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Research Article

Antioxidant activity and apparent digestibility of amino acids of three macroalgae meals in the diets of Pacific white shrimp (*Litopenaeus vannamei*)

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ABSTRACT. The antioxidant activity of meals, growth performance, dry matter, crude protein and amino acid apparent digestibility coefficients (ADC) were determined for three macroalgae in Pacific white shrimp juveniles: *Gracilaria vermiculophylla*, *Dictyota dichotoma* and *Ulva lactuca*. For the digestibility determination, test diets included 15% of the test ingredients and 85% of a control diet supplemented with 1% chromic oxide. The amino acid content in the ingredients, diets and feces were analyzed using HPLC. In general, nutrient digestibility values were far higher in the diets with macroalgae meals than in the control diet. In conclusion, diets with the macroalgae *U. lactuca* and *G. vermiculophylla* in particular showed high antioxidant activity, high amino acid digestibility and improved *Litopenaeus vannamei* growth.

Keywords: shrimp, macroalgae, digestibility, amino acid, antioxidant.

INTRODUCTION

Macroalgae represent a source of natural bioactive compounds (gallic acid, catechin, epicatechin and others) (López *et al*., 2011). It has been discovered that the antioxidant capacity of various species of brown algae, which are associated with high levels of phenolic compounds, contributes to the ability to neutralize the effects of oxidative stress that is associated with ageing in living organisms (Cano-Mallo, 2008).

Macroalgae have been considered a source of nutrients in the diet of aquatic organisms. Some studies have reported the growth levels of shrimp fed with diets containing macroalgae meal. Rodríguez-González *et al*. (2014) evaluated *Ulva lactuca* and *Gracilaria parvispora* meals, and they concluded that both macroalgae may be used as a source of protein in balanced diets for shrimp. However, except for a study that evaluated the crude nutrient digestibility of the macroalgae *Macrocystis pyrifera* and *Sargassum* sp. in diets for *Litopenaeus vannamei* (Gutiérrez-Leyva, 2006), no work has been carried out specifically to evaluate the amino acid digestibility of macroalgae in shrimp diets.

The genus of macroalgae most commonly studied in the field of aquaculture nutrition, effluent bioremediation and shrimp immunostimulation are *Gracilaria* sp. and *Ulva* sp. In this study, three macroalgae, *Gracilaria vermiculophylla*, *Dictyota dichotoma* and *Ulva lactuca*, were selected to evaluate the antioxidant activity and their effects on shrimp growth and amino acid digestibility.

MATERIALS AND METHODS

Macroalgae meals

Macroalgae (*G. vermiculophylla*, *D. dichotoma* and *U. lactuca*) were collected from Agiabampo Bay, Sonora, Mexico (26°22'31"N, 109°13'37"W), dried in a convection oven (Shell Lab CE3F) at 60°C for 24 h, milled in a pulveriser Pulvex 200, sifted through a 250 µm mesh strainer, and stored at 4°C until its use.

Analysis of macroalgae meals

The moisture, ash, crude fiber, crude protein using Kjeldahl method and crude fat for Soxhlet method according to Association of Official Analytical Chemist (AOAC, 2002). The Nitrogen Free Extract (NFE) was calculated by the difference, using the following equation: NFE = (100 - % crude protein - % crude fat - % crude fiber - % ash). Methanolic extracts of macroalgae were prepared according to Robles-Sánchez *et al*. (2011). The extraction was carried out

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for 12 h using 99% methanol (1:12, macroalgae meal:methanol). Mixture was filtered and the supernatant was used for total phenols, DPPH and TEAC assays.

Phenols were measured spectrophotometrically using the Folin–Ciocalteu reagent, with gallic acid as the standard. Briefly, 50 µL of macroalgae extract were added to 3 mL of deionized water plus 250 µL of Folin-Ciocalteu reagent (diluted 2-fold using water as diluent before use). After 5 min, 250 µL of a 7% Na_2CO_3 solution were added. The mixture was made up to 5 mL with deionized water and incubated for 90 min at room temperature. The absorbance was measured at 750 nm, and the results were reported as mg of gallic acid equivalents (GAE) per 100 g of meal (Robles-Sánchez *et al*., 2011).

Free radical scavenging activity of macroalgae was evaluated spectrophotometrically against the absorbance of the indicator 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution. Macroalgae extracts were diluted to a final concentration of 0.06 mg mL^{-1}, and 3.9 mL of a methanolic solution of DPPH (2.5 mg of DPPH.100 mL^{-1} MeOH) were mixed with 0.1 mL of each sample and shaken vigorously. The tubes were allowed to stand at 27°C for 20 min. The control was prepared as above without any extract, and MeOH was used for the baseline correction. Changes in the absorbance of the samples were measured at 515 nm. Radical scavenging activity (RSA) was expressed as mg Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) equivalents/g dry macroalgae meal (Robles-Sánchez *et al*., 2011).

The hydrophilic (TEAC) assay was performed using ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)). This stable radical cation was performed by mixing 10 mL of a 7 mmol L^{-1} ABTS solution with 10 mL of a 2.45 mmol L^{-1} $K_2S_2O_8$ solution. After 24 h at room temperature in the darkness, the ABTS stock solution was ready to use. An ABTS working solution was prepared daily by diluting the ABTS stock solution with a phosphate buffer (75 mmol L^{-1}, pH 7.4) to an absorbance of 0.70 ± 0.05 at 734 nm. Twenty microliters of extract were mixed with 200 mL of an ABTS working solution on a 96-well microplate. Trolox solutions (12.5-250 mmol L^{-1}) were used for constructing a regression line, water acted as blank, and the antioxidant capacities of the samples were expressed as mg Trolox equivalents g^{-1} dry macroalgae meal (Re *et al*., 1999, Müller *et al*., 2010).

Growth trial

Diet formulation and processing

Three experimental diets were elaborated, each with the inclusion of 15% of macroalgae meal and a control diet without macroalgae meal. Experimental diets were isoproteic and isocaloric, formulated with the Nutrion 5 Pro software. Ingredients such as fishmeal, soybean meal and whole-wheat flour were milled with a Cyclotec mill and strained at 250 µ. Subsequently they were mixed with an emulsion of soy lecithin and fish oil, enriched with a premix of vitamins and minerals and distilled water was added at 60°C to form a paste. This was passed through a conventional meat mill, using a 3 mm diameter die with which the pellets were formed.

Experimental diets were dried in an air convection oven at 45°C for 24 h and the product was used to feed the shrimp in culture. Table 1 shows the ingredient composition of the diets that were used in this experiment.

Selection of shrimp juvenile

Fifteen hundred juvenile of *L. vannamei* were obtained from a hatchery in Sonora south, a temperature of 20°C was maintained during the transfer to the bioassay laboratory at the Universidad Estatal de Sonora.

Juvenile white shrimp (*L. vannamei*), with a mean weight of 0.054 ± 0.012 g, were stocked in 60 L rectangular tanks at a density of 10 shrimp/tank. Three replicate tanks were randomly assigned for each diet. Shrimps were maintained in filtered seawater at 28 ± 1°C, 35 ± 0.04 of salinity, and 5.0 ± 0.11 mg L^{-1} dissolved oxygen. The shrimp were fed *ad libitum* three times daily (08:00, 13:00 and 18:00 h). At the beginning of the trial, the diet was introduced at a 10% rate of the biomass in each tank. During the following days, the amount was corrected based on the residual food. The shrimp were weighed every 15 days for a period of 45 days. The following response variables were determined for each experimental tank: mean body weight; growth rate expressed as percentage weight gain (%WG) = 100 × (initial weight-final weight)/(initial weight); feed conversion ratio (FCR) = (total feed intake)/(weight gain); and protein efficiency ratio (PER) = (weight gain)/(protein intake); and survival (%S) =100 × (final count)/(initial count).

Apparent digestibility trial

Diet formulation and processing

Four diets for the digestibility bioassay were formulated and elaborated with the same composition and methodology as those developed for the growth bioassay. In contrast, these diets were added 1% chromium oxide (Cr_2O_3) as an inert marker. Table 2 shows the ingredient composition of the diets that were used in this experiment.

Table 1. Ingredient composition (g kg^{-1} diet) of the diets used to determine growth in *L. vannamei* juveniles.

Ingredient (%)	Control	*G. vermiculophylla*	*D. dichotoma*	*U. lactuca*
Fishmeal (sardine)[1]	150.00	150.00	150.00	150.00
Wheat flour[2]	515.50	376.40	370.00	376.40
Soybean meal[2]	260.90	230.00	256.4	250.00
Macroalgae meal[3]	0.00	150.00	150.00	150.00
Sodium alginate[4]	25.00	25.00	25.00	25.00
Vitamin premix[5]	8.00	8.00	8.00	8.00
Mineral premix[6]	5.00	5.00	5.00	5.00
Choline chloride 62 %[7]	2.00	2.00	2.00	2.00
Vitamin C[8]	1.00	1.00	1.00	1.00
Dibasic sodium phosphate[9]	5.00	5.00	5.00	5.00
Fish oil (sardine)[1]	17.50	17.50	17.50	17.50
Soybean lecithin 1[10]	10.00	10.00	10.00	10.00
BHT[11]	0.10	0.10	0.10	0.10

[1]Industrias Barda, Yavaros, Sonora, Mexico. [2]Alimentos Colpac S.A. de C.V. [3]Prepared in the laboratory from macroalgae collected at Agiabampo Bay, Sonora, México. [4]Química Meyer Cat. Num. 6780. México, D.F. [5]Composition of the vitamin premix (g kg^{-1} premix): Vit. A (20,000 UI g^{-1}) 5.6, D_3 (850,000 UI g^{-1}) 0.001, DL-α-tocopheryl acetate (250 UI g^{-1}) 8.9, Menadione 2.2, Thiamin-HCl 0.6, Riboflavin 3.3, Pyridoxine-HCl 1.1, DL-Ca-Pantothenate 5.6, nicotinic acid 5.6, Biotin 0.1, Inositol 5.6, B_{12} 0.002, folic acid 0.2, alpha-cellulose 961.4. [6]Composition of the mineral premix (g 100 g^{-1} premix): $CoCl_2$ 0.004, $CuSO_4.5H_2O$ 0.25, $FeSO_4.7H_2O$ 4, $MgSO_4.7H_2O$ 28.398, $MnSO_4.H_2O$ 0.65, KI 0.067, Na_2SeO_3 0.01, $ZnSO_4.7H_2O$ 13.193, alfa-celulose 53.428. [7]SIGMA Cat. Num. C1879. SIGMA-ALDRICH chemical company, St. Louis, MO, USA. [8]Stay C 35% active agent. Roche, México, D.F. [9]SIGMA Cat No. S-0876. SIGMA-ALDRICH Chemical Company, St. Louis, MO, USA; [10]ODONAJI, distribuidora de alimentos naturales y nutricionales (distributor of natural and nutritional food) S.A. de C.V. México, D.F. [11]Butylated hydroxytoluene, ICN Cat. No.101162. Aurora, Ohio, USA.

Table 2. Ingredient composition (g kg^{-1} diet) of diets used to determine digestibility in *L. vannamei* juveniles.

Ingredient (%)	Control	*G. vermiculophylla*	*D. dichotoma*	*U. lactuca*
Fishmeal (sardine)[1]	150.00	148.5	148.5	148.5
Wheat flour[2]	480.00	376.20	366.40	372.60
Soybean meal[2]	280.00	247.50	258.40	247.50
Macroalgae meal[3]	0.00	148.50	148.50	148.50
Sodium alginate[4]	24.70	24.80	24.80	24.80
Vitamin premix[5]	7.80	7.80	7.80	7.80
Mineral premix[6]	5.00	5.00	5.00	5.00
Choline chloride 62%[7]	1.90	1.90	1.90	1.90
Vitamin C[8]	1.00	1.00	1.00	1.00
Dibasic sodium phosphate[9]	5.00	5.00	5.00	5.00
Fish oil (sardine)[1]	17.40	17.40	17.40	17.40
Soybean lecithin 1[10]	9.9	9.9	9.9	9.9
BHT[11]	0.10	0.10	0.10	0.10
Chromic oxide[12]	10.00	10.00	10.00	10.00

[1]Industrias Barda, Yavaros, Sonora, Mexico. [2]Alimentos Colpac S.A. de C.V. [3]Prepared in the laboratory from macroalgae collected at Agiabampo Bay, Sonora, México. [4]Química Meyer Cat. Num. 6780. México, DF, [5]Composition of the vitamin premix (g kg-1 premix): Vit. A (20,000 UI g-1) 5.6, D3 (850,000 UI g-1) 0.001, DL-α-tocopheryl acetate (250 UI g-1) 8.9, Menadione 2.2, Thiamin-HCl 0.6, Riboflavin 3.3, Pyridoxine-HCl 1.1, DL-Ca-Pantothenate 5.6, nicotinic acid 5.6, Biotin 0.1, Inositol 5.6, B12 0.002, folic acid 0.2, alpha-celulose 961.4, [6]Composition of the mineral premix (g 100 g-1 premix): CoCl2 0.004, CuSO4.5H2O 0.25, FeSO4.7H2O 4, MgSO4.7H2O 28.398, MnSO4.H2O 0.65, KI 0.067, Na2SeO3 0.01, ZnSO4.7H2O 13.193, alfa-celulose 53.428, [7]SIGMA Cat. Num. C1879. SIGMA-ALDRICH Chemical Company, St. Louis, MO, USA, [8]Stay C 35% active agent. Roche, México, DF, [9]SIGMA Cat No. S-0876. SIGMA-ALDRICH Chemical Company, St. Louis, MO, USA, [10]ODONAJI, distribuidora de alimentos naturales y nutricionales (distributor of natural and nutritional food) S.A. de C.V. México, DF, [11]Butylated hydroxytoluene, ICN Cat. No.101162. Aurora, Ohio, USA, [12]Aldrich Cat. No. 20,216-9. SIGMA-ALDRICH Chemical Company, St. Louis, MO, USA.

Selection of shrimp juvenile

Juvenile white shrimp (*L. vannamei*), with a mean weight of 8.2 ± 1.2 g, were obtained from a shrimp farm located in Sonora south and stocked in 60 L rectangular tanks at a density of 10 shrimp/tank. Three replicate tanks were randomly assigned for each diet. The shrimp were maintained for acclimation in filtered seawater at 29 ± 1°C, 35 of salinity and 6 mg L^{-1} dissolved oxygen. The shrimp were fed with the experimental diets *ad libitum* three times daily for 7 days before feces collection began. One hour after each feeding, fecal strands were siphoned, gently rinsed with distilled water, and frozen at -60°C. At the end of the trial, the feces collected from each tank were pooled and freeze-dried. Diets and fecal samples were analyzed for chromic oxide (Olvera-Novoa, 1994), crude protein (AOAC, 2002) and amino acids (Umagat *et al*., 1982; Jones & Gilligan, 1983). The apparent digestibility coefficients (ADC) for nutrients of test ingredient were determined according to Bureau & Hua (2006) using the following equation:

$$ADC\ test\ ingredient = ADC\ test\ diet + [(ADC\ test\ diet\text{-}ADC\ ref.diet) \times \left(\frac{0.85*D\ ref.}{0.15*D\ ing.}\right)]$$

where D ref. = % nutrient of reference diet; D ing. = % nutrient of test ingredient.

Amino acid composition

Amino acids were determined after hydrolysis of the macroalgae, fecal and diet samples were placed in 6 N HCl for 6 h at 150°C. Samples were then dried using a rotary evaporator (Yamato, RE301) and re-suspended in 2 mL of 6N HCl. The total amino acids were determined via HPLC (HITACHI L-8900 amino acid analyser) using an ion exchange column (HITACHI # 2622SC-PH) at a flow rate of 1 mL min^{-1} with a fluorescence detector (Umagat *et al*., 1982; Jones & Gilligan, 1983).

Statistical analysis

A one-way ANOVA was applied to determine significant differences among treatments. Tukey's multiple range test was used to identify differences among means. All statistical analyses were performed at the 0.05 significance level using Statistica™ 7.0 (StatSoft Inc., Tulsa, OK, USA).

RESULTS

Table 3 shows the results of the total phenol content of macroalgae meals. Concentration of total phenols in *G. vermiculophylla* was significantly higher than in *U. lactuca* and *D. dichotoma* (14.78, 11.59 and 11.68 mg GA100 g^{-1} dry wt, respectively) and exhibited the greatest antioxidant activity expressed as DPPH radical scavenging activities.

In the growth trial, significant differences were found between the means of the treatments, where the *U. lactuca* fed shrimp obtained a final weight higher than the rest of the treatments. The shrimp cultivated with the control diet, to which no macroalgae were added, grew below the rest of the treatments. On the other hand, in the survival and in the feed conversion factor, no significant differences ($P > 0.05$) were found between the treatments (Table 4).

The ADC of dry matter and crude protein for the experimental diet of 150 g kg^{-1} of macroalgae meal are shown in Table 5. The apparent digestibility of the diet changed from 41.6 to 72.1%, with *G. vermiculophylla* recording the best ADC for dry matter followed by *U. lactuca* and *D. dichotoma*. The ADC of crude protein was similar for diets containing macroalgae meal, which were higher than the results recorded for the control diet.

Table 6 shows the amino acid ADC of the diets containing macroalgae meal. The highest ADC was obtained by the diet formulated with *G. vermicullophylla* meal (92.8%), and the lowest was obtained by the control diet (67.3%), to which macroalgae meal was not added. Alanin (54.9%) was the least digestible amino acid in all samples, while arginine and lysine (87.4 and 85.7%, respectively) were generally the most easily digested.

DISCUSSION

Antioxidant activity

Wang *et al*. (2009) found that brown algae generally contained higher amounts of polyphenols than red and green algae, and in our study, we did not find this trend, as the red algae *G. vermiculophylla* presented the highest content of phenols.

Some authors have found a positive correlation between the content of total phenols and the antioxidant activity of macroalgae (López *et al*., 2011; Raja *et al*., 2016), and this behavior was also found in our study; *G. vermiculophylla* presented the highest content of total phenols as well as antioxidant activity, measured as DPPH radical scavenging activities and ABTS. The high scavenging property of *G. vermiculophylla* may be due to hydroxyl groups that exist in the phenolic compounds (Raja *et al*., 2016).

In general, the use of macroalgae as an ingredient in diets for *L. vannamei* has not been observed to affect negatively the survival (Peñaflorida & Golez, 1996;

Table 3. Antioxidant activity of macroalgae extracts. Mean ± SD of three replicates. Values in the same column with different superscripts are significantly different ($P < 0.05$).

Macroalgae	Total phenolic content (mg GA equivalents g^{-1} dry macroalgae meal)	DPPH radical scavenging activities (mg Trolox equivalents g^{-1} dry macroalgae meal)	ABTS (mg Trolox equivalents g^{-1} dry macroalgae meal)
Gracilaria vermiculophylla	$14.78^{a} \pm 1.47$	$0.21^{a} \pm 0.01$	$40.0^{a} \pm 0.6$
Dictyota dichotoma	$11.59^{b} \pm 1.16$	$0.13^{b} \pm 0.01$	$28.4^{b} \pm 2.5$
Ulva lactuca	$11.68^{b} \pm 2.19$	$0.14^{b} \pm 0.02$	$37.1^{a} \pm 4.7$

Table 4. Growth performance of juvenile *L. vannamei* fed on different macroalgae meals. Mean ± SD of three replicates. Numbers in the same column with different superscripts are significantly different ($P < 0.05$). [1]Factor conversion ratio, [2]Relative growth ratio.

Diet	Final weight (g)	Survival (%)	FCR[1]	RGR[2] (%)
Control	$2.41^{c} \pm 0.28$	$90^{c} \pm 0.03$	$2.57^{a} \pm 0.42$	$4.319^{a} \pm 569$
G. vermiculophylla	$3.37^{ab} \pm 0.28$	$97^{b} \pm 0.01$	$2.40^{a} \pm 0.19$	$5.469^{a} \pm 398$
D. dichotoma	$3.06^{bc} \pm 1.04$	$100^{a} \pm 0.00$	$2.77^{a} \pm 0.54$	$5.698^{a} \pm 1,89$
U. lactuca	$3.71^{a} \pm 0.25$	$100^{a} \pm 0.00$	$2.54^{a} \pm 0.16$	$6.804^{a} \pm 356$

Table 5. Apparent digestibility coefficients for dry matter and crude protein of diets containing macroalgae meals. Mean ± SD of three replicates. Values in the same column with different superscripts are significantly different ($P < 0.05$).

Diet	Dry matter (%)	Crude protein (%)
Control	$41.60^{c} \pm 0.45$	$56.44^{b} \pm 0.34$
G. vermiculophylla	$72.06^{a} \pm 3.68$	$81.07^{a} \pm 2.91$
D. dichotoma	$60.83^{b} \pm 0.82$	$78.80^{a} \pm 2.47$
U. lactuca	$61.72^{b} \pm 1.26$	$82.19^{a} \pm 1.43$

Cruz-Suárez *et al*., 2000; Gutiérrez-Leyva, 2006; Da Silva & Barbosa, 2008; Peña-Rodríguez *et al*., 2010).

Chemical composition of macroalgae meal and shrimp growth

The proximal chemical composition of the macroalgae studied is very similar to that reported by Cruz-Suárez *et al*. (2010) for *U. clathrata*, as well as for *U. pertusa* and *U. intestinalis* (Benjama & Masniyom, 2011). The proximal analysis of the algae meals studied shows that they have nutritional characteristics superior to those reported by Gutiérrez-Leyva (2006) for *Sargassum* sp. and kelp, where meals of these algae were used as diet ingredients for *L. vannamei* with levels of inclusion of 1, 4, 7 and 10% for both algae.

The final weight increased significantly ($P < 0.05$) in shrimp fed with *G. vermiculophylla* and *U. lactuca*. Gutiérrez-Leyva (2006) found similar results with *M. pyirifera* and *Sargassum* sp., where the final weight of shrimp was higher than that of shrimp fed with the control diet. Peña-Rodríguez *et al*. (2010) shows the benefits obtained by the shrimp when fed with macroalgae, especially those of the genus *Ulva*. Diets prepared with inclusion rates of less than 5% of macroalgae meals give favorable results with respect to untreated organisms, mainly in terms of weight gain and shrimp coloration after a cooking process.

Macroalgae have been associated with the presence of bioactive compounds with antioxidant, antimicrobial and anti-inflammatory effects (Banerjee *et al*., 2009; Plaza *et al*., 2010; Raja *et al*., 2016), an association that can also be made from the results of this study, considering that these macroalgae contained higher contents of total phenols and higher antioxidant activity.

Macroalgae of the genus *Gracilaria* are an important source of phytocolloid (Troell *et al*., 2003); the green macroalgae of the genus *Ulva* containing the polysaccharides ulvans (Sathivel *et al*., 2008). Algal polysaccharides have been demonstrated to play an important role as free-radical scavengers and antioxidants for the prevention of oxidative damage in living organisms (Kim *et al*., 2007; Wang *et al*., 2009; Souza *et al*., 2012). In the present study the macroalgae were supplied complete to organisms of *L. vannamei* and it is possibly that this components had an important effect in the growth and survival response.

The feed conversion and the relative growth ratios were unaffected, with Cruz-Suárez *et al*. (2000) finding similar results in their evaluation of *M. pyrifera*. While macroalgae represent an important source of protein

Table 6. Apparent digestibility coefficients for amino acids of diets containing macroalgae meals Mean ± SD of three replicates. Values in the same row with different superscripts are significantly different ($P < 0.05$).

	Control	*Gracilaria vermiculophylla*	*Dictyota dichotoma*	*Ulva lactuca*
Essential amino acids (EAA)				
Arginine	$76.82^{bc} \pm 3.31$	$95.28^{a} \pm 0.17$	$88.91^{b} \pm 0.69$	$86.92^{b} \pm 0.68$
Histidine	$67.89^{c} \pm 5.34$	$93.60^{a} \pm 1.22$	$83.68^{b} \pm 0.45$	$81.49^{b} \pm 1.73$
Isoleucine	$68.38^{c} \pm 1.01$	$93.33^{a} \pm 1.26$	$84.43^{b} \pm 0.33$	$78.36^{b} \pm 1.87$
Leucine	$71.85^{c} \pm 2.67$	$93.79^{a} \pm 1.17$	$85.28^{b} \pm 0.26$	$82.33^{b} \pm 1.76$
Lysine	$73.23^{c} \pm 0.16$	$94.91^{a} \pm 2.87$	$87.28^{b} \pm 3.75$	$85.31^{b} \pm 1.11$
Methionine	$59.95^{c} \pm 2.50$	$92.61^{a} \pm 1.37$	$81.71^{b} \pm 0.78$	$76.19^{b} \pm 1.10$
Phenylalanine	$69.36^{c} \pm 3.30$	$93.19^{a} \pm 1.50$	$84.13^{b} \pm 0.74$	$80.54^{b} \pm 1.62$
Valine	$70.04^{c} \pm 0.82$	$93.19^{a} \pm 1.45$	$85.11^{b} \pm 0.67$	$80.84^{b} \pm 2.96$
Non-essential amino acids (NEAA)				
Alanine	$54.87^{c} \pm 9.94$	$90.56^{a} \pm 1.98$	$80.59^{b} \pm 7.34$	$79.75^{b} \pm 4.27$
Glutamic acid	$68.68^{c} \pm 2.21$	$88.41^{b} \pm 0.00$	$94.24^{a} \pm 5.55$	$87.75^{b} \pm 1.20$
Glycine	$56.42^{c} \pm 8.50$	$90.96^{a} \pm 0.47$	$77.21^{b} \pm 4.72$	$78.06^{b} \pm 2.34$
Serine	$70.00^{c} \pm 0.00$	$93.14^{a} \pm 0.17$	$76.02^{b} \pm 0.00$	$82.27^{b} \pm 2.91$
Tyrosine	$68.08^{c} \pm 1.22$	$93.25^{a} \pm 1.26$	$82.60^{b} \pm 1.78$	$80.43^{b} \pm 1.35$

and energy, information about the availability of nutrients for aquatic organisms is scarce (Cruz-Suárez *et al*., 2008). Most researches focus on the use of vegetable matter as a substitute for fishmeal.

The protein and energy composition of macroalgae is a viable source to be used as an ingredient in shrimp diets. The few studies on its use make it necessary to know more about the bioavailability of these nutrients for aquatic organisms (Cruz-Suárez *et al*., 2008). Most of the research is focused on the use of terrestrial vegetable sources in the substitution of fishmeal.

The relationship between weight gain and survival (Table 4) with ADC of dry matter, protein (Table 5) and amino acids (Table 6) in diets, is something to be emphasized in this study, due to the shrimp that were treated with diet containing macroalgae meal as an ingredient, obtained better results than the untreated organisms. This suggests that the nutrient content of macroalgae (Cruz-Suárez *et al*., 2010) and the presence of antioxidant compounds and other bioactive compounds (Esquer-Miranda *et al*., 2016) improve the health and well-being of shrimp, which allows efficient nutrition, weight gain and survival greater than the control organisms.

Digestibility protein and amino acids

Several aspects determine the nutritional quality of an ingredient as a nutrient source specifically: its palatability, level of the nutrient, anti-nutritional factors and digestibility of the nutrients (Divakaran *et al*., 2000). Studies conducted to ascertain the nutrients digestibility coefficients are most commonly conducted by feeding a diet containing fixed levels of a nutrient source, the inclusion commonly used of test ingredients in experimental diets is 30%, however, some authors had used different levels of inclusion: 88% (Akiyama *et al*., 1989), 50% (Kumaraguru *et al*., 2007) and 15% (Divakaran *et al*., 2000). Found that the dietary soybean meal level did not consistently affect the ADC values calculated for this ingredient, in *Litopenaeus vannamei* diets. In the present study, the level of macroalgae meals is 15% and is assumed that the estimation of digestibility is not affected by the inclusion.

Non-significant differences among treatments were found for the amino acid ADC of the macroalgae meal. Terrazas-Fierro *et al*. (2010) evaluated marine foodstuffs and found that these ingredients are good sources of available protein and amino acids for juvenile white leg shrimp. Despite being a vegetable protein source, macroalgae presented high amino acid digestibility, in contrast with non-marine vegetable foodstuffs (Oujifard *et al*., 2012).

The in vivo ADC of dry matter and crude protein for the experimental diets added with 15% of meal from each studied macroalgae are shown in Table 5. The apparent digestibility of diets varied from 41.60 to 82.19%. It was found that the diets with macroalgae were significantly ($P < 0.05$) more digestible than the control diet, being concurrent with a higher growth of the cultivated organisms comparable to that reported by Oujifard *et al*. (2012), in diets used for *L. vannamei* by partially replacing fishmeal with rice protein concentrate. The results for ADC of dry matter and protein are similar to those reported by Oujifard *et al*.

Table 7. Apparent digestibility coefficients for essential and non-essential amino acids in macroalgae meals. Mean ± SD of three replicates. Only non-significant differences among treatments were found ($P > 0.05$).

	Gracilaria vermiculophylla	*Dictyota dichotoma*	*Ulva lactuca*
Essential amino acids (EAA)			
Arginine	68.6 ± 7.7	71.9 ± 9.1	66.1 ± 2.2
Histidine	88.5 ± 5.8	85.9 ± 1.5	71.8 ± 6.0
Isoleucine	75.4 ± 4.0	63.3 ± 2.3	68.9 ± 6.5
Leucine	84.2 ± 2.1	88.5 ± 0.9	70.6 ± 2.8
Lysine	82.8 ± 1.9	80.3 ± 4.4	69.3 ± 3.5
Methionine	74.7 ± 2.5	76.5 ± 1.2	82.3 ± 3.6
Phenylalanine	76.0 ± 2.1	92.0 ± 2.4	72.3 ± 3.4
Valine	82.4 ± 3.9	89.1 ± 2.0	65.2 ± 2.2
Non-essential amino acids (NEAA)			
Alanine	69.6 ± 2.6	68.8 ± 2.0	81.7 ± 1.1
Glutamic acid	83.6 ± 2.7	78.5 ± 1.4	79.1 ± 2.9
Glycine	84.6 ± 2.7	77.6. ± 2.0	76.6 ± 3.5
Serine	66.4 ± 0.9	71.6 ± 1.8	67.7 ± 1.5
Tyrosine	89.5 ± 1.6	92.1 ± 2.8	74.8 ± 1.3

(2012), with values for dry matter between 60 and 85% between the experiments.

Similar studies have been performed on shrimp culture using *M. pyrifera*, *Ascophylum nodosum* and *Ulva Clathrata* as ingredients in diets, using 3.33% inclusion (Cruz-Suárez *et al*., 2008), studying the zootechnical parameters of the culture. Wong *et al*. (2001) studied the *in vitro* protein digestibility and amino acid profile of three species of macroalgae (*Hypnea charoides, Hypnea japonica* and *Ulva lactuca*) where they found that the digestibility of the *U. lactuca* protein (85.7%) was very similar to what was found in this study (82.2%). In relation to the reports made by Ramos-Díaz *et al*. (2001), for a dry matter and protein CDA of 78.6% and 94%, respectively, for the diet formulated with the macroalga *Lessonia* sp., with a percentage of inclusion of 30.9%, in the present study, lower results were obtained, of 64.8% and 80.7%, respectively, when using an inclusion level of 15%.

The results obtained in the amino acid digestibility of the tested diets are shown in Table 6, the best CDA for amino acids, was obtained in the diet added with *G. vermiculophylla* meal, while the control diet had the lowest amino acid digestibility.

The amino acid ADC, reported by Nieto-López *et al*. (2011), was slightly lower than that found in the digestibility of the amino acids in this experiment for the diet formulated with *G. vermiculophylla* meal. For the diets added with *D. dichotoma* and *U. lactuca* CDA were equivalent, while the control diet of this experiment resulted in an amino acid digestibility below from that reported by the previously mentioned author. The reports by Oujifard *et al*. (2012), where *L. vannamei* was grown with balanced diets, in which fishmeal was partially replaced by a rice flour protein concentrate, show the CDA results of amino acids similar to those found in this study, (81.35 to 92.9%).

CONCLUSIONS

Macroalgae, especially *U. lactuca* and *G. vermiculophylla*, demonstrated high antioxidant activity, high amino acid digestibility and improved *L. vannamei* growth rates. The ADC of dry matter, proteins and amino acids found in this study show the benefit of using macroalgae as an ingredient in diet for *L. vannamei*, where percentages between 60 and 72% for dry matter, of 78 amounted to 82% for protein and 82 to 93% for the amino acids of the experimental diets.

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