR but no significant difference compared to other supplemented *L. plantarum* groups ($p > 0.05$).

***Blood parameters***. The total number of RBCs in all treatments was increased at W8 compared to W4, but the values did not differ significantly among the treatments ($p > 0.05$) (Figure 1). The highest number ($3.9 \times 10^6$ cells $mm^{-3}$) was observed in the treatment supplemented with $10^8$ CFU $g^{-1}$. The treatments supplemented with $10^7$ and $10^8$ CFU $g^{-1}$ had a higher number of RBCs compared to the control group at W8, whereas the treatment supplemented with $10^6$ CFU $g^{-1}$ has a smaller number of RBCs than the control group. The highest number of RBCs with the value of $4 \times 10^6$ cells $mm^{-3}$ was shown in treatment supplemented with $10^7$ CFU $g^{-1}$ at W8.

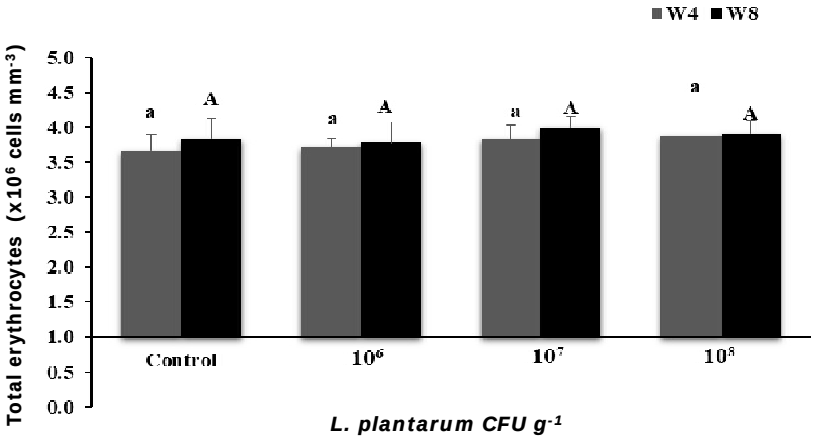


Figure 1. Total erythrocytes of striped catfish after 4 and 8 weeks of the experiment. Each bar represents the mean value with standard deviation (SD). Values with the same letters in each sampling are not significantly different, $p > 0.05$.

Total WBCs were significantly higher ($p < 0.05$) in all *L. plantarum* fed groups compared to the control group at W4 (Figure 2). The highest WBCs was $1.3 \times 10^5$ cells $mm^{-3}$ in the treatment fed with $10^7$ CFU $g^{-1}$, whereas the lowest WBCs ($0.8 \times 10^5$ cells $mm^{-3}$) were seen in the control group. Similarly, at the end of the experiment (W8), the total WBCs were significantly higher in the *L. plantarum* fed groups than in the control group ($p < 0.05$). Specifically, the highest numbers of total WBCs were observed in the group supplemented with $10^7$ CFU $g^{-1}$ at $1.5 \times 10^5$ cells $mm^{-3}$, followed by treatment supplemented with $10^8$ and $10^6$ CFU $g^{-1}$ at $1.4 \times 10^5$ cells $mm^{-3}$ and $1.3 \times 10^5$ cells $mm^{-3}$, respectively.

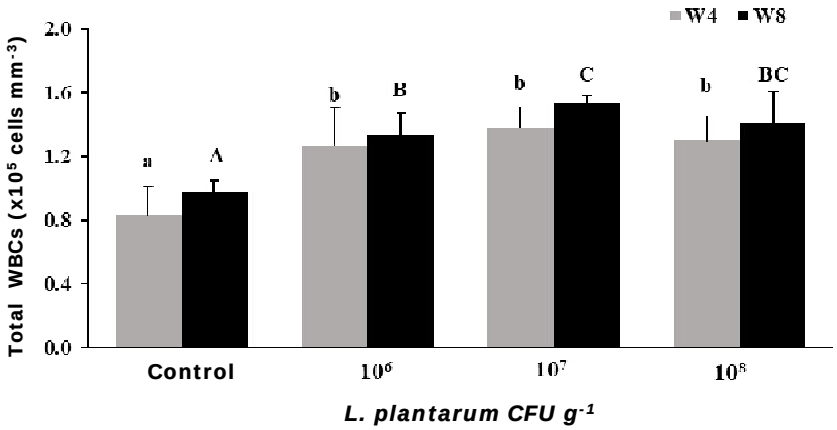


Figure 2. Total WBCs of striped catfish after 4 and 8 weeks of feeding trial. Each bar represents the mean value with standard deviation (SD). Values with the same letters are not significantly different, $p > 0.05$.

The hematological parameters with each WBC type are shown in Table 2. Neutrophil count in treatments supplemented with $10^7$ and $10^8$ CFU $g^{-1}$ was significantly higher ($p < 0.05$) compared to control at W4. Correspondingly same results were also observed at W8, with the highest neutrophil count in treatment supplemented with $10^8$ CFU $g^{-1}$ ($p < 0.05$).

Monocytes count in all *L. plantarum* fed treatments was significantly higher than the control group at W4 (Table 2). Specifically, a *L. plantarum* based diet with $10^7$ CFU $g^{-1}$ could stimulate to increase the highest monocyte numbers at W4 and the value was higher more than double compared to the control group ($p < 0.05$). The monocytes numbers were significantly higher ($p < 0.05$) in treatment fed with $10^7$ and $10^8$ CFU $g^{-1}$ compared to the control group, whereas group fed diet supplemented with $10^6$ CFU $g^{-1}$ was not significantly different from the control group at W8.

All the *L. plantarum* fed treatments showed a significant increase in the number of lymphocytes compared to the control at W4 (Table 2). However, there were only treatments supplemented with $10^7$ and $10^8$ CFU $g^{-1}$ showing a significantly higher number of lymphocytes (632 × $10^3$ cells $mm^{-3}$ and 644 × $10^3$ cells $mm^{-3}$ respectively) at W8 ($p > 0.05$) compared to control (515 × $10^3$ cells $mm^{-3}$).

Table 2

Hematological parameters of striped catfish at the end of 4th weeks (W4) of feeding trial

| *Parameters (×$10^3$ cells $mm^{-3}$)* | *$10^6$ CFU $g^{-1}$* | *$10^7$ CFU $g^{-1}$* | *$10^8$ CFU $g^{-1}$* | *Control* |
|---|---|---|---|---|
| Neutrophils | 20.3±3.4$^a$ | 26.7±2.2$^b$ | 26.8±1.5$^b$ | 18.4±2.7$^a$ |
| Monocytes | 24.0±7.6$^b$ | 29.7±4.0$^b$ | 24.8±4.0$^b$ | 13.9±4.1$^a$ |
| Lymphocytes | 57.7±6.6$^b$ | 58.6±6.6$^b$ | 59.4±7.8$^b$ | 43.8±5.3$^a$ |
| Thrombocytes | 6.5±0.8$^{bc}$ | 6.8±0.9$^c$ | 5.5±1.0$^b$ | 4.4±0.6$^a$ |

Values are means±SD of different treatments (n = 12). Within the columns, values with the same letters are not significantly different ($p > 0.05$).

The highest number of thrombocytes (6.8 × $10^3$ cells $mm^{-3}$) was observed at W4 in treatment $10^7$ CFU $g^{-1}$ which differs significantly from other treatments. But at W8, treatment $10^8$ CFU $g^{-1}$ does not have significant differences with the control group in thrombocytes number (Table 3). Treatment $10^6$ and $10^7$ CFU $g^{-1}$ have a significantly higher number of thrombocytes at W8 compared to the other two treatments.

Table 3

Hematological parameters of striped catfish at the end of 8th weeks (W8) of feeding trial

| *Parameters (×$10^3$ cells $mm^{-3}$)* | *$10^6$ CFU $g^{-1}$* | *$10^7$ CFU $g^{-1}$* | *$10^8$ CFU $g^{-1}$* | *Control* |
|---|---|---|---|---|
| Neutrophils | 20.8±3.3$^a$ | 26.3±5.2$^b$ | 27.2±1.3$^b$ | 18.8±4.8$^a$ |
| Monocytes | 23.4±2.2$^a$ | 31.0±3.0$^b$ | 27.7±1.7$^b$ | 22.2±3.5$^a$ |
| Lymphocytes | 59.8±7.6$^{ab}$ | 63.2±7.0$^b$ | 64.4±3.9$^b$ | 51.5±4.9$^a$ |
| Thrombocytes | 6.8±1.0$^b$ | 6.7±1.1$^b$ | 5.3±0.7$^{ab}$ | 4.3±0.9$^a$ |

Values are means±SD of different treatments (n = 12). Within the columns, values with the same letters are not significantly different ($p > 0.05$).

***Lysozyme activity***. Although treatment supplemented with $10^6$ CFU $g^{-1}$ did not differ significantly in lysozyme activity compared to the control group, the significantly higher values of lysozyme activity were observed in other treatments supplemented with $10^7$ and $10^8$ CFU $g^{-1}$ ($p < 0.05$) at W4 compared to the control group (Figure 3). The highest lysozyme activity (154 U $mL^{-1}$) was found in treatment fed with $10^7$ CFU $g^{-1}$ at W4. Similarly, the lysozyme activity was highest in treatment supplemented with $10^7$ CFU $g^{-1}$ (221 U $mL^{-1}$) at W8 with a significant difference compared to control. But $10^6$ and $10^8$ CFU $g^{-1}$ did not have any significant difference compared to the control group at W8.

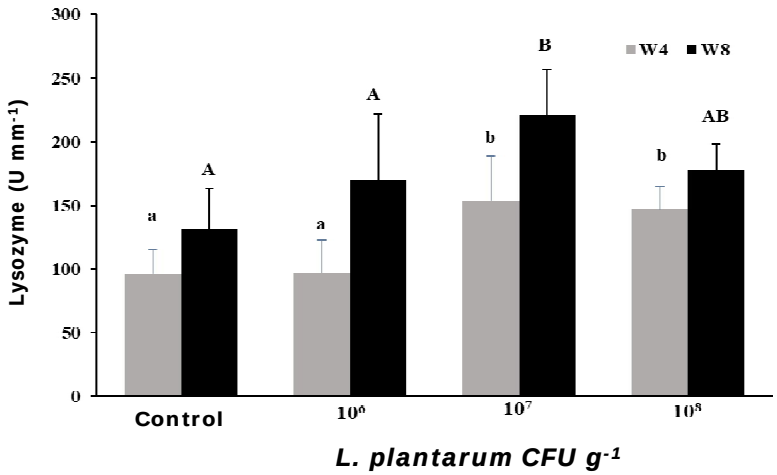


Figure 3. Plasma lysozyme activity of striped catfish at 4 and 8 weeks of feeding trial. Each bar represents the mean value with standard deviation (SD), values with the same letters are not significantly different, $p < 0.05$.

***Complement activity***. Complement activity ranged from 8.3 to 8.9 U $mL^{-1}$ at W8 in three treatments ($10^6$, $10^7$, and $10^8$ CFU $g^{-1}$) and did not show any significant difference compared to W4 (Figure 4). At W4, $10^6$ CFU $g^{-1}$ has significantly higher units of complement activity compared to other treatments, but at W8 no significant differences can be seen between the treatments.

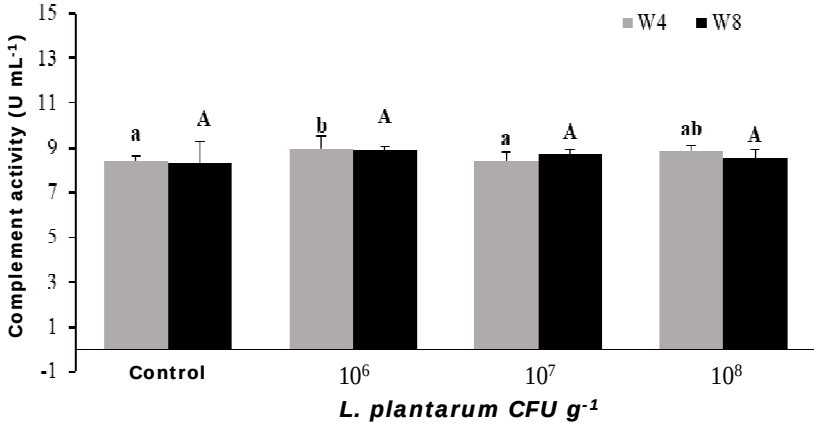


Figure 4. Complement activity of striped catfish at 4 and 8 weeks of the experiment. Each bar represents the mean value with standard deviation (SD), values with the same letters are not significantly different, $p < 0.05$.

***Fish mortality post-challenge***. Mortality of fish infected by *E. ictaluri* occurred from day 3 to day 7 post-challenge. The clinical signs like white spots on internal organs notably the liver, kidney, and spleen were observed in the dead fish (Figure 5); pale colored liver and spleen were also seen in the dead fish.

The mortality rates were significantly lower in the probiotic-fed groups than the control group ($p < 0.05$). The cumulative mortalities after bacterial challenge were 33.3, 20.0, 23.3, and 23.3% for treatment without probiotic supplementation (control), treatment supplemented with $10^6$, $10^7$, and $10^8$ CFU $g^{-1}$ of feed respectively (Figure 6). No mortality was found in negative control treatment without *E. ictaluri* infections.

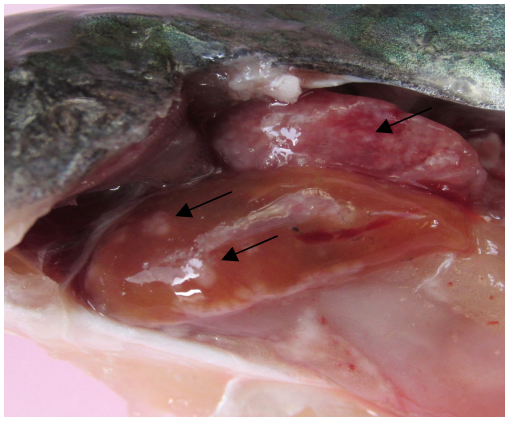

Figure 5. White spots on internal organs of infected fish (black arrow).

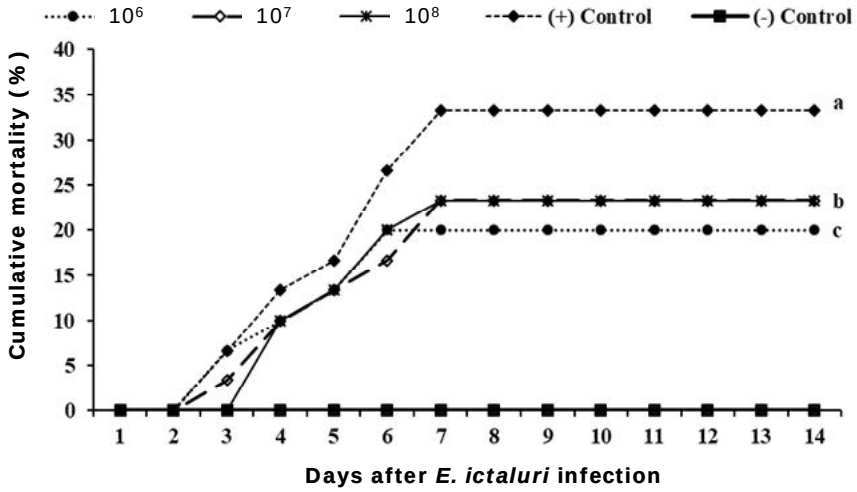


Figure 6. Accumulated mortality of supplemented *L. plantarum* fish post-challenge test with *E. ictaluri*. Different letters indicate differences among treatments, $p < 0.05$.

The results of bacteria identification showed that *E. ictaluri* were detected in all bacterial infection samples, while no signs of contamination in the control groups (Figure 7).

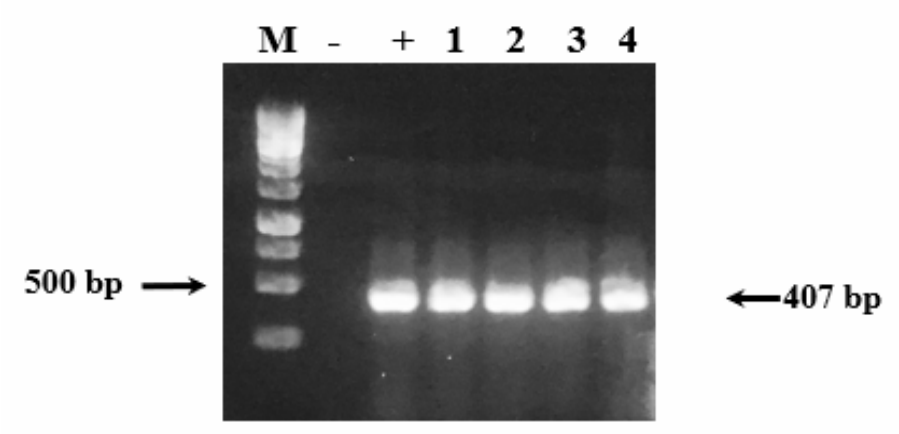


Figure 7. PCR amplification results of detecting *E. ictaluri* from infected striped catfish. Lane M: DNA marker; Lane (-): the negative control; Lane (+): positive control; Lane 1, 2, 3, and 4: DNA isolated from the infected fish of control, $10^6$ CFU $g^{-1}$, $10^7$ CFU $g^{-1}$, and $10^8$ CFU $g^{-1}$ treatment, respectively.

**Discussion**. Commercialization and intensification of fish culture are increasing year by year. The use of drugs, chemicals, and antibiotics to control fish diseases can result in the development of drug-resistant pathogens (Le et al 2005). Thus, searching for less harmful and environment-friendly treatments has become of premium importance in aquaculture management. Probiotics are living organisms that are given to enhance the feeding efficiency, growth or to protect against pathogens such as bacteria, viruses, fungi, and parasites (Balcázar et al 2006). Various studies have been done in different fishes using various species of probiotic bacteria to date (Kane et al 2016; Soltani et al 2017, 2019; Yu et al 2017) which resulted in the growth enhancement, increased survival and also augmented immune response as well as disease resistance development. Also, *L. plantarum* proved to colonize the intestines of fish, enhance growth performance and some hematological parameters in Nile tilapia (Yamashita et al 2017), and also showed decreased pathogenic bacteria in the gut of probiotic-fed groups. In addition, Yu et al (2017) concluded that dietary supplements of *L. plantarum* for fish can prevent aquaculture and food safety problems.

In the present study, the SR of treatment $10^8$ CFU $g^{-1}$ was higher than those of the control treatment, but no significant differences were found in SR among the treatments. However, the WG, DWG and SGR of the treatment supplemented with $10^7$ CFU $g^{-1}$ supplemented group were significantly higher than those of the control group in this study. In other study, 91% Nile tilapia survived until the end of the experiment when fed with *L. plantarum* whereas only 80% survival was seen on the control group (Abumourad et al 2013), which also supports the findings of the present study. Giri et al (2013) reported that the dietary administration of *L. plantarum* improved growth indices of *Labeo rohita.* The enhancement of the growth parameters can be the reason for some functions provided by the probiotics such as improvement in feed utilization by the synthesis of some growth factors including vitamins, co-factors, fatty acids, amino acids and digestive activity of the fed animal (Mohapatra et al 2018). Probiotics can also alter the architecture of the gut epithelium (Soltani et al 2019), improving nutrient absorption via increased the absorptive surface area (Pirarat et al 2011) and also the production of endogenous digestive enzymes enhances digestion of food which may affect the increment of growth parameters (Soltani et al 2017). Pathogens that cannot bind to and colonize the digestive tract are released through excretion as a result of *L. plantarum* colonization, resulting in microbial equilibrium and improved fish growth efficiency (Silarudee et al 2019). Similar growth improvements have been seen in different species like *Pangasius bocourti* (Doan et al 2014, 2015), *Pangasius larnaudii* (Piccolo et al 2015), *Oncorhynchus mykiss* (Soltani et al 2017) and *Oreochromis niloticus* (Yamashita et al 2017; Yu et al 2017) after feeding diet enriched with *L. plantarum*. From the present study, after the 8-week feeding trial, it was found that the dose of $10^7$ CFU $g^{-1}$ was the best concentration of *L. plantarum* for enhanced growth performance among all treatments.

The physiological state of fish health is indicated by the hematological parameter of the fish (Chauhan et al 2014). In this study, the RBCs in the treatments did not differ substantially, although treatment supplemented with $10^7$ CFU $g^{-1}$ had a higher number of RBCs than the other treatments in the end of experiment. The number of white blood cells in the *L. plantarum* supplemented groups is significantly higher than in the control group. The increase in total WBCs may be considered as a good indicator of activation response of fish cellular immunity. Fish use their immune system, which consists of physical barriers, humoral, and cellular elements, to defend themselves against pathogens. WBCs are the immune cells of an animal's body; they attack against foreign invaders and infectious pathogens (Akinwande et al 2005). Several fish species fed with the *L. plantarum* diets have also demonstrated an increase of WBCs (Kane et al 2016; Soltani et al 2017, 2019) which in turn indicates the increment of immune cells.

In the present study, the number of neutrophils, monocytes, lymphocytes, and thrombocytes in the treatments supplemented with *L. plantarum* were highest and significantly difference with control treatment. Neutrophils and monocytes are phagocytic cells that provide innate cellular immunity (Magnadóttir 2006). Physiological processes such as phagocytosis, internalization of viruses and bacteria, the killing of bacteria,

release of platelet microbicidal proteins, and superoxide production provide host protection in humans (Harrison 2005). The non-specific immune cells granulocytes (neutrophils) and monocytes (macrophages) are significant in fish (Dalmo et al 1997). Lymphocytes maintain some features of innate immunity and acquired immunity in fish. It regulates the efficacy of immune response (Scapigliati 2013). Likewise, thrombocytes are involved in the fish's immunological protection (De Carla Dias et al 2009). The increased number of total WBCs, monocytes, and thrombocytes in fish fed *L. plantarum* is associated to the stimulation of non-specific immune response, according to the literature.

Innate humoral immunity markers include lysozyme and complement function and these components may be used to assess the status of a fish's innate immune response (Magnadóttir 2006). Lysozyme is an anti-microbial protein associated with front-line innate immunity of invertebrates. This enzyme breaks the bond in the cell wall of gram-positive bacteria and is considered to be an indicator of pro-inflammatory phagocytic activity (Marsh & Rice 2010). In this study, the highest serum lysozyme activity was observed in treatment supplemented with $10^7$ CFU $g^{-1}$ and showed significantly higher than the control treatment. This result is similar to the experiment conducted by Butprom et al (2013), where hybrid catfish were fed with *L. plantarum* in different doses and after 60 days significantly increased lysozyme activity levels were observed. Similarly increased lysozyme activity levels were seen in different species like *P. bocourti* (Doan et al 2015), rainbow trout (Soltani et al 2017), and *Pangasius larnaudii* (Silarudee et al 2019) after feeding diet enriched with *L. plantarum*. Measurement of lysozyme activity is also a way of determining whether non-specific immunity could be improved by probiotic feeding. The range of immune effector activities like serum lysozyme is 192-388 U $mL^{-1}$ in healthy fish (Adikesavalu et al 2020). Relying on this information, it can be said that treatment supplemented with $10^7$ CFU $g^{-1}$ having 221 U $mL^{-1}$ of lysozyme activity can be considered healthier than other groups. Moreover, activation of the complement system helps in the development of acquired immunity (Boshra et al 2006). Complement can eliminate pathogens in the inflammatory process by lysis of cell membranes and activation of non-specific mediators (Holland & Lambris 2002). So, increase in the complement activity may be the indication of enhanced immunity in the fish. In this study, at W4 it was found that the treatment $10^6$ CFU $g^{-1}$ had the highest units of complement activity per milliliter of serum compared to the control group ($p < 0.05$). However, the complement activity of the other two groups was close to that of the control group, with no major differences. Similar indifferent complement activity was seen in the experiment conducted by Soltani et al (2019) in rainbow trout with *L. plantarum* supplemented diet.

*E. ictaluri* is gram-negative, long slender bacilli, motile, rod-shaped bacteria, infects the striped catfish and causes significant losses in commercial striped catfish production (Crumlish et al 2002, 2010). This study found that treating striped catfish with *L. plantarum* reduced mortality in *E. ictaluri* infected fish. According to the study conducted by Soltani et al (2019) on common carp, a clear indication that the post-challenge mortality rates was decreased in *L. plantarum* fed groups compared to the control group which was indicting the increase of fish disease resistance. Also, dietary administration of *L. plantarum* stimulated non-specific immunity and resistance against *A. hydrophila* infection on the *P. bocourti* (Doan et al 2015, 2016). In addition, *L. rohita* juveniles were fed *L. plantarum* increased the survival after challenge with *A. hydrophila* from 14.8% in control diets to 77.7% in $10^8$ CFU $mL^{-1}$ (Giri et al 2013). *L. plantarum* supplemented fish diet was known to decrease the mortality from 50% in the control group to 0% in the treated group in hybrid catfish challenged with *A. hydrophila* (Butprom et al 2013). In this study, higher post-challenge mortality rates were obtained in the control group than in probiotic-fed groups. All the previous results prove that *L. plantarum* has a good effect as an immunostimulant resulting in high immune response and bacterial resistance, but more future studies are needed to get insightful information of *L. plantarum* as a potential probiotic in larval striped catfish culture as a great prospective tool to improve productivity.

**Conclusions**. After 8 weeks of feeding the diet supplemented with *L. plantarum*, growth parameters of striped catfish such as weight gain, daily weight gain and specific growth rate were significantly increased. Certain hematological parameters such as total WBCs, neutrophils, monocytes, lymphocytes, and thrombocytes were significantly higher. Similarly, the immune parameter serum lysozyme activity and complement activity were also analyzed to be greater in the probiotic-fed group compared to the control group after 8 weeks of feeding experiment. Post-challenge mortality rates were higher in the control group compared to the *L. plantarum* fed groups indicating that the *L. plantarum* supplemented groups had considerably improved disease resistance with higher innate immunity response. Finally, dietary supplementation with *L. plantarum* improved growth performace, immune response, and provided protection of striped catfish against bacterial challenge. Nonetheless, with higher values of the above-listed variables, $10^7$ CFU *L. plantarum* per gram of feed was found to be the best dose.

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**Conflict of interest**. The authors declare that there is no conflict of interest.

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