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PRO RECTORIA DE INVESTIGACIÓN Y ESTUDIOS DE POSTGRADO
PROGRAMA DE POSTGRADO EN RECURSOS PESQUEROS Y ACUICULTURA

**EVALUACIÓN DE LA ACTIVIDAD ANTIBACTERIANA Y SUPLEMENTACIÓN
DIETÉTICA DE MACROALGAS VERDES EN (*Litopenaeus vannamei*).**

Jessie Marina Vargas Cárdenas

Tesis presentada al programa de Pos
Graduación de Recursos Pesqueros y
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Jessie Marina Vargas Cárdenas

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Resumen

Con el objeto de encontrar productos nutraceuticos y funcionales en ingredientes naturales para mejorar la calidad física (aglutinante, texturizante) y nutricional en dietas de juveniles de *L. vannamei*, se realizó el presente estudio con macroalgas verdes. Se realizaron dos experimentos: el primero para determinar cualitativamente el perfil fitoquímico en extractos metanólicos y el efecto antibacterial *in vitro* en extractos de metanol: diclorometano (1:1) de las especies *Ulva papenfussi* y *Ulva nematoidea* frente a la bacteria *Vibrio parahaemolyticus* mediante el método de discos de difusión. Los discos se impregnaron con 1.0, 1.5, 2.0, 3.0 y 4.0 mg del extracto. Se encontraron compuestos bioactivos con propiedades antibacterianas tales como fenoles, polifenoles, y flavonoides; las zonas de inhibición variaron significativamente ($P < 0.05$) de 8.33 ± 0.12 a 11.41 ± 0.73 mm para 1 a 3 mg de extractos respectivamente. En el segundo experimento se evaluó la calidad física y nutricional de tres dietas, una basal (dieta control) y dos dietas con 4(M4) y 8 (M8) % de inclusión de una mezcla de harina tres géneros de macroalgas: *Ulva*, *Enteromorpha* y *Caulerpa*. Las dietas fueron isonitrogenadas (300 g kg^{-1} proteína cruda) e isoenergéticas (3.3 Mcal kg^{-1}). Se determinaron el % retención de materia seca (RMS), porcentaje de pérdida de proteína de las dietas (%PP) y la capacidad de absorción de agua en el pellet. Para la evaluación nutricional se determinaron los parámetros zootécnicos en juveniles de *L. vannamei*, ($1.42 \pm 0.27 \text{ g}$) cultivados en acuarios a una densidad de $286 \text{ juveniles m}^{-3}$ (15 juveniles por acuario⁻¹) en un sistema de recirculación de agua por 29 días. La mejor retención de materia seca ($P < 0.05$) fue en el tratamiento M4 (97.06). No se tuvieron diferencias en los parámetros productivos a excepción de la sobrevivencia que fue significativamente mayor ($P < 0.05$) con la dieta M4, mientras que la mayor mortalidad fue registrada en el tratamiento M8. Se concluye que las macroalgas *U. papenfussi* y *U. nematoidea* tienen propiedades antibacterianas, frente al *V. parahaemolyticus* y la inclusión del 4% de la harina de macroalgas, mezcla de los géneros *Ulva*, *Enteromorpha* y *Caulerpa*, funciona como un buen aditivo funcional al ser un buen aglutinante y texturizante del alimento a la vez que mejora la sobrevivencia de juveniles de *L. vannamei*.

Palabras clave: Perfil fitoquímico, antibacterial, aglutinantes, harina de macroalgas verdes, *L. vannamei*

Abstract

To seek nutraceutical and functional products in natural ingredients, that could improve the physical (binder, texturizing) and nutritional quality of juvenile's *L. vannamei* feed, this study was carried out with green seaweed. Two experiments were performed: the first with the species *Ulva papenfussi* and *Ulva nematoidea* to qualitatively determine the phytochemical profile in methanolic extracts and the *in vitro* antibacterial test in methanol: dichloromethane (1: 1) extracts against the bacterium *Vibrio parahaemolyticus* using the disk diffusion method. The discs were impregnated with 1.0, 1.5, 2.0, 3.0, and 4.0 mg of the extract. Bioactive compounds with antibacterial properties such as polyphenol, phenols, and flavonoids were found, and the zones of inhibition varied significantly ($P < 0.05$) from 8.33 ± 0.12 to 11.41 ± 0.73 mm for 1 to 3 mg of seaweeds extracts respectively; In the second experiment, the physical and nutritional quality of 3 diets was evaluated, a basal one (control diet) and 2 diets with 4 (M4) and 8 (M8) % inclusion of a green seaweed meal, made from a mixture of three genera: *Ulva*, *Enteromorpha* and *Caulerpa*. The diets were isonitrogenated (300 g kg^{-1} crude protein) and isoenergetic (3.3 Mcal kg^{-1}). Water stability in % Dry matter Retention (DMR), protein loss (%), and water absorption capacity of the diets was determined. For the nutritional evaluation, the zootechnical parameters were evaluated in juveniles of *L. vannamei*, ($1.42 \pm 0.27 \text{ g}$) grown in aquariums at a density of $286 \text{ juveniles m}^{-3}$ (15 juveniles per aquarium) in a water recirculation system for 29 days. The best DMR ($P < 0.05$) was in M4 (97.06 %); There were no differences at the zootechnical parameters except for survival, which was higher ($P < 0.05$) in the M4 diet, while the highest mortality was observed in M8. It is concluded that *U. papenfussi* and *U. nematoidea* have antibacterial properties, against *V. parahaemolyticus*, and the inclusion of 4% of the green seaweed meal from the mixture of the genus *Ulva*, *Enteromorpha* and *Caulerpa*, is as a good functional additive as a good binder and texturizer of the pellet while adequately supports the survival of *L. vannamei* juveniles.

Keywords: Phytochemical screening, antibacterial, binders, green seaweed meal, *L. vannamei*, zootechnical parameters

Resumo

Com o objetivo de encontrar produtos nutracêuticos e funcionais em ingredientes naturais para melhorar a qualidade física (aglutinante, texturizante) e nutricional em dietas de juvenis de *L. vannamei*, realizou-se o presente estudo com macroalgas verdes. Foram realizados dois experimentos: o primeiro para determinar qualitativamente o perfil fitoquímico nos extratos metanólicos e o efeito antibacteriano *in vitro* nos extratos de metanol: diclormetano (1:1) das espécies *Ulva papenfussi* e *Ulva nematoidea* na presença da bactéria *Vibrio parahaemolyticus* mediante o método de discos de difusão. Os discos foram impregnados com 1.0, 1.5, 2.0, 3.0 e 4.0 mg do extrato. Foram encontrados compostos bioativos com propriedades antibacterianas tais como fenóis, polifenóis e flavanoides: as zonas de inibição variaram significativamente ($P < 0.05$) de 8.33 ± 0.12 a 11.41 ± 0.73 mm para 1 a 3 mg dos extratos respectivamente. No segundo experimento, avaliou-se a qualidade física e nutricional de três dietas, uma basal (dieta controle) e duas dietas com 4(M4) e 8(M8)% de inclusão de uma mistura de farinha de três gêneros de macroalgas verdes: *Ulva*, *Enteromorpha* e *Caulerpa*. As dietas foram isonitrogenadas (300 g kg^{-1} proteína crua) e isoenergéticas (3.3 Mcal kg^{-1}). Foram determinadas a % de retenção de matéria seca (RMS), a percentagem de perda de proteína das dietas (%PP), e a capacidade de absorção de água no pellet. Para a avaliação nutricional, foram determinados os parâmetros zootécnicos em juvenis de *L. vannamei*, ($1.42 \pm 0.27 \text{ g}$) cultivados em aquários a uma densidade de $286 \text{ juvenis m}^{-3}$ (15 juvenis por aquário⁻¹) num sistema de recirculação de água por 29 dias. A melhor retenção de matéria seca ($P < 0.05$) foi no tratamento M4 (97.06). Não houve diferenças nos parâmetros produtivos, à exceção da sobrevivência que foi significativamente maior ($P < 0.05$) com a dieta M4, enquanto a maior mortalidade foi registrada no tratamento M8. Conclui-se que as macroalgas *U. papenfussi* e *U. nematoidea* tem propriedades antibacterianas, ante ao *V. parahaemolyticus* e a inclusão de 4% da farinha de macroalgas, mistura dos gêneros *Ulva*, *Enteromorpha* e *Caulerpa*, funciona como um bom aditivo funcional ao ser um bom aglutinante e texturizante do alimento ao mesmo tempo que melhora a sobrevivência de juvenis de *L. vannamei*.

Palavras-chave: perfil fitoquímico, antibacteriano, aglutinantes, farinha de macroalgas verdes, *L. vannamei*, parâmetros zootécnicos.

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1- Introducción

1.1-Conceptualización de la Investigación

Según últimos reportes de la FAO (2020) la carnicultura está en incremento, y se observa que en 8 años casi ha duplicado su volumen de 5,478.8 toneladas métricas a 9,386.5T. Dentro de las especies comerciales, el camarón blanco (*Litopenaeus vannamei*) es la especie comercial más importante dentro de los crustáceos, entre otras cosas por su crecimiento rápido, soporta altas densidades y altas salinidades (CHAMORRO -LEGARDA et al., 2021;CORTÉS et al., 2021; ELIZONDO-REYNA et al., 2016; MANGOTT et al., 2020). En el 2018 la producción global de *L.vannamei* fue de 4 966 200 T que representó el 52.9% del cultivo de crustáceos y el 5.2% de la producción mundial de la acuicultura. (FAO, 2020).

En Perú, su producción alcanzó las 50 819T en el 2019, lo que corresponde al 31.51% de la producción nacional de acuicultura, facturando con el 63% del total de las exportaciones procedentes de la acuicultura (PRODUCE, 2019).

Se ha reportado que luego de años de cultivo de *L. vannamei* en altas densidades, han surgido numerosos problemas, como el deterioro del entorno agrícola, medioambiental y los frecuentes brotes de enfermedades (DENG et al., 2013; LÓPEZ-LEÓN et al., 2016) producidas por patógenos muchos de los cuales han sido introducidos a través del comercio internacional de nauplios, pos larvas y reproductores (FIGUEREDO et al., 2020). Aunque se considera a los virus como los más perniciosos a esta industria, las bacterias *Vibrio* también han causado pérdidas significativas tanto en etapas larvarias como de crecimiento (LÓPEZ-LEÓN et al., 2016). Así, la Enfermedad de Necrosis Hepatopancreática Aguda (AHPND), también llamada síndrome de muerte temprana (SME) (VARELA-MEJÍAS et al.,2017) es causada por una bacteria gram negativa, *Vibrio parahaemolyticus* y afecta a dos de las especies más cultivadas: el camarón tigre (*Penaeus monodon*) y el camarón blanco (*L.vannamei*) (NACA 2012; ESQUER MIRANDA et al.,2016; SOTO-RODRÍGUEZ et al., 2015). Para contrarrestar estos problemas, se han utilizado comúnmente antibióticos y desinfectantes. Actualmente, el uso de medicamentos veterinarios está cada vez más restringido debido a los numerosos efectos secundarios para el medio ambiente y la salud como el desarrollo de cepas bacterianas resistentes. Además, puede haber una acumulación química tanto en el agua como en los tejidos animales con efectos adversos al medio ambiente o a los consumidores o destruir las poblaciones microbianas normales en el entorno de la acuicultura. (DENG et al., 2013; REVERTER et al., 2014; THANIGAIVEL et al., 2016).

Otro problema encontrando en los cultivos de camarón son sus lentos hábitos de consumo, lo cual origina la lixiviación de nutrientes y la desintegración de los pellets en el agua (CRUZ-SUÁREZ et al., 2006; CUZON et al.,1994; LIM AND CUZON,1994). La pérdida de materia seca en el pellet es la razón más cuestionable de la mala calidad del agua, que a su vez provoca bajos rendimientos y altas mortalidades. (LIM AND CUZON,1994). Todos estos problemas de la carcinicultura mencionados en los párrafos anteriores, están relacionados con las estrategias planteadas del Objetivo 14 sobre Desarrollo Sostenible (ODS 14) de la Agenda 2030 (UNITED NATIONS, 2015), donde se pide conservar y utilizar de forma sostenible los océanos, los mares y los recursos marinos para el desarrollo sostenible, los que están en severo riesgo debido entre otras a la eutrofización y acidificación de los océanos. Es así y en este sentido que se tiene un reto y un compromiso pendiente en la carcinicultura.

Por lo tanto, los desafíos en el cultivo del camarón están orientados por un lado en la búsqueda de ingredientes no convencionales, de alta calidad (ELIZONDO-GONZÁLEZ et al., 2018; LITTLE, et al., 2016;), que puedan ayudar a controlar patógenos y que promuevan su estado sanitario, debido a que el hacinamiento, el manipuleo periódico, cambios bruscos de temperatura, mala calidad de agua entre otros, contribuyen a cambios fisiológicos que desencadenan el estrés oxidativo que los vuelve vulnerables a brotes de enfermedades. (COBOS AND FARÍAS, 2020; NAVARRO et al., 2020; REVERTER et al., 2014). Por otro lado, buscar aditivos que confieran características técnicas especiales como la hidroestabilidad que es crucial para asegurar la lixiviación mínima de los nutrientes como aminoácidos, vitaminas y minerales, minimizando la desintegración del pellet en el agua. (ARGUELLO-GUEVARA y MOLINA- POVEDA, 2013; NRC, 2011; OBALDO et al., 2002).

El uso de un enfoque ecológico con base biológica para controlar las infecciones y así promover el crecimiento ha atraído últimamente más atención (THANIGAIVEL et al., 2016). Las macroalgas sirven como una fuente prolífica de productos naturales con propiedades terapéuticas y nutritivas. Son capaces de producir exclusivos metabolitos secundarios, estructuralmente únicos y de importancia biomédica que son utilizados para el desarrollo de nuevos ingredientes funcionales. (ESQUER-MIRANDA et al., 2016; GARCIA-VAQUERO et al., 2017; GOMATHI Y SHEBA, 2017; VATSOS et al., 2015). Últimamente existe un creciente interés por las algas marinas como fuente de ingredientes para alimentos acuícolas (COSTA REZENDE et al., 2021; LIU et al., 2020; MANGOTT et al., 2020; MOHAN et al., 2019). Se ha reportado que las macroalgas estimulan el apetito, promueven el crecimiento,

poseen propiedades nutraceuticas en cantidades que pueden promover el sistema inmune debido a los compuestos bioactivos encontrados dentro de sus polisacáridos (LAHAYE Y ROVIC, 2007; MOHAN et al., 2019; REVERTER et al., 2014; RUSHDI et al., 2020; THANIGAIVEL et al., 2016).Igualmente Khalil et al.(2018) indican que la utilización comercial de las macroalgas ha tenido por años especial significado en su uso como hidrocoloides, aprovechando sus propiedades gelificantes, retención de agua y habilidad como emulsificantes.

En el caso de las macroalgas verdes (Clorofitas), los polisacáridos sulfatados (SPs) que conforman las paredes celulares son los ulvanos, los cuales poseen propiedades gelificantes (KIDGELL et al., 2019; LAHAYE y ROVIC, 2007; MOREIRA et al., 2021., TZIVELEKA et al., 2019). Estos SPs exhiben muchas actividades biológicas benéficas que incluyen inmunomoduladores, antivirales, antioxidantes, antihiperlipidémicos y anticáncer (KIDGELL et al., 2019; SMIT, 2004; THUY et al., 2016; WIJESEKARA et al., 2011). Estas macroalgas utilizan químicos antimicrobianos específicos y metabolitos secundarios importantes en la interacción ecológica entre macroorganismos y microorganismos, por lo que pueden ser fuente promisoría de compuestos bioactivos, (CHAKRABORTY et al., 2010; MOHAN et al., 2019). Moreira et al. (2021) mencionan que los ulvanos no son compuestos exclusivos de las especies del género *Ulva* y están presente en otros géneros como *Monostroma*, *Caulerpa*, *Codium* o *Gayralia* .

Por lo expuesto anteriormente, el objetivo de este trabajo es el estudio de la capacidad nutraceutica y funcional de algunas macroalgas de la clase *Ulvophyceae* del género *Ulva*, *Enteromorpha* y *Caulerpa* para el cultivo de *L. vannamei*

Hipótesis

HP1: Las macroalgas del género *Ulva* poseen compuestos bioactivos con propiedades antibacterianas frente al *Vibrio parahaemolyticus*

HP2: La adición de las harinas de macroalgas verdes de los géneros *Ulva*, *Caulerpa* y *Enteromorpha*, como aditivo en las dietas mejoran la calidad física del pellet y los parámetros productivos en los juveniles de *Litopenaeus vannamei*.

1.3 – Objetivos del trabajo (general y específicos)

✓ Objetivo General

Evaluar la capacidad nutracéutica y funcional de algunas macroalgas de la clase *Ulvophyceae* para el cultivo de *Litopenaeus vannamei*.

✓ Objetivos específicos

1. Evaluar y comparar el perfil de compuestos fitoquímicos y capacidad antibacterial frente a la presencia del *Vibrio parahaemolyticus* de las especies *Ulva papenfussi* y *Ulva nematoidea*;
2. Evaluar la harina de una mezcla de macroalgas verdes de los géneros *Ulva*, *Enteromorpha* y *Caulerpa* como aglutinante en dietas para *L. vannamei*.;
3. Evaluar la harina de una mezcla de macroalgas verdes de los géneros *Ulva*, *Enteromorpha* y *Caulerpa* como aditivo funcional sobre los parámetros productivos de *L. vannamei*

2. Phytochemical screening and antibacterial assessment of two seaweeds *Ulva papenfussi* and *Ulva nematoidea* (Chlorophyta) against the bacterium *Vibrio parahaemolyticus*.

Artículo científico enviado a la revista Food Science and Technology International

Phytochemical screening and antibacterial assessment of two seaweeds *Ulva papenfussi* and *Ulva nematoidea* (Chlorophyta) against the bacterium *Vibrio parahaemolyticus*

Vargas, J. et al., 2021

Abstract

This study was conducted to determine whether the seaweed extracts from *Ulva papenfussi* and *Ulva nematoidea* could be alternatives for preventing *Litopenaeus vannamei* vibriosis caused by the bacterium *Vibrio parahaemolyticus*. Phytochemical screening was performed on methanolic extracts to qualitatively determine the main groups of compounds. Bioactive compounds with antibacterial properties such as phenols, polyphenols, flavonoids, and large quantities of carbohydrates were found in almost the same amounts in both seaweeds. *U.papenfussi* showed higher quantities of lipids and alkaloids than *U.nematoidea*. Seaweeds extracts prepared (v:v) with a 1:1 methanol: dichloromethane solvent was used for the in vitro test using the disc diffusion method (MDD). Filter paper discs were impregnated with 1.0, 1.5, 2.0, 3.0, and 4.0 mg of the extracts. The results showed antibacterial activity against *V. parahaemolyticus* in a dose-dependent manner in both seaweeds. The zone of inhibition varied significantly from 8.33 ± 0.12 to 11.41 ± 0.73 mm for 1 to 3 mg of extract levels, respectively. In conclusion, both seaweeds have antibacterial activity in their crude extracts against this bacterium. This is the first report of phytochemical screening and antibacterial activity of these seaweeds against *V. parahemolyticus*.

Keywords: antibacterial activity, seaweed, phytochemical screening, *Ulva papenfussi*, *Ulva nematoidea*, *Vibrio parahaemolyticus*

1. Introduction

Litopenaeus vannamei is the most farmed crustacean worldwide. According to FAO (2020), white-leg shrimp (*L.vannamei*) production was 4 966 200 T, representing 52.9% of the total crustacean production by aquaculture. In Peru, where *L.vannamei* is a native species, its production accounted for 32 292 T in 2018, corresponding to 24% of the total aquaculture production in the country and increased to 14.86% more than in 2017 (PRODUCE, 2019). However, its farming is constantly threatened by disease outbreaks caused by viruses, fungi, bacteria, and pathogenic protozoa. Although viruses are considered to cause the greatest losses to this industry, *Vibrio* bacteria have also caused significant losses to shrimp aquaculture in both larval and growth out stages (LÓPEZ-LEÓN et al., 2016).

Thus, acute hepatopancreatic necrosis disease (AHPND) is caused by the bacteria, *Vibrio parahaemolyticus*, and affects the most cultivated shrimp specie: the white-leg shrimp (*L. vannamei*) (BOONSRI et al., 2017; ESQUER -MIRANDA et al., 2016; NACA, 2012; SOTO-RODRÍGUEZ et al., 2015). The outbreak of this disease has resulted in a huge reduction in shrimp production in countries such as Thailand where the loss of the shrimp food source impacted the global shrimp business worldwide (BOONSRI et al., 2017). This bacterium has moved from Asia to América (ALDAY-SANZ, 2019) and has been detected in shrimp farms in the Tumbes region of Peru (VICENTE, 2019). To prevent or treat disease outbreaks, antimicrobials and other veterinary drugs are regularly administered. Currently, the use of veterinary drugs is becoming more restricted because they present numerous side-effects to the environment and health. The massive use of antibiotics resulted in the development of resistant bacterial strains. Moreover, there can be a chemical accumulation both in water and animal tissues with adverse effects on the environment or consumers (REVERTER et al., 2014; THANIGAIVEL et al., 2016).

Therefore, it is necessary to seek and develop novel antimicrobial agents from renewable resources such as seaweeds. These organisms serve as a prolific source of natural products with therapeutic and nutritional properties. They are capable of producing exclusive secondary metabolites of biomedical importance that are used as functional ingredients (ESQUER-MIRANDA et al., 2016; GARCIA-VAQUERO and RAJAURIA, 2017; GOMATHI and SHEBA, 2017; VATSOS et al., 2017). Within these compounds are the marine polysaccharides isolated from seaweeds which have been probed to enhance the

immune system with important pharmacological properties. These can be influenced by macroalgal biology and by factors such as its biological cycle, geographic location, harvest, and extraction method (GARCIA-VAQUERO and RAJAURIA, 2017; MOHAN et al., 2019; SHEVCHENKO et al., 2009; THANIGAIVEL et al., 2016).

Within this big group of seaweeds belongs the green seaweeds, commonly referred to as “sea lettuces”, which are widely distributed in the seas. The sulfated polysaccharides of greatest interest in this genus are ulvans, which contributes to 9%–36% of the dry weight of *Ulva* biomass, and together with other polysaccharides such as cellulose, xyloglucan and glucuronate constitute >45% of the dry weight (KIDGELL et al., 2019; THUY et al., 2016). These seaweeds are of immense pharmaceutical and agricultural value (CHAKRABORTY et al., 2010; IN HAE KIM et al., 2007; KIDGELL et al., 2019).

Mangott et al., (2020) investigated the role of these seaweeds as a functional water bioremediation agent in *L. vannamei* aquaculture in a recirculating aquaculture system. They found that a 200 L sump with a filter of live *Ulva lactuca* stocked at a mean density of 0.5 g L⁻¹ suppressed or decreased potential pathogens, such as *Vibrio alginolyticus* and *Photobacterium sp.* Other researchers mentioned a positive response in *L.vannamei* juveniles co-cultivated with *U.lactuca* that were later challenged with white spot syndrome virus and *V. parahaemolyticus* (ANAYA et al., 2019). Even more, an antibacterial response was found in *U.lactuca* extracts against *V. parahaemolyticus* (IMMANUEL et al., 2004; IN HAE KIM et al., 2007; ESQUER-MIRANDA et al., 2016). Chakraborty et al., (2010) and Sivakumar et al., (2014) tested *U.faciata* extracts and found potent antibacterial activity against *V. parahemoliticus* and *V. harveyii* respectively, and Dussault et al., (2016) showed antibacterial activity of *Ulva* sp extracts against gram-positive bacteria.

Species of the genus *Ulva* also inhabit the Peruvian coast (LI and ZAFRA, 2009). On the Central Coast of Perú, specifically in the district of La Punta, Constitutional Province of Callao at Department of Lima, *Ulva papenfussi* and *Ulva nematoidea* are found. Nevertheless, there is a lack of information on the phytochemical constituents and antimicrobial properties of these seaweeds that could be exploited for benefits in aquaculture and to clarify if any antimicrobial activity could be different among these species. To date, there has been no study to elucidate this response.

Altogether, this study aimed to investigate and compare the phytochemical compounds in these seaweeds' structures and ascertain their capacity of producing compounds with antimicrobial activities against *V. parahaemolyticus*.

2. Materials and methods

2.1 Obtaining and preparing dry biomass from seaweeds

The microalga *U. papenfussi* was collected from the wetland “Poza de la Arenilla” (10 ° 04'30” S and 78 ° 10'30” W) (IMARPE report 41), whereas *U. nematoidea* was collected from Cantolao beach (12 ° 4'9.98 "S and 77 ° 9'45" W). Both samples were transferred under refrigeration (“cool box”) to the Aquaculture Laboratory of the Faculty of Fisheries at the National Agrarian University la Molina (UNALM) and the herbarium (Botanical Division) of the Natural History Museum that belongs to the National University of San Marcos (Lima, Peru) for identification and taxonomic classification.

The seaweeds were gently washed with tap water to remove sand and epibionts (IN HAE et al., 2007; DUMBRAWA et al., 2018). They were subsequently dried in a dryer—Klimatechnik dehydrator at 45°C until constant weight was achieved. The dried biomass was ground (pulverized) in an Oster blender and then screened through a 250-µm sieve to obtain the highest surface-to-volume ratio during extraction. The obtained powder was kept at 4°C until later use (ESQUER-MIRANDA et al., 2016).

2.2 Phytochemical Screening of *U. nematoidea* and *U. papenfussi*

For the phytochemical screening, 10 g of each dried seaweeds powder were crushed with 10% analytical grade methanol (w/v) and kept under constant stirring for 24 h (magnetic stirrer Benchmark model H4000-HS-E-US states) at room temperature and subsequently subjected to centrifugation (Centrifuge refrigerated model Rotofix 32, Hettich, Germany) at 4500 rpm. The supernatant obtained was collected and concentrated in a rotary evaporator (Heraeus - Labofuge 400R- UK) to 50% of its volume, forming the room temperature extract named E1. The residue called “frame” was oven-dried at 40°C (Binder stove-Germany) until the dry weight was obtained. Next, methanol was added to obtain a 10% (w/v) extract and was subjected to constant stirring for 24 h at 70°C. Similar to the previous step, it was centrifuged at 4500 rpm and the supernatant was collected, obtaining the extract at 70°C named E2.

To qualitatively determine the main group of compounds in seaweeds structures, methanolic extracts from both seaweeds were separated into several groups of chemical substances according to standard procedures of Rondina and Coussio (1989).

Both methanolic extracts, were divided into four equal fractions: A, B, C and D that were analyzed according Rondina and Coussio (1989). All these processes were performed at the Research Institute of Biochemistry and Molecular Biology of the National University Agraria La Molina (UNALM-IIBBM).

2.3 Seaweeds extracts for antibacterial testing

To obtain moderately polar components in the extract (CARVALHO et al., 2017; CHAKRABORTY et al., 2010; DUSSAULT et al., 2016), samples of 6 g of both dried seaweeds meal (*U. papenfussi* and *U. nematoidea*) were macerated for a week in a 1: 1 (v / v) solution of dichloromethane (CHCl₂) and methanol. The extraction was performed at room temperature and subjected to continuous agitation on a magnetic stirrer (magnetic stirrer Benchmark model H4000-HS-E -US states) for 7 days. The macerate was filtered and brought to volume with the solvent mixture for the determination of triterpenes/steroids (Liebermann–Burchard reaction) where chloroform was replaced with dichloromethane. The mixture was filtered, and the aliquot was concentrated in vacuum (rotary evaporator Heraeus- Labofuge 400R-Germany) at <45°C to obtain a dry crude extract for *U. nematoidea* (57.8mg) and *U. papenfussi* (63.1 mg) which were stored at 4°C (BOSCH refrigerator model had62vsa40 ixl - Germany) until use (CHAKRABORTY et al., 2010).

2.4 Antibacterial activity of *U. papenfussi* and *U. nematoidea* extracts against the gram-negative bacterium *V. parahaemolyticus*

The *V. parahaemolyticus* strain EB101 of the American Type Culture Collection (ATCC 17802 PK/5) was provided by SANIPES (National Fisheries Health Agency). This bacterium was reactivated in trypticase soy broth (Liofilchem: 610053) and incubated at 30°C for 18 h. Subsequently, it was seeded at 10⁹ CFU ml⁻¹ (Mc Farland 0.5) using cotton swabs on sterile petri dishes (90 mm diameter) containing Mueller Hinton agar (HIMEDIA M173) (GONZÁLEZ and PRADO, 2003). For susceptibility testing, all the stock solutions were

resuspended in dimethyl sulfoxide (DMSO). The in vitro antibacterial activity of the seaweeds compounds was tested by the disc diffusion method (MDD) described by Desbois and Smith (2015). Filter paper (Liofilchem-9999) 6-mm discs were impregnated with 1, 1.5, 2, 3, and 4 mg extracts of *U. nematoidea* and *U. papenfussi* (BRAND micropipette). As a negative control, 6-mm discs were impregnated with the maximum DMSO volume loaded onto the discs with the extracts. It was conducted in additional plates because there was no space in the plates with the extracts. As a positive control, a 5-mm paper disc impregnated with oxytetracycline (30 µg) was used. Subsequently, the plates were incubated at 30°C for 24 h (Memmert) until a clear zone of inhibition appeared. This evaluation was performed in triplicates.

2.5 Data analysis

The Cochran and Shapiro–Wilk tests were employed to determine the homogeneity of the variances and normality of the data, respectively. Two-way analysis of variance was used to determine the effect of the species (*U. papenfussi* and *U. nematoidea*) and extract levels (1.0, 1.5, 2.0, 3.0, and 4.0 mg ml⁻¹) and their interaction. Tukey’s test was used when differences between factors and treatments were detected ($P < 0.05$). Language R vs i386.3.3.3 was used as the software program.

2.6 Results

Qualitative phytochemical screening of the seaweeds

The content of the extracts (10 g of dried seaweeds) at room temperature was 0.011g ml⁻¹ for *U. papenfussi* and 0.00405g ml⁻¹ for *U. nematoidea*.

Table 1 shows the phytochemical screening of the four different fractions. It revealed the presence of carbohydrates, flavonoids, alkaloids, polar triterpenes, tannins, phenols, polyphenols, lipids, steroids and cardenolides in both seaweeds’ compounds.

In fraction “A” at room temperature, both seaweeds showed high concentration for carbohydrates and phenolic compounds as well as moderate concentration for flavonoids and tannins. *U. nematoidea* exhibited a higher amount of polyphenols and tannins, unlike *U. papenfussi* which showed moderate ones, but got higher amounts in lipids concentrations than *U. nematoidea*. Conversely, in fraction “B”, no differences were found among seaweeds for

cardenolide contents (high amount); steroids (moderate); triterpenes (low amount) and anthraquinones which did not appear. In fraction C, *U. papenfussi* showed a moderate amount for alkaloids, whereas *U. nematoidea* showed a low amount. Both seaweeds were negative for leucoanthocyanidins. Finally, in fraction D, both seaweeds did not show alkaloids, steroids or flavonoids, while being slightly positive for triterpenes; however, *U. papenfussi* showed few alkaloids and *U. nematoidea* was negative.

At 70°C, only fraction “A” showed phytochemicals compounds, displaying similar amounts in both seaweeds; high amounts for polyphenols, tannins, and phenolic compounds; and moderate amounts for carbohydrates, flavonoids and lipids.

The Liebermann–Burchard dichloromethane/methanol test showed an abundant presence of steroids for both seaweeds species, whereas the triterpene content was abundant for *U. papenfussi* and scarce for *U. nematoidea*.

Antibacterial assay

Factorial analyses revealed no significant difference in the inhibition zones (mm) against *V. parahaemolyticus* among seaweeds and in their interaction but, showed significance difference ($p < 0.05$) among extract levels ranging from 1 to 4 mg. (Table 2 and figure 1).

This zone varied from 8.0 to 12.0 mm for 1.5 to 3 mg of extract, respectively, showing an increasing dose-dependent trend in both seaweeds. The maximum inhibitory effect was reached with extracts levels of 3 and 4 mg for both seaweeds.

2.7 Discussion

Seaweeds are the most important source of carbohydrates and according to Garcia-Vaquero and Rajauria (2017), the extraction of polysaccharides is performed by treating the dry biomass of the seaweeds with solvents at different temperatures ranging from room temperature up to 70°C. In this experiment, high concentration of carbohydrates was found in the phytochemical screening in *U. papenfussi* and *U. nematoidea* at room temperature and at 70°C, denoting their abundance in both seaweeds. The cell walls of marine algae characteristically contain sulfated polysaccharides, with the ulvans being the water-soluble polysaccharides derived from *Ulva*. They possess anti-coagulant, anti-oxidant,

immunostimulant, anti-bacterial, anti-protozoal and anti-fungal capacity (PATEL, 2012; THUY et al., 2016; TZIVELEKA et al., 2019; YAICH et al., 2013). The high concentration of carbohydrates found in our research is consistent with that reported for *U. reticulata* by Gomathi and Sheba (2017) and in *U. lactuca* by Whankatte and Ambhore (2016) using methanol as solvent for phytochemical screening. Similarly, phenolic compounds, polyphenols and tannins were found in large quantities in both seaweeds species. Vinayak et al., (2011) indicated that phenolic compounds and flavonoids are known for their pharmacological properties. Our results are consistent with those of Gomathi and Sheba (2017), who found polyphenols in the chloroform extract and phenolic compounds in the acetone extract of *U. reticulata* ; with the findings of Whankatte and Ambhore (2016) who detected phenolic and tannin compounds in methanolic extracts of *U.lactuca* and those of Shankhadarwar (2015) who found high quantity of phenols in *U.lactuca* but to a lesser extent in *Enteromorpha intestinalis* (currently known as *Ulva intestinalis*) using thin-layer chromatography. In our study, moderate amounts of flavonoids were found in both species. In contrast, Shankhadarwar (2015) identified large quantities of flavonoids in *U.lactuca* and *E. intestinalis*, whereas Gomathi and Sheba (2017) found flavonoids in the acetone extract of *U.reticulata*. All these compounds have antioxidant, antibacterial, anti-cancer and antiviral activities.

Other compounds found in low (*U.nematoidea*) to moderate (*U.papenfussi*) amounts were alkaloids, which are used as antiseptics owing to their antibiotic activity (ROBERTS, 1998). Gomathi and Sheba (2017) found alkaloids in *U.reticulata* and Shankhadarwar (2015)found in moderate quantities in *U.lactuca* and *E.intestinalis*. However, they were not reported by Whankatte and Ambhore (2016) in *U.lactuca* evaluated with eight different types of solvents, including methanol.

Terpenes were found in both seaweeds but in small amounts. These substances are metabolites produced by marine organisms to ensure their survival. In this manner, they develop cytotoxic, antiproliferative, antifouling, antifungal and antimicrobial activity (ROCHA and DUARTE, 2014).

Lipids amounts were moderate in *U.nematoidea* but abundant in *U.papenfussi*. These molecules were also found by Whankatte and Ambhore (2016) in the methanolic extracts of *U.lactuca* and by Shankhadarwar (2015) who applied the Bligh and Dyer method for lipid extraction and reported them in greater amounts in *E.intestinalis* than in *U.lactuca*. Guihéneuf

et al., (2015) indicated that both macro and microalgae are capable of synthesizing large amounts of polyunsaturated long-chain fatty acids, such as eicosapentanoic acid (20: 5 n-3 and docosahexanoic acid (22: 6 n-3), with important nutraceutical and pharmaceutical properties. Therefore, it can be inferred that these compounds are within the lipids reported for both seaweeds.

Both seaweeds have high amounts of carbohydrates and a variety of simple and complex bioactive compounds, with variation or not in their amounts. However, most compounds with pharmaceutical properties. This variation could be due to the extraction method (VATSOS, 2017) (i.e. filtration, thin-layer chromatography) or by the solvent used which has an important role in these determinations (GOMATHI and SHEBA, 2017; VATSOS, 2017; WHANKATTE and AMBHORE, 2016). All these factors could affect the chemical composition, molecular weight, performance and rheological and textural properties of the ulvans (YAICH et al., 2013); similarly, the content of these bioactive compounds could be modified by environmental factors such as temperature, salinity, light and nutrients. Therefore, variations according to seasons or changes in ecological conditions can stimulate or restrict the biosynthesis of various compounds as indicated by Yaich et al., (2013), Michalak and Chojnacka (2015), Ryan Young et al., (2015) and Vatsos (2017).

The above mentioned could explain certain differences found in their phytochemical profile, since they were extracted from different ecosystems besides that they are different species. *U. papenfussi* was collected from a wetland ("Poza de la Arenilla"). According to IMARPE report (2014), in this wetland the average concentration of total suspended solids, and the microbial contamination exceeded the legal levels allowed by the ECA (National Environmental Quality Standards for Water), whereas *U. nematoidea* was harvested from Cantolao beach, which is a place for summer recreation purposes. Despite this, owing to the compounds found on the phytochemical screening, it can be assumed that both seaweeds have antibacterial activity.

The diffusion disc test is an in vitro test widely used to study the antimicrobial activity (VATSOS, 2017) and to select bioactive compounds for later evaluation in vivo. In this experiment, an antibacterial effect against *V. parahaemolyticus* was obtained in an extract of methanol and dichloromethane (1:1). The maximum inhibition zone of 12 mm for *U. nematoidea* and 11.6 mm in *U. papenfussi* were obtained with 3 and 4 mg, respectively, which are considered moderate values (CHAKRABORTY et al., 2010). These authors found similar

values (13-mm) at doses of 150 ug diterpenoid labdanes of *U. fasciata* obtained by chromatography from methanol/dichloromethane (1:1) extracts. However, they were in contrast to the higher values reported by Inmanuel et al., (2004) who obtained a 17-mm inhibition zone using N-butanol as a solvent to obtain extracts from *U. lactuca*. Similarly, In Hae et al., (2007) obtained 10 and 18-mm inhibition zones with doses of 0.2 and 0.4 mg ml⁻¹, respectively, applying several doses of ethyl ether extract of *U. lactuca*. They concluded that extracts were potent in activity against *V. parahaemolyticus* in a dose-dependent manner. Similarly, Esquer-Miranda et al. (2016) evaluated methanol extracts of *U. lactuca* and established the minimum inhibitory concentration with 1.5 mg of the extract.

These differences in the inhibition zone related to doses and the solvent used were investigated by Saritha et al., (2013) who applied 50 µl of *U. lactuca* extract (without specifying the weight) against *V. parahaemolyticus*. They reported a 19-mm inhibition zone with butanol extracts, 18-mm with acetone extract, 16-mm with ethyl alcohol, 12-mm with methanol, 10-mm with ethanol and 6-mm with chloroform. All these results are consistent with the findings of Vatsos (2017) in the sense that the extraction method and the solvent used can affect the effectiveness of the extracts.

In a same way, a dose-response effect was also observed in this research, where mean inhibition zones of 8.3, 9.7 and 11.07 mm were obtained for doses of 1, 2 and 3 mg ml⁻¹, respectively. This behavior was also described by Sivakumar et al., (2014), who evaluated the antibacterial effect of *U. fasciata* extracts in ethyl acetate against *V. harveyii*. They obtained inhibition zones of 3.3, 8.3 and 12.3 mm with 100, 200 and 300 µg of the extract, respectively.

This variation in the antibacterial activity against pathogenic microbes has also been observed by Tziveleka et al., (2019) who attributed it to the structural difference of ulvans among *Ulva* species. Several factors such as species metabolites, the season of collection, biological activity of ulvans, and type of ulvans' polysaccharide are suggested to affect their sugar composition, molecular weight and sulfate content. These characteristics are defined by genetics as well as environmental factors. However, no clear correlation has been established to date because biological studies are not supported by a systematic structure of the ulvans investigated.

Finally, the antibacterial activity not only can be attributed to differences in ulvans but to other compounds reported in the phytochemical screening. The effectiveness of the extracts depends on the extraction method and the solvent used which affects the antibacterial activity.

2.8 Conclusion

In conclusion, *U.papenfussi* and *U.nematoidea* have antibacterial activity against the *V. parahaemolyticus* strain EB-101 with methanol/dichloromethane extract without a significant difference among them. Further studies are required to elucidate the effectiveness of these antibacterial properties of the seaweeds in a multitrophic culture with *L. vannamei* to control the spread of the bacterium *V. parahaemolyticus*. This is the first report of a phytochemical screening of these species and the discovery of antibacterial activity in these species against *V. parahaemolyticus*.

2.9 References

ALDAY-SANZ, V. Aquaculture biosecurity seen from the ground. In: OIE Global Conference on Aquatic Animal Health. **World Organization for Animal Health**, Santiago, Chile (Abril 2019). 2019

ANAYA-ROSAS, R., RIVAS-VEGA, M., MIRANDA-BAEZA, A., PIÑA-VALDEZ, P., NIEVES-SOTO, M. Effects of a co-culture of marine algae and shrimp (*Litopenaeus vannamei*) on the growth, survival and immune response of shrimp infected with *Vibrio parahaemolyticus* and white spot virus (WSSV). **Fish and Shellfish Immunology** v.87, p.136 – 143. 2019 <https://doi.org/10.1016/j.fsi.2018.12.071>

BOONSRI, N., RUDTANATIP, T., WITHYACHUMNARNKUL, B. WONGPRASERT, K. Protein extract from red seaweed *Gracilaria fisheri* prevents acute hepatopancreatic necrosis disease (AHPND) infection in shrimp. **Journal of Apply Phycology** v.29, p.1597–1608.2017 DOI 10.1007/s10811-016-0969-2

CARVALHO A.P, BATISTA D., DOBRETSOV, S. and COUTINHO, R. Extracts of seaweeds as potential inhibitors of quorum sensing and bacterial growth. **Journal of Apply Phycology** v.29, p.789–797. 2017 DOI 10.1007/s10811-016-1014-1

CHAKRABORTY K, LIPTON A.P, RAJ R and VIJAYAN, K. Antibacterial labdane diterpenoids of *Ulva fasciata Delile* from southwestern coast of the Indian Peninsula. **Food Chemistry** v.119, p.1399–1408. 2010 doi:10.1016/j.foodchem.2009.09.019

DESBOIS, A. and SMITH, V. Disk diffusion assay to assess the antimicrobial activity of marine algal extracts. In: Stengel D. and Connan S. (eds). Natural products from Marine Algae. Methods and protocols. Springer New York ISSN 1940-6029 (electronic), p 403-410. 2015

DUSSAULT, D., DANG, VU K., VANSACH, T., HORGEN, F., LACROIX, M. Antimicrobial effects of marine algal extracts and cyanobacterial pure compounds against five foodborne pathogens. **Food Chemistry** v.199, p.114–118. 2016 <http://dx.doi.org/10.1016/j.foodchem.2015.11.119>

ELIZONDO-REYNA, E., MEDINA-GONZÁLEZ, R., NIETO-LÓPEZ, M., ORTIZ-LÓPEZ, R., ELIZONDO-GONZÁLEZ, R., POWELL, M., RICQUE-MARIE, D., CRUZ-SUÁREZ, E. Consumption of *Ulva clathrata* as a dietary supplement stimulates immune and lipid metabolism genes in Pacific white shrimp *Litopenaeus vannamei*. **Journal of Apply Phycology** v.28, p.3667–3677. 2016 <https://doi.org/10.1007/s10811-016-0889-1>

ESQUER-MIRANDA, E., NIEVES-SOTO, M., RIVAS-VEGA, M., MIRANDA-BAEZA, A. and PIÑA-VALDEZ, P. Effects of methanolic macroalgae extracts from *Caulerpa sertularioides* and *Ulva lactuca* on *Litopenaeus vannamei* survival in the presence of *Vibrio* bacteria. **Fish and Shellfish Immunology** v.51, p.346–350. 2016 <http://dx.doi.org/10.1016/j.fsi.2016.02.028>

FAO. (2020) The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome. Available at <http://www.fao.org/3/ca9229en/CA9229EN.pdf> (Accesed 06 june 2020).

GARCIA-VAQUERO, M., RAJAURIA, G., O'DOHERTY, J., SWEENEY, T. Polysaccharides from macroalgae: Recent advances, innovative technologies and challenges in extraction and purification. **Food Research International** v.99, p.1011–1020. 2017 <http://dx.doi.org/10.1016/j.foodres.2016.11.016>

GOMATHI, K. and SHEBA, A. Phytochemical screening and heavy metal analysis of *ulva reticulata*. **Asian Journal of Pharmaceutical and Clinical Research** v.11, n.4, p.84–88. 2018. DOI: 10.22159/ajpcr.2018.v11i4.23012

GONZÁLEZ, J. and PRADO, P. Técnicas de Bacteriología, Análisis en Fresco, Calidad de Agua y Buenas Prácticas de Manejo y Bioseguridad en Granjas Camaroneras. Programa de Capacitación. Comité Estatal de Sanidad Acuícola de Sinaloa. CESASIN. Mazatlán MX. 2003.p17-18

GUIHÉNEUF, F., SCHMID, M. and STENGEL, D. Lipids and Fatty Acids in Algae: Extraction, Fractionation into Lipid Classes, and Analysis by Gas Chromatography Coupled with Flame Ionization Detector (GC-FID). In: Stengel, D. and Connan, S. (eds). Natural products from Marine Algae. Methods and protocols. Springer New York, p. 173–190.

IMARPE Instituto del Mar del Perú. (2014) Calidad ambiental del humedal Poza La Arenilla-Callao, 2008. V.41, p. 1- 4. 2014

IMMANUEL, G., VINCYBAI, V., SIVARAM, V., PALAVESAM, A. and MARIAN, M. Effect of butanolic extracts from terrestrial herbs and seaweeds on the survival, growth and pathogen (*Vibrio parahaemolyticus*) load on shrimp *Penaeus indicus* juveniles. **Aquaculture** v.236, p.53–65. 2004 doi:10.1016/j.aquaculture.2003.11.033

IN HAE, K., DONG HUN, L., SANG HYUN, L., JONG MYUNG, H., BAE JIN, H., SUNG KOO, K. and JAE HWA, L. Antibacterial Activity of *Ulva lactuca* against methicillin-Resistant *Staphylococcus aureus* (MRSA). **Biotechnology and Bioprocess Engineering** v.12, p.579–582. 2007. <https://doi.org/10.1007/BF02931358>

KIDGELL, J., MAGNUSSON, M., DE NYS, R. and GLASSON, Ch. Ulvan: A systematic review of extraction, composition and function. Review article. **Algal Research** v.39, p.101–422. 2019. <https://doi.org/10.1016/j.algal.2019.101422>

LI, G. and ZAFRA, A. (2012). Composición, abundancia y diversidad de macroalgas en el litoral de puerto Malabrigo, La Libertad – Perú 2009. *Sciendo*. 15(1):33 - 42. Available at: <http://revistas.unitru.edu.pe/index.php/SCIENDO/article/view/448/384> (accessed 20 december 2020).

LÓPEZ-LEÓN, P., LUNA-GONZÁLEZ, A., ESCAMILLA-MONTES, R., FLORES-MIRANDA, M., FIERRO-CORONADO, J. and ÁLVAREZ-RUIZ, P. Isolation and characterization of infectious *Vibrio parahaemolyticus*, the causative agent of AHPND, from the whiteleg shrimp (*Litopenaeus vannamei*). *Latin America Journal Aquatic Research*, v.44, n.3, p.470–479, 2016 Available at:

https://scielo.conicyt.cl/scielo.php?script=sci_arttext&pid=S0718-560X2016000300005&lng=es (accessed 03 January 2020)

MANGOTT, A., NAPPI, J., DELLI PAOLI CARINI, A., GONCALVES, P., HUA K., DOMINGOS, J.A., DE NYS, R., and THOMAS, T. *Ulva lactuca* as a functional ingredient and water bioremediator positively influences the hepatopancreas and water microbiota in the rearing of *Litopenaeus vannamei*. **Algal Research** v.51, p.102040. 2020 <https://doi.org/10.1016/j.algal.2020.102040>

MICHALAK, I. and CHOJNACKA, K. Production of Seaweed Extracts by Biological and Chemical Processes. Cap 7. In: Se-Kwon K and Chojnacka K (eds). Marine Algae Extracts, Products, and Applications. Germany. Volume 1 Willey, 2015 p.121–143.

MOHAN, K., RAVICHANDRANA, S., MURALISANKARB, TH., UTHAYAKUMARC, V., CHANDIRASEKARC, R., SEEDEVID, P., GANESAN, R., KARTHICK, R. D. Application of marine-derived polysaccharides as immunostimulants in aquaculture: A review of current knowledge and further perspectives. **Fish and Shellfish Immunology** v.86, p.1177–1193. 2019 <https://doi.org/10.1016/j.fsi.2018.12.072>

NETWORK OF AQUACULTURE CENTRE'S IN ASIA-PACIFIC (NACA) Report of the Asia Pacific Emergency Regional Consultation on the Emerging Shrimp Disease: Early Mortality Syndrome (Ems)/Acute Hepatopancreatic Necrosis Syndrome (AHPNS), 9-10 Published by the Network of Aquaculture Centers in Asia and the Pacific, Bangkok, Thailand. August 2012. Available at: <http://library.enaca.org/Health/Publication/ahpns-emergency-consultation-report.pdf> (accessed 30 August 2019).

PATEL, S. Therapeutic importance of sulfated polysaccharides from seaweeds: Updating the recent findings. **3 Biotech** v.2, p.171–185. 2012 DOI 10.1007/s13205-012-0061-9

PRODUCE. ANUARIO ESTADÍSTICO PESQUERO Y ACUÍCOLA. Disponible en; <https://ogeiee.produce.gob.pe/index.php/en/shortcode/oe-documentos-publicaciones/publicaciones-anuales/item/949-anuario-estadistico-pesquero-y-acuicola-2019> > (Accessed 30 November 2020).

REVERTER, M., BONTEMPS, N., LECCHINI, D., BANAIGS, B. and SASAL, P. Review Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and

future perspectives. **Aquaculture** v.433 p.50–61. 2014
<https://doi.org/10.1016/j.aquaculture.2014.05.048>

ROBERTS, M. and WINK, M. Alkaloids Biochemistry, Ecology, and Medicinal Application. In: Roberts M (ed) Alkaloids. New York and London: Plenum Press, 1998. pp.1-8.

RONDINA, R. AND COUSSIO, J. Estudio fitoquímico de plantas medicinales Argentinas (1). Revista de Investigaciones Agropecuarias, INTA. Serie 2, **Biología y Producción Vegetal**. Buenos Aires-Argentina v.6, n.22, p.351-366. 1989.

SARITHA, K., ASWATHI, E., PRIYALAXMI, M. and PATTERSON, J. Antibacterial Activity and Biochemical Constituents of Seaweed *Ulva lactuca*. **Global Journal of Pharmacology** v.7, n.3, p.276–282. 2013 DOI: 10.5829/idosi.gjp.2013.7.3.75156

SATARUPA, R., NAYAK, B., MADHUMITA, R., ABHIJIT, M., DEVALINA, H., CHATTERJEE, A., CHAKRABORTY, S. and CHAKRABORTY, T. Antimicrobial and Phytochemical Screening of Seaweeds: *Enteromorpha intestinalis* and *Ulva lactuca* Collected from Indian Sunderbans Delta Region. IOSR. **Journal of Pharmacy and Biological Sciences** v.11, n.4, p.1-5. 2016 DOI: 10.9790/3008-1104020105

SHANKHADARWAR, S. Phytochemical screening of marine algae *Ulva lacuta* (Linn.) and *Enteromorpha intestinalis* (Linn.). **Journal of Chemical and Pharmaceutical Research** v.7, n.12, p.419–423. 2015 ISSN : 0975-7384

SHEVCHENKO, N., BURTSEVA, Y., ZVYAGINTSEVA, T., MAKAREVA, T., SERGEEVA, O., ZAKHARENKO, A., ISAKOV, V., THI LINH, N., XUAN HOA, N., MINH, L.Y. B., VAN HUYEN, P. Polysaccharides and sterols from green algae *Caulerpa lentillifera* and *C. sertularioides*. **Chemistry of Natural Compounds** v.45, n.1, p.1–5. 2009 <https://doi.org/10.1007/s10600-009-9223-3>

SIVAKUMAR, K., SUDALAYANDI KANNAPPAN, S., DINESHKUMAR, M., PRASANNAKUMAR, P. Evaluation of marine macroalga, *Ulva fasciata* against bioluminescent causing *Vibrio harveyi* during *Penaeus monodon* larviculture **African Journal of Microbiology Research** v.8, n.8, p.803–813. 2014 DOI: 10.5897/AJMR2013.6233

SOTO-RODRIGUEZ, S.A., GOMEZ-GIL, B., LOZANO-OLVERA, R., BETANCOURT-LOZANO, M., MORALES-COVARRUBIAS, M.S. Field and experimental evidence of *Vibrio parahaemolyticus* as the causative agent of acute hepatopancreatic necrosis disease of

cultured shrimp (*Litopenaeus vannamei*) in northwestern Mexico **Applied and Environmental Microbiology** v.81, p.1689–1699. 2015 doi: 10.1128/AEM.03610-14

THANIGAIVEL, S., CHANDRASEKARAN, N., MUKHERJEE, A. THOMAS, J. Seaweeds as an alternative therapeutic source for aquatic disease management. **Aquaculture** v.464, p. 529–536. 2016 <https://doi.org/10.1016/j.aquaculture.2016.08.001>

THUY, T., QUACH, T., NGUYEN, N., VU LUONG, D., BUI, L., VAN, T. Structure and cytotoxic activity of ulvan extracted from green seaweed. **International Journal of Biological Macromolecules** v.93, p.695–702. 2016. <https://doi.org/10.1016/j.ijbiomac.2016.09.040>

TZIVELEKA, L-A; IOANNOU, E; ROUSSIS, V. Ulvan, a bioactive marine sulphated polysaccharide as a key constituent of hybrid biomaterials: A review. **Carbohydrate polymers**, v. 21, p. 355-370. 2019 <https://doi.org/10.1016/j.carbpol.2019.04.074>

VATSOS, I. AND REBOURS, C. Seaweed extracts as antimicrobial agents in aquaculture. **Journal of Applied Phycology** v.27, p.2017–2035. 2015 DOI: 10.1007/s10811-014-0506-0

VICENTE VILLANUEVA, A. Detección de genes PirABVp en aislados del género *Vibrio* del langostino *Penaeus vannamei* de centros de cultivo del departamento de Tumbes. Master Thesis, Universidad Peruana Cayetano Heredia, PE. 2019 Available at: <http://repositorio.upch.edu.pe/handle/upch/6399> (accessed 30 march 2020).

VINAYAK, R.C., SUDHA, S.A., CHATTERJI, A. Bio-screening of a few green seaweeds from India for their cytotoxic and antioxidant potential. **Journal of the Science of Food and Agriculture** v.91, p.2471–2476. 2011 DOI: 10.1002/jsfa.4490

WHANKATTE, V., AMBHORE, J. Phytochemical screening and antioxidant activity of *Ulva lactuca*. **International Journal of Current Research** v.8, n.9, p.38265–38269. 2016 ISSN:2320-7817(p) 2320-964X (0)

YAICH, H., GARNA, H., BESBES, S., PAQUOT, M., BLECKER, CH., ATTIA, H. Effect of extraction conditions on the yield and purity of ulvan extracted from *Ulva lactuca*. **Food Hydrocolloids** v.31, p.375–382. 2013 <https://doi.org/10.1016/j.foodhyd.2012.11.013>

TABLE 1. Phytochemical screening of *U. papenfussi* and *U. nematoidea* seaweeds at room temperature and 70°C in four chemical fractions

Fraction	Chemical groups	Reactive	Room Temp.		70°C	
			<i>Ulva papenfussi</i>	<i>Ulva nematoidea</i>	<i>Ulva papenfussi</i>	<i>Ulva nematoidea</i>
A	Carbohydrates	Molisch	+++	+++	++	++
	Flavonoids	Shinoda	++	++	++	++
	Lípidos	Vapour I ₂	+++	++	++	++
	Polyphenols and tannins	K ₂ Cr ₂ O ₇	++	+++	+++	+++
	Tannins	jellow	++	++	+++	+++
	Phenolic compounds	FeCl ₃	+++	+++	+++	+++
B	Anthraquinones	Borntrager	-	-	-	-
	Esteroids	Lieberman	++	++	-	-
	Triterpenes	n-Burchard	+	+	-	-
	Cardenolides	Kedde	+++	+++	-	-
C	Alkaloids	Mayer	++	+	-	-
	Alkaloids	Wagner	++	+	-	-
	Leucoanthocyanins	Rosenheim	-	-	-	-
	Cardenolides	Kedde	++	++	-	-
D	Alkaloids	Mayer	+	-	-	-
	Alkaloids	Wagner	-	-	-	-
	Esteroids	Lieberman	-	-	-	-
	Triterpenes	n-Burchard	+	+	-	-
	Flavonoids	Shinoda	-	-	-	-

(-) Not present ; (+) level low; (++) Moderate Positive; (+++) High level.

Table 2. Inhibition zone (mm) of 1:1 methanol: dichloromethane extracts against the bacterium *V. parahaemolyticus*

Extract level (mg)	Zone of inhibition (mm)						Mean inhibition zone (mm)
	<i>U. papenfussi</i>			<i>U. nematoidea</i>			
	A1	A2	A3	A4	A5	A6	
1	8.40	8.40	8.00	8.40	8.40	8.20	8.3000 ^c
1.5	8.96	8.40	8.80	8.96	9.36	8.60	8.8467 ^{bc}
2	9.52	10.00	9.60	9.64	10.80	8.96	9.7533 ^b
3	10.00	11.20	11.00	10.60	11.64	12.00	11.0733 ^a
4	10.48	10.56	11.60	10.80	12.00	11.00	11.0733 ^a

A = experiment repetition

Means in rows with different lowercase letters are significantly different ($p < 0.05$, Tukey's test) among the inhibition zones

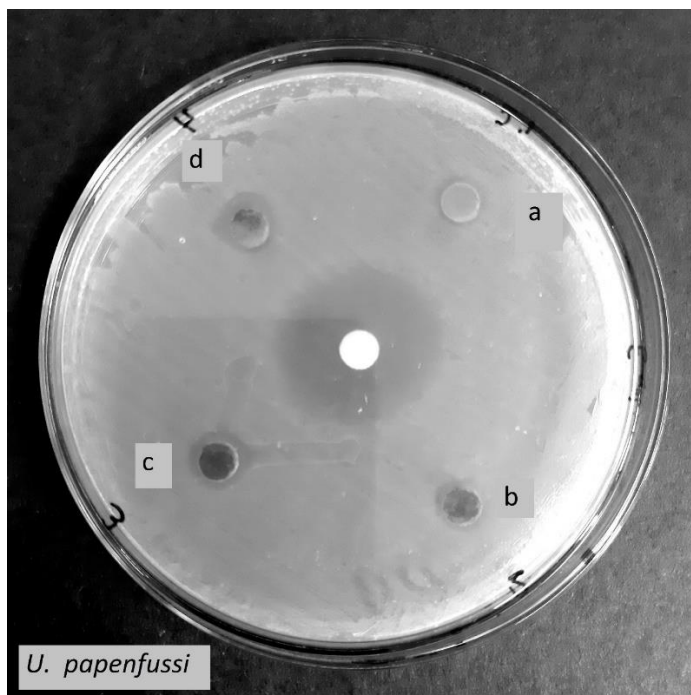
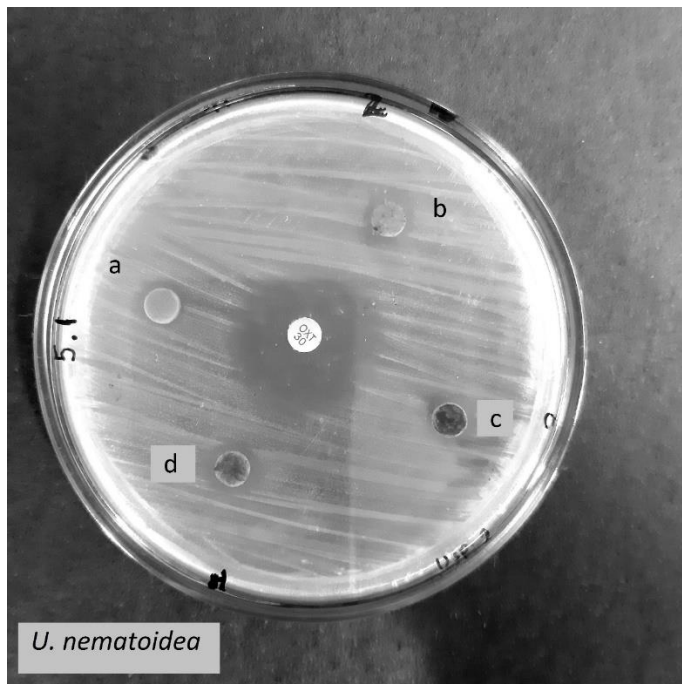


FIGURE 1 Antibacterial activity of *U. nematoidea* and *U. papenfussi* extracts against *V. parahaemolyticus* strain EB101 of the American Type Culture Collection (ATCC 17802 PK/5) a, 1.5 mg dry weight /ml. b, 2 mg dry weight /ml. c, 3mg dry weight /ml. d,4 mg dry weight/ml. At the center, 30 mg oxytetracycline disc as a positive control.

3- Effect of a blend of green seaweed meal (*Ulva spp.*, *Caulerpa spp.*, and *Enteromorpha spp.*) as a quality enhancer in juvenile's diets of *Litopenaeus vannamei*

Artículo Científico II

(Será sometido al Aquaculture Research)

Effect of a blend of green seaweed meal (*Ulva spp.*, *Caulerpa spp.*, and *Enteromorpha spp.*) as a quality enhancer in juvenile's diets of *Litopenaeus vannamei*

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Keywords: green macroalgae, *L.vannamei*, binders, zootechnical parameters

Abstract

This study aimed to assess a green seaweed meal mixture of three genera: *Ulva*, *Enteromorpha*, and *Caulerpa*, as a functional additive in *Litopenaeus vannamei* diets. Two experiments were conducted for determining the physical and nutritional quality of three experimental diets: a control diet (0% seaweeds) and two diets with 4 (M4) and 8 % (M8) of seaweed meal. The diets were isonitrogenous (300 g kg⁻¹ crude protein) and isoenergetic (3.3 Mcal kg⁻¹DE). The nutritional quality of the diets was evaluated through zootechnical parameters in *L. vannamei* juveniles (1.42±0.27g), and pellet quality was determined by percentages of dry matter retention (%DMR), protein loss (%PI), and water absorption capacity. They were reared at a density of 286 juveniles m⁻³ for 29 days in a water recirculation system. The best DMR was obtained with the M4 diet and higher water absorption capacity and less PI with the M8 diet. No differences were in the productive parameters except for survival (P<0.05), with the best in the M4 diet and the highest mortality in M8. In conclusion, 4% of green seaweed meal can be efficiently utilized as a feed additive like a suitable binder and texturizer while improving the survival of *L. vannamei* juveniles.

Keywords: *Caulerpa*, *Ulva*, *L.vannamei*, binders, zootechnical parameters

1. Introduction

Within the commercial species, the white shrimp (*Litopenaeus vannamei*) is the most important species within the crustaceans, primarily due to its rapid growth, supports high farming densities and high or lower salinities (ELIZONDO-REYNA et al., 2016; LEGARDA et al., 2021; MANGOTT et al., 2020). The development of shrimp farming is increasing, as shown by the latest Food and Agriculture Organization (FAO) reports (2020). It has almost doubled its volume in the last eight years from 5,478.8T to 9,386.5T. In 2018, *L. vannamei* production rose 4,966,200 T, representing 52.9% of the crustacean farming and 5.2% of the world's aquaculture production (FAO, 2020). In Peru, its production reached 50 819T in 2019, which corresponds to 31.51% of the national aquaculture production, billing with 63% of the total exports from aquaculture (Ministerio de la Producción Perú [PRODUCE], 2019).

Actual challenges in shrimp farming are to look for unconventional ingredients that allow high-quality foods (ELIZONDO-GONZÁLEZ et al., 2018; LITTLE et al., 2016). At this point, binders have both a substantial effect on the physical integrity of the pellet. On the one hand, it means finding foods with unique technical characteristics such as high-water stability, which expressed the retention of dry matter after a period of immersion in water (ARGÜELLO-GUEVARA Y MOLINA-POVEDA, 2013). Retention of dry matter is crucial to ensure minimal leaching of nutrients such as amino acids, vitamins, and minerals, and the pellet disintegration in the water due to the slow feeding habits of the shrimp (ARGÜELLO-GUEVARA and MOLINA-POVEDA, 2013; NRC, 2011., OBALDO et al., 2002). The bioavailability of nutrients since the loss of dry matter in the pellet is the most questionable reason for poor water quality, which in turn causes low yields and high mortality (COBOS and FARÍAS, 2020; VOLPE et al., 2010).

On the other hand, there is challenging to find functional additives in *L. vannamei* diets that can promote growth and survival, which are diminished due to crowding, handling, environmental changes, poor water quality, and other stressors. These contribute to physiological changes that trigger oxidative stress making them vulnerable to disease outbreaks (COBOS and FARÍAS, 2020., NAVARRO et al., 2019; REVERTER et al., 2014) since shrimp farming is exposed constantly to the presence of viruses, bacteria and fungi.

Exists interest in using seaweeds as a source of ingredients for aquaculture feed, which has been developing in recent years (COSTA REZENDE et al., 2021; MOHAN et al., 2019).

Seaweeds have been reported to stimulate appetite, promote growth and possess nutraceutical properties in amounts that can help against oxidative stress due to the bioactive compounds found within their polysaccharides (LAHAYE and ROBIC, 2007; MOHAN et al., 2019; NAIEL et al., 2020; REVERTER et al., 2014; THANIGAIVEL et al., 2016). Additionally, the commercial use of seaweeds has had for years special significance in its use as hydrocolloids, taking advantage of its gelling properties, water retention, and ability as emulsifiers (KHALIL et al., 2018).

In the green seaweeds (Chlorophytes), ulvans are the sulfated polysaccharides (SPs) that make up the cell walls, which have gelling properties (KIDGELL et al., 2019; LAHAYE and ROVIC, 2007; MOREIRA et al., 2021; TZIVELEKA et al., 2019). These SPs exhibit many beneficial biological properties, including immunomodulatory, antiviral, antihyperlipidemic, antioxidant, and anticancer activity (KIDGELL et al., 2019; WIJESEKARA et al., 2011). Moreira et al.,(2021) mentioned that ulvans are not exclusive compounds of the *Ulva* species and are present in another genus such as *Monostroma*, *Caulerpa*, *Codium*, or *Gayralia*.

There is scientific evidence of the nutraceutical effect of green seaweeds that works when they are incorporated as supplements in shrimp's feeds (AKBARY and AMINIKHOEI, 2017; COSTA REZENDE et al., 2021; ELIZONDO-GONZÁLEZ et al.,2018; ELIZONDO-REYNA et al., 2016; GE et al., 2018; LARAMORE et al., 2018; LIU et al., 2020; MANGOTT et al., 2020; PUSPITASARI et al., 2019; RODRÍGUEZ-GONZÁLEZ et al., 2014) or when they have been used in co-cultures with shrimp (ANAYA-ROSAS et al., 2019; ANH et al., 2021 ;BRITO et al., 2014., LEGARDA et al., 2021; LY et al., 2021).

Moreover, many studies mentioned above evaluated the isolated use of *Ulva*, *Caulerpa*, and *Enteromorpha* seaweed meal as an ingredient in commercial shrimp feed formulation with positive results in terms of performance; however, the effect of a green seaweed mix meal of three genera *Ulva*, *Enteromorpha*, and *Caulerpa*, as a functional additive in diets for *L. vannamei* and its role in the pellet quality parameters (stability of pellet after water immersion, percentage of protein loss and water feed absorption) remain unknown.

Therefore, this study aimed to evaluate a mixture of green seaweed meal composed of *Ulva spp*; *Caulerpa spp*, and *Enteromorpha spp*, as a feed additive in levels of 4 and 8% in

juvenile's diets of *L. vannamei* to improve the quality of the pellet, as well, to improve shrimp performance in growth and survival under laboratory conditions.

2 Materials and methods

2.1 Experimental conditions and tank facilities

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

L. vannamei (juveniles) were obtained from a commercial laboratory (Marina Azul SAC of Tumbes-Perú) and were distributed and acclimated for one week in a fiberglass tank (1 shrimp by 6 L) with a salinity of 33 g/ L. They were fed with commercial feed (28 % crude protein and 6 % crude lipid, Langostina-Purina) at 7 % of their body weight-adjusted daily, and twice a day: at 08:00 and 17:00 h. After acclimation, the juveniles (1.42 ± 0.27 g) were transferred into nine RAS glass aquariums (0.50 x 0.35 x 0.30 m) at a density of 286 shrimp m^{-3} (15 shrimp per aquarium). The RAS was filled with clean seawater filtered through 300 and then 0.050 mm-mesh and sterilized with 25 mg/L of formalin. The sides of the aquariums were covered with black plastic to provide a sense of “refuge” to the shrimp. They were provided additional aeration from a 0.5 HP blower to allow better oxygenation. A water flow of $4 L min^{-1} tank^{-1}$ was maintained.

2.2 Seaweed meals and experimental diets

The green seaweed meal (Chlorophyta), commercially known as Nutrigreen, came from PSW SAC. The seaweed meal is a blend of green seaweed of the genus *Caulerpa*, *Enteromorpha*, and *Ulva*. These were collected from natural grasslands in Paracas's Bay (Pisco-Ica) and Sechura's Bay (Piura). The meal composition came from the company, which is in Table 1. Two experimental diets were formulated to include 4 and 8 % green seaweed meals and tested along with a basal control diet without seaweed meal. The diets had similar levels of crude protein ($300 g kg^{-1}$) and digestible energy ($3.3 Mcal kg^{-1}$).

Should be Table 1

The basal diet (Control Diet - CD) contained as binder a mixture of sodium alginate and sodium hexametaphosphate. These ingredients, plus a part of the wheat meal, were replaced in the basal diet until they attained 4 and 8 % inclusion of the seaweed meal. The ingredients were mixed from the lowest to the highest concentration for its elaboration to accomplish a good blend. Then the premix, which includes vitamins, minerals, and all the ingredients in small quantities, were added, with the soy lecithin and fish oil plus hot water, obtaining a wet mixture, which was pressed twice through an electric meat grinder (Ferton) to increase ingredients agglomeration. The noodles obtained were dried at 60°C for 1 hour, in a dehydrator (Klimatech). When dry, they were broken and sieved to a particle size of two millimeters. They were bagged and kept refrigerated until later use. According to the standards methods (AOAC 2005), the proximate composition analysis of the diets was done in the nutritional assessment laboratories (LENA) at the School of Animal Husbandry, UNALM. The formulation of these experimental diets and the chemical composition are in Table 2.

Should be Table 2

The shrimp were fed three times a day (at 08:00, 12:00, and 16:00 h), 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 0.25mm mesh baskets by siphoning after each feeding. Before feeding, each morning feed waste and feces were removed.

At the beginning of the experiment, ten animals were sacrificed for body protein determination (N x 6.25); in the same way, at the end of the experiment, a composite sample of shrimps from each treatment (n = 3) was also analyzed for body protein content. The shrimps were sacrificed using the thermal shock technique.

2.3 Pellet quality

In order to assess the quality of the pellet, parameters of the stability of pellet after water immersion, percentage of protein loss in the pellet, and water feed absorption were evaluated.

Pellet stability after water immersion

The stability of the pellets was evaluated in terms of percentage of dry matter retention (DMR) after 2 hours, according to Argüello-Guevara and Molina-Poveda (2013), using three replicates per treatment. For this experiment, 3g of pellets (length average 3mm and 2 mm of diameter) of each experimental diet were weighed on an analytical balance, Sartorius, (4

decimals), and placed inside labeled and tared PVC tubes, with a 0.25-mm-mesh bottom. Then they were placed in a bath -Maria thermoregulated (Memmert), shaken at 30 rpm, 28 ° C, and 30 g/L of salinity. After immersion, the tubes were allowed to drain for 20 minutes and placed in an oven (Memmert) at 60°C until constant weight. This weight was registered for each sample. Feed stability was calculated using the following formula:

$$\text{DMR (\%)} = 100 - (\text{DW}_{\text{bi}} - \text{DW}_{\text{ai}}) / \text{DW}_{\text{bi}} \times 100$$

Where:

DW_{bi} = Dry weight of diet before water immersion

DW_{ad} = Dry weight of diet after drying

Protein loss

Dry leached pellet allows the calculation of the protein loss (%PL) that occurred during leaching, and was calculated according to Cruz -Suárez et al. (2006):

$$\% \text{PL} = ((100 * [\text{PP}_{\text{bi}}] - (100 - \text{PMS}) * [\text{PP}_{\text{al}}])) / \text{PP}_{\text{bi}}$$

Where:

PP_{bi} = Protein percentage before leaching

PMS = % loss dry matter

PP_{al} = Protein percentage after leaching

Water feed absorption

Water feed absorption was calculated following Argüello-Guevara & Molina-Poveda (2013) by gravimetric difference. Three grams of a sample of each treatment by triplicate were used. They were placed in tared PVC tubes with a 0.25-mm mesh bottom and placed for one hour of immersion in water at 30 g/L of salinity and 28 °C. The excess water was drained for 40 minutes. Then after each unit was weighed on an analytical balance (Sartorius) and registered the weight gained. The formula used was:

$$\text{Water absorption (\%)} = (\text{WP}_{\text{ai}} - \text{Tw} - \text{WP}_{\text{bi}}) / \text{WP}_{\text{bi}}$$

Where

WP_{ai} = Weight pellets after 60 minutes immersion

Tw = pvc tubes weight

WP_{bi} = pellets weight before immersion

2.4 Water quality

Dissolved oxygen (mg/L) and temperature (° C) values were daily monitored twice a day, at 08:00 and 16:00 with an oximeter (YSI model 55, Yellow Springs, Ohio, USA) to maintain values of 5.5mg/L and 28°C; Additionally, once a week at 4:00 p.m., salinity (g/L) was evaluated with an Atago refractometer (model 2493 Master S / MillM, Japan), total ammonia-nitrogen (TAN) with a spectrophotometer (Thermo Scientific, Helio Gamma Model, England) according to the standard method of APHA(1998), and pH with a potentiometer (Schott Model Lab 850, Germany) for maintaining values of 33 mg/L; 0.24 ± 0.02 mg/L and 7.7 respectively.

2.5 Zootechnical parameters

Individual weight (g) was recorded at 1, 7, 14, 21, and 29 days to determine zootechnical parameters. All zootechnical parameters were determined using the following equations: Biomass = sum of the individual weights (Kg) per m³; feed conversion rate (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 cultivation; weight gain (WG) = (average final weight–average initial weight); Individual consumption (IC) = $\sum 1^{29}$ (experimental tank consumption day¹/total of shrimps' day¹); survival rate (S) (%) = (final number of shrimp per treatment / initial number of shrimp per treatment) ×100 and body protein retention % = (final weight X final protein content) – (initial weight X initial protein content) X100/ protein intake.

2.6 Statistical analysis

Data were tested for homogeneity of variances (Brown–Forsythe test) and normality (Shapiro–Wilk test) before applying a one-way variance analysis (ANOVA; $P < 0.05$). A posterior two-way ANOVA was made among diets treatments and period time to determine an interaction. Significant differences between means were compared using Tukey's test ($P < 0.05$). The statistical software package used was Lawstat R version 2.4.1 (Statistical Analysis System software, New York, USA).

3. Results

3.1 Water Quality

The values of the monitored water quality parameters for 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) were not significantly different ($P > 0.05$) among the treatments.

3.2 Diet Quality

After the 2 hours of water immersion, the diets showed significant differences ($P < 0.05$) in dry matter retention (% DMR), with the lowest value of 92.53% for the M8 diet, followed by the CD diet with 94% (with 3% alginate) and the highest DMR value was for M4 diet with 97.06%. There were no significant differences ($P > 0.05$) upon protein loss (% PL) among treatments, although the values were between 1.79% (M8) to 3.92% (M4), denoting an inverse relationship to the inclusion of the seaweed meals. Also, there were no significant differences in the water absorption capacity; diets with the inclusion of green seaweed meal showed 183% to 185% (Table 3).

Should be Table 3

The physical appearance of the experimental diets can be seen before and after one hour of immersion in saltwater (33g/L) at the water absorption test (Figure 1), which can be seen the degree of turgid where diet M4 is the one that shows the best texture.

3.3 Zootechnical Parameters

Values for shrimp zootechnical parameters were assessed for the two green seaweed meal diets, and the control diet are shown in Table 4. The performance parameters of shrimp

Individual consumption (IC), Food Conversion Rate (FCA), Weight gain (G), Specific Growth Rate (SGR), and Protein Efficiency Rate (PER) in the experimental diets were no significant differences ($P > 0.05$) among treatments. However slightly better values in shrimp growth were in the seaweed's groups (Figure 2). After 29 days of the experiment, shrimps juveniles showed significant differences ($P < 0.05$) only for survival and final biomass parameters with the higher values for M4 treatment with 84.4% and 0.876 kg/m^3 , respectively, higher than the control group, while the lowest values were for the M8 treatment with 55.5% and 0.593 kg/m^3 respectively (table 4).

The IC value in the M8 diet probably was influenced by leaching; this idea is supported by the highest percentage of dry matter loss (Table 3), which overvalued and negatively influenced the PER and FCR in this treatment (Table 4); however, this diet obtained numerically more body protein content with 71.75% (Table 5), probably due to the lower loss of proteins that allowed to take advantage of it, and both diets with seaweed relatively show numerically more body protein retention (%) than de Control diet. The body protein retention (%) varied from 62.9 to 63.8. Relative higher values corresponded to the diets with seaweed meals (Table 5).

Should be Table 4 and figure 2

The ANOVA showed significant interaction ($P < 0.05$) between experimental days and the seaweed meal inclusion in the feed during experimental days. Shrimp fed with 4% seaweed meal survive almost twice shrimp fed 8% seaweed meal. Figure 3 shows that until the second week, no differences in biomass were among the three treatments. The biomass in the M8 treatment begins to decrease from day 14, while the biomass in M4 continues its rising trend above the M8 and CD treatments; Although the biomass of the CD treatment also follows an increasing trend throughout the experimental days, it is lower than that shown with the M4 treatment.

Should be Figure 3

Should be Table 5

4. Discussion

4.1 Pellet quality

The stability of aquatic feed is achieved with the inclusion of binders. These must improve the texture of the food and allow it to remain at least for 1 hour without disintegrating, maintaining the nutrients' bioavailability and reducing wastage (COBOS and FARÍAS, 2020;

VOLPE et al., 2010). Despite the significant number of studies carried out about immune response exerted by seaweed incorporation into diets, those describing effects on the quality of the pellet are still very scarce, and most published studies on decapod nutrition lack data on the physical characteristics of the feed types (AAQILLAH-AMR et al., 2021).

In 2 hours of water immersion, DMR in M4 and M8 were higher than 92%. These values are better than that of Ruscoe et al. (2005), who evaluated DMR in freshwater for 2 hours with 3 and 5% inclusion of carrageenan and agar, obtaining a DMR of 67.9 and 84.8 for carrageenan and 60.7 and 61.25 % for the agar respectively. The DMR in M4 (97%) was better than the 93.7% of Cruz Suárez et al. (2006) with a 3.5% kelp meal after one hour of immersion in seawater. Our value for M4 was also better than the values of Cruz-Suárez et al. (2009) that included 3.33 % of meal from the green *Ulva clathrata*, and the brown seaweeds *Ascophyllum nodosum* and *Macrocystis pyrifera*. They obtained 96.3, 95.6, and 94% of DMR, all evaluated in one hour in water immersion, while M8 was slightly lower (92%) from these values after two hours of water immersion. Our DMR values were also better than the 84.81 and 85.68% of Argüello-Guevara & Molina-Poveda, (2013), who evaluated 3 and 5% of Kelp meal after three hours of immersion in seawater. The DMR values for M4 and M8 will be considered very good by Cuzon et al. (1994), who pointed out that pellets for shrimp must maintain a minimum of 90% dry matter retention after a 1-h exposure in water. This statement is due to the peculiar feeding behavior, as slow feeders and the tendency to manipulate food before ingestion. Khalil et al. (2018) mentioned that alginate, agar, and carrageenan are three commercially valuable hydrocolloids derived from certain brown and red algae with gelling, stabilizing, and excellent properties. Nevertheless, in our experiment, despite the longer time of two hours in water immersion, the DMR was over 90%, strongly supporting the gelling, hydrocolloid, and binding property of the green seaweed meal for shrimp feeds.

Regarding the protein loss (PL), the values for M4 and M8 were 3.9% and 1.79, respectively, these values lowest than the 13% with the green seaweed *Ulva clathrata*, and the 13.9 and 8.1 % with the brown seaweeds *Ascophyllum nodosum* and *Macrocystis pyrifera* meals respectively, found by Cruz-Suárez et al., (2009); but higher than the 1.43 and 0.88% of loss protein by Argüello-Guevara and Molina-Poveda (2013) with three and 5% of Kelp. These last values were obtained using measuring protein leaching in the water during the pellet stability test and with brown seaweeds containing alginates like our control diet, which achieved 1.85% of protein loss with 3% sodium alginate. Alginates are known for their excellent stabilizing capacity (KHALIL et al.,2018); even so, our PL values are low.

With more seaweed meal inclusion (M8), it seems less protein loss despite the lower DMR found in this treatment. The seaweeds meal used in this experiment was composed of three genera: *Ulva*, *Caulerpa*, and *Enteromorpha*; this last is synonymous with *Ulva*, (MOREIRA et al., 2020; TZIVELEKA et al., 2019). These genera belong to the ulvophyceas (MOREIRA et al., 2020), having to the ulvans as the water-soluble sulfated polysaccharides composed of xylose, ramosa, glucuronic, and iduronic acid (ROBIC et al., 2009). In this way, ulvans exhibit a high cation exchange capacity, are soluble, and of low viscosity, but can form a very stable gel in an appropriate ionic environment (with the presence of boric acid and calcium). These gels have variable stiffness depending on the associated metal and the type of polysaccharide produced by the source species, and it thus appears that the mechanical properties of *Ulva* cells wall can easily be altered by changing the ionic and ph environment. (KIDGEL et al., 2019; LAHAYE et al., 1996., LAHAYE and ROBIC ,2007; MOREIRA et al., 2021). In *Caulerpa*, this polysaccharide is xyloarabinogalactan sulfate, while in *Ulva* and *Enteromorpha* is the glucuron-xyloramus sulfate (PERCIVAL, 1979). At the same time, Kidgel et al. (2021) mention an apparent disparity in ulvans from blade and filamentous species of *Ulva* (e.g., *Enteromorpha spp* and *Ulva spp*). Table 1 shows the high ash content in the seaweed meal, which probably affected the ion exchange in M8, while M4 got a suitable gelling property, obtaining the best DMR ($P < 0.05$). In Table 3, despite more DMR in M4 and Control diet than M8 and without no significant differences in protein loss (PL) among treatments, M4 and Control diet have more standard deviation (1.18 and 1.15) than M8 (0.61), suggesting that the polysaccharides in M8 might be favoring the protein retention. This value 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.

The water absorption capacity is related to the texture of the pellet; a strong agglutination could affect it. Therefore, the need to evaluate the ability of the binder to absorb or retain water, providing a soft and easy-to-chop granule for shrimp (CERECER-COTA et al., 2005). This property influences the polymers of seaweeds to form gels and produce viscous solutions and is regulated by the type and amount of polysaccharides (ARGÜELLO-GUEVARA and MOLINA-POVEDA, 2013; CRUZ-SUÁREZ et al., 2006; CRUZ-SUÁREZ et al., 2009;). The water absorption of 183.8 and 185.4% for M4 and M8 respectively, were higher than the 139 % reported by CRUZ-SUÁREZ et al. (2006), with 3.5 percent of brown seaweeds meal, and higher than the 132 % for *Ulva clathrata* meal and 112 % for the brown

seaweeds *Ascophyllum nodosum* and *Macrocystis pyrifera*, all in the inclusion of 3.33 % reported by CRUZ-SUÁREZ et al. (2009). Finally, they were higher than those reported by ARGÜELLO-GUEVARA and MOLINA-POVEDA, (2013) with 3 and 5% of brown seaweeds meal who obtained 70 and 68.4 % of water absorption, respectively.

Numerically to greater inclusion of seaweed meal belongs a greater water absorption capacity. Cruz-Suárez et al. (2006) mentioned that the water absorption capacity correlates with dry matter loss. This pattern probably corresponds to binders different from hydrocolloids as those of the green seaweeds have. Interestingly, M4 with more water absorption than the Control Diet has more DMR than this, but M8 with more water absorption capacity than M4 had the lowest DMR among treatments, although it had the less protein loss percentage. This finding may be explained by the idea that leaching in M8 came mainly from the more minerals and water-soluble sulfated polysaccharides content in this diet. Figure 1 shows differences through visual evaluation of the physical appearance in the experimental diets after the dry matter retention test. It can be seen as a different ‘inner skeleton’ due to the types of binders forming a rigid matrix (DOMINY et al.,2004). The degree of swelling in the seaweeds meal diets is of a greater degree with a uniform texture and without fines. This characteristic improves the physical and structural properties of the pellet.

Regarding cost, the control diet used sodium alginate and hexametaphosphate, which are necessary sequestrants when using fishmeal because calcium or other cations present in by-products of the fish can react prematurely with the alginate when adding water, causing poor stability (CUZON et al., 1972). Meyers and Zein-Eldin (1972) indicates that these two ingredients are necessary to bind the pellet but have a high cost and are relatively little used in aquaculture feeds. These alginate-bound rations provide an excellent tool for laboratory research in aspects of crustacean nutrition but will increase feed cost. In general, adding 2.5% alginate and 1% sequestrant to the dry mix will produce acceptable feed. In our research, we used 3% sodium alginate and 1% hexametaphosphate as a sequestrant.

Based on our results, we suggest the value of 4% of seaweed meals to improve the quality of the pellets in texture, higher DMR, and less protein loss after two hours in water immersion without disintegrating.

4.2 Zootechnical parameters

Several studies have been made with green seaweeds as an additive or dietary ingredient with shrimp. These have been administered as meal, with *Ulva* (CRUZ-SUÁREZ et al. 2009;

ELIZONDO-GONZÁLEZ et al., 2018; ELIZONDO-REYNA et al., 2016; LARAMORE et al., 2018; MANGOTT et al., 2020; RODRÍGUEZ-GONZÁLEZ et al., 2014;SERRANO et al.,2015) as associated IMTA with *Ulva* (ANAYA-ROSAS et al., 2019; BRITO et al., 2014),with *Caulerpa* (LY et al., 2021) or adding their extracts in the diets, as in the case of *Enteromorpha prolifera* (LIU et al., 2020), or *Ulva* spp (ABO-RAYA et al., 2020; AKBARY and AMINIKHOEI, 2018; GE et al., 2019), with very good results in improving growth and the immune system.

Our experimental diets were isoenergetic and, isonitrogenous and after 29 experimental days, no significant differences for growth parameters were determined among treatments, except for survival and final biomass with the best values for the M4 diet. The growth values obtained with the M4 diet are slightly lower compared with those of Cruz-Suárez et al. (2009), with 3.33 % of *Ulva clathrata* meal in juveniles of *L. vannamei*, obtaining a final weight of 4.8 g, FCR of 1.7, and a PER of 2.0. In our study, the M4 diet obtained 3.6 g, 1.77, and 1.86 for final weight, FCR, and PER, respectively. We used a density of 286 shrimps/m³ (15 tank-1), unlike the 117shrimps/m³ (7 tank-1) they used. The density, plus handling (we made weekly biometric controls) could be stressors that negatively affected the performance (AGUILAR et al., 2012; SAMOCHA et al., 2004). Elizondo-González et al.(2018) evaluate the inclusion of 1, 2, and 3% of *U. Lactuca* in diets of *L. vannamei* juveniles (0.30g), finding that the inclusion of 3% resulted in a significant improvement in the FCR and growth, achieving 100% survival; Similarly, Mangott et al.(2020) added 4% of *U. Lactuca* meal within *L. vannamei* diets (1.2g) reared in a RAS system obtaining a SGR of 4,92%, higher than the M4 and M8 diets (3.23 and 3.30%) and 93% survival, they concluded that both growth and survival increased with the seaweed diet, although they fed the shrimps with a 49% protein diet, and in our experiment we used a 30% protein diet. Contrary, Rodríguez-González et al. (2014) did not find significant differences when including 5% of *U. Lactuca* meal in *L. vannamei* juveniles (1.10g) with respect to the control diet, but levels of 10 and 15% led to lower growth, suggesting 5% as the inclusion limit; Ge et al. (2019) added extracts of *U. prolifera* on a basal diet of *L. vannamei* (4.08g), they found greater weight gain in the groups fed with the extracts compared to the control group; Akbary and Aminikhoei (2018) investigate the effect of 0.5, 1 and 1.5 g/kg water-soluble polysaccharides extract of *Ulva rigida* as a dietary supplement on growth performance in *L. vannamei* and suggest that doses of 1.5 g/kg of water-soluble improve growth. Liu et al. (2020) tested doses of 0, 1, 2, and 3 g / kg of *Enteromorpha*'s extracts in juveniles (2.18 g.) *Fenneropenaeus merguensis* feed, with

the better performance in 1 g / kg supplementation and concluded that 1 g/kg of EPS positively affected growth performance and manifested that lower dose of EPS is more effective than a higher doses.

The stress by handling is common in shrimp when they are weighed or transferred between ponds or when they are stocked in high densities, leading to increased reactive oxygen species (ROS) due to oxidative stress, which negatively influence growth and survival (GAO et al.,2017; NAVARRO et al.,2019). This can explain the lower growth values in our experiment, respect other authors. Even so, despite no significant differences, seaweeds treatments had relatively better growth values than the Control diet, and M4 survival was the best among treatments. All this, plus the findings mentioned above, support the hypothesis that specific components in *Ulva* positively affect the health of shrimps (MANGOTT et al., 2020).

In our experiment, M8 with more seaweed meal inclusion registered higher mortality which significantly obtained the lowest yield with 0.593kg / m³, due to the lowest survival (55.5%), contrary shrimp fed the M4 diet had the highest survival (84.4%) among all treatments ($P < 0.05$), revealing a higher seaweed meal dose, less survival. The two-way ANOVA indicates that mortality started in all treatments since the second experimental week, even in the Control diet, which continued until the end of the experiment. These results indicate that handling and some metabolites belonging to the green seaweeds meal affected survival in M8 and correlated with dosage.

Concerning the *Caulerpa* genus, Ly et al. (2021) co-culture *C. lentillifera* with *L. vannamei* using four seaweed densities (0.5, 1, 1.5, and 2 kg m⁻³) significantly improved the growth rate and survival compared to monoculture. They suggested 1 kg m⁻³ as the best density among the integrated systems. Unlike Ara et al. (1999) reported mortalities of 13% in brine shrimp nauplius when fed with 5ug of ethanolic extracts of *C. racemose* that rose to 56% with 1000ug., while no deaths were reported when fed with 5ug of extract of *U. lactuca* but incremented to 26% deaths at 1000mg dose. In the same way, Movahhedini et al. (2014) found a toxic effect of methanolic extracts of *C. peltate* in brine shrimp nauplii., and Mozzachiodi et al. (2001) determined that caulerpenyne affects the activity of nerve cells in the leech *H. medicinalis* in a dose dependents manner. Puspitasari et al. (2019) tested the inclusion of *C. racemosa* meal in levels of 10, 20, and 30% in *Penaeus monodon* and reported that growth performance with a diet containing 10% of *C. racemosa* was not significantly different from

the control. However, more than 20% decreased growth, without significant difference among survival rate.

Some *Caulerpa* species are considered a food source (*C. racemosa*, *C. lentillifera*), and others are considered deadly, like *C. Taxifolia* (CHENEY, 2016; KUMAR and SHARMA, 2021). The *Caulerpa* genus is resistant to herbivorous predators due to the high content of the toxin caulerpenyne, which has been identified from 11 species belonging to *Caulerpa* genus. There are reports of this toxic obtained from *C. racemosa* (KUMAR and SHARMA, 2021) and other chemicals that act as repellent substances which also are considered poisons (PAUL et al., 1987; RUSHDI et al., 2020). Chemical studies of various species of *Caulerpa* have shown that some of these seaweeds produce caulerpine, and caulerpicin, which are nitrogen-containing compounds that have ichthyotoxic effects (CHENEY, 2016), and the diterpenoid alcohol, caulerpol. (PAUL and FENICAL, 1982); while Kumar and Sharma (2020) indicated that a few mollusks feeding on *Caulerpa* also exhibited a significant increase in their toxic metabolites, resulting in toxicity to its predators.

Dietary supplementation of green seaweed meals and extracts has been studied and reported to improve growth performance, feed utilization and exhibits antioxidant activity in shrimp (MOHAN et al., 2019). However, it indicated that the effects of dietary marine polysaccharides on growth performance could be varied, and it may depend on some factors like shrimp species, supplemental dosage, the source of polysaccharides, and feeding conditions. (COSTA-REZENDE et al., 2020). One interpretation of these findings is that benefits in growth and antioxidant activity are reported when added at lower percentages. Another could be that growth and survival depend on the seaweed species. However, some adverse metabolites in the seaweeds could be present and be hazardous in high inclusion levels, which occurred in the present experiment. Without any significant differences in growth parameters in this experiment, biomass differences were due to the mortality of shrimps. It starts at the second week in all treatments (Figure 3), which may be due to handling and density. Afterward can be seen different behaviors among them through the experimental days: The biomass in the CD treatment began descending but less than M8, in which, not only the stress was but also a more amount of seaweed consumed; here the biomass began to diminish and continued dropping down until the last experimental day; while the biomass of M4 stayed ascending. For all mentioned above, we can infer that feeding high levels of some green seaweed species can produce mortality, which could be one reason why higher mortality

was found at a higher level of inclusion (8%). In the M4 diet, seaweed meal supports shrimp health and improves survival against stress conditions.

Certain limitations of this study could be addressed in future research. For example, to classify the species of seaweeds that composed the blend and separately test them to elucidate a degree of toxicity and establish an inclusion level for *L. vannamei*.

The control diet containing alginate did not show the best performance and survival. This effect agrees with Volpe et al. (2010), who mentioned that alginate has no nutritional or functional value, achieved with 4% of seaweed meals. Despite these limitations, this research can contribute to promoting green seaweeds as an eco-friendly feed additive option that can help under stress conditions while taking advantage of the gelling, hydrocolloid, and binding properties of these seaweeds.

5. Conclusions

The results of the present study support the hypothesis that a meal composed of green seaweeds from the genus *Ulva*, *Enteromorpha*, and *Caulerpa* can be used in 4% as an excellent functional additive in shrimp diets in terms of a suitable binder, allowing less protein loss and texturizer of the feed, giving solutions for less feed waste in the aquatic environment. At the same time improves survival in juveniles of *L. vannamei*, enhancing production. Further separate studies are necessary with *Ulva* and *Caulerpa* genus original from Perú as an ingredient to determine their best inclusion level and elucidate some toxicity for *L. vannamei*.

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7. References

AAQILLAH-AMR, M. A., HIDIR, A., AZRA, M. N., AHMAD-IDERIS, A. R., ABUALREESH, M. H., NOORDIYANA, M. N., IKHWANUDDIN, M. Use of Pelleted Diets in Commercially Farmed Decapods during Juvenile Stages: A Review. **Animals**, v 11, n. 6, p.1761. 2021. <https://doi.org/10.3390/ani11061761>

ABO-RAYA, M., ALSHEHRI, K., ABDELHAMEED, R., ELBIALY, Z., ELHADY,S., MOHAMED, R. . Assessment of growth-related parameters and immune-biochemical profile of Nile tilapia (*Oreochromis niloticus*) fed dietary *Ulva fasciata* extract. **Aquaculture Research** v52, p. 3233-3246. 2021 <https://doi.org/10.1111/are.15169>

AGUILAR, V., RACOTTA, I. S., GOYTORTÚA, E., WILLE, M., SORGELOOS, P., CIVERA, R., PALACIOS, E. (2012). The influence of dietary arachidonic acid on the immune response and performance of Pacific whiteleg shrimp, *Litopenaeus vannamei*, at high stocking density. **Aquaculture nutrition** v 18, n 3, p. 258 - 271. <https://doi.org/10.1111/j.1365-2095.2011.00892.x>

AKBARY, P., AMINIKHOEI, Z. Effect of polysaccharides extracts of algae *Ulva rigida* on growth, antioxidant, immune response and resistance of shrimp, *Litopenaeus vannamei* against *Photobacterium damsela*. **Aquaculture Research**, v.49, n 7, p. 2503-2510. 2018. <https://doi.org/10.1111/are.13710>

ANAYA-ROSAS, E., RIVAS-VEJA, M., MIRANDA-BAEZA, A., PIÑA-VALDEZ, P., NIEVES-SOTO, M. Effects of a co-culture of marine algae and shrimp (*Litopenaeus vannamei*) on the growth, survival and immune response of shrimp infected with *Vibrio parahaemolyticus* and white spot virus (WSSV). **Fish and Shellfish Immunology** v87, p. 136 – 143. 2019 <https://doi.org/10.1016/j.fsi.2018.12.071>

ANH, N. T. N., SHAYO, F. A., NEVEJAN, N., VAN HOA, N. Effects of stocking densities and feeding rates on water quality, feed efficiency, and performance of white leg shrimp *Litopenaeus vannamei* in an integrated system with sea grape *Caulerpa lentillifera*. **Journal of Applied Phycology**. v.33, n.5, p.3331-3345. 2021. <https://doi.org/10.1007/s10811-021-02501-4>

AOAC (2005). Official methods of analysis (18th ed.). Gaithersburg, MD: Association of Official Analytical Chemists.

ARA J, SULTANA V, EHTESHAMUL-HAQUE S, QASIM R, AHMAD VU. Cytotoxic activity of marine macro-algae on *Artemia salina* (brine shrimp). **Phytotherapy Research**. V.13, n.4, p.304-307. 1999 doi: 10.1002/(SICI)1099-1573(199906)13:4<304:AID-PTR439>3.0.CO;2-9. PMID: 10404536.

ARGÜELLO-GUEVARA, W. Y MOLINA-POVEDA, C. Effect of binder type and concentration on prepared feed stability, feed ingestion and digestibility of *Litopenaeus vannamei* broodstock diets. **Aquaculture Nutrition**. V.19, p.515–522, 2013. <https://doi.org/10.1111/anu.12003>

BRITO, LO.; ARANTES, R.; MAGNOTTI, C.; DERNER, R.; PCHARA,F.; OLIVERA, A.; VINATEA , L. Water quality and growth of Pacific white shrimp *Litopenaeus vannamei* (Boone) in co-culture with green seaweed *Ulva lactuca* (Linnaeus) in intensive system. **Aquaculture International**. v22, p.497–508. 2014. DOI 10.1007/s10499-013-9659-0

CERECER-COTA, E., Ricque-Marie, D., Mendoza-Cano, F., Nieto-López, M. G., Cruz-Suárez, L. E., Ramirez-Wong, B., Salazar.García, G. & Velasco-Escudero, M. Pellet stability, hardness influence feed consumption of Pacific white shrimp. April 1 Global Aquaculture Advocate. v.8, n.3, p.84-85. 2005. Disponible en: <https://www.aquaculturealliance.org/advocate/topic/estrella-e-cerecer-cota/> Consultado 30 de julio 2021

CHENEY, D.. Chapter 13 - Toxic and Harmful Seaweeds. In: Joël Fleurence, Ira Levine, (eds). Seaweed in Health and Disease Prevention, Academic Press, 2016. p.407-421. <https://doi.org/10.1016/B978-0-12-802772-1.00013-0>

COBOS, J. V., FARÍAS, C. V. Study about the use of aquaculture binder with tuna attractant in the feeding of white shrimp (*Litopenaeus vannamei*). **Revista Mexicana de Ingeniería Química**, v.19, n.1, p.355-361. 2020. <https://doi.org/10.24275/rmiq/Bio615>

COSTA REZENDE, P., SOARES, M., GUIMARÃES, A. M., DA ROSA COELHO, J., SEIFFERT, W. Q., DIAS SCHLEDER, D., VIEIRA, F. D. N. Brown seaweeds added in the diet improved the response to thermal shock and reduced *Vibrio* spp. in pacific white shrimp post-larvae reared in a biofloc system. **Aquaculture Research**, v.52, n.6, p. 2852-2861. 2021. <https://doi.org/10.1111/are.15136>

CRUZ-SUÁREZ, L; RUIZ, D.P; COTA-CERECER, E; NIETO, M; GUAJARDO-BARBOSA, C; VILLARREAL, D; RICQUE-MARIE, D. Revisión sobre algunas características físicas y control de calidad de alimentos comerciales para camarón en México. En: Avances en Nutrición Acuícola VIII. VIII Simposium Internacional de Nutrición

Acuícola. 15 - 17 noviembre. Universidad Autónoma de Nuevo León, Monterrey. Eds. L. E, Cruz-Suárez, D. Ricque-Marie; M. Tapia-Salazar, M; L. G. A; Nieto; C. D; Villarreal; C A. Puello y O. A. García. Nuevo León, MX p.330-370. 2006 En línea Accesado 2 de agosto de 2021. Disponible

https://www.uanl.mx/utilerias/nutricion_acuicola/VIII/archivos/21CruzSuarez.pdf

CRUZ-SUÁREZ, L; TAPIA, M; NIETO, M; GUAJARDO, C. AND RICQUE, D. Comparison of *Ulva clathrata* and the kelps *Macrocystis pyrifera* and *Ascophyllum nodosum* as ingredients in shrimp feeds. **Aquaculture Nutrition** v.15, p. 421 – 430. 2009. <https://doi.org/10.1111/j.1365-2095.2008.00607.x>

CUZON, G., GUILLAUME, J., CAHU, CH. Composition, preparation and utilization of feeds for Crustacea, **Aquaculture**, v.124, n.1-4, p.253-267. 1994. [https://doi.org/10.1016/0044-8486\(94\)90387-5](https://doi.org/10.1016/0044-8486(94)90387-5).

DOMINY, W. G., CODY, J. J., TERPSTRA, J. H., OBALDO, L. G., CHAI, M. K., TAKAMORI, T. I., LARSEN, B., FORSTER, I. P. A comparative study of the physical and biological properties of commercially-available binders for shrimp feeds. **Journal of Applied Aquaculture**, v.14, n.3-4, p.81-99. 2004. https://doi.org/10.1300/J028v14n03_07

ELIZONDO-GONZÁLEZ R, QUIROZ-GUZMÁN E, ESCOBEDO-FREGOSO C, MAGALLÓN-SERVÍN P, PEÑA-RODRÍGUEZ A. Use of seaweed *Ulva lactuca* for water bioremediation and as feed additive for white shrimp *Litopenaeus vannamei*. **PeerJ** v.6, p. e4459. 2018. <https://doi.org/10.7717/peerj.4459>

ELIZONDO-REYNA, E., MEDINA-GONZÁLEZ, R., NIETO-LÓPEZ, M., ORTIZ-LÓPEZ, R., ELIZONDO-GONZÁLEZ, R., POWELL, M., RICQUE-MARIE, D., CRUZ-SUÁREZ, E. Consumption of *Ulva clathrata* as a dietary supplement stimulates immune and lipid metabolism genes in Pacific white shrimp *Litopenaeus vannamei*. **Journal of Applied Phycology**, v.28, p.3667–3677. 2016, <https://doi.org/10.1007/s10811-016-0889-1>

FAO, Food and Agriculture Organization .El estado mundial de la pesca y la acuicultura 2020. Organización de las Naciones Unidas para la Alimentación y la Agricultura. Disponible en: <http://www.fao.org/documents/card/en/c/ca9229es> . Acceso Julio 22 del 2021

GAO, Y., HE, Z., ZHAO, B., LI, Z., HE, J., LEE, J., & CHU, Z.. Effect of stocking density on growth, oxidative stress and HSP 70 of pacific white shrimp *Litopenaeus vannamei*. **Turkish Journal of Fisheries and Aquatic Sciences**, v.17, n.5, p.877-884. 2017. https://doi.org/10.4194/1303-2712-v17_5_04

GE, H., NI, Q., CHEN, Z., LI, J., ZHAO, F. Effects of short period feeding polysaccharides from marine macroalga, *Ulva prolifera* on growth and resistance of *Litopenaeus vannamei* against *Vibrio parahaemolyticus* infection. **Journal of Applied Phycology**. v. 31, p.2085–2092. 2019. <https://doi.org/10.1007/s10811-018-1663-3>

KHALIL, H. P. S., LAI, T. K., TYE, Y. Y., RIZAL, S., CHONG, E. W. N., YAP, S. W., HAMZAH, A.A., FAZITA, M.R.N. & PARIDAH, M. T. A review of extractions of seaweed hydrocolloids: Properties and applications. **Express Polymer Letters**, v.12, n.4. 2018. <https://doi.org/10.3144/expresspolymlett.2018.27>

KIDGELL, J. T., CARNACHAN, S. M., MAGNUSSON, M., LAWTON, R. J., SIMS, I. M., HINKLEY, S. F., DE NYS, R. GLASSON, C. R. Are all ulvans equal? A comparative assessment of the chemical and gelling properties of ulvan from blade and filamentous *Ulva*. **Carbohydrate Polymers**, v.264, p.118010. (2021). <https://doi.org/10.1016/j.carbpol.2021.118010>

KIDGELL, J, MAGNUSSON M, DE NYS R, GLASSON CH. Ulvan: A systematic review of extraction, composition and function. Review article **Algal Research**, v.39, p.101- 422. 2019. <https://doi.org/10.1016/j.algal.2019.101422>

KUMAR, M. S., SHARMA, S. A. Toxicological effects of marine seaweeds: A cautious insight for human consumption. **Critical reviews in food science and nutrition**, v.61, n.3, p.500-521. 2021. <https://doi.org/10.1080/10408398.2020.1738334>

LAHAYE, M., RAY, B., BAUMBERGER, S., QUEMENER, B., AXELOS, M. A. Chemical characterisation and gelling properties of cell wall polysaccharides from species of *Ulva* (Ulvales, Chlorophyta). In **Fifteenth International Seaweed Symposium** (pp. 473-480). Springer, Dordrecht. (1996). <https://doi.org/10.1007/BF00047848>

LAHAYE, M. AND ROBIC, A. Reviews. Structure and Functional Properties of Ulvan, a Polysaccharide from Green Seaweeds. **Biomacromolecules** v.8, n.6, p.1765 – 1774. 2007 doi: 10.1021/bm061185q.

LEGARDA, E. C., DA SILVA, D., MIRANDA, C. S., PEREIRA, P. K. M., MARTINS, M. A., MACHADO, C., DE LORENZO, M.A., HAYASHI, L. & DO NASCIMENTO VIEIRA, F. Sea lettuce integrated with Pacific white shrimp and mullet cultivation in biofloc impact system performance and the sea lettuce nutritional composition. **Aquaculture**, v.534, p.736265. 2021. <https://doi.org/10.1016/j.aquaculture.2020.736265>

LITTLE, D. C., NEWTON, R. W., BEVERIDGE, M. C. M. Aquaculture: a rapidly growing and significant source of sustainable food? Status, transitions and potential. **Proceedings of the Nutrition Society**, p.75, n.3, p.274-286. 2016. <https://doi.org/10.1017/S0029665116000665>

LIU, W.CH., ZHOU, S.H., BALASUBRAMANIAN, B., ZENGA, F.Y., SUNA, CH.B., PANG, H.Y. Dietary seaweed (*Enteromorpha*) polysaccharides improve growth performance involved in regulation of immune responses, intestinal morphology and microbial community in banana shrimp *Fenneropenaeus merguensis*. **Fish and Shellfish Immunology**, v.104, p. 202-212. 2020. <https://doi.org/10.1016/j.fsi.2020.05.079>

LY, K. V., MURUNGU, D. K., NGUYEN, D. P., NGUYEN, N. A. T. Effects of Different Densities of Sea Grape *Caulerpa lentillifera* on Water Quality, Growth and Survival of the Whiteleg Shrimp *Litopenaeus vannamei* in Polyculture System. **Fishes**, v.6, n.2, p.19. 2021. <https://doi.org/10.3390/fishes6020019>

MANGOTT, A., NAPPI, J., DELLI PAOLI CARINI, A., GONCALVES, P., HUA K., DOMINGOS, J.A., DE NYS, R., THOMAS, T. *Ulva lactuca* as a functional ingredient and water bioremediator positively influences the hepatopancreas and water microbiota in the rearing of *Litopenaeus vannamei*. **Algal Research**. v.51, p.102040. 2020 <https://doi.org/10.1016/j.algal.2020.102040>

MEYERS, S. P., ZEIN-ELDIN, Z. P. Binders and pellet stability in development of crustacean diets 1. In **Proceedings of the annual workshop-World Mariculture Society** v. 3, n. 1-4, p. 351-364. 1972, <https://doi.org/10.1111/j.1749-7345.1972.tb00079.x>

MOHAN, K., RAVICHANDRAN, S., MURALISANKAR, T., UTHAYAKUMAR, V., CHANDIRASEKAR, R., SEEDEVI, P., GANESAN, R., RAJAN, D. K. Application of marine-derived polysaccharides as immunostimulants in aquaculture: A review of current knowledge and further perspectives. **Fish and shellfish immunology**, v.86, p.1177-1193. 2019. <https://doi.org/10.1016/j.fsi.2018.12.072>

MOREIRA, A., CRUZ, S., MARQUES, R. Y CARTAXANA, P. The underexplored potential of green macroalgae in aquaculture. **Reviews in Aquaculture**. v.00, p.1- 22. 2021. DOI: 10.1111/raq.12580

MOVAHHEDIN, N., BARAR, J., AZAD, F. F., BARZEGARI, A., NAZEMIYEH, H. Phytochemistry and biologic activities of *Caulerpa peltata* native to Oman Sea. **Iranian journal of pharmaceutical research: IJPR**, v.13, n.2, p.515. 2014

MOZZACHIODI, R., SCURI, R., ROBERTO, M., BRUNELLI, M. Caulerpenyne, a toxin from the seaweed *Caulerpa taxifolia*, depresses afterhyperpolarization in invertebrate neurons, **Neuroscience**, v.107, n.3, p.519 -526. 2001 [https://doi.org/10.1016/S0306-4522\(01\)00365-7](https://doi.org/10.1016/S0306-4522(01)00365-7).

NAIEL, M. A., ALAGAWANY, M., PATRA, A. K., EL-KHOLY, A. I., AMER, M. S., ABD EL-HACK, M. E. Beneficial impacts and health benefits of macroalgae phenolic molecules on fish production. **Aquaculture**, v. 534, p736186. 2020. <https://doi.org/10.1016/j.aquaculture.2020.736186>

NRC (National Research Council). Nutrient requirements of fish and shrimp. National Academy Press, Washington.2011

NAVARRO, C., CIVERA, R., ARJONA, O., RIVERA-PEREZ, C., GARCÍA, H. S., HURTADO-OLIVA, M. Á.,PALACIOS, E. Stress response and lipid composition in shrimp *Litopenaeus vannamei* fed diets enriched with squid or scallop viscera meal. **Aquaculture Research**, v.51, n.4, p.1602-1622. 2020. <https://doi.org/10.1111/are.14508>

OBALDO, L; DIVAKARAN, S. AND TACON, A. Method for determining the physical stability of shrimp feeds in water. **Aquaculture Research**. v.33, p.369 -377. 2002
<https://doi.org/10.1046/j.1365-2109.2002.00681.x>

PAUL, V. J., FENICAL, W. Toxic feeding deterrents from the tropical marine alga *Caulerpa bikiensis* (Chlorophyta). **Tetrahedron Letters**, v.23, n.48, p.5017-5020. 1982.
[https://doi.org/10.1016/S0040-4039\(00\)85561-6](https://doi.org/10.1016/S0040-4039(00)85561-6)

PAUL, V. J., LITTLER, M. M., LITTLER, D. S., FENICAL, W. Evidence for chemical defense in tropical green alga *Caulerpa ashmeadii* (Caulerpaceae: Chlorophyta): Isolation of new bioactive sesquiterpenoids. **Journal of chemical ecology**, v.13, n.5, p.1171-1185. 1987.
<https://doi.org/10.1007/BF01020547>

PERCIVAL, E. The polysaccharides of green, red and brown seaweeds: their basic structure, biosynthesis and function. **British Phycological Journal**, v.14, n.2, p.103-117. 1979.
<https://doi.org/10.1080/00071617900650121>

PRODUCE. ANUARIO ESTADÍSTICO PESQUERO Y ACUÍCOLA 2019. Disponible en:
<https://ogeiee.produce.gob.pe/index.php/en/shortcode/oe-documentos-publicaciones/publicaciones-anuales/item/949-anuario-estadistico-pesquero-y-acuicola-2019>
Acceso: 21 de julio del 2021

PUSPITASARI, W., JUSADI, D., SETIAWATI, M., EKASARI, J., NUR, A., SUMANTRI, I. Utilization of green algae *Caulerpa racemosa* as feed ingredient for tiger shrimp *Penaeus monodon*. **Jurnal Akuakultur Indonesia**, v.18, n.2, p.162-171. 2019.
<https://doi.org/10.19027/jai.18.2.162-171>

REVERTER, M., BONTEMPS, N., LECCHINI, D., BANAIGS, B., SASAL, P. Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives. **Aquaculture**, v.433, p. 50-61. 2014.
<https://doi.org/10.1016/j.aquaculture.2014.05.048>

ROBIC, A., GAILLARD, C., SASSI, J: F: LERAT, Y., LAHAYE, M. Ultrastructure of Ulvan: A Polysaccharide from Green Seaweeds. **Biopolymers** v.91, n.8, p.652-664. 2009
Disponible <https://pubmed.ncbi.nlm.nih.gov/19353644/>. Access August 7, 2021

RODRIGUEZ-GONZALEZ, H., ORDUÑA-ROJAS, J., VILLALOBOS-MEDINA, J., GARCÍA-ULLOA, M., POLANCO-TORRES, A., LÓPEZ-ÁLVAREZ, E., MONTOYA-MEJÍA, M., HERNÁNDEZ-LLAMAS, A. Partial inclusion of *Ulva lactuca* and *Gracilaria parvispora* meal in balanced diets for white leg shrimp (*Litopenaeus vannamei*), **Journal of Apply Phycology**. v.26, p.2453–2459. 2014 <https://doi.org/10.1007/s10811-014-0272-z>.

RUSCOE, I., JONES, C., JONES, P. CALEY, P. The effects of various binders and moisture content on pellet stability of research diets for freshwater crayfish. **Aquaculture Nutrition** v.11, p.87–93, 2005. <https://doi.org/10.1111/j.1365-2095.2004.00324.x>

RUSHDI, M., ABDEL-RAHMAN, I., ZEKRY ATTIA, E., WEDAD M. ABDELRAHEEM., SABER, H., AMIN, E., HASSAN, H., ABDELMOHSEN, U. A review on the diversity, chemical and pharmacological potential of the green algae genus *Caulerpa*, South African **Journal of Botany**, v.132, p. 226-241.2020 <https://doi.org/10.1016/j.sajb.2020.04.031>.

SAMOCHA, T; LAWRENCE, A; COLLIN, C; CASTILLE, F., BRAY, W; DAVIES, C; LEE, P. AND WOOD, G.S. Production of the pacific white shrimp, *Litopenaeus vannamei*, in high-density greenhouse-enclosed raceways using low salinity groundwater. **Journal of Applied Aquaculture**. v.15, n.3-4, p.1-19, 2004. https://doi.org/10.1300/J028v15n03_01

SERRANO JR, A. E., SANTIZO, R. B., TUMBOKON, B. L. M. Potential use of the sea lettuce *Ulva lactuca* replacing soybean meal in the diet of the black tiger shrimp *Penaeus monodon* juvenile. **Aquaculture, Aquarium, Conservation and Legislation**, v.8, n.3, p.245-252. 2015. <http://www.bioflux.com.ro/aac>

THANIGAIVEL, S., CHANDRASEKARAN, N., MUKHERJEE, A., THOMAS, J. Seaweeds as an alternative therapeutic source for aquatic disease management. **Aquaculture**, v.464, p.529-536. 2016. <https://doi.org/10.1016/j.aquaculture.2016.08.001>

TZIVELEKA, L. A., IOANNOU E, ROUSSIS V. Ulvan, a bioactive marine sulphated polysaccharide as a key constituent of hybrid biomaterials: A review. **Carbohydrate Polymers** v.218, p. 355–370. 2019 <https://doi.org/10.1016/j.carbpol.2019.04.074>

VOLPE, M.G., VARRICCHIO, E., COCCIA, E., SANTAGATA, G., DI STASIO, M., MALINCONICO, M., PAOLUCCI, M. Manufacturing pellets with different binders: Effect

on water stability and feeding response in juvenile *Cherax albidus*, **Aquaculture**, v.324, p.104-110. 2012 <https://doi.org/10.1016/j.aquaculture.2011.10.029>.

WIJESEKARA, I., PANGESTUTI, R., KIM, S. K. Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae. **Carbohydrate polymers**, v.84, n.1, p.14-21. 2011. <https://doi.org/10.1016/j.carbpol.2010.10.062>

Table 1. Proximate composition of the Seaweed meal *Nutrigreen* (% dry basis)

<i>Proximate composition (%)</i>	
Moisture	< 12
Crude protein	14 -17
Lipid	1 - 1.5
Ash	48 - 50
Fiber	4 - 7
NFE	30 -35
*ED (Mcal kg. ⁻¹)	1.66
Carotens	>200ppm

*ED digestible energy estimated on the basis of caloric values of 3.8, 8 and 3 Mcal kg⁻¹ for proteins, lipids and carbohydrates, respectively; NFE nitrogen-free extract.

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

Ingredients	seaweed meal levels (%)		
	Control diet	M4	M8
	0%	4	8
Wheat meal	41.76	43.26	39.51
Fish meal	30.5	29	29
Soybean meal	12	12	12
Soy lecithin	5	5	5
Sodium alginate	3	0	0
Seaweed's meal	0	4	8

Phosphate dicalcium	2.8	2.9	2.97
Calcium carbonate	2.35	2.25	2.07
Sodium hexametaphosphate	1	0	0
Fish oil	1	1	1
Cholesterol SF	0.25	0.25	0.25
Vitamin C	0.14	0.14	0.14
*Premix vitamins and minerals	0.1	0.1	0.1
Antioxidant	0.05	0.05	0.05
Molt inhibitor	0.05	0.05	0.05

Proximal Composition (%Dry matter)

Humidity	7.81	5.4	5.4
Crude protein	30.48	30.89	31.1
Crude lipid	9.7	9.27	9.5
Crude fiber	0.79	0.94	0.9
Ash	11.97	11.42	13.22
NFE	39.25	42.08	39.88
ED Mcal kg-1	3.31	3.32	3.27

DMS Nutritional Products Peru S.A.kg⁻¹ *Premix: Vit. A 9333.34 UI; Vit. D3 1333.34 UI; Vit. E 93.34 UI;Tiam 12.00 mg; (B2) 13.34 mg; Niacin 100.00 mg; Pantothenic Acid 33.34 mg; B6, 10.00 mg; Biotin 0.54 mg; Folic Acid 2.66 mg;Vit C. 400.00 mg ;Vitamin B12 0.02 mg ; Choline Clorure 400.00 mg; Mn, Manganese 16.66 mg; He, 13.34 mg; Zinc 13.34 mg; Cu, 1.00 mg ;I, 1.00 mg; Se, 0.20 mg; Co, 1.00 mg ;B.H.T 80.00 mg ,Excipients c.s.p 2000.00 mg. ED digestible energy estimated on the basis of caloric values of 4.24 / 3.8, 8 and 3 Mcal

kg-1 for proteins(animal /veg), lipids, and carbohydrates, respectively ; NFE nitrogen-free extract

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

	Control Diet	M4	M8	P value
DMR (%)	94.03±2.06 ^{ab}	97.06±0.69 ^a	92.53±0.25 ^b	0.044
Protein loss (% Pl)	1.85±1.15 ^a	3.92±1.18 ^a	1.79±0.61 ^a	ns
Water absorption capacity (%)	166.10±11.95 ^a	183.83±10.81 ^a	185.48±5.64 ^a	ns

Mean values and standard deviations. Different superscripts mean statistical difference (P <0.05) CD = Control diet; M4 = 4% seaweed meal; M8 = 8% seaweed meal

Table 4: Shrimp Zootechnical parameters of fed diets with two levels of seaweed meal in experimental diets for 29 days

	Control Diet	M4	M8	P value
Final weight (g)	3.42±0.16 ^a	3.61±0.17 ^a	3.71±0.46 ^a	0.8307
Initial Biomass(Kg/m ³)	0.458±0.16	0.458±0.16	0.458±0.16	0.850
Final Biomass (Kg/m ³)	0.711±0.08 ^{ab}	0.876±0.12 ^{ab}	0.593±0.12 ^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 ^{ab}	55.56±6.28 ^b	0.016

Mean values and standard deviations. Different superscripts mean statistical difference (P <0.05). CD = Control diet; M4 = 4% green seaweed meal; M8 = 8% green seaweed meal; IC (individual consumption), FCR -Feed conversion rate; SGR -Specific growth rate; PER - Protein Efficiency Rate.

Table 5. Body Protein Retention (%) in *L. vannamei* juveniles fed diets with two levels of seaweed meal in experimental diets for 29 days (% on dry basis)

	Control Diet	M4	M8
Initial protein content (%)	67.87	67.87	67.87
Final protein content (%)	70.68	69.12	71.75
Protein retention (%)	62.9	63.8	63.5

CD = Control diet; M4 = 4% green seaweed meal; M8 = 8% green seaweed meal



Figure 1. Dry (upper) and wet experimental diets (below) after 2 hours of immersion in saltwater (30gL^{-1}) and $28\text{ }^{\circ}\text{C}$. From left to right, CD: Control diet; M4: 4% seaweed meal; M8: 8% seaweed meal

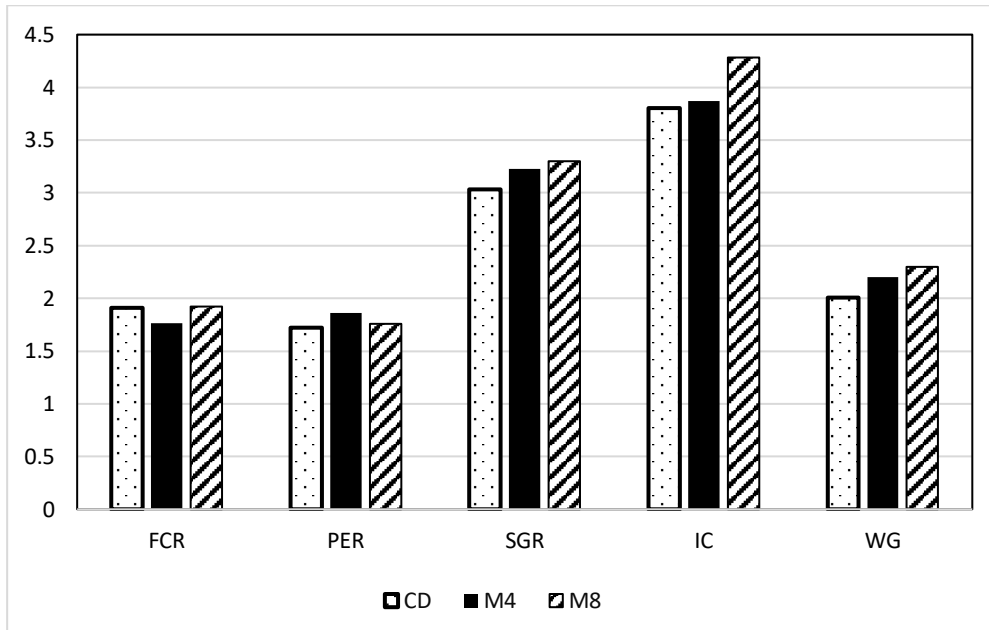


Figure 2. Zootechnical parameters after 29 experimental days with three different diets. FCR: Feed Conversion Rate; PER: Protein efficiency Ratio; SGR: Specific Growth Rate (%); IC: Individual consumption; WG: Weight Gain (g) CD: Control Diet; M4 :4% seaweed meal; M8= 8% seaweed meal.

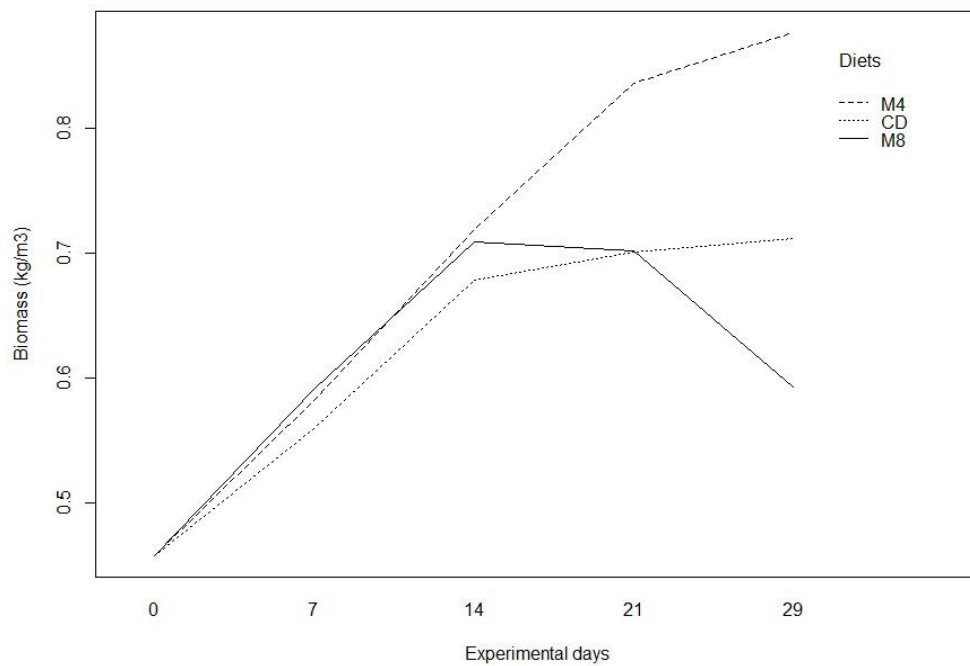


Figure 3. Interaction of shrimp biomass (kg/m³) between experimental diets and Experimental days CD: Control Diet; M4 :4% seaweed meal; M8= 8% seaweed meal.

4. Consideraciones finales

De los resultados obtenidos a partir de las investigaciones realizadas, se concluye que las especies *Ulva papenfussi* y *Ulva nematoidea* poseen una variedad de compuestos con actividad antibacteriana contra *V. parahaemolyticus*., Así también, que un 4% de inclusión de una mezcla de ulvofíceas (*Ulva* spp, *Enteromorpha* spp y *Caulerpa* spp) funcionan como un aditivo alimenticio que mejora la retención de materia seca, mayor capacidad de absorción de agua y menor pérdida de proteína del pellet, a la vez que mejora la supervivencia de los juveniles de *L. vannamei*.

Se recomienda realizar estudios posteriores en un sistema multitrófico con las macroalgas *U.papenfussi* y *U.nematoidea* para elucidar la efectividad de las propiedades antibacteriales frente al *V. parahaemolyticus*;

Es necesario realizar estudios por separado con los géneros *Ulva* y *Caulerpa* como aditivos dietéticos, para determinar su mejor nivel de inclusión y dilucidar algún grado de toxicidad para *L. vannamei*.,

Se recomienda evaluar en granja el efecto del 4% de inclusión de la mezcla de estas especies *Ulva*, *Caulerpa*, *Enteromorpha* en las dietas de *L. vannamei* sobre los parámetros productivos. De ser positiva será una excelente opción ecológica para preservar la calidad del agua a la vez de promover el sistema inmune, mejorando la supervivencia.

5. Referencias

ARGÜELLO-GUEVARA, W. Y MOLINA-POVEDA, C. Effect of binder type and concentration on prepared feed stability, feed ingestion and digestibility of *Litopenaeus vannamei* broodstock diets. **Aquaculture Nutrition**. V.19, p.515–522, 2013. <https://doi.org/10.1111/anu.12003>

CHAKRABORTY, K., LIPTON, A.P., RAJ R AND VIJAYAN, K. Antibacterial labdane diterpenoids of *Ulva fasciata Delile* from southwestern coast of the Indian Peninsula. **Food Chemistry** v.119, n,4, p.1399–1408. 2010 <https://doi.org/10.1016/j.foodchem.2009.09.019>

CHAMORRO-LEGARDA, E., DA SILVA, D., SOUZA MIRANDA, C., MOREIRA PEREIRA, P., ARANHA MARTINS, M., MACHADO, C., DE LORENZO, M., HAYASHI, L., DO NASCIMENTO VIEIRA, F. Sea lettuce integrated with Pacific white shrimp and mullet cultivation in biofloc impact system performance and the sea lettuce nutritional composition. **Aquaculture**, v.534, p.736265 . 2021 <https://doi.org/10.1016/j.aquaculture.2020.736265>.

CORTÉS, A., CASILLAS-HERNÁNDEZ, R., CAMBESES-FRANCO, C., BÓRQUEZ-LÓPEZ, R., MAGALLÓN-BARAJAS, F., QUADROS-SEIFFERT, W., FEIJOO, G., MOREIRA, M. Eco-efficiency assessment of shrimp aquaculture production in Mexico. **Aquaculture**, v.544, p.737145. 2021 <https://doi.org/10.1016/j.aquaculture.2021.737145>

COSTA REZENDE, P., SOARES, M., GUIMARÃES, A. M., DA ROSA COELHO, J., SEIFFERT, W. Q., DIAS SCHLEDER, D., VIEIRA, F. D. N. Brown seaweeds added in the diet improved the response to thermal shock and reduced *Vibrio* spp. in pacific white shrimp post-larvae reared in a biofloc system. **Aquaculture Research**, v.52, n.6, p. 2852-2861. 2021. <https://doi.org/10.1111/are.15136>

CRUZ-SUÁREZ, L; RUIZ, D.P; COTA-CERECER, E; NIETO, M; GUAJARDO-BARBOSA, C; VILLARREAL, D; RICQUE-MARIE, D. Revisión sobre algunas características físicas y control de calidad de alimentos comerciales para camarón en México. En: Avances en Nutrición Acuícola VIII. VIII Simposium Internacional de Nutrición Acuícola. 15 - 17 noviembre. Universidad Autónoma de Nuevo León, Monterrey. Eds. L. E, Cruz-Suárez, D. Ricque-Marie; M. Tapia-Salazar, M; L. G. A; Nieto; C. D; Villarreal; C A.

Puello y O. A. García. Nuevo León, MX p.330-370. 2006 En línea Accesado 2 de agosto de 2021. Disponible

<https://www.uanl.mx/utilerias/nutricion_acuicola/VIII/archivos/21CruzSuarez.pdf>

CUZON, G., GUILLAUME, J., CAHU, CH. Composition, preparation and utilization of feeds for Crustacea, **Aquaculture**, v.124, n.1-4, p.253-267. 1994. [https://doi.org/10.1016/0044-8486\(94\)90387-5](https://doi.org/10.1016/0044-8486(94)90387-5).

DENG, D., MEI, C., MAI, K., TAN, B. P., AI, Q., & MA, H. Effects of a yeast-based additive on growth and immune responses of white shrimp, *Litopenaeus vannamei* (Boone, 1931), and aquaculture environment. **Aquaculture research**, v.44, p.1348– 1357. 2013 doi:10.1111/j.1365-2109.2012.03139.x

ELIZONDO-GONZÁLEZ R, QUIROZ-GUZMÁN E, ESCOBEDO-FREGOSO C, MAGALLÓN-SERVÍN P, PEÑA-RODRÍGUEZ A. Use of seaweed *Ulva lactuca* for water bioremediation and as feed additive for white shrimp *Litopenaeus vannamei*. **PeerJ** v.6, p. e4459. 2018. <https://doi.org/10.7717/peerj.4459>

ELIZONDO-REYNA, E., MEDINA-GONZÁLEZ, R., NIETO-LÓPEZ, M., ORTIZ-LÓPEZ, R., ELIZONDO-GONZÁLEZ, R., POWELL, M., RICQUE-MARIE, D., CRUZ-SUÁREZ, E. Consumption of *Ulva clathrata* as a dietary supplement stimulates immune and lipid metabolism genes in Pacific white shrimp *Litopenaeus vannamei*. **Journal of Applied Phycology**, v.28, p.3667–3677. 2016, <https://doi.org/10.1007/s10811-016-0889-1>

ESQUER-MIRANDA, E., NIEVES-SOTO, M., RIVAS-VEGA, M., MIRANDA-BAEZA, A. & PIÑA-VALDEZ, P. Effects of methanolic macroalgae extracts from *Caulerpa sertularioides* and *Ulva lactuca* on *Litopenaeus vannamei* survival in the presence of *Vibrio* bacteria. **Fish and Shellfish Immunology** v.51, p.346–350. 2016 DOI: 10.1016/j.fsi.2016.02.028

FAO, El estado mundial de la pesca y la acuicultura. Organización de las Naciones Unidas para la Alimentación y la Agricultura. 2020 Disponible en: <http://www.fao.org/documents/card/en/c/ca9229es> . Acceso Julio 22 del 2021

FIGUEREDO, A., FUENTES, J. L., CABRERA, T., LEÓN, J., PATTI, J., SILVA, J., RON, E., PICHARDO, O. AND MARCANO, N. Bioseguridad en el cultivo de camarones penaeidos: una revisión. **AquaTechnica: Revista Iberoamericana de Acuicultura.**, v2, n.1, p.1-22. 2020 <https://doi.org/10.33936/at.v2i1.2409>

GARCIA-VAQUERO, M., RAJAURIA, G., O'DOHERTY, J. AND SWEENEY, T. Polysaccharides from macroalgae: Recent advances, innovative technologies and challenges in extraction and purification. **Food Research International** v.99, p.1011–1020. 2017 DOI: 10.1016/j.foodres.2016.11.016

GOMATHI, K. AND SHEBA, A. Phytochemical screening and heavy metal analysis of *ulva reticulata*. **Asian Journal of Pharmaceutical and Clinical Research** v.11, n.4, p.84–88. 2018. DOI: 10.22159/ajpcr.2018.v11i4.23012

KHALIL, H. P. S., LAI, T. K., TYE, Y. Y., RIZAL, S., CHONG, E. W. N., YAP, S. W., HAMZAH, A.A., FAZITA, M.R.N. & PARIDAH, M. T. A review of extractions of seaweed hydrocolloids: Properties and applications. **Express Polymer Letters**, v.12, n.4. 2018. <https://doi.org/10.3144/expresspolymlett.2018.27>

KIDGELL, J, MAGNUSSON M, DE NYS R, GLASSON CH. Ulvan: A systematic review of extraction, composition and function. Review article **Algal Research**, v.39, p.101- 422. 2019. <https://doi.org/10.1016/j.algal.2019.101422>

LAHAYE, M. AND ROBIC, A. Reviews. Structure and Functional Properties of Ulvan, a Polysaccharide from Green Seaweeds. **Biomacromolecules** v.8, n.6, p.1765 – 1774. 2007 doi: 10.1021/bm061185q.

.LIM, CH., & CUZON, G. Water Stability of Shrimp Pellet: A Review. **Asian Fisheries Science** v.7, p.115-127. 1994

LITTLE, D. C., NEWTON, R. W., BEVERIDGE, M. C. M. Aquaculture: a rapidly growing and significant source of sustainable food? Status, transitions and potential. **Proceedings of the Nutrition Society**, p.75, n.3, p.274-286. 2016. <https://doi.org/10.1017/S0029665116000665>

LIU, W.CH., ZHOU, S.H., BALASUBRAMANIAN, B., ZENGA, F.Y., SUNA, CH.B., PANG, H.Y. Dietary seaweed (*Enteromorpha*) polysaccharides improve growth performance involved in regulation of immune responses, intestinal morphology and microbial community in banana shrimp *Fenneropenaeus merguensis*. **Fish and Shellfish Immunology**, v.104, p. 202-212. 2020. <https://doi.org/10.1016/j.fsi.2020.05.079>

LÓPEZ-LEÓN, P., LUNA-GONZÁLEZ, A., ESCAMILLA-MONTES, R., FLORES-MIRANDA, M., FIERRO-CORONADO, J. AND ÁLVAREZ-RUIZ, P. Isolation and characterization of infectious *Vibrio parahaemolyticus*, the causative agent of AHPND, from the whiteleg shrimp (*Litopenaeus vannamei*). **Latin America Journal Aquatic Research**, v.44, n.3, p.470–479. 2016 Available at: https://scielo.conicyt.cl/scielo.php?script=sci_arttext&pid=S0718-560X2016000300005&lng=es (accessed 03 January 2020)

MANGOTT, A., NAPPI, J., DELLI PAOLI CARINI, A., GONCALVES, P., HUA K., DOMINGOS, J.A., DE NYS, R., THOMAS, T. *Ulva lactuca* as a functional ingredient and water bioremediator positively influences the hepatopancreas and water microbiota in the rearing of *Litopenaeus vannamei*. **Algal Research**. v.51, p.102040. 2020 <https://doi.org/10.1016/j.algal.2020.102040>

MOHAN, K., RAVICHANDRANA, S., MURALISANKARB, TH., UTHAYAKUMARC, V., CHANDIRASEKARC , R., SEEDEVID, P., GANESAN, R. AND KARTHICK, R. D. Application of marine-derived polysaccharides as immunostimulants in aquaculture: A review of current knowledge and further perspectives. **Fish and Shellfish Immunology** v.86, p.1177-1193.. 2019 <https://doi.org/10.1016/j.fsi.2018.12.072>

MOREIRA, A., CRUZ, S., MARQUES, R. Y CARTAXANA,P. The underexplored potential of green macroalgae in aquaculture. **Reviews in Aquaculture**. v.00, p.1- 22. 2021 DOI: 10.1111/raq.12580

NAVARRO, C., CIVERA, R., ARJONA, O., RIVERA-PEREZ, C., GARCÍA, H. S., HURTADO-OLIVA, M. Á., AND PALACIOS, E. Stress response and lipid composition in shrimp *Litopenaeus vannamei* fed diets enriched with squid or scallop viscera meal. **Aquaculture Research**, v.51, n.4, p.1602-1622. 2020 <https://doi.org/10.1111/are.14508>

NETWORK OF AQUACULTURE CENTRE'S IN ASIA-PACIFIC (NACA) Report of the Asia Pacific Emergency Regional Consultation on the Emerging Shrimp Disease: Early Mortality Syndrome (Ems)/Acute Hepatopancreatic Necrosis Syndrome (AHPNS), 9-10 Published by the Network of Aquaculture Centers in Asia and the Pacific, Bangkok, Thailand. August 2012. Disponible en : <<http://library.enaca.org/Health/Publication/ahpns-emergency-consultation-report.pdf>> Acceso en : 30 August 2020).

NRC (National Research Council). Nutrient requirements of fish and shrimp. National Academy Press, Washington. 2011

OBALDO, L; DIVAKARAN, S. AND TACON, A. Method for determining the physical stability of shrimp feeds in water. **Aquaculture Research**. v.33, p.369 -377. 2002 <https://doi.org/10.1046/j.1365-2109.2002.00681.x>

PRODUCE. ANUARIO ESTADÍSTICO PESQUERO Y ACUÍCOLA. Disponible en; <<https://ogeiee.produce.gob.pe/index.php/en/shortcode/oee-documentos-publicaciones/publicaciones-anuales/item/949-anuario-estadistico-pesquero-y-acuicola-2019>> Acceso: 21 de julio del 2021

REVERTER, M., BONTEMPS, N., LECCHINI, D., BANAIGS, B. AND SASAL, P. Review. Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives. **Aquaculture** v.433, p.50–61.2014 <https://doi.org/10.1016/j.aquaculture.2014.05.048>

RUSHDI, M. I., ABDEL-RAHMAN, I. A., ATTIA, E. Z., ABDELRAHEEM, W. M., SABER, H., MADKOUR, H. A., AMIN, E., HASSAN, H.M., ABDELMOHSEN, U.R. A review on the diversity, chemical and pharmacological potential of the green algae genus *Caulerpa*, **South African Journal of Botany**, v.132, p. 226-241, ISSN 0254-6299. 2020 <https://doi.org/10.1016/j.sajb.2020.04.031>.

SMIT, A. Medicinal and Pharmaceutical uses of seaweed natural products: A Review. **Journal of Applied Phycology**. v.16, p.245–262. 2004 <https://doi.org/10.1023/B:JAPH.0000047783.36600.ef>

SOTO-RODRIGUEZ, S.A., GOMEZ-GIL, B., LOZANO-OLVERA, R., BETANCOURT-LOZANO, M. AND MORALES-COVARRUBIAS, M.S. Field and experimental evidence of *Vibrio parahaemolyticus* as the causative agent of acute hepatopancreatic necrosis disease of cultured shrimp (*Litopenaeus vannamei*) in northwestern Mexico. **Applied and Environmental Microbiology** v.81, p.1689–1699. 2015 <https://doi.org/10.1128/AEM.03610-14>

THANIGAIVEL, S, CHANDRASEKARAN, N, MUKHERJEE, A. & THOMAS, J. Seaweeds as an alternative therapeutic source for aquatic disease management **Aquaculture** v.464, p.529–536. 2016 <https://doi.org/10.1016/j.aquaculture.2016.08.001>

THUY, T., QUACH, T., NGUYEN, N., VU LUONG, D., BUI, L. AND VAN, T. Structure and cytotoxic activity of ulvan extracted from green seaweed. **International Journal of Biological Macromolecules** v.93, p.695–702. 2016 <https://doi.org/10.1016/j.ijbiomac.2016.09.040>

TZIVELEKA, L-A; IOANNOU, E; ROUSSIS, V. Ulvan, a bioactive marine sulphated polysaccharide as a key constituent of hybrid biomaterials: A review. **Carbohydrate polymers**, v. 21, p. 355-370. 2019 <https://doi.org/10.1016/j.carbpol.2019.04.074>

UNITED NATIONS, 2015. Transforming our world: The 2030 agenda for sustainable development. In: A/RES/70/1. UN General Assembly. Disponible en <https://www.un.org/en/development/desa/population/migration/generalassembly/docs/globalcompact/A_RES_70_1_E.pdf>Acceso en agosto 7 del 2021

VARELA-MEJÍAS, ALEXANDER; PEÑA-NAVARRO, NELSON; ARANGUREN-CARO, LUIS FERNANDO. Necrosis aguda de la hepatopáncreas: una revisión de la enfermedad en *Penaeus vannamei*. **Agronomía Mesoamericana**, vo. 28, n 3, p. 735-745. 2017

VATSOS, I., REBOURS, C. Seaweed extracts as antimicrobial agents in aquaculture. **Journal of Applied Phycology** v.27, p.2017–2035. 2015. doi: [10.1007/s10811-014-0506-0](https://doi.org/10.1007/s10811-014-0506-0)

WIJESEKARA, I; PANGESTUTIA, R. KIMA, S.K. Biological activities and potential health

benefits of sulfated polysaccharides derived from marine algae. **Carbohydrate Polymers**. V.84, p.14–21. 2011. <https://doi.org/10.1016/j.carbpol.2010.10.062>