BF-1687
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RESEARCH ARTICLE Indian Journal of Animal Research

Effect of Oyster Mushroom (*Pleurotus ostreatus*) Extract on Survival, Growth Performances, Immune Responses and against *Aeromonas hydrophila* of Snakehead Fish (*Channa striata*)

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10.18805/IJAR.BF-1687

ABSTRACT

Background: The high density of farmed snakehead fish and the expansion of the farming area might cause a negative impact on water environment and an increased frequency of fish diseases. The use of plant-based extracts has been promised as an alternative method to antibiotic application in the controlling of fish diseases. This study was conducted to investigate the effect of *Pleurotus ostreatus* extract on survival, growth, immune responses and against *Aeromonas hydrophila* of snakehead fish (*Channa striata*).

Methods: During 2021-11 to 2022-10, the *P. ostreatus* extract was prepared using hot water method and the fish fed diets supplemented the extract at 0.0 g kg^{-1}, 1.5 g kg^{-1}, 2.5 g kg^{-1}, 3.5 g kg^{-1} and 4.5 g kg^{-1} was examined for survival rates, growth responses, white blood cell count, respiratory burst activity and mortality rates of fish in *A. hydrophila* infection.

Result: The results indicated snakehead fish fed five different diets showed no significant differences in survival and growth performances. However, the innate immune parameters were likely increased in fish fed diets supplemented *P. ostreatus* extract and the diet supplemented extract at 4.5 g kg^{-1} could reduce the accumulate mortality of snakehead fish challenged with *A. hydrophila*. *P. ostreatus* extract could be a promising material for enhancing the innate immunity of snakehead fish and can protect fish from *A. hydrophila* infection.

Key words: Immune, *Pleurotus ostreatus,* RBA, Snakehead, WBCs.

INTRODUCTION

Snakehead fish (*Channa striata*) is a commercially important fish, fast-growing and promising freshwater species for aquaculture development (Gustiano *et al*., 2021). In Vietnam, snakehead fish is one of the important commercial freshwater species. The production of farmed snakehead fish in Vietnam is about 40.000 tons per year and the area of snakehead fish farming has been expanded (Bich *et al*., 2020). It is likely that the intensive culture of snakehead fish leads to negative impacts on water quality and an increase in the frequency of fish diseases such as parasites and hemoharric diseases (Sinh and Chung, 2010). Antibiotics are commonly applied to prevent and control diseases in aquaculture. This practice can cause antibiotic residuals and antibiotic resistances in environment (Bondad-Reantaso *et al*., 2023). Recently, alternative methods have been introduced by using plant-based extracts as feed additives to enhance the immune system of aquatic animals and protect animals from pathogen infection (Bharathi *et al*., 2021; Jha *et al*., 2022; Hong To *et al*., 2022).

Mushrooms have been widely used because of high nutritional values and medicinal properties, particularly carbohydrates, proteins, minerals, dietary fibre and vitamins B, C and D (Nowakowski *et al*., 2021). *Pleurotus ostreatus* is considered as a good source of carbohydrates, mainly represented by polysaccharides including cellulose, chitin, α- and β- glucans, mannans, xylans and galactans which are known as a source of antitumor and immunomodulatory (Deepalakshmi and Mirunalini, 2014). Up to now, there are few reports on the effect of mushroom extract on snakehead fish culture in the Mekong Delta. This study examined the effect of *P. ostreatus* extract on survival, growth, immune parameters and against *Aeromonas hydrophila* of snakehead fish.

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How to cite this article: To, T.T.H., Lan, T.T.P. and Sinh, N.T. (2023). Effect of Oyster Mushroom (*Pleurotus ostreatus*) Extract on Survival, Growth Performances, Immune Responses and against *Aeromonas hydrophila* of Snakehead Fish (*Channa striata*). Indian Journal of Animal Research. doi: 10.18805/IJAR.BF-1687.

Submitted: 17-08-2023 **Accepted:** 04-10-2023 **Online:** 11-10-2023

MATERIALS AND METHODS

The experiment was conducted from 2021-11 to 2022-10 at the School of Agriculture and Aquaculture, Tra Vinh University, Tra Vinh province, Vietnam. Fresh *P. ostreatus* was collected from local market in Tra Vinh province. The extraction of *P. ostreatus* was carried out using the hot water method following the protocol described by Huang *et al*. (2022) and Uluköy *et al*. (2016). Briefly, mushroom was dried

at 50°C and ground into fine powder. After that, the dried powder was added to water at the ratio 1:20 and left in 70°C water bath for 4h. Next, the suspension was filled through a muslin cloth several times to remove debris. The sediment was extracted twice more. Then, the collected supernatant was subjected to vacuum concentrate till 1/5 of the original volume. Finally, the concentrate was freeze-dried to have powder and kept at 4°C until use. The basal diet was made following the formulas described by Hien *et al*. (2016). The ingredients including fish meal, defatted soybean meal, dried rice-bran, squid oil, soybean oil, premix vitamins, premix minerals, hydrolyzed protein and cassava meal were formulated to meet 45% protein level in the diet. Five different diets were prepared by adding *P. ostreatus* extract to the basal diet at 0.0 g kg^{-1}, 1.5 g kg^{-1}, 2.5 g kg^{-1}, 3.5 g kg^{-1} and 4.5 g kg^{-1}. All ingredients in each diet were thoroughly mixed by mixer machine. Water was added to the mixture to form a uniform paste. After that, the paste was added to meat grinder to form pellets. The pellet was air dried and stored at 4°C until use. Fingerling of snakehead fish was brought from fish hatchery in Dong Thap province and nursed at Aquaculture Department, school of Agriculture and Aquaculture, Tra Vinh university to read expected size for experiment. After nursing period, fish at 21.63±1.7 g was selected to place in experimental tanks. Fish was stocked in 1 m^3 composite tank with a density of 70 individuals per tank. Five treatments were examined in triplicates. Fish was fed two times a day. Water parameters in each tank were measured every day for pH, oxygen and temperature and every 3 days for amonia and hydrosulfic. During experimental period, environmental parameters were maintained at suitable levels for fish growth (oxygen above 4ppm, pH 7.5-8.0, temperature 28-29°C, amonia and hydrosulfic undetectable). Siphoning and water exchange were carried out at 20% every 2 days. After 56-days culture, survival, growth responses and immune parameters of experimental fish were examined. Survival rate (SR) and growth responses including weight gain (WG), length gain (LG) and specific growth rate (SGR) of experimental fish was calculated following the formulas described by Hien *et al*. (2016). Immune parameters such as white blood cell count (WBCs) and respiratory burst activity (RBA) were measured following the protocols described by Natt and Herrick (1952) and Bilen *et al*. (2016), respectively.

Regarding resistance against *A. hydrophila* infection, after 56-days culture, fish from five treatments were subjected to *A. hydrophila* challenge following the protocol described by Uluköy *et al*. (2016). *A. hydrophila* isolate previously isolated from diseased fish and stored at -80°C at the Laboratory of Aquaculture Disease Management, Tra Vinh University was recovered on trypsin soybean agar (TSA) (Himedia, India) and re-checked for identification by PCR method using the protocol described by Swaminathan *et al*. (2004). In our another experiment, LD50 of *A. hydrophila* on snakehead fish was calculated at 5.0×10^6 CFU ml^{-1}. This dose was applied to challenge snakehead fish in this study. Fifteen fish were placed in each plastic container containing 50 l freshwater. Each fish was injected with 0.1 ml *A. hydrophila* solution (5.0×10^6 CFU ml^{-1}). The experiment was observed over two weeks for the cumulative mortality and clinical signs of infected fish. Fish showing signs of disease such as red pot on body and fin surface was picked-up for re-isolation of *A. hydrophila*.

Before subjected to One-way ANOVA and Turkey HSD for comparison, the collected data was analyzed for normal distribution and variance homogeneity. Significant differences were considered at P<0.05. SPSS software (version 20. IBM, USA) was used for statistical analysis.

RESULTS AND DISCUSSION

Survival, growth and immune responses

Table 1 indicated that snakehead fish fed 5 different diets showed no significant difference (P>0.05) in survival rates and growth responses after the culture period of 56 days. Immune parameters of snakehead fish were shown in Table 2. There were no significant differences (P>0.05) in WBCs among fish fed diets supplemented *P. ostreatus* extract at 0.0 g kg^{-1}, 1.5 g kg^{-1}, 2.5 g kg^{-1} and 3.5 g kg^{-1}; however, WBCs of fish fed diet supplemented *P. ostreatus* extract at 4.5 g kg^{-1} (7.67×10^4 cells/mm^3) were significantly higher (P<0.05) than that of fish fed diets without extract supplementation (4.58×10^4 cells/mm^3) and with extract supplementation at 1.5 g kg^{-1} (5.0×10^4 cells/mm^3). There

Table 1: Growth performances and survival rates of snakehead fish fed five different diets after 56-days period.

Parameters	*P. ostreatus* extract supplementation (g kg^{-1})				
	0.0	1.5	2.5	3.5	4.5
Initial weight (g)	21.63±1.70	21.63±1.70	21.63±1.70	21.63±1.70	21.63 ±1.70
Initial length (cm)	13.81±0.53	13.81±0.53	13.81±0.53	13.81±0.53	13.81±0.53
Final weight (g)	63.70±0.52[a]	64.63±0.94[a]	64.78±0.96[a]	65.06±1.48[a]	65.13±0.20[a]
Final length (cm)	19.37±0.29[a]	19.58±0.19[a]	19.61±0.10[a]	19.73±0.29[a]	19.96±0.22[a]
WG (%)	194.70±2.4[a]	198.79±4.35[a]	199.48±4.56[a]	200.78±6.50[a]	201.11±0.93[a]
LG (%)	40.18±2.10[a]	41.67±1.38[a]	41.90±0.72[a]	42.77±2.13[a]	44.42±1.59[a]
SGR (% day^{-1})	1.93±0.17[a]	1.96±0.25[a]	1.96±0.26[a]	1.97±0.38[a]	1.97±0.06[a]
SR (%)	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]

Data are presented as mean±SD. Different lowercase letters in the same row indicate statistically significant differences (*P*<0.05).

Table 2: Non-specific immune parameters of snakehead fish fed five different diets after 56-days period.

Parameters	*P. ostreatus* extract supplementation (g kg^{-1})				
	0.0	1.5	2.5	3.5	4.5
WBCs (×10^4 cells/mm^3)	4.58±0.72^b	5.00±0.00^b	5.83±0.72^{ab}	6.22±0.38^{ab}	7.67 ±1.53^a
RBA (OD)	0.53±0.06^b	0.57±0.18^{ab}	0.62±0.05^{ab}	0.64±0.05^{ab}	0.77±0.05^a

Data are presented as mean±SD. Different lowercase letters in the same row indicate statistically significant differences (P<0.05).

Table 3: Accumulate mortality of *A. hydrophila* challenged snakehead fish.

P. ostreatus extract supplementation (g kg^{-1})	Accumulate mortality (%)
0.0	66.67±6.67^a
1.5	66.67±0.00^a
2.5	64.45±3.85^{ab}
3.5	64.45±3.85^{ab}
4.5	53.33±6.67^b

Different lowercase indicate statistically significant differences (P<0.05).

were also no significant differences (P>0.05) in RBA among fish fed diets supplemented *P. ostreatus* extract at 0.0g kg^{-1}, 1.5 g kg^{-1}, 2.5 g kg^{-1} and 3.5 g kg^{-1}. Notably, RBA of fish fed the extract at 4.5g kg^{-1} was significantly higher (P<0.05) than animals fed diet without *P. ostreatus* extract, although it showed no significant difference (P>0.05) to fish in other treatments with *P. ostreatus* extract supplementation.

This study showed a stimulation of innate immune parameters of snakehead fish fed *P. ostreatus* extract. Similar to this study, the previous studies stated that the supplementation of *P. ostreatus* extract could enhance phagocytic activity, lysozyme activity, myeloperoxide activity, *n*itroblue tetrazolium activity and WBC production of rainbow trout (*Oncorhynchus mykiss*) (Bilen *et al.,* 2016; Uluköy *et al*., 2016). Other edible mushroom species were also reported to stimulate immune response of fish. The administration of *Lentinula edodes* medicinal mushroom extract could enhance serum total immunoglobulin and RBA of rainbow trout (*O. mykiss*) (Baba *et al*., 2015; Baba and Uluköy, 2022). Another study by Rattanachan *et al*. (2020) stated that *Pleurotus sajo-caju* could improve monocyte number and increase the number and size of melanomacrophage centers in hemotopoietic organs, posterior kidney and spleen of fancy carp (*Cyprinus carpio*). As reported by Cruz-García (2022), the diet supplemented *P. djamor* var. roseus enhanced red blood cell and white blood cell production of Nile tilapia (*Oreochromis niloticus*).

Against *A. hydrophila*

The resistance against *A. hydrophila* of snakehead fish fed diets supplemented *P. ostreatus* extract was illustrated in Table 3. There were no significant differences in the accumulate mortality among fish fed diets supplemented the extract at 0.0 g kg^{-1}, 1.5 g kg^{-1}, 2.5 g kg^{-1} and 3.5 g kg^{-1}. Fish among treatments with the extract at 2.5 g kg^{-1}, 3.5 g kg^{-1} and 4.5 g kg^{-1} also showed no significant difference in the accumulate mortality. However, fish fed diet with the extract at 4.5 g kg^{-1} (53.33%) showed a significantly lower mortality rate (P<0.05) compared to fish fed diets without extract supplementation (66.67%) and with extract supplementation at 1.5g kg^{-1} (66.67%). The infected fish showed hemorrhagic signs on the body and fin surface and *A. hydrophila* was also isolated from diseased fish in the challenge experiment.

It is known that *A. hydrophila* is the pathogen of hemorrhagic disease in snakehead fish and has caused a large economic losses for fish farming (Duc *et al*., 2013). In this study, *P. ostreatus* extract was shown to reduce the mortality of *A. hydrophila* infected fish. Previous studies also noted that mushroom extracts can protect fish from bacteria infection. Rainbow trout (*O. mykiss)* fed diets supplemented *P. ostreatus* extract reduced mortality by 46.65 to 53.32% as challenged with *Lactococcus garvieae (*Uluköy *et al*., 2016). Other studies reported that rainbow trout (*O. mykiss*) fed *L. edodes* mushroom extract could protect fish from *L. garvieae* and *A. hydrophila* infection *(*Baba *et al*., 2015; Baba and Ulukoy, 2022).

CONCLUSION

In conclusion, *P. ostreatus* extract likely improved innate immune parameters of snakehead fish such as increasing WBCs and RBA and reduced the mortality of fish challenged with *A. hydrophila*. Therefore, *P. ostreatus* extract could be a potential material for improving innate immunity of snakehead fish and against *A. hydrophila* infection.

ACKNOWLEDGMENT

This study was funded by the Tra Vinh University as in contract number 255/2021/HĐ.HĐKH&ĐT-ĐHTV. We expressour deep thanks to Ms. Lam Thi Yen Trinh, Ms. Huynh BaoNgoc and Ms. Nguyen Ngoc Anh for their great support of this study

Conflict of interest: None.

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