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Original Research Articles

Effect of ginger, *Zingiber officinale* extract on growth performance, digestive enzyme and stress tolerance of whiteleg shrimp, *Litopenaeus vannamei* juveniles

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This study evaluated the effects of ginger extract as a feed additive on the growth performance, digestive enzymes, and resistance to environmental stress of the whiteleg shrimp, *Litopenaeus vannamei*. 1200 juveniles were randomly allocated into four groups with three replicates. Shrimps were fed diets supplemented with 0 (T0-control), 0.5 (T1), 1 (T2), and 1.5 g kg^{-1} of ginger extract (T3) for 56 days. The results indicated that shrimp fed with ginger extract had a lower feed conversion ratio (FCR) than the control. In addition, shrimp fed with 0.5 g kg^{-1} of ginger extract had significantly greater weight gain and protease activity in the intestine than those in the control group. However, dietary supplementation with ginger extract did not significantly affect survival rates. After 56 days of culture, shrimp fed with 0.5 and 1 g kg^{-1} of ginger extract had higher survival rates than the controls after 72 – 96 h exposure to high ammonia stress (40 mg L^{-1}). Based on the study's findings, ginger extract could be recommended for shrimp feed to enhance growth and resistance against stress factors, and the optimal level is 0.5 g kg^{-1}.

INTRODUCTION

Aquaculture production has increased rapidly in recent years and supplies the major food source for human consumption. In brackish water aquaculture, the whiteleg shrimp is reported to be the most important commercial species in the world, accounting for 51.7% of the total production of crustaceans in 2020.[1,2] In Vietnam, the whiteleg shrimps' farming area reached 121,000 hectares, with 655 thousand tons produced in 2021, occupying second place among the major crustacean species.[3] In commercial aquaculture farms, feed is the main factor in the total production costs and comprises at least 50% of operational costs.[4,5] Many aquaculture farmers have used natural products as feed additives that promote the growth and health of aquatic species without causing any harmful effects. In addition, Doyle[6] reported that natural products like herbs could be used as an alternative source for antibiotic growth promoters.

Medical plants have been increasingly applied in aquaculture because they are safe, easy to prepare, have a lower cost of production, and are environmentally friendly. Medical plant extracts are rich in many bioactive components such as alkaloids, flavonoids, phenolics, saponins, tannins, and vitamins that enhance growth and immune parameters in fish and shrimp aquaculture.[7] For example, tilapia *Oreochromis mossambicus* showed significant improvement in specific growth rate and immune response after administration of a diet supplemented with extracts of Bermuda grass, beal, winter cherry, and ginger.[8] Moreover, the positive effect of herb extract *Artemisia annua* has been demonstrated in largemouth bass, *Micropterus salmoides* juvenile, where 0.05% concentration showed an improved growth due to the increase in the number of beneficial intestinal bacteria (*Lactobacillus* and *Enterococcus*).[9] Maurus et al.[10] reported that *Scutellaria baicalensis* extract (1%) was effective as a growth promoter for whiteleg shrimp, where a significant increase in weight gain and specific growth rate was observed after two weeks of feeding. Also, shrimp fed with 1% *Scutellaria baicalensis* extract enhanced the immune response as well as increased resistance against *Vibrio parahaemolyticus*.[10,11]

Ginger, *Zingiber officinale*, used as a spice and traditional medicine in many countries worldwide for a long time, belongs to the family Zingiberaceae. Ginger rhizomes contain various nutrients such as carbohydrates, protein, fiber, lipids, minerals, and vitamins. This plant is also rich in bioactive constituents, such as gingerols, shogaols,

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paradols, zingerone, terpenes, and zingiberene that play an important role as anti-oxidant, anti-stress, anti-bacterial, anti-fungal, anti-inflammatory, and immunostimulation.[11-14] The supplementation of ginger extract in aquatic animal's diets has shown positive effects on growth performance in benni fish, *Mesopotamichthys sharpeyi*,[15] common carp, *Cyprinus carpio*,[14] black rockfish, *Sebastes schlegelii*,[16] and Nile tilapia, *Oreochromis niloticus*.[17] Although ginger is widely researched in fish species, to the best of our knowledge, few studies have reported the effects of its extracts on shrimp as a feed additive.[18-20] The objective of the present study was to investigate the effects of ginger extract on growth parameters, activity of digestive enzymes, and tolerance to high ammonia and low salinity conditions of the whiteleg shrimp.

MATERIALS AND METHODS

GINGER EXTRACT AND DIET PREPARATION

Fresh ginger was obtained from a local market in Can Tho City (Vietnam). They were cleaned and cut into small pieces. After being sun-dried for one week, the ginger was powdered and mixed with distilled water at a ratio of 1:9 (w:v) and then placed at room temperature for 24 h. The mixture was filtered through Whatman No. 1 filter paper, and the liquid was evaporated using a rotary evaporation at 50°C. The powder ginger extract was kept at 4°C until further use.

The commercial shrimp diets (Grobest Landfound Co. Ltd., Vietnam: 40% protein, 4% lipid, 5% fiber and 11% moisture) were supplemented with different levels of 0.5, 1 and 1.5 g kg^{-1} ginger extract. To prepare experimental diets, ginger extract was mixed with distilled water and sprayed on the commercial diets. Then the prepared diets were dried at room temperature and kept in plastic bags at 4°C until use.

EXPERIMENTAL DESIGN

Whiteleg shrimp juveniles were supplied by the Faculty of Marine Science and Technology, College of Aquaculture and Fisheries, Can Tho University. They were acclimated to the wetlab conditions for 5 days before the start of the experiment. Shrimp were fed a commercial diet three times a day. After the acclimation period, 1200 healthy shrimps weighing 0.6 ± 0.01 g were randomly allocated into 12 500-L composite tanks in recirculating aquaculture systems. Each treatment was conducted in triplicate. The shrimps were fed three experiment diets or the control diet (commercial diet) (at 3-5% of body weight) four times daily at 6:00, 12:00, 17:00, and 20:00 h for 56 days. During the culture period, the water quality parameters including temperature, pH, dissolved oxygen and alkalinity were monitored daily using a multi-parameter meter (HANNA HI9828, Romania). Total ammonium nitrogen, nitrite and nitrate were measured weekly according to the method of APHA.[21]

GROWTH PERFORMANCES AND SURVIVAL RATE

At the end of the experiment, growth parameters and survival of shrimp in all treatments were measured as follows:

Survival rate (%) = (Final numbers/ Initial numbers) × 100

Feed conversion rate (FCR) = Feed intake / Weight gain

Weight gain (g) = Final weight – Initial weight

Daily weight gain (DWG) = (Final weight – Initial weight)/ days

Specific growth rate (SGR) (% day^{-1}) = ([Ln final weight - Ln initial weight]/ days) × 100

Biomass (kg m^{-3}) = [final weight × final population]/volume of water

DIGESTIVE ENZYME ACTIVITY

At the end of the feeding trial, 6 shrimps were randomly collected from each tank and starved at 24 h to collect the intestine samples. The samples were then homogenized in a sterile saline solution and centrifuged at 3000 rpm for 15 min at 4°C. The supernatant was collected and stored at -20°C to analyze enzymes. The amylase activity was measured according to Bernfeld et al.[22] method by using maltose as a standard. Enzyme concentrations were determined using a spectrophotometer by the absorbance at 540 nm and the level was expressed as U mg $protein^{-1}$.

Protease activity was determined as in the previous study of Lowry et al.[23] by casein solution as the substrate. For the reaction, 100 µL of enzyme extract was mixed with the same amount of substrate and incubated at 37°C for 10 min. Add trichloroacetic acid (500 L of 5%, v/v) stopped the reaction. It was then centrifuged at 3000 x g for 20 min, the supernatant was collected, and the absorbance was measured at 580 nm. Protease activity was expressed as U mg $protein^{-1}$.

LOW SALINITY CHALLENGE TEST

After 56-day feeding with different experiment diets, thirty shrimps from each treatment were collected and transferred to a 20 L tank for the salinity stress experiment. Each treatment was performed in three replicates, and each replicate consisted of 10 shrimps. The salinity levels gradually decreased from 15 ppt to freshwater by adding dechlorinated tap water. The survival rate of shrimp was calculated every 24 h for 96 h.

AMMONIA CHALLENGE TEST

The experimental design was similar to the low salinity test as described above. Based on the results of Ciji et al.,[24] total ammonia nitrogen in intensive shrimp ponds can reach concentrations of 46.1 mg/L. The shrimps were exposed to high concentrations of ammonia (40 mg L-1) for 96 hours. Ammonium chloride (NH4Cl) was diluted in distilled water to prepare the stock ammonia solution. The test solution was renewed every 24 h, and shrimp were not fed during the experiment. During the experiment, the average pH and temperature were 8.3 ± 0.05 and 29.1 ± 0.12°C, respectively.

Table 1. Water quality parameters during the culture period

Parameters	Treatments			
	T0 (control)	T1	T2	T3
Temperature (°C)	27.2 ± 0.2	27.4 ± 0.2	27.2 ± 0.1	27.3 ± 0.2
pH	8.1 ± 2.7	8.3 ± 2.6	8.3 ± 2.6	8.3 ± 2.7
DO (mg L^{-1})	6.1 ± 2.0	5.97 ± 2.0	6.15 ± 2.0	6.2 ± 1.9
Alkalinity (mg$CaCO_3$ L^{-1})	105.1 ± 5.3	110.9 ± 5.6	98.1 ± 5.3	101.2 ± 4.5
Hardness (mg$CaCO_3$ L^{-1})	2018.4±93.4	2042.3±85.3	2004.6±120.7	1956.3±110.8
TAN (mg L^{-1})	0.257 ± 0.038	0.178 ± 0.036	0.201 ± 0.039	0.201 ± 0.037
NO_2^--N (mg L^{-1})	0.277 ± 0.060	0.186 ± 0.049	0.210 ± 0.050	0.208± 0.039
NO_3^--N (mg L^{-1})	3.003 ± 0.618	2.851 ± 0.604	2.861 ± 0.577	2.416 ± 0.516

STATISTICAL ANALYSIS

After conducting a homogeneity of variance test using Levene's test and normality with Shapiro-Wilk's test, statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's Multiple Range. A significance level of 0.05 was applied. All data were expressed as mean ± standard deviation (SE). Statistical analyses were performed using the statistical software SPSS version 22.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

WATER QUALITY AND GROWTH PERFORMANCE

Water quality from all treatments was maintained within acceptable ranges for shrimp growth during the culture period.[25] There were no significant differences in all the parameters for shrimp fed with different ginger extract levels compared to the control ($p > 0.05$) (Table 1).

Growth performance parameters and the survival of shrimp during a 56-day feeding trial are shown in Table 2. The values of final weight, WG, SGR, and DWG were significantly higher in shrimp fed T1 group than the control group; however, no significant differences were found among the control, T2 and T3 groups for all parameters. Shrimp fed T1 group had the highest biomass value (2.51 ± 0.03 kg m^{-3}). Meanwhile, the FCR value in T1 group was 1.22 ± 0.7, which was significantly the lowest ($p < 0.05$). Although the FCR revealed no significant differences between the T2 and T3 groups ($p > 0.05$), these values were significantly lower compared to the control group ($p < 0.05$). Moreover, there were no significant differences in the survival rate among all treatment groups ($p > 0.05$).

DIGESTIVE ENZYME ACTIVITY

The digestive enzyme activities in the intestine of shrimp were shown in Figure 1. There was no significant difference in amylase activity among all experimental groups ($p > 0.05$). Shrimp fed T1 group showed a notable increase in protease level when compared with the control and T3 groups ($p<0.05$); however, no significant difference was found in protease activity between the control and T3 groups.

SURVIVAL OF SHRIMP AFTER CHALLENGE

During the 96-h exposure to low salinity stress, there were no significant differences in survival rates among all experimental groups ($p > 0.05$) (Table 3). As for ammonia stress tolerance, the survival of shrimp in T1 and T2 groups at 72 h and 96 h had a significant effect compared with the control and T3 groups ($p < 0.05$), whereas no significant differences were found between T1 and T2 groups or between the control and T3 groups ($p > 0.05$) (Table 4).

DISCUSSION

To develop alternative products for promoting growth in aquaculture, the administration of herbal extracts has been extensively studied in recent years due to its potential benefits with fewer side effects. Ginger extract contains active compounds that could enhance the growth of fish and shrimp.[19] In the present study, the addition of 0.5 g kg^{-1} ginger extract to the feed significantly increased weight gain and specific growth rate of the whiteleg shrimp. This finding is similar to the report of Venkataramalingam et al.,[18] where ginger-enriched artemia increased significantly the growth parameters of black tiger shrimp, *Penaeus monodon* postlarvae. In another study, Shahraki et al.[20] reported that supplementing diets with aqueous extract from ginger had a positive effect on the growth performance of *L. vannamei* postlarvae. The enhanced growth performance could be related to increased nutrient utilization. These functions are attributed to the bioactive compounds of ginger extract which includes gingerols, zingerone, zingiberene, and so on which increased the activity of digestive enzymes.[18,26] For example, gingerols containing ginger can function as an appetizer that stimulates the palatability of food and the secretion of digestive enzyme, leading to increase food consumption.[27] Chang et al.[19] also reported that shrimp fed diets supplemented with different levels of zingerone had a significant increase in the growth and feed efficiency of *L. vannamei* because of promoting appetite in shrimp. In addition, the increase in population of beneficial bacteria in the intestine is considered as one of the reasons for enhancing the growth of aquatic species.[9] Previous studies demonstrated that ginger extract is a good source of phytochemical constituents that help to improve intestinal

Table 2. Growth performance of whiteleg shrimp after 56 days of culture

Parameters	Treatments			
	T0 (control)	T1	T2	T3
Initial weight	0.793 ± 0.005[a]	0.788 ± 0.005[a]	0.798 ± 0.007[a]	0.787 ± 0.003[a]
Final weight	14.21 ± 0.38[a]	15.72 ± 0.1[b]	14.5± 0.15[ab]	14.4 ± 0.43[ab]
WG (g)	13.42 ± 0.38[a]	14.93 ± 0.11[b]	13.44 ± 0.14[ab]	13.61± 0.43[ab]
DWG (g day^{-1})	0.224 ± 0.006[a]	0.249 ±0.004[b]	0.224 ± 0.005[ab]	0.227 ± 0.002[ab]
SGR (% day^{-1})	4.81 ± 0.051[a]	4.989 ± 0.019[b]	4.802 ± 0.015[ab]	4.842 ± 0.053[ab]
Survival rate (%)	76 ± 1.53[a]	80 ± 0.58[a]	77.7 ± 1.2[a]	77 ± 1[a]
Biomass (kg m^{-3})	2.04 ± 0.03[a]	2.51 ± 0.03[c]	2.13 ± 0.03[b]	2.22 ± 0.05[b]
FCR	1.62 ± 0.94[a]	1.22 ± 0.7[c]	1.39 ± 0.8[b]	1.41 ± 0.81[b]

Values shown are mean ± SE (n = 3). Mean values within a column followed by the same letters show that there is no significant difference among the groups (p > 0.05).

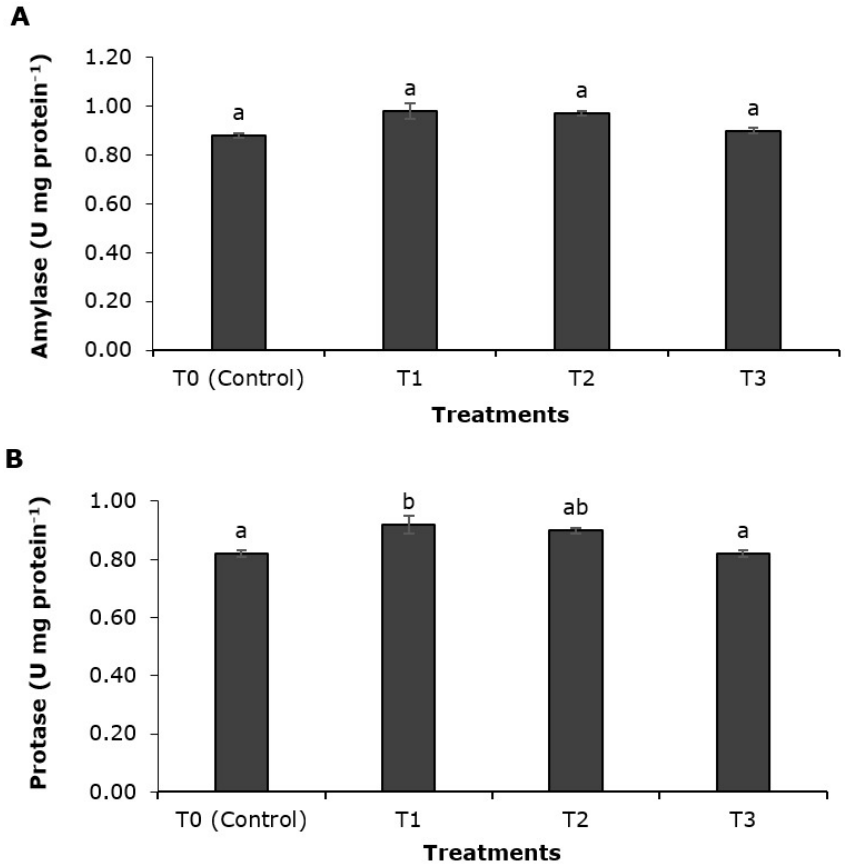

Figure 1. Activities of (A) amylase and (B) protease in *Litopenaeus vannamei* during a 56-day feeding trial with ginger extract supplementation. Values shown are mean ± SD (n = 3). Different lowercase letters above bars are significantly different among the groups (p < 0.05).

health of animals by decreasing the abundance of undesirable bacteria.[28-30] The results of the present study showed that the highest protease activity was recorded in shrimp fed with the diet containing 0.5 g kg^{-1} of ginger extract. Similar results were found in other shrimp species, including *P. monodon*[18] and *Macrobrachium rosenbergii*.[31] The increase in the level of protease activity in the intestine resulted in promoting digestion of dietary protein. However, the activity of amylase in the intestine of shrimp did not correlate with ginger extract supplementation. This finding is in contradiction with the results of study in *P. monodon* showing amylase levels significantly increased following the supplementation of ginger in the feed.[18] This could be explained that the effect of each plant also depends on species-specific, dose supplements, and size and age of shrimp.

Shrimp fed the control diet showed a higher FCR value than those fed supplemented with ginger extract, suggesting that the supplementation of shrimp with ginger extract resulted in reduced FCR. According to Syafirah et al.,[32] ginger contains a fragrant essential oil, which could arouse appetite. Moreover, ginger extract has high mineral and nu-

Table 3. Survival of *L. vannamei* after exposure to low salinity stress during 96 h

Treatments	Survival rate (%)			
	24 h	48 h	72 h	96 h
T0 (control)	77.5 ± 1.4	73.8 ± 2.4	61.3 ± 2.4	51.3 ± 2.4
T1	76.3 ± 1.3	75.0 ± 2.0	60.0 ± 2.0	52.5 ± 1.4
T2	80.0 ± 2.0	75.0 ± 2.0	62.5 ± 1.4	52.5 ± 1.4
T3	76.3 ± 1.3	73.8 ± 2.4	60.0 ± 2.0	51.3 ± 1.3

Values shown are mean ± SE. Mean values within a column show that there is no significant difference among the groups ($p > 0.05$).

Table 4. Survival of *L. vannamei* after exposure to high ammonia stress during 96 h

Treatments	Survival rate (%)			
	24 h	48 h	72 h	96 h
T0 (control)	80.0 ± 2.0^{a}	67.5 ± 1.4^{a}	48.8 ± 1.3^{a}	38.8 ± 1.3^{a}
T1	81.3 ± 2.4^{a}	70.0 ± 2.0^{a}	62.5 ± 3.2^{b}	46.3 ± 3.8^{b}
T2	83.8 ± 1.3^{a}	71.3 ± 2.4^{a}	61.3 ± 2.4^{b}	50.0 ± 2.0^{b}
T3	81.3 ± 2.4^{a}	67.5 ± 3.2^{a}	50.0 ± 2.0^{a}	37.5 ± 3.2^{a}

Values shown are mean ± SE. Mean values within a column show that there is no significant difference among the groups ($p > 0.05$).

trient components that stimulate the digestion of animals and absorption properties.[33] Determining how to enhance tolerance towards environmental stress (salinity, ammonia, pH, and temperature fluctuations) is necessary for the shrimp culture industry. A drastic change in salinity and ammonia may directly impact the growth and survival of penaeid shrimp throughout the culture period.[34,35] Several studies have demonstrated that plant extracts contain active compounds that help to cope with stress.[36] Ginger has been reported to have powerful antioxidant activity, effective against stress.[37] In the present study, adding 0.5 g·kg^{-1} ginger extract to the diet enhanced the survival of shrimp after exposure to high ammonia stress. Citarasu et al.[38] reported that *P. monodon* postlarvae (PL1-30) fed five herbal extracts (*Hygrophila spinosa, Withania somnifera, Zingiber officinalis, Solanum trilobatum,* and *Andrographis paniculata*) showed higher tolerance to stress conditions (pH, formalin and salinity) compared to the control group. In addition, the medicinal plant *Mucuna pruriens* extract effectively improved resistance against high thermal stress in ornamental fish *Botia rostrata*.[39]

In conclusion, the present study indicated that dietary supplementation of ginger extract could be used as a feed additive to improve growth performance and ammonia stress tolerance of juvenile *L. vannamei,* and the optimum dose is 0.5 g kg^{-1}. However, ginger extract did not affect the survival and resistance to low salinity of whiteleg shrimp. Future research should evaluate ginger extract's effect on the whiteleg shrimp's immunological parameters.

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AUTHORS' CONTRIBUTION PER CREDIT

Conceptualization: Phan Thi Cam Tu (Equal), Nguyen Thi Kim Lien (Equal). Methodology: Phan Thi Cam Tu (Equal), Nguyen Thi Kim Lien. Formal Analysis: Phan Thi Cam Tu (Equal), Doan Xuan Diep (Equal). Investigation: Phan Thi Cam Tu (Equal), Doan Xuan Diep (Equal). Writing – original draft: Phan Thi Cam Tu (Lead). Writing – review & editing: Doan Xuan Diep (Equal), Tien Hai Ly (Equal). Supervision: Tien Hai Ly (Lead).

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