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Evaluation of green seaweed *Ulva* sp. as a replacement of fish meal in plant-based practical diets for Pacific white shrimp, *Litopenaeus vannamei*

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Abstract A growth trial and a digestibility trial were conducted to evaluate seaweed *Ulva* sp. as a substitution for fish meal (FM) in commercial-type feed formulation for Pacific white shrimp, *Litopenaeus vannamei*. Towards this goal, the 6-week growth trial utilized increasing levels (0, 6.35, 12.7, 19.05, and 25.4%) of the first batch of *Ulva* meal (UM1) to replace up to 8% FM in a plant-based feed formulation. At the end of the growth trial, shrimp offered diets containing 12.7, 19.05, and 25.4% UM1 exhibited significantly reduced weight gain. Apparent net protein retention (ANPR) was significantly decreased, while feed conversion ratio (FCR) was significantly increased when shrimps were fed with diets containing 19.05 and 25.4% UM1. Crude lipid content of whole shrimp samples were significantly decreased when UM1 was supplemented in the diets. Apparent digestibility coefficients of dry matter, energy, protein, and amino acids of two batches of *Ulva* meal (UM1 and UM2) were determined using chromic oxide as an inert maker and the 70:30 replacement technique. Energy and protein digestibility of UM1 and UM2 were significantly lower than FM and soybean meal (SBM) which were run at the same time. As a result of relatively low protein availability, individual amino acids digestibility of UM1 and UM2 are also significantly lower than those of FM and SBM. Results of the present study indicate that UM1 can be included in the shrimp diet up to 6.35% to replace 2% fish meal without resulting in growth depression. The low nutrients availability and high mineral contents of *Ulva* meal may explain a portion of the observed reduction in shrimp growth.

Keywords Apparent digestibility coefficients · Fish meal replacement · Apparent net protein retention · *L. vannamei* · *Ulva* sp.

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Introduction

Traditionally, fish meal (FM) has been utilized as a major ingredient in commercial shrimp feed formulations (Tacon and Metian 2008). However, this valuable source is becoming scarce compared to the growing demand, resulting in a drastic increase in the market price (Qiu and Davis 2016a). Terrestrial plant-based protein ingredients especially soybean meal have been well defined as alternative protein sources in shrimp feeds (Davis and Arnold 2000; Samocha et al. 2004; Amaya et al. 2007a, b; Roy et al. 2009; Qiu and Davis 2016a, b, 2017; Qiu et al. 2017). Although these resources will continue to be the mainstay in shrimp feed formulation in terms of their nutritional preponderances, it is still necessary for us to explore new alternative ingredients to ensure sustainability and economy of shrimp culture.

Seaweeds have been utilized for nutritional purposes in human and animal diets since a very early date, and recently,

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there are increasing interests in applying seaweed meals as protein sources in aquaculture feeds (Wassef et al. 2013). The popularity of seaweeds as feed ingredients in aquaculture feeds is a result of favorable levels of amino acids, fatty acids, minerals, vitamins, carotenoid pigments, and bioactive compounds. With respect to the use of land and water, seaweeds are more productive than terrestrial plants such as soy and canola with yields of 10–20 t ha^{-1} $year^{-1}$ dry weight being the norm in the industry, and several reports have described annual yields of 50 t ha^{-1} $year^{-1}$ (Neori et al. 2004).

In an integrated cultivation system, the seaweed uses the metabolic residues of animals as nutrients, absorbs CO_2, and produces O_2 for the environment (Marinho-Soriano et al. 2007). The interaction allows the excretion of an organism to serve as food for another. Presently, significant improvements in growth and survival have been observed when Pacific white shrimp, *Litopenaeus vannamei* (Cruz-Suárez et al. 2010; Brito et al. 2014a, b), giant tiger shrimp, *Penaeus monodon* Fabr (Tsutsui et al. 2010; Izzati 2012), and yellowleg shrimp, *Farfantepenaeus californiensis* (Portillo-Clark et al. 2012), are co-cultured with seaweeds.

A number of studies have demonstrated that dietary *Ulva* meal inclusion at low levels (< 5% of the diet) did not affect the growth performance in a variety of species including African catfish *Clarias gariepinus* (Abdel-Warith et al. 2016), gilthead seabream *Sparus aurata* (Emre et al. 2013), Pacific white shrimp (Rodríguez-González et al. 2014; Cárdenas et al. 2015), Nile tilapia *Oreochromis niloticus* (Güroy et al. 2007; Ergün et al. 2009), and rainbow trout *Oncorhynchus mykiss* (Güroy et al. 2013). However, the growth responses of different aquatic animal species to the moderate and high supplementation levels of *Ulva* meal are somewhat inconsistent.

Although there are several studies looking at the efficacy of *Ulva* sp. in shrimp feeds, the information about *Ulva* sp. as a replacement for fish meal in shrimp feeds and nutrients digestibility of *Ulva* sp. for shrimp is limited. Hence, the purpose of this study was to evaluate the biological responses of Pacific white shrimp, *L. vannamei* to dietary *Ulva* sp. supplementation as a replacement for fish meal and determine the apparent digestibility values of *Ulva* sp. as compared to other protein sources.

Materials and methods

Ingredients

Two pooled samples or batches (UM1 & UM2) were obtained in the present study. *Ulva* sp. (probably *U. lactuca* L) was obtained from National Center for Mariculture, Israel Oceanographic and Limnological Research, Eilat, Israel. The *Ulva* sp. was brought in from a neighboring algal pond and grown on a spray-drip irrigated system, modified slightly from that in Msuya and Neori (2010). Briefly, the algae were placed on several 1 m^2 plywood boards and held by plastic 4-mm mesh netting, creating flat square mattresses 3 to 5 cm thick, inclined from the horizontal at 5.7° due west. The boards were each placed above a plastic tank unit, to which water was drained. Water from fishponds, enriched with additional nutrients to a level of 10 g ammonia-N (TAN), 15 g nitrate-N, and 2 g phosphate-P m^{-2} day^{-1} (plus micronutrients, from Shefer 7-3-7+3, Fertilizers & Chemicals, Haifa, Israel), was dripped onto the seaweed mattresses by perforated plastic pipes at the tops of the boards. Each of the units received about 5 (± 5%) m^3 m^{-2} day^{-1} of water.

Experimental diets

All test diets were formulated to be isonitrogenous and isolipidic (35% protein and 8% lipid). Primary ingredients and pooled batches of sun-dried *Ulva* meal were analyzed at the University of Missouri Agricultural Experimental Station Chemical Laboratories (Columbia, MO, USA) and Auburn University Soil Laboratory (Auburn, AL, USA) for proximate composition, amino acids profile, and mineral contents (Table 1 and Table 2). Prior to pooling to create the second *Ulva* meal (UM2), the seven samples were analyzed for proximate composition and mineral contents (Table 3) at Midwest Laboratories (Omaha, NE, USA). In the growth trial, five experimental diets were formulated to contain increasing levels (0, 6.35, 12.70, 19.05, and 25.40%) of the first batch of *Ulva* meal (UM1) as a replacement of fish meal (Table 4). Additionally, a reference diet was utilized to determine digestibility coefficients in conjunction with 1% chromic oxide as an inert marker and 70:30 replacement strategy (Table 5).

All experimental diets were produced at the Aquatic Animal Nutrition Laboratory at the School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University (Auburn, AL, USA) using the standard procedures for the shrimp feeds (Qiu and Davis 2017). Briefly, diets were prepared by mixing the pre-ground dry ingredients in a food mixer (Hobart, USA) for 10–15 min. Hot water was then blended into the mixture to obtain a consistency appropriate for pelleting. Diets were pressure-pelleted using a meat grinder with a 2.5-mm die. The wet pellets were then placed into a fan-ventilated oven (< 50 °C) overnight in order to attain a moisture content of less than 10%. Dry pellets were crumbled, packed in sealed bags, and stored in a freezer until use. The diets were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA) for proximate composition and amino acid profile (Table 6).

Table 1 Proximate composition, phosphorus content, and amino acid profile of the fish meal (FM), soybean meal (SBM), and two batches *Ulva* meal (UM1 and 2)

Composition (% as is)	UM1	UM2	FM	SBM
Crude protein	20.64	27.24	62.78	44.89
Moisture	8.89	13.74	7.99	10.97
Crude fat	0.53	0.12	10.56	3.78
Crude fiber	5.17	2.93	0.00	3.20
Ash	46.01	22.18	18.75	6.67
Phosphorus	0.43	0.30	3.15	0.66
Alanine	1.64	2.03	3.91	2.04
Arginine	0.99	1.39	3.68	3.35
Aspartic acid	2.12	2.67	5.34	5.10
Cysteine	0.34	0.39	0.47	0.62
Glutamic acid	2.02	2.59	7.47	8.24
Glycine	1.17	1.59	4.88	2.04
Histidine	0.25	0.40	1.63	1.2
Hydroxylysine	0.17	0.12	0.2	0.05
Hydroxyproline	0.2	0.30	1.03	0.05
Isoleucine	0.8	1.06	2.42	2.17
Leucine	1.22	1.87	4.21	3.57
Lysine	0.95	1.22	4.67	3.06
Methionine	0.26	0.44	1.61	0.66
Phenylalanine	0.98	1.37	2.39	2.35
Proline	0.76	1.17	3.08	2.39
Serine	0.91	1.05	2.11	1.90
Taurine	0.15	0.18	0.73	0.13
Threonine	0.94	1.17	2.41	1.75
Tryptophan	0.16	0.20	0.62	0.62
Tyrosine	0.48	0.77	1.67	1.64
Valine	1.17	1.56	2.99	2.34

Table 2 Mineral composition of the two batches *Ulva* meal (UM1 and 2)

Minerals	UM1	UM2
Quantity elements (% as is)		
Calcium	2.29	0.49
Potassium	1.99	2.21
Magnesium	2.57	2.93
Sodium	4.79	1.63
Phosphorus	0.4	0.32
Sulfur	3.46	4.54
Trace elements (mg kg^{-1} as is)		
Aluminum	4173.2	380.5
Arsenic	1.6	1.3
Boron	76.2	38.8
Barium	13.8	2.6
Cadmium	50.4	8.3
Cobalt	3.0	0.8
Chromium	9.7	1.8
Copper	26.5	17.5
Iron	9086.7	581.6
Manganese	112.4	21.1
Nickel	7.7	2.1
Lead	10.8	2.0
Selenium	5.3	3.9
Silicon	70.3	68.4
Zinc	63.1	34.6
Zirconium	1.0	1.0

Growth trial

The growth trial was conducted at the E.W. Shell Fisheries Research Station, Auburn University (Auburn, AL, USA). Pacific white shrimp post larvae (PL) were obtained from Shrimp Improvement Systems (Islamorada, FL, USA) and nursed in an indoor recirculating system. PLs were fed a commercial feed (Zeigler Bros., Inc., Gardners, PA, USA) using an automatic feeder for ~ 1 week, and then switched to crumbled commercial shrimp feed (Zeigler Bros., Inc.) for ~ 1–2 weeks.

In the growth trial, the recirculating aquaculture system consisted of 35 aquaria (135 L) connected to a common reservoir, biological filter, bead filter, fluidized biological filter, and recirculation pump. A sub-sample of 20 shrimp from the initial stocking was retained for whole body chemical analysis to be utilized for later apparent net protein retention analysis.

Seven replicate groups of shrimp (0.46 g initial mean weight, 10 shrimp $tank^{-1}$) were offered diets using our standard feeding protocol over 6 weeks. Based on historic results, feed inputs were pre-programmed assuming the shrimp would double their weight weekly up to 1 g then gain 0.8–1.1 g weekly (expected growth) with an estimate feed conversion ratio (FCR) of 1.8. Daily allowances of feed were adjusted based on observed feed consumption, weekly counts of the shrimp, and mortality. The formula used to calculate the feed inputs was presented as follows: daily feed input (g) = (estimated FCR × expected growth × number of shrimp)/7. Consequently, for each tank in trial 1, a fixed ration of 0.67 g day^{-1} for the first week, 1.45 g day^{-1} for the second week, 2.06 g day^{-1} for the third week, and 2.31 g day^{-1} for the fourth week, 2.57 g day^{-1} for the fifth week, and 2.83 g day^{-1} for the sixth week was offered over four feedings.

Dissolved oxygen (DO), water temperature, and salinity were measured twice daily using a YSI 650 multi-parameter instrument (YSI, USA). Hydrogen potential (pH) was measured twice weekly by using a waterproof pHTestr30 (Oakton

Table 3 Proximate and mineral composition of *Ulva* meal collected from seven different dates and then pooled into UM2. The analysis of the pooled sample is presented in Tables 1 and 2

Proximate composition (% as is)	Collection dates (2015)						
	21 Jul	30 Jul	16 Aug	20 Aug	23 Aug	25 Aug	30 Aug
Moisture	83.99	87.14	85.59	82.78	83.32	82.44	85.07
Crude protein	28.2	19.4	29.0	28.3	27.3	28.0	26.9
Crude fat	0.46	n.d.	0.2	n.d.	n.d.	0.62	n.d.
Fiber	10	13.9	13.4	10.9	11.3	10.5	10.5
Ash	17.3	39.2	19.8	15.6	17.1	18.4	20.9
Quantity elements (% as is)							
Calcium	0.42	2.01	0.86	0.47	0.44	0.42	0.46
Magnesium	3.12	3.07	3.00	3.25	3.21	3.25	3.12
Phosphorus	0.32	0.37	0.38	0.35	0.31	0.31	0.31
Potassium	2.71	2.26	1.82	2.2	1.95	2.31	2.49
Sodium	1.26	2.74	1.46	0.89	1.55	1.96	2.05
Sulfur	4.38	3.64	3.78	4.19	4.16	4.33	4.24
Trace elements (mg kg^{-1} as is)							
Copper	7.7	28.2	11.0	7.8	7.6	8.9	8.8
Iron	331	6780	2040	424	450	356	510
Manganese	21.1	99.2	47.0	22.9	22.2	21.9	24.3
Zinc	37.6	79.3	64.0	49.0	38.4	38.9	38.8

n.d. not detected

Instrument, USA). Total ammonia-nitrogen (TAN) and nitrite were evaluated every week using the methods described by Solorzano (1969) and Spotte (1979). During the growth trial, DO, temperature, salinity, pH, TAN, and nitrite were maintained within acceptable ranges for *L. vannamei* at 6.19 ± 0.25 mg L^{-1}, 28.4 ± 0.8 °C, 11.8 ± 0.4 ppt, 7.23 ± 0.22, 0.079 ± 0.041 mg L^{-1}, and 0.039 ± 0.021 mg L^{-1}, respectively.

At the end of the experiment, shrimps were counted and group weighed. Final mean weight, FCR, weight gain, biomass, and survival were determined (Table 7). After obtaining the final total weight of shrimps in each aquarium, four shrimps from each tank were randomly selected and frozen at − 20 °C for subsequent determination of whole body composition. All results were rounded to two decimal places. Proximate composition (Table 8) of whole shrimp body was analyzed by the University of Missouri-Columbia, Agriculture Experiment Station Chemical Laboratory (Columbia, MO, USA). Apparent net protein retention (ANPR) was calculated as follows:

ANPR (%) = (final weight × final protein content) − (initial weight × initial protein content) × 100/protein offered.

Digestibility trial

The digestibility trial was conducted in the previously mentioned recirculation system and utilized six shrimps (~ 12 g mean weight) per aquarium with six aquaria per dietary treatment. Once acclimated for 3 days to the test diets, feces from two aquaria were pooled ($n = 3$) and collected over a 5-day period or until adequate samples were obtained. To obtain fecal samples, the aquaria were cleaned by siphoning before each feeding with the first collection of the day discarded. After cleaning, the shrimp were offered an excess of feed and then about 1 h later feed was removed and feces were collected by siphoning onto a 500-μm mesh screen. Collected feces were rinsed with distilled water, dried at 95 °C until a constant weight was obtained, and then stored in freezer (− 20 °C) until analyzed. Apparent digestibility coefficient for dry matter, protein, energy, and amino acids were determined by using chromic oxide (Cr_2O_3, 10 g kg^{-1}) as an inert marker. Chromium concentrations were determined by the method of McGinnis and Kasting (1964) in which, after a colorimetric reaction, absorbance is read on a spectrophotometer at 540 nm. Gross energy of diets and fecal samples were analyzed with a Semi micro-bomb calorimeter (Model 1425, Parr Instrument Co., USA). Protein was determined by micro-Kjeldahl analysis (Ma and Zuazaga 1942). Amino acids were analyzed by the University of Missouri-Columbia, Agriculture Experiment Station Chemical Laboratory (Columbia, MO, USA). The apparent digestibility coefficient of dry matter (ADM), protein (APD), energy (AED), and amino acids (AAAD) of the test diets (D) were calculated according to Cho et al. (1982) as follows:

ADMD (%) = 100 − [100 × (% Cr_2O_3 in feed/% Cr_2O_3 in feces)]

Table 4 Formulation of test diets utilized in the growth trial

Ingredient (% as is)	D_1	D_2	D_3	D_4	D_5
Fish meal[1]	10.00	8.00	6.00	4.00	2.00
Soybean meal[2]	48.70	48.70	48.70	48.70	48.70
Corn protein concentrate[3]	8.00	8.00	8.00	8.00	8.00
Ulva meal 1[10]	0.00	6.35	12.70	19.05	25.40
Fish oil[2]	5.65	5.75	5.86	5.97	6.07
Trace mineral premix[5]	0.50	0.50	0.50	0.50	0.50
Vitamin premix[6]	1.80	1.80	1.80	1.80	1.80
Choline chloride[4]	0.20	0.20	0.20	0.20	0.20
Stay C[7]	0.10	0.10	0.10	0.10	0.10
Mono-dicalcium phosphate[8]	1.62	1.90	2.15	2.40	2.65
Lecithin[9]	1.00	1.00	1.00	1.00	1.00
Cholesterol[4]	0.05	0.05	0.05	0.05	0.05
Corn starch[4]	22.54	17.78	13.05	8.31	3.58

[1] Omega Protein Inc., Huston, TX, USA

[2] De-hulled solvent extract soybean meal, Bunge Limited, Decatur, AL, USA

[3] Empyreal® 75, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA

[4] MP Biomedicals Inc., Solon, OH, USA

[5] Trace mineral premix (g (100 g)$^{-1}$ premix): cobalt chloride, 0.004; cupric sulfate pentahydrate, 0.550; ferrous sulfate, 2.000; magnesium sulfate anhydrous, 13.862; manganese sulfate monohydrate, 0.650; potassium iodide, 0.067; sodium selenite, 0.010; zinc sulfate heptahydrate, 13.193; alpha-cellulose, 69.664

[6] Vitamin premix (g kg^{-1} premix): thiamin.HCL, 4.95; riboflavin, 3.83; pyridoxine.HCL, 4.00; Ca-pantothenate, 10.00; nicotinic acid, 10.00; biotin, 0.50; folic acid, 4.00; cyanocobalamin, 0.05; inositol, 25.00; vitamin A acetate (500,000 IU g^{-1}), 0.32; vitamin D3 (1,000,000 IU g^{-1}), 80.00; menadione, 0.50; alpha-cellulose, 856.81

[7] Stay C, (L-ascorbyl-2-polyphosphate 35% Active C), DSM Nutritional Products., Parsippany, NJ, USA

[8] J. T. Baker, Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA

[9] The Solae Company, St. Louis, MO, USA

[10] First batch *Ulva* meal experimentally produced

APDD, AEDD, and AAADD (%) = 100 − [100 × (% Cr_2O_3 in feed/% Cr_2O_3 in feces) × (% nutrient in feces/% nutrient in feed).

The apparent digestibility coefficients of dry matter (ADM), protein (APD), energy (AED), and amino acids (AAAD) of the test ingredients (I) were calculated according to Bureau and Hua (2006) as follows:

$$ADMI = ADMD + \left[(ADMD{-}ADMD_{\mathrm{ref}.diet}) \times \left(0.7 \times D_{\mathrm{ref}}/0.3 \times D_{ingr}\right)\right]$$

$$APDI = APDD + \left[(APDD{-}APDD_{\mathrm{ref}.diet}) \times \left(0.7 \times D_{\mathrm{ref}}/0.3 \times D_{ingr}\right)\right]$$

$$AEDI = AEDD + \left[(AEDD{-}AEDD_{\mathrm{ref}.diet}) \times \left(0.7 \times D_{\mathrm{ref}}/0.3 \times D_{ingr}\right)\right]$$

$$AAADI = AAADD + \left[(AAADD{-}AAADD_{\mathrm{ref}.diet}) \times \left(0.7 \times D_{\mathrm{ref}}/0.3 \times D_{ingr}\right)\right]$$

where D_{ref} = % nutrient (or kJ g^{-1} gross energy) of reference diet mash (dry weight); D_{ingr} = % nutrient (or kJ g^{-1} gross energy) of test ingredient (dry weight).

Table 5 Composition of reference diet for the determination of digestibility coefficients of the first and second batch *Ulva* meal, fish meal, and soybean meal

Ingredients	% as is
Soybean meal[1]	10.00
Fish meal[2]	32.50
Fish oil[2]	3.20
Whole wheat[3]	47.60
Trace mineral premix[4]	0.50
Vitamin premix[5]	1.80
Choline cloride[6]	0.20
Stay C[7]	0.10
Corn starch[3]	1.00
Lecithin[8]	1.00
Chromic oxide[9]	1.00

[1] De-hulled solvent extract soybean meal, Bunge Limited, Decatur, AL, USA

[2] Omega Protein Inc., Houston TX, USA

[3] MP Biomedicals Inc., Solon, OH, USA

[4] Trace mineral premix(g (100 g)$^{-1}$ premix): cobalt chloride, 0.004; cupric sulfate pentahydrate, 0.550; ferrous sulfate, 2.000; magnesium sulfate anhydrous, 13.862; manganese sulfate monohydrate, 0.650; potassium iodide, 0.067; sodium selenite, 0.010; zinc sulfate heptahydrate, 13.193; alpha-cellulose, 69.664

[5] Vitamin premix (g kg^{-1} premix): thiamin.HCL, 4.95; riboflavin, 3.83; pyridoxine.HCL, 4.00; Ca-pantothenate, 10.00; nicotinic acid, 10.00; biotin, 0.50; folic acid, 4.00; cyanocobalamin, 0.05; inositol, 25.00; vitamin A acetate (500,000 IU g^{-}1), 0.32; vitamin D3 (1,000,000 IU g^{-1}), 80.00; menadione, 0.50; alpha-cellulose, 856.81

[6] VWR, Radnor, PA, USA

[7] Stay C, (L-ascorbyl-2-polyphosphate 35% Active C), DSM Nutritional Products., Parsippany, NJ, USA

[8] The Solae Company, St. Louis, MO, USA

[9] Alfa Aesar, Haverhill, MA, USA

Statistical analysis

All the data were analyzed using SAS (V9.4. statistical software). Data from growth trial and digestibility trial were analyzed using one-way ANOVA to determine significant differences ($P < 0.05$) among treatments followed by the Tukey's multiple comparison test to determine difference between treatments in each trial. Arcsine square root transformation was used prior to analysis for the proportion data. False discover rate (FDR) controlling procedures were applied to adjust the P value to control the FDR for amino acid data. Linear, second-, or third-order polynomial regressions were performed to investigate the relationship between the supplemental *Ulva* meal levels and weight gain, FCR, survival, and lipid content of whole shrimp body. To identify the most appropriate regression model, we compared P value of the model components, R^2 value, adjust R^2 value,

Table 6 Proximate composition and amino acid profile of the test diets used in the growth trial

Composition (as is %)	D_1	D_2	D_3	D_4	D_5
Crude protein	36.83	36.52	36.60	36.28	35.65
Moisture	5.46	6.56	5.12	7.15	8.70
Crude fat	10.09	8.94	9.06	8.22	7.51
Crude fiber	2.92	3.08	3.48	3.22	3.33
Ash	6.54	8.92	11.80	14.40	16.58
Alanine	2.03	2.00	2.08	2.08	2.04
Arginine	2.24	2.21	2.23	2.19	2.14
Aspartic acid	3.56	3.53	3.62	3.58	3.53
Cysteine	0.48	0.47	0.48	0.49	0.49
Glutamic acid	6.39	6.18	6.32	6.11	6.01
Glycine	1.65	1.63	1.63	1.61	1.55
Histidine	0.92	0.89	0.89	0.85	0.82
Hydroxylysine	0.04	0.06	0.08	0.09	0.06
Hydroxyproline	0.13	0.12	0.11	0.10	0.09
Isoleucine	1.68	1.64	1.70	1.68	1.6
Leucine	3.43	3.29	3.41	3.34	3.21
Lysine	2.13	2.08	2.06	1.99	1.91
Methionine	0.66	0.64	0.63	0.62	0.63
Phenylalanine	1.85	1.86	1.94	1.92	1.81
Proline	2.09	2.08	2.12	2.09	1.98
Serine	1.51	1.50	1.53	1.47	1.51
Taurine	0.18	0.17	0.16	0.14	0.13
Threonine	1.36	1.35	1.38	1.37	1.35
Tryptophan	0.42	0.39	0.39	0.39	0.37
Tyrosine	1.45	1.44	1.49	1.47	1.39
Valine	1.78	1.77	1.82	1.80	1.76

and the sum of squares for error (SSE) with different regression models.

Table 8 Proximate analysis of whole shrimp body offered varying levels of the first batch *Ulva* meal (UM1) as a replacement of fish meal over a 6-week growth trial

Diet	UM1 (%)	Moisture (%)	Crude protein (%)	Crude lipid (%)
T_1D_1	0	76.88	72.77	8.04^{a}
T_1D_2	6.35	76.29	73.63	6.12^{b}
T_1D_3	12.70	76.99	74.27	5.73^{b}
T_1D_4	19.05	76.37	72.83	5.99^{b}
T_1D_5	25.40	76.83	74.11	5.09^{b}
P value		0.7933	0.2576	0.0006
PSE[1]		0.1340	0.2240	0.1613

Values within a column with different superscripts are significantly different based on Tukey's multiple range test

[1] Pooled standard error

Results

Ingredient composition

The proximate and amino acid composition of soybean meal, fish meal, and two *Ulva* meals are presented in Table 1. Most notable is the range of protein (20.64 to 27.24%) and ash (22.18 to 46.01%) contents found in the *Ulva* meals. As UM2 contained high ash, the mineral content of the meals were determined and presented in Table 2. Most notable about this data is the high aluminum and iron contents detected in UM1. To provide data on variation of the *Ulva* meals, individual collections that were pooled to produce UM2 were analyzed and the data presented in Table 3. In general, UM2 collecting from different dates except for the one collected at 30 July 2015 shared similar protein and ash contents. UM2 sample collecting at 30 July 2015 contained the highest ash

Table 7 Performance of juvenile Pacific white shrimp (initial weight 0.26 g) offered diets with different levels of first batch *Ulva* meal (UM1) as a fish meal replacement over a 6-week growth trial

Diet	UM1 (%)	Final biomass (g)	Final mean weight (g)	WG[3] (%)	FCR[2]	Survival (%)	ANPR[4] (%)
D_1	0	44.63^{a}	5.01^{a}	1792.8^{a}	1.83^{b}	88.6	25.70^{ab}
D_2	6.35	45.45^{a}	5.09^{a}	1830.9^{a}	1.81^{b}	88.6	27.16^{a}
D_3	12.70	39.58^{ab}	4.30^{ab}	1555.1^{b}	2.15^{ab}	91.4	23.07^{ab}
D_4	19.05	36.10^{ab}	3.88^{b}	1389.1^{b}	2.36^{a}	92.9	20.20^{b}
D_5	25.40	32.26^{b}	3.87^{b}	1407.4^{b}	2.43^{a}	82.9	20.36^{b}
P value		0.0175	0.0006	0.0003	0.0039	0.2451	0.0073
PSE[1]		1.1253	0.0868	28.9568	0.0491	1.2074	0.5699

Values within a column with different superscripts are significantly different based on Tukey's multiple range test

[1] Pooled standard error

[2] FCR: feed conversion ratio = feed offered / (final weight − initial weight)

[3] WG: weight gain = (final weight − initial weight)/initial weight × 100%

[4] ANPR: apparent net protein retention = (final weight × final protein content) − (initial weight × initial protein content) × 100/protein intake

(39.2%) content, while the lowest protein level (19.4%) among the seven daily samples.

Growth trial

Performance of shrimp offered diets containing different UM1 levels is presented in Table 7. UM1 can be utilized up to 6.35% to replace 2% FM without causing growth depression. Significantly reduced final biomass, final mean weight, and ANPR as well as increased FCR were detected in shrimp fed with diets containing 19.05 and 25.4% UM1 compared to the reference diet. Weight gain in shrimp fed diets containing 12.7, 19.05, and 25.4% UM1 was significantly lower than those fed with reference diet. No significant difference was detected in the survival across the treatments (82.9 to 92.9%).

Proximate composition of whole body samples from offered diets contained different levels of UM1 is presented in Table 8. Crude lipid of whole shrimp body was significantly reduced when shrimp fed with diets contained various levels of UM1. No significances were observed in the moisture (76.29 to 76.99%) and protein (72.77 to 74.27%) contents of shrimp.

Regression analysis

Dietary UM1 levels significantly correlated with weight gain, FCR, and lipid content of whole shrimp body in the growth trial (Fig. 1a–c). There is a decreasing trend of weight gain (y) as UM1 (x) inclusion levels increased. The regression lines are described by $y = 0.1686x^3 - 6.2114x^2 + 34.409x + 1797.8$ ($R^2 = 0.492$, $P < 0.0001$). There is an increasing trend of FCR (y) as UM1 (x) inclusion levels increased. The regression line is described by $y = 0.0276x + 1.7708$ ($R^2 = 0.358$, $P = 0.0001$). Lipid content of whole shrimp body is negatively correlated with UM1 inclusion levels. The regression line is described by $y = -0.0962x + 7.4$ ($R^2 = 0.350$, $P = 0.0002$).

Digestibility trial

Apparent dry matter (ADM), apparent energy (AED), and apparent protein (APD) digestibility values for the diet (D) and ingredient (I) using 70:30 replacement technique offered to shrimp are presented in Table 9. The digestibility trial contained a range of ingredients; hence, we have provided a few other ingredients as a reference. In order to confirm the results, fecal samples for basal diets and FM diet were recollected. The results turned out to be quite similar, which indicated that the feces collection and sample analysis methods were utilized in the digestibility study are consistent. Dry matter, protein, and energy digestibility of UM1 and UM2 were significantly lower than those of FM and SBM. In terms of the two batches of *Ulva* meal, energy digestibility of UM1 was significantly higher than that of UM2. However, protein digestibility of UM1 was significantly lower than that of UM2.

Apparent amino acid digestibility values of UM1, UM2, FM, and SBM are presented in Table 10. Because of low protein digestibility, total and individual amino acid digestibility of UM1 and UM2 were also significantly lower than those of FM and SBM.

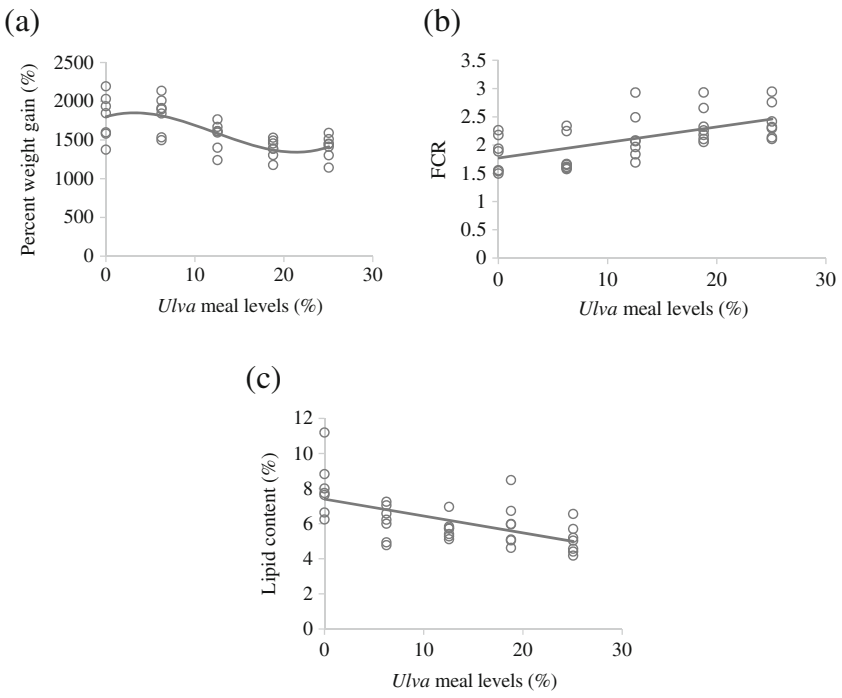

Fig. 1 **a** Relationship between weight gain (y) of shrimp and incorporation levels of *Ulva* meal 1 levels (x) in the diets. The regression line is described by $y = 0.1686x^3 - 6.2114x^2 + 34.409x + 1797.8$ ($R^2 = 0.4922$, $P < 0.0001$). **b** Relationship between FCR (y) and supplemental *Ulva* meal levels (x) in the diets. The regression line is described by $y = 0.0276x + 1.7708$ ($R^2 = 0.3582$, $P = 0.0001$). **c** Relationship between lipid content (y) of shrimp body and supplemental *Ulva* meal levels (x) in the diets. The regression line is described by $y = -0.0962x + 7.4$ ($R^2 = 0.3503$, $P = 0.0002$)

Table 9 Apparent dry matter (ADM), apparent energy (AED), and apparent protein (APD) digestibility values for the diet (D) and ingredient (I) using 70:30 replacement technique offered to Pacific white shrimp (*L. vannamei*)

Means	ADMD	AEDD	APDD	ADMI	AEDI	APDI
Basal diet 1	76.38 ± 0.37^{a}	82.65 ± 1.20^{a}	92.08 ± 0.55^{a}			
Soybean meal	77.02 ± 0.87^{a}	82.63 ± 1.05^{ab}	94.76 ± 0.49^{a}	78.51 ± 2.89^{a}	82.56 ± 3.79^{a}	97.03 ± 0.83^{a}
Fish meal 1	68.21 ± 3.80^{b}	78.31 ± 3.21^{bc}	80.86 ± 1.80^{b}	49.15 ± 2.67^{b}	69.77 ± 9.51^{a}	67.07 ± 4.02^{b}
Ulva meal 1	62.19 ± 1.26^{c}	71.96 ± 0.89^{d}	75.14 ± 1.19^{d}	29.10 ± 4.19^{c}	40.39 ± 3.52^{b}	15.17 ± 5.41^{d}
Basal diet 2	75.69 ± 0.52^{a}	81.51 ± 0.41^{ab}	92.04 ± 0.03^{a}			
Fish meal 2	67.99 ± 0.17^{b}	76.44 ± 0.78^{c}	82.34 ± 0.31^{b}	49.45 ± 0.56^{b}	65.78 ± 2.23^{a}	71.30 ± 0.68^{b}
Ulva meal 2	64.63 ± 1.08^{bc}	69.99 ± 0.64^{d}	78.33 ± 0.42^{c}	38.26 ± 3.61^{bc}	19.11 ± 3.33^{c}	43.51 ± 1.49^{c}

Ulva meal 1 and *Ulva* meal 2 represent first and second batch *Ulva* meal. Fish meal 1 and fish meal 2 represent the first and second collection of fish meal diet, respectively. Basal diet 1 and basal diet 2 represent the first and second collection of basal diet, respectively. Values are presented as mean ± standard deviation. Values within a column with different superscripts are significantly different on Tukey's multiple range test

Discussion

Seaweeds are valuable sources of protein, fiber, vitamins, macro and trace elements, as well as important bioactive compounds (Ortiz et al. 2006). The chemical composition of seaweeds can be influenced by both physical and chemical factors such as temperature, salinity, light (Lobban and Harrison 1994) or nutrient supply (Björnsäter and Wheeler 1990; Floreto et al. 1996; García-Ferris et al. 1996) during cultivation. Under nitrogen-enriched conditions, such as the effluents of fish or shrimp farms where seaweeds are used as bio-filters, the protein content of seaweeds can be enhanced (Cohen and Neori 1991; Lahaye et al. 1995; Pinchetti et al. 1998).

Interestingly, the batches that were less enriched with protein showed higher contents of useful minerals. Seaweeds contain a broad mineral composition including Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, S, Se, Si, and Zn, which has not been observed in edible terrestrial plants (Lee et al. 2014). Under different culture conditions, mineral contents of UM1 were superior to UM2. Difference in chemical composition of *Ulva* spp. is also tied to the variations in culturing periods (Fleurence 1999). Within the same batch of *Ulva* meal (Table 3), the chemical composition varied at different collection dates.

In the growth trial, UM1 can replace up to 2% fish meal in shrimp feed without causing negative effects on the growth of

Table 10 Apparent amino acids (AA) digestibility value of the soybean meal (SBM), fish meal (FM), *Ulva* meal 1 (UM1), and *Ulva* meal 2 (UM2) using 70:30 replacement technique offered to Pacific white shrimp (*L. vannamei*)

AA digestibility (%)	SBM	FM	UM1	UM2
Alanine	93.75 ± 2.02^{a}	69.09 ± 4.09^{b}	36.90 ± 6.56^{d}	50.77 ± 1.10^{c}
Arginine	96.91 ± 1.44^{a}	75.35 ± 3.78^{b}	42.20 ± 6.60^{c}	47.21 ± 0.77^{c}
Aspartic acid	95.39 ± 1.36^{a}	69.23 ± 3.70^{b}	35.87 ± 5.69^{c}	38.09 ± 1.70^{c}
Cysteine	91.29 ± 1.68^{a}	54.39 ± 7.06^{b}	13.44 ± 10.85^{c}	6.66 ± 7.45^{c}
Glutamic acid	95.69 ± 1.52^{a}	70.84 ± 3.70^{b}	33.85 ± 7.24^{c}	23.25 ± 3.17^{c}
Glycine	95.06 ± 2.05^{a}	66.55 ± 6.26^{b}	29.84 ± 8.78^{c}	34.04 ± 4.96^{c}
Histidine	94.33 ± 1.69^{a}	74.26 ± 2.86^{b}	7.10 ± 1.87^{d}	43.52 ± 0.22^{c}
Isoleucine	93.23 ± 1.72^{a}	68.72 ± 3.99^{b}	39.15 ± 5.74^{c}	46.33 ± 0.79^{c}
Leucine	92.23 ± 1.96^{a}	71.29 ± 3.16^{b}	34.65 ± 8.50^{d}	50.43 ± 0.80^{c}
Lysine	95.03 ± 1.84^{a}	76.97 ± 2.24^{b}	40.65 ± 6.50^{c}	38.07 ± 3.04^{c}
Methionine	95.20 ± 1.54^{a}	70.63 ± 3.30^{b}	44.13 ± 5.12^{c}	40.89 ± 3.18^{c}
Phenylalanine	93.41 ± 1.90^{a}	65.28 ± 4.13^{b}	27.23 ± 7.02^{d}	47.25 ± 0.76^{c}
Proline	94.68 ± 1.92^{a}	67.21 ± 5.39^{b}	15.81 ± 10.45^{c}	18.20 ± 2.42^{c}
Serine	93.11 ± 1.91^{a}	58.31 ± 4.65^{b}	10.76 ± 11.00^{c}	43.41 ± 0.82^{b}
Threonine	91.99 ± 1.94^{a}	66.33 ± 3.35^{b}	32.83 ± 6.84^{c}	42.57 ± 0.26^{c}
Tryptophan	95.37 ± 1.92^{a}	80.31 ± 1.53^{b}	65.58 ± 2.46^{c}	70.84 ± 3.26^{c}
Tyrosine	95.28 ± 1.22^{a}	73.62 ± 3.40^{b}	36.51 ± 4.10^{d}	59.02 ± 0.45^{c}
Valine	90.78 ± 2.39^{a}	67.06 ± 3.75^{b}	29.94 ± 6.89^{d}	54.20 ± 0.42^{c}
Total AA	94.31 ± 1.67^{a}	69.91 ± 3.89^{b}	29.80 ± 6.68^{d}	41.67 ± 0.51^{c}

Values are presented as mean ± standard deviation. Values within a row with different superscripts are significantly different on Tukey's multiple range test

shrimp. Growth and FCR were significantly compromised when shrimp fed with diets replacing more than 2% fish meal. Similarly, a number of studies demonstrated that low inclusion levels (≤ 5%) of seaweed meals generally did not result in growth reduction in African catfish *Clarias gariepinus* (Abdel-Warith et al. 2016; Al-Asgah et al. 2016), European sea bass (Valente et al. 2006), gilthead seabream *Sparus aurata* (Emre et al. 2013), Nile tilapia (Güroy et al. 2007; Marinho et al. 2013; Valente et al. 2016), red tilapia *Oreochromis* sp. (El-Tawil 2010), Pacific white shrimp (Rodríguez-González et al. 2014; Cárdenas et al. 2015), and rainbow trout (Soler-Vila et al. 2009; Güroy et al. 2013).

However, the results of the moderate and high inclusion levels of seaweed meals are somewhat inconsistent. Dietary supplementation of *U. lactuca* meal at both 10 and 15% as a substitution for FM significantly reduced the weight gain of Pacific white shrimp, whereas shrimp fed with diets containing similar levels of *Gracilaria parvispora* meal did not exhibit growth depression (Rodríguez-González et al. 2014). In another study, the dietary inclusion of raw *U. lactuca* meal at 10, 20, and 30% resulted in depressed growth performance in giant freshwater prawn *Macrobrachium rosenbergii*, but the fermentation of the *U. lactuca* meal before supplementation at the same levels did not result in the growth depression (Felix and Brindo 2014). A third study indicated no difference in terms of growth performance with the supplementation by 10% of raw or autoclaved *Ulva rigida* meal in the diet for rainbow trout (Güroy et al. 2013. A fourth study, on the contrary, has detected significant reductions in weight gain in rainbow trout fed with diets contained 10% *U. lactuca* and *Enteromorpha (Ulva) linza* meal (Yildirim et al. 2009). In addition, dietary inclusion of *U. lactuca* and *Gracilaria arcuata* meals at 9 and 13.5% significantly reduced the weight gain and increased FCR in African catfish (Abdel-Warith et al. 2016; Al-Asgah et al. 2016).

Variations among these researches could be attributed to the utilization of different kinds of seaweed meals and aquatic animal species as well as the over-formulation of reference diet. If a feed is designed to have an excess of nutrients or is over formulated then the nutritional value of ingredient replacement will be masked. In this experiment, diets were designed to meet the nutritional requirements of shrimp without being excessively over formulated. Protein, lipid, phosphorus, and amino acids compositions were balanced in the diets for the growth trial (Table 6). Differences in performance would be due to nutrient availability, shifts in palatability, anti-nutrients, or excesses of minerals.

The nutrient digestibility of a feed ingredient is an important factor to evaluate the overall nutritive value of the ingredient because it is related to the quantity of the nutrient absorbed by the animals. SBM had the highest APDI (97.03%), AEDI (82.56%), and AAADI (90.78–96.91%) among the ingredients tested in the current study. Similar ranges of results for APDI, AEDI, and AAADI were reported in multiple shrimp studies (Cruz-Suárez et al. 2009; Yang et al. 2009; Liu et al. 2013; Zhou et al. 2015; Fang et al. 2016). APDI and AEDI of FM1 were 67.07 and 69.77%, respectively. Similar results were acquired in FM2 (APDI and AEDI 71.3 and 65.78%, respectively). The analogous results of basal diet and FM diet from the collections at two occasions pointed to the consistency in the feces collection and sample analysis methods. Similar ranges of APDI of FM have been reported in many studies (Lemos et al. 2009; Yang et al. 2009; Terrazas-Fierro et al. 2010; Liu et al. 2013).

In the present study, APDI and AEDI of UM1 and UM2 were significantly lower than those of FM and SBM, which should be one of the factors resulting in the growth reduction in the growth trial. The low APDI of UM1 and UM2 translated to poor AAADI. Total amino acids and most individual amino acids availability in UM1 and UM2 were significantly lower than those of FM and SBM. With regard to the two batches of *Ulva* meal, UM1 exhibited significantly higher AEDI but lower APDI than those of UM2, indicating there are significant differences in nutrient availability between batches of *Ulva* meal. There are relatively few studies looking at the nutrient availability of seaweed meals in aquatic animal feeds particularly with regards to shrimp. Cárdenas et al. (2015) documented that APDI of Nutrikelp (a brown seaweed meal is comprised of mixtures of *Macrocystis*, Lessoniaceae and *Lessonia*) and Nutrigreen (a green seaweed meal contains mixtures of *Ulva*, *Caulerpa*, and *Enteromorpha*) for *L. vannamei* were 85.37 and 86.81%, respectively. Moreover, Pereira et al. (2012) reported that AEDI and APDI of four seaweeds (*Ulva* spp., *Porphyra dioica*, *Gracilaria vermiculophylla*, and *Sargassum muticum*) in rainbow trout *Oncorhynchus mykiss* were 72.7 and 75.6%, 66.8 and 79.5%, 62.4 and 87.8%, and 58 and 65.5%, respectively. In the same study, the AEDI and APDI of the identical four seaweeds in Nile tilapia *Oreochromis niloticus* were 57.1 and 63.4%, 39.6 and 58.5%, 27.8 and 51.4%, and 54.9 and 65.1%, respectively (Pereira et al. 2012). The variations in the nutrient availability results among these researches could be mainly attributed to the use of multiple seaweed species and different aquatic animals in the experiment.

To further investigate the effects of *Ulva* sp. on the body composition, four shrimps from each tank were randomly selected at the end of growth trial to be analyzed for proximate composition (Table 8). No significant differences were detected in crude protein and moisture contents of whole shrimp body. However, the lipid content of whole shrimp body was significantly reduced in shrimp fed with diets containing UM1. Similarly, lipid content of African catfish carcass was significantly reduced when fed diets that were supplemented with over 20% of *Gracilaria arcuata* meal (Al-Asgah et al. 2016. However, other authors did not report differences in

lipid content of whole body in a range of fish species (Valente et al. 2006; Güroy et al. 2007, 2013; Soler-Vila et al. 2009; Yildirim et al. 2009; Emre et al. 2013;; Marinho et al. 2013; Felix and Brindo 2014; Rodríguez-González et al. 2014; Abdel-Warith et al. 2016; Valente et al. 2016). The significantly reduced lipid content in shrimp in the current study may result from significantly lower energy availability of UM1 compared to that of FM for which it replaced.

Apparent net nutrient retention (ANPR) was determined by a number of factors including dietary protein levels, feed intake, final weight, and initial weight of animals as well as the final and initial protein content of animals (Halver and Hardy 2002). The ANPR was significantly reduced in the shrimp fed with diets containing over 12.7% UM1, indicating the supplementation of UM1 over 12.7% resulted in reduced protein deposition in the shrimp body. Similarly, a number of studies also documented the negative effects of seaweed meals on the protein retention in Nile tilapia (Marinho et al. 2013), rainbow trout (Soler-Vila et al. 2009; Yildirim et al. 2009; Güroy et al. 2013) and European sea bass (Valente et al. 2006). The feed intake was estimated by using feed offered to shrimp because the exact feed intake was not able to be measured as apparent satiation feeding was not adopted in the current study. The FCR of the diets containing 19.05 and 25.4% UM1 were 2.36 and 2.43, respectively, which were significantly higher than the estimate FCR (1.8), indicating shrimp in these two treatments may be overfed. Therefore, the results of ANPR in these two treatments may be masked.

In conclusion, under the reported conditions of this study, the *Ulva* meal can be supplemented up to 6.35% to replace 2% FM without compromising the growth of shrimp. However, growth performance of shrimp was depressed when more than 6.35% of UM1 was supplemented in the diets. The reductions in the growth performance may result from nutrient availability, shifts in palatability, anti-nutrients, or excesses of minerals. Future research regarding determination of the reasons for growth depression in shrimp and exploration of the biological response of shrimp to the inclusions of other batches *Ulva* is warranted.

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