

Potential effects of organic acid- and essential oil-supplemented diets on growth performance and disease resistance against the *Aeromonas hydrophila* infection in snakehead fish (*Channa striata*)

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ARTICLE INFO	ABSTRACT
Research Paper Received: September 28, 2024 Revised: November 18, 2024 Accepted: November 22, 2024 Keywords <i>Aeromonas hydrophila</i> Essential oil Growth performance Organic acid Snakehead fish * Corresponding author Vo Van Tuan Email: vovantuan@hcmuaf.edu.vn	This study was carried out to evaluate the potential effects of organic acid and essential oil supplemented diets on growth performance and resistance to infection with <i>Aeromonas hydrophila</i> of snakehead fish (<i>Channa striata</i>). A total of 600 healthy snakehead fish (15 ± 2 g) were randomly divided into four experimental variants with three replicates, and fed diets supplemented with different levels of organic acid and essential oil (T1: 0 mL/kg of feed; T2: 2.5 mL organic acid/kg of feed; T3: 1.0 mL essential oil/kg of feed; and T4: 2.5 mL organic acid and 1 mL essential oil/kg of feed) for 10 weeks. At the end of the trial, the challenge test with <i>Aeromonas hydrophila</i> was conducted for 2 weeks. The results indicated that growth performance and survival rate of fish were not significantly improved by the organic acid and essential oil supplement ($P > 0.05$). However, there was a tendency to have an increased survival rate in treatments supplemented with the organic acid and essential oil. For the challenge test, the mortality rates in treatments supplemented with the combination of the organic acid and essential oil were lower and significant difference than those of the control treatment ($P < 0.05$). These results suggested that the organic acid and essential oil supplements in diets may help to improve health status of snakehead fish through reducing pathogenic bacteria <i>Aeromonas hydrophila</i> in the intestinal tract.

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1. Introduction

Snakehead fish (*Channa striata*) is a carnivorous, freshwater species and considered as an important commercial fish in the Mekong Delta, Vietnam. Snakehead fish has been intensively cultured in ponds with high stocking density, resulting in a severe impact on water quality. Consequently, a frequency of infectious diseases such as hemorrhage and white spot disease (Liu et al., 2012; Tu et al., 2019; Pham et al., 2023), severe ulcerative disease (John & George, 2012) has been increased. These bacterial infectious diseases are the main agents that can cause high mortality in snakehead fish, resulting in a significant economic loss.

To control the infectious diseases in snakehead fish, several approaches such as chemotherapeutics and antibiotics have been applied (Pulipat et al., 2023), but the application of these chemotherapeutics and antibiotics is strictly regulated due to their negative impacts, such as development of antibiotic-resistant bacteria and immune suppression in the fish (Lim et al., 2013; Hossain et al., 2022). Hence, developing effective alternative therapies to conventional chemotherapeutics for controlling infectious diseases affecting cultured fish is considered as a global critical concern (Wang et al., 2022; Bondad-Reantaso et al., 2023).

In recent years, there is an increasing interest in the use of functional dietary supplements in commercial fish feed such as organic acids (Heshmatfar et al., 2023; Chen et al., 2024; Khoris et al., 2024), essential oils (Ngugi et al., 2017; Gheytsi et al., 2021; Magouz et al., 2022), plant herbal extracts (Rajendiran et al., 2008; Zhang et al., 2022; Baghalian et al., 2023), to improve fish growth and control infectious diseases. A dietary with organic acids and essential oils, alone or in combination, are considered as a promising solution in preventing and managing aquatic pathogenic agents, and has been studied in many

aquatic species, such as tilapia (Magouz et al., 2022), carp (Khoris et al., 2024), shrimp (He et al., 2017). Besides, organic acids or essential oils supplemented diets can enhance specific and non-specific immune protective mechanisms of fish.

Therefore, the aims of this study were to evaluate the potential effects of the organic acid and essential oil supplemented diets on growth performance and resistance to infection with *Aeromonas hydrophila* of snakehead fish (*Channa striata*).

2. Materials and Methods

2.1. Experimental diets

Four experimental diets including a basal diet were formulated to contain different levels of commercial organic acid (MEGACID: 65% formic acid) and commercial essential oil (OREGO-STIM: 5% carvacrol and 0.05% thymol) (T1: 0 mL/kg of feed; T2: 2.5 mL organic acid/kg of feed; T3: 1.0 mL essential oil/kg of feed; and T4: 2.5 mL organic acid and 1 mL essential oil/kg of feed). The basal diet had approximately 40% crude protein and 6% lipid with different ingredients, such as fishmeal, soybean meal, poultry meal, cassava, rice bran, soy oil, vitamin and mineral premix. All feed ingredients were thoroughly mixed, extruded and pelleted to produce a floating feed.

From the basal diet, three treatment diets were produced in the laboratory by coating with the organic acid and essential oil at different dosages. Then all four diets were finally coated with 0.5% soy oil to prevent the product leaking. All prepared feed was preserved in storage at -20°C and labelled as T₁, T₂, T₃ and T₄.

2.2. Experimental animals

Snakehead fish fingerlings were purchased from private fish farm at Dong Thap Province and transported to the Experimental Farm

of Nong Lam University, Ho Chi Minh City, Vietnam. The fish are acclimated and cultured for 2 weeks and fed on a basal diet twice daily to apparent satiation.

Fish with initial weight of 15 ± 5 g were selected for the trial. The trial was carried out in 12 cement tanks that contained 50 fish per tank ($1.0 \times 2.0 \times 1.5$ m). Fish were fed two times a day (8:00 AM and 16:00 PM) to apparent satiation for a period of 10 weeks. One hour after feeding, the unconsumed feed was collected and air-dried to calculate daily consumed feed for each tank. Feed intake was recorded daily to compare the feeding intake of four diets at the end of the experiment.

During the feeding trial, water temperature was maintained between 28 - 30°C, pH 6.5 - 7.5, dissolved oxygen above 4 mg/L. Regular water changes kept total ammonia-N below 0.5 mg/L and nitrite-N below 0.2 mg/L.

At the end of the trial, consumed feed and survival rate of fish were measured to determine the effect of organic acid and essential oil supplementation on fish growth and feed utilization. Parameters were analyzed as follows:

- Survival rates (%):

$SR (\%) = (\text{Number of survival fish} / \text{Number of initial fish}) \times 100$

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

$DWG = (\text{Final weight} - \text{Initial weight}) / \text{Cultured days}$

- WG percent (WG): %

$WG = [(\text{Final weight} - \text{Initial weight}) / \text{Initial weight}] \times 100$

- Specific growth rate (SGR).

$SGR (\% \cdot \text{day}^{-1}) = [(\ln(\text{final weight}) - \ln(\text{Initial weight})) / \text{Cultured days}] \times 100$

- Feed conversion ratio (FCR)

$FCR = \text{Consumed feed} / \text{Weight gain}$

- Feed intake (FI)

$FI (\text{g/day}) = (\text{Consumed feed in tank} / \text{Number fish of tank}) / \text{Cultured days}$

2.3. Bacterial challenge

To evaluate the potential effects of organic acid and essential oil supplements on fish health status, fish were challenged with pathogenic bacteria *Aeromonas hydrophila* through injection. At the end of the feeding period, 10 fish in each tank were randomly selected and transferred to 100 L plastic tanks. Fish were intraperitoneally injected with 0.1 mL of *A. hydrophila* suspension with a dosage of 2.0×10^6 CFU/fish. 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 for 336 h and mortality of fish was daily monitored. At the end of the trial, survival rate and intestinal microbial (*A. hydrophila* counts) were recorded.

2.4. Analysis of bacteria in fish intestine

At the end of the bacterial trial, two fish per tank 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 *Aeromonas hydrophila* agar (Rimler Shott). 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 *A. hydrophila* counts. The results were expressed as CFU/g sample.

2.5. Statistical analysis

Results were presented as means $\pm$ 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. Growth and feed utilization

Growth performance of the snakehead fish expressed as final body weight (FBW), weight gain (WG), specific growth rate (SGR) and survival rate (SR) was presented in Table 1.

Table 1. Effect of the organic acid and essential oil supplemented diets on the growth performance and survival rates of the snakehead fish

Treatment	IBW (g/fish)	FBW (g/fish)	DWG (g/day)	WG percent (%)	SGR (%/day)	SR (%)
T ₁	17.4 $\pm$ 0.1	87.5 $\pm$ 2.7	1.00 $\pm$ 0.04	3.66 $\pm$ 0.30	2.31 $\pm$ 0.05	92.7 $\pm$.1
T ₂	17.5 $\pm$ 0.2	81.2 $\pm$ 4.2	0.91 $\pm$ 0.06	3.47 $\pm$ 0.19	2.19 $\pm$ 0.09	96.7 $\pm$ 2.3
T ₃	17.6 $\pm$ 0.2	79.7 $\pm$ 7.5	0.89 $\pm$ 0.11	3.47 $\pm$ 0.36	2.16 $\pm$ 0.15	98.7 $\pm$ 2.3
T ₄	17.7 $\pm$ 0.5	86.7 $\pm$ 14.2	0.99 $\pm$ 0.20	3.75 $\pm$ 0.74	2.25 $\pm$ 0.21	97.3 $\pm$ 3.1
<i>P</i>	0.092	0.162	0.241	0.510	0.182	0.131

Results are mean $\pm$ standard deviation ($n = 3$). IBW: initial body weight; FBW: final body weight; DWG: daily weight gain; WG: weight gain; SGR: specific growth rates; SR: survival rate.

The results showed that the survival rates of the snakehead fish were quite high in all treatments (ranging from 92 to 98%), but no significant difference was recorded among treatments. However, it was a tendency to increase the survival rate of fish in the organic acid and essential oil supplemented diets. For the growth performance, after 10 weeks of

the feeding trial, no significant difference was recorded in terms of final body weight, daily weight gain, weight gain percent and specific growth rate between the fish fed the organic acid and essential oil supplemented diets and the control diet ($P > 0.05$). The feed utilization of the fish is expressed as feeding intake (FI) and feed conversion ratio (FCR). Results are presented in Table 2.

Table 2. Feeding intake and feed conversion ratio of fish fed four experimental diets

Treatment	FI (g/fish/day)	FCR
T ₁	1.75 $\pm$ 0.04	1.92 $\pm$ 0.13
T ₂	1.68 $\pm$ 0.02	1.92 $\pm$ 0.08
T ₃	1.74 $\pm$ 0.10	1.97 $\pm$ 0.05
T ₄	1.68 $\pm$ 0.02	1.82 $\pm$ 0.37
<i>P</i>	0.300	0.406

Results are mean $\pm$ standard deviation ($n = 3$). FI: feed intake; FCR: feed conversion ratio.

The results demonstrated that the feed intake (FI) and feed conversion ratio (FCR) of fish were lowest in the combination of organic acid and essential oil supplemented diet comparison with the control diet. The FCR was not significantly different among treatments; however, the T4 treatment gave the lowest value (1.82) compared to the control diet (1.92).

3.2. Cumulative mortality of the snakehead fish after bacterial challenge

At the end of the feeding trial, the snakehead fish were injected with *Aeromonas hydrophila*, and the results were illustrated in Figure 1.

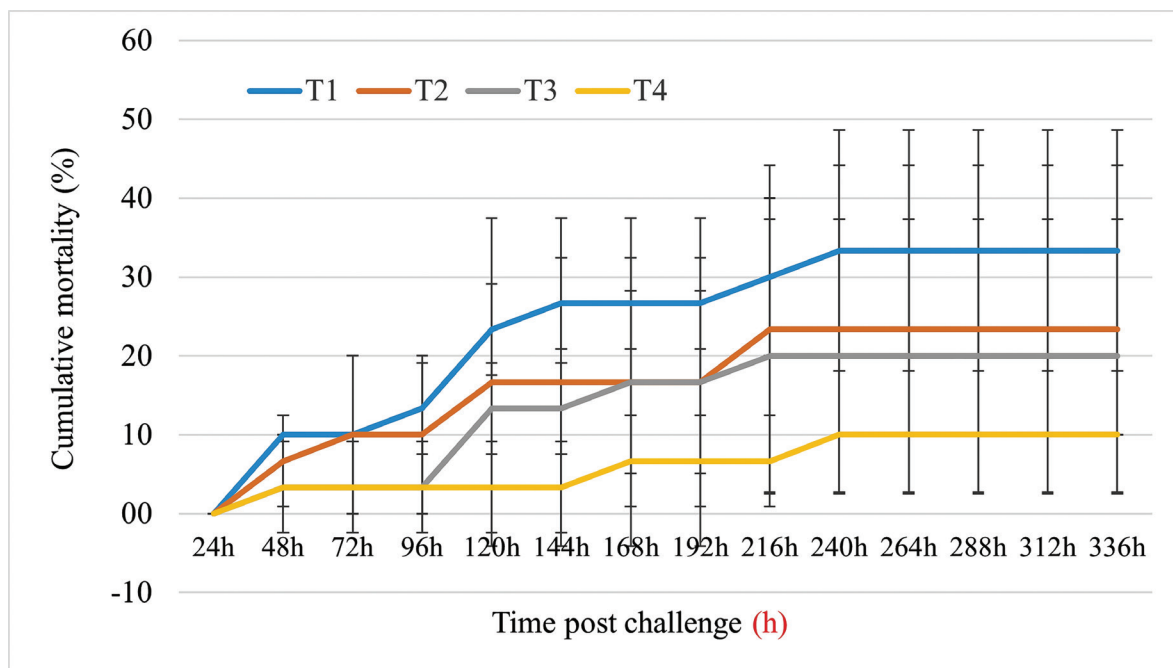


Figure 1. Cumulative mortality of the snakehead fish after bacterial challenge. T₁: 0 mL/kg of feed; T₂: 2.5 mL organic acid/kg of feed; T₃: 1.0 mL essential oil/kg of feed; T₄: 2.5 mL organic acid and 1 mL essential oil/kg of feed.

The death of fish in the *A. hydrophila* challenge was initially recorded on the second day post infection (48 h post infection) in all test groups. The cumulative mortality of fish in the organic acid and essential oil supplemented groups, especially in T₄ (combination of organic acid and essential oil) was lower and significant difference in comparison with the control group ($P < 0.05$). No significant difference in cumulative mortality of the snakehead fish was recorded between the treatment groups.

To evaluate the effect of the organic acid and essential oil supplement on gut health status of the snakehead fish, fish intestine was sampled at the end of the bacterial challenge. Total *Aeromonas hydrophila* counts were determined, and the result was illustrated in Table 3.

Table 3. Effect of the organic acid and essential oil supplement on intestinal microbial of fish

Parameters	Experimental diets			
	T ₁	T ₂	T ₃	T ₄
<i>A. hydrophila</i> (CFU/g)	2513.3 ± 1397.2 ^a	1469.1 ± 611.6 ^{ab}	1155.4 ± 426.4 ^{ab}	664.8 ± 152.3 ^b

Results are mean ± standard deviation (n = 3). The row with the different superscripts is significant differences (P < 0.05).

The current study showed that lower counts of *Aeromonas hydrophila* were observed in the organic acid and essential oil supplemented diets in comparison to the control diet. Significant differences of the number of *Aeromonas hydrophila* counts were recorded between the control diet (T₁) and the combination of organic acid and essential oil supplemented diet (T₄) (P < 0.05).

4. Discussion

The results of the current study showed that the growth performance and feed utilization of fish were not significantly different between the organic acid and essential oil supplemented diets and the control diet. Dietary with a combination of the organic acid and essential oil supplemented diet (T₄) had a lower FCR than other diets (1.82 versus 1.92), but no difference was recorded between diets. Our results were similar to the results of He et al. (2017) in Pacific white shrimp. These authors found that no statistical difference in final weight and weight gain of shrimp fed the organic acids and essential oils supplemented diets. Similarly, Chuchird et al. (2015) could not find any significant difference of formic acid supplemented diets on growth performance of shrimp. Concerning essential oil supplementation in fish diets, reports of Baba et al. (2016) on tilapia (*Oreochromis niloticus*), Monteiro et al. (2021) on juvenile tambaqui fish (*Colossoma macropomum*) also indicated that no effect of essential oil on the growth performance of fish.

Organic acid and essential oil can be used as feed additive in aqua-feed fish (Dawood et al., 2017; Gheytsi et al., 2021; Addam et al., 2024). Dietary with the organic acid or essential oil supplement have positive effects on aquatic health status. They possibly possess antimicrobial activity against several pathogenic bacteria such as *Streptococcus agalactiae* (Koh et al., 2014), *Vibrio parahaemolyticus* (Chuchird et al., 2015; Khoris et al., 2024), *Aeromonas hydrophila* (Monteiro et al., 2021), *Pseudomonas putida* (Alzahrani et al., 2022). The challenge test in this study showed that supplementation of the organic acid and essential oil (T₄) in feed did increase the survival rate of *Aeromonas hydrophila* infected snakehead fish significantly compared with the control fish (P < 0.05). It pointed out that fish fed a combination of the organic acid and essential oil had significantly lower *A. hydrophila* in the intestinal tract compared with the control diet. Results of Addam et al. (2018) on tilapia also demonstrated that a significant decrease in total heterotrophic bacteria and *Pseudomonas* sp. in the intestine of fish fed the organic acid and essential supplemented diets. Antibacterial activity of the organic acid and essential oil is possible direct mechanism that involved in this process. Reducing intestinal bacteria may be related to not only the diffusion of organic acid into bacterial cells but also the bacterial membrane damage caused by the essential oil. A combination of the essential oil and organic acid may influence the intestinal bacteria because the essential oil damages the bacterial membrane that makes them more susceptible to the action of the organic acid.

5. Conclusions

Supplementation of the organic acid and essential oil, alone or in combination, has no effect on the growth performance and feed utilization of the snakehead fish. However, a combination of the organic acid and essential oil (T₄) significantly improved survival rate of the bacterial challenged fish.

Conflict of interest

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

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