Effects of dietary supplementation with probiotics on growth performance, gut health and disease resistance of striped catfish (*Pangasianodon hypophthalmus*)

Can T. Vo, Binh T.T. Vo, Dang H. Mai, Binh A. Bach, Hung T. Le, & Tuan V. Vo*
Faculty of Fisheries, Nong Lam University, Ho Chi Minh City, Vietnam

ARTICLE INFO

Research Paper

Received: August 21, 2024
Revised: October 01, 2024
Accepted: October 08, 2024

Keywords

Edwardsiella ictaluri
Growth performance
Probiotics
Striped catfish

***Corresponding author**

Vo Van Tuan
Email:
vovantuan@hcmuaf.edu.vn

ABSTRACT

This study was conducted to investigate the effects of the probiotic (*Bacillus* Liq-Vtech)-supplemented diets on growth performance, intestinal microbe, morphology of the intestine, and resistance to *Edwarsiella ictaluri* infection in striped catfish (*Pangasianodon hypophthalmus*). A total of 800 healthy striped catfish (12 ± 2 g) were randomly divided into four experimental groups with four replicates each, and were fed diets supplemented with different levels of *Bacillus* Liq-Vtech (T_1: 0 cfu/g; T_2: 0.5×10^6 cfu/g; T_3: 1.0×10^6 cfu/g; and T_4: 1.5×10^6 cfu/g) for 8 weeks. At the end of the trial, a challenge test with *Edwardsiella ictaluri* was conducted for 2 weeks. The results showed no statistically significant improvement in the growth performance and survival rate of fish with *Bacillus* Liq-Vtech supplementation ($P > 0.05$). However, there was a tendency for the improved growth performance in treatments supplemented with *Bacillus* Liq-Vtech. Additionally, the density of *Bacillus* spp. increased in the intestinal tract of fish fed probiotic-supplemented diets compared with the control. All probiotic treatments exhibited positive effects for different histomorphological features of the intestine. Mucus secreting goblet cells and villi increased in probiotic-supplemented groups. Mortality rates in treatments supplemented with *Bacillus* Liq-Vtech were lower than those of the control. These results suggested that the *Bacillus* Liq-Vtech supplementation in diets has the potential to improve growth performance, increase beneficial bacteria in the intestinal tract of the fish, and reduce the mortality of striped catfish after being challenged with *Edwardsiella ictaluri.*

Cited as: Vo, C. T., Vo, B. T. T., Mai, D. H., Bach, B. A., Le, H. T., & Vo, T. V. (2024). Effects of dietary supplementation with probiotics on growth performance, gut health and disease resistance of striped catfish (*Pangasianodon hypophthalmus*). *The Journal of Agriculture and Development* 23(Special issue 1), 88-99**.**

1. Introduction

Striped catfish, also known as tra catfish (*Pangasianodon hypophthalmus,* Sauvage, 1878) is a freshwater species and considered as an important commercial aquaculture fish in Vietnam. According to MARD (2024), the production of striped catfish reached 1.61 million tons and an export income of approximately 1.8 billion dollars in 2023. Striped catfish has been intensively cultured in ponds with high stocking density so that the disease outbreak is a main risk for the fish farming. Bacterial infectious diseases are the main agents that caused huge economic loss for striped catfish industry in Vietnam (Ferguson et al., 2001; Tu et al., 2008; Dang & Truong, 2022). Several approaches such as chemotherapeutics and antibiotics have been applied to control the infectious diseases in striped catfish culture, but the application of these antibiotics and chemotherapeutics is strictly regulated due to their negative impacts (Lim et al., 2013; Ramos et al., 2018).

In recent years, there is an increasing interest in the use of functional feeds that contain natural supplements such as prebiotics, probiotics, synbiotics, herbal extracts to improve fish growth and health, in which probiotics are considered as a promising solution in preventing and managing aquatic pathogenic agents (Ringø & Song, 2016; Bui et al., 2022; Liaqat et al., 2024). Application of probiotics in aqua-farming is evident in enhancing growth performance through ensuring better digestion with the proliferation of useful bacterial colony in intestine of fish (Ringø, 2020). A dietary with probiotics supplementation has been studied in many aquatic species, such as striped catfish, channel catfish, tilapia (Nguyen et al., 2022; Liaqat et al., 2024; Youssef et al., 2024).

The probiotics *Bacillus* Liq-Vtech (provided by UV company, Vietnam) is composed of *Bacillus subtilis* and *Bacillus licheniformis* with a density of 10^9 cfu/mL. A preliminary experiment has been proved that it plays an important role in inhibiting intestinal harmful bacteria and stimulating immune responses, leading to prevent diseases and enhance aquatic animal survival rate. Therefore, the objective of the present study was to evaluate effects of the probiotics *Bacillus* Liq-Vtech-supplemented diets on growth performance, intestinal microbe and morphology of the intestine, and resistance of striped catfish (*Pangasianodon hypophthalmus*).

2. Materials and Methods

2.1. Experimental diets

Four experimental diets including a basal diet were formulated to contain different levels of probiotics *Bacillus* Liq-Vtech (T_1: 0 cfu/g; T_2: 0.5 × 10^6 cfu/g; T_3: 1.0 × 10^6 cfu/g; and T_4: 1.5 × 10^6 cfu/g). The basal diet contained approximately 28% crude protein and 5% lipid, formulated from different ingredients. All feed ingredients were thoroughly mixed, extruded and pelleted to produce a floating feed at Godaco feed mill. The feed processing involved several stages as grinding, mixing, conditioning, extrusion and drying in which the extrusion lasted 5 - 10 min at 120°C and followed by drying at high temperature of 120 - 150°C. The ingredients and proximate analyses of the experimental diets was illustrated in Table 1.

The analyses of proximate composition of feed ingredients were determined by standard methods (AOAC, 1995). Crude protein content was determined by the Kjeldahl method. Crude lipid content was extracted by n-hexane using the Soxhlet method. Ash content was determined by combustion method. Moisture content was determined by the drying method using an oven at 105°C.

Table 1. Feed formulation of the basal diet and its estimated nutrient analysis

Ingredients	Percentage (%)
Rice bran	33.20
Fishmeal 60%	4.00
Soybean meal	47.09
Cassava	14.00
L-Lysine	0.03
DCP	0.63
DL Methionine	0.36
Choline Chloride	0.15
Stay Vitamin C	0.04
Premix	0.50
Nutrient level	% Dry matter
Crude protein (%)	28.00
Crude fat (%)	5.60
Fiber (%)	4.02
Ash (%)	8.90
Lysine (%)	1.55
Methionine (%)	0.70
Gross energy	4200 Kcal/kg

From the basal diet, three probiotics *Bacillus* Liq-Vtech treatment diets were produced in the laboratory by coating with probiotics at different dosages. Then all four diets were finally coated with 0.5% soy oil to prevent the probiotics leaching. All prepared feed were stored at 20°C and labelled as T_1, T_2, T_3 and T_4.

2.2. Experimental animals

Striped catfish fingerlings were purchased from private fish farm at An Giang province and transported to the Experimental Farm of Nong Lam University, Ho Chi Minh City, Vietnam. The fish were acclimated and cultured for 2 weeks and fed on a basal diet twice daily to apparent satiation.

Fish with initial weight of 12 ± 2 g were selected for the trial. The trial was carried out in 16 hapas that contained 50 fish per hapa (1.0 m × 2.0 m × 1.5 m). Fish were fed two times a day (8 am and 16 pm) to apparent satiation for a period of 8 weeks. One hour after feeding, the unconsumed feed was collected and dried to calculate the daily consumed feed for each hapa. Feed intake was recorded daily to compare the feeding intake (consumptions) of four diets at the end of the experiment.

During the feeding trial, water quality was monitored in order to evaluate the water quality. Water temperature, dissolved oxygen (DO) and pH were daily monitored using a multi-parameters photome (Hanna, Italy). Total ammonium nitrogen was weekly checked by TAN meter (Hanna, Italy).

At the end of the trial, consumed feed and survival rate of fish were measured to determine the effect of *Bacillus* Liq-Vtech supplementation on fish growth and feed utilization. Besides, intestinal microbial and histology of striped catfish intestine were also measured. Parameters were analyzed as follows:

- SR (Survival rates): %

SR (%) = (Number of survival fish/Number of initial fish) × 100

- DWG (Daily weight gain): g/fish per day

DWG = (Final weigh - Initial weight)/ Cultured days

- WG (Weight gain): %

WG = (Final weight - Initial weight)/Initial weight

- Specific growth rate (SGR).

SGR (%.day^{1}) = [(Ln(final weight) - Ln(Initial weight))/Cultured days)] × 100

- Feed conversion ratio (FCR)

FCR = Consumed feed/Weight gain

- Feed intake (FI)

- FI (g/fish per day) = (Consumed feed in tank/Number fish of tank)/Cultured days

2.3. Bacterial challenge

To evaluate the effect of *Bacillus* Liq-Vtech supplements on fish health status, fish were subjected to an immersion challenge with pathogenic bacteria *E. ictaluri*. At the end of the feeding period, 20 fish in each hapa were randomly selected and transferred to 100 L plastic tanks. Fish were subjected to a two-hour immersion challenge with *E. ictaluri* at a dosage of 2.6×10^6 cfu/mL. The concentration of bacteria in the suspension was determined through spectrophotometry at an absorbance of 600 nm and serial plate count method. Fish were housed in plastic tanks supplied with aeration and mortality of fish was monitored. At the end of the trial, survival rate and intestinal microbial (total bacteria counts and *Bacillus* sp. counts) were recorded.

2.4. Analysis of bacteria in fish intestine

At the end of the trial, two fish per hapa were randomly sampled and anaesthetized with tricaine methanesulfonate (MS - 222) with a dosage of 80 mg/L. Approximately 6 - 8 cm of fish hindgut was aseptically excised, then the inside of the intestine was gently removed and washed two times with sterile distilled saline water. The intestine samples were then homogenized, suspended in 9 mL of sterile distilled saline (0.9%), and serially diluted of suspension to 10^{-5} in 9 mL of sterile distilled saline. 0.1 mL aliquot from each dilution was spread onto duplicate PCA (plate count agar, Himedia) and HiCrome *Bacillus* Agar Base (Himedia). Plates were incubated at 28°C for 24 h. Plates containing between 30 and 300 colony-forming units (cfu) were used to enumerate the number of total cultivable bacteria and *Bacillus* sp. The results were expressed as cfu/g sample.

2.5. Histological analysis

To analyze the histo-morphological structure of the stripped catfish intestine, fragments of the middle portion of the intestine were removed with sterilized surgical instruments and fixed in 10% buffered formalin solution for 48 h, then

dehydrated in graded ethanol concentrations, cleared in xylene, and embedded in paraffin wax. Sections of 5 μM thickness were stained with Alcian blue (AB) and periodic-acid stain (PAS), and analyzed under light microscope.

To determine the height of the intestinal epithelium, five random villi per histological section were measured according to the structural characteristics. The number of goblet cells was counted and expressed in number per μM^2 of villi. The images were obtained with digital camera attached to a photomicroscope and analyzed by the program Image J.

2.6. Statistical analysis

Results were presented as means ± standard deviation (SD). All data were firstly examined for homogeneity of variance using SPSS statistic 20.0 software (IBM, New York, USA). One-way ANOVA was used to test the main effect of different diets to fish growth. The Duncan test was used to determine the significant differences among treatment groups. The probability values of $P < 0.05$ were applied to confirm the statistical difference.

3. Results

3.1. Water quality

Water quality which directly affect to reproduction, growth, and survival of aquatic organisms are considered as the most important factors in aquaculture. Aquatic organisms are susceptible to suffer stress when ecological conditions are not adequate. High stress levels generate low feeding and low growing rates as well, resulting in the appearing of sickness in the organisms (Chainark & Boyd, 2010). Striped catfish are also aquatic species but can use their swim bladder to breath air, so the demand of dissolved oxygen is not strictly required high like other fish.

During the trial, water quality parameters such as temperature, pH, DO, total ammonia nitrogen (TAN) were sampled and the results were illustrated in Table 4.

Table 4. Water quality parameters of the trial

Water quality parameters						
Temperature (°C)		pH		Dissolved oxygen (mg/L)		TAN (mg/L)
Morning	*Afternoon*	*Morning*	*Afternoon*	*Morning*	*Afternoon*	
29.7 ± 0.7	31.5 ± 0.9	7.1 ± 0.3	8.3 ± 1.1	2.6 ± 0.5	4.0 ± 1.6	0.10 ± 0.21

Results are mean of 8 weeks; TAN: total ammonia nitrogen.

The results showed that the water temperature in the morning was lower, but not significant difference in compared to the one in the afternoon ($P > 0.05$). pH and dissolved oxygen (DO) are the most important factors affecting the growth and the health status of aquatic animals. They are also important limiting factors for cultivation activities of all aquatic organisms to survive and grow (Dandruff & Dean, 1967; Boyd, 2001). During the culture season, pH should be

controlled in the suitable range for each aquatic animal. If the pH is too low or too high, it will cause negative effects to cultured species such as slow growth, stunting, and reduce ability to resist pathogens from water environment. The results of the current study showed that the average pH value was varied from 7.1 to 8.3, and was within the appropriate limits for freshwater fish growth and development (Boyd, 2001). The dissolved oxygen (DO) in the morning was 2.6 ± 0.5 mg/L and 4.0 ± 1.6 mg/L in the afternoon. The mean TAN was 0.10 ± 0.2 mg/L. In general, the values of water parameters during the trial time exhibited slight fluctuations, but still remained within the appropriate range of fish.

3.2. Growth and feed utilization

Growth performance of striped catfish expressed as final body weight (FBW), specific growth rate (SGR) and weight gain (WG) were presented in Table 5.

Table 5. Effect of *Bacillus* Liq-Vtech supplement on the growth performance of striped catfish

Treatment	IBW (g/fish)	FBW (g/fish)	DWG (g/fish per day)	WG (%)	SGR (%/day)	Survival (%)
T_1	13.44 ± 0.03	164.56 ± 14.93	2.52 ± 0.24	11.25 ± 1.10	4.17 ± 0.14	99.50 ± 1.00
T_2	13.42 ± 0.02	164.78 ± 13.22	2.52 ± 0.22	11.28 ± 0.97	4.18 ± 0.13	97.50 ± 3.76
T_3	13.39 ± 0.04	170.93 ± 9.85	2.63 ± 0.16	11.76 ± 0.72	4.24 ± 0.09	97.00 ± 2.58
T_4	13.38 ± 0.03	181.39 ± 7.71	2.80 ± 0.12	12.55 ± 0.55	4.34 ± 0.07	99.50 ± 1.00
P	0.093	0.297	0.295	0.263	0.260	0.280

Results are mean ± standard deviation (n = 4). IBW: initial body weight. FBW: final body weight. DWG: daily weight gain. WG: weight gain. SGR: specific growth rates.

The results show that the survival rates of striped catfish were quite high across all four treatments (ranging from 97% to 99.5%), but no significant difference was recorded among treatments. It means that the supplementation of *Bacillus* Liq-Vtech did not affect the survival rates of fish in normal living conditions.

The growth performance of striped catfish tended to increase from T_1 to T_4 treatments. After 8 weeks of the feeding trial, the best values in terms of final body weight, daily weight gain, weight gain percent and specific growth rate were observed in the treatment T_4 supplement compared with the control treatment. No significant difference was recorded between the fish fed probiotics supplemented diets and the control diet. However, there was a clear tendency for improved the growth performance with supplemented *Bacillus* Liq-Vtech in its diet (Figure 1).

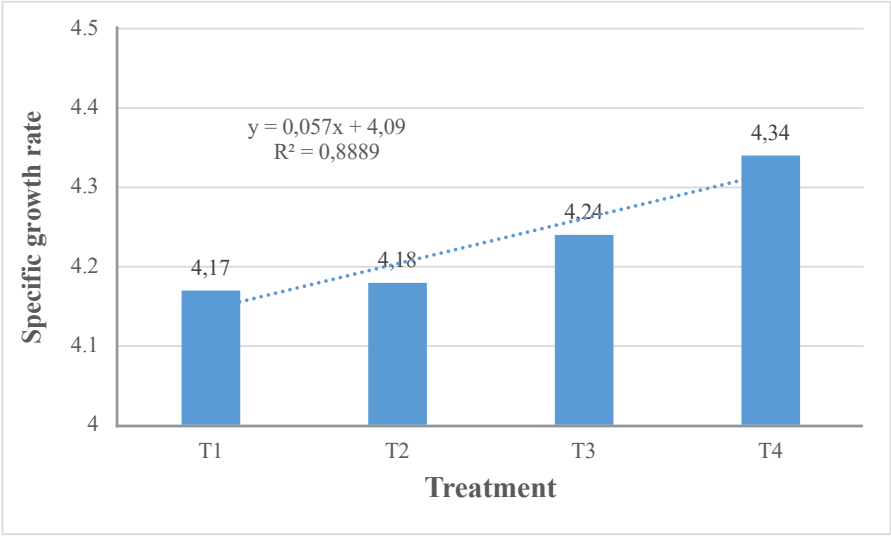

Figure 1. Specific growth rates of fish fed four experimental diets with trend line.

The feed utilization of the fish is expressed as feeding intake (FI) and feed conversion ratio (FCR). Results are presented in Table 6.

Table 6. Feeding intake and Feed conversion ratio of fish fed four experimental diets

Treatment	FI (g/fish per day)	FCR
T_1	$3.33^{a} \pm 0.23$	1.26 ± 0.01
T_2	$3.31^{ab} \pm 0.08$	1.26 ± 0.01
T_3	$3.33^{b} \pm 0.14$	1.27 ± 0.07
T_4	$3.03^{b} \pm 0.16$	1.18 ± 0.05
P	0.06	0.263

Results are mean ± standard deviation (n = 4). FI: feed intake. FCR: feed conversion ratio. Values in the same columns having the same superscript letter are not significantly different (P > 0.05) by Duncan test.

The results demonstrated that the feed intake of fish was lowest in *Bacillus* Liq-Vtech supplemented diets and significantly different from the control diet ($P < 0.05$). It indicated that *Bacillus* Liq-Vtech supplementation significantly improved the feeding intake of striped catfish ($P < 0.05$). The FCR was not significantly different among treatments; however, the T_4 treatment gave the lowest value (1.18) compared to the control diet (1.26). Considering the feed intake and FCR, it seems that the T_4 diet gave the highest efficiency in feed use. Combined growth performances and feed use efficiency (Tables 5 and 6), we can conclude that the application of probiotics *Bacillus* Liq-Vtech in the striped catfish might induce the growth rates and feeding intake.

3.3. Effects of *Bacillus* Liq-Vtech supplement on intestine morphology of striped catfish

At the end of feeding trial, intestine of fish (hind gut) was collected to determine the intestinal microbial (total bacteria and *Bacillus* sp. counts), and the structure of intestinal histo-morphology. The results are demonstrated in Table 7 and Figure 6.

Table 7. Effect of *Bacillus* Liq-Vtech supplement on intestinal bacteria and histology of the striped catfish

Parameters	Experimental diets			
	T_1	T_2	T_3	T_4
TBC ($\times 10^7$ cfu/g)	2.77 ± 1.67[a]	2.59 ± 2.03[a]	2.00 ± 1.52[a]	2.23 ± 1.20[a]
Bacillus sp. ($\times 10^7$ cfu/g)	1.27 ± 0.81[a]	2.54 ± 1.54[a]	1.73 ± 2.37[a]	2.00 ± 1.31[a]
GCs ($\times 10^{-4}$ cells/ μM^2)	5.49 ± 2.51[a]	7.83 ± 4.18[a]	6.29 ± 2.44[a]	8.35 ± 4.72[a]
Villi height (µM)	361.3 ± 96.7[a]	326.5 ± 38.6[a]	335.3 ± 54.6[a]	368.4 ± 49.8[a]
Villi width (µM)	67.8 ± 6.4[a]	75.6 ± 2.3[a]	73.8 ± 6.7[a]	74.4 ± 9.3[a]

Results are mean ± standard deviation (n = 4). TBC: total bacterial counts. GCs: goblet cells. The row with the same superscripts is not significant differences (P > 0.05).

The results showed that the total bacteria count had a tendency to decrease from the control diet to the probiotics *Bacillus* Liq-Vtech supplemented diets, while the total number of *Bacillus* sp. increased. The number of goblet cells, villi height and villi width in fish intestine showed a slightly better result in the probiotics *Bacillus* Liq-Vtech supplemented diets, but was not significantly different when compared with the control diet (Figure 2).

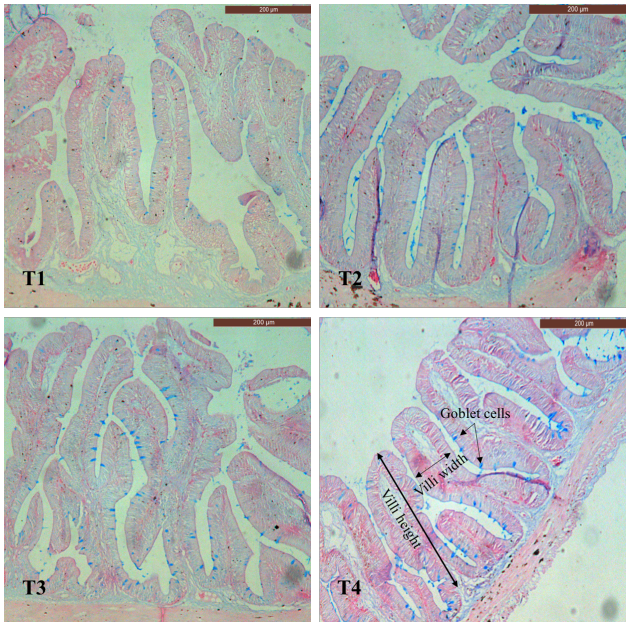

Figure 2. Histology of the striped catfish mid-intestine fed probiotics *Bacillus* Liq-Vtech supplemented diets.

3.4. Cumulative mortality of striped catfish after bacterial challenge

At the end of the feeding trial, the striped catfish were subjected to the immersion challenge with *Edwardsiella ictaluri*, and the results are illustrated in Figure 3.

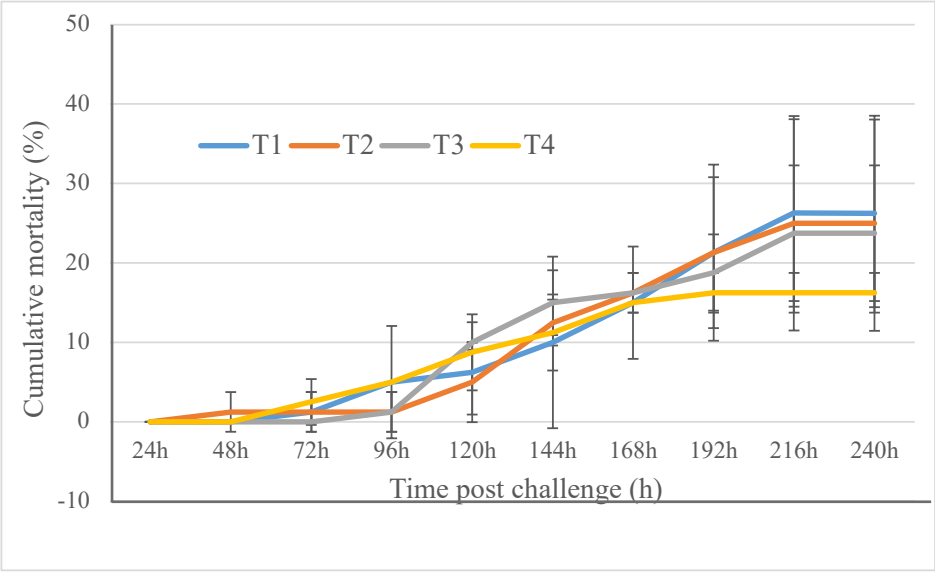

Figure 3. Cumulative mortality of the striped catfish after bacterial challenge.

The death of fish in the *E. ictaluri* challenge was initially recorded on the third day post infection (72 h post infection) in all groups. The cumulative mortality of fish in the probiotics *Bacillus* Liq-Vtech supplemented groups, especially in T_4 (1.5×10^6 cfu/g of feed) was reduced after 192 h post challenge, while it occurred continuously until 216 h post challenge in the control group. Although no significant difference in survival rate of the striped catfish challenged with pathogenic bacteria *E. ictaluri* was recorded, fish in the probiotics *Bacillus* Liq-Vtech treatments showed higher survival rate.

To evaluate the effect of *Bacillus* Liq-Vtech supplement on gut health status of the striped catfish, fish intestine was sampled at the end of bacterial challenge. Total bacterial counts and *Bacillus* sp. were determined, and the result was illustrated in Table 8.

Table 8. Effect of *Bacillus* Liq-Vtech supplement on intestinal microbial of fish

Parameters	Experimental diets			
	T_1	T_2	T_3	T_4
TBC ($\times 10^7$ cfu/g)	5.69 ± 5.41^a	4.46 ± 2.46^a	4.70 ± 3.74^a	2.73 ± 0.31^a
Bacillus sp. ($\times 10^7$ cfu/g)	3.84 ± 1.86^a	3.05 ± 1.62^a	2.75 ± 1.28^a	2.58 ± 0.91^a

Results are mean ± standard deviation (n = 4). TBC: total bacterial counts. The row with the same superscripts is not significant differences (P > 0.05).

Bacillus Liq-Vtech is a probiotic product that contains high levels of beneficial populations of bacteria. It plays an important role in supporting gut health status of fish. The current study showed that lower counts of bacteria (total bacterial counts and total *Bacillus* sp. counts) were observed in the probiotics *Bacillus* Liq-Vtech supplemented diets in comparison to the control diet. However, no significant differences of the total bacteria populations between diets were reported.

4. Discussion

The current study was carried out to evaluate the effect of the probiotics *Bacillus* Liq-Vtech supplemented diets on the growth performance, intestinal microbe, morphology of the intestine, and resistance to infection with *E. ictaluri* of the striped catfish (*Pangasianodon hypophthalmus*). The results of the current study showed that the growth performance and feed utilization of fish were not significantly different between the control diet and the probiotics *Bacillus* Liq-Vtech supplemented diets. However, fish fed diet with a dose of *Bacillus* Liq-Vtech of 1.5×10^6 cfu (T_4) had a better results in terms of growth performance, feed utilization, and survival rate compared to the control diet. After feeding in 8 weeks, there was a tendency to have an increased the growth performance of striped catfish. Dietary with 1.5×10^6 cfu/g (T_4) *Bacillus* Liq-Vtech supplement had a lower FCR than other diets (1.18 versus 1.26), but no difference was recorded between diets. The result seems not to meet the expectation of probiotics used in the striped catfish feed. A possible explanation is that the dosage of probiotics *Bacillus* Liq-Vtech was not sufficient to significantly enhance the growth performance of striped catfish. Our results align with the results of Yazici et al. (2016) in rainbow trout, Niamphithak et al. (2017) in bocourti catfish, and Nguyen et al. (2022) in channel catfish. Besides, results of the current study are in line with the results of Shelby et al. (2006) and Sirbu et al. (2022) in tilapia. These authors also concluded that supplementation of probiotics in fish diets did not produce significant effect on growth parameters; however, fish fed with probiotics diets showed potential for improved the growth performance and survival rate.

Total bacterial flora in fish intestine is considered as a key indicator to evaluate fish health status. It was found in our study that higher count of bacteria was observed in the control diet in comparison to the probiotics *Bacillus* Liq-Vtech supplemented diets; however, no significant differences of the total bacterial counts among diets was reported. Besides, the *Bacillus* sp. levels in the treatment diets were higher and showed a tendency to be increased in diets supplemented with probiotics *Bacillus* Liq-Vtech. It means that *Bacillus* sp. in the probiotics *Bacillus* Liq-Vtech has a potential to colonize and survival in the intestine of striped catfish. Our results are in line with the results of Chang & Liu (2002). These authors demonstrated that higher number of probiotic bacterial survival in the intestine of European eel (*Anguilla anguilla*).

Goblet cells or intestinal mucus-secreting goblet cells, are very important for the nutrition of the fish and its health. They have both absorptive and secretory functions in fish (Pereira et al., 2020). In the current study, the goblet cells and the size of villi have a tendency to be increased in diets supplemented with probiotics *Bacillus* Liq-Vtech. Linked to the modulate of the microbial gut communities, it might responsible for the increased goblet cells numbers. Increased goblet cells had also reported in Artic charr (*Salvelinus alpinus* L.) and tilapia (*Oreochromis niloticus*) (Lødemel et al., 2001; Liu et al., 2016).

Probiotics can be applied in aqua-farming for several purposes such as promoting growth performance, enhancing feed digestion, maintaining normal intestinal microbial communities. Besides, it has ability to control

the infectious diseases. The challenge test in this study showed that supplementation of the probiotics *Bacillus* Liq-Vtech in feed of striped catfish tended to reduced mortality caused by *E. ictaluri*. There are two possible direct mechanisms that may be involved in the process. First is the competitive ability of *Bacillus* sp. against other harmful bacteria in the intestine of fish. The other could be the ability to produce antimicrobial substances that inhibited and suppressed bacterial infection.

5. Conclusions

Supplementation of the probiotics *Bacillus* Liq-Vtech in striped catfish diets shows a potential to increase the growth performance and feed utilization. Dietary with 1.5×10^6 cfu/g feed (T_4) *Bacillus* Liq-Vtech supplement resulted a lower FCR than other diets.

Probiotics used in diets is expected to improve fish health status, and the results of the current study have showed that a tendency to increase the *Bacillus* sp. counts, goblet cells, villi height and villi width of fish intestine.

In the bacterial challenge test, higher survival rate of striped catfish was observed in the *Bacillus* Liq-Vtech supplemented diets, especially in the treatment T_4 (1.5×10^6 cfu/g feed). These findings suggest that the *Bacillus* Liq-Vtech has a positive effect on improving fish health.

Conflict of interest

The authors (corresponding and co-authors) declare no personal conflicts of interest in the present research.

Acknowledgements

This study was made with financial support from Nong Lam University (Ref. no. CS-SV24-TS-01) and from UV Joint Stock Company.

References

AOAC (Association of Official Analytical Chemists). (1995). *Official methods of analysis* (16th ed.) Washington, USA: Association of Official Analytical Chemists.

Boyd, C. E. (2001). Water quality standards: total ammonia nitrogen. *The Advocate* 4(4), 84-85.

Bui, H. B. T., Balami, S., & Nguyen, P. T. (2022). Effect of *Lactobacillus plantarum* on growth performance, immune responses, and disease resistance of striped catfish (*Pangasianodon hypophthalmus*). *Aquaculture, Aquarium, Conservation and Legislation - International Journal of the Bioflux Society* 15(1), 174-187.

Chainark, S., & Boyd, C. E. (2010). Water and sediment quality, phytoplankton communities and channel catfish production in sodium nitrate-treated ponds. *Journal of Applied Aquaculture* 22(2), 171-185. https://doi.org/10.1080/10454431003736524.

Chang, C. I., & Liu, W. Y. (2002). An evaluation of two probiotic bacterial strains, *Enterococcus faecium* SF68, and *Bacillus toyoi*, for reducing edwardsiellosis in cultured European eel, *Anguilla anguilla* L. *Journal of Fish Diseases* 25(5), 311-315. https://doi.org/10.1046/j.1365-2761.2002.00365.x.

Dandruff, P., & Dean, L. S. (1967). Dissolved oxygen criteria for the production of fish. *Special Publications of The American Fisheries Society* 4, 13-19.

Dang, O. H. T., & Truong, P. Q. (2022). Occurrence of diseases and selected water quality parameter in culture striped catfish (*Pangasianodon hypophthalmus*) ponds in the Mekong delta of Vietnam. *Aquaculture, Aquarium, Conservation and Legislation - International Journal of The Bioflux Society* 15(3), 1333-1343.

Ferguson, H., Turnbull, J., Shinn, A., Thompson, K., Dung, T. T., & Crumlish, M. (2001). Bacillary necrosis in farmed *Pangasius hypophthalmus* (Sauvage) from the Mekong Delta, Vietnam. *Journal of Fish Diseases* 24, 509-513. https://doi.org/10.1046/j.1365-2761.2001.00308.x.

Liaqat, R., Fatima, S., Komal, W., Minahal, Q., Kanwal, Z., Suleman, M., & Carter, C. G. (2024).

Effects of *Bacillus subtilis* as a single strain probiotic on growth, disease resistance and immune response of striped catfish (*Pangasius hypophthalmus*). *Plos One* 19(1), e0294949. https://doi.org/10.1371/jounal.pone.0294949.

Lim, S. J., Jang, E., Lee, S. H., Yoo, B. H., & Kim, S. K. (2013). Antibiotic resistance in bacteria isolated from freshwater aquacultures and prediction of the persistence and toxicity of antimicrobials in the aquatic environment. *Journal of Environment Sciences and Health* 48(6), 495-504. https://doi.org/10.1080/03601234.2013.761911.

Liu, W., Ran, C., Liu, Z., Gao, Q., Xu, S., Ringo, E., Myklebust, E., Gu, Z., & Zhou, Z. (2016). Effects of dietary *Lactobacillus plantarum* and AHL lactonase on the control of *Aeromonas hydrophila* infection in tilapia. *Microbiology Open* 5(4), 687-699. https://doi.org/10.1002/mbo3.362.

Lødemel, J. B., Mayhew, T. M., Myklebust, R., Olsen, R. E., Espelid, S., & Ringø, E. (2001). Effect of three dietary oils on disease susceptibility in Arctic charr (*Salvelinus alpinus* L.) during cohabitant challenge with *Aeromonas salmonicida* ssp. *salmonicida*. *Aquaculture Research* 32(12), 935-945. https://doi.org/10.1046/j.1365-2109.20.

MARD (Ministry of Agriculture and Rural Development). (2024). An overview of Pangasius industry conference in 2023. Retrieved May 14, 2023, from https://mard.gov.vn/Pages/xuat-khau-ca-tra-uoc-dat-1-8-ty-usd-trong-nam-2023.aspx.

Nguyen, K. Q., Bruce, T. J., Afe, O. E., Liles, M. R., Beck, B. H., & Davis, D. A. (2022). Growth performance, survival, blood chemistry, and immune gene expression of channel catfish (*Ictalurus punctatus*) fed probiotic-supplemented diets. *Veterinary Sciences* 9(12), 701. https://doi.org/10.3390/vetsci9120701.

Niamphithak, P., Chareonwattanasak, S., & Doolgindachbaporn, S. (2017). Effect of dietary supplement of probiotic (*Lactobacillus plantarum*) on growth performance, feed utilization and survival rate in bocourti catfish (*Pangasius bocourti* Sauvage, 1880). *Journal of Pure and Applied Microbiology* 11(2), 803-809. http://dx.doi.org/10.22207/JPAM.11.2.19.

Pereira, R. T., Nebo, C., Paula, N. L., Fortes-Silva, R., Oliveira, I., Paulino, R. R., Drummond, C. D., & Rosa, P. V. (2020). Distribution of goblet and endocrine cells in the intestine: A comparative study in Amazonian freshwater Tambaqui and hybrid catfish. *Journal of Morphology* 281(1), 55-67. https://doi.org/10.1002/jmor.21079.

Ringø, E. (2020). Probiotics in shellfish aquaculture. *Aquaculture and Fisheries* 5(1), 1-27. https://doi.org/10.1016/j.aaf.2019.12.001.

Ringø, E., & Song, S. K. (2016). Application of dietary supplements (synbiotics and probiotics in combination with plant products and beta glucans) in aquaculture. *Aquaculture Nutrition* 22(1), 4-24. https://doi.org/10.1111/anu.12349.

Shelby, R. A., Lim, C., Yildirim-Aksoy, M., & Delaney, M. A. (2006). Effects of probiotic diet supplements on disease resistance and immune response of young Nile tilapia, *Oreochromis niloticus*. *Journal of Applied Aquaculture* 18(2), 23-34. https://doi.org/10.3390/fishes7050273.

Tu, D. T., Haesebrouck, F., Nguyen, T. A., Sorgeloos, P., Baele, M., & Decostere, A. (2008). Antimicrobial susceptibility pattern of *Edwardsiella ictaluri* isolates from natural outbreaks of bacillary necrosis of *Pangasianodon hypophthalmus* in Vietnam. *Microbial Drug Resistance* 14(4), 311-316. https://doi.org/10.1089/mdr.2008.0848.

Yazici, I. S., Hisar, O., Yilmaz, S., & Yigit, M. (2016). Effects of different probiotic bacteria on growth, body composition, immune response and hematological parameters of rainbow trout (*Oncorhynchus mykiss*) under sublethal water temperature. *Marine Science and Technology Bulletin* 4(2), 21-28.

Youssef, M. Y. I., Saleem, A. S. Y., Ahmed, F. A., Said, E. N., Abdel-Hamid, S. E., & Gharib, H. S. A. (2024). The impact of dietary probiotic supplementation on welfare and growth performance of Nile tilapia (*Oreochromis niloticus*). *Open Veterinary Journal* 14(1), 360-369. https://doi.org/10.5455/OVJ.2024.v14.i1.33.