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Effect of green seaweed meal blend on feed quality and zootechnical performance in shrimp (*Penaeus vannamei*) juveniles

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Abstract

Aim of study: To evaluate a green seaweed meal in the diets of *Penaeus vannamei* juveniles, comprising *Ulva* spp., *Caulerpa* spp. and *Enteromorpha* spp. as a feed blend at inclusion levels at 4% and 8%.

Area of study: Universidad Nacional Agraria La Molina, Lima, Perú.

Material and methods: Analyses were conducted to determine the pellet quality through percentages of dry matter retention (DMR), protein loss and water absorption capacity; and to evaluate the effect of this seaweed meal in the digestibility and zootechnical shrimp performance. Three treatments (diets) were formulated to contain 0% (control diet), 4% (M4) and 8% (M8 of green seaweed meals (blend)), in isonitrogenous (crude protein; 300 g kg^{-1}) and isocaloric (3.3 Mcal kg^{-1}) diets. The shrimps were reared at a density of 286 juveniles m^{-3} for 29 days in a recirculating aquaculture system (RAS).

Main results: Among the diets, M4 had the highest DMR value (97.06%), whereas M8 had highest water absorption capacity (185.48%) with lower % of protein loss between the treatments diets. No differences were observed in the zootechnical performance, except for survival ($p<0.05$), with the M8 diet having highest mortality rate (44.4%) between the treatments diets.

Research highlights: Incorporating 4% green seaweed meal in shrimp feed supported adequate growth and survival of juvenile *P. vannamei* with adequate DMR values, water absorption capacity, protein loss and high apparent dry matter digestibility and apparent digestibility of the reference diet.

Additional key words: *Ulva* spp; *Enteromorpha* spp; *Caulerpa* spp; growth; digestibility; feed quality.

Abbreviations used: ADMD (apparent dry matter digestibility); ADR (apparent digestibility of the reference diet); ADT (apparent digestibility of the test diet); APD (apparent protein digestibility); CD (control diet); DE (digestible energy); DMR (dry matter retention); DW_{ad} (dry weight of diet after drying); DW_{bi} (dry weight of diet before water immersion); FCR (feed conversion ratio); IC (individual consumption); M4 (4% the green seaweed meal (blend) in a basal control feed); M8 (8% the green seaweed meal (blend) in a basal control feed); PER (protein efficiency ratio); PL (protein loss); PP_{al} (% of protein after leaching); PP_{bl} (% of protein before leaching); RAS (recirculating aquaculture system); SGR (specific growth rate); TAN (total ammonia nitrogen); T_w (PVC tube weight); WG (weight gain); WP_{ai} (pellet weight after 60-min immersion); WP_{bi} (pellet weight before immersion).

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Introduction

Among the commercial aquaculture of crustacean species, the white shrimp (*Penaeus vannamei*) is the most important, with 5,812.2 thousand tonnes produced in 2020 (FAO, 2022). One serious challenge in shrimp farming is the quality and availability of unconventional raw materials for high-quality feed (Little et al., 2016; Elizondo-González et al., 2018). The loss of nutrients and dry matter in the pellet are a disadvantage of the shrimp feed, due to its slow-feeding habits. For this reason, using feed with poor water stability leads to low yields and high mortality due to inadequate nutrient supply (Volpe et al., 2012; Valenzuela-Cobos & Vargas-Farías, 2020).

Dry matter retention (DMR) is crucial to ensure minimal leaching of nutrients, such as amino acids, vitamins and minerals. Furthermore, shrimp's feed requires the selection of pellets with improved water stability; without this attribute, the pellets may disintegrate in the water before being consumed by the shrimp (Obaldo et al., 2002; NRC, 2011; Argüello-Guevara & Molina-Poveda, 2013; Aaqillah-Amr et al., 2021). Thus, foods with high-water stability, which reflects DMR after water immersion, need to be identified (Argüello-Guevara & Molina-Poveda, 2013).

It is noteworthy that blends have a substantial effect on the physical integrity of pellets. They can be synthetic, such as polymethyl carbamide and urea formaldehyde, or natural, such as starch and its by-products, including dextrin, cellulose compositions and carboxymethylcellulose, alongside alginates obtained from seaweed (Pastore et al., 2012).

These seaweed phycocolloids (alginate, agar and carrageenan) have high viscosity and unique stabilising, emulsifying and gelling properties. The alginate production process involves pre-treatment with HCl, extraction with Na_2CO_3, dilution and filtration in a rotary vacuum filter. For agar, the production process involves pre-treatment, extraction, filtration, concentration and dehydration. This process is relatively expensive, with agar as the most expensive colloid at US$18 kg^{-1}, followed by alginate from brown seaweed at US$12 kg^{-1} and carrageenan from red algae at US$10.5 kg^{-1} (Hernandez-Carmona et al., 2013; Fleurence, 2016; Qin, 2018). Seaweed meal is cheaper and has an easier extraction process than seaweed phycocolloids (alginate, agar and carrageenan) for aquaculture.

There is interest in using seaweeds as a source of ingredient for aquaculture feed, which has gained momentum recently (Buschmann et al., 2017; Mohan et al., 2019; Costa Rezende et al., 2021). About 35.8 million tons of seaweed were produced in 2019 by 49 countries/territories, 97% of which came from Asia. Production in the Americas and Europe is dominated by a wild collection, while cultured seaweeds are predominant in Asia, Africa and Oceania (Cai et al., 2021). China and Indonesia are by far the largest seaweed producers (farmed and wild), with over 30 million tons, while Chile and Peru produce more than 0.5 million tons, especially wild (Cai et al., 2021). World seaweed production in 2019 concentrated in three groups: red, brown, and green. Green seaweeds (excluding microalgae) were produced by 12 countries with 32.926 tons (wet weight basis), a 0.09% of the total algae production (Cai et al., 2021).

Seaweeds stimulate appetite, promote growth and possess nutraceutical properties in amounts that can help against oxidative stress due to the presence of bioactive compounds in their polysaccharides (Lahaye & Robic, 2007; Reverter et al., 2014; Thanigaivel et al., 2016; Mohan et al., 2019; Naiel et al., 2020). In green seaweeds (Chlorophytes), ulvans are the sulphated polysaccharides (SPs) which have gelling properties and make up the cell walls (Lahaye & Rovic, 2007; Kidgell et al., 2019; Tziveleka et al., 2019; Moreira et al., 2021). These SPs have many beneficial biological properties, including immunomodulatory, antiviral, antihyperlipidemic, antioxidant and anticancer activities (Wijesekara et al., 2011; Kidgell et al., 2019). Ulvans are not exclusive compounds of the *Ulva* species, they are also present in other genera, such as *Monostroma*, *Caulerpa*, *Codium* or *Gayralia* (Moreira et al., 2021).

This study aimed to evaluate a mixture of green seaweed meal composed of *Ulva* spp., *Caulerpa* spp. and *Enteromorpha* spp. as a feed blend in the diets for *P. vannamei* juveniles to improve the quality of the pellet quality parameters (stability of pellet after water immersion, percentage of protein loss and water feed absorption) and shrimp performance.

Table 1. Proximate composition of the seaweed meal Nutrigreen (% dry basis). Data provided by PSW-SAC (Peruvian Seaweeds).

Proximate composition (%) [1]	**Mean ± SD**
Moisture	10 ± 2
Crude protein	15.5 ± 1.5
Lipid	1.25 ± 0.25
Ash	49 ± 1
Fibre	5.5 ± 1.5
NFE	32.5 ± 2.5
Carotenes	> 200 ppm
DE (Mcal kg^{-1}) [1]	1.66

NFE: nitrogen-free extract (carbohydrates). DE: digestible energy estimated based on caloric values of 3.8, 8 and 3 Mcal kg^{-1} for proteins, lipids and carbohydrates, respectively.

Material and methods

Seaweed samples and experimental blend

The green seaweed meal (composed of *Caulerpa*, *Enteromorpha* and *Ulva* – Chlorophyta), commercially known as Nutrigreen, was obtained from PSW SAC (Peruvian Seaweeds). PSW SAC also supplied the proximate composition of the green seaweed meal, as presented in Table 1.

Three treatments (diets) were formulated to be isoenergetic (3.3 Mcal kg^{-1}) and isoproteic (300 g kg^{-1}). They contained 0% (control diet [CD]), 4% (M4) and 8% (M8) of the green seaweed meal (Table 2).

The basal diet (CD) contained a mixture of sodium alginate and sodium hexametaphosphate. Before mixing, all the ingredients were milled in a disc mill and sieved until particles of 100-µm were obtained.

To accomplish a good blend, the ingredients were mixed from the highest to the lowest quantities, including the pre-

Table 2. Formulation and proximate composition of the experimental diets (% on a dry basis).

Ingredients	Green seaweed blend levels (%)		
	CD [4]	M4 [5]	M8 [6]
Wheat meal	41.76	43.26	39.51
Fish meal	30.50	29.00	29.00
Soybean meal	12.00	12.00	12.00
Soy lecithin	5.00	5.00	5.00
Dicalcium phosphate	2.80	2.90	2.97
Calcium carbonate	2.35	2.25	2.07
Sodium alginate	3.00	0.00	0.00
Sodium hexametaphosphate	1.00	0.00	0.00
Seaweed meal	0.00	4.00	8.00
Fish oil	1.00	1.00	1.00
Cholesterol SF [1]	0.25	0.25	0.25
Vitamin C	0.14	0.14	0.14
Premix (vitamins and minerals) [2]	0.10	0.10	0.10
Antioxidant	0.05	0.05	0.05
Mold inhibitor	0.05	0.05	0.05
Proximate composition (%dry matter) [3]			
Crude protein	30.48	30.89	31.10
Crude lipid	9.70	9.27	9.50
Crude fiber	0.79	0.94	0.90
Ash	11.97	11.42	13.22
NFE	39.25	42.08	39.88
DE, Mcal kg^{-1} [3]	3.31	3.32	3.27

[1] Cholesterol SF is made from wool grease by extraction and refining processes; insoluble in water and approximately 1% Aerosil to improve flowability. [2] DMS Nutritional Products Peru S.A. (Peru) kg^{-1} Premix: Vit. A, 9,333.34 UI; Vit. D3, 10333.34 UI; Vit. E, 93.34 UI; Thiamin, 12.00 mg; Vit. B2, 13.34 mg; Niacin, 100.00 mg; Pantothenic acid, 33.34 mg; Vit. B6, 10.00 mg; biotin, 0.54 mg; folic acid, 2.66 mg; Vit. C, 400.00 mg; Vit. B12, 0.02 mg; choline chloride, 400.00 mg; Mn, 16.66 mg; Fe, 13.34 mg; Zn, 13.34 mg; Cu, 1.00 mg; I, 1.00 mg; Se, 0.20 mg; Co, 1.00 mg; B.H.T. (butyl hydroxy toluene), 80.00 mg; excipients, c.s.p 2,000.00 mg. [3] DE: digestible energy estimated based on caloric values of 4.24, 3.8, 8 and 3 Mcal kg^{-1} for proteins (animal/veg), lipids and NFE: nitrogen-free extract (carbohydrates), respectively. [4] CD = control diet. [5] M4 = 4% green seaweed meal. [6] M8 = 8% green seaweed meal.

mix of vitamins, minerals, and all the ingredients in very small quantities. Then the soy lecithin, fish oil, and finally enough quantity of hot water (70°C) were added, to get a wet dough to allow pressing in the meat grinder through a 2-mm die. This dough was pressed twice, to increase ingredient agglomeration. The noodles obtained were dried at 60°C for 1 hour in a dehydrator. When dry, they were broken and sieved to 2-mm particle size. They were bagged and kept refrigerated until later use. According to the standard methods (AOAC, 2005), the proximate composition of the diets was analysed in the nutritional assessment laboratories (LENA) at the School of Animal Husbandry in the National Agrarian University La Molina (UNALM).

Feed water stability

The stability of the pellets was evaluated in terms of DMR percentage after immersion in a shaking water bath for 2 hours (Argüello-Guevara & Molina-Poveda, 2013). Each diet had three replicates. For this analysis, 3-g of pellets from each experimental diet (83 pellets g^{-1}, 3 mm average length and 2 mm average diameter) were weighed on an analytical balance (Sartorius, four decimals) and placed inside labelled and tared polyvinyl chloride (PVC) tubes, with a 0.25-mm mesh bottom. Then, they were placed in a thermoregulated bain-marie and shaken at 30 rpm at 28°C and 30 g L^{-1} salinity. After immersion, the tubes were allowed to drain for 20 min and placed in an oven at 60°C until a constant weight was obtained. This weight was registered for each sample. Feed stability was calculated using the following formula:

$$DMR\ (\%) = 100 - [(DW_{bi} - DW_{ad})/DW_{bi}] \times 100,$$

where DW_{bi} = dry weight of diet before water immersion and DW_{ad} = dry weight of diet after drying.

Water absorption (%)

Pellet water absorption was calculated by gravimetric difference (Argüello-Guevara & Molina-Poveda, 2013). A sample (3 g) of each treatment by triplicate was placed in tared PVC tubes with a 0.25-mm mesh bottom and immersed in water for 1 h at 30 g L^{-1} of salinity and 28°C. The excess water was drained for 40 min. Subsequently, each unit was weighed on an analytical balance (Sartorius), and the weight was registered. The formula used was as follows:

$$\text{Water absorption } (\%) = (WP_{ai} - T_w) - (WP_{bi} - T_w)/WP_{bi} - T_w,$$

where WP_{ai} = pellets weight plus PVC tube weight after a 60-min immersion, T_w = PVC tubes weight and WP_{bi} = pellets weight plus PVC tubes weight before immersion.

Feed protein loss

Dry leached pellet obtained for the DMR (%) determination on the 3-g sample, allowed the calculation of the protein loss (%PL) that occurred during leaching; %PL was obtained using the following formula (Cruz -Suárez et al., 2006):

$$\%PL = ((100 \times [PP_{bl}] - (100 - PMS) \times [PP_{al}]))/PP_{bl},$$

where PP_{bl} = % protein before leaching, PMS = % loss dry matter and PP_{al} = % protein after leaching.

Feeding trial

A 29-days indoor trial was conducted at the Aquaculture Laboratory of the Faculty of Fisheries of the Universidad Nacional Agraria La Molina (Peru). The experimental design was completely randomized with three experimental diets: M4 and M8 (with respectively 4% and 8% inclusions of commercial green seaweed meal blend, in shrimp diet), and a control diet (CD) without seaweed meal, all with three replicates.

P. vannamei (juveniles) were obtained from a commercial laboratory (Marina Azul SAC of Tumbes, Peru), distributed and acclimated for one week in a fiberglass tank (1 shrimp by 6 L) with a salinity of 33 g L^{-1}. They were fed with commercial feed (28% crude protein and 6% crude lipid [Agribrands Purina Peru S.A.] at 7% of their body weight per day, divided into two portions given at 8 am and 5 pm). After acclimation, the juveniles (1.42 ± 0.27 g) were transferred into nine glass recirculating aquaculture systems (RAS) (0.50 × 0.35 × 0.30 m) at a density of 286 shrimp m^{-3} (15 shrimps per aquarium). The RAS was filled with clean seawater filtered through a 300-mm and then a 0.050-mm mesh, then sterilised with 25 mg L^{-1} formalin. To provide a sense of 'refuge' to the shrimps, the sides of the aquariums were covered with black plastic. The shrimps were provided additional aeration from a 0.5-HP blower for better oxygenation. A water flow of 4 L min^{-1} $tank^{-1}$ was maintained.

The shrimps were fed three times a day (at 08 am, 12 pm and 4 pm), with the test diets at 7% of their body weight and adjusted daily according to the estimated shrimp consumption, mortality rate and leftover feed. Uneaten feed was collected into 0.25-mm mesh baskets by siphoning after each feeding. Every morning, feed waste and faeces were removed before feeding.

Water quality parameters, as dissolved oxygen (mg L^{-1}) and temperature (°C) values, were monitored twice a day (at 08 am and 4 pm) using an oximeter (YSI model 55, Yellow Springs, OH, USA); salinity (g L^{-1}) was evaluated using an Atago refractometer (model 2493 Master S/MillM, Japan); total ammonia nitrogen (TAN) using a spectrophotometer Thermo Scientific, Helio Gamma Model, England (APHA, 1998); and pH using a potentiometer Schott Mod-

el Lab 850, Germany, once a week (at 4 pm). The values were: dissolved oxygen, 5.8 ± 0.15 mg L^{-1}; salinity, 33 ± 0.05 g L^{-1}; temperature, 28°± 1.02°C; pH 7.7 ± 0.1; and TAN, 0.24 ± 0.02 mg L^{-1}.

Digestibility trial

P. vannamei juveniles (4.0 ± 0.52 g) that were grown in the previous experiment were randomly mixed and redistributed to allow a homogeneous population. They were maintained for one week in two 1,000-L and one 500-L fibreglass tanks, and fed with the CD diet at 10% of their body weight adjusted daily, three times a day (at 8 am, 11 am and 5 pm). After this time, to effectively cleaning the gut from ingestion of the previous diets, they were randomly distributed into fifteen 60-L glass aquariums (0.50 × 0.35 × 0.30 m) in a RAS at a stocking density of 7 juveniles per aquarium during 15 days. The apparent digestibility coefficient of the diets (M4%, M8% and CD) was determined using the indirect method in diets containing chromium oxide (Cr_2O_3) as an inert marker. This purpose was achieved withdrawing 300 g in each of the three experimental diets, and grounding them to dust, 1% of which was weighed and replaced by the same weight of Cr_2O_3. They were processed as describe before to obtain the pellets. An especially constructed digestibility system was used to collect faeces (Choubert et al., 1982).

To determine the coefficient of apparent digestibility of the seaweed, the 70:30 ratio protocol was followed (Takeuchi, 1988; NRC, 2011). A 300 g total of the control diet (used as a reference diet) was completely ground, replacing 90 g (30%) with the seaweed meal; then 1% of this blend was weighed and replaced by the same weight of Cr_2O_3. Hot water was added, the dough was pressed again with a meat grinder and then dried at 60°C.

The coefficients of apparent digestibility of dry matter (%ADMD) and the apparent digestibility of protein (%APD) were calculated as described by Guillaume & Choubert (2004):

$$\%ADMD = 100 \times [1 - (\text{diet } Cr_2O_3 / \text{faeces } Cr_2O_3)$$

$$\%APD = [(\text{diet } Cr_2O_3 / \text{faeces } Cr_2O_3) \times \%\text{feces protein} / \%\text{diet protein}) \times 100]$$

The digestibility of the seaweed was calculated following Takeuchi (1988):

$$\%\text{Digestibility of the ingredient} = (ADT - 0.7\ ADR) / 0.3$$

where ADT = % apparent digestibility of the test diet and ADR = % apparent digestibility of the reference diet. Cr_2O_3 (experimental diets and faeces) was calculated in the Soils Laboratory, UNALM, via atomic absorption spectrophotometry (AOAC, 2005).

Shrimp zootechnical performance

Shrimp weight was monitored weekly to determine the shrimps' growth and adjust the feed amount. All zootechnical parameters were determined using the following formulas:

- Biomass = sum of the individual weights (kg) m^{-3};
- Feed conversion ratio (FCR) = feed supplied (dry weight) / weight gain (g);
- Protein efficiency ratio (PER) = weight gain (g) / protein consumption;
- Specific growth rate (SGR) (%) = 100 × (ln final weight (g) – ln initial weight (g)/days of culture;
- Weight gain (WG) = (average final weight – average initial weight);
- Individual consumption (IC) = $\sum 1^{29}$ (experimental tank consumption day^{-1} / total of shrimp's day^{-1}); and
- Survival rate (S) (%) = (final number of shrimps per treatment/initial number of shrimps per treatment) × 100.

Shrimp body protein retention

The shrimps were sacrificed using the thermal shock technique, which induces insensibility within a few seconds (Piana et al., 2018), at the end of the experiment time. An analysis of crude protein (whole shrimp) using standard methods (AOAC, 2005) was performed, with protein content being determined by measuring nitrogen (N × 6.25).

Body protein retention (%) = (final weight × final protein content) – (initial weight × initial protein content) × 100/ protein intake.

Statistical analyses

Statistical analyses were conducted using the Lawstat R software vers. 2.4.1 (Statistical Analysis System software, NY, USA). Data were checked for homogeneity of variances using the Brown-Forsythe test and for normality using the Shapiro-Wilk test. The parametric one-way analysis of variance was used, and when differences were observed, Tukey's mean comparison test was adopted ($p < 0.05$).

Results

Feed water stability, water absorption and protein loss

After 2 h of water immersion, the feed exhibited significant differences ($p < 0.05$) in %DMR, with M8 having

Table 3. Dry matter retention values (%DMR), water absorption capacity (%) and percentage of protein loss (%PL) in the experimental feed.

	CD [1]	M4 [2]	M8 [3]	p value
%DMR	94.03 ± 2.06^{ab}	97.06 ± 0.69^{a}	92.53 ± 0.25^{b}	0.044
Water absorption capacity (%)	166.10 ± 11.95	183.83 ± 10.81	185.48 ± 5.64	ns
%PL	1.85 ± 1.15	3.92 ± 1.18	1.79 ± 0.61	ns

Mean values and standard deviations. In the same line, values with different superscripts are significantly different ($p < 0.05$). [1] CD = control diet. [2] M4 = 4% green seaweed meal. [3] M8 = 8% green seaweed meal.

the lowest value (92.53%), followed by the CD (94%), with 3% alginate; M4 had the highest value (97.06%). No significant differences were observed in the feed water absorption capacity. The feed with green seaweed meal exhibited a 183–185% capacity. Furthermore, there were no significant differences ($p > 0.05$) in %PL among the treatment diets (Table 3).

Digestibility trial

Table 4 presents the coefficients of ADMD and APD of the test diets and the seaweed meal. No significant differences ($p > 0.05$) were observed in the ADMD among the treatment diets; however, the APD of M4 was significantly lower ($p < 0.05$) than those of the CD and M8 ($p < 0.05$).

Shrimp zootechnical performance

The values of shrimp zootechnical performance in Table 5 were assessed for the two green seaweed meal treatments plus the CD. The performance parameters IC, FCR, WG, SGR and PER showed no significant differences ($p > 0.05$) among the treatment diets. After 29 days of the experiment, the juveniles exhibited significant differences ($p < 0.05$) in survival and yield parameters. M4 had 84.4% survival and 0.876 kg m^{-3} yield, higher than the CD group, whereas M8 had the lowest values, 55.5% and 0.593 kg m^{-3}, respectively (Table 4).

The values for shrimp body protein retention were assessed for the two green seaweed meal and the CD (Table 5). No significant differences ($p > 0.05$) were observed in the final protein content and protein retention.

Discussion

Aquatic feed stability can be achieved with the inclusion of binders. Thus, the texture of the feed needs to be improved so that it can remain immersed in the water for at least 1 h without disintegrating and decreasing the bioavailability of nutrients; this will also help reduce wastage (Volpe et al., 2012; Valenzuela-Cobos & Vargas-Farías, 2020; Aaqillah-Amr et al., 2021).

After 2 h of immersion in seawater, the DMR in M4 (97%) was higher than that in 3.5% kelp meal (93.7%) (Cruz-Suárez et al., 2006), but the values were similar with the inclusion of 3.33% meal from the green *Ulva clathrata* and the brown seaweeds *Ascophyllum nodosum* and *Macrocystis pyrifera* (Cruz-Suárez et al., 2009). The DMR value of up to 90% in M4 and M8 may be considered excellent, according to Cuzon et al. (1994), who pointed out that pellets for shrimp must maintain a minimum of 90% DMR after a 1-h immersion in water. Despite the longer time of 2-h in water immersion, the DMR was over 90%, strongly supporting the gelling, hydrocolloid and binding properties of the green seaweed meal for shrimp feed. These results are important given the shrimps' peculiar feeding behaviour of slow eating and tendency to manipulate food before ingestion.

The water absorption capacity of the pellet is related to its texture; a strong agglutination could affect it. Therefore, the ability of the blend to absorb or retain water is necessary to provide a soft and easy-to-eat meal for the shrimps (Cerecer-Cota et al., 2005). This property of the water absorption capacity of the pellet influences seaweed polymers to form gels and produce viscous solutions and is regulated by the type and quantity of polysaccharides (Cruz-Suárez et al., 2006, 2009; Lahaye & Robic, 2007; Argüello-Guevara & Molina-Poveda, 2013).

Table 4. Apparent digestibility coefficients for dry matter (%ADMD) and protein (%APD) of the test feed and ingredient in *Penaeus vannamei* juveniles and the digestibility of the seaweed meal.

Parameter	Diets				Digestibility of the seaweed meal
	CD [1]	M4 [2]	M8 [3]	p value	
%ADMD	83.11 ± 0.42^{a}	82.32 ± 0.36^{a}	81.33 ± 0.45^{a}	0.8812	80.97±0.77
%APD	91.56 ± 1.07^{a}	85.52 ± 0.24^{b}	89.44 ± 0.73 ^{a}	0.0000	

Values are the mean ± SD of three replicate samples. In the same line, values with different superscripts are significantly different ($p < 0.05$). [1] CD = control diet. [2] M4 = 4% green seaweed meal. [3] M8 = 8% green seaweed meal.

Table 5. Shrimp zootechnical performance when fed with diets containing different levels of seaweed meal Nutrigreen in experimental diets for 29 days.

	CD [1]	M4 [2]	M8 [3]	p value
Initial weight	1.42 ± 0.27	1.42 ± 0.27	1.42 ± 0.27	
Final weight (g)	3.42 ± 016^{a}	3.61 ± 0.17^{a}	3.71 ± 0.46^{a}	0.830
Initial biomass (kg m^{-3})	0.458 ± 016	0.458 ± 016	0.458 ± 016	0.850
Final biomass (kg m^{-3})	0.711 ± 0.08^{ab}	0.876 ± 0.12^{a}	0.593 ± 0.1^{2}b	0.028
Weight gain (g)	2.01 ± 0.19^{a}	2.20 ± 0.21^{a}	2.30 ± 0.56^{a}	0.820
IC (g)	3.80 ± 0.21^{a}	3.87 ± 0.27^{a}	4.29 ± 0.31^{a}	0.414
PER	1.72 ± 0.05^{a}	1.86 ± 0.02^{a}	1.76 ± 0.29^{a}	0.900
FCR	1.91 ± 0.05^{a}	1.77 ± 0.01^{a}	1.92 ± 0.03^{a}	0.797
SGR (% day^{-1})	3.03 ± 0.16^{a}	3.23 ± 0.16^{a}	3.30 ± 0.41^{a}	0.832
Survival (%)	73.33 ± 10.88^{ab}	84.44 ± 10.18^{a}	55.56 ± 6.28^{b}	0.016
Protein retention (%)	62.9± 0.12^{a}	63.8± 0.15^{a}	63.5± 0.12^{a}	0.851

Mean values and standard deviations. In the same line, values with different superscripts are significantly different ($p < 0.05$). IC: individual consumption; PER: protein efficiency ratio; FCR: feed conversion ratio; SGR: specific growth rate. [1] CD = control diet. [2] M4 = 4% green seaweed meal. [3] M8 = 8% green seaweed meal.

The water absorption capacities of 183.8% and 185.4% for M4 and M8, respectively, were higher than in the earlier report of 139% (Cruz-Suárez et al., 2006) with the inclusion of 3.5% brown seaweed meal. Also, these values were higher than the 132% observed with *U. clathrata* meal and the 112% with the brown seaweeds *A. nodosum* and *M. pyrifera*, all them with the inclusion of 3.33% (Cruz-Suárez et al., 2009). Furthermore, our values were higher than those of 70% and 68.4% in water absorption capacities with the 3% and 5% inclusion of brown seaweed meal, respectively, obtained by Argüello-Guevara & Molina-Poveda (2013). We can say that this blend of green seaweeds has a high-water absorption capacity to the pellet which is the property of retaining moisture under dry conditions (Percival, 1979; Lahaye & Rovic, 2007).

Numerically in this experiment, the greater the inclusion of this green seaweed meal, the greater the water absorption capacity, parameter which is also correlated with the dry matter loss in the diets (Cruz-Suárez et al., 2006). This pattern probably corresponds for blends different from hydrocolloids, as those of the green seaweeds which possess specifical characteristics linked to their type and composition of polysaccharides (Lahaye & Rovic, 2007).

Interestingly, M4 had a high-water absorption capacity but also showed the highest DMR, while the M8 diet, with more water absorption capacity than M4 (without significant differences), had the lowest DMR among treatments. At the same time, treatment M8 with the greatest green seaweed meal inclusion, had a lower loss of proteins (1.79%), despite the low DMR ($p < 0.05$). Observing Table 3, M4 shows greater standard deviation in %PL (1.18) than M8 (0.61), suggesting that some type of sulphated polysaccharides that were in more quantity in M8 might be favouring in some way the protein retention. Anyway, the %PL in both treatments (M4 and M8) were < 3.9%, a better value than the 13% observed after 1 h of water immersion in a previous study (Cruz-Suárez et al., 2009) and higher than the 1.43% and 0.88% reported in 3% and 5% of the brown kelp meal, respectively (Argüello-Guevara & Molina-Poveda, 2013). The lowest DMR (%) may be explained because leaching in M8 came mainly in one way from the additional minerals, since the seaweed meal had around 49% ash (Table 1) as the genus *Ulva* is rich in minerals (Tziveleka et al., 2019), and by the higher water-soluble sulfated polysaccharides content in this diet.

This %PL plus the DMR is a quantitative measure of the physical and chemical integrity of the food in the water (Cruz-Suárez et al., 2006). At the same time, both values are responsible for less feed waste in the aquatic environment. Cruz-Suárez et al. (2000) mentioned that the gel produced by seaweeds was affected by several factors, including composition of ingredients and nutrients. The seaweed meal used in this experiment was composed of three genera: *Ulva* spp., *Caulerpa* spp. and *Enteromorpha* spp., synonymous with *Ulva* and belonging to the ulvophytes (Tziveleka et al., 2019; Moreira et al., 2021; Kigdell et al., 2021), and have water-soluble sulfated polysaccharides composed of xylose, rhamnose, arabinose, galactose and glucuronic acid, distributed in repeating several combinations of disaccharide units (Robic et al., 2009; Synytsya et al., 2015). Lahaye & Robic (2007), Kigdell et al. (2019) and Moreira et al. (2021) indicated that ulvans exhibit a

high cation exchange capacity (with the presence of boric acid and calcium), are soluble and have low viscosity, but can form a stable gel with variable stiffness depending on the associated metal and the type of polysaccharide. Calcium has been reported to help boric acid ester formation with carboxylates and with rhamnogalacturonan II-borate cross-link formation. Borate, divalent cations, and pH may play important roles in promoting and/or stabilizing ulvan to promote the formation of hydrogel that depends of ulvan types. The role of borate and cations in ulvan gelation mechanism appears unique among polysaccharide hydrogel. These chemical, physicochemical and gelling properties of ulvan offer potential applications where texture need to be precisely controlled by cations, pH, or temperature (Percival, 1979; Lahaye & Robic, 2007; Lakshimi et al., 2020). The polysaccharides type in the seaweed meal used in this experiment was different because the blend of species: in *Caulerpa* this was the xyloarabinogalactan sulfate with positive optical rotation containing only small to trace amounts of uronic acids and rhamnose, but high levels of D-galactose, arabinose and, in some cases D-xylose; while in *Ulva* and *Enteromorpha* it was the glucuron-xyloramus sulfate with negative optical rotation –uronic acid-rich polysaccharides also containing rhamnose, xylose, and sometimes galactose–; in vivo they exist as mucilage or gels of varying stiffness. This family of polysaccharides are attractive candidates for novel functional and biologically active polymers for the food/feed, pharmaceutical, chemical aquaculture, and agriculture domains (Percival, 1979; Synytsya et al., 2015; Lakshimi et al., 2020). Table 1 shows the high ash content in the seaweed meal, which probably interfered negatively with the ion exchange in M8, where they were in more quantity; this fact plus the higher content of some sulfated polysaccharides with water-soluble properties probably affected negatively the DMR ($p < 0.05$) in M8.

The CD included sodium alginate and hexametaphosphate, which are necessary sequestrants when using fishmeal, as the calcium or other cations present in the by-products of the fish can prematurely react to the alginate when adding water, causing poor stability (Cuzon et al., 1994). The two ingredients necessary to bind the pellet are expensive but less profitable than the seaweed meal. This statement was supported by Cruz-Suárez et al. (2000), who stated that 'pure' alginates have been rarely used in aquaculture feeds, especially in experimental and larval feeds, owing to their high cost. However, the inclusion levels are generally lower than 5% when used.

Good quality ingredients, minimal nutrient leaching and pellet disintegration contribute to an accuracy in digestibility test, since leaching can overestimate values of digestibility. ADMD of diets or ingredients above 80% indicates good digestibility and can be used to select ingredients that optimise the nutritional value and cost of the formulated diet test ingredients (Guillaume & Ceccaldi, 2004; Yang et al., 2009). In this study, the ADMD (>80%) and APD (>85%) were relatively high in the test diets and ingredients (Table 4). The results indicate that using seaweed blends does not interfere with the digestibility of nutrients and proteins. This suggests that *P. vannamei* has the enzymes necessary to digest the carbohydrates in the green seaweed blend.

The most serious reports of illness and death related with seaweed come from the direct consumption of just three genera (*Caulerpa, Gracilaria, Acanthophora*) found in Pacific Rim countries (Cheney, 2016). Some species are considered a food source (*C. racemosa*, *C. lentillifera*) and others are considered deadly (*C. taxifolia*). It has been reported that *Caulerpa* is resistant to herbivorous fish due to the high content of sesquiterpenes and other chemicals that act as repellent substances, which are also considered poisons (Paul et al., 1987; Mohamed et al., 2020).

Caulerpa species appear to possess chemical deterrents to reduce predation. Chemical studies of various species of *Caulerpa* have shown that some of these seaweeds produce triterpenoids, caulerpine and caulerpicin, which are N-containing compounds with ichthyotoxic effects and anti-fat activity (Cheney, 2016), as well as diterpenoid alcohol, caulerpol (Paul & Fenical, 1982). Therefore, feeding certain levels of some green seaweed can produce toxicity, which could be one of the reasons why at a higher level of inclusion (M8) higher mortality was found ($p < 0.05$).

The M4 diet showed the lowest numerical value for FCR (1.77), while the highest value was for the M8 diet, which also obtained the lowest DMR value ($p < 0.05$) among all diets. This fact, in conjunction with the unconsumed losses, increased the IC value, which included significant leaching of nutrients such as polysaccharides and water-soluble vitamins, as the DMR greatly influence the performance parameters (Cruz-Suárez et al., 2000; Arguello-Guevara & Molina-Poveda, 2013). However, the M8 diet had the numerically highest weight gain (2.30 g), which could be explained by the significantly lower survival ($p < 0.05$), that would result in a lower stocking density, favoring greater individual weight gain.

In summary, the inclusion of a 4% green seaweed meal blend in shrimp feed supported growth and survival of juvenile *P. vannamei* without impairment in shrimp performance when compared to the CD. The M4 diet provided adequate values of DMR, water absorption capacity, protein loss and high ADMD and APD. Further work is required to explore separately with meals from *Caulerpa* spp, *Ulva* spp and *Enteromorpha* spp to elucidate which one contributes to better enhance for shrimp pellet.

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