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Research Article

Effectiveness of dietary *Moringa oleifera* leaf powder and extract in the Pacific white shrimp (*Litopenaeus vannamei*)

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Abstract

Moringa oleifera is an important herbal plant with a valuable source of major essential nutrients and nutraceuticals. In this study, we supplemented the diet of *Litopenaeus vannamei* with different levels of *M. oleifera* leaf powder (MLP) and extract (MLE) and evaluated the growth, survival, body and fatty acid composition, hemolymph biochemistry, antioxidant status, and salinity stress resistance after six weeks of feeding. In total, 840 shrimp (2.6±0.02 g) were divided into seven groups including the shrimp fed with the basal diet (control), and the diets containing different levels of MLP [25 (MLP25), 50 (MLP50), and 100 (MLP100) g kg^{-1}] and MLE [0.25 (MLE0.25), 0.5 (MLE0.5), and 1.0 (MLE1.0) %]. The results showed a lower FCR value in MLP25 and MLE0.5 fed shrimp as well as a higher survival rate in MLP100 and MLE0.5 fed shrimp than those of the control group. Dietary MLP enhanced the body contents of lipid and fatty acids (*i.e.*, the pentadecanoic acid and the omega-3 and -6 polyunsaturated fatty acids). The MLP100 diet remarkably enhanced the hemolymph total protein, albumin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels of shrimp. On the other hand, the AST and ALT activities were significantly reduced in the shrimp fed with MLP25, MLE0.25 and MLE0.5 diets. MLP and MLE in the diet of shrimp also led to a significant increase in the hemolymph antioxidant enzymes activity. Moreover, the shrimp fed with MLP50 diet showed a significantly higher survival rate in response to the high salinity stress compared to the control group. In conclusion, the supplementation of both MLP and MLE in the diet of *L. vannamei* showed beneficial effects on the performance of the shrimp farming industry.

Keywords: Shrimp, Moringa, Growth, Fatty acids, Antioxidant capacity

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Introduction

The shrimp farming industry is contributing a major income to several countries in tropical and subtropical countries, especially in Asia (Burford *et al*., 2004, Zhang *et al.*, 2013). The Pacific white shrimp, *Litopenaeus vannamei*, is the most commonly shrimp cultured species in the global economy (Duan *et al*., 2017a). Given the advantages of fast-growing, euryhalinity, high-density culture, etc., *L. vannamei* is considered as the most important economic crustacean species, accounting for 53% of the total crustacean production worldwide (FAO, 2018).

Enhancing the performance of farmed shrimp using natural products is believed to be a beneficial approach to the health management of shrimp aquaculture (Duan *et al.*, 2017b). Using natural and safe feed additives in the shrimp diet could be an eco-friendly approach in shrimp aquaculture to enhance production and prevent disease (Chen *et al.*, 2019). Many herbs and plants and their products are known as important sources of multifunctional curing agents and bioactive compounds (Saini *et al.*, 2016a).

Moringa oleifera (MO) is known as an important herbal plant with a valuable source of major essential nutrients and nutraceuticals (Kunyanga *et al.*, 2013). The leaf of MO is characterized by a rich source of vitamins and minerals (e.g., ascorbic acid, tocopherol vitamin A, calcium, phosphorus, magnesium and potassium), carotenoids and folate, (e.g., β-carotene), and various phenolics and glucosinolates. It also contains low amount of anti-nutritional compounds such as phytic acid, tannins, oxalic acid and lectins (Nouala *et al.*, 2006; Amaglo *et al.*, 2010; Asare *et al.*, 2012; Egwui *et al.*, 2013; Saini *et al.*, 2014a; Saini *et al.*, 2014b; Saini *et al.*, 2014c; Saini *et al.*, 2014d; Karthivashan *et al.*, 2015; Saini *et al.*, 2016b).

The medicinal properties of MO are known as cardiovascular stimulant, antitumor, antipyretic, anti-inflammatory, anti-coagulant, anti-spasmodic, antihypertensive, cholesterol-lowering, antioxidant, anti-diabetic, hepato-protective, antibacterial, and antifungal effects (Anwar *et al.*, 2007). The positive effects of MO on flesh quality and omega-3 poly unsaturated fatty acids (PUFAs) contents have been also reported in animals (Nkukwana *et al.*, 2014; Zhang *et al.*, 2019; Selim *et al.*, 2021).

In aquaculture, the leaf powder and extract of MO have been supplemented to the diet of some fish and shellfish species. The positive effects of MO leaf powder (MLP) on growth performance, antioxidant activity, resistance to starvation stress, immunity system, and lipid metabolism have been demonstrated in Nile tilapia, *Oreochromis niloticus* (Richter *et al.*, 2003; Bbole *et al.*, 2016; Elabd *et al.*, 2019; El-Kassas *et al.*, 2020). Furthermore, MLP enhanced the growth performance in fingerlings of Indian carp, *Labeo rohita* (Hussain *et al.*, 2018). In Bocourti's catfish, *Pangasius*

bocourti up to 100 g kg^{-1} supplementation of MLP did not show any harmful effects on growth, nutrient digestibility, feed utilization, and serum biochemistry (Puycha *et al.*, 2017). The beneficial effects of dietary MLP on antioxidant activity have been also demonstrated in gilthead Seabream, *Sparus aurata* (Jiménez-Monreal *et al.*, 2021). The effect of Moringa leaf extract (MLE) has also been investigated in the diet of farmed shrimp. The MLE showed beneficial effects on growth performance, physiological and immune function, and resistance to *Vibrio anguillarum* infection and ammonia stress in freshwater prawn, *Macrobrachium rosenbergii* (Brilhante *et al.*, 2015; Kaleo *et al.*, 2019). Moreover, the administration of MLE improved growth performance, antioxidant activity, fatty acid composition, and resistance of shrimp *P. vannamei* against *Photobacterium damselae* (Akbary *et al.*, 2021).

Although, the MLE has been administrated in the diet of farmed shrimp, little is known about the effect of MLP on farmed shrimp performance. Therefore, in the present study, different levels of MLP and MLE were supplemented to the diet of the Pacific white shrimp (*L. vannamei*) and the growth performance, body and fatty acid composition, hemolymph biochemicals and antioxidant activity and resistance to salinity stress were evaluated and compared.

Materials and methods

Diet preparation

In this study, the diets with different levels of MLP [25 (MLP25), 50 (MLP50) and 100 g kg^{-1} (MLP100)] and MLE [0.25 (MLE0.25), 0.5 (MLE0.5) and 1.0 (MLE1.0) %] were prepared. A control diet without MO supplementation was also made. The main protein resource formulated in the diets were fish meal, soybean meal, shrimp powder, and rapeseed meal (Table 1). To prepare the diets containing MLP, the leaf of MO was washed and dried at room temperature. Then, the dried leaf was ground into a fine powder. All other ingredients were also powdered and mixed with oil and different levels of MLP and then pelleted (about 2.0 mm in diameter). The pellets were dried to contain nearly 10% moisture, wrapped in plastic bags, and kept at −4°C until consumed by shrimp. To prepare diets containing MLE, the dry powder of MO (100 g) was soaked in 600 mL of ethanol (70%) for 48 h in a percolator. Then, the liquid phase was separated and concentrated several times by filter. The obtained extracts were sterilized and stored in an airtight container at a cooled temperature and then added to the basal diets at the levels described above.

The major chemical and biochemical composition of both MLP and MLE (Table 2) were measured by GC-MS method using a 7890A gas chromatograph with a 5975C Network Mass Selective Detector (Agilent Technologies).

Experimental shrimp and conditions

Following two weeks of acclimation, the shrimp with an initial weight of 2.6±0.02 g were assigned into seven groups (one control and six treated groups), each with three replicates. 40 shrimp were randomly positioned in 300 L circular fiberglass tanks.

Table 1: The ingredients and chemical composition of the experimental diets (g/kg).

Ingredients (g/kg)	Control	MLP 25	MLP 50	MLP 100	MLE 0.25	MLE 0.5	MLE1.0
Fish meal	280	288	297	314	280	280	280
Soybean meal	190	190	190	190	190	190	190
Wheat flour	200	185.5	170	142	199.2	198.5	197
Wheat gluten	96	96	96	96	96	96	96
Corn gluten	134.0	116.5	99.0	64.0	132.3	130.5	127.0
Bentonite	10	10	10	10	10	10	10
Binder	10	10	10	10	10	10	10
Permix (Vitamin & Mineral additives)[1]	20	20	20	20	20	20	20
Fish oil	40.0	39.3	38.7	36.0	40.0	40.0	40.0
Soybean oil	20.0	19.7	19.3	18.0	20.0	20.0	20.0
Moringa leaves powder	0	25	50	100	0	0	0
Moringa leaves extract	0	0	0	0	2.5	5	10
Chemical composition (g/kg)							
Crud protein	424.8	407.5	432.2	420.2	433.9	430.3	423.2
Crud lipid	81.8	95.9	88.0	82.3	81.5	80.2	81.7
Carbohydrate	281.0	298.7	276.1	260.6	266.4	274.3	271.9
Ash	106.6	97.1	100.3	116.7	101.4	104.9	111.2
Dry matter (%)	894.2	899.2	896.6	879.8	883.2	889.7	888.0
Moisture (%)	105.8	100.8	103.4	120.2	116.8	110.3	112.0
Energy (kcal kg^{-1})	18089.6	18542.7	18424.8	17649.9	18041.4	18040.9	17891.4

[1] Permix (Creve Tec shrimp feed concentrate 2%): wheat protein, vitamins minimum value: (inositol, biotin, folic acid, nicotinic acid, panthothenic acid, vit B2 (riboflavin), vit B1 (thiamine), vit B6 (pyridoxine), vit B12 (cyanocobalamine), vit A1000, vit D3, vit K, vit C (L-ascorbic acid), choline, organic trace minerals: (Fe, Cu, Mn, Zn, Se, I), Phosphates, digestibility enhancer, cholesterol.

Table 2: Chemical and biochemical composition of *Moringa oleifera* leaf powder (MLP) and *M. oleifera* leaf extract (MLE).

Parameters in MLE	Value
K (mg L^{-1})	1174.9
Mg (mg L^{-1})	230.00
Ca (mg L^{-1})	36.80
Zn (mg L^{-1})	0.74
Fe (mg L^{-1})	<2.00
Protein (%)	1.24
Stigmasterol (%)	63.05
β-Amyrin (%)	17.75
Stigmast-7-en-3-ol (%)	8.60
4,22-Cholestadien-3-one (%)	5.80
Heptacosane (%)	4.80
Crude protein (%)	25.10
Crude lipid (%)	7.90
Carbohydrate (%)	40.45
Fiber (%)	8.37
Ash (%)	10.90
Dry matter (%)	92.72
Moisture (%)	7.28
Energy (kcal kg^{-1})	1600.15

One-third of the water in each tank was changed every day. Shrimp were fed with the experimental diets at a rate of 6.0–8.0% body weight at 8:00–8:30, 14:00–14:30, and 18:00–18:30. for six weeks daily. The amount of daily feed was readjusted every 2 weeks by

measuring the shrimp total weight in each tank. During the period of feeding trial, the range of temperature, pH, and DO were 30-32±2°C, 7.8–8.5, and >6 mg/L, respectively. After six weeks of the feeding trial, the shrimp from each tank were sampled and the body mass was measured.

Growth indices

Using the following formulae, the shrimp growth indices i.e., weight gain (WG), specific growth rate (SGR), protein efficiency ratio (PER), and feed conversion ratio (FCR) were calculated as follows:

Specific growth rate (SGR %/day)=100×[(ln final body weight–ln initial body weight)/feeding days]
Weight gain rate (WG, %)=100×[(final body weight-initial body weight)/ initial body weight] Feed conversion ratio (FCR)=[feed intake (g) / weight gain (g)]
Protein efficiency ratio (PER)=WG (g)/protein intake (g)
Survival rate (%)=[(initial shrimp number-dead shrimp number)/(initial shrimp number)]×100

Body proximate and fatty acid composition

The whole-body proximate composition of shrimp (three shrimp per tank) was assessed using the AOAC method (Cunniff, 1995). The amount of moisture was measured by drying in an oven (Binder, USA) at 105°C for 24 h. The Crude protein and lipid levels were measured using a Kjeldahl system (Gerhardt, type VAP.40, Germany), and ether extraction in a Soxhlet extractor (Gerhardt, type SE-416, Germany), respectively. The amount of ash was measured using a muffle furnace (Nabertherm, Germany) at 550°C for 8 h.

The fatty acid profile of three shrimp per tank for the control and MLP treatments were evaluated using an adapted method of Lepage and Roy (1984) as formerly described in Pakravan *et al.* (2017). Briefly, the fatty acids were quantified by an Agilent gas chromatograph (Agilent 7890A GC System, USA) using a BP×70 capillary glass column (0.32 mm×50 m, SGE Analytical Science Australia) after esterification and preparation of fatty acid methyl esters.

Hemolymph biochemistry and antioxidant status

Hemolymph was taken from three specimens per tank at the end of the feeding period as formerly described by Niroomand *et al.* (2020). The obtained hemolymph samples were retained in the refrigerator for 1–2 h at 4°C, and then, centrifuged at 4600 ×g for 10 min at 4°C. The biochemical and antioxidant parameters including total protein, cortisol, albumin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels were determined using the Roche Kits and an automatic blood analyzer (COBAS Integra 400 Plus, Germany). The activities of antioxidant enzymes including glutathione peroxidase (GP_x) superoxide dismutase (SOD), and catalase (CAT) were measured by a

spectrophotometric method using Zell Bio GmbH (Germany) kits as previously described (Rice-Evans and Miller, 1994).

Salinity challenge test

At the end of the experimental period, the shrimp from all treatments were exposed to the salinity stress. For both low and high salinity stress tests, 15 shrimp (9.7±0.16 g) from each experimental group were randomly taken and placed into three 300-L tanks. The shrimp were held off feed 24 h prior to the onset of the stress trial and gradually exposed to both low and high salinity stresses separately at a rate of 5 and 55 ppt, respectively (Akbarzadeh *et al*., 2019). The survival rate of the exposed shrimp was recorded 24 h after exposure and measured as follows:

Survival rate (%)=100×initial shrimp number−dead shrimp number/initial shrimp number

Statistical analysis

The obtained from different parameters were subjected to the normality test (Kolmogorov–Smirnov) and heterogeneity for variance (Leven's test) at the level of $p<0.05$. Then, differences in all the data among the treatments were analyzed using the one-way analysis of variance (ANOVA) followed by the Tukey posthoc test. Values are expressed as mean ± standard deviation (SD). The statistical analyses were conducted by SPSS and the figures were drawn using Sigma Plot software.

Results

Growth performance

The results of growth indices and survival rate of the shrimp fed with different levels of MLP and MLE are shown in Table 3. No significant differences were observed in the initial weight, final weight, weight gain, SGR, and PER among the treatments. However, the FCR value of the shrimp fed with MLE0.5 diet was significantly lower than the control group ($p<0.05$). The survival rate of the shrimp fed with MLP100 and MLE0.5 diets was significantly higher than the control group ($p<0.05$; Table 3).

Table 3: Growth performance and feed utilization of *Litopenaeus vannamei* fed with different levels of *Moringa oleifera* leaf powder (MLP) and *M. oleifera* leaf extract (MLE).

Parameters	Control	MLP 25	MLP 50	MLP 100	MLE 0.25	MLE 0.5	MLE 1.0
Initial Weight(g)	2.53±0.08	2.58±0.01	2.61±0.01	2.49±0.04	2.68±0.02	2.55±0.07	2.50±0.05
Final Weight (g)	9.93±0.53	10.38±0.38	9.54±0.72	9.15±0.37	9.60±0.12	9.62±0.01	10.17±0.41
SGR	3.03±0.19	3.09±0.88	2.86±0.17	2.88±0.06	2.83±0.02	2.94±0.69	3.11±0.04
WG (g)	7.39±0.61	7.80±0.39	6.92±0.71	6.66±0.33	6.92±0.11	7.06±0.07	7.66±0.36
FCR	1.70±0.11 ^{a}	1.46±0.06^{b}	1.73±0.18 ^{a}	1.56±0.03 ^{ab}	1.56±0.06 ^{ab}	1.40±0.05 ^{b}	1.63±0.03 ^{ab}
PER	3.06±0.39	2.77±0.42	2.82±0.20	2.64±0.09	2.70±0.22	2.93±0.16	2.82±0.49
Survival rate (%)	80.83±2.20^{b}	90.83±7.94^{ab}	90.00±1.44^{ab}	96.66±3.33^{a}	91.66±3.33^{b}	100.00±0.00^{a}	83.33±3.00^{b}

Data (mean±SD) with different letters are significantly different among treatments according to the Tukey posthoc test ($p<0.05$). WG: weight gain; SGR: specific growth ratio; FCR: feed conversion ratio; PER: protein efficiency rate.

Proximate body composition

The proximate chemical composition of whole-body of *L. vannamei* fed diets containing different levels of MLP and MLE are presented in Table 4. The MLE 0.25 fed shrimp showed a significantly lower amount of protein compared to other treatments ($p < 0.05$). Moreover, the content of body lipid in the shrimp fed with different levels of MLP was significantly higher than the control group ($p<0.05$), but MLE showed no significant effect on the lipid content of shrimp ($p>0.05$). The MLE0.25 and MLE0.5 treatments showed a significantly lower amount of ash compared to the control group, but the MLE0.5 and MLE1.0 treatments revealed significantly higher amounts of carbohydrates than the control treatment ($p<0.05$). The carbohydrate content of shrimp fed with different levels of MLP was significantly lower than the control group ($p<0.05$).

Table 4: Proximate body composition (g kg^{-1} wet weight) of *Litopenaeus vannamei* fed with different levels of *Moringa oleifera* leaf powder (MLP) and *M. oleifera* leaf extract (MLE).

Parameters	Treatments						
	Control	MLP 25	MLP 50	MLP 100	MLE 0.25	MLE 0.5	MLE 1.0
Protein	198.14 ± 2.11^{a}	195.55 ± 0.67^{a}	202.77 ± 8.18^{a}	201.77 ± 0.18^{a}	177.41 ± 5.32^{b}	196.43 ± 1.58^{a}	200.85 ± 0.69^{a}
Lipid	4.52 ± 0.15^{d}	5.73 ± 0.15^{b}	6.50 ± 0.38^{a}	4.99 ± 0.15^{c}	4.29 ± 0.27^{d}	4.65 ± 0.40^{cd}	4.46 ± 0.12^{d}
Ash	14.16 ± 0.54^{a}	13.96 ± 4.93^{a}	13.94 ± 0.11^{a}	13.58 ± 0.23^{ab}	12.12 ± 0.01^{c}	13.04 ± 0.65^{b}	14.21 ± 0.71^{a}
Carbohydrate	38.45 ± 0.15^{b}	36.47 ± 0.14^{c}	35.03 ± 1.52^{c}	35.14 ± 1.10^{c}	35.33 ± 1.33^{c}	40.19 ± 0.15^{a}	41.42 ± 0.55^{a}
Moisture	744.74 ± 2.66^{b}	748.30 ± 1.15^{b}	741.76 ± 10.48^{b}	745.22 ± 0.54^{b}	770.85 ± 6.93^{a}	745.68 ± 2.05^{b}	739.06 ± 0.72^{b}

Data (mean±SD) with different letters are significantly different among treatments according to the Tukey posthoc test ($p<0.05$). Carbohydrate (CHO) was calculated by the formula: CHO=100-(moisture+crude ash+crude lipid +crude protein)

Fatty acid composition

The composition of body fatty acids in *L. vannamei* fed with diets containing different amounts of MLP and control are shown in Table 5. The contents of C15:0 (pentadecanoic acid) and C17:0 (heptadecanoic acid) were significantly higher in MLP treatments compared to those of the control group. However, the amount of saturated fatty acids (SFA) was significantly higher in the shrimp fed control diet compared to that of MLP treatments ($p<0.05$). The linoleic acid (LA) and alpha-linolenic acid (ALA) fatty acids were significantly higher in MLP100 and MLP25 treatments compared to the control group, respectively ($p<0.05$). Moreover, the amount of docosahexaenoic acid (DHA) was significantly higher in MLP50 fed shrimp compared to the control group ($p<0.05$). The body content of arachidonic acid (ARA) significantly elevated in MLP25 and MLP50 treatments compared to the control group ($p<0.05$). The amount of PUFAs was also significantly higher in both MLP50 and MLP100 treatments compared to the control group ($p<0.05$). The n3/n6 ratio was significantly higher in MLP50 treatment compared to the control group ($p<0.05$).

Hemolymph's biochemical and antioxidant indices

The results of hemolymph biochemical parameters and antioxidant activity of

L. vannamei fed diets supplemented with different levels of MLE and MLP are shown in Table 6. There were significant differences among the experimental treatments (p<0.05). The levels of serum total protein, albumin, and the activities of ALT and AST were significantly higher in MLP100 treatment compared to the control group (p<0.05). On the other hand, the activities of ALT and AST enzymes were significantly lower in MLP25, MLE0.25, and MLE0.5 treatments than the control group. The SOD and GP_x activities were higher in MLP50 and MLP100 groups compared to the control group. Moreover, the shrimp fed with MLE1.0 diet showed a significant increase in the hemolymph CAT compared to the control group (p<0.05). The amount of hemolymph cortisol showed a significant decrease in the shrimp fed with MLP50, MLP100, and MLE1.0 diets compared to the control group (p<0.05).

Table 5: Fatty acid composition of *Litopenaeus vannamei* fed different levels of *Moringa oleifera* leaf powder (MLP).

Parameter	Control	MLP 25	MLP 50	MLP 100
C14:0	0.00±0.00	0.00±0.00	00±0.00	0.00±0.00
C15:0	0.22±0.00^{c}	0.96±0.12^{b}	0.82±0.01^{ab}	1.5±0.34^{a}
C16:0	22.6±0.7^{a}	22.4±0.02^{ab}	20.5±0.71^{bc}	19.5±0.62^{c}
C17:0	1.25±0.01^{ab}	1.08±0.03^{b}	1.6±0.22^{a}	1.3±0.07^{ab}
C18:0	11.01±0.2	10.2±0.1	10.8±0.19	10.56±0.45
C20:0	0.71±0.01^{a}	0.62±0.00^{a}	0.51±0.05^{b}	0.45±0.02^{b}
SFA	35.88±1.02	35.34±0.01	34.32±1.09	33.47±1.52
C16:1n	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
C17:1n	0.27±0.02^{b}	0.49±0.01^{a}	0.63±0.06^{a}	0.5±0.09^{a}
C18:1n-9	27.1±0.67^{a}	23.49±0.00^{b}	22.44±0.31^{b}	22.79±0.96^{b}
C20:1n	1.17±0.03^{bc}	1.33±0.02^{a}	1.25±0.03^{ab}	1.09±0.03^{c}
MUFA	28.57±0.69^{a}	25.32±0.04^{b}	24.32±0.28^{b}	24.38±1.09^{b}
C18:2n-6 (LA)	11.61±0.31^{b}	12.36±0.03^{b}	11.15±0.08^{b}	18.39±3.55^{a}
C18:3n-6	0.00±0.00^{b}	0.15±0.01^{a}	0.00±0.00^{b}	0.00±0.00^{b}
C18:3n-3(ALA)	0.49±0.00^{b}	0.68±0.00^{a}	0.5±0.00^{b}	0.48±0.02^{b}
C20:4n-6 (ARA)	2.87±0.07^{b}	3.36±0.00^{a}	3.21±0.02^{a}	2.63±0.09^{c}
C20:5n-3 (EPA)	10.64±0.24^{a}	9.08±0.01^{b}	11.13±0.19^{a}	9.02±0.34^{b}
C22 :4n-6 (DTA)	0.18±0.01^{ab}	0.22±0.02^{ab}	0.15±0.03^{b}	0.25±0.00^{a}
C22 :5n-6	0.48±0.01^{a}	0.36±0.02^{b}	0.00±0.00^{c}	0.38±0.02^{b}
C22:5n-3 (DPA)	1.08±0.02^{a}	1.14±0.01^{a}	1.15±0.05^{a}	0.84±0.05^{b}
C22:6n-3 (DHA)	8.16±2.4^{b}	11.94±0.02^{ab}	14.02±0.41^{a}	10.1±0.38^{ab}
PUFAs	35.55±1.72^{b}	39.34±0.03^{ab}	41.35±0.81^{a}	42.14±2.62^{a}
HUFAs	21.68±2.09^{b}	24.4±0.04^{b}	28.38±0.63^{a}	21.77±0.82^{b}
Total n-3	20.39±2.13^{b}	22.87±0.06^{b}	26.81±0.66^{a}	20.46±0.8^{b}
Total n-6	15.15±0.41^{b}	16.47±0.09^{ab}	14.53±0.14^{b}	21.68±3.42^{a}
n-3/n-6	1.35±0.17^{b}	1.39±0.01^{b}	1.84±0.02^{a}	1.00±0.2^{b}
DHA/EPA	0.78±0.24^{b}	1.31±0.00^{a}	1.26±0.01^{a}	1.12±0.00^{ab}
ARA/EPA	0.27±0.00^{c}	0.37±0.00^{a}	0.28±0.00^{b}	0.29±0.00^{b}
n-3 LCPUFA	18.8±2.16^{b}	21.03±0.04^{b}	25.16±0.6^{a}	19.13±0.73^{b}
n-3 LCPUFA/ARA	6.59±0.92	6.25±0.02	7.8±0.12	7.25±0.01

Data (mean±SD) with different letters are significantly different among treatments according to the Tukey posthoc test (p<0.05). SFA: saturated fatty acid, MUFA: mono unsaturated fatty acid, LA: linoleic acid, ALA: alpha linolenic acid, ARA: arashidonic acid, EPA: eicosapentaenoeic acid, DHA: docosahexaenoic acid, PUFA: polyunsaturated fatty acid, HUFA: highly unsaturated fatty acids.

Salinity challenge test

The survival rate (%) of *L. vannamei* fed diets containing different amounts of MLP after exposure to low and high salinity stress are presented in Table 7. After 24 h exposure to the low salinity stress, the survival rate did not show any significant differences among all treatments (p>0.05), but the shrimp fed with MLP50 diet showed a significantly higher survival rate in response to the high salinity stress compared to the control treatment (p<0.05).

Table 6: Plasma biochemical and antioxidant parameters of *Litopenaeus vannamei* fed with different levels of *Moringa oleifera* leaf powder (MLP) and *M. oleifera* leaf extract (MLE).

Parameter	Treatments						
	Control	MLP 25	MLP 50	MLP 100	MLE 0.25	MLE 0.5	MLE 1.0
Albumin (mg mL^{-1})	4.65 ± 0.16^{b}	4.36 ± 0.30^{b}	4.87 ± 0.32^{b}	5.02 ± 0.13^{a}	4.93 ± 0.32^{b}	4.47 ± 0.35^{b}	4.65 ± 0.55^{b}
Alanine aminotransferase (ALT) (U ml^{-1})	215.33 ± 12.66^{a}	183.33 ± 3.79^{b}	218.67 ± 21.55^{a}	219.67 ± 16.65^{a}	191.00 ± 4.58^{b}	183.33 ± 11.93^{b}	199.33 ± 8.50^{ab}
Aspartate aminotransferase (AST) (U ml^{-1})	152.67 ± 18.72^{bc}	136.33 ± 4.93^{cd}	170.33 ± 16.26^{ab}	183.67 ± 12.58^{a}	128.00 ± 5.57^{d}	128.33 ± 3.79^{d}	154.67 ± 13.65^{bc}
Cortisol (ng mL^{-1})	23.37 ± 0.61^{a}	24.00 ± 0.36^{a}	20.60 ± 0.79^{b}	20.37 ± 1.33^{b}	23.03 ± 0.40^{a}	22.70 ± 1.11^{a}	20.87 ± 0.65^{b}
Total Protein (mg mL^{-1})	46.00 ± 1.00^{bc}	44.00 ± 1.00^{bc}	47.67 ± 1.53^{ab}	51.00 ± 2.65^{a}	48.00 ± 3.61^{ab}	43.00 ± 1.00^{c}	47.00 ± 3.00^{abc}
Catalase (CAT) (U mL^{-1})	140.00 ± 10.58^{bc}	125.67 ± 7.02^{c}	127.00 ± 10.58^{c}	122.67 ± 5.16^{c}	149.33 ± 2.52^{ab}	149.00 ± 4.58^{ab}	162.67 ± 16.62^{a}
superoxide dismutase (SOD) (U mL^{-1})	57.00 ± 2.00 ab	53.33 ± 1.53^{b}	61.33 ± 5.13^{ab}	64.67 ± 7.37^{a}	57.00 ± 4.36^{ab}	59.33 ± 2.52^{ab}	59.67 ± 4.62^{ab}
Glutathione peroxidase (GPx) (U mL^{-1})	1220.00 ± 65.57^{ab}	1120.00 ± 30.00^{b}	1370.67 ± 82.11^{a}	1361.67 ± 80.36^{a}	1338.33 ± 137.14^{ab}	1226.67 ± 40.41^{ab}	1290.67 ± 79.37^{ab}

Data (mean±S.D.) with different letters are significantly different among treatments according to the Tukey posthoc test (p<0.05).

Table 7: Survival rate (%) of *Litopenaeus vannamei* fed with different levels of *Moringa. oleifera* leaf powder (MLP) and *M. oleifera* leaf extract (MLE) after 24 h exposure to low (8 g L^{-1}) and high (55 g L^{-1}) salinity stress.

Items	Treatments						
	Control	MLP 25	MLP 50	MLP 100	MLE 0.25	MLE 0.5	MLE 1.0
Survival rate (%) at low salinity	73.3±11.6	80.0±0.0	93.3±11.6	80.0±0.0	86.7±11.6	86.7±11.6	73.3±11.6
Survival rate (%) at high salinity	60.0±0.0^{b}	46.7±11.6^{b}	86.7±11.6^{a}	60.0±0.0^{b}	46.7±11.6^{b}	60.0±0.0^{b}	60.0±0.0^{b}

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

Discussion

The leaf of *M. oleifera* is a valuable and encouraging source of nutrients for supplementation in aquafeed (Tagwireyi *et al.*, 2017). Given the nutritional value of MO leaf and its extensive availability all over the tropical and subtropical regions, it can be considered as a potential feed ingredient for farmed shrimp feed. Therefore, the present study examined the effectiveness of different levels of MLP and MLE in the diet of the Pacific white shrimp (*L. vannamei*). Our results showed no remarkable effect of dietary MO on the growth indices. However, the supplementation of both MLP and

MLE improved the survival rate of the shrimp. Moreover, the body contents of lipids and fatty acids (*i.e.*, pentadecanoic acid and omega-3 and omega-6 polyunsaturated fatty acids), and the amount of hemolymph biochemical and antioxidant parameters were significantly improved in response to the dietary supplementation of MLP.

After six weeks of the feeding trial, the growth indices of the shrimp were not remarkably affected by dietary MLP and MLE, however, dietary MLE 0.5 showed a significant positive effect on the FCR value. Moreover, both MLP and MLE could significantly improve the survival rate. Previous studies in both freshwater and seawater shrimp farming demonstrated the positive effects of dietary MLE on growth performance. Dietary MLE could improve the growth indices of freshwater prawn, *M. rosenbergii* (Kaleo *et al.* 2019). Similarly, Akbary *et al.* (2021) showed that 1.0 g kg^{-1} MLE significantly enhanced the growth indices of *L. vannamei*. The effectiveness of MLE on the growth performance of shrimp is reported to be attributed to the improved digestive enzymes activity and physiological functions due to the increased energy flows in the host (Kaleo *et al.*, 2019).

Our results also revealed that the supplementation of both MLP and MLE affected the body composition of the shrimp. Interestingly, the shrimp fed with different levels of MLP showed a higher amount of lipids in the body compared to the control group. The enhancement of body lipid content could be attributed to the high amount of lipid in Moringa leaf. It has been known that *M. oleifera* leaf contains more than 7% lipids (Teixeira *et al.*, 2014; Su and Chen, 2020) and this amount is higher than the lipids amounts in other woody plants. Previous studies also showed that dietary MLP increased the body lipid contents in African sharptooth catfish, *Clarias gariepinus* (Idowu *et al.*, 2017), fingerlings of Indian carp, *Labeo rohita* (Hussain *et al.*, 2018), and abalone, *Haliotis asinina* (Reyes and Fermin, 2003). Therefore, dietary MLP can have beneficial effects on farmed aquatic animals by increasing the amount of lipid contents in the body.
Given that the effect of MLE on the fatty acid composition of *L. vannamei* has been already reported (Akbary *et al.*, 2021), in this study, we only evaluated the effectiveness of MLP on the fatty acid contents of the shrimp.

Our results showed beneficial effects of MLP on fatty acid contents in *L. vannamei*. Interestingly, the supplementation of MLP could significantly enhance the content of C15:0 fatty acid (pentadecanoic acid) in shrimp. Pentadecanoic acid has been recently found as a potential essential fatty acid with remarkable health benefits for humans (Venn-Watson *et al.*, 2020). Pentadecanoic acid is believed to provide worthwhile health benefits against several cardiometabolic, liver, and aging-associated conditions (Venn-Watson *et al.*, 2020). Pentadecanoic acid has the antifibrotic, anti-inflammatory, red

blood cell-stabilizing and mitochondrial-reparative properties that can reduce inflammation, anemia, and liver fibrosis (Venn-Watson *et al.,* 2020). Akbary *et al.* (2021) also showed that 1.0 g Kg^{-1} inclusion of MLE in the diet remarkably enhanced the pentadecanoic acid content of *L. vannamei.* On the contrary, dietary MLP showed no effects on the Pentadecanoic acid content of fish such as gilthead seabream, *S. aurata* (Jiménez-Monreal *et al.,* 2021) and finishing pigs (Zhang *et al.,* 2019). Our data also showed that dietary MLP could enhance the contents of both omega-3 and omega-6 PUFAs in the body of *L. vannamei*. Their increment in the MLP-treated shrimp may be related to high levels of the PUFA fatty acids and the high antioxidant contents (*e.g.*, phenolic compounds) in MLP (Lalas and Tsaknis, 2002). The enhancement of PUFA in response to the supplementation of MLE has been reported in *L. vannamei* (Akbary *et al.*, 2021). The results of body fatty acid composition also showed that the dietary MLP increased the n-3/n-6 ratio in the shrimp. Therefore, MLP can be considered a beneficial herbal supplement that increases the omega-3 PUFA contents and increases the quality of the shrimp for consumers. Notably, it is known that over 50 % of fatty acids in *M. oleifera* leaf are unsaturated fatty acids, and α-linolenic acid is the most abundant *unsaturated* fatty acid in *M. oleifera* leaf (Su and Chen, 2020). Therefore, dietary MLP could improve the fatty acid composition of the shrimp due to the presence of unsaturated fatty acids in Moringa leaf. The improvement of fatty acid profile and the increase of omega-3 PUFAs in response to the inclusion of MLP in the diet has been also reported in other animals such as broiler chickens (Nkukwana *et al.,* 2014), pig (Zhang *et al.,* 2019), rabbits (Selim *et al.,* 2021), and goats (Kholif *et al.,* 2019).

In this study, the supplementation of MLP and MLE in the diet of shrimp remarkably improved the hemolymph biochemistry. Our results showed that the levels of total protein and albumin were significantly higher in MLP 100 treatment compared to the control group and other experimental treatments. It has been known that the haemolymph biochemical indicators including the haemolymph protein and albumin contents can be used in determining the health status of the shrimp. In line with our results, dietary MLP significantly enhanced the plasma protein and albumin contents in Nile tilapia (El-Kassas *et al.,* 2020; Elgendy *et al.,* 2021) and other animals (Lu *et al.,* 2016; Meel *et al.,* 2018; Kholif *et al.,* 2019; Afzal *et al.,* 2021). The administration of MLE did not enhance the haemolymph protein and albumin contents in *M. rosenbergii* (Kaleo *et al.,* 2019) which was consistent with our results in *L. vannamei*. However, the addition of MLE to the diet of Nile tilapia, *O. niloticus,* enhanced the levels of plasma total protein and albumin (Shourbela *et al.,* 2020). The positive effects of the dietary MLP on the

plasma protein and albumin contents of the shrimp could be attributed to the high contents of proteins in MLP.

Our data also showed that the activities of ALT and AST were significantly influenced by the dietary MLP and MLE. The serum ALT and AST activities are known as indicators of the health status of hepatopancreas, so that the increased serum ALT and AST activities could be associated with the impaired hepatopancreas cells (Yu *et al.,* 2021). The significant decrease of the serum ALT and AST activities in shrimp fed with MLP25, MLE0.25 and MLE0.5% diets observed in the present study could be related to the protective effects of the MLP and MLE on the shrimp hepatopancreas. In line with our data, a previous study also showed the decrease in activities of ALT and AST in freshwater prawn, *M. rosenbergii* fed with MLE supplemented diets (Kaleo *et al.*, 2019).

The results of this study also showed that the MLP and MLE supplementation improved some plasma antioxidant indices in the shrimp. The positive effects of MLP and MLE on the antioxidant activities of the shrimp could be attributed to the bioactive and biological compounds in Moringa, especially antioxidant substances such as flavonoids (myricetin, quercetin, and kaempferol), phenolic acids (gallic, chlorogenic, and ellagic acid), proanthocyanidins, vitamin E, vitamin C, selenium, zinc, and β-carotene (Makkar and Becker, 1997; Wei and Shibamoto, 2007; Kaleo *et al.,* 2019; Afzal *et al.,* 2021). This positive effect has been already reported in fish and shellfish including *O. niloticus* (Shourbela *et al.,* 2020), *S. aurata* (Jiménez-Monreal *et al.,* 2021), shellfish, i.e., *M. rosenbergii* (Kaleo *et al.,* 2019), *L. vannamei* (Akbary *et al.,* 2021) when fed diets supplemented with MLP and MLE.

In the present study, low and high salinity were chosen as common environmental stressors that are widespread in the shrimp culture systems. Although, *L. vannamei* is a euryhaline shrimp species, however, any changes in the ambient salinity can influence the metabolism, growth, oxygen consumption, feeding rate, molting, and survival (Li *et al*., 2007; Chen *et al*., 2014; Akbarzadeh *et al*., 2019). Our results showed that exposure to low salinity stress did not cause any significant differences in the survival rate of the shrimp. Furthermore, in all feeding treatments, the shrimp could survive more successfully in the low salinity stress compared to the higher one. On the other hand, the survival rate of the shrimp fed diet containing MLP 50 was significantly higher than the control group, when they were exposed to the high salinity stress. It is well-known that the metabolites in Moringa leaf such as proline can improve the plasma membrane functions due to their ability to scavenge reactive oxygen species (ROS), buffer redox potential, and stabilize the membrane. Therefore, the metabolites in Moringa leaf might help the shrimp to mitigate the adverse

effects of the salinity stress (Hassan *et al*., 2021).

In conclusion, the results of the present study showed that the supplementation of both MLP and MLE improved the survival rate of *L. vannamei*. Moreover, the body contents of lipid and fatty acid composition of the shrimp were significantly improved in response to dietary MLP. Both MLP and MLE also improved serum biochemistry and antioxidant activity. Considering the beneficial effects of dietary Moringa on the performance of shrimp observed in this study, up to 100 g kg^{-1} MLP or 1.0 % MLE can be recommended as a supplementation to the diet of farmed shrimp.

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