2026 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 16, Issue 1: 01-09; January 30, 2026

ISSN 2228-7701

DOI: https://dx.doi.org/10.51227/ojafr.2026.1

EFFECT OF SUPPLEMENTED *Cordyceps militaris* EXTRACT ON DISEASE RESISTANCE AND SURVIVAL RATE OF STRIPED CATFISH (*Pangasius hypophthalmus*) FINGERLINGS

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Supporting Information

RESEARCH ARTICLE
PII: S222877012600001-16
Received: November 25, 2025
Revised: January 20, 2026
Accepted: January 22, 2026

ABSTRACT: The effects of supplemented *Cordyceps militaris* extract in diets has been studied on the survival and immune response of striped catfish (*Pangasius hypophthalmus*) fingerlings with bacterial challenge conditions. The experiment was conducted for 60 days with six treatments and three replicates, including negative and positive controls with four dietary supplementation levels of *C. militaris* extract (0.18%, 0.24%, 0.36%, and 0.47%). After the feeding trial, striped catfish were challenged with *Edwardsiella ictaluri* at a dose of 10^5 CFU/mL. Results showed that survival rates of striped catfish increased significantly with higher extract levels, reaching the highest value (70%) at 0.47% supplementation compared with only 16.67% in the positive control group. Hematological indices such as red blood cell (RBC) and white blood cell (WBC) including lymphocytes, monocytes, and neutrophils increased proportionally with extract concentration, which can indicate enhanced immune responses. In conclusion, the findings suggest that dietary supplementation of *C. militaris* extract at 0.47% improves the disease resistance and health status of striped catfish fingerlings against *E. ictaluri* infection. This result highlights the potential application of *C. militaris* extract as a natural immunostimulants to enhance survival and reduce antibiotic use in catfish aquaculture.

Keywords: *Cordyceps militaris*, *Edwardsiella ictaluri*, Immune response, *Pangasius hypophthalmus*, Survival rate.

INTRODUCTION

Cordyceps species are parasitic fungi that grow on insect larvae, pupae, or adult forms. The most well-known species, *Cordyceps sinensis*, was first identified in the high-altitude regions of the Tibetan Plateau, parasitizing larvae of moths (*Thitarodes* spp.) (Lo et al., 2013; Sheng and Gerasimova, 2024; Kumar and Sharma, 2025). Another related species, *Cordyceps militaris*, also parasitizes insects of the same genus and has attracted great attention for its rich bioactive compounds, particularly cordycepin and adenosine (Lo et al., 2013; Park, 2025; Jędrejko et al., 2021).

There is substantial evidence showing that *Cordyceps* fungi possess medicinal properties and contain a variety of bioactive compounds with therapeutic potential (Das et al., 2021). These compounds have been used in traditional medicine for treating malaria, palpitations, cancer, fever, diabetes, chronic kidney disease, and other disorders (Sharma et al., 2023). Beyond human medicine, the application of *Cordyceps militaris* as a dietary supplement has been extended to terrestrial animals such as pigs (Li et al., 2024; Cheng et al., 2016; Boontiam et al., 2020) and chickens (Wang et al., 2025; Barido et al., 2024; Lan et al., 2021), where it has shown positive effects on immune response and health status.

In aquaculture, *Cordyceps* supplementation has been investigated for several aquatic species (Tian et al., 2025; Sun et al., 2021; Lan et al., 2025; Da-oh et al., 2024; Choosong et al., 2025; Van Doan et al., 2018). Van Doan et al. (2017a); Van Doan et al. (2017b) demonstrated that adding powdered *C. militaris* at 10 g/kg feed improved the growth and health of Nile tilapia (*Oreochromis niloticus*). Similarly, Vietnamese aquaculture practitioners have reported beneficial effects of *Cordyceps* supplementation on shrimp hatchery performance, including improved post-larvae immunity, reduced stress in broodstock, and enhanced larval survival (Deng et al., 2015).

The striped catfish (*Pangasius hypophthalmus*) is one of Vietnam's most economically important aquaculture species, particularly in the Mekong Delta. However, its production faces major challenges from bacterial diseases such as bacillary necrosis of the kidney and liver (BNKL), caused by *Edwardsiella ictaluri*. This disease can lead to substantial mortality in nursery and grow-out stages, threatening farm productivity (Hoa et al., 2021; Tran et al., 2025). Therefore, the search for safe and natural immunostimulants to enhance disease resistance is critical for sustainable production.

Citation: Lan T.T., Hang N.T.T., Hanh N.T.B., Mai V.P., Vy P.N.T., Nhi N.H.Y. (2026). Effect of supplemented *Cordyceps militaris* extract on disease resistance and survival rate of striped catfish (*Pangasius hypophthalmus*) fingerlings. *Online J. Anim. Feed Res.*, 16(1): 01-09. DOI: https://dx.doi.org/10.51227/ojafr.2026.1

Given the proven bioactivity of *Cordyceps militaris* in promoting immunity and growth in terrestrial animals, this study aimed to evaluate the effects of dietary supplementation with *C. militaris* extract on the survival and immune responses of striped catfish fingerlings challenged with *Edwardsiella ictaluri*. The findings are expected to provide scientific evidence for the application of *Cordyceps* extracts as natural immunostimulants in catfish aquaculture.

MATERIALS AND METHODS

Experimental design

The experiment was conducted following a completely randomized design with six treatments and three replicates per treatment. Two control groups were used:

- Negative control: fish fed a commercial diet without extract and not challenged.
- Positive control: fish fed a commercial diet without extract and challenged with *E. ictaluri*.

Four other treatments included diets supplemented with *C. militaris* extract at 0.18%, 0.24%, 0.36%, and 0.47%, corresponding to 1.8 g, 2.4 g, 3.6 g, and 4.7 g extract per kg feed. Fish were reared in 500-L composite tanks at a density of 200 fish per tank for 60 days.

Feed preparation and feeding management

The *C. militaris* extract was dissolved in 100 mL distilled water and sprayed onto the feed to achieve the desired inclusion level for each treatment. The feed was then mixed thoroughly, air-dried at 60°C for 6 hours (mixed every 2 hours), and stored at −20°C until use. Fish were fed ad-libitum twice daily (07:30 and 17:00).

Bacterial challenge

After 60 days of feeding, fish were challenged with *Edwardsiella ictaluri* (isolated in An Giang University) by immersion in a 500 mL bacterial suspension at a dose of LD_{50} 10^5 CFU/mL (Hang et al., 2013; Hang and Phuong, 2020) for 30 minutes (except the negative control). After challenge exposure, fish were returned to their respective tanks for 14 days of observation. Mortality was recorded daily, and moribund fish were collected for bacterial re-isolation to confirm infection.

Hematological analysis

Ten fish per tank were randomly sampled before infection (0 h), 48 h post-infection, and 14 days post-infection for hematological analysis. Red blood cells (RBC), white blood cells (WBC), lymphocytes, monocytes, and neutrophils were counted using standard hemocytometer techniques following methods by Hrubec et al. (2000); Dung (2010) and Hang et al. (2025a).

Statistical analysis

Data were analyzed using the General Linear Model (GLM) in Minitab 16.0 (Minitab, 2010). One-way ANOVA was applied to compare mean differences among treatments, followed by Duncan's multiple range test at a significance level of $P < 0.05$. Results were expressed as mean ± standard error (SE).

RESULTS AND DISCUSSION

Survival rate

The survival rate of fingerlings significantly increased with higher levels of *C. militaris* extract ($P < 0.05$) (Table 1). The survival rate of *Pangasius* fingerlings was increased correspondingly with the increase in the rate of supplementing cordyceps extract. The highest survival (70%) was observed at 0.47% extract treatment, compared to 16.67% in the positive control treatment. Meanwhile, in the negative control treatment, the survival rate reached 100%, because the infection method and the setup conditions did not affect the experimental results. This proves that the diseased and dead fish in the remaining experiments were caused by *E. ictaluri* bacteria. Therefore, it can be concluded that 0.47% *Cordyceps* extract can be used to supplement the diet of *Pangasius* fry to help increase the survival rate when there is pathogenic *E. ictaluri* bacteria. The improvement in survival indicates that *C. militaris* extract enhances the host's resistance against *E. ictaluri*. Similar immunoprotective effects have been observed with inulin (Hang and Phuong, 2020) and *Lactobacillus plantarum* supplementation (Hang et al., 2022).

Research on *Cordyceps* extract on aquatic animals is still very new and can be said to be the first research at An Giang University and the Mekong Delta region. The results have shown that *Cordyceps* extract has great potential in the application to prevent diseases in *Pangasius* fry today. Compared with other studies on the effects of herbal extracts, probiotics, prebiotics or other immune stimulants, *Cordyceps* extract also has similar potential.

Many studies have demonstrated the effectiveness of supplementing beneficial biological substances and microorganisms to the diet to enhance resistance and reduce mortality in fish species when infected with bacteria.

Specifically, Adeshina et al. (2024) reported that supplementing alpha-lipoic acid at a dose of 1.5 to 2 g/kg of feed to the diet of catfish infected with *Edwardsiella tarda* significantly reduced mortality, to only 3.3% compared to 86.7% in the control group. In addition, the use of *Lactobacillus plantarum* at a density of 10^{12} CFU/kg of feed also showed positive effects, helping fish recover health after infection and achieving a survival rate of 56.7%.

Meanwhile, tests on *Pangasius* showed that inulin is a potential additive in improving growth and immunity. According to Hang and Phuong (2020) supplementing 1% inulin every two weeks helps fish grow well, while improving resistance to *Edwardsiella ictaluri* bacteria. Another study by Kattakdad et al. (2025) reported that supplementing 0.8% inulin in an effective functional feed additive for enhancing growth, immune defense, and tissue integrity in striped catfish culture. In addition, the survival rate of *Pangasius* fingerlings in this study was lower than the results of the trial of supplementing EPS to the diet of whiteleg shrimp (97.3%) and swamp eel fingerlings (96.67%) (Thanh et al., 2025; Hang et al., 2025a). Similarly, the study added 0.2% MOS and 0.6% MOS to the diet of carp (98.08%, 83.3%) (Staykov et al., 2005; Culjak et al., 2006).

Compared with the above results, the survival rate of *Pangasius* fingerlings in the present study reached 70%. This shows the obvious effectiveness of increasing the survival rate of the method of supplementing *Cordyceps sinensis* extract into the diet of *Pangasius* fingerlings. At the same time, it opens up new potential for practical application in *Pangasius* fingerlings.

Table 1 - The survival rate of catfish.

Treatments	Survival rate (%)
Negative control	100.00[a]
Positive control	16.67[d]
0.18%	56.67[c]
0.24%	60.00[c]
0.36%	60.00[c]
0.47%	70.00[b]
SEM	1.92
p-Value	0.000

[a,b,c,d]Different letters following the mean values in the same column are statistically different ($p<0.05$)

Red blood cells (RBC)

RBC counts showed no significant differences among treatments before infection with *Edwardsiella ictaluri* ($P > 0.05$). However, 48 hours and 14 days post-challenge, RBC counts were significantly higher in all extract-supplemented groups compared to the positive control, suggesting that *C. militaris* mitigated infection-induced anemia. The RBC concentration in fish fed 0.47% extract ($2.35–2.38 \times 10^6$ cells/mm^3) was consistent with healthy catfish values reported by Dung (2010). The elevation in RBCs indicates better oxygen transport and physiological status, consistent with reports by Wilhelm Filho et al. (1992) and Hang and Hoa (2020).

Table 2 - Red blood cells of *Pangasius* before and after infection with *Edwardsiella ictaluri* bacteria (10^6 cell /mm^3)

Treatments	RBC0H	RBC48H	RBC14D
Negative control	2.00	1.96[b]	1.95[a]
Positive control	2.27	1.16[b]	1.32[b]
0.18%	2.29	2.24[a]	2.28[a]
0.24%	2.29	2.19[a]	2.24[a]
0.36%	2.30	2.15[a]	2.27[a]
0.47%	2.31	2.35[a]	2.38[a]
SEM	0.08	0.08	0.12
P-value	0.135	0.000	0.001

RBC0H: red blood cell before infection; RBC48H: red blood cell after infection 48 hours; RBC14D: red blood cell after infection 14 days; [a,b,c,d]Different letters following the mean values in the same column are statistically different ($p<0.05$).

Red blood cell (RBC) counts showed no significant differences among treatments before infection with *Edwardsiella ictaluri* ($P > 0.05$). This also shows that the red blood cell density before infection did not affect the experimental results. However, 48 hours and 14 days post-challenge with bacteria causing pyogenic liver and kidney disease, the red blood cell density results fluctuated and were different between the treatments, especially the positive control treatment ($P < 0.05$).

After 48 hours and 14 days of infection, the positive control treatment had the lowest red blood cell density, respectively 1.16 x 10^6 cells /mm^3 and 1.32 x 10^6 cells /mm^3, which was different from the remaining treatments. In particular, the experiments supplemented with *Cordyceps sinensis* extract showed that the red blood cell density did not differ between the treatments, but at the highest supplement concentration of 0.47%, the red blood cell density reached the highest level of 2.35 x 10^6 cells/mm^3 and 2.38 x 10^6 cells/mm^3. RBC counts were significantly higher in all extract-supplemented groups compared to the positive control, the red blood cell density increased correspondingly with the concentration of *Cordyceps sinensis* extract, suggesting that *C. militaris* mitigated infection-induced anemia. The RBC concentration in fish fed 0.47% extract (2.35–2.38 × 10^6 cells/mm^3) was consistent with healthy catfish values reported by Dung (2010).

Red blood cells play a very important role in the metabolism of fish, transporting oxygen and nutrients to nourish cells in the body and eliminating CO_2 from the fish body (Tu, 2000). The elevation in RBCs indicates better oxygen transport and physiological status, consistent with reports by Wilhelm Filho et al. (1992) and Hang and Hoa (2020). According to Wilhelm Filho et al. (1992), the red blood cell density of bony fish is determined to be 2.289 x 10^6 cells/mm^3. Meanwhile, according to the research results of Hii et al. (2007) on freshwater eels (*Monopterus albus*), the red blood cell density is only about 1.05 - 1.10 x 10^6 cells/mm^3. For healthy catfish, the red blood cell density is determined to be 1.79-2.75 x 10^6 cells/mm^3 (Galagarza et al., 2017). At the same time, the number of red blood cells in the blood of freshwater fish varies greatly, depending on the stage of development, physiological status and activity of the fish, ranging from 1 - 3.5 million cells/mm^3 (Tu, 2000).

Compared with other studies on supplementing products that help stimulate fish immunity, the results of red blood cell density in this study were also higher and still within the appropriate range. When *Pangasius* had white liver and white gill disease, the red blood cell density decreased to only 0.1 x 10^6 cells/mm^3 (Hrubec et al., 2000). Compared with the study of adding 1% inulin to *Pangasius* feed, the red blood cell density only reached 1.99 x 10^6 cells/mm^3 (Hang and Phuong, 2020). Supplementation vitamin C in feed on the hematology parameters of Mekong Giant Catfish (*Pangasianodon gigas*) got 2.34 - 2.64 x 10^6 cells/mm^3 (Pimpimol et al., 2012). Or the study of adding 3% pomegranate extract to *Pangasius* feed, the red blood cell density reached 2.99 x 10^6 cells/mm^3 (Hang and Hoa, 2020), which is equivalent to the results of adding cordyceps extract in this result. However, the red blood cell density when supplementing *Cordyceps sinensis* extract was lower than the study of supplementing probiotic *Lactobacillus acidophilus* on catfish fry at 3.10 x 10^6 cells/mm^3. At the same time, it was lower than the research results of Hang et al. (2025b) when adding 8 g/kg EPS to the feed of swamp eel fingerling at 3.03 x 10^6 cells/mm^3. Besides, the red blood cell results in this study were also much lower than the study of Hu et al. (2020) when adding vitamin C to the diet of eel, the red blood cell density reached 7.6 x 10^{12} cells/mm^3.

White blood cells (WBC) and immune indices

White blood cells (WBC)

WBC counts increased significantly with extract supplementation after infection ($P < 0.05$). Fish fed 0.47% extract recorded WBC counts of 201.43 × 10^3 cells/mm^3 at 48 hours and 644.35 × 10^3 cells/mm^3 at 14 days, the highest among all treatments (Table 3).

Table 3 - White blood cells of *Pangasius* before and after infection with *Edwardsiella ictaluri* bacteria (10^3 cell /mm^3).

Treatments	WBC0H	WBC48H	WBC14D
Negative control	68.32^{c}	67.96^{c}	78.32^{c}
Positive control	86.29^{bc}	65.33^{c}	100.48^{c}
0.18%	91.99^{ab}	141.83^{b}	303.57^{b}
0.24%	90.92^{ab}	136.67^{b}	369.19^{b}
0.36%	94.15^{ab}	152.34^{b}	525.46^{a}
0.47%	106.89^{a}	201.43^{a}	644.35^{a}
SEM	3.89	5.72	27.26
P-value	0.0001	0.0001	0.0001

WBC0H: white blood cell before infection; WBC48H: white blood cell after infection 48 hours; WBC14D: white blood cell after infection 14 days; ^{a,b,c,d}Different letters following the mean values in the same column are statistically different ($p<0.05$).

Similar to the results of red blood cell density, white blood cell density also fluctuated when infected with bacteria causing pyogenic liver and kidney disease after 48 hours and 14 days. The results showed that the positive control experiment had the lowest white blood cell density and was different from the remaining experiments ($P<0.05$). Meanwhile, the experiments supplemented with *Cordyceps* extract tended to increase gradually according to the concentration of the extract, so at a concentration of 0.48%, it reached the highest level after 48 hours and 14 days of bacterial infection, respectively (201.43 x 10^3 cells/mm^3 and 644.35 x 10^3 cells/mm^3). This result shows that adding *Cordyceps* extract to the diet of *Pangasius* fish at a concentration of 0.48% after only 48 hours of exposure to the pathogen has been effective in preventing the disease. The function of white blood cells in fish blood is to destroy pathogens that attack the fish body and the number of white blood cells in fish blood is 10-100 times smaller than the number of red blood cells (Tu, 2000). According to Dung (2010), the density of white blood cells in the blood of healthy catfish is determined to be 1.0 x 10^6 cells/mm³. But when catfish have white liver and white gill disease, the white blood cell density is only 0.01 x 10^3 cells/mm³ (Hrubec et al., 2000). Compared with the results of the study on supplementing probiotic *Lactobacillus acidophilus* into the catfish diet, the white blood cell density is only 25 x 10^3 cells/mm³ (Al-Dohail et al., 2009), much lower than the addition of 0.48% Cordyceps extract in this study. But it is much lower than the result of adding 68.60 mg/kg of vitamin to the feed of freshwater eels, reaching 16.68 x 10^{10} cells/mm³ (Hu et al., 2020). In addition, the result of leukocyte density when adding 0.48% of cordyceps extract is also much lower than the result of the study adding 8 g/kg EPS to the diet of freshwater eels, reaching 1.4 x10^6 cells/mm³ (Hang et al., 2025b). At the same time, it is also lower than the study of adding 1% inulin, reaching 232.45 x10^3 cells/mm³ (Zhou et al., 2020). At the same time, the results of determining the white blood cell density of this study were also higher than the results of the study adding 3% pomegranate extract to *Pangasius* feed (Hang and Hoa, 2020) or the study adding 1.2% inulin in diets of striped catfish (Kattakdad et al., 2025). The results recorded in this study show that 0.48% of cordyceps extract can be used to supplement the diet of *Pangasius* to bring high disease prevention efficiency.

Immune indices

Lymphocytes, monocytes, and neutrophils followed similar trends of WBC, indicating enhanced cellular immunity (Table 4). The increase in lymphocytes implies a stronger adaptive immune response (Houston, 1997; Groff and Zinkl, 1999); , while elevated monocyte and neutrophil counts reflect activation of innate defenses (Yano, 1992; Magnadóttir, 2006). Comparable immune enhancements were reported in fish fed β-glucan (Hoang et al., 2024) and *Eclipta alba* extract (Harikrishnan et al., 2009).

Table 4 - Immune indices (lymphocytes, monocytes, and neutrophils) of *Pangasius* before and after infection with *Edwardsiella ictaluri* bacteria (10^3 cell /mm³)

Immune indices \ Treatments	Negative control	Positive control	0.18%	0.24%	0.36%	0.47%	SEM	P-value
Lymphocytes								
Lym0H	6.62^d	4.75^d	11.00^{cd}	23.9^{bc}	33.14^b	53.50^a	2.90	0.0001
Lym48H	8.89^d	8.61^d	32.50^c	36.29^c	53.53^b	81.95^a	3.17	0.0001
Lym14D	16.64^d	16.03^d	84.07^{cd}	117.40^c	223.60^b	315.50^a	18.61	0.0001
Monocytes								
Mono0H	6.45^{bc}	5.90^c	10.52^{bc}	10.21^{bc}	12.78^b	21.80^a	1.37	0.0001
Mono48H	8.74^c	7.33^c	16.96^b	17.79^b	21.11^b	33.44^a	1.40	0.0001
Mono14D	18.96^d	18.70^d	98.08^{cd}	136.97^c	260.87^b	368.08^a	21.71	0.0001
Neutrophils								
Neu0H	10.92^c	14.21^c	20.22^c	20.23^c	48.89^b	68.79^a	3.97	0.0001
Neu48H	12.93^d	9.65^d	36.40^c	40.64^c	59.96^b	91.79^a	3.53	0.0001
Neu14D	23.46^d	21.38^d	112.10^{cd}	156.50^c	298.10^b	420.70^a	24.81	0.0001

Lym0H: Lymphocytes before infection; Lym48H: Lymphocytes after infection 48 hours; Lym14D: Lymphocytes after infection 14 days; Mono0H: Monocytes before infection; Mono48H: Monocytes after infection 48 hours; Mono14D: Monocytes after infection 14 days; Neu0H: Neutrophils before infection; Neu48H: Neutrophils after infection 48 hours; Neu14D: Neutrophils after infection 14 days; [a,b,c,d] Different letters following the mean values in the same row are statistically different ($p<0.05$).

Lymphocytes play an essential role in the immune defense mechanism of fish, especially in recognizing and destroying pathogens such as bacteria and parasites. They are one of the key cell types involved in specific immunity, contributing to stimulating macrophages to produce antibodies and enhancing the overall immune response (Houston, 1997). In some fish species, lymphocytes account for a high proportion of the total leukocytes, up to 85%, indicating their prominent role in the immune system of fish (Groff and Zinkl, 1999). The increase in the number of lymphocytes may be related to the ability to adapt and better respond to adverse environmental conditions. In addition, the increase in the number of lymphocytes after infection is a normal physiological phenomenon in fish species, demonstrating the core role of this cell in the specific immune mechanism (Magnadóttir, 2006).

The results from Table 4 show that the number of lymphocytes fluctuated in a significant increasing trend corresponding to the concentration of *Cordyceps* extract supplementation and gradually increased over time. In the positive control group, the number of lymphocytes remained low at 48 hours and 14 days after infection. In contrast, the treatment supplemented with 0.47% Cordyceps extract gave outstandingly high results, especially at the time after 12 days of infection, reaching the highest level of 315.5 × 10^3 cells/mm^3, proving that this concentration strongly stimulates the immune system and has a high disease prevention effect.

For healthy *Pangasius* , the number of lymphocytes is 81 x 10^3 cells/mm^3, but in diseased *Pangasius* with white liver and white gills, the number was only 0.25 x 10^3 cells/mm^3 (Dung, 2010). This result shows that the number of lymphocytes in healthy *Pangasius* fish will be equivalent to the number of lymphocytes in *Pangasius* fish supplemented with 0.47% cordyceps extract after only 48 hours of infection with bacteria causing pyogenic liver and kidney disease. However, the lymphocyte results in this study were much lower than the study of supplementing 8 g/kg EPS to the diet of young eels, reaching 2.5 x 10^6 cells/mm^3. In addition, Oligochitosan (COS) and oligo-β-glucan (βOG) show strong potential as immunostimulants and growth-enhancing additives for *Pangasianodon hypophthalamus*, as they are capable of stimulating innate immune parameters, improving disease resistance, and promoting growth performance in aquaculture systems (Nguyen et al., 2017). At the same time, the results of this study are also consistent with the results of (Rattanachaikunsopon and Phumkhachorn, 2009), when using *Andrographis paniculata* extract on tilapia (*Oreochromis niloticus*), also showed a significant increase in the number of lymphocytes in the blood of the fish. Similarly, the study by (Harikrishnan et al., 2009) on *Sparus aurata* fish when using *Eclipta alba* plant extract showed a sharp increase in the number of lymphocytes and enhanced antibody production, helping the fish to improve their ability to prevent diseases caused by *Vibrio harveyi*.

Thus, the results of lymphocytes in this study have opened up the potential for applying *Cordyceps* extract to stimulate immunity in *Pangasius* fish with the most effective addition concentration of 0.47%. The results in Table 4 showed that the number of monocytes increased significantly according to the concentration of supplementation after 48 hours and 12 days of infection. In which, the positive control group had the lowest number of monocytes and was different from the remaining treatments, while the 0.47% supplemented treatment reached the highest number at 48 hours and 12 days, respectively, at 33.44 x 10^3 cells/mm^3 and increased sharply at 14 days after infection to 368.08 x 10^3 cells/mm^3. This clearly reflects the stimulation of the fish's immune system. Because monocytes are white blood cells that play an important role in the fish's immune system when pathogens invade (Tu, 2000). When compared with other research results on aquatic species, the quantity and function of monocytes also tend to increase when there is a pathogen attack or when there is an addition of immunostimulants in the fish diet (Aly et al., 2008; Hang et al., 2025b; Misra et al., 2006). For example, according to the results of Aly et al. (2008), tilapia (*Oreochromis niloticus*) when supplemented with the immunostimulant β-glucan was recorded to not only increase the number of monocytes, but also increase the ability of macrophages. Similarly, Misra et al. (2006) reported that common carp (*Labeo rohita*) after infection with *Aeromonas hydrophila* had a significant increase in monocytes, reflecting the core role of this cell line in the cellular immune response. In addition, the results of (Harikrishnan et al., 2011) also showed that the use of plant extracts such as *Withania somnifera* in sea bream (*Sparus aurata*) increased the density of monocytes, thereby enhancing the ability to fight against pathogenic bacteria. This result was also consistent with the increasing trend of monocytes, especially in the treatments with high concentrations of 0.36% and 0.47%.

The results of neutrophil counts also showed a similar trend as monocytes, lowest in the positive control and highest in the 0.47% treatment (Table 4). This suggests that, when supplemented at a concentration of 0.47%, fish may have strongly mobilized the number of neutrophils. This is the first important cell type in detecting and destroying pathogens (Tu, 2000). This result is consistent with previous studies such as those of Reyes-Becerril et al. (2011), which recorded a significant increase in the number of neutrophils in sea bream *(Sparus aurata*) when injected with *Aeromonas hydrophila*, a component of Gram-negative bacteria. Similarly, Magnadóttir (2006) described the key role of neutrophils and macrophages in the acute inflammatory response in fish, especially the ability to phagocytose and secrete enzymes to destroy bacteria. Studies on grass carp (*Ctenopharyngodon idella*) supplemented with β-glucan also showed an increase in the number and activity of neutrophils, contributing to enhanced non-specific defense (Yano, 1992).

These findings suggest that *C. militaris* extract stimulates both specific and non-specific immune mechanisms, leading to improved disease resistance against *E. ictaluri*. The dose-dependent pattern also supports the hypothesis that bioactive compounds such as cordycepin and adenosine contribute to hematological and immunostimulatory effects.

CONCLUSION

The results of the study showed that the addition of *Cordyceps militaris* extract to the diet of *Pangasius* fingerlings significantly improved the survival rate and enhanced the immune response when the fish were infected with *Edwardsiella ictaluri* bacteria causing pyogenic liver and kidney disease. At the addition concentration of 0.47%, the fish achieved the highest survival rate (70%) and had significantly increased red blood cell, white blood cell, lymphocyte, monocyte and neutrophil indices compared to the positive control. This proves that *Cordyceps militaris* extract has the ability to stimulate the immune system and enhance the natural resistance of *Pangasius* fish. Therefore, it is recommended to use *Cordyceps militaris* extract at a rate of about 0.47% in the diet of *Pangasius* fingerlings to increase disease prevention and reduce dependence on antibiotics in aquaculture.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution

Conceptualization, resources, supervision and funding acquisition: Lan T.T. and Nhi N.H.Y.;
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Acknowledgments

This research is funded by Vietnam National University HoChiMinh City (VNU-HCM) under grant number C2024-16-09.

Funding

The authors declare that no funds, grants, or other support were received during the preparation or publication of this manuscript.

Competing interests

The authors declare no competing interests in this research and publication.

REFERENCES

Adeshina I, Paray BA, Bhat EA, Ibrahim AD and Tiamiyu LO (2024). Stimulatory effect of dietary alpha-lipoic acid on growth performance, antioxidant capacity, liver enzymes, immunity and protection of African catfish, Clarias gariepinus (B.), Edwardsiella tarda infection. Journal of Animal Physiology and Animal Nutrition 108(1): 163-173. https://doi.org/10.1111/jpn.13876

Al-Dohail MA, Hashim R and Aliyu-Paiko M (2009). Effects of the probiotic, Lactobacillus acidophilus, on the growth performance, haematology parameters and immunoglobulin concentration in African Catfish (Clarias gariepinus, Burchell 1822) fingerling. Aquaculture Research 40(14): 1642-1652. https://doi.org/10.1111/j.1365-2109.2009.02265.x

Aly SM, Ahmed YA-G, Ghareeb AA-A and Mohamed MF (2008). Studies on Bacillus subtilis and Lactobacillus acidophilus, as potential probiotics, on the immune response and resistance of Tilapia nilotica (Oreochromis niloticus) to challenge infections. Fish & shellfish immunology 25(1-2): 128-136. https://doi.org/10.1016/j.fsi.2008.03.013

Barido FH, Hertanto BS, Cahyadi M, Kartikasari LR, Sujiwo J, Kim J, Kim H-Y, Jang A and Lee SK (2024). Enzymatic hydrolyzation of Cordyceps militaris mushroom extracts and its effect on spent hen chicken. Animal Bioscience 37(7): 1277. https://doi.org/10.5713/ab.23.0518

Boontiam W, Wachirapakorn C and Wattanachai S (2020). Growth performance and hematological changes in growing pigs treated with Cordyceps militaris spent mushroom substrate. Veterinary world 13(4): 768. https://doi.org/10.14202/vetworld.2020.768-773

Cheng Y, Wen C, Dybus A and Proskura W (2016). Fermentation products of Cordyceps militaris enhance performance and modulate immune response of weaned piglets. South African Journal of Animal Science 46(2): 121-128. https://doi.org/10.4314/sajas.v46i2.2

Choosong K, Kiriratnikom S, Pewhom A and Nitipan S (2025). Enhancing digestive and immune functions in Nile tilapia: Effects of Pediococcus acidilactici and Cordyceps militaris spent mushroom substrate extracts and their combination. Aquaculture Reports 45: 103093. https://doi.org/10.1016/j.aqrep.2025.103093

Culjak V, Bogut I, Has-Schön E, Milaković Z and Canecki K (2006). Effect of Bio-Mos* on performance and health of juvenile carp. Nutritional Biotechnology in the Feed and Food Industries. 90-90. https://www.croris.hr/crosbi/publikacija/prilog-skup/544728

Da-oh H, Kiriratnikom S, Choosong K and Nitipan S (2024). Evaluation of spent mushroom substrate extracts from Cordyceps militaris: Impact on growth Performance, Immunomodulation, and intestinal microbiota of Nile tilapia. Egyptian Journal of Aquatic Research 50(4): 577-584. https://doi.org/10.1016/j.ejar.2024.11.001

Das G, Shin H-S, Leyva-Gómez G, Prado-Audelo MLD, Cortes H, Singh YD, Panda MK, Mishra AP, Nigam M and Saklani S (2021). Cordyceps spp.: A review on its immune-stimulatory and other biological potentials. Frontiers in Pharmacology 11: 602364. https://doi.org/10.3389/fphar.2020.602364

Deng B, Wang Z, Tao W, Li W, Wang C, Wang M, Ye S, Du Y, Wu X and Wu D (2015). Effects of polysaccharides from mycelia of C ordyceps sinensis on growth performance, immunity and antioxidant indicators of the white shrimp L itopenaeus vannamei. Aquaculture Nutrition 21(2): 173-179. https://doi.org/10.1111/anu.12147

Dung TT (2010). Research on hematology of striped catfish (Pangasianodon hypophthalmus) affected by white liver and white gill disease. Can Tho University Journal of Science (15b): 81-90. https://ctujsvn.ctu.edu.vn/index.php/ctujsvn/article/view/818

Galagarza OA, Kuhn DD, Smith SA and Hrubec TC (2017). Hematologic and plasma chemistry RIs for cultured striped catfish (Pangasius hypophthalmus) in recirculating aquaculture systems. Veterinary Clinical Pathology 46(3): 457-465. https://doi.org/10.1111/vcp.12513

Groff JM and Zinkl JG (1999). Hematology and clinical chemistry of cyprinid fish: common carp and goldfish. Veterinary clinics of North America: Exotic animal practice 2(3): 741-776. https://doi.org/10.1016/S1094-9194(17)30120-2

Hang BT, Balami S and Phuong NT (2022). Effect of Lactobacillus plantarum on growth performance, immune responses, and disease resistance of striped catfish (Pangasianodon hypophthalmus). Aquaculture, Aquarium, Conservation & Legislation 15(1): 174-187. https://bioflux.com.ro/docs/2022.174-187.pdf

Hang BTB and Hoa TTT (2020). Effects of Pomegranate (*Punica granatum*) extract on growth performance and immune responses of striped catfish (*Pangasianodon hypophthalmus*). CTU Journal of Science 56(Special issue on Aquaculture and Fisheries): 161-169. https://doi.org/10.22144/ctu.jsi.2020.018

Hang BTB, Milla S, Gillardin V, Phuong NT and Kestemont P (2013). In vivo effects of Escherichia coli lipopolysaccharide on regulation of immune response and protein expression in striped catfish (Pangasianodon hypophthalmus). Fish & shellfish immunology 34(1): 339-347. https://doi.org/10.1016/j.fsi.2012.11.025

Hang BTB and Phuong NT (2020). Effects of dietary inulin supplementary duration on immune responses of striped catfish (*Pangasianodon hypophthalmus*). CTU Journal of Science 56(2): 100-109. https://doi.org/10.22144/ctu.jvn.2020.036

Hang NTT, Lan TT and Nhi NHY (2025a). Evaluation of the effect of extracellular polymeric substance (EPS) from Lactobacillus plantarum (LP) on the disease resistance of rice field eel (Monopterus albus) fingerlings. Journal of Global Innovations in Agricultural Sciences: 1095-1101. https://doi.org/10.22194/JGIAS/25.1700

Hang NTT, Lan TT, Nhi NHY, Hanh NTB and Thanh NH (2025b). Evaluation of the effects of extracellular polymeric substance (EPS) produced from *Lactobacillus plantarum* on growth performance and survival rate of ricefield eel (*Monopterus albus*) fingerlings. Egyptian Journal of Aquatic Biology & Fisheries 29 (4)(4). https://doi.org/10.21608/ejabf.2025.445041

Harikrishnan R, Balasundaram C and Heo M-S (2011). Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish. Aquaculture 317(1-4): 1-15. https://doi.org/10.1016/j.aquaculture.2011.03.039

Harikrishnan R, Balasundaram C, Kim M-C, Kim J-S, Han Y-J and Heo M-S (2009). Innate immune response and disease resistance in Carassius auratus by triherbal solvent extracts. Fish & shellfish immunology 27(3): 508-515. https://doi.org/10.1016/j.fsi.2009.07.004.

Hii YS, Lee MY and Chuah TS (2007). Acute toxicity of organochlorine insecticide endosulfan and its effect on behaviour and some hematological parameters of Asian swamp eel (Monopterus albus, Zuiew). Pesticide biochemistry and physiology 89(1): 46-53. https://doi.org/10.1016/j.pestbp.2007.02.009

Hoa TTT, Boerlage AS, Duyen TTM, Thy DTM, Hang NTT, Humphry RW and Phuong NT (2021). Nursing stages of striped catfish (Pangasianodon hypophthalmus) in Vietnam: Pathogens, diseases and husbandry practices. Aquaculture 533: 736114. https://doi.org/10.1016/j.aquaculture.2020.736114

Hoang D-H, Thuy NTT and Ky PX (2024). A synergistic effect of dietary β-glucan and mannan oligosaccharide on growth performance, haematology, body composition, nutrient utilisation, and intestinal morphology in pompano, Trachinotus ovatus. Regional Studies in Marine Science 73: 103494. https://doi.org/10.1016/j.rsma.2024.103494

Houston AH (1997). Are the classical hematological variables acceptable indicators of fish health? Transactions of the American Fisheries Society 126(6): 879-894. https://doi.org/10.1577/1548-8659(1997)126%3C0879:RATCHV%3E2.3.CO;2

Hrubec TC, Cardinale JL and Smith SA (2000). Hematology and plasma chemistry reference intervals for cultured tilapia (Oreochromis hybrid). Veterinary clinical pathology 29(1): 7-12. https://doi.org/10.1111/j.1939-165X.2000.tb00389.x

Hu Y, Zhang J, He L, Hu Y, Zhong L, Dai Z and Zhou D (2020). Effects of dietary vitamin C on growth, antioxidant activity, and immunity in ricefield eel (*Monopterus albus*). Journal of the World Aquaculture Society 51(1): 159-170. https://doi.org/10.1111/jwas.12636

Jędrejko KJ, Lazur J and Muszyńska B (2021). Cordyceps militaris: An overview of its chemical constituents in relation to biological activity. Foods 10(11): 2634. https://doi.org/10.3390/foods10112634

Kattakdad S, Phungam N, Pongket U, Muangmala W, Udduang S, Aripin SA and Yuangsoi B (2025). Microencapsulated inulin as a prebiotic: Enhancing growth, digestive enzyme activity, and immune response in striped catfish (Pangasianodon hypophthalmus). Aquaculture Reports 43: 102986. https://doi.org/10.1016/j.aqrep.2025.102986

Kumar G and Sharma A (2025). The Parasitic Power of Cordyceps. https://vigyanvarta.in/adminpanel/upload_doc/VV_0225_47.pdf

Lan J, Chen G, Cao G, Tang J, Li Q, Zhang B and Yang C (2021). Effects of α-glyceryl monolaurate on growth, immune function, volatile fatty acids, and gut microbiota in broiler chickens. Poultry Science 100(3): 100875. https://doi.org/10.1016/j.psj.2020.11.052

Lan TT, Hang NTT, Hanh NTB, Dang ND, Mai VP and Nhi NHY (2025). Improving the growth performance of striped catfish (Pangasius hypophthalmus) fingerlings through supplementation with Cordyceps militaris extract. Livestock Research for Rural Development 37 (3) 2025. https://lrrd.cipav.org.co/lrrd37/3/3731ttla.html

Li Y, Lu Y, Yu B, Huang Z, Luo Y, Zheng P, Mao X, Yu J, Luo J and Yan H (2024). Effect of Cordyceps militaris on growth performance, antioxidant capacity, and intestinal epithelium functions in weaned pigs. Journal of Animal Science 102: skae194. https://doi.org/10.1093/jas/skae194

Lo H-C, Hsieh C, Lin F-Y and Hsu T-H (2013). A systematic review of the mysterious caterpillar fungus ophiocordyceps sinensis in DongChongXiaCao (冬蟲夏草 dōng chóng xià cǎo) and related bioactive ingredients. Journal of traditional and complementary medicine 3(1): 16-32. https://doi.org/10.1016/S2225-4110(16)30164-X

Magnadóttir B (2006). Innate immunity of fish (overview). Fish & shellfish immunology 20(2): 137-151. https://doi.org/10.1016/j.fsi.2004.09.006

Minitab (2010). Minitab 16 Statistical Software 16.2.0. https://www.minitab.com/en-us/products/minitab/

Misra CK, Das BK, Mukherjee SC and Pattnaik P (2006). Effect of multiple injections of β-glucan on non-specific immune response and disease resistance in Labeo rohita fingerlings. Fish & shellfish immunology 20(3): 305-319. https://doi.org/10.1016/j.fsi.2005.05.007

Nguyen ND, Van Dang P, Le AQ, Nguyen TKL, Pham DH, Van Nguyen N and Nguyen QH (2017). Effect of oligochitosan and oligo-β-glucan supplementation on growth, innate immunity, and disease resistance of striped catfish (Pangasianodon hypophthalmus). Biotechnology and applied biochemistry 64(4): 564-571. https://doi.org/10.1002/bab.1513

Park H-J (2025). Influence of Culture Conditions on Bioactive Compounds in Cordyceps militaris: A Comprehensive Review. Foods 14(19): 3408. https://doi.org/10.3390/foods14193408

Pimpimol T, Phoonsamran K and Chitmanat C (2012). Effect of dietary Vitamin C supplementation on the blood parameters of Mekong Giant Catfish (*Pangasianodon gigas*). International Journal of Agriculture & Biology 14(2). https://www.cabidigitallibrary.org/doi/full/10.5555/20123099879

Rattanachaikunsopon P and Phumkhachorn P (2009). Prophylactic effect of Andrographis paniculata extracts against Streptococcus agalactiae infection in Nile tilapia (Oreochromis niloticus). Journal of bioscience and bioengineering 107(5): 579-582. https://doi.org/10.1016/j.jbiosc.2009.01.024

Reyes-Becerril M, López-Medina T, Ascencio-Valle F and Esteban MÁ (2011). Immune response of gilthead seabream (Sparus aurata) following experimental infection with Aeromonas hydrophila. Fish & Shellfish Immunology 31(4): 564-570. https://doi.org/10.1016/j.fsi.2011.07.006

Sharma H, Sharma N and An SSA (2023). Unique bioactives from zombie fungus (Cordyceps) as promising multitargeted neuroprotective agents. Nutrients 16(1): 102. https://doi.org/10.3390/nu16010102

Sheng J and Gerasimova K (2024). *Sustainable Management of Cordyceps: Supply Chains and Resource Management Policies*: Taylor & Francis. https://www.routledge.com/Sustainable-Management-of-Cordyceps-Supply-Chains-and-Resource-Management-Policies/Sheng-Gerasimova/p/book/9781032548159

Staykov Y, Spring P and Denev S (2005). Influence of dietary Bio-Mos® on growth, survival and immune status of rainbow trout (Salmo gairdneri irideus G.) and common carp (Cyprinus carpio L.), 333-343.. https://www.cabidigitallibrary.org/doi/full/10.5555/20063210034

Sun Y, Rabbi MH, Ma S, Wen Z, Li X, Mi R, Meng N, Li Y, Wang Q and Du X (2021). Effect of dietary Cordyceps polysaccharide supplementation on intestinal microflora and immune response of Apostichopus japonicus. Aquaculture Research 52(11): 5198-5212. https://doi.org/10.1111/are.15389.

Thanh NH, Lan TT, Hang NTT, Nhi NHY and Tho NP (2025). Effect of dietary Extracellular polymeric substances from Lactic acid bacteria on disease resistance and immune response in the whiteleg shrimp (Litopenaeus vannamei). Veterinary Integrative Sciences 23(3): 1-16. https://doi.org/10.12982/VIS.2025.061

Tian F, Li S, Xiao H, Li W, Wang H, Wang Y, Han L, Zhao C, Chang Y and Ding J (2025). The effects of dietary Cordyceps militaris substrate on growth, immunity, gonadal quality, and gut microbiota of the sea urchin Strongylocentrotus intermedius. Animal Feed Science and Technology: 116428. https://doi.org/10.1016/j.prevetmed.2025.106527

Tran VL, Barnes AC, Samsing F, Vu UN and Wiley K (2025). Striped catfish (Pangasianodon hypophthalmus) farmers' perspectives on challenges and health management practices in the Mekong Delta, Vietnam: A qualitative study. Preventive Veterinary Medicine 239: 106527. https://doi.org/10.1016/j.prevetmed.2025.106527

Tu NV (2000). Textbook of Aquatic Animal Physiology. https://caf.ctu.edu.vn/en/images/upload/AUN2022/AQ211.pdf

Van Doan H, Hoseinifar SH, Dawood MA, Chitmanat C and Tayyamath K (2017a). Effects of Cordyceps militaris spent mushroom substrate and Lactobacillus plantarum on mucosal, serum immunology and growth performance of Nile tilapia (Oreochromis niloticus). Fish & shellfish immunology 70: 87-94. https://doi.org/10.1016/j.fsi.2017.09.002

Van Doan H, Hoseinifar SH, Tapingkae W, Chitmanat C and Mekchay S (2017b). Effects of Cordyceps militaris spent mushroom substrate on mucosal and serum immune parameters, disease resistance and growth performance of Nile tilapia,(Oreochromis niloticus). Fish & shellfish immunology 67: 78-85. https://doi.org/10.1016/j.fsi.2017.05.062

Van Doan H, Mekchay S and Chitmanat APDC (2018). The use of prebiotics (from corncobs and Cordyceps militaris mushroom) and probiotics as alternative strategies for antibiotics in culturing of tilapia, Oreochromis niloticus. Thailand Science Research and Innovation. https://elibrary.tsri.or.th/fullP/MRG5980127/MRG5980127_full.pdf

Wang X, Zhang J, Zhang K, Xu G, Guo Z, Gu X, Ahmad S, Huang L, Liu S and Li J (2025). Effects of Cordyceps militaris solid medium on the growth performance, immunity parameters and intestinal health of broilers. Animal Bioscience 38(9): 1959. https://doi.org/10.5713/ab.24.0791

Wilhelm Filho D, Eble GJ, Kassner G, Caprario FX, Dafré AL and Ohira M (1992). Comparative hematology in marine fish. Comparative Biochemistry and Physiology Part A: Physiology 102(2): 311-321. https://doi.org/10.1016/0300-9629(92)90141-C

Yano T (1992). Assay of hemolytic complement activity. Techniques in fish immunology: 131-141. https://cir.nii.ac.jp/crid/1574231875535834880

Zhou L, Li H, Qin JG, Wang X, Chen L, Xu C and Li E (2020). Dietary prebiotic inulin benefits on growth performance, antioxidant capacity, immune response and intestinal microbiota in Pacific white shrimp (Litopenaeus vannamei) at low salinity. Aquaculture 518: 734847. https://doi.org/10.1016/j.aquaculture.2019.734847