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Effect of Dietary Supplementation with Fermented Betel Leaves (*Piper betle*) on Growth Performance, Feed Utilization, and Disease Resistance in Common Carp (*Cyprinus carpio*)

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Abstract

This study evaluated the effects of dietary supplementation with a fermented betel leaf preparation on the growth performance, feed utilization, and resistance to *Aeromonas veronii* infection in common carp (*Cyprinus carpio*). A six-week feeding trial was conducted using four diets: PF0 (control), PF2 (2 g kg^{-1}), PF5 (5 g kg^{-1}), and PF10 (10 g kg^{-1}). After the feeding period, fish were challenged with *A. veronii,* and the cumulative mortality rates were recorded over 14 days to assess disease resistance. The results showed that supplementation at 10 g kg^{-1} significantly enhanced growth performance, while the feed utilization indices did not differ among treatments. Survival during the feeding period remained 100%, indicating that the fermented preparation was safe for fish. The PF10 group exhibited the lowest cumulative mortality (22.73%), followed by PF5 (35.38%), with both values significantly lower than the control (46.67%), demonstrating the immunoprotective potential of the fermented product. Overall, the findings indicate that the dietary inclusion of fermented betel leaves, especially at 10 g kg^{-1}, can improve growth, maintain the feed conversion ratio, and enhance resistance to *A. veronii* infection in common carp, contributing to safer and more sustainable aquaculture practices.

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Keywords

Fermented herbal feed additive, common carp, disease resistance, sustainable aquaculture nutrition, *Aeromonas veronii*

Introduction

In recent years, the use of antibiotics in aquaculture for disease prevention and treatment has been increasingly restricted due to the risk of promoting antibiotic-resistant bacterial strains, as well as

concerns regarding food safety and environmental sustainability (Mohammed *et al.*, 2025). Consequently, the search for natural, environmentally friendly alternatives to antibiotics has become a major focus in the development of sustainable aquaculture practices (Far *et al*., 2024). Among these alternatives, medicinal plants and fermented herbal preparations have been recognized as promising options, owing to their ability to stimulate growth, enhance immune responses, and improve disease resistance in fish (Sumana *et al*., 2025).

Betel leaf (*Piper betle* L.) is a widely distributed plant species in Southeast Asia and is enriched in a variety of bioactive compounds, including phenolics, flavonoids, eugenol, chavicol, and essential oils, which exhibit antimicrobial, anti-inflammatory, antioxidant, and immunostimulatory properties (Anh *et al*., 2025). Recent studies have demonstrated that betel leaf extract, decoction, or powder can improve gut health, enhance digestive enzyme activity, and stimulate growth performance in several aquaculture species, such as Nile tilapia (*Oreochromis niloticus* L., 1758) (Mohtar *et al*., 2021), golden fish (Saputra *et al*., 2020), koi fish (Aslamiah *et al*., 2023), and white-leg shrimp (*Litopenaeus vannamei* Boone, 1931) (Guzman *et al*., 2021). However, many of the bioactive constituents in betel leaves are characterized by low stability and limited bioavailability within the gastrointestinal tract of aquatic animals. Microbial fermentation technology has been shown to enhance the biological efficacy of medicinal plants by degrading and biotransforming secondary metabolites, while simultaneously generating beneficial enzymes, vitamins, and organic acids that support digestion and immune function (Luo *et al*., 2024). Accordingly, the incorporation of fermented betel leaf preparations into aquafeeds may confer dual benefits: supplying natural bioactive compounds and beneficial microorganisms that improve feed utilization efficiency, and strengthening immune responses in cultured aquatic species.

Common carp (*Cyprinus carpio* L., 1758) is an economically important freshwater species that is widely cultured in Vietnam and many other Asian countries. Its broad adaptability, high market demand, and importance in small- and large-scale farming systems make common carp a key species for food security. As with many economically important aquaculture species, common carp are increasingly affected by infectious bacterial diseases. Among these, *Aeromonas veronii* has emerged as a particularly virulent pathogen and is currently regarded as a significant threat to carp health and production (Nguyen Thi Mai *et al*., 2021). Growth performance and disease resistance are key determinants influencing productivity and profitability in commercial aquaculture. However, studies investigating the effects of fermented betel leaf preparations on the growth, feed utilization efficiency, and health status in common carp remain very limited. Therefore, the present study was conducted to evaluate the effects of fermented betel leaf supplementation on the growth performance, feed utilization, and disease resistance in common carp. The findings of this study are expected to provide a scientific basis for the application of fermented herbal products in aquaculture nutrition and health management, contributing to safer and more sustainable aquaculture practices.

Materials and Methods

Experimental fish and rearing conditions

Juvenile common carp with an average body weight of approximately 15g were obtained from the Research Institute for Aquaculture No. I (RIA I), Tu Son, Bac Ninh. Fish were then acclimated for two weeks in a glass tank system at the wet laboratory of the Department of Aquaculture Nutrition and Feed, Faculty of Fisheries, Vietnam National University of Agriculture (VNUA). During the acclimation period, fish were fed a commercial pelleted diet (Nutrigo, MF41), and environmental conditions were maintained comparable to those applied during the subsequent experimental period. After acclimation, healthy fish with no visible signs of disease and of uniform size were selected for the growth performance trial.

Fermented betel leaf preparation

The fermented preparation was formulated using fresh betel leaves, corn flour, sugarcane

molasses, distilled water, and a consortium of beneficial microorganisms consisting of *Bacillus* sp., *Lactobacillus* sp., and *Saccharomyces* sp. at a ratio of 1:1:1. Fresh betel leaves were thoroughly washed, finely chopped, and homogenized using a blender, with an appropriate amount of water added to obtain a uniform slurry. The resulting mixture was then thoroughly blended with corn flour, followed by the addition of molasses and the microbial inoculum to create favorable conditions for fermentation. Fermentation was carried out under aerobic conditions at 36°C for three days (Zhou *et al*., 2022). After fermentation, the pH of the product reached a value of 6.5. Upon completion of fermentation, the product was dried at 40°C until the moisture content was reduced to below 5%. The dried material was subsequently ground into a fine powder with a light brown color and used as a dietary additive in the feeding trial.

Feeding trial

Experimental diet preparation: The fermented betel leaf preparation was mixed with fresh chicken eggs (12mL egg per 100g feed) and incorporated into the diet at inclusion levels of 0, 2, 5, and 10 g kg^{-1} feed, corresponding to the experimental diets PF0, PF2, PF5, and PF10, respectively. The mixtures were then evenly coated onto commercial pelleted feed (Nutrigo, MF41) and air-dried at room temperature. The experimental diets were prepared daily.

Experimental design and fish care: After the acclimation period, juvenile common carp were randomly distributed into glass tanks with an effective volume of 100L at a stocking density of 20 fish per tank. The tanks were randomly assigned to the respective experimental diets PF0, PF2, PF5, and PF10, with each treatment conducted in triplicate. Continuous aeration was provided throughout the experiment, and a 12 h light/12 h dark photoperiod was maintained. Fish were fed the experimental diets twice daily at 3% of their body weight during the first two weeks and 4% during the subsequent four weeks. Feed intake was monitored for 30 min after feeding, after which uneaten feed remaining on the water surface was carefully collected using a hand net, dried to a constant weight, and weighed. Fish were weighed and measured weekly to assess growth performance and to adjust feed rations accordingly. Approximately 30% of the tank water volume was renewed twice daily prior to feeding to remove fecal waste. Water quality parameters were monitored and maintained within suitable ranges for common carp culture, including temperature (27-31°C), pH (7.5-8.0), dissolved oxygen (4-5 mg L^{-1}), nitrite (NO_2^-), and total ammonia nitrogen (NH_3/NH_4^+) concentrations (below 0.5 mg L^{-1}).

Husbandry and intestinal indices: Fish were weighed at the beginning and end of the feeding trial, as well as weekly throughout the experiment, to monitor growth performance and calculate the feed conversion ratio. At the end of the feeding period, somatic indices were evaluated, namely the visceral somatic index (VSI, %), hepatosomatic index (HSI, %), intestinal-somatic index (ISI, %), and relative intestinal length (%).

The monitored parameters were calculated using the following equations:

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

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

Where, IBW and FBW are the initial and final body weights of the experimental fish; and T is the number of experiment days.

Feed conversion ratio (FCR) = Consumed feed/(Final biomass – initial biomass)

Visceral somatic index (VSI, %) = 100 × visceral weight/wet fish weight

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

Intestinal somatic index (ISI, %) = 100 × intestinal weight/wet fish weight

Relative intestine length (%) = 100 × intestine length/fish length

Bacterial challenge experiment

At the end of the feeding trial, fish were challenged with *Aeromonas veronii* isolated and preserved at the Faculty of Fisheries, Vietnam National University of Agriculture, at the LD_{50}

dose (1.58×10^8 CFU mL^{-1}, 0.1 mL $fish^{-1}$). The LD_{50} determination, bacterial preparation procedures, and verification of the actual bacterial density were conducted according to the methodologies described in a previous publication by the research group of Nguyen Thi Mai *et al*. (2021).

Data analysis

Data were analyzed using one-way analysis of variance (ANOVA) with STATISTICA software version 10.0. Differences among treatment means were assessed using the least significant difference (LSD) post-hoc test. For the growth performance parameters and survival rate, replicate tanks were considered as the experimental units (n = 3), whereas for the intestinal indices, the number of sampled fish per treatment was used as the statistical unit (n = 9). Statistical significance was accepted at $P < 0.05$.

Results and Discussion

Growth performance and survival rate

After six weeks of the feeding trial, the growth performance parameters and survival rate of the common carp were calculated and are presented in **Table 1**, showing clear differences among the treatments supplemented with fermented betel leaf at different inclusion levels.

At the end of the experimental period, the final body weight (FBW) of fish in the PF10 treatment reached the highest value (28.21 ± 0.24 g $fish^{-1}$) compared to the other treatments ($P < 0.05$). In contrast, no significant differences were observed among the PF0, PF2, and PF5 treatments, with the FBW values ranging from 25.95 to 26.63 g $fish^{-1}$. Daily weight gain (DWG), specific growth rate (SGR), and weight gain (WG) followed a similar trend, with fish in the PF10 treatment achieving values of 0.42 g $fish^{-1}$ day^{-1}, 1.93% day^{-1}, and 71.47%, respectively, which were significantly higher than those of the control group and the diets supplemented at 2 and 5 g kg^{-1} ($P < 0.05$). These results indicate that supplementation with fermented betel leaves at lower inclusion levels did not result in a marked improvement in growth performance. Notably, the survival rate was 100% across all treatments, indicating suitable rearing conditions and demonstrating that the dietary inclusion of fermented betel leaves had no adverse effects on the survival of common carp throughout the experimental period.

The pronounced improvement in the growth performance of common carp observed in the PF10 treatment may be attributed to an optimal concentration of bioactive compounds released during the fermentation of betel leaves, such as polyphenols, flavonoids, and other biologically active constituents (Ho *et al*., 2020). These compounds are known to stimulate metabolic activity, enhance gut health, and create favorable conditions for growth processes (Khayatan *et al*., 2024). As such, the bioactive compounds produced by beneficial bacteria during betel leaf fermentation may have contributed to the improved digestive capacity and growth

Table 1. Growth parameters and survival rates of experimental fish after a six-week trial

Variables	Diet			
	PF0	PF2	PF5	PF10
IBW (g $fish^{-1}$)	$16.22^a \pm 0.06$	$16.23^a \pm 0.06$	$16.30^a \pm 0.15$	$16.45^a \pm 0.13$
FBW (g $fish^{-1}$)	$26.47^a \pm 0.54$	$25.95^a \pm 0.59$	$26.63^a \pm 0.80$	$28.21^b \pm 0.24$
DWG (g $fish^{-1}$ day^{-1})	$0.37^a \pm 0.02$	$0.35^a \pm 0.02$	$0.37^a \pm 0.03$	$0.42^b \pm 0.01$
SGR (% day^{-1})	$1.75^a \pm 0.08$	$1.68^a \pm 0.09$	$1.75^a \pm 0.13$	$1.93^b \pm 0.03$
WG (%)	$63.26^a \pm 3.83$	$59.88^a \pm 4.17$	$63.41^a \pm 6.14$	$71.47^b \pm 1.23$
Survival rate (%)	100	100	100	100

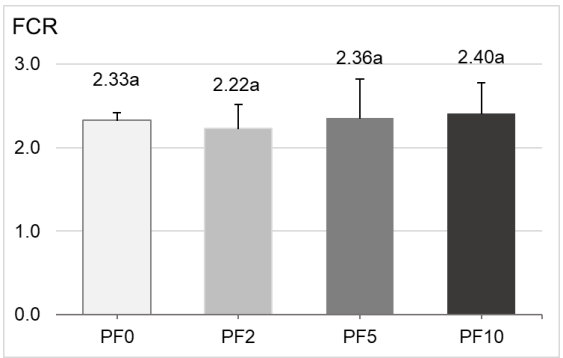
Note: PF0, PF2, PF5, and PF10: experimental diets supplemented with fermented Piper betle *leaves at 0, 2, 5, and 10 g kg^{-1} feed, respectively. IBW and FBW: initial and final body weight; DWG: daily weight gain; SGR: specific growth rate; WG: weight gain. The data are presented as mean ± SD. Data with the same superscript letter represent the non-significant differences (P >0.05).*

performance in the experimental fish. Similar effects were reported in previous studies on common carp fed fermented herbal supplements, in which fermented formulations resulted in higher growth compared to the control group (Zou *et al*., 2025). The absence of significant growth differences at lower supplementation levels suggests that the efficacy of the fermented preparation is dose-dependent. The present findings are consistent with previous studies demonstrating that fermented herbal products can promote fish growth by improving the intestinal microbial environment and enhancing physiological functions (Chen *et al*., 2023; Siddik *et al*., 2024). Betel leaves are recognized as rich sources of phenolic compounds and bioactive essential oils, whose availability and biological activity are further enhanced through microbial fermentation. The achievement of a 100% survival rate across all treatments further confirms the safety of the fermented betel leaf preparation when included in the diet of common carp.

Feed utilization

The feed conversion ratios (FCR) of common carp after six weeks of the feeding trial are presented in **Figure 1**. The FCR values among the treatments ranged from 2.22 to 2.40 and did not differ significantly (P >0.05). This indicates that supplementation with the fermented betel leaf preparation at inclusion levels of 2, 5, and 10 g kg^{-1} diet did not exert a pronounced effect on the feed utilization efficiency of common carp under the present experimental conditions. Although the PF2 treatment tended to exhibit a lower FCR compared with the other treatments, this difference was not sufficient to reach statistical significance.

The absence of significant differences in the FCR among the treatments may be attributed to the digestive physiology of common carp, which is well adapted to efficiently utilize commercial diets under intensive culture conditions. In addition, the effects of fermented betel leaf supplementation may be more strongly associated with improvements in growth performance and the overall physiological status rather than directly enhancing the feed conversion efficiency. While microbial fermentation facilitates the release and biotransformation of beneficial bioactive compounds, the magnitude and duration of their effects may not have been sufficient to induce a clear improvement in the FCR within the experimental period. Moreover, FCR is influenced by multiple interacting factors, including diet composition, feeding regime, environmental conditions, and stocking density, which may obscure the individual effects of herbal additives. These findings are consistent with previous studies reporting that dietary supplementation with herbal products or fermented herbs does not always result in reduced FCR (Nguyen Thi Mai et al., 2025). In the case of betel leaves, phenolic compounds and essential oils primarily exert antimicrobial activity and modulate gut microbiota, thereby indirectly supporting growth rather than directly improving the feed conversion efficiency.



Note: PF0, PF2, PF5, and PF10: experimental diets supplemented with fermented Piper betle leaves at 0, 2, 5, and 10 g kg^{-1} feed, respectively. The data are presented as mean ± SD. Data with the same superscript letter represent the non-significant differences (P >0.05).

Figure 1. Feed conversion ratio of fish fed on fermented betel leaves for 6 weeks

Therefore, the lack of significant differences in the FCR observed in the present study is reasonable and does not diminish the practical applicability of fermented betel leaf preparations in common carp nutrition.

Somatic indices

The results presented in **Table 2** indicate that dietary supplementation with fermented betel leaves at inclusion levels of 2, 5, and 10 g kg^{-1} feed did not significantly affect the somatic indices of common carp after six weeks of feeding. The relative somatic index (VSI) ranged from 6.0 to 6.5%, the hepatosomatic index (HSI) from 1.5 to 1.8%, and the intestinal somatic index (ISI) from 3.4 to 3.9%, with no significant differences observed among the treatments ($P > 0.05$). Similarly, the relative intestinal length varied between 127.7 and 144.5% and did not differ significantly among the experimental groups. These results suggest that supplementation with fermented betel leaves at the tested levels does not exert adverse effects on the morphological development of the digestive organs or liver in common carp.

The absence of significant changes in the somatic indices indicates that the fermented betel leaf preparation is biologically safe for common carp and does not induce hepatic hypertrophy or abnormalities in the development of the digestive system. This finding is consistent with previous studies reporting that many herbal additives and fermented herbal products, when applied at appropriate inclusion levels, do not alter organ structure or relative organ indices, while still enhancing the growth performance and immune responses in fish (Zhou *et al.*, 2022). Several studies have shown that the use of unprocessed herbs or herbs administered at excessive doses may exert adverse effects on the digestive system and hepato-intestinal function in fish, as reflected by increased HSI values, intestinal hypertrophy, or intestinal epithelial damage due to the presence of anti-nutritional compounds such as tannins, saponins, and alkaloids (Banaee *et al.*, 2025). These compounds can reduce digestive enzyme activity, irritate the intestinal mucosa, and disrupt nutrient absorption. However, herbal fermentation has been demonstrated to effectively degrade or substantially reduce anti-nutritional factors, while simultaneously generating beneficial metabolites such as organic acids, enzymes, and bioactive compounds that promote gut health (Luo *et al.*, 2024). A previous study even reported significant improvements in gut health in common carp fed diets supplemented with a fermented herbal product (Han *et al.*, 2025). In the present study, the lack of significant differences in the somatic indices among treatments suggests that fermented betel leaf supplementation does not exert negative effects on the digestive system of common carp, thereby confirming the role of fermentation in enhancing both the safety and biological efficacy of herbal feed additives.

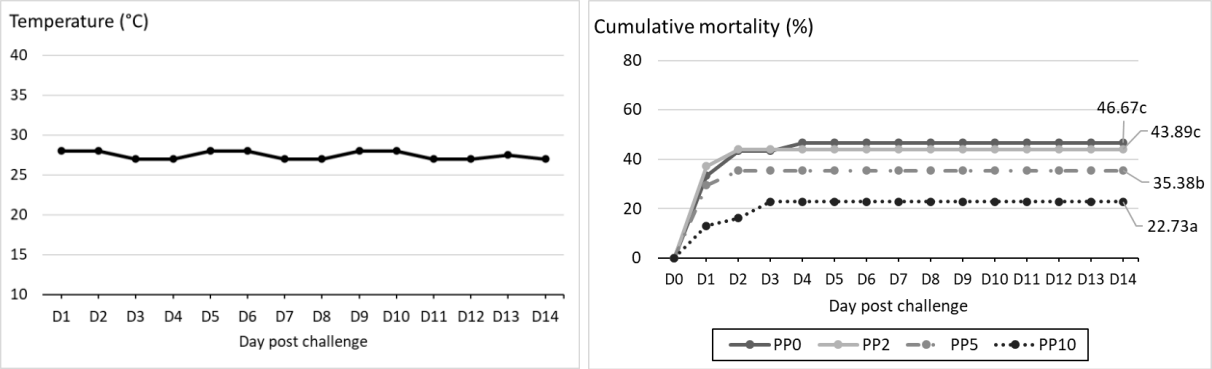
Fish mortality in the infected experiment

During the 14-day post-challenge period with *Aeromonas veronii*, water temperature in all the experimental tanks ranged from 27 to 28°C (**Figure 2**), which is suitable for common carp as

Table 2. Visceral indices of common carp after six weeks of feeding on diets supplemented with fermented betel leaves

Parameters	Experimental diets			
	PF0	PF2	PF5	PF10
VSI (%)	$6.1^{a} \pm 0.5$	$6.5^{a} \pm 0.7$	$6.0^{a} \pm 0.4$	$6.4^{a} \pm 0.8$
HSI (%)	$1.5^{a} \pm 0.3$	$1.8^{a} \pm 0.3$	$1.6^{a} \pm 0.4$	$1.7^{a} \pm 0.2$
ISI (%)	$3.4^{a} \pm 0.3$	$3.6^{a} \pm 0.3$	$3.5^{a} \pm 0.2$	$3.9^{a} \pm 0.5$
Relative intestine length (%)	$136.9^{a} \pm 12.5$	$143.6^{a} \pm 13.1$	$127.7^{a} \pm 14.3$	$144.5^{a} \pm 13.1$

Note: PF0, PF2, PF5, and PF10: experimental diets supplemented with fermented Piper betle leaves at 0, 2, 5, and 10 g/kg feed, respectively. VSI: visceral somatic index, HSI: hepato-somatic index, ISI: intestinal-somatic index. The data are presented as mean ± SD. Data with the same superscript letter represent the non-significant differences (P >0.05).



Note: PF0, PF2, PF5, and PF10: experimental diets supplemented with fermented Piper betle leaves at 0, 2, 5, and 10 g kg^{-1} feed, respectively. The data are presented as mean ± SD. Data with the same superscript letter represent the non-significant differences (P >0.05).

Figure 2. Variation in water temperature and cumulative mortality of common carp in the experimental treatments during the 14 days after bacterial challenge with *Aeromonas veronii*

well as the infected pathogenic bacteria. Cumulative mortality of the common carp in all treatments increased rapidly during the first three days after the bacterial challenge and then stabilized from day 4 to day 14. The control group without fermented betel leaf supplementation (PF0) exhibited the highest cumulative mortality, reaching 46.67%, which was comparable to that observed in the PF2 group (43.89%). This was followed by the PF5 group, which showed a lower mortality rate of 35.38% compared with the control. Notably, the PF10 group demonstrated the highest disease resistance, with the lowest cumulative mortality of only 22.73%. These results indicate that dietary supplementation with fermented betel leaf preparations, particularly at an inclusion level of 10 g kg^{-1} feed, significantly enhances the survival capacity of common carp following infection with *A. veronii*.

The lack of a significant difference in mortality between the PF0 and PF2 groups indicates that supplementation at 2 g kg^{-1} of the fermented betel leaf preparation did not reach the biological threshold required to fully activate the defensive mechanisms of common carp against A. veronii infection. At higher inclusion levels (PF5 and PF10), mortality was markedly reduced, highlighting the positive role of fermented betel leaves in enhancing disease resistance. Betel leaves are rich in phenolic compounds and essential oils such as eugenol and chavicol, which have been demonstrated to inhibit Aeromonas spp., while also mitigating oxidative stress and inflammatory responses in the host (Aslamiah *et al.*, 2023; Dewi *et al.*, 2025). In addition, microbial fermentation of herbal materials disrupts plant cell wall structures, thereby increasing the release and bioavailability of bioactive compounds, while simultaneously generating organic acids and enzymes that are beneficial to fish digestion (Hussain *et al.*, 2016). According to Syawal *et al.* (2022), diets supplemented with fermented products can improve the gut microbial balance and stimulate non-specific immune responses, including lysozyme activity, phagocytic capacity, and antioxidant defenses, ultimately enhancing resistance to pathogenic infections. The lowest mortality observed in the PF10 group suggests that a supplementation level of 10 g kg^{-1} feed is optimal under the present experimental conditions, further confirming the potential of the fermented betel leaf preparation as a safe biological additive and a promising alternative to antibiotics in intensive common carp culture.

Common carp challenged with *Aeromonas veronii* exhibited clear pathological signs compared with the unchallenged control fish (**Figure 3**). Externally, infected fish showed lethargic behavior, reduced responsiveness, hemorrhaging at the base of the fins and in the abdominal region, and abdominal distension in some individuals. Upon necropsy, the internal organs of challenged fish displayed pronounced congestion and hemorrhaging; the liver appeared pale and enlarged, while the kidney and spleen were hypertrophied, and abnormal fluid



Figure 3. Comparison of external clinical signs and gross pathology between control fish (upper panel) and infected fish (lower panel), and histological staining of kidney tissue from fish challenged with *Aeromonas veronii*

accumulation was observed in the abdominal cavity. Microscopic examination of Gram-stained kidney tissue from challenged fish revealed the presence of rod-shaped, Gram-negative bacteria, characterized by pink to red staining. These pathological and histological features are consistent with descriptions reported in previous studies on *A. veronii* infection in fish (Nguyen Thi Mai *et al*., 2021).

Conclusions

The current study demonstrated that dietary supplementation with fermented betel leaves at a level of 10 g kg^{-1} feed resulted in improved growth performance and enhanced disease resistance in common carp. These beneficial effects were achieved without adverse impacts on the feed utilization efficiency, visceral indices, or morphology of the digestive system, indicating good tolerance of the supplemented diet. The observed improvements suggest that fermented betel leaf supplementation can positively influence fish performance and health under experimental conditions, supporting its potential role as a functional feed additive in common carp culture. However, the present findings are primarily based on growth responses and survival following bacterial challenge. Further studies are therefore warranted to elucidate the underlying physiological and immunological mechanisms, to assess the long-term effects under practical farming conditions, and to evaluate the optimal inclusion levels. Such investigations will be essential to confirm the consistency and applicability of fermented betel leaves as a sustainable alternative to antibiotics in common carp farming.

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