Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Dietary sweet potato leaf extract enhances growth performance, antioxidant gene expression, and health status of whiteleg shrimp (Litopenaeus vannamei)

Thi Cam Tu Phan
Can Tho University
Thanh Men Tran
Can Tho University
Thi Phuong Thao Truong
truongthiphuongthao@ctu.edu.vn
Can Tho University

Research Article

Keywords: Litopenaeus vannamei, sweet potato, leaf extract, antioxidant gene

Posted Date: November 24th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11262822/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

This study evaluated the efficacy of sweet potato leaf extract (SPE) on growth performance, antioxidant activity and health of *Litopenaeus vannamei*. In experiment 1, juvenile whiteleg shrimp were fed diets supplemented with 0 (control), 0.25, 0.5, and 1% SPE for 56 days. The diet containing 0.5% SPE showed the highest values of growth parameters ($p = 0.001$) compared to other treatments. Similarly, digestive enzyme activities, including amylase and leucine aminopeptidase, were also significantly higher in the 0.5% SPE group than in the other treatments ($p = 0.002$; $p = 0.003$). Moreover, shrimp-fed diets supplemented with 0.25, 0.5, and 1.0% SPE exhibited significantly higher survival rates than the control group ($p = 0.001$). Additional benefits included increased whole-body protein content and reduced whole-body lipid content in the 0.5% SPE group, suggesting enhanced shrimp product quality. Dietary supplementation with SPE significantly influenced antioxidant-related transcriptional responses, with 0.5% SPE eliciting the highest relative expression of CAT, SOD, and GSH-Px. In experiment 2, after four weeks of feeding the experimental diets, shrimp were challenged by immersion in *Vibrio parahaemolyticus* (1×10^5 CFU/mL) for 14 days. The results showed that cumulative mortality was significantly lower in all SPE-supplemented groups than in the positive control group ($p = 0.005$). Overall, the findings suggest that dietary SPE supplementation can improve shrimp growth performance and health status, potentially by modulating antioxidant-related gene expression. These results support the potential application of SPE as a functional feed additive for sustainable shrimp aquaculture.

Introduction

Currently, whiteleg shrimp (*Litopenaeus vannamei*) has been widely cultured as one of the major aquaculture species in Vietnam, playing a pivotal role in the country's seafood industry and export economy (Phan et al. 2026). However, the increasing demand for higher production has led to the widespread adaption of intensive farming practices, which have exacerbated environmental stress, deteriorated water quality, and increased the incidence of disease outbreaks. These challenges often result in oxidative stress, immune suppression, and substantial production losses. To address these issues, various sustainable strategies have been developed to improve water quality, enhance disease resistance, and reduce the dependence on antibiotics in aquaculture. Among these strategies, the use of natural sources, particularly agricultural by-products, has gained considerable attention as a sustainable approach to improving aquaculture productivity and animal health. Several studies demonstrated the efficiency of plant extracts as dietary supplements in aquatic animals. For example, dietary supplementation with plant extracts improved growth performance, antioxidant capacity, immune system and disease resistance in several fish species, including catfish, *Ictalurus punctatus* (Lochmann et al. 2013), Senegalese Sole, *Solea senegalensis* (Xavier et al. 2021), common carp, *Cyprinus carpio* (Abdel Rahman et al. 2022), and largemouth bass, *Micropterus salmoides* (Yue et al. 2024). In shrimp aquaculture, numerous plant-derived feed additives have shown promising biological effects. For example, dietary supplementation with noni (*Morinda citrifolia*) leaf extract enhanced growth performance, digestive enzyme activity, and resistance to *Vibrio parahaemolyticus* infection in whiteleg shrimp, *Litopenaeus vannamei* (Phan et al. 2023). Likewise, supplementation with medicinal plant

extracts has been reported to significantly promote growth, immune competence, antioxidant status, and disease resistance in the same species (Fadel et al. 2025).

Sweet potato (*Ipomoea batatas* L.) is an important food crop cultivated worldwide for its nutritional and functional properties. In Vietnam, sweet potato (*Ipomoea batatas* L.) is widely cultivated across the Mekong Delta, Red River Delta, Central Highlands, and other agricultural regions due to its adaptability to diverse agro-ecological conditions, relatively low production cost, and high nutritional value. While the storage roots are extensively utilized for human consumption, the leaves remain an underexploited agricultural resource despite being rich in proteins, dietary fiber, polyphenols, flavonoids, chlorogenic acid, caffeoylquinic acids, carotenoids, vitamins C and E, and essential minerals (Nguyen et al. 2021; Tang et al. 2021). Sweet potato leaves possess remarkable antioxidant capacity and have been reported to exhibit antimicrobial, anti-inflammatory, antidiabetic, and immunomodulatory activities, primarily due to their high levels of phenolic compounds (Sun et al. 2014). These biological properties suggest that sweet potato leaf extract could serve as an effective phytogenic feed additive in aquaculture. However, information regarding its dietary additive in whiteleg shrimp remains limited, particularly concerning its effects on antioxidant defenses, innate immunity, and disease resistance. Therefore, the present study evaluated the effects of dietary sweet potato leaf extract (SPE) supplementation on the antioxidant capacity and innate immune responses of whiteleg shrimp (*Litopenaeus vannamei*). In addition, a bacterial challenge test using *Vibrio parahaemolyticus*, one of the major bacterial pathogens affecting shrimp culture, was carried out to assess the effect of dietary SPE supplementation on disease resistance.

Materials and Methods

Sweet potato extract and phytochemical screening

Sweet potato leaves were collected and weighed immediately to determine the wet weight (m_0). The samples were washed and then dried at 40–50°C to constant weight and subsequently weighed to obtain the dry weight (m_1). The dried materials were ground into a fine powder and extracted with ethanol 99% during 24h. The solvent extracts were filtered and concentrated under reduced pressure using a vacuum rotary evaporator (Phung 2007). The extraction yield was calculated using the following equation, with an extraction yield of 6.76%:

$H\% = [(m_0 - m_1) / m_0] \times 100\%.$

Total polyphenol content was determined according to the method of Singleton et al. (1999) with minor modifications. Briefly, 250 µL of sample extract was diluted with 250 µL of distilled water and mixed with 250 µL of Folin–Ciocalteu reagent. Subsequently, 250 µL of 10% Na_2CO_3 was added, and the mixture was incubated at 40°C for 30 min in a thermostatic water bath. The absorbance was measured at 765 nm using a spectrophotometer. The total polyphenol content was calculated based on a calibration curve constructed using gallic acid and expressed as mg GAE/g extract. Total flavonoid and saponin contents

were determined according to the methods described by Bag et al. (2015) and Anh et al. (2018), respectively. Total polysaccharide content was determined using D-glucose (anhydrous) as the standard, with absorbance measured at 488 nm following He et al. (2018).

Feed preparation and shrimp culture

A commercial feed (42% crude protein, 11% moisture, 4% crude lipid, 10% crude fiber, TongWei VietNam Co., Ltd., Vietnam) was used as the control diet, whereas three experimental diets were formulated by incorporating sweet potato leaf extract (SPE) into the commercial feed at inclusion levels of 0.25%, 0.5%, and 1.0%. The appropriate concentrations of sweet potato leaf extract (SPE) were diluted in distilled water and uniformly sprayed onto the surface of the feed pellets. To ensure consistency in diet preparation across all experimental groups, the control diet was sprayed with an equivalent volume of distilled water. All coated pellets were then air-dried at room temperature for 24–48 h and subsequently stored at 4°C until use in the feeding experiment.

Healthy post-larvae of *Litopenaeus vannamei* (PL12) were obtained from a private company in Ca Mau Province, Vietnam, and transported to the wet laboratory of the College of Aquaculture and Fisheries, Can Tho University. Upon arrival, shrimp post-larvae were acclimated and subsequently divided into two groups, which were reared separately in two 4-m^3 composite tanks for Experiment 1 and Experiment 2, respectively. The pre-experimental rearing period lasted 4 weeks for Experiment 1 and 2 weeks for Experiment 2, allowing to attain the desired initial body weight for their respective experiments. During this period, shrimp were hand-fed four times daily (07:00, 11:00, 15:00, and 19:00 h) at a feeding rate of 7–8% of their body weight using a commercial feed (48% crude protein; 11% moisture, 4% crude lipid, 10% crude fiber, TongWei VietNam Co., Ltd., Vietnam) of the same type as that used in the subsequent feeding experiments. Throughout the rearing period, water quality parameters, including salinity, alkalinity, pH, dissolved oxygen (DO), and nitrite, were monitored and maintained within optimal ranges for *L. vannamei* culture (salinity: 15.0 ± 0.1 ppt; alkalinity: 140 ± 5 mg $CaCO_3$ L^{-1}; pH: 8.2 ± 0.1; DO: > 5.0 mg L^{-1}). The tank was siphoned every three days to remove accumulated detritus.

Experiment 1. Effect of sweet potato extract on growth performance, antioxidant activity and proximate composition of the whiteleg shrimp

A total of 1200 shrimp, with an initial weight of 0.57 ± 0.15 g, were divided into four groups with three replicates per group. The experiment was conducted using a completely randomized design (CRD) with four dietary treatments: a control diet and three diets supplemented with 0.25%, 0.5%, and 1% SPE. The experiment lasted for 8 weeks. The shrimp were weighed before being randomly assigned to one of 500-L tanks (100 shrimp per tank). Shrimp were fed four times daily at a feeding rate of 3–5% of their body weight. Water temperature and pH were measured twice daily using a pH meter, whereas total ammonia nitrogen (TAN), alkalinity, and nitrite concentrations were determined weekly according to standard methods described by APHA (2017). Additionally, uneaten feed was collected and recorded daily

throughout the experimental period to calculate the feed conversion ratio (FCR) at the end of the experiment.

Experiment 2. Effect of sweet potato extract the disease resistance of whiteleg shrimp against Vibrio parahaemolyticus

The whiteleg shrimp (initial weight 0.15 ± 0.02 g) were randomly allocated to four dietary treatments following a completely randomized design: a control diet without supplementation and diets supplemented with 0.25%, 0.5%, and 1% SPE. After 4 weeks of the feeding experimental diets, 60 shrimp (2.10 ± 0.05 g) from each treatment were randomly distributed into three fiberglass tanks, with 20 shrimp per tank, for the subsequent challenge experiment. The challenge experiment consisted of five treatments: a negative control, a positive control, and three experimental groups corresponding to the dietary supplementation levels. Subsequently, shrimp were challenged with *Vibrio parahaemolyticus* (VP_{AHPND}) at a concentration of 10^5 CFU/mL by immersion method, based on the result of 24h-LD_{50} (data not shown). The VP_{AHPND} strain was cultured in Tryptic Soy Broth (TSB) supplemented with 1.5% NaCl at 30°C under continuous shaking (200 rpm) until the desired cell density was reached. Daily mortality was recorded for 14 days to determine cumulative mortality. To confirm successful VP_{AHPND} infection, the hepatopancreas of dead shrimp in each group were sampled and analyzed by PCR following the method described by Oanh et al. (2018). During the challenge test, shrimp were fed the experimental diets supplemented with SPE twice daily. Water quality parameters including temperature (28–30°C), salinity (14–15 ppt), pH (7.5–8.5), dissolved oxygen (> 5.5 mg/L), and ammonia (< 0.1 mg/L) were maintained within suitable ranges.

Determination of growth performance and digestive enzyme activities

After the 8-week feeding trial, 30 surviving shrimp were randomly selected from each of the three replicate tanks (90 shrimp per treatment). All shrimp were individually weighed to assess growth performance. The replicate tank was considered the experimental unit, and tank means were used for statistical analyses (n = 3). Growth indices, including final weight (FW), weight gain (WG), daily weight gain (DWG), specific growth rate (SGR), and survival rate (SR), were calculated using the equations presented by Phan et al. (2023). Feed intake was recorded daily throughout the experimental period to determine the feed conversion ratio (FCR).

Three shrimp were randomly sampled from each replicate, resulting in nine intestinal samples per treatment (n = 9). These shrimp were anesthetized using eugenol (1:10,000) and subsequently dissected on ice to collect intestinal samples for further analyses. Following dissection, the intestinal samples were immediately homogenized in 1:9 (w/v) ice-cold 0.85% physiological saline and centrifuged at 2,800 × g for 10 min at 4°C. The supernatants were collected and used to determine the activities of amylase (Bernfeld 1955), protease (Lowry et al. 1951), and leucine aminopeptidase (Ezquerra et al. 1999).

Determination of antioxidant gene response

At the end of the experiment, three shrimp were randomly selected from each tank (9 shrimp per treatment). The shrimp were also anesthetized with eugenol (1:10,000) and subsequently frozen at −80°C until analyzing. Equal amounts of tissue (60 mg) from each shrimp were pooled for RNA extraction. Each pooled sample represented one biological replicate, resulting in three biological replicates per treatment (n = 3). Total RNA was extracted from tissue samples using the Quick-RNA™ Miniprep Kit (R1054/R1055; Zymo Research, USA), according to the manufacturer's instructions. RNA concentration and purity were assessed spectrophotometrically prior to RT-qPCR analysis.

The relative mRNA expression levels of three antioxidant-related genes, catalase (CAT), manganese superoxide dismutase (Mn-SOD), and glutathione peroxidase (GSH-Px), were determined by real-time quantitative reverse-transcription PCR (RT-qPCR). Primers for CAT, Mn-SOD, GSH-Px, and β-actin were selected based on published *Litopenaeus vannamei* sequences and the corresponding sequences deposited in GenBank (Table 1). The expected amplicon sizes were 147 bp for CAT (AY518322.1), 122 bp for Mn-SOD (DQ005531), 117 bp for GSH-Px (AY973252), and 121 bp for β-actin (AF300705). The primer sequences and expected amplicon sizes were cross-checked against previously published *L. vannamei* RT-qPCR assays.

RT-qPCR was performed using the ZymoScript™ One-Step RT-qPCR Kit with SYBR Green chemistry on a StepOnePlus™ Real-Time PCR System (Applied Biosystems, USA). The thermal cycling program consisted of an initial step at 95°C for 10 min, followed by 40 cycles of 95°C for 20 s and 60°C for 1 min. A melt-curve analysis was performed immediately after amplification to evaluate the specificity of the PCR products. The melt-curve profiles of β-actin, CAT, Mn-SOD, and GSH-Px were examined for a single predominant melting peak, indicating a single major amplification product. Representative melt curves for all four genes are provided in Supplementary Figure S1.

β-actin was used as the reference gene for normalization. Because a single reference gene was used in the original experimental design, its expression stability across dietary treatments was evaluated statistically before normalization. β-actin Ct values did not differ significantly among the control, 0.25%, 0.5%, and 1.0% SPE groups (one-way ANOVA, $p = 0.147$), indicating no detectable treatment-associated variation in β-actin expression within the present dataset. Nevertheless, the use of a single reference gene is acknowledged as a methodological limitation.

Relative gene expression was calculated using the $2^{\wedge -\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). For each biological replicate, ΔCt was calculated as Ct_target − Ct_β-actin. The mean ΔCt of the control group was used as the calibrator to calculate ΔΔCt, and relative expression ($2^{\wedge -\Delta\Delta Ct}$) was calculated independently for each biological replicate. Thus, biological variability within the control group was retained rather than assigning an identical relative-expression value of 1 to all control replicates. Relative expression data are presented as mean ± SE of three independent biological replicates (n = 3).

Table 1
Primer sequences used for RT-qPCR analysis of antioxidant-related gene expression in *Litopenaeus vannamei*.

Gene	Primer sequence (5'-3')	GenBank accession no.	Amplicon size (bp)
β-actin-F	GCCCATCTACGAGGGATA	AF300705	121
β-actin-R	GGTGGTCGTGAAGGTGTAG		
Mn-SOD-F	CTGGTTCCGTTGCTTGGC	DQ005531	122
Mn-SOD-R	CGCTCATTCACGTTCTCCC		
CAT-F	TCAGCGTTTGGTGGAGAA	AY518322.1	147
CAT-R	GCCTGGCTCATCTTTATC		
GSH-Px-F	AGGGACTTCCACCAGATG	AY973252	117

Determination of proximate composition

Six shrimp from each tank were randomly selected for crude protein, crude lipid, moisture and ash of shrimp muscle. The proximate analysis of shrimp was determined according to the standard methods of AOAC (2000). Moisture content was measured by oven drying at 105°C to constant weight. Crude protein was analyzed using the Kjeldahl method. Crude lipid was determined by Soxhlet extraction using petroleum ether, while ash content was measured by incineration in a muffle furnace at 550°C for 24h.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's multiple range test to determine significant differences among treatments at $p < 0.05$. The normality of the data was evaluated using the Shapiro–Wilk test, while homogeneity of variances was assessed using Levene's test prior to conducting parametric analyses. Results are presented as mean ± standard error (SE). All statistical analyses were performed using SPSS software (version 22.0).

Results

Quantitative phytochemical characteristics of sweet potato leaf extract

Quantitative colorimetric assays were used to characterize selected phytochemical indices of sweet potato leaf extract (SPE). The total polyphenol content was 487.50 ± 1.79 mg gallic acid equivalents (GAE)/g extract, while the total flavonoid, polysaccharide, and saponin contents were 390.95 ± 8.99 mg quercetin equivalents (QE)/g extract, 387.50 ± 8.33 mg glucose equivalents (GE)/g extract, and 324.41 ± 1.03 mg aescin equivalents (ACE)/g extract, respectively (Table 2).

Table 2
Quantitative phytochemical indices of sweet potato leaf extract

Phytochemical index	Content
Polyphenols	487.5 ± 1.79 mg GAE/g extract
Flavonoids	390.95 ± 8.99 mg QE/g extract
Saponins	324.41 ± 1.03 mg ACE/g extract
Polysaccharides	387.5 ± 8.33 mg GE/g extract

Values are expressed as mean ± SE of three replicates (n = 3) obtained from a pooled extract sample prepared by combining extracts from separate extraction batches. GAE, gallic acid equivalents; QE, quercetin equivalents; GE, glucose equivalents; ACE, aescin equivalents. Values were obtained from separate colorimetric assays using different calibration standards and represent assay-specific equivalent concentrations rather than mutually exclusive mass fractions. Therefore, values across phytochemical classes should not be summed to estimate total phytochemical content.

Importantly, these values represent assay-specific equivalent concentrations derived from separate colorimetric assays and calibration standards, rather than direct gravimetric concentrations of mutually exclusive chemical fractions. Therefore, the numerical values obtained for polyphenols, flavonoids, polysaccharides, and saponins should not be summed to estimate the total phytochemical mass per gram of extract. In particular, flavonoids constitute a subclass within the broader polyphenolic fraction and therefore are not chemically independent of the total polyphenol measurement.

Physicochemical factors of water

Throughout the feeding trial, water quality parameters remained within the acceptable ranges for *L. vannamei* culture (Table 3). The recorded ranges of temperature, pH, dissolved oxygen, alkalinity, TAN, and NO_2^- were 26.6–28.4°C, 8.0-8.4, 5.06–6.07 mg/L, 110.7-177.3 mg$CaCO_3$/L, 0.090–1.552 mg/L, and 0.038–1.906 mg/L, respectively.

Table 3
Water quality parameters monitored during the culture period

Water parameters	Treatments			
	Control	**SPE 0.25%**	**SPE 0.5%**	**SPE 1%**
Temperature (^oC)	26.8 ± 0.2	26.9 ± 0.1	26.8 ± 0.1	26.9 ± 0.1
pH	8.32 ± 0.17	8.33 ± 0.13	8.30 ± 0.10	8.33 ± 0.14
DO (mg/L)	5.95 ± 0.4	5.85 ± 0.67	5.92 ± 0.51	6.00 ± 0.32
Alkalinity ($mgCaCO_3/L$)	147.6 ± 8.0	150.3 ± 6.1	147.5 ± 8.7	148.3 ± 8.33
TAN (mg/L)	1.253 ± 0.027	1.488 ± 0.060	1.247 ± 0.091	1.415 ± 0.072
NO_2^--N (mg/L)	0.855 ± 0.046	1.057 ± 0.074	0.742 ± 0.039	1.037 ± 0.065

Data are presented as mean ± standard error (SE) of three replicates (n = 3). No significant differences were observed among treatments for any of the measured parameters (p > 0.05).

Growth performance and survival

On day 56, shrimp fed diets containing 0.25%, 0.5% and 1.0% SPE exhibited significantly higher survival rates than those in the control group ($p < 0.05$) (Table 4). However, no significant differences in survival rate were observed among the three SPE-supplemented groups ($p > 0.05$). Growth parameters including final weight, WG, DWG and SGR, showed significant improvement in shrimp fed diets containing 0.5% SPE compared to the other treatment groups ($p < 0.05$). The greatest mean FCR was recorded for shrimp fed diet 0.25%, while the lowest value was found for shrimp fed diet 0.5% SPE.

Table 4
Growth performance of *Litopenaeus vannamei* fed diets supplemented with different levels of sweet potato extract

Parameters	Treatments			
	Control	**SPE 0.25%**	**SPE 0.5%**	**SPE 1%**
Initial weight (g)	0.57 ± 0.007^{a}	0.57 ± 0.012^{a}	0.57 ± 0.007^{a}	0.57 ± 0.011^{a}
Final weight (g)	10.69 ± 0.1^{a}	10.88 ± 0.04^{a}	12.39 ± 0.11^{b}	10.68 ± 0.03^{a}
Final weight (g)	10.69 ± 0.1^{a}	10.88 ± 0.04^{a}	12.39 ± 0.11^{b}	10.68 ± 0.03^{a}
WG (g)	10.12 ± 0.1^{a}	10.31 ± 0.05^{a}	11.82 ± 0.1^{b}	10.11 ± 0.04^{a}
DWG (g/day)	0.169 ± 0.002^{a}	0.172 ± 0.002^{a}	0.197 ± 0.002^{b}	0.169 ± 0.001^{a}
SGR (%/day)	4.887 ± 0.004^{a}	4.91 ± 0.042^{a}	5.134 ± 0.007^{b}	4.876 ± 0.037^{a}
Survival rate (%)	83.0 ± 1.0^{a}	94.0 ± 2.0^{b}	91.7 ± 2.1^{b}	93.3 ± 2.9^{b}
FCR	1.35 ± 0.04^{a}	1.21 ± 0.05^{ab}	1.16 ± 0.02^{b}	1.25 ± 0.03^{ab}

Data are presented as mean ± standard error (SE) of three replicates (n = 3). Different superscript letters (a, b, c) indicate significant differences among treatments ($p < 0.05$).

Digestive enzyme activity

Dietary SPE supplementation significantly enhanced digestive enzyme activities in shrimp (Table 5). Amylase activity increased significantly in all SPE-supplemented groups compared with the control, with the highest value in shrimp fed the 0.5% SPE diet. Protease activity was also significantly higher in all SPE treatments compared to the control, although no significant differences were detected among the SPE-supplemented groups. Similarly, leu-aminopeptidase activity was significantly increased by SPE supplementation, with the greatest activity recorded in shrimp fed the 0.5% SPE diet.

Table 5
Digestive enzyme activities of *Litopenaeus vannamei* fed diets supplemented with different levels of sweet potato extract

Parameters	Treatments			
	Control	SPE 0.25%	SPE 0.5%	SPE 1%
Amylase (U/mg protein)	0.312 ± 0.057^{a}	0.388 ± 0.036^{b}	0.467 ± 0.036^{c}	0.412 ± 0.027^{b}
Protease (U/mg protein)	0.097 ± 0.019^{a}	0.212 ± 0.023^{b}	0.217 ± 0.040^{b}	0.196 ± 0.016^{b}
leu-Aminopeptidase (U/mg protein)	0.108 ± 0.019^{a}	0.172 ± 0.022^{bc}	0.196 ± 0.018^{c}	0.152 ± 0.016^{b}

Data are presented as mean ± standard error (SE) (n = 9). Different superscript letters (a, b, c) indicate significant differences among treatments (p < 0.05).

Body composition analysis of shrimp

Total protein and moisture contents in shrimp varied significantly among the dietary treatments (Table 6). Specifically, shrimp fed the diet supplemented with 0.5% SPE exhibited the highest protein content (19.29%), which was significantly higher than that of shrimp in the other dietary treatment groups. In contrast, the same group exhibited the lowest moisture content, which was significantly lower than that of shrimp in the other dietary treatment groups ($p < 0.05$). Moreover, shrimp fed the SPE-supplemented diets showed significantly lower whole-body lipid content than those fed the control diet ($p < 0.05$). However, no significant differences in ash content were observed among the four dietary treatments.

Table 6
Whole-body chemical composition of *Litopenaeus vannamei* fed diets supplemented with different levels of sweet potato extract

Parameters	Treatments			
	Control	SPE 0.25%	SPE 0.5%	SPE 1%
Moisture (%)	78.0 ± 0.12^{a}	78.65 ± 0.04^{b}	77.11 ± 0.14^{c}	78.45 ± 0.18^{b}
Protein (%)	18.67 ± 0.15^{a}	18.77 ± 0.24^{a}	19.29 ± 0.20^{b}	18.73 ± 0.25^{a}
Lipid (%)	0.70 ± 0.04^{a}	0.43 ± 0.01^{b}	0.48 ± 0.06^{b}	0.46 ± 0.05^{b}
Ash (%)	1.28 ± 0.03^{a}	1.30 ± 0.07^{a}	1.38 ± 0.04^{a}	1.30 ± 0.08^{a}

Data are presented as mean ± standard error (SE) of three replicates (n = 3). Different superscript letters (a, b, c) indicate significant differences among treatments (p < 0.05).

Effects of dietary SPE on antioxidant-related gene expression

Dietary supplementation with sweet potato leaf extract (SPE) significantly affected the relative mRNA expression of CAT, SOD, and GSH-Px in *Litopenaeus vannamei* ($p < 0.05$; Fig. 1). The expression patterns were treatment-dependent and non-linear, with the highest mean expression of all three genes observed in shrimp fed 0.5% SPE. CAT expression differed significantly among all four dietary treatments ($p < 0.05$; Fig. 1a). Compared with the control, CAT expression increased in shrimp fed 0.25% SPE and reached its highest level at 0.5% SPE, approximately 2.4-fold higher than the control. At 1.0% SPE, CAT expression was significantly lower than that at 0.5% SPE but remained significantly higher than the control and 0.25% SPE treatments.

For SOD, no significant difference was observed between the control and 0.25% SPE treatments ($p > 0.05$; Fig. 1b). Expression increased significantly at 0.5% SPE, reaching approximately 3.1-fold relative to the control and representing the highest level among the four treatments ($p < 0.05$). At 1.0% SPE, SOD expression was significantly lower than at 0.5% SPE but remained significantly higher than in the control and 0.25% SPE groups. Similarly, GSH-Px expression did not differ significantly between the control and 0.25% SPE groups ($p > 0.05$; Fig. 1c). The highest expression was observed at 0.5% SPE, approximately 1.8-fold relative to the control and significantly higher than the other treatments ($p < 0.05$). Expression at 1.0% SPE was significantly lower than at 0.5% SPE but remained significantly higher than in the control and 0.25% SPE groups. Summarily, these results show that dietary SPE altered the transcription of the three antioxidant-related genes examined, with 0.5% SPE producing the highest relative expression under the experimental conditions. Increasing the inclusion level from 0.5% to 1.0% did not further increase transcript abundance.

Mortality after challenge with Vibrio parahaemolyticus

Following the challenge test, cumulative mortality increased progressively in all challenged groups (Fig. 2). The positive control exhibited the highest cumulative mortality, reaching 50% by day 11 and remaining unchanged until the end of the experiment. Dietary supplementation with SPE significantly decreased cumulative mortality compared with the positive control group ($p < 0.05$). Among the SPE treatments, the 0.5% supplementation resulted in the lowest cumulative mortality, followed by the 1% (28%) and 0.25% (36%) supplementation groups. However, no significant differences were observed between the SPE 0.5% and SPE 1% groups or between the SPE 0.25% and SPE 0.5% groups ($p > 0.05$). Cumulative mortality in the negative control remained low throughout the experimental period, with a final value of only 5%.

Discussion

This study demonstrated that the ethanol extract of sweet potato leaves contains several bioactive compounds, including polyphenols, flavonoids, saponins, and polysaccharides, with polyphenols being

the most abundant. These findings are consistent with those of Sun et al. (2014), who reported high polyphenol contents in the leaves of forty sweet potato cultivars grown in China. These phytochemical compounds may contribute to improved growth performance and enhanced antioxidant capacity in crustaceans, including shrimp (Ghosh et al. 2021; Wei et al. 2024). For example, both the growth performance and antioxidative capacity in *L. vannamei* were improved by adding polysaccharide from *Jania adharens* and *Amphiroa fragilissima* extracts into the diet (Muttharasi et al. 2021; Akbary et al. 2020). Several studies have demonstrated that growth performance of shrimp might be related to the presence of polyphenol compounds in plant extracts, as well as the flavonoids and saponins contained in its (Qi et al. 2017; Li et al. 2023; Li et al. 2024). The present study demonstrated that dietary supplementation with sweet potato leaf extract effectively enhanced growth performance, as evidenced by improved growth parameters and a reduced feed conversion ratio (FCR). These improvements may contribute to greater economic viability and profitability in shrimp farming.

The enhanced growth performance of whiteleg shrimp fed diets supplemented with SPE may be attributed to promoting nutrient metabolism and absorption, thereby enhancing better growth. Among the SPE-supplemented diets, SPE at 0.5% significantly improved the growth performance of whiteleg shrimp. Similarly, supplementation of guava leaf extract at 5 g/kg diet for 56 days significantly improved growth performance in whiteleg shrimp (Dewi et al. 2021). AftabUddin et al. (2021) also reported enhanced growth performance in *L. vannamei* fed diets supplemented with *Padina tetrastromatica* extract at 5 g/kg. In another study, dietary supplementation with *Moringa oleifera* leaf extract at 2.5 g/kg positively affected growth performance in the same species (Abidin et al. 2021). These findings suggest that the optimal concentration of leaf extracts may vary in Pacific white shrimp depending on the plant species from which the extract is derived. In the present study, higher concentrations of SPE (1%) did not further improve growth performance or FCR, suggesting that the optimal inclusion level of SPE may differ from those of other leaf extracts due to differences in their phytochemical composition and biological activity. This lack of improvement may be associated with the presence of antinutritional factors in sweet potato, as in many plants, including polysaccharides, polyphenols, flavonoids, saponins, and alkaloids. These compounds can bind to digestive enzymes, thereby reducing nutrient digestibility and availability (Mbokane and Moyo 2022). Büyükçapar et al. (2007) also reported that excessive intake of antinutritional factors may adversely affect diet palatability and nutrient balance, intestinal morphology, and the composition of beneficial gut microbiota. Moreover, the polyphenolic compounds present in SPE may have contributed to enhanced nutrient digestion and absorption, thereby supporting growth performance in shrimp.

Digestive enzyme activity is a critical indicator of nutrient digestion and utilization, which is closely associated with growth performance in aquatic animals (Xia et al. 2018; Lin et al. 2023; Klahan et al. 2023). In the present study, supplementation with sweet potato leaf extract, which is rich in polysaccharides (387.5 ± 8.33 mgGE/g extract), enhanced the activities of amylase, protease, and aminopeptidase in *L. vannamei*, suggesting improved digestive capacity and nutrient utilization. In consistent with these findings, the administration of optimal levels of moringa (*Moringa oleifera*) (Abidin et al. 2022), olive (*Olea europaea*) (Yang et al. 2026), and noni (*Morinda citrifolia*) (Phan et al. 2026) leaf

extracts has been reported to enhance digestive enzyme activity in *L. vannamei*. These enhancements in digestive enzyme activities may have facilitated more efficient nutrient digestion and utilization, contributing to the improved growth performance and survival rates observed in this study. In addition, Lu et al. (2020) reported that enhanced digestive enzyme activities may have improved nutrient utilization, thereby contributing to increased dry matter, crude protein, and ash content and greater protein deposition observed in shrimp. Indeed, the whole-body crude protein content was significantly higher in shrimp fed a diet supplemented with 0.5% SPE than in those fed the control diet. Wu et al. (2021) reported that the proportion of crude protein in *L. vannamei* ranged from 17.70–18.71%. The relatively high protein content of shrimp contributes substantially to its nutritional value as a seafood product (Liu et al. 2021).

Dietary SPE supplementation significantly altered the relative expression of CAT, SOD, and GSH-Px in *L. vannamei*, with the highest transcript abundance of all three genes observed at 0.5% SPE. In contrast, increasing SPE inclusion to 1.0% resulted in significantly lower expression than at 0.5%. These findings indicate a treatment-dependent, non-linear transcriptional response and suggest that the response of these antioxidant-related genes did not increase proportionally with dietary SPE concentration. The three genes investigated are associated with complementary components of cellular antioxidant defense in aquatic animals (Ye et al. 2024). SOD catalyzes the dismutation of superoxide radicals into hydrogen peroxide (H_2O_2) (Trasviña-Arenas et al. 2013), whereas CAT and GSH-Px catalyze the removal of H_2O_2 and other peroxides (Brigelius-Flohé and Maiorino 2013; Ge et al. 2022). Thus, the simultaneous changes in SOD, CAT, and GSH-Px transcript abundance indicate that dietary SPE influenced the transcription of genes associated with cellular redox regulation. However, these findings represent changes at the mRNA level and do not, by themselves, demonstrate an improvement in functional antioxidant status. The phytochemical characteristics of SPE provide a plausible biological context for the observed transcriptional responses. Sweet potato leaves contain diverse bioactive constituents, particularly phenolic acids and flavonoids, including caffeoylquinic acid derivatives, that have been associated with antioxidant and other biological properties (Johnson and Pace 2010; Wang et al. 2016; Zhang et al. 2019; Nguyen et al. 2021). In the present study, SPE also exhibited substantial polyphenols, flavonoids, polysaccharides, and saponin-equivalent assay responses. These phytochemicals may therefore be associated with the transcriptional responses observed in SPE-fed shrimp. Nevertheless, the present experimental design does not establish a causal relationship between any individual phytochemical or phytochemical class and the expression of CAT, SOD, or GSH-Px.

Notably, the highest expression of all three genes occurred at 0.5% SPE rather than at the highest dietary inclusion level. The reduction in transcript abundance at 1.0% SPE indicates that the molecular response was non-linear within the tested concentration range. A similar pattern was observed in growth performance, with the 0.5% SPE treatment also producing the most favorable responses. This correspondence suggests that 0.5% SPE represented an effective dietary inclusion level under the conditions of the present experiment. However, the association between growth performance and gene expression should not be interpreted as evidence that the transcriptional changes caused the improved growth. Likewise, the mechanism responsible for the lower response at 1.0% SPE cannot be established

from the present data. Importantly, increased CAT, SOD, and GSH-Px transcript abundance should not be considered equivalent to enhanced antioxidant capacity or improved antioxidant status. RT-qPCR quantifies relative transcript abundance, whereas functional antioxidant defense depends additionally on translation, protein abundance, enzyme activity, post-translational regulation, substrate availability, and cellular redox conditions. Because antioxidant enzyme activities and direct oxidative stress biomarkers were not measured in the present study, the current results should be interpreted specifically as modulation of antioxidant-related gene expression rather than as evidence of enhanced antioxidant function. Future studies combining transcriptional analysis with CAT, SOD, and GSH-Px enzyme activities and oxidative-stress indicators, such as reactive oxygen species and lipid peroxidation products, would be necessary to determine whether these transcriptional changes translate into functional antioxidant effects.

Interestingly, the present study showed that shrimp fed SPE-supplemented diets exhibited lower mortality than those fed the control diet following immersion challenge with *V. parahaemolyticus*. In particular, cumulative mortality in shrimp fed 0.5% SPE was reduced by 25% compared with that in the positive control group on day 14. This finding suggests that dietary supplementation with 0.5% SPE may enhance the resistance of *L. vannamei* to *V. parahaemolyticus* infection and may have potential applications in shrimp aquaculture. This protective effect is consistent with previous studies reporting the beneficial effects of plant extracts against bacterial infections in *L. vannamei*. For instance, Maurus et al. (2023) reported a protective effect of *Scutellaria baicalensis* extract in *L. vannamei* challenged with *V. parahaemolyticus*. Similarly, Fadel et al. (2025) found that dietary supplementation with extracts of *Artemisia herba-alba*, *Lonicera japonica*, and *Lilium candidum* significantly reduced cumulative mortality in *L. vannamei* following *V. parahaemolyticus* challenge. Tadese et al. (2022) also reported that extracts of *Acalypha indica*, *Adhatoda vasica*, *Anacardium occidentale*, *Azadirachta indica*, *Lawsonia inermis*, *Psidium guajava*, *Rosa damascena*, and *Tridax procumbens* exhibited antibacterial activity against *Vibrio* species, including *V. anguillarum*, *V. alginolyticus*, and *V. harveyi*, which are important bacterial pathogens in *P. vannamei* aquaculture. The protective effect of SPE observed in the present study may be associated with its bioactive constituents, particularly flavonoids, polyphenols, and saponins. These compounds possess pharmacological, antioxidant, and antimicrobial properties that may enhance the resistance of whiteleg shrimp to bacterial infections by inhibiting pathogen growth and supporting host defense mechanisms (Fadel et al. 2025).

Conclusion

The present study demonstrated that dietary supplementation with sweet potato extract (SPE) improved growth performance, digestive enzyme activity, antioxidant-related gene expression, and disease resistance in *L. vannamei*. Shrimp fed SPE exhibited higher weight gain (WG) and specific growth rate (SGR), as well as lower cumulative mortality following the *Vibrio parahaemolyticus* challenge. In addition, the upregulation of antioxidant-related genes was more pronounced at higher SPE supplementation levels, particularly in shrimp fed 0.5% SPE. Overall, these findings suggest that SPE has potential as a functional feed additive to enhance shrimp health and productivity, thereby contributing to more

sustainable aquaculture practices. Further studies are needed to investigate the effects of SPE supplementation on the composition and regulation of the gut microbiota in *L. vannamei*.

CRediT authorship contribution statement

Thi Cam Tu Phan: Conceptualization, Methodology, Writing -original draft, Writing - review & editing. **Thanh Men Tran**: Methodology, Formal analysis, Writing - review & editing. **Thi Phuong Thao Truong**: Conceptualization, Methodology, Investigation, Writing - review & editing, Funding acquisition. All authors read and approved the final manuscript.

Ethical declarations

The study was conducted in accordance with the animal care regulations of Can Tho University (CTU-AEC 25011 dated 09/5/2025) and relevant policies and guidelines of Vietnam.

Data availability

The data are available from the corresponding author upon reasonable request.

Declarations

Declaration of Competing Interest

The authors have no competing interest

Declaration of Generative AI Use

Generative AI was used solely to assist with language editing and improve the clarity of the manuscript. All content was reviewed and verified by the authors, who take full responsibility for the final manuscript.

Funding statement

This work was supported by Vietnamese Ministry of Education and Training (Code: B2025-TCT-06).

Author Contribution

Thi Cam Tu Phan: Conceptualization, Methodology, Writing -original draft, Writing - review & editing. Thanh Men Tran: Methodology, Formal analysis, Writing - review & editing. Thi Phuong Thao Truong: Conceptualization, Methodology, Investigation, Writing - review & editing, Funding acquisition. All authors read and approved the final manuscript.

Acknowledgement

We would like to thank BSc. Nguyen Kim Huyen and Mr. Nguyen Chi Linh for their support during the experiment.

Data Availability

Data will be made available on request.

References

1. Abdel Rahman AN, Doan HV, Elsheshtawy HM, Dawood A, Salem SMR, Sheraiba NI, Masoud SR, Abdelnaeim NS, Khamis T, Alkafafy M, Mahboub HH (2022) Dietary *Salvia officinalis* leaves enhance antioxidant-immune-capacity, resistance to *Aeromonas sobria* challenge, and growth of *Cyprinus carpio*. Aquaculture 586:740731
2. Abidin Z, Huang HT, Hu YF, Chang JJ, Huang CY, Wu YS, Nan FH (2022) Effect of dietary supplementation with *Moringa oleifera* leaf extract and *Lactobacillus acidophilus* on growth performance, intestinal microbiota, immune response, and disease resistance in whiteleg shrimp (*Penaeus vannamei*). Fish Shellfish Immunol 127:876–890. https://doi.org/10.1016/j.fsi.2022.07.007
3. Abidin Z, Huang HT, Liao ZH, Chen BY, Wu YS, Lin YJ, Nan FH (2021) *Moringa oleifera* leaves' extract enhances nonspecific immune responses, resistance against *Vibrio alginolyticus*, and growth in whiteleg shrimp (*Penaeus vannamei*). Animals 12(1):42. https://doi.org/10.3390/ani12010042
4. AftabUddin S, Siddique MAM, Habib A, Akter S, Hossen S, Tanchangya P, Abdullah Al M (2021) Effects of seaweeds extract on growth, survival, antibacterial activities, and immune responses of *Penaeus monodon* against *Vibrio parahaemolyticus*. Ital J Anim Sci 20:243–255. https://doi.org/10.1080/1828051X.2021.1878943
5. Akbary P, Adeshina I, Jahanbakhshi A (2020) Growth performance, digestive enzymes, antioxidant activity and immune responses of *Litopenaeus vannamei* fed with *Jania adhaerens* J.V. supplemented diet against *Photobacterium damselae* infection. Anim Feed Sci Technol 270:114696. https://doi.org/10.1016/j.anifeedsci.2020.114696
6. Anh VL, Sophie EP, Minh HN, Minh P, Paul DR (2018) Improving the Vanillin-Sulphuric Acid Method for Quantifying Total Saponins. Technologies 6(3):84–95
7. AOAC (2000) Official methods of analysis, 17th edn. The Association of Official Analytical Chemists, Gaithersburg, MD, USA
8. APHA (2017) Standard methods for the examination of water and wastewater. 19th ed. American Public Health Association, 1015 Fifteenth Street, NW, Washington, DC, USA
9. Bag GC, Devi PG, Bhaigyabati T (2015) Assessment of total flavonoid content and antioxidant activity of methanolic rhizome extract of three *Hedychium* species of Manipur valley. Int J Pharm Sci

Res 30:154–159

10. Bernfeld P (1955) Amylases, α and β. In: Abelson J, Simon M, Verdine G, Pyle S (eds) Methods in Enzymology. Academic, pp 149–158
11. Brigelius-Flohé R, Maiorino M (2013) Glutathione peroxidases. Biochim Biophys Acta Gen Subj 1830(5):3289–3303
12. Büyükçapar HM, Yanar M, Yanar Y (2007) Pigmentation of rainbow trout (*Oncorhynchus mykiss*) with carotenoids from marigold flower (*Tagetes erecta*) and red pepper (*Capsicum annuum*). Turk J Vet Anim Sci 31:7–12
13. Dewi NR, Huang HT, Wu YS, Liao ZH, Lin YJ, Lee PT, Nan FH (2021) Guava (*Psidium guajava*) leaf extract enhances immunity, growth, and resistance against *Vibrio parahaemolyticus* in white shrimp *Penaeus vannamei*. Fish Shellfish Immunol 118:1–10. https://doi.org/10.1016/j.fsi.2021.08.017
14. Ezquerra JM, Garcia-Carreno FL, Guillermo AM, Haard NF (1999) Effect of feed diet on aminopeptidase activities from the hepatopancreas of white shrimp (*Penaeus vannamei*). J Food Biochem 23:59–74. https://doi.org/10.1111/j.1745-4514.1999.tb00005.x
15. Fadel A, Khafage A, Abdelsalam M, Abdel-Rahim MM (2025) Comparative evaluation of three herbal extracts on growth performance, immune response, and resistance against *Vibrio parahaemolyticus* in *Litopenaeus vannamei*. BMC Vet Res 21:166. https://doi.org/10.1186/s12917-025-04588-0
16. Ge HX, Ni Q, Liu JL, Dong ZG, Chen SB (2022) Effect of chronic ammonia nitrogen stress on the SOD activity and interferon-induced transmembrane protein 1 expression in the clam *Cyclina sinensis*. Front Mar Sci 9:1034152. https://doi.org/10.3389/fmars.2022.1034152
17. Ghosh AK, Panda SK, Luyten W (2021) Anti-vibrio and immune-enhancing activity of medicinal plants in shrimp: A comprehensive review. Fish Shellfish Immunol 117:192–210. https://doi.org/10.1016/j.fsi.2021.08.006
18. He TB, Huang YP, Huang Y, Wang XJ, Hu JM, Sheng J (2018) Structural elucidation and antioxidant activity of an arabinogalactan from the leaves of *Moringa oleifera*. Int J Biol Macromol 112:126–133
19. Johnson M, Pace RD (2010) Sweet potato leaves: properties and synergistic interactions that promote health and prevent disease. Nutr Rev 68(10):604–615. https://doi.org/10.1111/j.1753-4887.2010.00320.x
20. Klahan R, Deevong P, Wiboonsirikul J, Yuangsoi B (2023) Growth performance, feed utilisation, endogenous digestive enzymes, intestinal morphology, and antimicrobial effect of Pacific white shrimp (*Litopenaeus vannamei*) fed with feed supplemented with pineapple waste crude extract as a functional feed additive. Aquac Nutr 2023:1160015. https://doi.org/10.1155/2023/1160015
21. Li JT, Jing Z, Chen HQ, Zheng PH, Lu YP, Zhang XX, Zhang ZL, Lv Y, Xu JR, Wang DM, Zhao YH, Xian JA (2024) Effects of *Alpinia officinarum* stems and leaves extract on growth performance, non-specific immunity, and intestinal microflora of *Litopenaeus vannamei*. Aquac Rep 34:101893. https://doi.org/10.1016/j.aqrep.2023.101893
22. Li JT, Zhao YH, Lv Y, Su X, Mei WL, Lu YP, Zheng PH, Zhang ZL, Zhang XX, Chen HQ, Dai HF, Xian JA (2023) Evaluating the antioxidant properties of the leaves and stems of *Alpinia oxyphylla* in vitro and

its growth-promoting, muscle composition change, and antioxidative stress function on juvenile *Litopenaeus vannamei*. Antioxidants 12:1802. https://doi.org/10.3390/antiox12101802

23. Lin H, Deng Y, Zhu D, Yang Q, Zhou X, Tan B, Feng L, Chi S (2023) Effects of partially replacing fishmeal with corn gluten meal on growth, feed utilization, digestive enzyme activity, and apparent nutrient digestibility for juvenile white shrimp, *Litopenaeus vannamei*. Front Vet Sci 10:1–11. https://doi.org/10.3389/fvets.2023.1162599
24. Liu Z, Liu Q, Zhang D, Wei S, Sun Q, Xia Q, Shi W, Ji H, Liu S (2021) Comparison of the proximate composition and nutritional profile of byproducts and edible parts of five species of shrimp. Foods 10(11):2603. https://doi.org/10.3390/foods10112603
25. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2 – ΔΔCT method. Methods 25:402–408. https://doi.org/10.1006/meth.2001.1262
26. Lochmann RT, Islam S, Phillips H, Adam Z, Everette J (2013) Effects of dietary sweet potato leaf meal on the growth, non-specific immune responses, total phenols and antioxidant capacity in channel catfish (*Ictalurus punctatus*). J Sci Food Agric 93(6):1365–1369. https://doi.org/10.1002/jsfa.5898
27. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193(1):265–275. https://doi.org/10.1016/S0021-9258(19)52451-6
28. Lu X, Peng D, Chen X, Wu F, Jiang M, Tian J, Liu W, Yu L, Wen H, Wei K (2020) Effects of dietary protein levels on growth, muscle composition, digestive enzymes activities, hemolymph biochemical indices and ovary development of pre-adult red swamp crayfish (*Procambarus clarkii*). Aquac Rep 18:100542
29. Maurus G, Ho TH, Lee PT (2023) Effects of dietary *Scutellaria baicalensis* extract on growth performance, immune-related genes expression, and resistance against *Vibrio parahaemolyticus* in white shrimp (*Litopenaeus vannamei*). Res Vet Sci 159:160–170
30. Mbokane EM, Moyo NAG (2022) Use of medicinal plants as feed additives in the diets of Mozambique tilapia (*Oreochromis mossambicus*) and the African Sharptooth catfish (*Clarias gariepinus*) in Southern Africa. Front Vet Sci 9:1–14. https://doi.org/10.3389/fvets.2022.1072369
31. Muttharasi C, Gayathri V, Muralisankar T, Mohan K, Uthayakumar V, Radhakrishnan S, Kumar P, Palanisamy M (2021) Growth performance, digestive enzymes and antioxidants activities in the shrimp *Litopenaeus vannamei* fed with *Amphiroa fragilissima* crude polysaccharides encapsulated *Artemia* nauplii. Aquaculture 737263. https://doi.org/10.1016/j.aquaculture.2021.737263
32. Nguyen HC, Chen CC, Lin KH, Chao PY, Lin HH, Huang MY (2021) Bioactive compounds, antioxidants, and health benefits of sweet potato leaves. Molecules 26(7):1820. https://doi.org/10.3390/molecules26071820
33. Oanh DTH, Nghia NT, Tien TV, Bondad-Reantaso MG (2018) Identification and characterization of *Vibrio* bacteria isolated from shrimp infected with early mortality syndrome/acute hepatopancreatic

necrosis syndrome (EMS/AHPNS) in Vietnam. Asian Fish Sci 31S:283–292. https://doi.org/10.33997/j.afs.2018.31.S1.0

34. Phan TCT, Huynh TG, Nguyen TKL, Tran TTH (2026) Effects of synbiotic from noni leaf extract supplementation on survival, growth, and resistance to *Vibrio harveyi* in the whiteleg shrimp, *Litopenaeus vannamei*. J Anim Plant Sci 36(5)
35. Phan TCT, Nguyen TKL, Huynh TG, Doan XD (2023) Effects of noni fruit extract on the growth performance, digestive enzymes, and stress tolerance of juvenile whiteleg shrimp (*Litopenaeus vannamei*). Egypt J Aquat Res 49:549–554. https://doi.org/10.1016/j.ejar.2023.08.003
36. Phung NKP (2007) Methods for the isolation of organic compounds. Vietnam National University Ho Chi Minh City, p 528. (in Vietnamese)
37. Qi MS, Wang T, Chen RW, Wang CF, Ao CJ (2017) Effects of flavonoids from *Allium mongolicum* Regel on growth performance and growth-related hormones in meat sheep. Anim Nutr 3:33–38. https://doi.org/10.1016/j.aninu.2017.01.003
38. Singleton VL, Orthofer R, Lamuela-Raventós RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteau reagent. In: Packer L (ed) Methods in Enzymology, Oxidant and Antioxidant (Part A). Academic, San Diego, CA, pp 159–178
39. Sun H, Mu T, Xi L, Zhang M, Chen J (2014) Sweet potato (*Ipomoea batatas* L.) leaves as nutritional and functional foods. Food Chem 156:380–389. https://doi.org/10.1016/j.foodchem.2014.01.079
40. Tadese DA, Song C, Sun C, Liu B, Liu B, Zhou Q, Xu P, Ge X, Liu M, Xu X, Tamiru M, Zhou Z, Lakew A, Kevin NT (2022) The role of currently used medicinal plants in aquaculture and their action mechanisms: A review. Rev Aquac 14:816–847. https://doi.org/10.1111/raq.12626
41. Tang CC, Ameen A, Fang BP, Liao MH, Chen JY, Huang LF, Zou HD, Wang ZY (2021) Nutritional composition and health benefits of leaf-vegetable sweet potato in South China. J Food Compos Anal 96:103714. https://doi.org/10.1016/j.jfca.2020.103714
42. Trasviña-Arenas CH, Garcia-Triana A, Peregrino-Uriarte AB, Yepiz-Plascencia G (2013) White shrimp *Litopenaeus vannamei* catalase: Gene structure, expression and activity under hypoxia and reoxygenation. Comp Biochem Physiol B Biochem Mol Biol 164(1):44–52. https://doi.org/10.1016/j.cbpb.2012.10.004
43. Wang S, Nie S, Zhu F (2016) Chemical constituents and health effects of sweet potato. Food Res Int 89:90–116. https://doi.org/10.1016/j.foodres.2016.08.032
44. Wei LS, Sukri SAM, Tahiluddin AB, Kari ZA, Wee W, Kabir MA (2024) Exploring beneficial effects of phytobiotics in marine shrimp farming: A review. Heliyon 10:e31074. https://doi.org/10.1016/j.heliyon.2024.e31074
45. Wu S, Zhao M, Gao S, Xu Y, Zhao X, Liu M, Liu X (2021) Change regularity of taste and the performance of endogenous proteases in shrimp (*Penaeus vannamei*) head during autolysis. Foods 10:1020. https://doi.org/10.3390/foods10051020
46. Xavier MJ, Conceição LEC, Valente LMP, Colen R, Rodrigues ACM, Rocha RJM, Custódio L, Carballo C, Manchado M, Engrola S (2021) Dietary natural plant extracts can promote growth and modulate

oxidative status of Senegalese sole post larvae under standard/challenge conditions. Animals 11(5):1398. https://doi.org/10.3390/ani11051398

47. Xia S, Zhao W, Li M, Zhang L, Sun L, Liu S, Yang H (2018) Effects of dietary protein levels on the activity of the digestive enzyme of albino and normal *Apostichopus japonicus* (Selenka). Aquac Res 49(3):1302–1309. https://doi.org/10.1111/are.13585
48. Yang Y, Mao M, Lin H, Tan B, Chi S, Yang Q (2026) The effect of low-fishmeal feed including an olive leaf extract (*Olea europaea*) additive on growth, intestinal health and disease resistance in *Litopenaeus vannamei*. Animals 16(15):2418. https://doi.org/10.3390/ani16152418
49. Ye Y, Zhu B, Yun J, Yang Y, Tian J, Xu W, Du X, Zhao Y, Li Y (2024) Comparison of antioxidant capacity and immune response between low salinity tolerant hybrid and normal variety of Pacific white shrimp (*Litopenaeus vannamei*). Aquac Int 32(2):1879–1894. https://doi.org/10.1007/s10499-023-01248-8
50. Yue R, Dong W, Feng Z, Jin T, Wang W, He Y, Chen Y, Lin S (2024) Effects of three tested medicinal plant extracts on growth, immune function and microflora in juvenile largemouth bass (*Micropterus salmoides*). Aquac Rep 36:102075. https://doi.org/10.1016/j.aqrep.2024.102075
51. Zhang C, Liu D, Wu L, Zhang J, Li X, Wu W (2019) Chemical characterization and antioxidant properties of ethanolic extract and its fractions from sweet potato (*Ipomoea batatas* L.) leaves. Foods 9(1):15

Figures

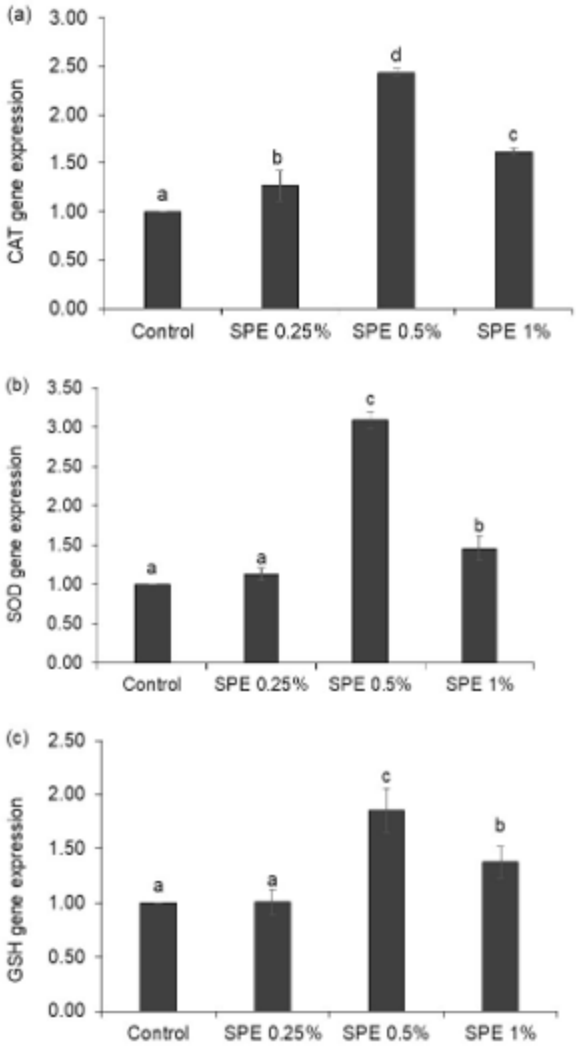

Figure 1

Effects of dietary sweet potato leaf extract (SPE) supplementation on antioxidant-related gene expression in *Litopenaeus vannamei*. Relative expression of (a) catalase (CAT), (b) superoxide dismutase (SOD), and (c) glutathione peroxidase (GSH-Px) in shrimp fed diets containing 0 (control), 0.25, 0.5, or 1.0% SPE. Gene expression was normalized to β-actin and expressed relative to the control group. Data are presented as mean ± SE. Different lowercase letters above bars indicate significant differences among dietary treatments ($p<0.05$), whereas bars sharing the same letter are not significantly different.

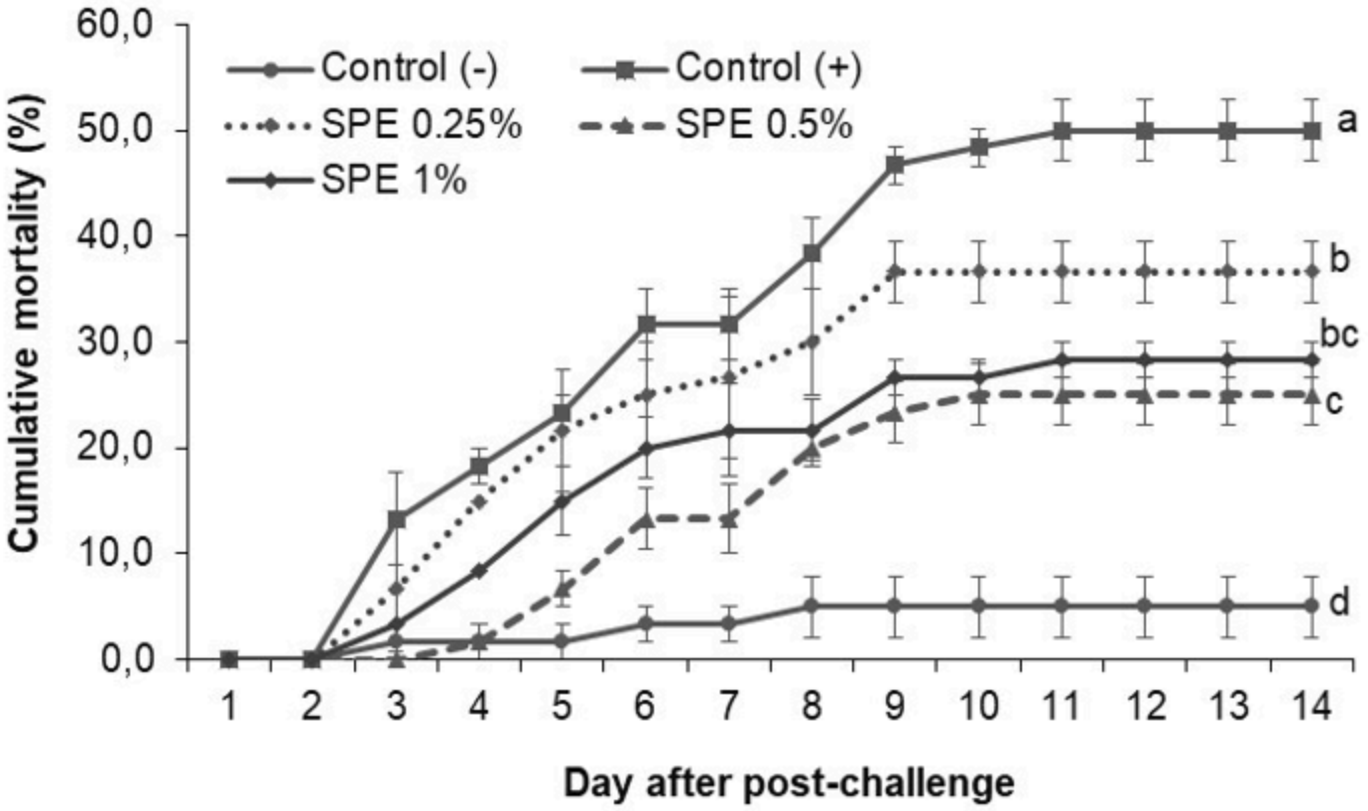

Figure 2

Cumulative mortality of *Litopenaeus vannamei* following challenge with *Vibrio parahaemolyticus* after dietary supplementation with different levels of sweet potato extract. Data are presented as mean ± SE.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementData.docx