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Research Article

Dietary effect of *Artemia urmiana* enriched with a brown macroalgae premix (*Padina australis, Sargassum ilicifolium,* and *Stoechospermum marginatum*) on the growth performance, nutritional value, phytochemical, and antioxidant properties of *Litopenaeus vannamei*

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Abstract

The aim of this investigation is to evaluate the effects of *Artemia urmiana* metanauplii enriched with a premix extract of brown macroalgae including *Padina australis*, *Sargassum ilicifolium*, and *Stoechospermum marginatum* (macroalgae premix extract; MPE) on growth performance, survival, carcass quality traits, and antioxidant properties of *Litopenaeus vannamei* (PL_{15}). A total of 1200 PL_1(19±0.97 mg) were randomly divided into 4 groups (100 individuals in each group (in triplicate)). The control group was fed only with non-enriched metanauplii (MPE_0) and other groups were fed with metanauplii enriched with 200 (MPE_{200}), 400 (MPE_{400}), and 600 (MPE_{600}) mg L^{-1} for 15 days. Our findings revealed that the highest specific growth ratio (SGR), percentage weight gain (WG), protein efficiency ratio (PER) and dry matter were recorded in the group fed with MPE_{600}-enriched metanauplii. The feed conversion ratio (FCR) in the group fed with MPE_{600} was less than in other experimental treatments (p<0.05). The highest levels of polyunsaturated fatty acid (PUFA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), arachidonic acid(ARA), total sterols, and total amino acid content were observed in the shrimp fed with metanauplii enriched with MPE_{600} (p<0.05). The phenol, flavonoid, superoxide dismutase (SOD), and catalase (CAT) contents were increased by increasing levels of MPE in themetanauplii diet (p<0.05). This study demonstrates the positive effect of metanauplii enrichment with 600 mg L^{-1}MPE on growth performance, carcass quality, and antioxidant properties of *L.vannamei*.

Keywords: *Litopenaeus vannamei*, Artemia enrichment, seaweed, Live feed, Growth

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Introduction

Whiteleg shrimp, *Litopenaeus vannamei*, has been introduced as one of the most important aquaculture shrimp species worldwide due to its high tolerance to a wide range of salinity (5-40 ppt), rapid growth, and low protein requirement. Nevertheless, the development of the shrimp farming industry has confronted environmental and pathological issues and challenges (Ghaednia *et al.*, 2011). Therefore, natural immune stimulants are highly recommended to add in aquafeed to improve health and safety performance compared to the administration of antibiotics or vaccines to control or prevent infectious diseases (Meenakshi *et al.*, 2019). On the other hand, strengthening shrimp immunity is considered as an important point in the current support policies for disease prevention in shrimp (Ghaednia *et al.*, 2011).

Macroalgae are rich in various bioactive compounds (Reverter *et al.*, 2014; Thanigaivel *et al.*, 2014). Algae (red, brown, and green species) are characterized by a wide variety of properties including nutritional, growth-enhancing, cytostatic, antioxidant, immunomodulatory, neuroprotective, hepatic protective properties as well as antibacterial, antifungal, and antiviral activities (Milledge *et al.*, 2016; Sanjeewa *et al.*, 2016; Bolaños *et al.*, 2017; Schleder *et al.*, 2018; Rushdi *et al.*, 2020).

Macroalgae species have recently been considered as alternative protein sources in aquaculture (Nakagawa and Montgomery, 2007) due to essential amino acid content, vitamins, and trace metals. In 2016, the annual production of seaweed was reported to be 31.2 million tons (Buschmann *et al.*, 2017; Ferdouse *et al.*, 2018) and the annual value of nearly of the industry is estimated to have an US$6 billion (FAO, 2018). Nevertheless, employing a practical and sustainable approach to algae farming which is economical and productive in different parts of the world is still challenging. In recent years, several studies have been conducted in Iran on macroalgae species found on the shores of the Persian Gulf and the Sea of Oman (Kokabi and Yousefzadi, 2015). In the last study in 2015, a checklist of the country's marine seaweeds was prepared, which resulted in 309 species of algae (78 species in15 families of green algae, 70 species in 7 families of brown algae, and 161 species in30 families of red algae) (Sohrabipour and Rabiei, 2007; Kokabi and Yousefzadi, 2015). Distribution and relative abundance of red, brown, and green algae species vary depending on latitude and seawater temperature (Bocanegra *et al*., 2009; Tabarsa *et al*., 2012). Brown macroalgae including *Padina australis*, *Sargassum ilicifolium*, and *Stoechospermum marginatum* are found on the Chabahar coast located in southeastern Iran and rich in polysaccharides, proteins, fatty acids, vitamins, and minerals(Ashour *et al.*, 2020). The nutritional value of seaweed food supplements in aquaculture industry is mainly evaluated with factors such as growth performance, feed

consumption, and survival rate (Ortiz *et al.*, 2006;Garcia-Casal *et al.*, 2007). It has also been shown that the inclusion of seaweed in aquafeed leads to positive changes in factors such as gut microbiota, immune system enhancement, growth performance, feed utilization, and disease resistance in a large number of aquatic animals (Ashour *et al.*, 2020).

Macroalgae offer a novel and added-value dietary ingredient in formulated diets for fish and shellfish. In some studies, the growth performance of shrimps fed with diets containing macroalgae have been reported by many researchers (Cruz-Suarez *et al.*, 2008; Rodríguez-González *et al.*, 2014; Cárdenas *et al.*, 2015; Akbary and Aminikhoei, 2018b; Akbary *et al.*, 2020a; Akbary *et al.*, 2021a). Rodríguez-González *etal*. (2014) reported that *Ulva lactuca* and *Gracilaria parvispora* macroalgae may be utilized as protein sources in balanced diets for *L.vannamei*. Although some studies have investigated the use of seaweed as a protein source in the shrimp diet, there is no study conducted on the premix brown macroalgae as a feed additive in shrimp diets. As far as we know, there is no preliminary study accomplished concerning the inclusion of premix of macroalgae including *Padina australis*, *Sargassum ilicifolium*, and *Stoechospermum marginatum* as a feed additive in whiteleg shrimp diets. In the present study, we aimed to determine the dietary effects of *Artemia urmiana* enriched with a brown macroalgae premix (*P. australis*, *S.ilicifolium*, and *S. marginatum*on) on growth, survival, nutritional value (approximate composition, amino acid, and fatty acid), and phytochemical components (sterol, Phenols, and flavonoids), and antioxidant status (superoxide dismutase; SOD, catalase; CAT, glutathione peroxidase; GPx, and malondialdehyde; MDA) in *L.vannamei*.

Material and methods

Preparation of premix extract of macroalgae

The whole seaweed samples of three species, *P.australis, S. marginatum*, and *S. ilicifolium* were collected from Chabahar coast (Iran) during low tide in November-December 2021. The classification details and habitats of the three species were displayed in Table1.

Table 1: Classification details and Habitat on the Chabahar coasts of the three macroalgae studied.

Class	Order	Family	Species	Habitat
Phaeophyceae	Dictyotales	Dictyotaceae	*Padina australis* Hauck	Lower shore
	Dictyotales	Dictyotaceae	*Stoechospermum marginatum* (C.Agardh) Kützing	Lower shore
	Dictyotales	Dictyotaceae	*Sargassum ilicifolium* (Turner) C.Agardh	Lower shore

They were rinsed several times with freshwater to remove mud, grass, and epiphytes and then dried away from sun at room temperature (25°C). To prepare the extract, the dried macroalgae were initially ground into fine pieces by an electrical mill (20 µm). Next, 200g of the powdered sample containing three

macroalgae species (1:1:1) were added to 200 ml ethanol (90%) in triplicate and the bottles were shaken vigorously for 20 min. Then, they were kept in a closed dark container. The supernatants were carefully collected and passed through a filter paper (Whatman paper No. 1). To concentrate the extract, a rotary evaporator (vacuum distillation) was employed at 45°C for 6 h. Then, the obtained extract was placed in a clean petri dish using a laminar flow hood to promote evaporation of the residual solvent. The extracts obtained from the replicates were mixed before using the rotary evaporator and maintained at -20°C until further use (Choudhury *et al.*, 2005).

The evaluation of nutritional value, phytochemical, and antioxidant properties of macroalgae premix extracts (MPE)

The nutritional value, phytochemical, and antioxidant properties of macroalgae premix extracts (MPE) are presented in Table 2. The assessment of nutritional value of MPE includes chemical composition, amino acid and fatty acid were measured by Lindroth and Mopper (1979), AOAC (2020), and Pal *et al.* (2013) methods respectively. The phytochemical compounds were appraised using various reagent testing. Sterols were analyzed by gas chromatography (Unicam 4600, Germany) (Liu *et al.*, 2007), and phenol and flavonoid (Ebrahimzadeh *et al.*, 2008) at 765 and 415 nm, respectively by the spectrophotometery (Shimadzu, uv-1800, Japan). Investigation of the antioxidant activity was conducted employing a free radical scavenging capacity assay kit based on the DPPH assay (Zytox kit, KavoshAzma Co.) at 515 nm by a micromole spectrophotometer (Shimada *et al.*, 1992).

Table 2: Nutritional value, phytochemical, and antioxidant activity of ethanol extract of the brown macroalgae extract premix (*Padina australis, Sargassum ilicifolium*, and *Stoechospermum marginatum*).

Analyzed compounds	Value
Crude protein (g.100 g^{-1})	68.01±0.93
Crude fat (g.100 g^{-1})	0.24±8.43
Ash(g.100 g^{-1})	0.12±2.21
Carbohydrate (g.100 g^{-1})	3.21±19.05
Raw energy (cal g^{-1})	352±4158.39
Moisture (g.100 g^{-1})	0.93±68.01
Total amino acid (g amino acid.100 g^{-1})	13.93 ±3.62
Saturated fatty acid (% of total fatty acid)	39.10 ±12.05
Monounsaturated fatty acid (% of total fatty acid)	22.95 ±9.13
polyunsaturated fatty acid (% of total fatty acid)	36.1 ±5.87
Sterols (g.100 g^{-1})	368.78 ±24.12
Phenol (mg GAE. g^{-1} extract)	132.46±11.07
Flavonoid (mg QE. g^{-1} extract)	25.51±0.67
diphenyl picrylhydrazyl (DPPH, µmol trolex. g^{-1} extract)	1967.32±31.07

Preparation and hatching of Artemia Cysts

Artemia cysts were obtained from the Konarak Shrimp Breeding Center, Iran and were hatched according to the standard hatching protocol (Sorgeloos *et al.*, 2001). Disinfected with sodium hypochlorite (200mgL^{-1}) for 20 min, 24 g cysts were rinsed with fresh water, placed in containers filled with 10 L of autoclaved seawater (35g L^{-1}) and hatching occurred in conical tanks at 28-30°C with continuous aeration and the light intensity regime of 2000lux for 24 h. After 12 h, instar II *Artemia* nauplii were isolated and pooled from the shell employing the positive photosensitive properties. They were then passed through a 120µ filter and washed with fresh water for further use.

Preparation of the macroalgae premix extracts (MPE) emulsion and enrichment of Artemia nauplii

The enrichment material was dissolved in 1000mL sterile lukewarm seawater (45°C) according to the protocol done by Leger *et al*. (1986) method with minor modifications. Algae extracts were added to the solution in different ratios including 200, 400, and 600 mg L^{-1} (MPE_0, MPE_{200}, MPE_{400}, and MPE_{600}) and homogenized on an electric mixer to assure complete homogeneity. For enrichment, 12 plastic bottles (1.5L) containing 1L sterile seawater and instar II *Artemia* at a density of 150 to 200 metanauplii mL^{-1} were utilized. Then, 2mL of enrichment material was added to the bottle and gentle aeration accompanied by supplying the required oxygen led to appropriate and sufficient distribution of nutrients in the media. *Artemia urmiana* nauplii enriched with MPE_0, MPE_{200}, MPE_{400}, and MPE_{600} respectively. All experiments were performed in triplicate for each condition and the enrichment process was lasted up to 8 h. The control group was only treated with 2mL of a mixture containing 0.1g lecithin in1000mL of sterile seawater (45°C). *Artemia* were rinsed in a sieve (120 µ) and maintained at -20°C until further use.

Shrimp and experimental design

One thousand and two hundred 1-day-old post-larvae (PL_1) of *L.vannamei* with an average body weight of 19±0.97 mg were purchased from Konarak Shrimp Breeding Center, Iran. Then, the shrimps were randomly divided into four groups (100 shrimps in each group (three replications). The control group was fed only with unenriched metanauplii (MPE_0) and the other groups were fed with metanauplii enriched with 200 (MPE_{200}), 400 (MPE_{400}), and 600 (MPE_{600}) mg L^{-1} for 15 days. *Artemia* metanauplii with a density of 1 to 19 m L^{-1} at a rate of 1 to 4 mg L^{-1}, based on post larval stages, were fed every 4 h (6 times a day) until satiety (Støttrup and McEvoy, 2003). One-third of the water in each container was replaced every other day, and the tank wastes were removed using a siphon. During the experiments, physical and chemical properties of water, notably temperature, dissolved oxygen (DO), and pH were measured by using a mercury thermometer with a resolution of 0.1˚C

accuracy, a Digital dissolved oxygen meter (TECPEL DO-1609), and digital pH meter (Ebro, PHT-3140), based on a daily basis, respectively. The average water temperature, oxygen, acidity, and salinity were maintained at 30±2°C, 8.2±0.5 mgL^{-1}, 7.5 and 35±0.47 gL^{-1}, respectively.

Assessment of shrimp growth performance

At the end of the trial period (15 days), the length and weight of all shrimps were calculated with an accuracy of 1mm and 0.001g, respectively. then by using the biometry data, final weight (FW), percentage weight gain (WG), survival, feed conversion ratio (FCR), specific growth ratio (SGR), and protein efficiency ratio (PER) have been represented in equations1 to 5 (Harikrishnan *et al.*, 2011):

$SGR(\%.day^{-1})=[(L_nW_f–L_nW_i)/t]\times100$

W_I :initial weight (g),W_f :final weight (g) ,t :day

$WG(\%)=(Wf-Wi/Wi)\times100$

Wf :final weight (g), Wi :initial weight(g)

$FCR=WG/F$

F:the amount of food consumed (g),WG: weight gained (g)

$PER=(BW_f–BW_i)/AP$

BW_f :final weight (g),BW_i :initial weight (g),AP :protein fed (g)

Survival rate= (The number of larvae remained at the end of the period/ The number of larvae stored at the beginning of the period) ×100

Nutritional value of shrimp

Chemical composition

The shrimp's chemical composition was examined using the AOAC standard procedure (2020). In a nutshell, three shrimp were chosen at random from each replication and oven dried at 60°C for 24 h (AOAC, 2020). After that, the dry mass was taken out of the oven, crushed with a mill and pestle into fine powders, and stored in freezer-safe containers until needed again. The Kjeldahl method was used to test the total protein in the carcass, the Soxhlet extraction method and ether solvent was used to measure the total fat, and the ash from burning the sample at 550°C for six hours was used to measure the moisture content. The sample was first heated to 105°C and then dried before being weighed.

Fatty acid profile

Initially, 100-200mg of dried shrimp powder was placed into a sealed glass container. Subsequently, each container received 1mL of a solution comprising 2.5% H_2SO_4 and 98% methanol in a 1:40 ratio (v/v) and was heated to 80°C for 1 h. To extract fatty acid methyl esters, the samples were allowed to cool to room

temperature, after which 500µl of hexane was combined with 1.5mL of 9% NaCl solution and added to each sample. Upon centrifugation of the samples, the supernatant was collected and injected into a gas chromatography (GC) device (4600-Unicam company, England) for fatty acid composition analysis. For the isolation and identification of different fatty acids, a Unicam 4600 gas chromatograph equipped with a Bp×10 type column measuring 30 m in length and 0.1 mm in diameter was utilized. The detector employed was of FID type, and the carrier gas used was helium, with hydrogen and oxygen pressures set at 30 mL s^{-1} and 300 mL s^{-1}, respectively. Temperature settings for the detector, injector, and column were 250°C, 240°C, and 200°C, respectively. A Hamilton syringe was utilized to inject 1µL of the extracted sample into the device. The retention time of each fatty acid was compared with a standard curve, enabling the determination of fatty acid types and quantities as a percentage of the total fatty acids present (Pal *et al*., 2013).

Amino acid composition

Lindroth and Mopper's (1979) approach was used to assess the amino acid profile with minor adjustments. To the digesting tube in a freeze dryer (FDU-7012, Operon, South Korea), 0.1g of dried shrimp from each replicate was first added. Next, 7.5mL of 6.00 N hydrochloric acid (HCl) was added. It was diluted to 25mL with distilled water and baked at 110°C for 24 h after the air inside the tube was expelled using nitrogen gas. 10µL of the filtered solution was transferred into glass containers after the solution was filtered via syringe filters with a 0.45µm filter. Before being placed in a refrigerator, the samples were vacuum-sealed. Lastly, using HPLC equipment syringes (1290 infinity of England), 20µL of the finished mixture was injected into the column (18RP OPA specific column 100×4 mm, column temperature=30ºC).

Phytochemical composition

Sterol extraction and measurement

First, 1g of dried shrimp powder and 20mL dichloromethane (DCM) were combined, well mixed, and homogenized using an ultrasonic homogenizer in order to extract free sterols. Subsequently, the mixture was allowed to stand at room temperature for thirty minutes before being filtered through Whatman™ qualitative filter paper, grade 1. Lastly, the solvent was eliminated entirely using nitrogen gas and vacuum evaporation. After the shrimps' free sterols were extracted, nitrogen gas was used to dry the extracts. For additional examination, 1µL of every sample was put into the gas chromatograph. Every extraction technique was run through three times. The sterols in the extracts were purified using a gas chromatography (4600-Unicam business, England). The following are the column specifications: FID, 30 m × 0.1 mm, Bp10, detector Gas carrier: 6 Pa of helium filled.The column's thermal program is as follows: After two min at 150°C, the temperature increased by 5°C every minute until

300°C, where it stayed for fifteen minutes.The injection volume was 1µL, and both the injection chamber and the detector had a temperature of 300°C. Retention time was utilized to identify the sample components in the chromatogram. Cholesterol served as an internal standard for quantitative analysis, and the retention times of various components were determined using data from the injection of phytosterol standards (campesterol, stigmasterol, and β-sitosterol) (Sigma-Aldrich Company) under identical conditions to the injection of samples (Liu *et al*., 2007).

Shrimp homogenization

In order to evaluate the total phenolic content, flavonoid content, and antioxidant activity, ten shrimps were taken out of each replicate. Following that, they were homogenized in phosphate buffer solution (PBS) comprising 8 g of NaCl, 0.2 g of KCl, 1.42 g of Na_2HPO_4, and 0.24 g of KH_2PO_4 at a pH of 7.2 in a 1:10 (w/v) ratio. Then, total phenolics, flavonoids, and antioxidant activity were measured using the supernatant fraction that was left over after the homogenized samples were centrifuged for 10 minutes at 4°C (1500×g) (Akbary *et al*., 2021b).

Total phenolics content

Only slight alterations were made to the total phenolic contents (Ebrahimzadeh *et al*., 2008). To sum up, 200 µg of shrimp extracts were combined with 20 µL of Folin–Ciocalteu reagent (1:10 ratio) and allowed to sit at room temperature for four hours. After adding and combining 2µL of 6% sodium bicarbonate, the mixture was allowed to sit for 15 minutes at room temperature. A Shimadzu UV-1800 spectrophotometer (Shimadzu, UV-1800, Japan) was then used to detect optical absorption at 705.5 nm. The total phenolic content was reported in terms of gallic acid equivalent (mg of GAE g^{-1} of extract), and total phenolics were determined using a standard curve.

Total flavonoids content

The flavonoid content was ascertained using a slightly modified version of Ebrahimzadeh *et al*. (2008)'s methodology. The procedure involved mixing 0.5 mL of each extract with 1.5 mL of 95% methanol, 0.1 mL of 10% aluminum chloride hexahydrate ($AlCl_3$), 0.1 mL of 1M potassium acetate (CH_3CO_2K), and 2.8 mL of distilled water. Following 30 minutes at room temperature, optical absorption was measured at 415 nm using a Shimadzu UV-1800 spectrophotometer. The results were computed using a quercetin standard curve and reported as mg of quercetin equivalent (mg QEg^{-1}) per gram of dry sample.

Antioxidant activity measurement

A reaction mixture of HEPES-KOH pH7.8 including 0.1 mM EDTA, 50 mM sodium carbonate buffer, pH7.2, 12.7 mM metallothionein, 75 µM nitro blue tetrazolium, 1 µM riboflavin, and 200µL of extracts was used for the superoxide dismutase (SOD) assay. A spectrophotometer was used to measure

the samples' absorbance for 15 min at 560 nm. Winterbourn *et al*. (1975) used a test tube as a control that included every component of the reaction mixture save the enzyme extract.The evaluation of catalase (CAT) activity was done using hydrogen peroxide analysis, which involved using 20µL of 30% hydrogen peroxide and 20mM sodium phosphate buffer, pH7, as electron acceptors for 30 seconds of decreased light absorbance at 240 nm (Dazy et al., 2008). The activity of glutathione peroxidase (GPx) was measured using the Lawrence and Burk (1976) procedure, and a spectrophotometer was used to measure light absorbance at 440 nm. The Malondialdehyde (MDA) assay was performed using Baluchnejadmojarad *et al*. (2010) technique. After centrifuging the samples for 10 minutes at 1000 x g, a spectrophotometer was used to measure the absorbance at 535 nm.

Statistical analysis

One-way ANOVA was used for data analysis, and Duncan's Multiple Range Test was used to compare treatment means at a significance level of 0.05. The Kolmogorov-Smirnov normalcy test was used to investigate if the data were normal. Additionally, Levene's test was employed to evaluate the equality of variances, and Microsoft Excel 2010 and the statistical software package SPSS (SPSS Inc., version 19, Chicago, IL, USA) were utilized to analyze all of the data.

Results

Growth performance

As presented in Table 3, the inclusion of MPE in the diet improved shrimp growth in comparison to the control group (MPE_0). The FW of the shrimp fed metanauplii unenriched (MPE_0, 289.8±24.9 mg) was significantly lower compared to shrimp fed with metanauplii enriched with MPE_{600} (552.6±24.5 mg).

Table 3: Growth performance and survival rate (mean± SE, n=3) in *Litopenaeus vannamei* post-larvae after being fed 15 days on *Artemia urmiana* nauplii enriched with three different levels of a brown macroalgae premix extracts (MPE).

Growth indices	MPE content (mg L^{-1})			
	0	200	400	600
Initial weight (mg)	18.93±0.26	19.13±0.91	18.86 ±1.06	19.06 ±0.96
Initial length(mm)	0.53±0.04	0.51±0.02	0.52±0.02	0.53 ±0.03
Final weight (mg)	289.77±24.95^{c}	300.66 ±20.81^{c}	416.88 ±31.41^{b}	552.66 ±24.47^{a}
Final length (mm)	9.43±1.53^{d}	11.87 ±0.96^{c}	13.70 ±1.01^{b}	18.74± 0.75^{a}
Weight gain	14.46±1.44^{c}	14.70 ±1.08^{c}	21.15 ±1.69^{b}	28.07 ±1.35^{a}
Weight gain (%)	1466.29±144.58^{c}	2115.61 ±169.73^{b}	1620.96 ±35.82^{c}	2807.08 ±135.91^{a}
Specific growth ratio (%)	4.46±0.63^{c}	4.51 ±0.53^{c}	5.09 ±0.05^{b}	5.95±0.30^{a}
Feed conversion ration	1.84±0.59^{a}	1.31 ±0.42^{b}	1.33 ±0.56^{b}	1.20± 0.28^{b}
Survival rate (%)	97±0	98± 0	98± 0	100 ± 0
protein efficiency ratio	3.09 ± 0.30^{c}	3.14± 0.23^{c}	4.53± 0.36^{b}	6.01± 0.29^{a}

Different letters in the same row indicate significant differences between treatments (p<0.05).

Furthermore, significant higher SGR, WG and PER values were observed in the shrimp fed with metanauplii enriched with MPE_{600} compared to those fed with the unenriched metanauplii (MPE_0). The FCR in the group fed with

metanauplii enriched with MPE_{600} was lower than in other study groups (p<0.05). In term of survival rate, no significant differences were observed between experimental groups (p>0.05).

Nutritional value

Proximate composition

Proximate composition of the shrimp body fed with metanauplii enriched with different levels of macroalgae premix extract (MPE) has been represented in Table 4. The highest level of crude protein was observed in the groups fed with metanauplii enriched with MPE_{400} and MPE_{600}, which indicated a significant difference compared with the MPE_{200} and MPE_0 groups (p<0.05).The lowest and highest levels of crude fat were observed in the shrimps fed with metanauplii enriched with MPE_0 and MPE_{600}, respectively (p<0.05). Increased MPE concentrations in *Artemia* enrichment led to an increase in ash and dry matter in the shrimps. The highest level was recorded in the group fed with metanauplii enriched with MPE_{600} (p<0.05).

Table 4: Comparing chemical composition (means± SE (n=3)) in *Litopenaeus vannamei* post-larvae after being fed 15 days on *Artemia urmiana* nauplii enriched with three different levels of a brown macroalgae premix extract (MPE).

Chemical (%) composition	MPE content (mg L^{-1})			
	0	200	400	600
Crude protein	70.46±0.12^{c}	71.95 ±0.75^{b}	75.78 ±1.09^{a}	76.15 ± .21^{a}
Crude fat	2.22±0.03^{a}	1.96 ±0.08^{b}	1.62 ±0.34^{c}	1.20 ±0.12^{d}
Ash	5.62±0.11^{c}	6.16 ±0.45^{b}	6.24 ±0.54^{b}	6.41 ±0.27^{a}
Dry matter	38.40±3.09^{d}	39.43 ±2.16^{c}	40.26 ±4.54^{b}	40.76 ±1.08^{a}

Different letters in the same row indicate significant differences between treatments (p<0.05).

Fatty acid composition

Fatty acid composition of the shrimps fed with metanauplii enriched with different levels of MPE is presented in Table 5. The highest levels of lauric acid (0.98±0.01%) were observed in the group fed with metanauplii enriched with MPE_{600}. Increased MPE concentration in nauplii enrichment led to the decrease in Myristic acid, Palmitic acid, stearic acid, and total saturated fatty acids in the groups fed with metanauplii enriched with MPE indicating a significant difference compared with the control group (MPE_0, p<0.05).The saturated fatty acids mostly included palmitic acid (C16:0) and stearic acid (C18:0), respectively. Among monounsaturated fatty acids, the highest levels of myristoleic acid (0.49±0.01%) and palmitoleic acid (4.37±0.02%) were observed in the group fed with unenriched metanauplii (MPE_0). Total monounsaturated fatty acids (MUFA) demonstrated no significant difference among the groups fed with metanauplii enriched with different levels of MPE (p<0.05). Oleic acid was the predominant fatty acid found in the samples. The highest total level of poly unsaturated fatty acid (PUFA), eicosapentaenoic acid (EPA), docosahexanoic acid (DHA), Arachidonic acid (ARA), was found in

shrimp fed with metanauplii enriched with MPE_{600}, which demonstrated a significant difference compared to other groups (p<0.05).

Table 5: Comparison of fatty acid composition (% of total fatty acids, (mean±SE) in *Litopenaeus vannamei* post-larvae after being fed 15 days on *Artemia urmiana* nauplii enriched with three different levels of a brown macroalgae premix extract (MPE).

Fatty acids	MPE content (mg L^{-1})			
	0	200	400	600
C12:0	0.5 ±0.01^{d}	0.72 ±0.01^{c}	0.84 ±0.05^{b}	0.98 ±0.01^{a}
C14:0	1.00 ±0^{a}	0.97 ±0^{b}	0.81 ±0.01^{c}	0.68 ±0.01^{d}
C16:0	24.30 ±0.10^{a}	22.70 ±0.10^{b}	20.51 ±0.07^{c}	18.67 ±0.14^{d}
C18:0	9.39 ±0.05^{a}	9.20 ±0.01^{b}	8.47 ±0.50^{c}	8.32 ±0.05^{d}
C20:0	0.48 ±0^{b}	0.61 ±0.01^{a}	0.60 ±0^{a}	0.49 ±0.01^{b}
SFA*	35.19 ±0.10^{a}	33.46 ±0.08^{b}	30.57 ±0.02^{c}	28.17 ±0.10^{d}
C14:1n-5	0.49 ±0.01^{a}	0.37 ±0.02^{b}	0.27 ±0.02^{c}	0.20 ±0^{d}
C16:1n-7	4.37 ±0.02^{a}	4.28 ±0.01^{a}	3.97 ±0.06^{c}	4.10 ±0.10^{b}
C18:1n-7	0.49 ±0.01 ^{b}	0.49 ±0.01^{b}	0.53 ±0.01^{a}	0.50 ±0.02^{b}
C18:1n-9	16.12 ±0.02^{d}	16.31 ±0.01^{c}	16.65 ±0.01^{b}	16.60 ±0.01^{a}
MUFA **	21.48 ±0.06	21.45 ±1.01	21.43 ±1.04	24.41 ±2/17
C18:2n-6	9/10 ±0.04^{c}	10.98 ±0.01^{b}	10.97 ±0.65^{a}	10.99 ±1.01^{a}
C18:3n-3	4.48 ±0.08^{b}	5.41 ±0.04^{a}	5.42 ±0.06^{a}	5.42 ±0.07^{a}
C20:4n-6	14.40 ±0.24^{d}	15.62 ±0.87^{c}	16.32 ±0.26^{b}	16.80 ±0.43^{a}
C20:5n-3	13.32 ±0.12^{d}	15.42 ±6.04^{c}	16.16 ±0.15^{b}	17.92 ±0.75^{a}
C22:6n-3	2.23 ±0.06^{c}	4.31 ±0.08^{b}	4.29 ±0.09^{b}	4.46 ±0.03^{a}
PUFA***	34.49 ±0.19^{d}	40.78 ±3.34^{c}	42.21±2.03^{b}	44.61 ±2.04^{a}
n-3	20.08 ±0.16^{d}	25.15 ±2.04^{c}	25.88±0.28^{b}	27.81 ±0.16^{a}
n-6	23.50 ±2.01^{d}	26.52 ± 1.08^{c}	27.29±1.05^{b}	27.79 ±2.21^{a}
n-6/.n-3	1.71 ±0^{a}	1.05±0.01^{b}	1.05±0.03^{b}	0.99 ±0.01^{c}

The values (mean ± SE; n=3) with non-similar letters in each row indicate significant difference between the treatments (p<0.05). The mean values of the data were compared based on the one-way ANOVA SFA* saturated fatty acid** MUFA Monounsaturated fatty acid PUFA*** polyunsaturated fatty acid.

Amino acid (AA) composition

As presented in Table 6, increased MPE concentrations in metanauplii enrichment led to enhanced amino acid composition in the shrimp body. The highest average total of essential amino acids (7.26±0.07 g AA100 g^{-1} sample), non-essential amino acids (5.03±0.04 g AA100 g^{-1} sample) and total amino acid (12.30±0.11 g AA100 g^{-1} sample) were found in the group fed with metanauplii enriched with MPE_{600}, indicating a significant difference compared with other experimental groups (p<0.05).

Phytochemical composition

Sterols

As presented in Table 7, the predominant sterols in the shrimp fed with metanapolys enriched with different levels of MPE were cholesterol, campesterol and sitostanol, respectively. The highest levels of predominant sterols and total sterols were detected in the shrimp fed with metanauplii enriched with MPE_{600}, demonstrating a significant difference compared to other trial groups (p<0.05).

Table 6: Amino acid composition (g amino acid 100 g^{-1} sample, mean±SE) in *Litopenaeus vannamei* post-larvae after being fed 15 days on *Artemia urmiana* nauplii enriched with three different levels of a brown macroalgae premix extract (MPE).

Amino acids (mg g^{-1} samples)	MPE content (mg L^{-1})			
	0	200	400	600
Essential amino acids(EAA)				
Arginine	0.69 ± 0.01^{c}	0.76 ± 0.02^{b}	0.77 ± 0.04^{b}	0.88 ± 0.02^{a}
Histidine	0.47 ± 0.02^{c}	0.53 ± 0.01^{b}	0.58 ± 0.02^{b}	0.66 ± 0.03^{a}
Isoleucine	0.56 ± 0.02^{d}	0.64 ± 0.01^{c}	0.76 ± 0.01^{b}	0.97 ± 0.02^{a}
Leucine	0.67 ± 0.02^{d}	0.86 ± 0.02^{c}	0.91 ± 0.01^{b}	0.99 ± 0.07^{a}
Lysine	0.67 ± 0.02^{c}	0.75 ± 0.01^{b}	0.79 ± 0.01^{b}	1.02 ± 0.06^{a}
Methionine	0.48 ± 0.03^{d}	0.78 ± 0.01^{c}	0.83 ± 0.01^{b}	0.97 ± 0.01^{a}
Phenylalanine	0.56 ± 0.01^{c}	0.77 ± 0.01^{b}	0.80 ± 0.01^{b}	0.88 ± 0.02^{a}
Valine	0.63 ± 0.02	0.73 ± 0.01^{b}	0.75 ± 0.01^{b}	0.86 ± 0.03^{a}
Non-essential amino (NEAA) *acids*				
Alanine	0.45 ± 0.01^{d}	0.59 ± 0.01^{c}	0.68 ± 0.01^{b}	0.74 ± 0.02^{a}
Threonine	0.16 ± 0.01^{bc}	0.14 ± 0.05^{c}	0.20 ± 0.02^{b}	0.29 ± 0.02^{a}
Glutamic acid	0.43 ± 0.05^{d}	0.87 ± 0.01^{c}	0.89 ± 0.01^{b}	1.00 ± 0.02^{a}
Glycine	0.51 ± 0.02^{d}	0.60± 0.01^{c}	0.68 ± 0.01^{b}	0.73 ± 0.01^{a}
Aspartic acid	0.16 ± 0.01^{bc}	0.14 ± 0.05^{c}	0.20 ± 0.02^{b}	0.29 ± 0.02^{a}
Serine	0.62 ± 0.05^{c}	0.73 ± 0.01^{b}	0.75 ± 0.02^{b}	0.86 ± 0.04^{a}
Tyrosine	0.56 ± 0.01^{c}	0.61 ± 0.02^{b}	0.62 ± 0.02^{b}	0.70 ± 0.02^{a}
Total amino acids	4.76 ± 0.07^{d}	5.83 ± 0.05^{c}	6.20 ± 0.03^{b}	7.26 ± 0.07^{a}
Total essential Amino acids	3.11 ± 0.04^{d}	3.98 ± 0.02^{c}	4.39 ± 0.01^{b}	5.03 ± 0.04. ^{a}
Total non-Essential amino acids	7.87 ± 0.11^{d}	9.82 ± 0.08^{c}	10.59 ± 0.03^{b}	12.30 ± 0.11

The values (mean ± SE, n=3) with non-similar letters in each row indicate significant difference between the treatments (p<0.05).

Table 7: Mean (±SE) values of free sterols and total sterols (mg 100 g^{-1} dry weight basis) (mean±SE) in *Litopenaeus vannamei* post-larvae after being fed 15 days on *Artemia urmiana* nauplii enriched with three different levels of a brown macroalgae premix extract (MPE).

Sterol content(mg 100 g^{-1} dry weight basis)	MPE content (mg L^{-1})			
	0	200	400	600
Cholesterol	62.72±0.97^{c}	94.85±1.31^{b}	125.20±3.12^{a}	127.20±1.04^{a}
Ergosterol	0.92± 0.06^{d}	1.35 ± 0.02^{c}	1.46 ± 0.01^{b}	1.63± 0.05^{a}
24-Methylcholesterol	0.40±0.01^{a}	0.40±0.01^{a}	0.40±0.02^{a}	0.15± 0.01^{b}
Campestrol	10.28± 0.23^{c}	14.70± 0.15^{b}	15.31± 0.01^{a}	15.34 ± 0.04^{a}
Sitostanol	0± 0^{d}	9.43±0.05.^{c}	11.36± 0.15^{b}	12.33± 0.15.^{a}
Delta-5-avenasterol	0± 0^{c}	2.03±0.01^{b}	2.16± 0.05^{ab}	2.23± 0.20^{a}
Delta-7-Campesterol	0.18± 0.05^{c}	0.21±0.01^{b}	0.28± 0.03^{a}	0.16±0.04^{b}
Stigmasterol	0.34± 0.07^{d}	3.86± 0.06^{c}	4.36± 0.01^{b}	6.41± 0.16^{a}
Total sterols	74.36 ± 0.16^{d}	126.53± 1.02^{c}	160.48± 3.21^{b}	165.84± 1.48^{a}

Different letters in the same row indicate significant differences between treatments (p<0.05).

Total phenols and flavonoids

The average changes of total phenolic content and flavonoids of *L. vannamei* fed with metanauplii enriched with different levels of MPE are shown in Table 8.There was a positive correlation between increased MPE concentration and phenolic content. The highest levels of phenolic content were detected in the shrimp fed with metanauplii enriched

with MPE_{600} (6.75±0.02 mg GAE g^{-1} dry extract), indicating a significant difference compared to MPE_{400} (6.62±0.05 mg GAE g^{-1} dry extract) and MPE_{200} (5.26±0.01 mg GAE g^{-1} dry extract) (P<0.05).The highest levels of flavonoid (1.2±0.01 mg QE g^{-1} of sample) were observed in the group fed with metanauplii enriched with MPE_{600}, demonstrating a significant difference compared to other groups (p<0.05).

Table 8: Concentration of total phenols (a) and flavonoids (b) (mean±SE) in *Litopenaeus vannamei*post-larvae after being fed 15 days on *Artemia urmiana* nauplii enriched with three different levels of a brown macroalgae premix extract (MPE).

Sterol content (mg 100 g^{-1} dry weight basis)	MPE content (mg L^{-1})			
	0	200	400	600
Total phenol (mg gallic acidg^{-1}extract)	0.05±5.05^{d}	0.01±5.26^{c}	0.05±6.62^{b}	0.02±6.75^{a}
Total flavonoid (mg querceting^{-1} extract)	0.03±0.1^{d}	0.04±0.31^{c}	0.06±0.82^{b}	0.01±1.20^{a}

Different letters in each row demonstrate that differences are significant (p<0.05).

Antioxidant concentration

The concentration changes in of antioxidant enzymes (SOD, GPx and CAT, and MDA) of the shrimps fed with metanauplii enriched with MPE have been summarized in Table 9. A significant difference among the shrimps feeding with metanauplii enriched with MPE was observed (p<0.05). The highest SOD level was observed in group fed with metanauplii enriched with MPE_{600}. Increased concentration of MPE in metanauplii enrichment led to an increase in SOD content and CAT level and the difference among the shrimps fed with metanauplii enriched with MPE was significant (p<0.05).The lowest MDA content (4.83±0.15 $nmolmin^{-1}$ mg^{-1} protein) was reported in the shrimps fed with metanauplii enriched with MPE_{600}, indicated a significant difference (p<0.05) in comparison to the control group (MPE_0). The highest level of GPX was detected in the groups fed on metanauplii enriched with MPE_{400} and MPE_{600} (p<0.05).

Table 9: concentration of antioxidant enzymes (SOD, GPx and CAT) and (mean±SE) in *Litopenaeus vannamei* post-larvae after being fed 15 days on *Artemia urmiana* nauplii enriched with three different levels of a brown macroalgae premix extract (MPE).

Antioxidant status	MPE content (mg L^{-1})			
	0	200	400	600
Superoxide Dismutase (SOD) (Units mL^{-1})	1.08±38.21^{c}	0.08±38.19^{c}	0.01±45.42^{b}	0.15±45.76^{a}
Glutathione Peroxidase (GPx) (Units mL^{-1})	1.31±130.40^{c}	2.62±189^{b}	3.05±233.33^{a}	9.01±239.33^{a}
Malondialdehyde (MDA) **(***nmol min^{-1} mg of protein***)**	0.03±6.16^{a}	0.01±5.68^{b}	0.05±5.16^{c}	0.15±4.83^{d}
Catalase (CAT) (Units mL^{-1})	0.06±3.76^{b}	0.43±3.79^{b}	0.01±4.18^{b}	0.18±4.76^{a}

Different letters in each row demonstrate that differences are significant (p<0.05).

Discussion

Our findings revealed that including MPE in the diet of the shrimp increases growth. Abdel-Rahim *et al*. (2021) claimed that using 500 mg kg^{-1} *Sargassum polycystum* dietary supplementation for *L. vannamei* resulted in improved growth performance and enhanced feed utilization indicators (FCR and PER). Arizo *et al*. (2015) concluded that fucoid, a dietary supplement extracted from *Sargassum polycystum*, indicated the highest growth enhancement for *Macrobrachium rosenbergii* when included in the diet at a concentration of 500 mg kg^{-1} of *S. polycystum* extract. Growth performance indices in PL_{15}-PL_{35} of the black tiger shrimp, *P. monodon*, fed on *Artemia* Instar II nauplii enriched with different concentrations of Brown algae (*Sargassum* sp) extract (250, 500, and 750 mg extract L^{-1}) was significantly higher than the control group and the best growth performance was found at the concentration of 750mg extract L^{-1} (Immanuel *et al*., 2010). Moreover, Indian white shrimp, *Penaeus indicus* juveniles fed with *Artemia* metanauplii enriched with the brown alga *Sargassum wightii* showed increased WG and higher SGR than the control group after 30 days (Ghaednia *et al.*, 2011), which was similar to our finding. The highest final weight, specific growth rate, and protein efficiency were detected in white shrimp fed on diets supplemented with 1 and 1.5g $kg^{-1}$the brown seaweed *Iyengaria stellata* extract and 1g $kg^{-1}$the red alga *Jania adhaerens* extract (Akbary *et al.*, 2020a; Akbary *et al.*, 2021a). The cause of improvement in shrimp growth can be attributed to the presence of active substances like flavonoids and tannins in the diet containing macroalgae of interest, which enhanced feed consumption. Moreover, compounds including amino acids and essential fatty acids are perceived as the positive mechanisms affecting the growth, playing a crucial role in the growth and basic functions of shrimp (Akbary *et al.*, 2021a). In addition, in term of survival rate, no significant differences were observed between experimental groups, which was consistent with the findings obtained from the research accomplished by Mazlum *et al*. (2020). Mazlum *et al*. (2020) reported that growth performance was enhanced in red swamp crabs (*Procambarusclarkii*) fed with a concentration of 10% two macroalgae, *U.lactuca*, *J.rubens.* The highest rate of protein efficiency in white shrimps fed diet with 1 and 1.5g kg^{-1}*J. adhaerens* (red algae) extract was detected (Akbary *et al.*, 2020a), which was similar to our finding.

Our results indicated that the highest amount of crude protein in the group fed with metanauplii enriched with MPE_{400} and MPE_{600}. Increased concentration of MPE in *Artemia* enhanced the amount of ash and dry matter in the shrimp. While the lowest level of the crude fat was observed in the groups fed with metanauplii enriched with MPE_{600}. Cholesterol and lipid composition of the shrimp carcass can be reduced due to the consumption of *Sargassum* sp

macroalgae (Casas-Valdez *et al.*, 2006) and *U.clathrata*macroalgae (Cruz-Suarez *et al.*, 2008). Moreover, by investigating the effect of seaweed extract (*Ulva*) on the growth and biochemical composition of Nile tilapia (*Oreochromis niloticus*), Ergün*et al*.(2008) indicated that fish fed with 5% algae extract contained less carcass fat compared to the control group, which was in agreement with our study. Also, Nakagawa *et al.* (1997) reported that *U. lactuca* alga can alter the metabolic pathway and fat storage in sea bass (*Pagrus major*), which results in the more effective use of fat reserves, and subsequently, weight loss reduces during winter. These can at least partially be attributed to the presence of cysteinolic acid as a non-protein amino acid that is similar to taurine. Cysteinolic acid, like taurine, can contribute to the formation of bile salts, at least in marine species, conjugates with cholesterol (Yone *et al.*, 1986), and this could be perceived as the underlying mechanism through which cysteinolic acid affect lipid and cholesterol metabolism (Cruz-Suarez *et al.*, 2008). Cysteinolic acid plays more roles in metabolism. For instance, it converts into cysteine (Akbary *et al.*, 2020b). Safavi *et al*.(2019) indicated that the concentration 1.5g sulfated polysaccharides extract of *Gracilariopsis persica* kg^{-1} diet of rainbow trout (*Oncorhynchus mykiss*) resulted in a significant increase in protein content and a significant reduction in crude fat level in fish muscle compared to the control group. Similar to our findings, Akbary and Shahraki (2020) indicated that incorporation 15g kg^{-1} of *Padina australis* extract in grey mullet (*Mugil cephalus*) feed significantly increased protein content compared to the control group. It can be saidthat the use of metanauplii enriched with MPE has directed the protein toward the major route of anabolism during the process of metabolism (Shalaby *et al.*, 2006).

In this study, increased MPE concentration in metanauplii enrichment led to a significant reduction in myristic acid, palmitic acid, stearic acid, and total saturated fatty acids in the groups fed with enriched metanauplii in comparison to the control group (MPE_0). Shrimp fed metanauplii enriched with MPE_{600} had the highest levels of PUFA, EPA, DHA, ARA, and total sterols. ARA and EPA were the predominant PUFAs, in line with a study by Choi *et al*. (2015), which found that saturated fatty acids play a significant role in raising the amount of PUFA in the muscles of fish fed algae extract. According to Choi *et al*. (2014), PUFA levels, such as DHA, ARA, linoleic acid (LIA), and EPA, changed when olive flounder, *Paralichthys olivaceus*, was fed *Hizikia fusiformis* algal glycoprotein (Choi and al., 2014). Choi *et al*. (2015) suggested that the incorporation 20 $gkg^{-1}$of *Pyropia yezoensis* extract in olive flounder feed increased DHA, ARA and LIA in the muscle, which is in line with our findings. While the precise mechanism behind the alteration in lipid metabolism following the inclusion of algae in the diet remains unclear, relevant studies

suggest that incorporating seaweed into the diet positively influences lipid metabolism, leading to an increase in PUFA levels and enhancing lipid storage efficiency. Moreover, aquatic species consuming diets high in protein and carbohydrates may experience an excess energy intake, which is subsequently converted into fatty acids and stored as lipids (Choi *et al*., 2014). Consistent with our findings, Akbary *et al*. (2021a) examined the fatty acid composition in *L. vannamei* muscle when fed with *I. stellate* brown algae extract and noted that diets containing 1 and 1.5 g kg-1 I. stellate extract resulted in the highest levels of PUFA and EPA in shrimps. This suggests that the premix extract derived from the macroalgae in our study serves as a rich source of ω3 fatty acids, such as DHA and EPA, and effectively enhances muscle lipid profiles in proportion to the concentration of ω3 fatty acids (Güroy *et al*., 2007; Akbary *et al*., 2020a), thereby reducing the 3-n/6-n ratio.In this study, including MPE in the diet improved the mean whole-body amino acid levels of *L. vannamei* shrimp than the groups fed with non-enriched metanauplii (MPE_0). As compared to non-enriched *Artemia nauplii*, Kalaiswlvi et al. (2018) found that *Artemia nauplii* enriched with ether extract, acetone extract, and particularly the ethanolic extract of *Phyllanthus amarus* significantly increased the total protein level as well as the amino acid and lipid concentration of freshwater shrimp larvae, *Macrobrachium rosenbergii*. Furthermore, diets including *G. vermiculophylla* and *U. lactuca* increased the ability of *L. vannamei* to digest amino acids, which is in line with our findings (Anaya-Rosas *et al*., 2019). It is possible to argue that an imbalance in the amount of amino acids in the diet causes an increase in amino acid oxidation, which in turn reduces fish development and conversion efficiency. Thus, using macroalgae that contain essential amino acids is the best strategy to make up for the lack of essential amino acids (Rajapakse and Kim, 2011).

As the most well-known animal sterol, which is a vital and functional component for animal cell structure, cholesterol is utilized as an essential precursor for fat-soluble vitamins and steroid hormones (Phillips *et al.*, 2012). Our findings revealed that cholesterol, campesterol, and sitostanol were the most abundant sterols found in the groups fed with metanauplii enriched with MPE, respectively. To the best of our knowledge, no research has been conducted to investigate these indexes. It should be mentioned that sterols may possess extensive biological activities, such as anti-inflammatory, anti-oxidative, and antibacterial properties (Fernandes and Cabral, 2007). Main phytosterols including campesterol, stigmasterol, and beta-sitosterol are introduced as the major components to inhibit intestinal cholesterol absorption (Ozogul *et al.*, 2015). Phenolics are one of the most abundant secondary metabolites in macroalgae, which contribute to the stimulation of growth, appetite, and amplification the immune system of aquatic organisms. These

compounds are involved in mechanisms aiming to defend against pathogens and repair damage (Santos *et al.*, 2019; Cotas *et al.*, 2020). Our results demonstrated that there is a direct relationship between the increased MPE concentration and total phenolic content and flavonoids. The highest level was found in the groups fed with MPE_{600}.Therefore, enhanced the growth performance and antioxidant properties in the groups fed with metanauplii enriched with MPE can be attributed to the phenolics and flavonoids found in the macroalgae premix extract. Wood and Enser (1997) indicated that diets containing antioxidants can enhance the fatty acid profile of meat. This may be additional reason for the enhancement of the fatty acids content recorded in the current study, because phenolic compounds of algae which are responsible for antioxidant activity (Hongayo *et al*., 2012). For instance, Tenorio-Rodriguez *et al*. (2017) reported that among the 17 large algae including green, red and brown algae, the brown macroalgae extract was found to have the highest antioxidant activity and sources of natural bioactive compounds, and the antioxidant activities of algae can be attributed to the presence of various secondary metabolites such as phenolic compounds and carotenoids (Hongayo *et al*., 2012). In addition, the phenolic compounds of macroalgae are important as potential factors in improving the health and performance of aquatic animals (Naiel *et al*., 2020; Naiel *et al*., 2021). Also, These compounds are typically used to prevent lipid per oxidation and increase shelf life (Holdt and Kraan, 2011).

According to a large number of previous studies, extracts obtained from marine macroalgae are rich sources of natural antioxidants, since they are capable of neutralizing free radicals (Goiris *et al.*, 2012). SOD and CAT are regarded as the key antioxidant enzymes since they serve important functions in neutralizing free radicals and in protecting against drug and chemical toxicity (Farombi *et al.*, 2007). In the current investigation, the level of SOD and CAT increased in shrimps fed with metanauplii enriched with MPE_{600}. While the lowest level of MDA was reported in the groups fed with metanauplii enriched with MPE_{600}. Akbary and Aminikhoei (2018) pointed out that employing 1.5g kg^{-1}*U. rigida* extract dietary supplementation for *L.vannamei* resulted in a significant increase in reduced GPx and SOD activity. Furthermore, Akbary *et al.* (2021a) displayed that *L.vannamei* fed on diet supplemented with 1g kg^{-1}*I. stellata* extract in *L.vannamei* led to increased levels of SOD (19.32 Units mg^{-1} protein), GPX (249.06 Units mg^{-1} protein), CAT (1.12 Units mg^{-1} protein) and phenol oxidase (31.19 Units mg^{-1} protein). Similarly, SOD activity in grouper (*Epinephelus coioides*) fed on a diet supplemented with sodium alginate significantly enhanced compared to the group fed on the control diet (Yeh *et al.*, 2008; Lee *et al.*, 2017). Phenolics in MPE can decrease oxidative stress by neutralizing free radicals (Shi *et al.*, 2005). MDA concentrations

demonstrate the toxic processes resulting from free radicals, and MDA level is considered as a reliable indicator of the level of lipid peroxidation (Peixoto *et al.*, 2016; Akbary *et al.*, 2021a). The results of this assessment indicated that the lowest levels of MDA were observed at the group fed with metanauplii enriched with MPE_{600}, which is in line with the study of Peixoto *et al.*(2016) and Akbary *et al*.(2021a).

Overall, our results demonstrate the positive effects of metanauplii enriched with 600 mg L^{-1} macroalgae premix extracts (*Padina australis*, *Sargassum ilicifolium*, and *Stoechospermum marginatum*) on growth performance, nutritional values, phytochemical, and antioxidant properties of *Litopenaeus vannamei*, so this is strongly recommended to be included in the diet of *Litopenaeus vannamei*.

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