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Research Article

Effects of dietary mangrove leaf powder on growth performance, survival, body and fatty acid composition, and resistance to pH stress in Pacific white shrimp (*Litopenaeus vannamei*)

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Abstract

This study aimed to examine the effectiveness of mangrove (*Avicennia marina*) leaf powder (MLP) in the diet of Pacific white shrimp (*Litopenaeus vannamei*). The shrimp were fed with diets containing different amounts of MLP including 0 (control), 25 (MLP25), 50 (MLP50), and 100 (MLP100) g/kg MLP for 13 weeks. The results showed a significant improvement in the growth performance and survival rate of the shrimp fed different levels of MLP compared to the control group (p<0.05). Diets containing up to 50 g/kg MLP revealed significantly higher amounts of lipids and linoleic acid (LA), alpha linolenic acid (ALA) fatty acids compared to those of the control diet, while the carcass of shrimp fed diets containing MLP showed significant higher contents of polyunsaturated fatty acids (PUFAs) compared to the control diet, particularly DHA and ARA compared to those of the control group (p<0.05). After 48 hours of exposure to both low (4) and high (10) pH stress, the shrimp fed with different levels of MLP showed higher survival than that of the control group. The results of this study demonstrated the positive effect of dietary MLP on shrimp growth performance and resistance to pH stress.

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Introduction

Median projections suggest global human population will grow to over 9.7 billion by 2050 (United Nations, 2015). Global demands for food from aquatic environments are expected to increase in future decades because these foods will help to meet the needs and preferences of a growing human population. Aquatic white meat has a very high nutritional value. Aquaculture represents the fastest overall growth in the food industry, and it is an important global seafood source (Nadaranjah and Flaaten, 2017). The aquaculture industry, by conducting new research activities including proper nutrition, seeks to improve and enhance (Gephart *et al.*, 2020).

Shrimp culture is a major activity in aquaculture due to its special flavor, high nutritive value, and continuous demand in the global market (Pushparajan and Soundarapandian, 2010). The Pacific white shrimp, *Litopenaeus vannamei*, is the most common shrimp cultured species in the global economy (Duan *et al.*, 2017a). Due to its advantages of fast growth, euryhalinity, high-density culture, etc., *L. vannamei* is now the most important economic crustacean species, accounting for 53% of the total crustacean production worldwide (FAO, 2018). To decrease the high cost of shrimp production and to deliver inexpensive feeds for farmers, substituting organic-based plants has been a prioritized field of study during the last few decades (Yousif *et al.*, 1996; Amisah *et al.*, 2009; Abarike *et al.*, 2012; Yao *et al.*, 2020). The use of plant-derived food ingredients has led to an increase in the volume of production in aquatic animals. For instance, soybean, rapeseed, sunflower powder and oil, legumes and many other plant-derived proteins have been widely and successfully incorporated into aquaculture. Various studies have been conducted on the use of plant-derived compounds in shrimp feeding (Citarasu *et al.*, 1999).

Mangrove forests are distributed in tropical and subtropical areas (Safdari and Shadman, 2015). They are known as the primary nursery habitat for many species of fish, crustaceans, birds, and marine mammals (Shepard *et al.*, 2011). Many mangrove species contain bioactive metabolites and compounds that can control microbial growth. Given the antiviral, antibacterial, antifungal and insecticide properties of mangroves and their effective inhibitory activity against pathogens, they are considered an alternative medicine in aquaculture (Vadlapudi and Naidu, 2015). *Avicennia marina* is an important mangrove species because it can grow and reproduce across a wide range of climatic, edaphic, and tidal conditions. The antiviral, antibacterial, and antifungal activities of *A. marina* extracts have already been reported (Khafagi *et al*., 2003).

Limited studies have investigated the effects of dietary mangrove leaves on shrimp performance (Hai and Yakupitiyage, 2005; Avenido and Serruno, 2012a, b,c; Hajian *et al.*, 2017). The positive effect of dietary mangrove leaf powder (MLP) on some immune parameters of *L. vannamei* was reported (Eshagh Nimvari *et al*., 2019). The extracts of mangrove leaf, *Sonneratia alba* and *Acanthus ilicifolius* could improve the

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resistance of tiger shrimp *Penaeus monodon* against White Spot Syndrome Virus (WSSV) and *Vibrio harveyi* bacterial disease, respectively (Saptiani *et al.,* 2021*;* Susianingsih, 2021). The use of mangrove extract could also increase the level of digestive enzymes and consequently cause growth increase in shrimp post-larvae (Avenido and Serrano, 2012c). Antibacterial and antifungal compounds in mangroves could also reduce the harmful microbial population of the gastrointestinal tract, and promote health, safety, and growth performance in shrimp (Platel *et al.,* 2002). The use of MLP and its effect on cultured shrimp performance have not been studied. Therefore, the present study aimed to investigate the effect of dietary MLP (*A. marina*) on growth performance, survival, body biochemical and fatty acid composition, and tolerance of pH stress in *L. vannamei*.

Materials and methods

Fresh mangrove leaves of *A. marina* were collected from an artificially developed mangrove forest (Lat. 27°8′N and Long. 56°44′E) located in Tiab, Hormozgan province Iran. The collected leaves of *A. marina* were washed with tap water and distilled water to remove the necrosis, adhering salts and other associated animals (Balakrishnan *et al.,* 2016). Then, drought in the shade at room temperature and the leaves were powdered by an electric mill (Kumar *et al.,* 2011). Other ingredients of the diet were prepared from the Hormoz Dam factory, Eisin, Hormozgan, Iran. The dry materials were powdered by an electric mill, and each material was passed through a sieve with pores of 50 μm. The ingredients of the diets were mixed and the pellet was made by an extruder (2 mm diameter), then the diets were dried and preserved as previously described (Pakravan *et al.,* 2017). The proximate composition and fatty acid contents of mangrove leaf powder (MLP) are presented in Table 1.

Table 1: Proximate composition and fatty acid contents of leaf powder (*Avicennia marina*) (% total fatty acid).

Item	Value
Proximate composition (dry basis; g/kg)	
Protein	184.2
Lipid	31.3
Ash	187.7
Fiber	148.7
Moisture	79.6
Fatty acid profile (%)	
C14:0	2.18 ± 0.09
C16:0	32.65 ± 1.77
C16:1	1.92 ± 0.28
C18:0	9.84 ± 0.39
C18:1 (n-9)	22.20 ± 0.47
C18:1 (n-6)	2.04 ± 0.76
C18:3 (n-3)	8.14 ± 0.23

Four diets were prepared with the incorporation of 0 (control), 25 (MLP25), 50 (MLP50) and 100 (MLP100) g/kg MLP. The ingredients and chemical composition of the experimental diets are presented in Table 2.

Experimental shrimp and feeding

L. vannamei specimens were purchased from a shrimp farm in Kolahi, Hormozgan province, Iran, and transferred to the Kolahi Shrimp Development and Training Center located in Hormozgan province, Iran. After 1 week of acclimation to laboratory conditions and feeding with a commercial diet, healthy shrimp with a mean weight of

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5.26±0.21 g, and a total length of 7.59±0.75 cm were distributed into four groups with three replicates. For each replicate, 40 shrimp were randomly placed in circular fiber glass tanks (300 L volumes with about 200 L water). Daily water replacements were about 50%. Shrimp were manually fed by experimental diets at a rate of 5% body weight per day at 8:00, 14:00, and 20:00 h during a period of 13 weeks. Every 2 weeks, the amount of daily feed was readjusted according to the total weight of shrimp in each tank. During the experimental period, temperature, salinity, pH and dissolved oxygen (DO) were about 23.78±0.16°C, 42.86±0.03 g/L, 7.46±0.02 mg/ L and 5.24±0.01mg/ L, respectively.

Table 2: The ingredient and chemical composition of the experimental diets with 0 (control), 25, 50, and 100 g/kg mangrove leaf powder (MLP).

Composition	Diets			
	Control	MLP25	MLP50	MLP100
Ingredients (g/kg)				
Fish meal	300.0	300.0	300.0	300.0
Soybean meal	180.0	175.0	170.0	160.0
Wheat flour	154.0	158.0	161.0	168.0
Shrimp meal	64.0	64.0	64.0	64.0
Rice flour	106.0	86.0	66.0	26.0
Wheat Glotin	96.0	96.0	96.0	96.0
Bentonit	10.0	10.0	10.0	10.0
Biender	10.0	10.0	10.0	10.0
Vitamin mixture[a]	10.0	10.0	10.0	10.0
Mineral mixture[b]	10.0	10.0	10.0	10.0
Fish oil	40.0	37.3	35.3	30.7
Soybean meal	20.0	18.7	17.7	15.3
Mangrove meal	00.0	25.0	50.0	100.0
Chemical composition (g/kg)				
Crud protein	336.5	333.8	344.8	340.1
Crud lipid	48.5	51.0	59.0	60.5
Ash	119.0	112.0	120.5	122.0
Dry matter	905.5	902.5	904.5	895.5
Moisture	94.5	97.5	95.5	104.5

[a] Supplied (IU or mg/kg diet): vitamin A, 1800 IU; vitamin D3, 1200 IU; vitamin E, 120 mg; vitamin B12, 24mg; riboflavin, 15 mg; niacin, 90 mg; D-pantothenic acid, 27 mg; menadione, 3 mg; folic acid, 4.8 mg; pyridoxine, 9 mg; thiamine, 9 mg; D-biotin, 0.48 mg; choline chloride 360 mg; cobalamin 24 mg; ascorbic acid 156 mg; nicotinic acid 90 mg; inositol 72; antioxidant 15 mg.

[b] Supplied (mg kg^{-1} diet): Zn, 18 mg; I, 0.6 mg; Mg, 7.8 mg; Co, 0.15 mg; Se, 0.15 mg; CU, 1.8 mg; Fe, 12 mg.

After 13 weeks of the feeding trial, shrimp from each tank were sampled and weighed. Afterward, eight individuals from each tank were used for analyses of body proximate composition and fatty acid profile.

Growth parameters

Growth parameters of shrimp were measured as follows (Immanuel *et al.*, 2001):

$WG\ (g) = W_f - W_i$

$SR\% = 100 \times (S - D) / S$

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$SGR = 100 \times (\ln W_f - \ln W_i) / t$
FCR = Feed intake (g) / Weight gain (g)
$ADG = 100 \times (W_f - W_i) / W_i \times t$
$TLI\ (cm) = L_f - L_i$

Where, WG is weight gain, W_i is initial body weight, W_f is final body weight. SR% is Survival rate % = 100 × (initial shrimp number−dead shrimp number) / (initial shrimp number), SGR is specific growth rates, t is number of culture days. FCR is feed conversion ratios. ADG is Average daily growth. TLI is total length increase, L_f is final length and L_i is initial length.
%Survival rate= 100 × (initial shrimp number−dead shrimp number) / (initial shrimp number)

Body proximate and fatty acid composition
Three samples from each tank were evaluated for the whole body proximate composition of shrimp using the AOAC method (1995) as previously described. (Pakravan *et al.*, 2017). Also, three samples from each tank and the experimental diets were evaluated for the fatty acid profile using a modified method of Lepage and Roy (1984) as previously described (Pakravan *et al.*, 2017).

pH stress
At the end of the feeding period, shrimp were challenged to pH stress. The water pH was adjusted by adding HCl to reach a pH of 4 and adding NaOH to reach a pH of 10 (Li and Chen, 2008). Twenty shrimp (10 shrimp in each tank) were taken for each treatment and placed into the 300-L tanks. Shrimp were gradually exposed to low and high pH stress. The survival of the exposed shrimp was recorded up to 48 h and measured as the following:

Statistical analysis
Data obtained from this experiment was analyzed using one-way ANOVA and multiple comparisons were performed by Turkey's post hoc based on significant effects at (p<0.05). All the statistical analyses were conducted by the SPSS program (version16). Values are expressed as mean±standard deviation (SD).

Results

Growth parameters
The growth parameters of *L. vannamei* fed diets containing different levels of MLP are presented in Table 3. Almost all the shrimp accepted the diet. The results after 13 weeks showed that there were significant differences in W_f, WG, FCR, ADG, and TLI between the control group and the treatments fed diets containing different levels of MLP (p<0.05). There were no significant differences among treatments fed diets containing 25, 50, and 100 g/kg MLP. The survival rate of the shrimp fed diets containing MLP was significantly higher than that of the control group (p<0.05).

Body proximate composition
The body proximate compositions (wet weight basis) of *L. vannamei* fed diets with different amounts of MLP are shown in Table 4. The highest amount of protein was observed in the control group and showed a significant difference compared to other treatments (p<0.05). There was no significant difference in fat content between treatments (p>0.05). The hrimp

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fed diets containing 50 g/ Kg MLP and 100 g/Kg MLP showed significantly higher ash content compared to the control group and MLP25 (p<0.05). The dry matter content in the treatment of MLP50 was significantly higher than the control group (p<0.05).

Table 3: Growth parameters of *Litopenaeus vannamei* fed with 0 (control), 25, 50, and 100 g/kg mangrove leaf powder (MLP).

Growth parameters	Treatments			
	Control	MLP25	MLP50	MLP100
W_i (g)	5.14 ± 0.34	5.50 ± 0.17	5.36 ± 0.33	5.03 ± 0.18
W_f (g)	11.7 ± 0.20^b	13.62 ± 0.83^a	13.64 ± 0.41^a	13.55 ± 0.24^a
WG (g)	6.56 ± 0.38^b	8.12 ± 0.64^a	8.27 ± 0.44^a	8.52 ± 0.07^a
L_i (cm)	8.59 ± 0.12	8.63 ± 0.03	8.61 ± 0.08	8.53 ± 0.04
L_f (cm)	10.77 ± 0.05^b	11.30 ± 0.08^a	11.48 ± 0.12^a	11.43 ± 0.06^a
TLI (cm)	2.17 ± 0.15^b	2.67 ± 0.12^a	2.86 ± 0.20^a	2.90 ± 0.70^a
FCR	3.02 ± 0.13^a	2.71 ± 0.04^b	2.54 ± 0.05^b	2.69 ± 0.03^b
ADG	0.07 ± 0.00^b	0.09 ± 0.05^a	0.09 ± 0.10^a	0.09 ± 0.00^a
SGR (g/day)	0.91 ± 0.07	1.00 ± 0.07	1.03 ± 0.04	1.10 ± 0.02
SR (%)	70.00 ± 11.02^b	87.50 ± 5.20^{ab}	87.50 ± 0.00^{ab}	95.83 ± 1.16^a

$W_{i=\text{ initial weight}}$, $W_{f=\text{ final weight}}$, WG= weight gain, L_i= initial length, $L_{f=\text{ final length}}$, TLI= total length increase, FCR= feed conversion ratio, ADG= avarage daily growt , SGR= specific growth rate and SR= survival rate %.
Data (mean ± SD) with different letters are significantly different among treatments according to ANOVA test (p<0.05).

Table 4: Body proximate composition (wet weight basis) of *L. vannamei* fed with 0 (control), 25, 50, and 100 g/Kg mangrove leaf powder (MLP).

Items (g/kg)	Treatments			
	Control	MLP25	MLP50	MLP100
Protein	247.4 ± 9.00^a	225.2 ± 2.20^b	216.6 ± 3.40^{bc}	203.7 ± 3.40^c
Lipid	12.3 ± 0.70	16.8± 0.40	17.6 ± 1.30	18.7 ± 0.70
Ash	13.5 ± 0.60^b	13.4 ± 0.00^b	14.9 ± 0.00^a	14.8 ± 0.20^a
Moisture	753.3 ± 8.80^a	740.0 ± 5.70^{ab}	730.0 ± 0.00^b	736.6 ± 3.30^{ab}
Dry matter (%)	246.4 ± .8.80^b	260.0 ± 5.70^{ab}	270.0 ± 0.00^a	263.4 ± 3.30^{ab}

Data (mean ± SD) with different letters are significantly different among treatments according to ANOVA test (p<0.05)

Fatty acid profile

The fatty acid composition of experimental diets and body of *L. vannamei* fed diets containing different amounts of MLP are presented in Tables 5 and 6, respectively. Diets containing MLP revealed significantly lower amounts of polyunsaturated fatty acids (PUFAs) compared to control diet (p<0.05, Table 5). Moreover, significantly higher amounts of linoleic acid (LA), alpha linolenic acid (ALA), and arachidonic acid (ARA) were observed in a diet containing up to 50 g/kg MLP compared to those of the control diet (p<0.05). A diet containing 100 g/kg MLP showed a significantly higher amount of docosahexaenoic acid (DHA) compared to that of the control (p<0.05). The body of shrimp fed diets containing different amounts of MLP showed significantly higher levels of PUFAs, particularly DHA and ARA compared to those of the control group (p<0.05, Table 6). However, the shrimp fed control diet revealed higher amounts of monounsaturated fatty acids (MUFAs) compared to the shrimp fed diets containing different amounts of MLP.

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Table 5: Fatty acid profile of experimental diets incorporated with 0 (control), 25, 50, and 100 g/kg mangrove leaf powder (MLP) (% total fatty acid)

Items	Treatments			
	Control	MLP25	MLP50	MLP100
C12:0	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.01
C14:0	3.45 ± 0.02	3.48 ± 0.02	3.45 ± 0.01	3.36 ± 0.08
C15:0	0.71 ± 0.00^{b}	0.74 ± 0.00^{a}	0.73 ± 0.00^{ab}	0.69 ± 0.01^{c}
C16:0	21.59 ± 0.16^{a}	21.64 ± 0.36^{a}	20.68 ± 0.07^{b}	21.44 ± 0.17^{a}
C17:0	1.00 ± 0.01^{ab}	1.03 ± 0.00^{a}	0.96 ± 0.00^{b}	0.98 ± 0.00^{b}
C18:0	4.81 ± 0.77^{ab}	6.58 ± 0.05^{a}	6.45 ± 0.01^{ab}	6.09 ± 0.07^{b}
C20:0	0.42 ± 0.01	0.41 ± 0.00	0.40 ± 0.00	0.40 ± 0.03
C22:0	0.32 ± 0.00	0.37 ± 0.02	0.36 ± 0.01	0.35 ± 0.02
C24:0	0.62 ± 0.00^{b}	0.51 ±0.02^{c}	0.52 ± 0.01^{c}	0.70 ± 0.01^{a}
SFA	32.96 ± 0.64^{b}	34.81 ± 0.19^{a}	33.59 ± 0.03^{b}	34.06 ± 0.12^{ab}
C16:1	4.48 ± 0.01^{a}	4.43 ± 0.05^{a}	4.50 ± 0.00^{a}	4.23 ± 0.00^{b}
C17:1	0.58 ± 0.00^{a}	0.57 ± 0.00^{a}	0.53 ± 0.00^{b}	0.59 ± 0.00^{a}
C18:1(n-9) C	22.84 ± 0.34^{b}	23.81 ± 0.18^{a}	23.37 ± 0.01^{ab}	23.83 ± 0.14^{a}
C18:1(n-9) t	0.11 ± 0.00	ND	ND	ND
C20:1	0.86 ± 0.00	0.82 ± 0.00	0.87 ± 0.00	0.84 ± 0.10
C22:1	0.20 ± 0.00^{b}	0.20 ± 0.00^{b}	0.22 ± 0.00^{a}	0.22 ± 0.00^{ab}
MUFA	29.29 ± 0.19^{b}	29.85 ± 0.13^{a}	29.52 ± 0.01^{ab}	29.80 ± 0.15^{a}
C18:2(n-6) LA	16.46 ± 0.06^{c}	16.86 ± 0.02^{b}	17.39 ± 0.00^{a}	14.70 ± 0.00^{d}
C18:3(n-6)	0.15 ± 0.01	0.13 ± 0.04	0.10 ± 0.01	0.18 ± 0.01
C18:3(n-3) ALA	2.44 ± 0.01^{c}	2.54 ± 0.00^{b}	2.82 ± 0.00^{a}	2.13 ± 0.02^{d}
C20:2	0.25 ± 0.00^{ab}	0.26 ± 0.01^{b}	0.24 ± 0.00^{b}	0.31 ± 0.01^{a}
C20:3 (n-6)	0.95 ± 0.37^{a}	0.25 ± 0.05^{b}	0.21 ± 0.00^{b}	0.29 ± 0.03^{a}
C20:3 (n-3)	0.10 ± 0.03^{a}	0.09 ± 0.01^{ab}	0.07 ± 0.00^{b}	0.10 ± 0.00^{a}
C20:4(n-6) ARA	1.26 ± 0.01^{b}	1.20 ± 0.00^{c}	1.38 ± 0.00^{a}	1.15 ± 0.00^{d}
C20:5(n-3) EPA	4.09 ± 0.00^{a}	3.74 ± 0.02^{b}	4.07 ± 0.00^{a}	4.09 ± 0.01^{a}
C22:4 (n-6)	0.22 ± 0.00^{b}	0.18 ± 0.00^{ab}	0.17 ± 0.00^{d}	0.20 ± 0.02^{c}
C22:5 (n-6)	0.40 ± 0.00^{b}	0.37 ± 0.03^{b}	0.34 ± 0.02^{b}	0.50 ± 0.03^{a}
C22:5 (n-3)	0.73 ± 0.00^{b}	0.69 ± 0.00^{c}	0.79 ± 0.00^{a}	0.74 ± 0.01^{b}
C22:6(n-3) DHA	10.66 ± 0.01^{b}	9.01 ± 0.06^{c}	9.30 ± 0.01^{c}	12.29 ± 0.21^{a}
PUFA	37.75 ± 0.45^{a}	35.34 ± 0.06^{b}	36.88 ± 0.02^{a}	36.81 ± 0.36^{a}
Total n-3	18.02 ± 0.00^{b}	16.08 ± 0.09^{d}	17.04 ± 0.00^{c}	19.37 ± 0.27^{a}
Total n-6	19.47 ± 0.45^{a}	19.00 ± 0.14^{a}	19.59 ± 0.02^{a}	17.13 ± 0.08^{b}
n-3/n-6	0.92 ± 0.02^{b}	0.85 ± 0.01^{c}	0.87 ± 0.00^{c}	1.13 ± 0.01^{a}

LA linoleic acid, ALA alpha linolenic acid, ARA arachidonic acid, EPA eicosapentaenoeic acid, DHA docosahexaenoic acid, SFA saturated fatty acid, MUFA mono unsaturated fatty acid, PUFA polyunsaturated fatty acid. ND, not detected.

Data (mean) with different letters are significantly different among treatments according to ANOVA test (p<0.05).

pH stress

Shrimp fed diets with different levels of MLP exhibited higher survival rates than the control group when exposed to both low (pH 4) and high (pH 10) pH stress. Specifically, incorporating 100 g/kg MLP into the diet resulted in 100% survival in shrimp exposed to low pH stress, which was significantly higher than the survival rate observed in the control group (P<0.05, Table 7).

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Table 6: Fatty acid profile of whole body of *Litopenaeus vannamei* fed with 0 (control), 25, 50, and 100 g/kg mangrove leaf powder (MLP) (% total fatty acid).

Items	Treatments			
	Control	MLP25	MLP50	MLP100
C12:0	0.39 ± 0.08^{b}	0.56 ± 0.00^{a}	0.45 ± 0.01^{ab}	0.37 ± 0.04^{b}
C14:0	0.37 ± 0.01^{b}	0.38 ± 0.00^{ab}	0.41 ± 0.00^{a}	0.30 ± 0.00^{c}
C15:0	0.93 ± 0.05^{b}	1.01 ± 0.01^{b}	1.15 ± 0.00^{a}	0.99 ± 0.04^{b}
C16:0	18.81 ± 0.15^{ab}	18.90 ± 0.05^{ab}	18.89 ± 0.03^{a}	18.34 ± 0.23^{b}
C17:0	1.36 ± 0.01^{a}	1.29 ± 0.00^{b}	1.34 ± 0.02^{ab}	1.36 ± 0.01^{a}
C18:0	11.57 ± 0.01^{c}	11.10 ± 0.00^{d}	11.78 ± 0.02^{b}	12.09 ± 0.01^{a}
C20:0	0.48 ± 0.01^{a}	0.33 ± 0.00^{b}	0.36 ± 0.00^{b}	0.47 ± 0.01^{a}
C22:0	0.49 ± 0.02^{a}	0.39 ± 0.00^{c}	0.40 ± 0.00^{ab}	0.44 ± 0.00^{b}
C24:0	0.42 ± 0.02^{a}	0.34 ± 0.00^{bc}	0.29 ± 0.00^{c}	0.35 ± 0.02^{b}
SFA	34.84 ± 0.21^{ab}	34.32 ± 0.08^{b}	35.09 ± 0.01^{a}	34.69 ± 0.23^{ab}
C16:1	1.63 ± 0.00	1.64 ± 0.02	1.52 ± 0.08	1.54 ± 0.01
C17:1	0.27 ± 0.00^{a}	0.21 ± 0.00^{c}	0.21 ± 0.00^{c}	0.22 ± 0.00^{b}
C18:1(n-9)	16.69 ± 0.11^{a}	15.27 ± 0.18^{b}	15.31 ± 0.28^{b}	15.24 ± 0.06^{b}
C18:1(n-9)	3.14 ± 0.22	3.52 ± 0.05	3.46 ± 0.02	3.40 ± 0.02
C20:1	0.27 ± 0.12	0.37 ± 0.01	0.38 ± 0.01	0.43 ± 0.04
C22:1	0.08 ± 0.00^{b}	ND	ND	0.14 ± 0.02^{a}
MUFA	22.34 ± 0.22^{a}	21.02 ± 0.16^{b}	20.88 ± 0.38^{b}	21.03 ± 0.07^{b}
C18:2(n-6) LA	9.69 ± 0.13	10.01 ± 0.14	9.93 ± 0.14	9.77 ± 0.09
C18:3(n-6)	0.08 ± 0.02	ND	0.06 ± 0.00	0.07 ± 0.00
C18:3(n-3) ALA	0.45 ± 0.00^{b}	0.46 ± 0.00^{b}	0.52 ± 0.01^{a}	0.46 ± 0.01^{b}
C20:3 (n-6)	1.14 ± 0.04	1.26 ± 0.11	1.21 ± 0.02	1.32 ± 0.09
C20:3 (n-3)	0.06 ± 0.00	0.06 ± 0.00	ND	0.007 ± 0.01
C20:4(n-6) ARA	4.63 ± 0.01^{b}	4.80 ± 0.03^{a}	4.81 ± 0.08^{a}	4.83 ± 0.04^{a}
C20:5(n-3) EPA	11.74 ± 0.03^{ab}	11.91 ± 0.12^{a}	11.75 ± 0.19^{ab}	11.43 ± 0.05^{b}
C22:4 (n-6)	0.17 ± 0.03	0.11 ± 0.00	0.12 ± 0.01	0.15 ± 0.01
C22:5 (n-6)	0.27 ± 0.05^{a}	0.13 ± 0.02^{bc}	0.07 ± 0.01^{c}	0.19 ± 0.02^{ab}
C22:5 (n-3)	0.43 ± 0.02^{b}	0.41 ± 0.00^{b}	0.45 ± 0.01^{b}	0.55 ± 0.02^{a}
C22:6(n-3) DHA	14.42 ± 0.12^{b}	15.50 ± 0.14^{a}	15.10 ± 0.29^{a}	15.40 ± 0.14^{a}
PUFA	43.05 ± 0.19^{b}	44.65 ± 0.24^{a}	44.04 ± 0.39^{a}	44.27 ± 0.31^{a}
Total n-3	27.11 ± 0.18^{b}	28.35 ± 0.26^{a}	27.82 ± 0.48^{ab}	27.93 ± 0.23^{ab}
Total n-6	15.98 ± 0.03^{b}	16.30 ± 0.02^{a}	16.21 ± 0.08^{a}	16.34 ± 0.08^{a}
n-3/n-6	1.69 ± 0.00	1.74 ± 0.01	1.71 ± 0.03	1.71 ± 0.00

LA linoleic acid, ALA alpha linolenic acid, ARA arashidonic acid, EPA eicosapentaenoeic acid, DHA docosahexaenoic acid, SFA saturated fatty acid, MUFA mono unsaturated fatty acid, PUFA polyunsaturated fatty acid. ND, not detected.

Data (mean) with different letters are significantly different among treatments according to ANOVA test (p<0.05).

Table 7: Survival rate (%) of *Litopenaeus vannamei* fed with 0 (control), 25, 50, and 100 g/kg mangrove leaf powder (MLP) at the end of 48 h after pH=10 and pH=4

Items	Treatments			
	Control	MLP25	MLP50	MLP100
pH=10	60 ± 11.54	80 ± 11.54	70 ± 5.77	70 ± 17.32
pH=4	60 ± 11.54^{b}	80 ± 0.00^{ab}	80 ± 0.00^{ab}	100 ± 0.00^{a}

Data (mean ± SD) with different letters are significantly different among treatments according to ANOVA test (p<0.05).

Discussion

These compounds also make mangrove plants a valuable supplement in aquaculture feed (Gao and Lee, 2022; Wu *et al*., 2023). Notably, the leaves of *A. marina* are particularly high in flavonoids and phenolic compounds (Wu *et al*., 2023). This study aimed to evaluate the effects of dietary *A.*

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marina leaf powder on the performance of *L. vannamei* shrimp, particularly focusing on growth, survival, fatty acid composition, and resistance to pH stress. Over a 13-week period, various doses of MLP were incorporated into the shrimp diet. The results demonstrated that MLP supplementation improved shrimp growth performance, survival, fatty acid composition, and resilience to pH stress.

The results of this study showed that the addition of different levels of MLP to the diet of cultured shrimp had positive effects on growth indices and survival of shrimp. The higher growth rate in the MLP fed treatments might be related to the presence of novel metabolites and bioactive compounds, *i.e.*, terpenoids, alkaloids, phenolics, saponins, flavonoids, and steroids in the mangrove leaf (Patra and Thatoi, 2011; Wu *et al*., 2023). It is known that mangrove leaf, particularly *A. marina* is a rich source of novel natural products that have promising biological activities such as gastroprotective, cytotoxic, antioxidant, antibacterial, antifungal, antiviral, enzyme activation and inhibition, immunosuppressive, anti-inflammatory, and antifeedant effects (Jassim and Naji, 2003; Dhayanithi *et al.*, 2013; Sur *et al.*, 2015; Parthiban *et al.* 2022; Wu *et al.*, 2023). Therefore, these positive effects might cause the improved growth indices of shrimp. The positive effects of decomposed mangrove leaf and extract on shrimp growth performance and survival have already been reported by different authors. However, our study reported the positive effects of mangrove leaf powder for the first time on shrimp. Hajian *et al*. (2017) reported positive effects of alcoholic extract of *A. marina* leaves on the growth and survival of shrimp, *L. vannamei*. Moreover, the positive effects of dietary decomposed mangrove leaf litter (*Rhizophora apiculate*, *A. marina*, *A. officinalis*, *Heritiera fomes*, *Sonneratia apetala and S. caseolaris*) on growth performance have been demonstrated in giant tiger shrimp, *P. monodon* (Hai and Yakupitiyage, 2005; Rejeki *et al.*, 2019; Alam *et al.*, 2022). In another study, the leaf extract of mangrove species, *Acanthus ilicifolius* improved the survival and non-specific immune response of *P. monodon* (Saptiani *et al.*, 2021).

Nutrition is the most important factor affecting the fat content and fatty acid composition of aquatic animals and, indeed, body fat and fatty acid composition completely depend on the animal's diet (Buchtova *et al.*, 2007; Tocher and Glencross, 2015). The results of this study showed that dietary MLP could significantly increase the body fat, and n-3 and n-6 PUFAs, *i.e*., LA, ALA, EPA, DHA and ARA in carcasses of shrimp. The carcass PUFA contents in shrimp fed with different levels of MLP were significantly higher than the control group. Fatty acid composition of dietary lipids plays an essential role in the maintenance of proper metabolic and many physiological processes which lead to better survival and growth (Colvin, 1976; Zeng *et al.*, 2023). Particularly, PUFAs, *i.e.*, EPA and DHA have been identified as important nutrients for animal growth and reproduction (Caers *et al.*, 2000; Navarro and Villanueva, 2000; Izquierdo *et al.*, 2000; Jeffs *et al.*, 2002; Nelson *et al.*, 2002). They play important roles in the normal development of the

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body, the reproductive system, the function of the cardiovascular and immune systems, and the prevention of certain human diseases such as depression (Schmidt *et al.*, 2005). Similar to our results, the inclusion of some medicinal plants in the diet of shrimp enhanced the PUFA contents of the carcass. *Alteranthera sessilis*, *Cissus quadrangularis*, *Eclipta alba*, *Ocimum sanctum*, *Phyllanthus amarus* and *Solanum trilobatum* were supplemented to the diet of freshwater shrimp, *M. rosenbergii* and *M. malcolmsonii* and could increase the PUFA contents in shrimp carcasses (Radhakrishnan *et al.*, 2014; Muralisankar *et al.*, 2017). The fatty acid contents of the experimental diets in the present study showed significantly higher levels of LA and ALA in the diets inoculated with 25 and 50 g/kg MLP and the DHA and ARA contents in tissues of shrimp were significantly higher in shrimp fed with MLP inoculated diets. It is well known that Long chain PUFAs (LC-PUFAs) in aquatic species is endogenously biosynthesized from ALA and LA precursors through fatty acid desaturation and carbon chain elongation (Morais *et al.*, 2015; Monroig *et al.*, 2016; Feng *et al.*, 2021; Zhu *et al.*, 2023). It has been demonstrated that *L. vannamei* has the potential ability to convert ALA to DHA, EPA and ARA (Chen *et al.*, 2014; Li *et al.*, 2015; Pakravan *et al.*, 2017; Feng *et al.*, 2021). Therefore, it is likely that higher levels of LA and ALA in diets containing MLP could increase the LC-PUFA contents in the carcass of shrimp and improve the conversion of LA and ALA to LC-PUFAs in *L. vannamei*.

The improved growth performance and the higher survival of shrimp fed dietary MLP compared to the control group could be associated with the positive effect of MLP on fatty acid composition. In the current study, the increased levels of LC-PUFAs contents in MLP-fed shrimp were consistent with the higher growth indices and survival of shrimp fed with MLP-supplemented diets. It is well known that dietary PUFAs play crucial roles in growth performance, development, non-specific immunity and reproduction of *L. vannamei* (Feng *et al.*, 2021; Zhu *et al.*, 2023). Supplementation of DHA and ARA in the diet is found to be effective in promoting the growth and survival of *L. vannamei* (González-Félix *et al.*, 2003). The appropriate diet of LA and ARA could also positively improve the growth, antioxidant ability and non-specific immunity of *L. vannamei* ; Zhu *et al.*, 2023).

In the present study, the survival of shrimp was improved in response to low and high pH stress when fed with dietary MLP. It is known that low pH stress can make the exoskeletons and carapace of shrimp remarkably softer than the shrimp exposed to normal pH (Geoff and Greg, 1992). When exposed to acute low and high pH stress, oxidative damage occurs in shrimp, with apoptosis and changes in cell viability (Wang *et al.*, 2009). The mangrove plants possess several biological activities, *i.e.*, antioxidants that might help shrimp tolerate pH stress when it is inoculated into the diet of shrimp. It is well known that mangrove leaves contain a strong antioxidant activity as they grow under environmental stress conditions (Patra *et al.*, 2009). The extract of mangrove leaves showed promising antioxidant properties when tested for

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various antioxidant assays (Konishi *et al.*, 1998, 2000; Masuda *et al.*, 1999; Chen *et al.*, 2000; Patra *et al.*, 2009). These strong antioxidant properties are believed to be due to the phenolic and lignan type compounds, and a benzofuran derivative in mangrove leaves (Jong and Chau, 1998). In addition to the antioxidant capacity of MLP, our results showed higher amounts of carcass lipid and PUFAs in MLP-fed shrimp compared to the control group, which might help shrimp to survive better in a pH stress environment. Shrimp might use body lipids and fatty acid storage in muscle to break them down to glucose (Pascual *et al.*, 2006) and provide more energy to deal with the adverse effects of stress. In general, various studies have shown that dietary fatty acid composition is an important environmental variable that can have a significant effect on adaptive responses to physiological stresses (McKenzie *et al.*, 1997; Huang *et al.*, 2019). Previous studies on shrimp have shown that supplementation with plant extracts can enhance their ability to tolerate environmental stress. For example, a dietary ethanolic extract of *Prosopis juliflora* improved cold water stress resistance in *L. vannamei* (Zabolinia *et al*., 2024). Additionally, *L. vannamei* fed a diet containing *Moringa oleifera* leaf extract exhibited a significantly improved survival rate under high salinity stress (Baniesmaeili *et al*., 2023).

Conclusions

The results of this study indicated the positive effects of MLP on growth performance, survival, body biochemical and fatty acid composition, and pH stress resistance *L. vannamei*. Significant improvements in growth performance and survival were observed in shrimp fed different levels of MLP compared to the control group. Moreover, the body of shrimp fed diets containing different amounts of MLP showed significantly higher contents of LC-PUFAs *i.e.,* DHA and ARA compared to the control diet. The shrimp fed different levels of MLP also showed a higher survival rate than the control group. These results demonstrate the positive effects of dietary MLP on growth performance and resistance to pH stress in *L. vannamei*.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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