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Effect of dietary macro-algae in diet of juvenile Pacific white shrimp *Litopenaeus vannamei*

Fan Yang[1] · Shiwei Xie[1] · Jin Niu[1] · Yongjian Liu[1] · Lixia Tian[1]

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Abstract An 8-week feeding trial was conducted to evaluate the effect of dietary macro-algae in diet of juvenile Pacific white shrimp, *Litopenaeus vannamei*. The five macro-algae ingredients, including *Saccharina japonica*, *Porphyra dioica*, *Gracilariopsis lemaneiformis*, *Ulva lactuca*, and *Undaria pinnatifida*, were used for test diets, named SJD, PDD, GLD, ULD, and UPD, respectively. A reference diet (RD) and five test diets (30% test ingredients and 70% RD) were formulated. Each diet was randomly assigned to triplicate groups of 40 shrimp (initial body weight, 2.00 ± 0.01 g) and the shrimp were hand fed four times a day to apparent satiation. During the last 2 weeks, the fecal samples were collected by siphoning. The results showed that among five test diets, shrimp fed the SJD had the highest weight gain and the lowest feed coefficient, shrimp fed the SJD and GLD shared the same survival ratio and were significantly higher than shrimp fed the PDD and UPD ($p < 0.05$). For the composition of whole body, shrimp fed the SJD had the highest protein and lipid content compared with the shrimp fed other test diets. Apparent dry matter digestibility from high to low was: *P. dioica*, *S. japonica*, *G. lemaneiformis*, *U. lactuca*, and *U. pinnatifida*. Apparent protein digestibility of five macro-algae was highest in *U. pinnatifida* and lowest in *G. lemaneiformis*. Additionally, the highest apparent digestibility of gross energy was found in *P. dioica*. Apparent digestibility of total phosphorous in five ingredients was generally low and highest in *S. japonica* which was significantly higher than other ingredients ($p < 0.05$). These results indicated that *S. japonica* is more suitable for feed ingredient than other four kinds of macro-algae.

Keywords *Litopenaeus vannamei* · Macro-algae · Growth performance · Body composition · Apparent digestibility

✉ Lixia Tian
edls@mail.sysu.edu.cn

[1] Nutrition Laboratory, Institute of Aquatic Economic Animals, School of Life Science, Sun Yat-sen University, Guangzhou 510275, China

Introduction

The yield of Pacific white shrimp, *Litopenaeus vannamei*, is highest among all the cultured shrimp species in the world. Rapid growth and high survival rate make it a good choice for intensive aquaculture (Williams et al. 1996; Ponce-Palafox et al. 1997). Especially in recent years, the unprecedented development of shrimp farming has demonstrated its increasing vital role in fishery economy.

To promote sustainable aquaculture development, the search for fish meal substitutes and alternative dietary protein sources has been an international research priority that could be of considerable economic advantage (Lee 2002). Many studies have been conducted to replace fish meal with plant-derived protein sources such as soybean meal, corn gluten meal, cottonseed meal, and so on (Kikuchi 1999; Albrektsen et al. 2006; Fordeskjaervik et al. 2006; Amaya et al. 2007; Hernandez et al. 2007; Lim and Lee 2009). These studies concluded that minimizing the use of fish meal in aquaculture diets is essential for the sustainability of the aquaculture industry (Naylor et al. 2009).

Marine algae, an important marine biological resource, contain an abundance of nutrition and a variety of bioactive compounds, not only rich in carbohydrates, proteins, lipids, vitamins, and fibers (Pulz and Gross 2004; Silva and Barbosa 2009), but also contain all essential amino acids in various concentrations (Galland-Irmouli et al. 1999; Macartain et al. 2007) and all essential minerals (Cabrita et al. 2016). A small amount of algae supplementation in aquafeeds has been shown to improve immune systems (Turner et al. 2002, Schleder et al. 2017), increase lipid metabolism (Nakagawa 1997; Güroy et al. 2011), virus and bacyerial resistance (Boonsri et al. 2017), improve gut function (Michiels et al. 2012), and enhance stress resistance (Nath et al. 2012; Sheikhzadeh et al. 2012). Algae resource is extremely rich in China; there are more than 1000 different types of macro-algae and over 100 kinds are considered to have economic value. Therefore, used as a new feed ingredient, macro-algae have great potential. At present, several species of marine algae have already been used for partial replacement as complementary protein source in the diet of *L. vannamei*, such as *Hypnea cervicornis* J. Agardh and *Cryptonemia crenulata* (J. Agardh) J. Agardh (Silva and Barbosa 2009), *Ulva clathrata* (Roth) C. Agardh (Cruz-Suárez et al. 2009a, b), *Gracilaria parvispora* I.A. Abbott, and *Ulva lactuca* Linnaeus (Rodríguez-González et al. 2014). Cruz-Suárez et al. 2009a, b, 2010) and Peña-Rodríguez et al. (2011) had found that inclusion of *U. clathrata* in the diet of *L. vannamei* improves growth, food conversion ratio, and pigmentation and modifies fatty acid and sterol content in muscle. Schleder et al. (2017) concluded that *Undaria pinnatifid*a enhances shrimp immunity and specifically inhibit *Vibrio* spp., while *Sargassum filipendula* increases shrimp resistance to thermal variation. Pallaoro et al. (2016) demonstrated that substitution of up to 50% of *Ulva lactuca* in commercial feed for *L. vannamei* does not interfere with the growth performance of shrimp.

In addition to quantify the gross composition of feed ingredients, knowledge of their digestibility is needed in order to assess the nutritional value (Shields and Lupatsch 2012). Apparent digestibility coefficients (ADC) reflect the percentage of a feed ingredient sample that is absorbed by an animal's intestinal tract (Lin et al. 2005). In the past decades, although many studies have been carried out on the application of macro-algae as a potential ingredient in the diets of *L. vannamei* (Briggs and Funge-Smith 1996; Gamboadelgado et al. 2007; Ju et al. 2009, 2012; Basri et al. 2015), basic research data on the apparent digestibility of macro-algae ingredients for *L. vannamei* is still insufficient. The accurate determinations of chemical composition of macro-algal ingredients and the precise assessment of digestibility and absorption of nutrients are the basis for developing macro-algae as feed ingredients and feed additives. Therefore, detailed research on the apparent digestibility of macro-algae for *L. vannamei* is particularly important.

The objective of the present study was to evaluate the potential of five macro-algae as feed ingredients in formulated diets for juvenile Pacific white shrimp, *L. vannamei.*

Materials and methods

Five test ingredients

The five test ingredients were *Saccharina japonica*, *Porphyra dioica*, *Gracilariopsis lemaneiformis*, *Ulva lactuca*, and *Undaria pinnatifida*, all of which were bought from specialized stores.

Diet preparation

The reference and test diets were formulated according to Table 1. Five test diets were prepared using the method of "nested algorithm" (Cho and Kaushik 1990) by incorporation of 30% test ingredients into the reference diet (RD). Yttrium oxide (Y_2O_3) was used as an external indicator and was incorporated into the RD as well as test diets. Nutrient and amino acids composition of feed ingredients are shown in Tables 2 and 3. Nutrient and amino acids composition of the RD and test diets are presented in Tables 4 and 5. All dry ingredients were finely ground and sieved with a 60-mm mesh, then weighed precisely and mixed thoroughly with the oils and

Table 1 Composition of the reference and test diet

Ingredients	Amount (g kg^{-1}) Reference diet	Test diet
Fish meal	300.00	210.00
Soybean meal	150.00	105.00
Peanut meal	122.90	86.03
Wheat flour	250.00	175.00
Beer yeast	50.00	35.00
Krill meal	70.00	49.00
Soybean lecithin	10.00	7.00
Fish oil	10.00	7.00
Soybean oil	10.00	7.00
Choline chloride (50%)	5.00	3.50
Monocalcium phosphate	1.00	0.70
Vitamin premix[1]	10.00	7.00
Mineral premix[2]	10.00	7.00
Ascorbic acid polyphosphate	1.00	0.70
Yttrium oxide Y_2O_3[3]	0.10	0.07
Test ingredient	–	300.00

[1] Vitamin (mg kg^{-1} diet): thiamin, 500; riboflavin, 1500; pyridoxine, 800; cyanocobalamin, 2; retinol, 500,000 IU; cholecalciferol, 2000 IU; all-rac-α-tocopherol, 7500; menadione, 500; Ascorbic Acid,14,000; nicotinic acid, 4000; folic acid, 250; Ca pantothenate, 2500; biotin, 8; inositol, 12,000

[2] Mineral ((mg kg$_{-1}$ diet): KCl, 60,000 IU; KI, 500; $MgSO_4 \cdot 7H_2O$, 6000; $ZnSO_4 \cdot 7H_2O$, 4000; $FeSO_4 \cdot 7H_2O$, 1000; $MnSO_4 \cdot H_2O$, 25; $CoSO_4 \cdot 7H_2O$, 500; $CuSO_4 \cdot 5H_2O$, 1500; Na_2SeO_3, 25

[3] Y_2O_3 (yttria): analytical grade, made from Guangzhou Weibo Chemical co., LTD

Table 2 Nutrient composition (%) in dry matter of feed ingredients

Feed ingredients	Dry matter	Crude protein	Crude lipid	Crude ash	Gross energy (J g^{-1})	Phosphorus
S. japonica	97.28	10.75	2.12	29.31	17.68×10^3	0.46
P. dioica	97.04	32.98	1.23	11.45	29.36×10^3	0.75
G. lemaneiformis	98.67	18.96	0.84	31.73	14.32×10^3	0.28
U. lactuca	97.69	18.94	2.19	31.84	21.32×10^3	0.42
U. Pinnatifida	97.93	19.11	1.55	29.28	17.80×10^3	0.08

distilled water. The 1.2-mm diameter pellets were cold-extruded using a pelletizer (Institute of Chemical Engineering, South China University of Technology, Guangdong, China) and heated in an electric oven at 90 °C for 60 min, then air-dried to approximately 10% moisture, sealed, and stored at − 20 °C until fed.

Feeding trial

This experiment was carried out in an indoor flow-through system of the Guangdong Evergreen Group (Zhanjiang, China), and juvenile shrimp were obtained from a commercial hatchery. Prior to the experiment, the shrimp were stocked in a 1000-L fiberglass tank for 2 weeks to acclimate to the internal culture environment and fed with a commercial diet (44% crude protein, 8% crude lipid, Guangdong Evergreen Group, China). At the beginning of the experiment, 20 shrimp were randomly sampled and stored frozen (− 20 °C) until analysis of the initial proximate composition. A total of 720 uniform-sized shrimp (the mean weight, 2 g) in good health and condition were selected and distributed randomly into 18 500-L cylindrical fiberglass tanks, at a density of 40 shrimp per tank. Each diet was randomly assigned to triplicate tanks. All shrimp were hand fed four times daily at 7:00, 12:00, 17:00, and 21:00 for 8 weeks. The shrimp were initially fed at 8% of biomass, and the feeding rate was adjusted weekly in a range of 6–10% based on biomass by using an estimated growth rate established in the laboratory. The uneaten feed, feces, and molts were removed by siphoning immediately from tanks every day.

During the experimental period, filtered seawater (salinity, 29–30 g L^{-1}) was constantly supplied to each tank at a flow rate of 1.0 L min^{-1}. Water temperature, pH, dissolved oxygen, and ammonia nitrogen ranged from 27 to 30 °C, 8.0–8.4, 5–6, and 0.02–0.05 mg L^{-1}, respectively. Natural light-dark cycle was used during the trial (5 August to 30 September).

Table 3 Amino acids composition (g per 100 g dry matter) of feed ingredients

Amino acid	*S. japonica*	*P. dioica*	*G. lemaneiformis*	*U. lactuca*	*U. pinnatifida*
Essential amino acid					
Lys	0.33	1.60	0.67	1.02	0.51
Phe	0.33	1.27	0.68	0.96	0.67
Met	0.15	0.53	0.28	0.49	0.26
Thr	0.38	1.55	0.70	0.86	0.78
Ile	0.27	1.15	0.65	0.85	0.49
Val	0.40	2.07	0.82	1.16	0.87
Leu	0.48	2.26	0.99	1.50	0.81
His	0.08	0.39	0.18	0.35	0.17
Arg	0.30	1.79	0.83	0.99	1.54
Non-essential amino acid					
Asp	2.88	3.05	1.48	1.75	1.68
Ser	0.29	1.41	0.71	0.77	0.71
Glu	3.93	3.52	1.60	2.09	2.82
Pro	0.24	1.06	0.50	0.66	0.37
Gly	0.39	1.84	0.79	1.06	0.90
Ala	0.64	3.00	0.86	1.29	1.19
Cys-Cys	0.04	0.10	0.03	0.05	0.04
Tyr	0.16	0.69	0.35	0.50	0.37
Total	11.31	27.34	12.16	16.38	14.19

Table 4 Nutrient composition of the RD and test diets (DM basis)

	The RD	The test diets				
		SJD	PDD	GLD	ULD	UPD
Nutrient composition (% dry matter)						
Dry matter	92.35	91.91	91.94	91.39	89.23	89.85
Crude protein	47.00	35.58	42.64	37.77	38.39	38.18
Crude lipid	9.45	6.97	7.04	6.33	9.31	6.52
Crude ash	11.01	16.95	11.37	17.72	17.75	17.82
Gross energy ($J\ g^{-1}$)	28.56×10^3	19.96×10^3	32.12×10^3	25.24×10^3	24.80×10^3	24.24×10^3
Total phosphorus	1.52	1.22	1.06	1.01	1.13	1.11

Sample collection

During the last 2 weeks of experiment, fresh fecal samples with integrated envelope were siphoned and collected from each tank into separate bottles 1 h after feeding, then partially dried, overnight, in a convection oven at 90 °C. Dried samples were stored at − 20 °C until analyzed. At the end of the whole feeding trial, shrimp in each tank were weighed and sampled 24 h after the last feeding. Six shrimp were selected randomly from each tank for the analysis of the proximate composition of the whole body.

Table 5 Amino acids composition of the RD and test diets

	The RD	The test diets				
		SJD	PDD	GLD	ULD	UPD
Amino acid composition(% dry matter)						
Essential amino acid						
Lys	3.05	2.19	2.62	2.24	2.43	2.26
Phe	2.09	1.52	1.84	1.63	1.75	1.68
Met	0.93	0.67	0.80	0.69	0.81	0.70
Thr	1.81	1.34	1.68	1.38	1.47	1.44
Ile	1.98	1.43	1.72	1.53	1.63	1.50
Val	2.38	1.75	2.32	1.89	2.03	1.94
Leu	3.36	2.44	3.02	2.57	2.80	2.56
His	1.46	1.00	1.13	1.04	1.11	1.05
Arg	3.12	2.22	2.80	2.34	2.45	2.63
Non-essential amino acid						
Asp	4.44	3.97	4.08	3.47	3.65	3.61
Ser	1.68	1.19	1.41	1.20	1.27	1.27
Glu	7.74	6.35	6.42	5.74	6.04	6.27
Pro	1.89	1.33	1.73	1.48	1.51	1.47
Gly	2.52	1.83	2.32	1.97	2.10	2.03
Ala	2.48	1.89	2.65	1.94	2.11	2.08
Cys-Cys	0.23	0.20	0.23	0.16	0.16	0.14
Tyr	1.13	0.84	1.01	0.83	0.95	0.87
Total	42.34	32.21	37.74	32.17	34.29	33.50

Chemical analysis

Moisture, crude protein, crude lipid, and ash content in the diets were determined following the methods described by AOAC (1995). Moisture was determined by oven-drying at 105 °C for 24 h. Crude protein content (N × 6.25) was determined by the Kjeldahl method after acid digestion using an Auto Kjeldahl System (1030-Auto-analyzer, Tecator, Sweden). Crude lipid content was determined by the ether-extraction method using a Soxtec System HT (Soxtec System HT6, Tecator, Sweden). And ash content was determined using a muffle furnace at 550 °C for 24 h. Gross energy was determined using an adiabatic bomb calorimeter (HR-15A Adiabatic Bomb Calorimeter, Changsha, China).

Amino acid composition of ingredients and feces were determined using an automatic amino acid analyzer (Hitachi, Model 835-50, Hitachi, Japan) with the column (Hitachi custom ion exchange resin no.2619). Samples were hydrolyzed in 6 N HCl at 110 °C for 22 h, then were separated by the ion exchange column and reaction with ninhydrin solution, and the amino acid concentrations were obtained by spectrophotometry. Yttrium oxide content of diets and feces were determined by ICP atomic emission spectrophotometry [ICP; model: IRIS Advantage (HR), Thermo Jarrel Ash Corporation, U.S.A] at the Centralized Analytical Laboratory at Sun Yat-sen University, China.

Calculations and statistical analysis

Percent weight gain (WG), feed coefficient (FC), and survival ratio (SR) were calculated as follows:

$$\text{WG}\ (\%) = (W_2 - W_1)/W_1 \times 100,$$

$$\text{FC}\ (\%) = \textit{feed consumed}\ (\text{g, wet}\ \textit{weight})/\textit{weight gain}\ (\text{g, wet}\ \textit{weight}),$$

Table 6 Growth performance of juvenile *L. vannamei* fed with the RD and test diets

	The RD	The test diets				
		SJD	PDD	GLD	ULD	UPD
IBW(g)	2.00 ± 0.01	2.01 ± 0.01	2.00 ± 0.01	2.00 ± 0.01	2.01 ± 0.01	2.00 ± 0.01
FBW(g)	7.36 ± 0.04^{d}	5.57 ± 0.02^{c}	4.58 ± 0.06^{a}	4.69 ± 0.13^{a}	4.76 ± 0.14^{a}	5.00 ± 0.16^{b}
WG(%)	268.04 ± 1.98^{d}	178.35 ± 0.86^{c}	128.87 ± 2.89^{a}	134.31 ± 6.83^{a}	137.88 ± 6.86^{a}	149.87 ± 7.87^{b}
SR(%)	96.67 ± 1.44^{c}	97.50 ± 2.50^{c}	86.67 ± 1.44^{a}	97.50 ± 2.50^{c}	95.83 ± 1.44^{bc}	91.67 ± 3.82^{b}
FC	1.16 ± 0.03^{a}	1.73 ± 0.06^{b}	2.68 ± 0.09^{d}	2.29 ± 0.07^{c}	2.27 ± 0.11^{c}	2.18 ± 0.05^{c}

Data represent mean ± SEM of three replicates. Mean values within a row with unlike superscript letters were significantly different ($p < 0.05$)
IBW initial body weight, *FBW* final body weight, *WG* weight gain rate, *SR* survival rate, *FC* feed coefficient

$$\text{SR }(\%) = 100 \times (\textit{final number of shrimp})/(\textit{initial number of shrimp}).$$

where the W_2 is final weight (g) of shrimp and W_1 is initial weight (g).

Apparent digestibility coefficients (ADC) for dry matter, protein, amino acid, energy and phosphorus of each diet were calculated using the following equations:

$$\text{ADC } \textit{of} \text{ dry } \textit{matter} = 100-[100\ (Y\ \textit{in feed}/Y\ \textit{in feces})],$$

$$\text{ADC } \textit{of nutrients} = 100-[100\ (Y\ \textit{in feed}/Y\ \textit{in feces}) \times (N\ \textit{in feces}/N\ \textit{in feed})].$$

where the concentrations of nutrients (*N*) and Y_2O_3 (*Y*) are in % dry matter.

The results are presented as means±standard deviation. All data were statistically analyzed by SPSS 20.0 (SPSS, USA). The data were first tested for homogeneity. If the data had similar variances, then one-way analysis of variance (ANOVA) was used to made statistical comparisons. When there were significant differences ($p < 0.05$) among groups, multiple comparisons among means were made with LSD and Duncan's new multiple-range test (Duncan 1955).

Results

Growth performance

Growth performance of juvenile *L. vannamei* fed with the reference and test diets are shown in Table 6. Shrimp fed the reference diet had the highest WG and lowest FC compared with five test diets. Among the five macro-algae diets, shrimp fed the SJD and GLD shared the same SR (97.5%) and were significantly higher than shrimp fed the PDD and UPD ($p < 0.05$), the lowest SR (86.67%) was observed in shrimp fed the PDD. For the WG, shrimp fed the SJD (178.35%) was significantly higher than other test diets ($p < 0.05$), and the lowest WG (128.87%) was found in shrimp fed the PDD. FC of five test diets ranged from 1.73 to 2.68, and lowest in shrimp fed the SJD, followed by UPD, ULD, GLD, and PDD.

Body composition

Body composition of juvenile *L. vannamei* fed with the reference and test diets is shown in Table 7. The protein and lipid contents of shrimp fed the reference diet were significantly higher than the five test diets ($p < 0.05$). The moisture and ash content of shrimp fed the reference diet were significantly lower than the five test diets ($p < 0.05$). Among the five macro-algae diets, shrimp fed the SJD had the highest protein and lipid content. The lowest protein content was observed in shrimp fed the PDD and the lowest lipid content was found in shrimp fed the ULD. Comparing the shrimp fed five test diets, shrimp fed the PDD had the highest ash content while shrimp fed the GLD had the lowest ash content, the moisture content of shrimp ranged from 78.70% (ULD) to 80.58% (UPD).

Apparent digestibility of five test ingredients

Apparent digestibility coefficients (ADC) for dry matter, crude protein, energy, and phosphorus of feed ingredients for *L. vannamei* are given in Table 8. Apparent dry matter digestibility (ADMD) of five test ingredients ranged from 35.3 to 60.4%; *P. dioica* had the highest ADMD and *U. pinnatifida* had the lowest ADMD. Apparent protein digestibility (APD) of ingredients ranged from 44.9 to 60.5%, and the highest and lowest APD were observed for *U. pinnatifida* and *U. lactuca*, respectively. Apparent energy digestibility ranged from 31.3% (*S. japonica*) to 68.2% (*P. dioica*). The total phosphorus apparent digestibility of five test ingredients is highest in *S. japonica* (29.5%) and lowest in *P. dioica* (9.1%).

Amino acid profiles of five feed ingredients

Amino acid analyses of five feed ingredients are presented in Table 3. For nine essential amino acids, the highest concentration was observed in *P. dioica* and lowest in *S. japonica*. In addition, high concentrations of valine and leucine were found in *P. dioica* and *U. lactuca*. The five macro-algae ingredients all have low concentrations of methionine and histidine. For

Table 7 Body composition of juvenile *L. vannamei* fed with the RD and test diets (DM basis)

Whole shrimp	The RD	The test diets				
		SJD	PDD	GLD	ULD	UPD
Moisture	78.18 ± 0.82^{a}	79.57 ± 0.13^{bc}	80.28 ± 1.50^{c}	79.68 ± 0.02^{bc}	78.70 ± 0.06^{ab}	80.58 ± 0.39^{c}
Protein	74.26 ± 0.05^{c}	72.14 ± 0.15^{b}	69.53 ± 1.40^{a}	71.21 ± 0.29^{b}	72.02 ± 0.30^{b}	71.24 ± 0.41^{b}
Lipid	4.32 ± 0.03^{d}	3.39 ± 0.22^{c}	3.27 ± 0.03^{c}	2.54 ± 0.01^{b}	2.19 ± 0.05^{a}	2.65 ± 0.11^{b}
Ash	13.32 ± 0.04^{a}	16.17 ± 0.21^{b}	17.09 ± 0.04^{d}	16.04 ± 0.03^{b}	16.10 ± 0.00^{b}	16.78 ± 0.04^{c}

Data represent mean ± SEM of three replicates. Mean values within a row with unlike superscript letters were significantly different ($p < 0.05$)

eight non-essential amino acids, aspartic and glutamic acids constitute together a large part of the amino acid fraction of the five ingredients.

Apparent digestibility of amino acids

Apparent digestibility coefficients (ADC) of amino acids of feed ingredients for *L. vannamei* are given in Table 9. The ADC of total amino acids, *S. japonica* and *P. dioica* were significantly higher than the other three macro-algae ($p < 0.05$). The highest ADC of amino acids was found in *S. japonica* (73.5%) and the lowest ADC of amino acids was observed in *U. lactuca* (50.0%). For the ADC of individual amino acids, there were significant differences ($p < 0.05$) among the five macro-algae. The highest ADC of essential amino acids was found in *U. pinnatifida* and *S. japonica* had the lowest ADC of lysine and methionine, two limiting amino acids. Additionally, the ADCs were very different among various amino acids within the same ingredient.

Discussion

Saccharina japonica is more suitable as a feed ingredient than the other four macro-algae. Additionally, some follow-up experiments need to be done to explore further the optimal addition of macro-algae in feeds, and formulate a reasonable feed formula to develop new cost-efficient compound feed used for shrimp.

The present results on growth performance indicated that shrimp fed the reference diet had the highest WG and were significantly higher than shrimp fed the five macro-algae diets. The better performance of shrimp fed the reference diet was partly due to the higher protein content. It is well known that the growth performance and survival of the shrimp reflects the quality of the dietary ingredients, particularly the protein source (Sudaryono et al. 1995). In contrast, increasing macro-algae meal in the test diets could increase the fiber content, lower the feed digestibility, and thus affect the growth performance of the shrimp. Briggs and Funge-Smith (1996) also observed a significant reduction in growth of *P. monodon* fed diets containing 30% red seaweed *Gracilaria* spp. Negative effects could be attributed to the high ash content, low dietary protein content, or the high levels of soluble fiber present in the test diets caused by high algae inclusion levels. Among the five test diets, shrimp fed the SJD had the highest WG and the lowest FC, which indicated that the nutrients in *S. japonica* can be better utilized by *L. vannamei* than those of the other four macro-algae.

Whole-body proximate composition of shrimp was significantly affected by the macro-algae inclusion. In the present study, the inclusion of macro-algae in the diet resulted in lower protein content and higher ash content in shrimp compared to the control group, which caused low feed digestibility and poor growth performance.

So far, research on the apparent digestibility of algae ingredient for aquatic animals are limited. Xu et al. (2011) added 33% dried *Gracilaria lemaneiformis* (DGL) as an ingredient instead of 10% fish meal and 24% starch in diets for teleost fish *Siganus canaliculatus*, and the results showed that although the growth performance and feed utilization efficiency of fish fed the DGL-diet were inferior to fish fed the control

Table 8 Apparent digestibility coefficients (ADC) for dry matter, crude protein, energy, and phosphorus of feed ingredients for *L. vannamei*

ADC (%)	*S. japonica*	*P. dioica*	*G. lemaneiformis*	*U. lactuca*	*U. pinnatifida*
Dry matter	54.30 ± 2.06^{c}	60.40 ± 2.28^{d}	51.80 ± 1.44^{c}	45.50 ± 1.47^{b}	35.30 ± 1.26^{a}
Protein	58.40 ± 1.71^{bc}	57.20 ± 1.14^{b}	45.40 ± 1.10^{a}	44.90 ± 2.08^{a}	60.50 ± 2.00^{c}
Energy	31.30 ± 2.05^{a}	68.20 ± 3.18^{d}	39.40 ± 2.25^{b}	38.00 ± 0.45^{b}	52.60 ± 0.72^{c}
Phosphorus	29.50 ± 1.56^{c}	9.10 ± 0.74^{a}	12.30 ± 0.86^{b}	10.50 ± 0.56^{ab}	11.30 ± 0.61^{b}

Data represent mean ± SEM of three replicates. Mean values within a row with unlike superscript letters were significantly different ($p < 0.05$)

Table 9 Apparent digestibility coefficients (ADC, %) of amino acids of feed ingredients for *L. vannamei*

Amino acid	*S. japonica*	*P. dioica*	*G. lemaneiformis*	*U. lactuca*	*U. pinnatifida*
Lys	27.80 ± 0.78[a]	64.00 ± 1.75[d]	46.90 ± 0.39[b]	56.10 ± 4.74[c]	69.20 ± 4.21[d]
Phe	32.30 ± 3.11[a]	53.60 ± 1.00[c]	36.20 ± 2.37[ab]	39.00 ± 1.99[b]	59.20 ± 4.23[d]
Met	38.40 ± 1.95[a]	61.90 ± 4.42[b]	44.50 ± 3.91[a]	40.40 ± 1.69[a]	68.40 ± 4.46[c]
Thr	32.30 ± 1.56[b]	31.70 ± 0.83[b]	18.10 ± 1.55[a]	15.60 ± 2.95[a]	33.20 ± 2.07[b]
Ile	85.50 ± 3.64[c]	54.90 ± 2.79[b]	47.60 ± 1.53[a]	47.40 ± 2.09[a]	86.00 ± 2.00[c]
Val	65.60 ± 3.55[a]	63.90 ± 5.86[ab]	50.40 ± 1.95[a]	48.80 ± 3.02[a]	75.00 ± 3.00[b]
Leu	74.20 ± 1.34[c]	63.70 ± 0.26[b]	53.30 ± 1.41[a]	53.00 ± 1.78[a]	80.40 ± 1.92[d]
His	17.70 ± 1.54[a]	35.70 ± 2.25[b]	55.10 ± 4.11[c]	57.10 ± 3.14[c]	72.00 ± 0.75[d]
Arg	56.60 ± 1.24[b]	55.40 ± 0.51[ab]	57.80 ± 2.04[b]	53.30 ± 1.14[a]	73.00 ± 2.05[d]
Asp	89.70 ± 1.91[e]	61.90 ± 3.67[d]	42.70 ± 0.65[a]	48.70 ± 0.87[b]	54.90 ± 4.24[c]
Ser	35.10 ± 2.09[a]	48.30 ± 3.10[c]	41.10 ± 1.32[b]	34.50 ± 0.74[a]	48.70 ± 1.53[c]
Glu	84.60 ± 3.41[c]	63.40 ± 6.39[b]	50.60 ± 1.94[a]	46.20 ± 2.26[a]	81.80 ± 1.38[c]
Pro	54.50 ± 2.30[c]	62.70 ± 2.64[d]	37.80 ± 1.71[b]	32.10 ± 3.90[a]	78.70 ± 3.51[e]
Gly	8.50 ± 0.52[a]	56.10 ± 6.29[d]	28.00 ± 1.00[b]	42.70 ± 0.31[c]	55.70 ± 4.70[d]
Ala	57.30 ± 2.05[c]	71.90 ± 1.00[d]	48.00 ± 0.40[b]	43.60 ± 3.88[a]	57.60 ± 1.68[c]
Cys-Cys	62.50 ± 0.80[c]	51.00 ± 2.25[b]	53.20 ± 2.94[b]	20.10 ± 2.56[a]	58.80 ± 2.29[c]
Tyr	24.6 ± 1.52[a]	48.8 ± 5.61[c]	30.1 ± 6.18[ab]	35.9 ± 1.51[b]	64.91.09[d]
Total	73.5 ± 2.38[c]	70.7 ± 1.51[c]	51.1 ± 1.86[a]	50.0 ± 0.61[a]	65.0 ± 3.61[b]

Data represent mean ± SEM of three replicates. Mean values within a row with unlike superscript letters were significantly different ($p < 0.05$)

diet, there were no significant differences in the ADC of dry matter, protein between DGL-diet and control diet. This suggested that *G. lemaneiformis* can partly replace fish meal and starch in diets for the teleost fish, *S. canaliculatus*. In the present study, the ADCs of nutrients for five test macro-algae were generally low and this may be related to the high fiber contents in the macro-algae.

ADMD provides a measure of the total quantity of an ingredient that is digested and absorbed. Because all components of an ingredient are not digested equally, apparent dry matter digestibility coefficients can provide a better estimate of the quantity of indigestible material presented in feed ingredients than digestibility coefficients for individual nutrients, which, in some cases, are minimally affected by indigestible material in the diet (Brunson et al. 2015). In the present study, shrimp fed the PDD had the highest ADMD, and shrimp fed the UPD had the lowest ADMD. The relatively higher ADMD of *P. dioica* could be explained by the more digestible starch compounds in the ingredient which could be better utilized by the animals. By contrast, the relatively lower ADMD of *U. pinnatifida* maybe attributed to high content of polysaccharides and ash which are difficultly digested by *L. vannamei* as shown in other studies (Sullivan and Reigh 1995; Stone et al. 2000; Lee 2002; Zhou et al. 2004). Appler (2010) found that the carbohydrate content of algae is usually over 40%, of which contains most non-peptic or non-nutritional carbohydrate with complicated structure that may result in poor palatability. Therefore, apparent dry matter digestibility of algae for animals is generally low.

Generally, the protein quality of dietary ingredients is a leading factor affecting aquaculture performance and APD is the effective evaluation index of availability of protein in feedstuff utilized by aquatic animals. In the present study, the highest APD was observed in shrimp fed the UPD and the lowest APD was observed in shrimp fed the ULD. The higher APD of UPD may be due to its well-balanced amino acid profile (Andrews and Page 1974), while the lower APD of ULD was probably due to poor protein quality in *U. lactuca*. In addition, lower protein digestibility of in high-lipid ingredients might be related to the formation of protein- and oxidized-fat complexes during drying (Ufodike and Matty 1983; Anderson et al. 1995).

Energy digestibility (ED) of ingredients tends to be inversely related to their fiber content (Hilton and Hodson 1983; Kirchgessner et al. 1986). In this study, The ED of shrimp fed the PDD was significantly higher than other test groups, indicating that the energy of *P. dioica* was better utilized by *L. vannamei* than the other four macro-algae.

Phosphorus is not only an important constituent of nucleic acids and cell membranes, but also an essential mineral nutrient for growth (Baeverfjord et al. 1998), skeletal development (Åsgård and Shearer 2015), and reproduction of aquatic animals (Hardy and Shearer 2011). However, as a critical pollutant in the aquatic environment, excessive excretion of phosphorus into water can stimulate the growth of algae and phytoplankton, thus reducing dissolved oxygen and causing water pollution (Miller et al. 1974; Sugiura et al. 1999). In the present study, shrimp fed the SJD had the highest ADC of phosphorus and shrimp fed the PDD had the lowest. This result indicated that phosphorus in *S. japonica* can be better used by *L. vannamei* than the other four macro-algae ingredients, and *S. japonica* as feed ingredient could more effectively protect the aquaculture water from pollution.

In general, macro-algae are considered as high nutritional value food, but most of them contain a low proportion of essential amino acids, especially lysine, a limiting factor in feedstuff (Brown et al. 1997). According to Fox et al. (1995), diets deficient in lysine, methionine, and arginine limited the growth of juvenile *L. vannamei*. In this study, shrimp fed the SJD diet had the best growth performance despite the lowest contents of these three amino acids in *S. japonica* and SJD. By contrast, *P. dioica* and PDD had the highest contents of these three limiting amino acids, but the shrimp had the worst growth performance. This result was in disagreement with the findings of Fox et al. (1995) and this could be partially a consequence of the higher fiber content in *P. dioica* and PDD.

The apparent amino acid digestibility of feed ingredient depends on not only its content, but also on the physical and chemical properties of the ingredient itself, the structural properties of amino acid itself, and other factors (Terrazas-Fierro et al. 2010; Oujifard et al. 2012). In the present study, shrimp fed the SJD had the highest ADC of total amino acid, whereas shrimp fed the ULD had the lowest. However, the ADCs were significantly different among various amino acids within the same ingredient. Therefore, the availability of amino acid cannot be estimated directly from the ADC of crude protein, as also reported by Masumoto et al. (1996), Lupatsch et al. (1997), Wilson et al. (1981), and Silva et al. (2000). By contrast, other studies have observed that the amino acid availability tended to reflect protein digestibility—an ingredient with high protein digestibility usually has high amino acid digestibility (Mu et al. 2000; Zhou et al. 2004; Yang et al. 2009).

Above all, as a new compound feed ingredient used for aquatic animals, macro-algae have enormous value. The high level of carbohydrates in macro-algae can reduce the amount of supplemental starch in formulated diets and minimize competition with human food sources. Furthermore, application of macro-algae in formulated diets can promote the coordinated development of aquaculture. However, due to the presence of a large amount of anti-nutritional factors (such as non-starch polysaccharides) in algae that may affect the palatability of diets, the digestibility of feeds and growth performance for aquatic animals will decrease when the addition of algae in feeds is too high. Therefore, appropriate additives that are highly palatable and digestible to animals are recommended to be added in formulated diets.

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