Supplementing Green Alga *Ulva intestinalis* Linnaeus in Feed of *Litopenaeus vannamei* Boone, 1931 Reduced Growth Performance and Red Color of Cooked Shrimp

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ABSTRACT

Supplementing shrimp feed with algae has captured interest because it may benefit their growth and health, and even enhance the color of cooked shrimp. In the present study the effects of supplementing *Ulva intestinalis* in the diet of Pacific white shrimp *Litopenaeus vannamei* were investigated. Diets of 40 % protein and 10 % lipid were prepared with six different concentrations (0, 6, 12, 18, 24, and 30% [w/w]) of *U. intestinalis* powder. The experimental diets were given to satiation to stage-30 shrimp post larvae (mean body weight of 0.12±0.01 g) five times per day. After a 12-week cultivation period, the shrimp fed the 0% algae diet (control) had higher final body weight (12.27±0.19 g), daily weight gain (0.42±0.18 g·day^{-1}) and specific growth rate (8.25±2.16 %·day^{-1}) than the groups fed with *U. intestinalis*. However, the feed conversion ratio (1.80±0.63) and protein efficiency ratio of the control and the groups fed with *U. intestinalis* diets were not significantly different except for the 30% algae treatment, in which these two parameters were higher than the control. Feeding the alga to the shrimp resulted in reduction of redness (a*) ($p<0.05$) after cooking, while lightness (L*) and yellowness (b*) were unaffected in all but one case (b* was significantly reduced in the 30% alga treatment). The shrimp fed 12-24% algae had significantly higher Ca, P and Mg in cooked shrimp than those of the other groups. We conclude that supplementation of *U. intestinalis* powder in the shrimp diet reduced their growth, while the cooked shrimp had reduced redness

Keywords: Cooked shrimp color, Dietary inclusion, *Litopenaeus vannamei*, Pacific white shrimp, *Ulva intestinalis*

INTRODUCTION

The Pacific white shrimp *Litopenaeus vannamei* is widely cultured and economically important worldwide, particularly in Asia (Jin *et al*., 2018). With global production of 5 million metric tonnes in 2018, *L. vannamei* ranked as the second highest species for aquaculture production (FAO, 2018). The shrimp has fast growth, high salinity tolerance, and resilience during high-density cultivation (Alfiansah *et al*., 2018). Diets for cultured shrimp have been developed to optimize levels of protein, minerals, lipids, sterols, carbohydrates and vitamins at several growth stages. Commercially manufactured feed contains varying levels protein from animal and plant sources, including fish meal, poultry by-product meal, meat and bone meal, and soybean meal (Amaya *et al*., 2007). Although the nutritional quality of the feed is of primary importance in the shrimp diet, a growing trend towards "green water" systems attempts to minimize ingredients of animal origin. Use of environmentally friendly practices for shrimp culture and development of diets to reduce feed costs are top priorities for the shrimp industry (Qiu and Davis, 2017).

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Ulva intestinalis is a green alga with worldwide distribution (Hayden *et al*., 2003). The seaweed can grow to nuisance levels in extensive shrimp culture and causes the phenomenon of green tide in some areas. Culture techniques and utilization of this species have also been reported. The alga has been used as supplementary feed for fish, shrimp, and other animals as a source of protein, carotenoids, minerals, polysaccharides, and vitamins (Kumar *et al*., 2011; Pena-Rodriguez *et al*., 2011; Syad and Kasi, 2013). *Ulva intestinalis* contains 98 % digestible protein, 2.0-3.6 % fat, and 32-36 % ash (Lin *et al*., 2008). This alga also contains essential fatty acids, with n-3 and n-6 fatty acids accounting for 10.4 and 10.9 $g \cdot 100\ g^{-1}$ of total fatty acid, respectively, but it is low in antioxidants (Aguilera-Morales *et al*., 2005). Furthermore, algae in the *Ulva* genera contain of 15.6-30.9% chlorophyll-*a*, 12.2-14.9% chlorophyll-*b*, and 11.4-29.7% β-carotene (Aguilera-Morales *et al*., 2005). These data suggest that the alga could be used as an alternative ingredient in diets for herbivorous animals. In addition, a previous study indicated that chlorophyll, carotenoid, and phenolic compounds extracted from *Ulva lactuca* have antioxidant and antibacterial properties (El-Baky *et al*., 2008).

Several authors have reported on *Ulva* in shrimp diets, and those studies were mostly carried out on the juvenile stages. For example, the digestibility in the diet as a whole of juvenile Pacific white shrimp reached 82.19 % when adding 15% *Ulva* meal (Anaya-Rosas *et al*., 2017). Santizo *et al*. (2014) examined bioavailability of 30% raw *Ulva* powder and *Ulva* protein concentrate extract in test diets of juvenile black tiger prawn *Penaeus monodon* using *in vivo* assays. Dry matter was 98.76 % in the test diet containing *Ulva* protein concentrate and 71.53 % for raw *Ulva* powder, while apparent digestibility of ingredients was 99.13 % for the concentrate and 71.17 % for raw *Ulva* powder. Additionally, Qiu *et al*. (2018) showed that *Ulva* meal can be supplemented up to 6.35 % to replace 2% fish meal without compromising the growth of juvenile *L. vannamei*, and that adding *Ulva clathrata* to the feed of juveniles could cause the shrimp to turn reddish when cooked (Cruz-Suarez *et al*., 2009).

However, no published studies have examined using algal supplements at the post-larval stage of *L. vannamei*. In a study of *P. monodon*, Serrano and Tumbokon (2015) found the optimum inclusion of *U. intestinalis* to be 13.9 to 14.7 % in the post-larval diet, and they showed that up to 21.0 % can be incorporated into the diet without deleterious effects on growth and feed efficiency. The replacement of *U. intestinalis* in post larvae *L. vannamei* diets should be similarly investigated. Our goal is to identify and develop new sources of raw materials for shrimp feed to increase the qualitative and quantitative yield of Pacific white shrimp. This research aims to define the optimal amount of *U. intestinalis* supplementation to the diet of *L. vannamei* to promote growth and appearance of this species.

MATERIALS AND METHODS

Source of Ulva intestinalis and preparation of diets

Ulva intestinalis was collected from Pattani Bay, located in Pattani Province, Thailand. The alga was cleaned using washing machine (Washing machine model Panasonic-NA-W17XG1BRC) with the algae (kg) to water (liters) ratio of 1:10 for 15 min or until sediment and other attached materials were completely removed. The alga was washed three times, spun (with spin cycle), and then dried under sunlight. Finally, the alga was ground into powder with an herb grinder (Grinder 1000-1200G WF-10 model) and stored at 4 °C for proximate analysis (AOAC, 1995). Six experimental diets based on Tamtin *et al*. (2016) were prepared, containing different proportions of the powdered *U. intestinalis*: 0 (control diet), 6, 12, 18, 24, and 30%. Proximate composition of each experimental diet formula was set to 40 % protein and 10 % lipid. As shown in Table 1, the experimental diets consisted of fish meal, squid meal, soybean meal, shrimp head meal, wheat flour, rice bran, fish oil, vitamin mix, mineral mix, and varying concentrations of *U. intestinalis* powder. All powdered ingredients were sieved using a 200 μm sieve, and the ingredients were thoroughly mixed. Fish oil was added, then the mixture was ground twice with a meat grinder

Table 1. Ingredient quantities and proximate composition of experimental shrimp diets.

Ingredients (g·100 g^{-1})	Percentage of *Ulva intestinalis*					
	0	6	12	18	24	30
Fish meal	35.00	35.00	35.00	35.00	35.00	35.00
Ulva intestinalis	0.00	6.00	12.00	18.00	24.00	30.00
Squid meal	11.00	11.00	11.00	11.00	11.00	11.00
Soybean meal	12.00	12.00	12.00	12.00	12.00	12.00
Shrimp head	5.00	5.00	5.00	5.00	3.00	3.00
Wheat flour	29.00	23.00	17.00	11.00	7.00	1.00
Rice bran	1.00	1.00	1.00	1.00	1.00	1.00
Fish oil	6.00	6.00	6.00	6.00	6.00	6.00
Vitamin mix[a]	0.50	0.50	0.50	0.50	0.50	0.50
Mineral mix[b]	0.50	0.50	0.50	0.50	0.50	0.50
	Proximate composition (%, dry weight)					
Crude protein	40.94±0.61^a	41.05±0.56^a	41.15±0.46^a	41.18±0.81^a	40.97±0.19^a	41.55±0.58^a
Crude fat	10.38±0.23^a	9.71±0.25^a	9.83±0.54^a	10.17±0.16^a	10.25±0.27^a	9.78±0.12^a
Crude fiber	2.63±0.29^a	2.94±0.16^a	2.14±1.86^a	2.30±1.99^a	2.35±2.04^a	3.71±0.11^a
Moisture	1.76±0.05^a	1.65±0.02^a	2.09±0.14^b	1.93±0.11^b	1.95±0.09^b	1.75±0.10^a
Ash	12.68±0.04^a	14.00±0.01^b	15.36±0.15^c	17.12±0.11^d	18.16±0.04^f	19.42±0.07^e

Note: Values are the mean±SD of three replications; Means within each row superscripted with different letters are significantly ($p<0.05$) different; [a]Vitamin mix (Immu Vite) (mg·kg^{-1} premix): 500,000 mg·kg^{-1} Vitamin A, 100,000 mg·kg^{-1} Vitamin D3, 1,000 mg·kg^{-1} Vitamin E, 3,000 mg·kg^{-1} Vitamin C, 1,000 mg·kg^{-1} Vitamin B1, 200 mg·kg^{-1} Vitamin B2, 600 mg·kg^{-1} Vitamin B6, 800 mg·kg^{-1} Pantothenic acid, 0.06 mg·kg^{-1} Vitamin B12, 200 mg·kg^{-1} Nicotinic acid, 50 mg·kg^{-1} Folic acid, 500 mg·kg^{-1} Inositol, 10,000 mg·kg^{-1} Methionine, 18,000 mg·kg^{-1} Glycine, 3,000 mg·kg^{-1} Lysine, 5,000 mg·kg^{-1} Glutamic acid, 50 mg·kg^{-1} Astaxanthin; [b]Mineral mix (ORC-Cal) (mg·kg^{-1} premix): 20,000 mg·kg^{-1} Ca, 10,625 mg·kg^{-1} P, 500 mg·kg^{-1} Cu, 1,700 mg·kg^{-1} F, 200 mg·kg^{-1} Mn, 900 mg·kg^{-1} Zn, 250 mg·kg^{-1} K, 100 mg·kg^{-1} Mg, 10 mg·kg^{-1} Co, 20 mg·kg^{-1} Se, 19,980 mg·kg^{-1} Na, 400 mg·kg^{-1} I, 32,000 mg·kg^{-1} Vitamin C

to obtain 3-mm food pellets. The diet was dried in an oven for 8-12 h at 60 °C until the moisture reached 1.85 %, and then stored in a refrigerator at 4 °C prior to feeding. Samples of prepared food pellets from each formulation were subjected to proximate analysis.

Proximate analysis of shrimp diets and mineral analysis

The six diets were dried at 80 °C before determining the proximate composition according to the standard methods of analysis (AOAC, 1995). The moisture content was determined by oven treatment at 105 °C until a constant weight was obtained. Crude protein content was analyzed by the Kjeldahl method with a conversion factor of 6.25 to convert total nitrogen into crude protein. Ash content was acquired by heating the sample overnight in a furnace at 525 °C, and the content was determined gravimetrically. Crude lipid was extracted by using petroleum ether at 160 °C and its content was measured gravimetrically after oven-drying (80 °C) the extract overnight. Crude fiber was extracted using 1.25% of H_2SO_4. Samples for mineral analysis were subjected to acid digestion and analyzed through inductively coupled plasma optical emission spectrometry (ICP-OES) (AOAC, 2000).

Experimental setting and feeding trial

Post-larvae 15th stage (PL15) of Pacific white shrimp *Litopenaeus vannamei* were obtained from Tabon Shrimp Farm in Ranot district, Songkhla Province. The shrimp larvae were maintained in a tank for one day, then packed in plastic bags and transferred to the laboratory of Fishery Science and Technology Division, Faculty of Science and Technology, Prince of Songkla University, Pattani Campus. At the laboratory, the shrimp larvae were acclimated in a tank prepared with 15 psu water and fed with the control diet (0% *Ulva*). Shrimp feeding was done by hand five times a day (6.00 am, 10.00 am, 02.00 pm, 06.00 pm and 10.00 pm).

The experiment was started when the shrimp larvae reached post-larvae 30 stage (0.12± 0.01 g mean body weight). The experiment was conducted in triplicate, in 50-L (60×30×30 cm) glass aquaria following a completely randomized design. The post larvae 30 were stocked at the density of one individual per liter (50 individuals per aquarium). Satiate feeding was done by hand five times a day as previously described, and sludge was removed by suction before every feeding. The diet assigned to each aquarium was weighed separately before and after feeding. Water exchange of 50-70 % was done weekly. Bulk weight and density of the shrimp were measured every two weeks until the end of the experiment, at week 12. Water quality parameters were maintained in the following ranges: salinity of 15-17 psu, temperature of 27-28 °C, dissolved oxygen of 5-9 mg·µL^{-1}, pH of 7.2-8.6, alkalinity of 60-70 mg·µL^{-1}, and total ammonia <1 mg-N·L^{-1}.

Proximate analysis and color measurement of cooked shrimp

Proximate analysis of the shrimp was done after 12 weeks of cultivation. The whole shrimp from each treatment were harvested and then boiled in distilled water for 5 min before being placed in iced water until the temperature decreased. Colorimetry measurement was conducted on the third abdominal segment (both sides) with a colorimeter (HunterLab MiniScan EZ 4500) in accordance with the CIE L*a*b* (CIELAB) system (Choubert and Heirich, 1993). The remaining boiled shrimp was dried in hot air oven at 70 °C until constant weight, then kept in plastic bags for proximate and mineral analysis using the same method as described above.

Growth parameter calculations

Weight gain, specific growth rate, food conversion ratio, protein efficiency ratio and survival rate of the experimental shrimp were calculated according to the formulae below:

1. Average daily gain, ADG (g·day^{-1}) = FBW-IBW/D

2. Specific growth rate, SGR (% daily) = (ln FBW-ln IBW)/D×100 (Brett, 1979)

3. Feed consumption (g-individual) = $\sum_{n=1}^{14}$ (consumption on the nth day/number of shrimp on the nth day) (Cruz-Suarez *et al*., 2009)

4. Food conversion ratio, FCR = feed consumed (g)/wet weight gain (g) (Dupree and Sneed, 1966)

5. Protein efficiency ratio, PER = weight gain (g)/protein fed (g) (Zeitoun *et al*., 1973)

6. Survival rate, SR (%) = 100×(final number of shrimp/initial number of shrimp)

Note: FBW = Final body weight
IBW = Initial body weight
D = culture period in days

Data analysis

One-way analysis of variance (ANOVA) was used to verify effects of the treatments. When ANOVA results showed significant differences, Duncan's multiple range test was performed to rank the means of the responses at alpha = 0.05 (Steel and Torrie, 1980). All statistical analysis was performed using SPSS Statistic Bass 17.0 for Windows EDU S/N 5065845 (SPSS Inc, Chicago, USA).

RESULTS

Nutrient composition in Ulva intestinalis and the experimental shrimp diets

The algae used in the present study was mature, mostly 12-37 cm long, 2.0-4.5 mm wide, and light green in color. The *Ulva intestinalis* in the experimental diets contained 19.13±0.25 % protein, 0.57±0.03 % lipid, 3.76±0.02 % fiber, 9.72±0.04 % moisture, and 23.58±0.05 % ash based on dry weight. The alga also contained the mineral elements P, Zn, Fe, Cr, Mg, Ca, Cu, Na, K, Pb, and Cd at concentrations of 2,736±1,472.43, 15.60±2.60,2,006±240.26, 8.03±0.53, 12,615±1,061.30, 22,955±1,181.06, 7.73±3.02, 2,739±169.93, 9,303.33±119.19, 3.00±0.19 and 0.43±0.08 $mg \cdot kg^{-1}$ dry weight, respectively (Table 2). Most minerals (except P, Cu, Pb and Cd) including Zn, Fe, Cr, Mg, Ca, Na, and K increased with the proportion of alga in the diet with some exception; however, Pb was not found in the experimental diet despite its presence in the alga.

Growth performance of shrimp

The maximum final bodyweight of the shrimp (12.27±0.19 g) was found for the control diet (0% algae), which was significantly higher ($p<0.01$) than the other treatments (Table 3). Growth with the control diet, as shown by average daily gain (ADG) and specific growth rate (SGR), was significantly higher than for the other diets. These values tended to decrease with an increased proportion of algae, although there were no significant differences among the treatments containing algae. In addition, FCR and PER of the shrimp fed with 0% algae (FCR = 1.80±0.63; PER = 1.85±0.88) tended to be lower than in the algae treatments, but without statistical support ($p>0.05$); one exception was for the 30% algae group (FCR = 4.33±2.64; PER = 1.06±0.81), whereby the control group showed significantly better FCR and PER ($p<0.05$). Survival rates (SR) of the experimental shrimp ranged between 70.58±22.93 and 81.86±15.66 %, and there were no significant differences among treatments ($p>0.05$).

Proximate composition and color of shrimp after cooking

Table 4 shows the proximate composition of the whole shrimp body after the 12-week feeding trial with the experimental diets. Protein, moisture, and ash contents were significantly different among treatments (ranges were 65.68-69.70 % protein;

Table 2. Mineral concentrations in experimental shrimp diets.

Minerals ($mg \cdot kg^{-1}$ dry weight)	Percentage of *Ulva intestinalis* in diet					
	0	6	12	18	24	30
P	7,311±6331.85[a]	7,351±6368.65[a]	11,428±1830.25[a]	13,615±1396.61[a]	14,087±4830.19[a]	14,520±736.67[a]
Zn	41.57±2.94[a]	43.47±1.72[a]	51.37±5.81[b]	56.00±1.00[bc]	56.67±1.53[bc]	59.00±4.00[c]
Fe	241±52.55[a]	413±46.20[b]	687±36.70[c]	994±70.84[d]	1,482±166.85[e]	1,490±70.46[e]
Cr	2.77±0.35[a]	3.37±0.61[a]	4.47±0.55[b]	5.70±0.52[c]	5.70±0.10[c]	6.70±0.20[d]
Mg	1,405±186.32[a]	1,821±156.69[a]	2,627±293.05[b]	3,853±536.00[c]	4,604±442.53[d]	6,615±635.35[e]
Ca	22,374±1383.56[ab]	19,924±1145.36[a]	23,634±2846.49[bc]	27,202±3327.04[bc]	29,283±4736.48[cd]	33,973±4160.16[d]
Cu	12.27±1.81[a]	13.53±2.10[a]	16.70±3.65[a]	16.43±0.87[a]	13.43±0.71[a]	22.90±13.89[a]
Na	5,429±593.43[b]	4,427±122.14[a]	5,517±447.26[b]	6,561±487.71[c]	6,460±874.63[c]	7,676±252.20[d]
K	3,054±436.82[a]	2,578±823.69[a]	4,149±400.56[b]	4,437±122.87[b]	5,848±294.00[c]	7,716±216.68[d]
Pb	ND	ND	ND	ND	ND	ND
Cd	0.53±0.12[a]	0.67±0.21[a]	0.60±0.56[a]	0.60±0.56[a]	0.67±0.58[a]	0.67±0.58[a]

Note: ND = not detected; LOD = 0.5 $mg \cdot kg^{-1}$ dry weight; values are mean±SD of three replications; means within each row superscripted with different lowercase letters are significantly ($p<0.05$) different.

10.36-7.29 % moisture, and 12.87-10.89 % ash). However, the variation was not clearly related to the level of algae in the feed. For example, protein content tended to increase as the percentage of algae in the diet increased, but the shrimp fed with the highest percentage of algae did not differ from the control group (0% algae). Moisture and ash contents fluctuated without any noticeable trends.

Mineral content in shrimp

Figure 1 shows mineral contents in whole shrimp at the end of the feeding trial. Of the eight minerals measured, concentrations of Zn, Cr, Na, and K were not significantly different among treatments (p>0.05). Among the remaining minerals, a marked difference was seen only for Fe, whereby feeding with algae appeared to reduce Fe in shrimp tissue compared to the control; however, there was no difference between the control and the group fed with 18% algae. The concentration of Ca did not differ between the control and shrimp fed 6% algae or 30% algae, but it was significantly higher in the groups fed 12-24% algae than the control. P, Cu, and Mg showed slight variation among the treatment groups but without clear patterns.

Table 3. Growth performance parameters of Pacific white shrimp fed with different percentages of *Ulva intestinalis* during a 12-week feeding trial.

Parameters	**Percentage of *Ulva intestinalis* in diet**					
	0	**6**	**12**	**18**	**24**	**30**
Initial body weight (g)	$0.12{\pm}0.00^{a}$	$0.12{\pm}0.00^{a}$	$0.12{\pm}0.00^{a}$	$0.12{\pm}0.02^{a}$	$0.12{\pm}0.01^{a}$	$0.12{\pm}0.01^{a}$
Final body weight (g)	$12.27{\pm}0.19^{c}$	$8.46{\pm}2.18^{b}$	$6.11{\pm}0.70^{a}$	$7.38{\pm}0.77^{ab}$	$6.23{\pm}1.09^{ab}$	$6.29{\pm}0.69^{ab}$
ADG (g·day^{-1})	$0.42{\pm}0.18^{b}$	$0.31{\pm}0.19^{a}$	$0.25{\pm}0.14^{a}$	$0.20{\pm}0.19^{a}$	$0.21{\pm}0.16^{a}$	$0.15{\pm}0.25^{a}$
SGR (%·day^{-1})	$8.25{\pm}2.16^{b}$	$7.04{\pm}1.74^{a}$	$6.52{\pm}1.46^{a}$	$6.78{\pm}1.53^{a}$	$6.55{\pm}1.66^{a}$	$6.40{\pm}1.30^{a}$
FC (g-individual)	$3.72{\pm}2.38^{a}$	$2.87{\pm}1.81^{a}$	$2.78{\pm}1.62^{a}$	$3.14{\pm}1.87^{a}$	$3.45{\pm}2.35^{a}$	$5.60{\pm}4.32^{b}$
FCR	$1.80{\pm}0.63^{b}$	$2.12{\pm}0.92^{b}$	$2.91{\pm}1.94^{ab}$	$2.78{\pm}1.10^{ab}$	$3.04{\pm}1.85^{ab}$	$4.33{\pm}2.64^{a}$
PER	$1.85{\pm}0.88^{b}$	$1.58{\pm}0.87^{ab}$	$1.40{\pm}0.92^{ab}$	$1.31{\pm}0.78^{ab}$	$1.48{\pm}1.24^{ab}$	$1.06{\pm}0.81^{a}$
SR (%)	$76.31{\pm}16.32^{a}$	$81.86{\pm}15.66^{a}$	$79.46{\pm}15.28^{a}$	$75.95{\pm}12.73^{a}$	$79.34{\pm}20.51^{a}$	$70.58{\pm}22.93^{a}$

Note: ADG: average daily gain; SGR: specific growth rate; FC: feed consumption; FCR: food conversion ratio; SR: survival rate; PER: protein efficiency ratio; Values are mean±SD of three replications; Means within each row superscripted with different lowercase letters are significantly (p<0.05) different.

Table 4. Proximate composition of whole Pacific white shrimp fed diets with different percentages of *Ulva intestinalis.*

Proximate composition	**Percentage of *Ulva intestinalis* in diet**					
(%, dry weight)	**0**	**6**	**12**	**18**	**24**	**30**
Protein	$69.01{\pm}0.65^{bc}$	$65.68{\pm}1.60^{a}$	$66.57{\pm}2.65^{ab}$	$69.70{\pm}0.85^{c}$	$68.84{\pm}1.16^{bc}$	$69.12{\pm}1.17^{bc}$
Lipid	$3.20{\pm}0.29^{a}$	$2.81{\pm}0.09^{a}$	$3.06{\pm}0.75^{a}$	$2.83{\pm}0.10^{a}$	$3.09{\pm}0.43^{a}$	$3.15{\pm}0.22^{a}$
Fiber	$6.80{\pm}0.31^{a}$	$6.53{\pm}0.22^{a}$	$6.62{\pm}0.19^{a}$	$6.76{\pm}0.11^{a}$	$6.80{\pm}0.07^{a}$	$6.71{\pm}0.10^{a}$
Moisture	$9.72{\pm}0.32^{d}$	$10.36{\pm}0.36^{e}$	$7.77{\pm}0.07^{ab}$	$8.86{\pm}0.29^{c}$	$8.17{\pm}0.11^{b}$	$7.29{\pm}0.39^{a}$
Ash	$11.47{\pm}0.04^{b}$	$12.03{\pm}0.03^{c}$	$12.87{\pm}0.09^{f}$	$12.25{\pm}0.19^{d}$	$12.58{\pm}0.03^{e}$	$10.89{\pm}0.12^{a}$

Note: Values are mean±SD of three replications; means within each row superscripted with different lowercase letters are significantly (p<0.05) different.

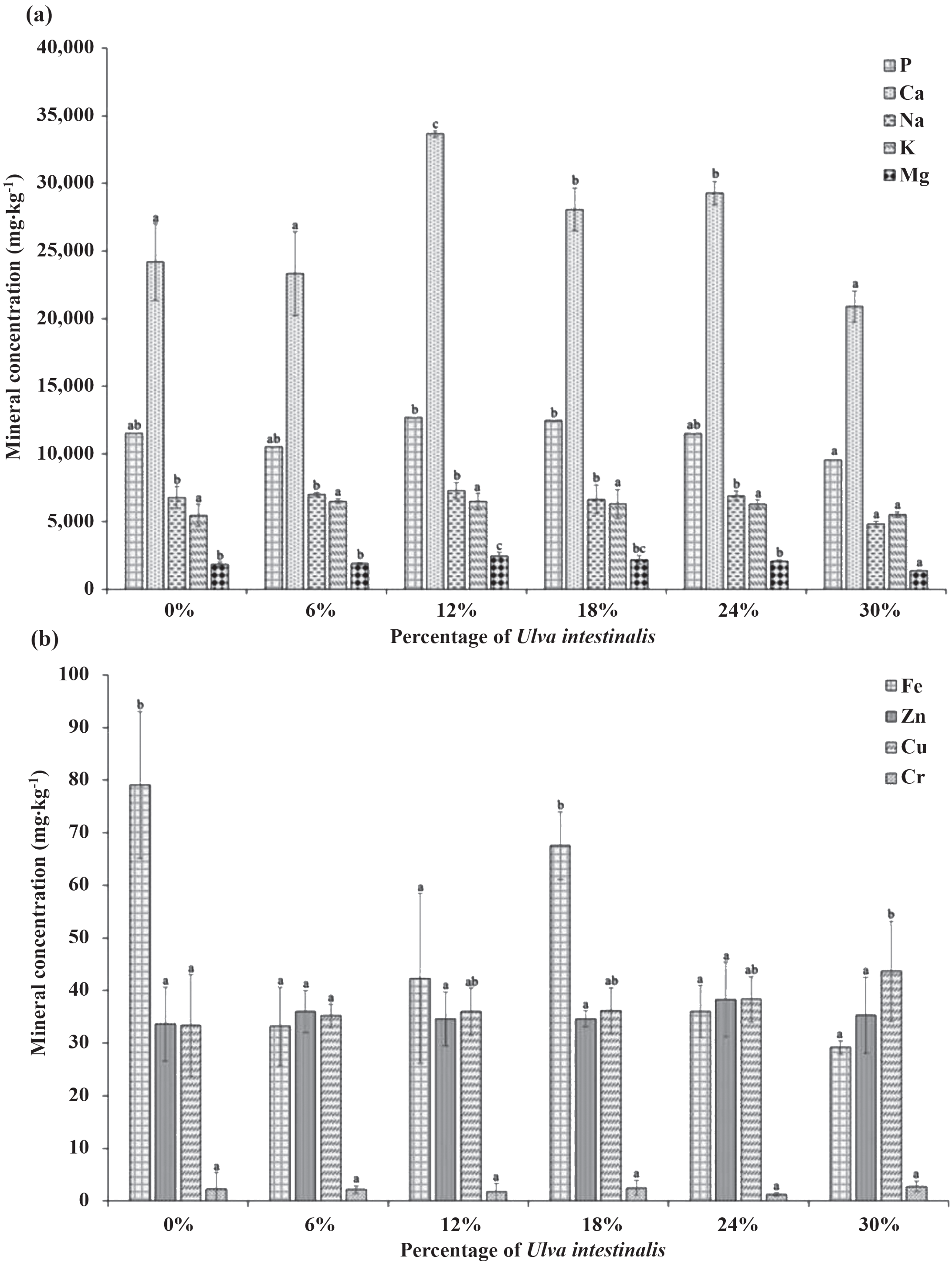

Figure 1. Mineral content in whole shrimp *Litopenaeus vannamei* fed diets with different percentages of *Ulva intestinalis*. Different letters above bars indicate significant ($p<0.05$) difference between treatments. Error bars represent SD.

Color of cooked shrimp

The color of the cooked shrimp shell was significantly different ($p<0.05$) among treatments in redness (a*) and yellowness (b*) values, but not in lightness (L*) ($p>0.05$) (Table 5 and Figure 2). Interestingly, the shrimp fed the control diet had the highest a* (redness) value for the shell (16.00±3.54), whereas the yellowness was more similar to the groups that received algae-supplemented diets.

Table 5. Color measurements of cooked shrimp *Litopenaeus vannamei* fed diets with different percentages of *Ulva intestinalis*.

Color value	**Percentage of *Ulva intestinalis* in diet**					
	0	**6**	**12**	**18**	**24**	**30**
L*	70.80±1.30^{a}	70.60±3.13^{a}	69.80±0.84^{a}	69.40±1.52^{a}	67.40±4.04^{a}	70.60±2.61^{a}
a*	16.00±3.54^{c}	11.00±2.35^{b}	7.80±2.39^{ab}	7.80±0.84^{ab}	9.60±3.13^{b}	4.80±1.10^{a}
b*	17.00±5.24^{ab}	19.40±2.41^{b}	16.80±2.39^{ab}	16.40±0.89^{ab}	17.60±0.55^{ab}	15.20±1.64^{a}

Note: Values are mean±SD of three replications; means within each row superscripted with different lowercase letters are significantly ($p<0.05$) different.

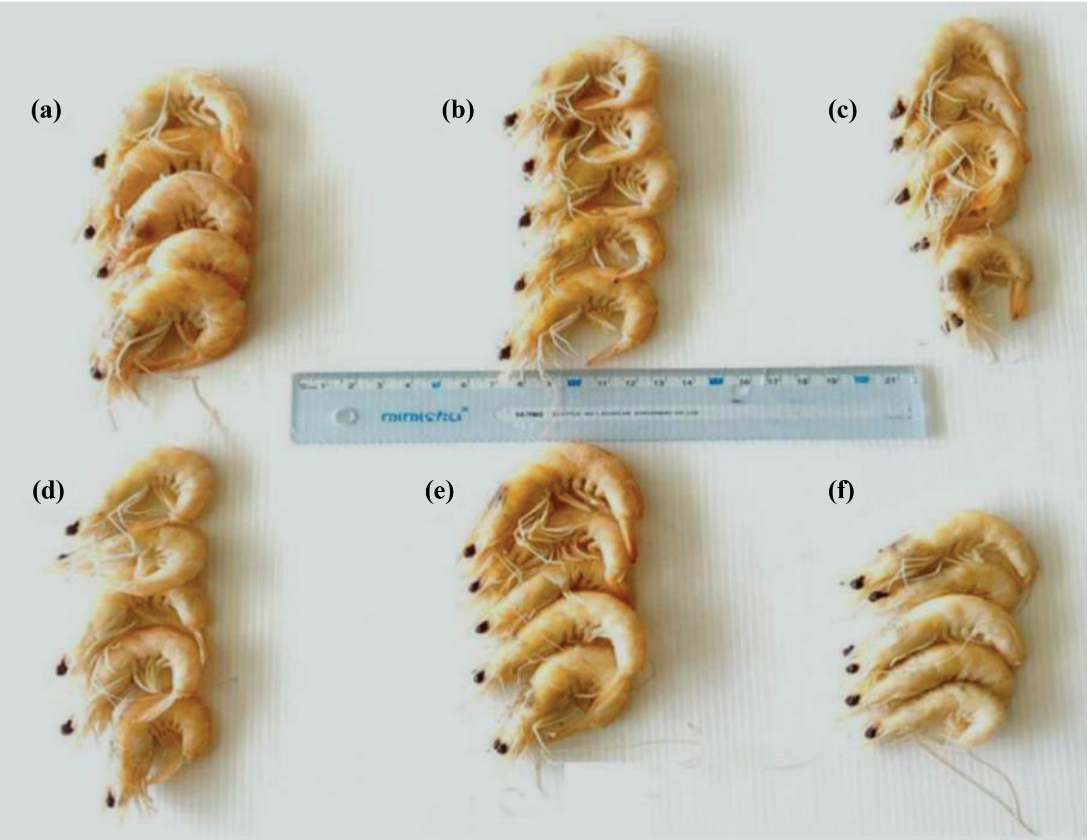

Figure 2. Color of cooked shrimp *Litopenaeus vannamei* fed diets with different percentages of *Ulva intestinalis*: (a) 0%; (b) 6%; (c) 12%; (d) 18%; (e) 24%; (f) 30%

DISCUSSION

In the present study, Pacific white shrimp (PL30) were fed diets supplemented with 0-30% *Ulva intestinalis* for 12 weeks. The results revealed that shrimp fed with *Ulva* diets performed worse in terms of growth and feed utilization than the control (0% *Ulva*). Our findings contradict many previous reports on *Litopenaeus vannamei* (Rodriguez-Gonzalez *et al*., 2014; Tamtin *et al*., 2016; Qiu *et al*., 2018) and other penaeid shrimps (Serrano and Tumbokon, 2015). For example, Qiu *et al*. (2018) reported that up to 8% *Ulva* sp. could be used as a substitute to commercial fish meal in *L vannamei* feed. Rodriguez-Gonzalez *et al*. (2014) included 5-15% *Ulva lactuca* in diets of juvenile *L. vannamei* and found that the highest growth was for shrimp fed 5% *U. lactuca*, although this was not different from shrimp fed a diet without algae ($p>0.05$). Similarly, Cardenas *et al*. (2015) reported that mixing 4% green seaweed meal from *Ulva* spp. in the diet of juvenile *L. vannamei* produced the best SGR, but without significant difference from 0% and 8% seaweed treatments. However, Cruz-Suarez *et al*. (2009) concluded that 3.3% meal of the green macroalga *Ulva clathrata* provided better performance (FC, FCR, weight gain, PER) in juvenile Pacific white shrimp when compared with those fed with brown seaweed meals of *Macrocystis pyrifera* and *Ascophyllum nudosum*.

Our findings are similar to those of Felix and Brindo (2014), who found that 10, 20, and 30% *Ulva lactuca* in diets depressed growth performance in giant freshwater prawn *Macrobrachium rosenbergii*. The growth reduction of *L. vannamei* in the present study was likely due to the presence of a wide variety of anti-nutritional substances such as saponins, tannins and phytic acid, which are commonly found in plant-derived feed materials (Guillaume and Choubert, 2001). Although information is lacking on such substances in *U. intestinalis*, Azaza *et al*. (2008) reported that *Ulva rigida* meal contained antinutrients such as 1.13% saponins, 0.61% tannins, and 0.47% phytic acid. Moreover, Oliveira *et al*. (2009) raised a concern that antinutrients and/or toxic compounds such as trypsin and α-amylase inhibitors, polyphenol compounds (tannins), lectins, phytic acid, and toxic contaminants (heavy metals) in seaweed may result in the reduction of nutrient availability of feed mixed with seaweed.

Further evidence of the adverse effects of feeding *U. intestinalis* to shrimp was seen in FCR, which was higher for shrimp fed algae than for the control. This might be explained by the presence of antinutritional substances in the algae. Notably, growth rates of shrimp in the present study were higher than the aforementioned reports (e.g., Rodriguez-Gonzalez *et al*., 2014; Qiu *et al*., 2018), and this might be due to different stages of shrimp used in the experiments. The present study used post larvae-30, while those experiments used juveniles.

Increasing *Ulva* meal in the experimental diets of the present study increased the concentration of some minerals in shrimp, but at the expense of reduced growth performance. However, Hua *et al*. (2019) mentioned that macroalgae were still suitable when incorporated as a functional feed ingredient at low levels, and that the substance ulvan contained in the algae indirectly boosts protein intake. In contrast, it was found that adding 13.9-14.7% *U. intestinalis* was optimal in the diet of *P. monodon* in the post larva stage for providing enhanced growth (Serrano and Tumbokon, 2015). The increased levels of minerals in diets supplemented with increasing levels of *U. intestinalis* in the present study did not elevate mineral concentrations in the shrimp, despite similar feed consumption/individual across the treatments (except for the 30% algae group). It should be noted that even though the levels of minerals in shrimp did not show apparent relationships with growth parameters, mineral ratios might impact growth performance of shrimp. Optimal ratios have been suggested for some minerals in shrimp feed, e.g., Ca:P ratio of 1:1 (Bautista and Baticados, 1989) and Mg:Ca ratio of 3:1 (NRC, 2011).

Although growth of post-larval shrimp was reduced by feeding with *U. intestinalis*, it did not affect their survival rate. During the experiment, shrimp appeared active and healthy based on external features, and without pathogens. Water quality was maintained in favorable ranges for shrimp, especially ammonium nitrogen, which was less than 1.0 mg·L^{-1}. Appelbaum *et al*. (2002) stated that water free of pollutants and marine pathogens is highly suitable for the culture of a number of species of aquatic organisms. Yang *et al*. (2018) found good survival for juvenile Pacific white shrimp that were fed a diet supplemented with macroalgae (*Saccharina japonica, Porphyra dioica, Gracilariopsis lemaneiformis, Ulva lactuca,* and *Undaria pinnatifida*). Similarly, survival rate was not affected when feeding black tiger shrimp (*P. monodon*) with *U. intestinalis* (Serrano and Tumbokon, 2015).

Shrimp products must have a desirable appearance for consumers in the market. The color of cooked shrimp is an important attribute in the food and bioprocessing industries, and producers should be aware of consumers' preferences (Pathare *et al*., 2013). However, in the present study, feeding shrimp with *U. intestinalis* resulted in a reduction of redness. In contrast, Cruz-Suarez *et al*. (2009) reported that shrimp fed with a diet containing 3.3% *Ulva clathrata* showed red color more than shrimps fed with other diets after cooking.

CONCLUSION

Growth performance of Pacific white shrimp decreased when supplementing their feed with 6-30% powdered *Ulva intestinalis*. The redness level of the cooked shrimp also decreased, which might affect its marketability. However, adding the algae to the diet provided a favorable balance of major minerals including Ca, P and Mg.

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