Revista Internacional de Investigación e Innovación Tecnológica
Página principal: www.riiit.com.mx

Nutraceutical potential of nejayote as a supplement to the conventional diet for feeding Pacific white shrimp (*Penaeus vannamei*) infected with *Vibrio parahaemolyticus*

Potencial nutracéutico del nejayote como suplemento a la dieta convencional para la alimentación del camarón blanco del Pacífico (*Penaeus vannamei*) infectado con *Vibrio parahaemolyticus*

[1]Gámez-Bayardo, S., [1]Castañeda-Ruelas, G.M., [2]Mora-Rochín, S., [1]López-López, E., [3]Valdez-González, F.J., [4]Gutierrez-Dorado, R., [1*]Jiménez-Edeza, M.

[1] Laboratorio de Investigación y Diagnóstico Microbiológico. Posgrado Integral en Biotecnología. Facultad de Ciencias Químico-Biológicas. Universidad Autónoma de Sinaloa. Culiacán, Sinaloa. CP 80013, México.
[2] Laboratorio de Nutracéuticos. Posgrado Integral en Biotecnología. Facultad de Ciencias Químico-Biológicas. Universidad Autónoma de Sinaloa. Culiacán, Sinaloa. CP 80013, México.
[3] Escuela Nacional de Ingeniería Pesquera. Universidad Autónoma de Nayarit. San Blas, Nayarit. CP 63740, México.
[4] Laboratorio de Bioprocesos y Alimentos Funcionales. Posgrado Integral en Biotecnología. Facultad de Ciencias Químico-Biológicas. Universidad Autónoma de Sinaloa. Culiacán, Sinaloa. CP 80013, México.
sergiogbayardo@gmail.com; gloria.ruelas@uas.edu.mx; smora@uas.edu.mx; est.esau.lopezl@uas.edu.mx; pacoblof43@gmail.com; robe399@hotmail.com; *mjimeneze@uas.edu.mx

Technological innovation: Implementation of corn by-products as an immunostimulant in shrimp diet.

Industrial Application Area: Biotechnology, Aquaculture and Microbiology.

Received: november 19th, 2020
Accepted: april 22th, 2021

Resumen

Los inmunoestimulantes han sido utilizados para prevenir enfermedades en cultivo de *Penaeus vannamei*. El nejayote es un residuo de la nixtamalización del maíz, cuyo perfil fitoquímico provee propiedades bioactivas. Este estudio evaluó el efecto de la adición de nejayote (2%, 4%, 8%, 16% p/p) al alimento comercial para camarón (AC) sobre las propiedades nutracéuticas y nutricionales. La respuesta fisiológica del camarón infectado con *Vibrio parahaemolyticus* y alimentado con la mejor formulación (ACN) fue evaluada. Los alimentos se caracterizaron por análisis físicoquímico, proximal, fenólico y actividad antioxidante (AOX). Se realizaron bioensayos para determinar el factor de conversión alimenticia (FCR) y la respuesta celular (CR) del camarón alimentado con

ACN y AC. Para FCR, el peso del grupo de camarones se registró durante 14 días de alimentación. Para CR, el grupo alimentado con ACN fue infectado con *V. parahaemolyticus* y el contenido de hemocitos fue registrado por 6 h. La calidad del agua (fisicoquímicos y microbiológicos) fue monitoreada para determinar su influencia. El contenido de fenoles totales y AOX se incrementó conforme la adición del nejayote en AC ($p \leq 0.05$). La formulación AC-4% presentó el mejor perfil nutrimental con potencial bioactivo ($p \leq 0.05$). Los organismos alimentados con AC-4% expresaron un mejor FCR ($p < 0.05$), la cual promueve la respuesta celular en poblaciones de camarón. Se descartó la relación de la calidad del agua con la respuesta fisiológica de los grupos ($p \leq 0.05$). El nejayote es un subproducto con alto potencial uso en la sanidad del cultivo camaronícola.

Palabras clave: *Penaeus vannamei*, Nejayote, Alimento nutraceútico, *Vibrio parahaemolyticus*.

Abstract

Immunostimulants have been used to prevent *Penaeus vannamei* culture diseases. Nejayote is a residue from the nixtamalization of corn, whose phytochemical profile provides bioactive properties. This study evaluated the effect of adding nejayote (2%, 4%, 8%, 16% w/w) to conventional shrimp feed (AC) on the nutraceutical and nutritional properties. The physiological response of shrimp infected with *Vibrio parahaemolyticus* and fed with the best formulation (ACN) was evaluated. Shrimp feed was characterized by physicochemical, proximal, phenolic and antioxidant activity (AOX). Two bioassays were performed to determine the feed conversion radio (FCR) and the cellular response (CR) of the shrimp fed with ACN and AC. For FCR, the weight of the shrimps was recorded during 14 days of feeding. For CR, the group fed with ACN was infected with *V. parahaemolyticus* and the hemocyte content was recorded for 6 h. The quality of the water (physicochemical and microbiological) was monitored to determine its influence. The content of total phenols and AOX increased according to the addition of nejayote in AC ($p \leq 0.05$). The AC-4% formulation has the best nutritional profile with bioactive potential ($p \leq 0.05$). The organisms fed with AC-4% expressed a better FCR ($p \leq 0.05$), which promotes the cellular response in shrimp populations. The relationship of the water quality with the physiological response of the groups was discarded ($p \leq 0.05$). Nejayote is a by-product with high potential use in the health of shrimp farming.

Key Words: *Penaeus vannamei*, Nejayote, Nutraceutical feed, *Vibrio parahaemolyticus.*

1. Introduction

The production of food from the sea has increased due to the growth and demand of the world population with an estimated value of profits of USD $ 250 billion [1]. The Pacific white shrimp (*Penaeus vannamei*) is the species that represents >50% of the total world production of food from marine origin [1]. The high production performance of Pacific white shrimp is due to the ability of the organism to grow at high production densities and its adaptation to various conditions of pH (6.8-8.7), salinity (1-50 ppm), dissolved oxygen (3-6 mg / L), NO_2 (1.8-6.4 mg / L), among other abiotic factors [2].

Shrimp production demands requirements that guarantee the health and safety of the product. However, an important aspect of Pacific white shrimp culture is its vulnerability to infectious diseases caused by

pathogenic bacteria of the genus *Vibrio* (*V. harveyi, V. alginolyticus, V. parahaemolyticus, V. campbellii* and *V. penaecida*) [2]. *Vibrio parahaemolyticus* is considered as one of the main disease-causing agents in shrimp production and it is challenge for aquaculture due to health and economic impacts. *Vibrio parahaemolyticus* is responsible for the acute hepatopancreatic necrosis disease (AHPND) or mostly known as early mortality syndrome (EMS), causing total loss of culture within <30 days of production [3]. In this context, the implementation of good aquaculture practices in conjunction with feeding protocols that improve shrimp health and sanitation have aroused interest [4, 5, 6].

The efficiency of shrimp farming is based on nutrition through shrimp feed, which it is estimated to represent the highest cost of production [4]. To satisfy the nutritional demand of shrimp, fish protein is traditionally used, which is characterized by being digestible, having an adequate protein and lipid profile with the shrimp growth stage [7]. However, fishmeal has a high cost, and the growing exploitation of aquaculture has promoted the search for other food sources that partially or totally replace fishmeal sustainably [4].

The physiological response of shrimp fed with several formulation of shrimp feed of animal and plant origin have been evaluated to determine biomass gain, survival and phenotypic attributes [8, 9, 10]. Even the incorporation of nutraceutical compounds (peptides, glucans, sterols, alkaloids, saponins, pigments, essential oils and phenols) to the formulations favors the optimal development and the immune system of the shrimp has been documented [11, 12]. The search for sources of nutraceutical substances and their use in food is an area of interest [13].

Nixtamalization is a thermal-alkaline process for the transformation of corn into edible products, from which two fractions are obtained: the nixtamal (mass) and the nejayote (waste by-product) [14, 15]. Nejayote is an alkaline residual liquid by-product that is produced in large quantities (16 to 22 million m^3) by the corn industry, representing an important source of environmental pollution. [16, 17, 18]. This waste contains high concentrations of organic matter, which their nutritional, nutraceutical, antioxidant and antimicrobial properties have been determined [19, 20, 21, 22]. The nejayote nutraceutical compounds include arabinoxylans, additives for baking products, source of phytochemical compounds such as hydroxycinnamic acid, ferulic acid, *p*-coumaric acid, dehydrodiferulic and dehydrotriferulic acid, making it a promising by-product in the biotech industry [23, 24].

The necessity of the aquaculture industry to guarantee the production and health of Pacific white shrimp, together with the reuse of by-products of the corn industry with biotechnological interest, implies demand to be addressed. Therefore, the aim of this study was to evaluate the nutraceutical potential of nejayote as a supplement to the commercial formulation for feeding shrimp (*Penaeus vannamei*) infected with *Vibrio parahaemolyticus*.

2. Materials and methods

2.1 Materials and equipment

For the formulation of the shrimp feed supplemented with nejayote, a commercial formulation for shrimp based on fish protein (CAMARONINA PLUS®) was used. The nejayote was donated by a regional company that produces corn flour. For the compliance of the bioassays, 120 L plastic fish tanks and seawater from the Sea of Cortez (24°39'01.8"N 107°59'38.7"W) were used.

The adult *Penaeus vannamei* organisms used were donated from the 2019-2020 production cycle from a local shrimp farm. Analytical reagents and culture media were purchased from authorized distributors.

2.2 Elaboration of nejayote flour

To obtain nejayote flour, the procedure was as it follows according to [21] with modifications. A total of 300 L of nejayote were left to stand for 24 h, at 4 °C. Subsequently, the supernatant was removed, and the resulting precipitate was filtered with a smooth organza cloth to decrease the percentage of moisture in the precipitate. The eluate was subjected to drying in the oven at 50 °C for 24 h to remove the moisture completely. Finally, the dry eluate was processed by grinding in a manual mill and then sieved until reaching a suitable pore size (No. 70 sieve, 212 microns). The flour obtained was stored in hermetic bags (4ºC) until its use. The yield for obtaining nejayote flour was calculated using Eq. 1.

$$\text{Yield}=\left(\frac{\text{Sedimented fraction (g)}}{\text{Solid phase (g)}}\right)\cdot 100 \qquad \textbf{(Eq. 1)}$$

2.3 Formulation of shrimp feed by cold extrusion

For the development of the formulation for shrimp supplemented with nejayote as an immunostimulant, the methodology described in [7] was followed with some modifications. The commercial formulation was ground and sieved (mesh no. 8), and the nejayote was added in proportions of 2, 4, 8, and 16% w/w per kg of commercial shrimp feed. For each kilogram of processed shrimp feed, 410 mL of distilled water, 40 g of gelatin were added, and the remainder corresponded to commercial shrimp feed and the percentage of nejayote flour supplement. Once the mixture was homogenized, the mass was subjected to the cold extrusion process, using a model 20N simple screw (Brabender Inc, NJ, USA), temperature of 85 ºC and a screw speed of 240 RPM. Subsequently, the prepared formulation was subjected to a drying process at room temperature with constant ventilation for 24 h. The elaborated formulation was stored at -20 ºC until its later use. The elaborated formulation samples were identified as follows: formulation without nejayote (AC), formulation + 2% of nejayote (AC-2%), formulation + 4% of nejayote (AC-4%), formulation + 8% of nejayote (AC-8%) and formulation + 16% of nejayote (AC-16%) and nejayote flour (HN).

2.4 Physical evaluation and determination of physicochemical parameters of shrimp feed formulations

The physicochemical parameters of the formulations prepared were determined as established in [25] and NMX-F-317-NORMEX-2013. The panel of physicochemical parameters included: diameter, length, yield (pellets/g), density, percentage of fines, water absorption capacity and pH. All analyzes were performed in triplicate on each made formulation.

2.5 Proximal analysis of processed shrimp feed

According to the specifications by the AOAC 2000 (Association of Official Analytical Chemists) the proximal properties of the shrimp feed were determined. For the analysis of humidity (AOAC 950.46), ash (AOAC 920.153), non-protein nitrogen (AOAC 960.52), and lipids (AOAC 4.5.01 Method 920.39). All tests were carried out in triplicate. The carbohydrate content was determined by difference in nutritional content. The caloric content was determined by converting grams to Kcal, thus being 1 g of carbohydrates = 9 Kcal, 1 g of lipids = 9 Kcal, and 1 g of protein = 4 Kcal.

2.6 Extraction of soluble phenolic compounds

For the extraction of soluble phenolic compounds, the method described in [26] was followed. Briefly, 0.01 g of flour was weighed and 1 mL of 80% (v/v) ethanol was added. This suspension was stirred in a rotator (OVAN Noria R, USA, 2010) at a speed of 25 RPM for 10 min. Subsequently, it was placed in the centrifuge (Thermo Fisher Scientific®, USA) at 3000 g at 4 °C for 10 min. The supernatant was brought to a volume of 200 µL in a concentrator (Speed Vac Concentrator, Thermo Electron Corporation) at 35 °C at low pressures. The concentrate was stored at -20 °C until use. The extractions were performed by quadruplicate.

2.7 Extraction of insoluble phenolic compounds

The extraction of insoluble phenolic compounds was performed using the method described in [26] and modified by [27]. The soluble extract residue was digested with 1 mL of 2 M NaOH and the sample was subjected to heat treatment for 30 min in a water bath (Labnet®, USA) at 90 °C. Subsequently, the sample was stirred for 60 min at 25 °C. The mixture was acidified with 0.2 µL of concentrated HCl and 500 µL of hexane was added for lipid removal. The resulting mixture was extracted four times with 500 µL of ethyl acetate. The ethyl acetate fraction was collected and evaporated to dryness (SpeedVac Concentrator, Thermo Electron Corporation). The compounds that were extracted were reconstituted with 200 µL of 50% methanol and stored at -20 °C until use. The extractions were performed by quadruplicate.

2.8 Determination of total phenolic compounds

To determine the concentration of total phenolics, the Folin-Ciocalteu colorimetric method described in [28], was used. In a 96-well plate, 20 µL of the soluble and insoluble phenolic compound extract was added. Subsequently, 180 µL of Folin-Ciocalteu reagent and 50 µL of Na_2CO_3 at 7% w/v were added. The plate was left to incubate for 90 min at room temperature in the absence of light to avoid photodegradation of the compounds. After the incubation time, the absorbance at 750 nm was recorded in a microplate reader (Synergy HT, Biotek Instrument). A calibration curve was constructed with reagent grade gallic acid as a standard. The results were expressed as mg equivalents of gallic acid (AGE) in 100 g of sample on a dry sample (ds) (mg AGE / 100 g, ds). The content of total phenolic compounds was calculated by adding the phenolic compounds present in the soluble and insoluble extracts.

2.9 Antioxidant activity of shrimp feed formulation by $ABTS^{+\bullet}$ method

For the evaluation of the reduction of the radical $ABTS^{+\bullet}$ by the antioxidants present in the extracts, the methodology proposed in [29] was followed. An aliquot of the solution with the radical $ABTS^{+\bullet}$ was diluted in phosphate buffer solution (PBS) until reaching an absorbance of 0.7±0.02 at a wavelength of 734 nm. A reading of the blank and extracts was taken. The test consisted in adding 20 µL of the sample and 1980 µL of $ABTS^{+\bullet}$ solution. The absorbance at 734 nm was measured 15 min after initial mixing and the absorbance loss of $ABTS^{+\bullet}$ was calculated relative to a blank. A Trolox equivalent calibration curve was prepared and data was expressed as µmol TE/100 g (ds).

2.10 Experimental design of the performed bioassays

To determine the effect of the prepared shrimp feed formulation on the growth and cellular response of shrimp, two bioassays (I and II) were performed. These bioassays were carried out in fish tanks with a capacity of 120

L of water and were filled with 20 L of seawater obtained from the Sea of Cortez (24°39'01.8"N 107°59'38.7"W). Each one of the fish tanks had constant aeration, cleaning by removing organic matter by siphoning and the lost water was recovering daily during the two bioassays. For both bioassays, the culture conditions described below were standardized.

2.10.1 Physicochemical monitoring of water

To monitor the physicochemical conditions, measurements of the parameters of pH, temperature, concentration of total ammonia nitrogen (TAN) and dissolved oxygen in the water were made during the acclimatization period and during the performance of the bioassays using a water quality kit, according to [30] (LaMotte, USA). Evaluations of these parameters were performed once a day in duplicate.

2.10.2 Microbiological water monitoring

The monitoring of microbiological parameters on water was performed according to [31]. From each of the fish tanks, a 50 mL water sample was taken in aseptic conditions in a falcon tube. The tube was submerged in the middle of the tank and was closed under the water. Subsequently, from this sample, one mL aliquot was taken and inoculated in 9 mL 1X PBS to make serial dilutions (10^{-1}–10^{-5}). An aliquot of each dilution (10 µL) was inoculated on TCBS agar added with 2.5% NaCl (p/v) and the bacterial concentration was quantified using the drop counting technique. The counting tests were performed by duplicate and the results were expressed in CFU/mL.

2.11 Bioassay I: Biological response of *Penaeus vannamei*

The comparison of the biological response of the organisms fed with a commercial shrimp feed formulation versus the formulation supplemented with 4% nejayote was carried out using 8 fish tanks with 6 organisms each with a starting weight of 11.00 ± 2.91 g. In 4 fish tanks, the organisms were fed with commercial shrimp feed formulation and in the remaining 4 tanks with the formulation supplemented with nejayote with the best nutritional characteristics. The organisms were fed in relation to 4% of their biomass, with rations of 30% in the morning, 30% in the afternoon and 40% at night. This evaluation was performed over a period of 14 days. During this period, the feed conversion radio (FCR) [32] and the biometry of the organisms [33] of each experimental group were determined.

2.12 Bioassay II: Cellular response of *Penaeus vannamei*

2.12.1 Preparation of suspension of *Vibrio parahaemolyticus*

The selected *V. parahaemolyticus* strain was previously isolated from *Penaeus vannamei* shrimp samples and characterized [34]. The growth and concentration of the bacteria were performed according to [35]. The selected strain was cultured in 500 mL of sterile medium of peptone water added with 3.5% NaCl, and it was incubated at 30 °C for 24 h. Subsequently, the bacterial culture was centrifuged (Sorvall™ ST 16 Model, Thermo Scientific, Germany) at 4500 RPM for 10 min at 4 °C. The pellet was recovered and adjusted to a concentration range of $1x10^8$ CFU/mL by serial dilutions (10^{-1}–10^{-8}). The concentration was verified by total count on TCBS agar added with 2.5% NaCl (p/v).

2.12.2 Determination of the lethal dose (LD_{50}) of *V. parahaemolyticus* in *Penaeus vannamei*

To determine the LD_{50}, the procedure established in [36] was followed, with some modifications. Four plastic fish tanks were used, each containing six organisms that were subjected to an acclimatization period of

seven days. During this period the organisms were fed with a commercial formulation in relation to 4% of their biomass, with rations of 30% in the morning, 30% in the afternoon and 40% at night. Following the acclimation period, the organisms in each group were challenged intramuscularly with 30 µL *V. parahaemolyticus* concentrations of $1x10^8$ CFU/mL, $1x10^7$ CFU/mL, $1x10^6$ CFU/mL, respectively, and 1X PBS for the control group. From this, the mortality and survival of the organisms was recorded. The LD_{50} was determined by Probit analysis [36]. Following this, the linear regression of the Probit values vs the logarithm of the dose was calculated. Once these values were known, it was possible to calculate the LD_{50} value. Additionally, the survival of the organisms infected with *V. parahaemolyticus* was determined.

2.12.3 Phisiological evaluation of *Penaeus vannamei*

The external evaluation of the shrimp consisted of taking a specimen from all the fish tanks (n=4) to visually detect the coloration in the cuticle, the pleopods and periopods, the facial deformations, the characteristics of the exoskeleton and the necrosis or melanization in the shrimp cuticle. For internal evaluation of the shrimp, an organism was taken from each fish tank and the gills, intestine, cecum and hepatopancreas were aseptically removed. The organs were observed fresh under the microscope (40x, ONYX, Mexico) with 0.85% saline solution and lesions caused by bacterial infection were determined according to the established criteria [37]. These attributes were measured at 0, 3 and 6 h.

2.12.4 Evaluation of the cellular response of *Penaeus vannamei*

The evaluation of the cellular response was performed by following a previously described protocol [38]. From a bacterial suspension of *V. parahaemolyticus* ($1x10^3$, $1x10^4$ and $1x10^5$ CFU/mL), two organisms per fish tank were injected with 30 µL intramuscularly. An equal volume of PBS (30 µL) was injected into the organisms for the control group. Hemolymph samples were taken at 0, 3 and 6 h after injection from the cardiocele of each shrimp in the ventral part of the organism, just in the area of the second pair of pleopods with insulin syringes (27G x 13 mm). The syringe was pre-filled with 20 µL of anticoagulant solution (30 mM $Na_3C_6H_5O_7$, 0.34 M NaCl and 10 mM EDTA at pH 7.55). The circulating hemocyte count was performed with the use of a hematocytometer (Neubauer improved) in an optical microscope (ONYX, Mexico). The number of hemocytes (cell/mL) was determined according to Eq. 2.

$$CR=\left(\frac{\text{Counted hemocytes}}{\text{Number of counted quadrants}}\right)\cdot 10000 \quad \textbf{(Eq. 2)}$$

2.13 Statistic analysis

The data were analyzed using a one-factor analysis of variance (ANOVA) to determine if there were significant differences ($p\leq 0.05$) between the means of the shrimp feed effect over the organisms. The Tukey-Kramer test was used for multiple comparison of means. Additionally, a Pearson correlation test was performed for the relationship of phenolic compounds and antioxidant activity. All analyzes were performed using Minitab statistical software version 19.2020.1.0.

3. Results and discussion

3.1 Yield of nejayote flour production

From the processing of the residual liquid from nixtamalization (nejayote) donated by the regional corn flour producer company, 745.36 g of nejayote flour were obtained on a dry sample. The yield obtained from this flour was 7.2±1.2%.

3.2 Physicochemical evaluation of the shrimp feed

Table 1 describes the physical characterization of the experimental formulations. The statistical analysis showed significant differences ($p \leq 0.05$) in the mean values of the diameter, yield, percentage of fines and water retention capacity among the formulations evaluated. The addition of nejayote to AC increases the diameter (2.80±0.40) and the percentage of fines (0.087±0.004), but the yield (27.83±0.93) and water retention capacity (51.59±4.49). The values of length (2.88±0.70), density (1.25±0.00) and pH (6.30±0.10) were not significantly between the formulations ($p \geq 0.05$).

The selection of ingredients for the development of shrimp feed formulations determines the texture, water retention capacity, uniformity, cost and nutritional quality of the feed [39]. The addition of HN to AC modifies some physical properties (Table 1), but they are maintained with values according to that reported in the literature [25, 40]. The evaluation of the physical quality in feed formulations after their immersion in water allows to establish critical points in the maintenance of nutrients and the effect on the feeding response of shrimp [25].

Table 1. Physicochemical properties of the elaborated formulation supplemented with nejayote.

Parameters	Commercial feed shrimp supplemented with nejayote				
	AC	AC-2%	AC-4%	AC-8%	AC-16%
Diameter (mm)	2.00±0.32^{b}	2.80±0.41^{a}	2.80±0.41^{a}	2.80±0.41^{a}	2.80±0.41^{a}
Length (mm)	2.50±0.88^{a}	2.90±0.64^{a}	2.90±0.64^{a}	3.00±0.56^{a}	3.10±0.64^{a}
Yield (pellets/g)	73.00±1.00^{a}	26.33±1.15^{b}	27.33±0.57^{b}	26.33±0.57^{b}	27.33±1.15^{b}
Density (g/mL)	1.25±0.00^{a}	1.25±0.00^{a}	1.25±0.00^{a}	1.25±0.00^{a}	1.25±0.00^{a}
Percentage of fines ($1x10^{-3}$)	8.86±1.52^{a}	8.06±0.57^{b}	8.76±2.08^{a}	8.86±1.15^{a}	8.93±3.21^{a}
Water retention capacity	62.57±0.54^{a}	45.92±0.24^{c}	49.44±0.08^{d}	53.81±1.01^{c}	57.18±0.70^{b}
pH	6.45±0.01^{a}	6.19±0.34^{a}	6.24±0.38^{a}	6.28±0.22^{a}	6.36±0.03^{a}

Row literals represent significant differences ($p \leq 0.05$) between the means by one-factor analysis of variance (ANOVA) and the establishment of mean differences using the Tukey-Kramer test.

The diameter and length in a shrimp feed formulation are indicators in the quality control of product elaboration [25, 39]. The adequate size of the pellet depends on the age of the organism, the suggested optimal size for adult organisms is 2.5 mm x 5 mm [40], as observed in our results. The yield of a shrimp feed must be small enough, this in order to be available for all organisms in culture, ensuring a uniform intake and growth [25, 41]. The values obtained in our study proved that the addition of nejayote does not influence the size and that it is within the optimal size (2.88 ± 0.68). Water retention capacity (WRC) is a relevant attribute for shrimp culture, due the shrimp feed formulation that is submerged and remains intact for a period of 4-6 h, that the shrimp feed is needed for it to be consumed [39]. The WRC values of the formulations ranged from 49-57%, guaranteeing the conservation of their properties [42]. Other factors must be considered on the WRC of the shrimp feed formulation such as the surface tension between the shrimp feed formulation and the water, the displacement of the pellet, the temperature and the salinity of the water [43, 44].

3.3 Determination of the proximal composition of the shrimp feed formulations prepared

The ideal shrimp feed formulation for optimal shrimp growth is in accordance with the stage they are in and the proximal composition of the elaborated product [4, 7, 12]. The

proximal analysis of the commercial formulation (AC), nejayote flour (HN) and the combination of both is presented in Table 2. The nutritional content varied between the experimental formulations elaborated and the AC ($p \leq 0.05$). The protein content (30.41±5.06) of AC improves with the addition of nejayote in the range of 2-4% (30.80-37.98%), and the lipid content is gradually decreased with the addition of HN to AC. However, the carbohydrate content does not reflect a defined pattern with the addition of HN in AC. The AC-4% formulation has a content of proteins (30.80±0.23), lipids (5.73±0.17) and carbohydrates (39.26±0.18) that corresponds to the ideal shrimp feed to be administered to shrimp in the juvenile/adult stage [4, 12].

During the cultivation of juvenile shrimp, its nutritional demand must be satisfied by requiring a food that contains 30% protein, 10% lipids, 43% carbohydrates, 1.5% fiber, 9% ash and 3.5% moisture [7]. Meanwhile, in the adult stage, the nutritional requirements are 30% protein, 4% lipids, 42% carbohydrates, 4% fiber, 10% ash and 10% moisture [4, 12]. In this sense, the values of the formulation with 4% added nejayote contain nutrients similar to those reported as adequate for shrimp intake. The protein content is relevant because the amino acids present in the formulation are adapted to the requirements of the organism and its state of development [44].

Table 2. Proximal composition of the shrimp feed added with nejayote.

Shrimp feed	Percentage of proximal composition (%)					
	Moisture	**Ashes**	**Lipids**	**Proteins**	**Carbohydrates**	**Kcal/kg**
AC	5.50 ± 0.05^{c}	16.46 ± 0.05^{a}	8.49 ± 0.06^{a}	30.41 ± 1.42^{bc}	40.00 ± 0.12^{cd}	354.77 ± 0.50^{b}
HN	4.57 ± 0.07^{d}	1.92 ± 0.10^{e}	6.47 ± 0.03^{b}	10.33 ± 0.07^{e}	76.69 ± 0.06^{a}	406.35 ± 0.32^{a}
AC-2%	8.92 ± 0.11^{a}	15.93 ± 0.29^{b}	6.44 ± 0.10^{b}	37.98 ± 0.10^{a}	30.68 ± 0.32^{e}	332.54 ± 2.77^{c}
AC-4%	8.28 ± 0.14^{b}	15.90 ± 0.05^{b}	5.73 ± 0.17^{cd}	30.80 ± 0.23^{b}	39.26 ± 0.18^{d}	331.87 ± 1.39^{c}
AC-8%	8.53 ± 0.15^{b}	14.96 ± 0.10^{c}	5.94 ± 0.18^{bc}	28.14 ± 0.46^{c}	42.40 ± 0.58^{c}	335.68 ± 0.75^{c}
AC-16%	8.91 ± 0.03^{a}	13.95 ± 0.19^{d}	5.40 ± 0.17^{d}	23.02 ± 1.40^{d}	48.69 ± 0.69^{b}	333.53 ± 1.47^{c}

Row literals represent significant differences ($p \leq 0.05$) between the means by one-factor analysis of variance (ANOVA) and the establishment of mean differences using the Tukey-Kramer test.

Regarding the lipid content in the formulation, its relevance converges on the storage of these in the shrimp hepatopancreas, a functional organ that stores this type of components and transforms it into energy when necessary [44]. In our results with the different additions of nejayote, the protein content was different, oscillating (37 to 28%) as the concentration of nejayote was increased. The nature of cold extrusion could be influencing protein denaturation by temperature during the manufacturing process [45], and thus complexing it with other components of the shrimp feed formulation. Nejayote as a supplement in animal feed formulations has been documented [15]. A previous study shows that the contribution of proteins and Ca^{+2} of the nejayote added to a cattle feed improve the nutrition and weight gain of the animals [16]. The tendency of shrimp farmers is to provide shrimp feed with adequate protein levels, considering that this makes the growth of organisms in culture more efficient without increasing the cost of production [1, 2].

3.4 Content of phenolic compounds in shrimp feed formulations

Figure 1 shows the phenol content of the experimental shrimp feed. The concentration of soluble and insoluble phenolic compounds varies between AC and HN ($p \leq 0.05$). The quantification and behavior of soluble and insoluble phenolic compounds of the shrimp

feed formulations prepared shows that the increase in the concentration of HN favors the content of phenolic compounds ($p \leq 0.05$). The shrimp feed AC-8% and AC-16% show the highest content of soluble phenols (376.56±12.59 and 461.72±27.95 mg AGE/100 g ds, respectively) compared to AC (298.01±0.79 mg AGE/100 g ds). The formulation AC-4% (261.28±6.59 mg AGE/100 g ds) does not modify the content of soluble phenolic compounds compared to AC (298.01±0.79 mg AGE/100 g ds). The increase in insoluble phenolic compounds in the formulations made increases gradually with the addition of HN, observing an increase of 134% for AC-2%, 175% for AC-4%, 217% for AC-8% and 325% for AC-16% compared to AC.

The use that has been given to nejayote in recent years is aimed at recovering value-added components [15]. It has been suggested that nejayote phenolic compounds can be used as a natural alternative to promote animal growth and additionally take advantage of their antioxidant effect in industry [44]. The evaluation of the content of hydroxynamic acids, the composition of sugars and the antioxidant capacity of corn and nejayote has been reported [46]. Ferulic acid (FA) and ethyl ferulate (EF) are compounds of natural origin from corn that have bioactive properties and act as synthetic β-adrenergic agonists (β-AA), providing biological effects such as antioxidant and anti-inflammatory activity in different animal study models [47, 48, 49, 50].

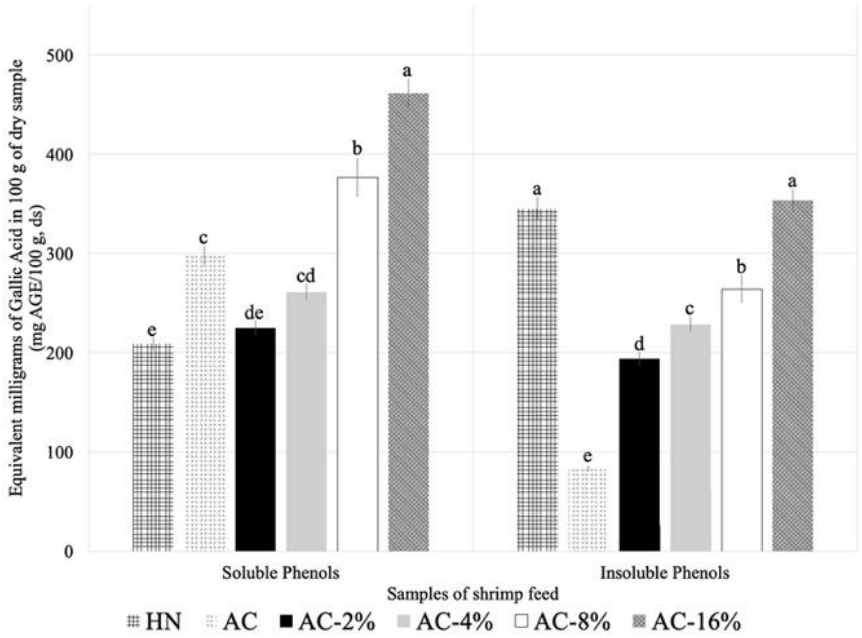

Figure 1. Content of soluble and insoluble phenolic compounds in shrimp feed formulations with nejayote. Literals in histogram bars represent significant differences ($p \leq 0.05$) between the means by one-factor analysis of variance (ANOVA) and the establishment of mean differences using the Tukey-Kramer test.

Furthermore, the prebiotic and immunomodulatory properties of nejayote against degenerative diseases have been demonstrated [51]. Importantly, foods rich in bioactive compounds have become an important alternative to reduce the risk of diseases [51].

3.5 Antioxidant capacity in shrimp feed formulations

The antioxidant capacity between the different shrimp feed formulations is observed in Figure 2. The ABTS method

supports that the addition of nejayote favors the antioxidant activity of AC (48.681±1.329 µmol ET/100 g ds) ($p \leq 0.05$). The values of the formulations were 67,389±2,667 in AC-2%, 73,906±773 in AC-4%, 77,401±2,633 in AC-8% and 81,441±1,991 in AC-16% µmol ET/100 g ds, respectively.

The correlation analysis between phenolic content and antioxidant activity was determined. The results of this test showed that there is a positive statistical relationship of the content of total phenols ($r=0.989$, $p \leq 0.005$), a total antioxidant activity ($r=0.790$ $p \leq 0.005$) as the increase of the percentage of supplement of nejayote to the formulations elaborated. Additionally, the total phenol content is positively related ($r=0.800$, $p \leq 0.005$) as the nejayote content in shrimp feed formulations increases.

Certain biological properties such as the antioxidant activity of nejayote are attributed to the presence of bioactive compounds, such as arabinoxylans (AX) and phenols. The former are polymers of xylose substituted by an arabinofuranosyl, which are present in cereals and grasses without cellulose. The latter are derived compounds that function as a structural link between the pericarp and the endosperm of the grain [15]. Phenols are secondary metabolites that have been attributed the antioxidant character of some cereals [27, 52].

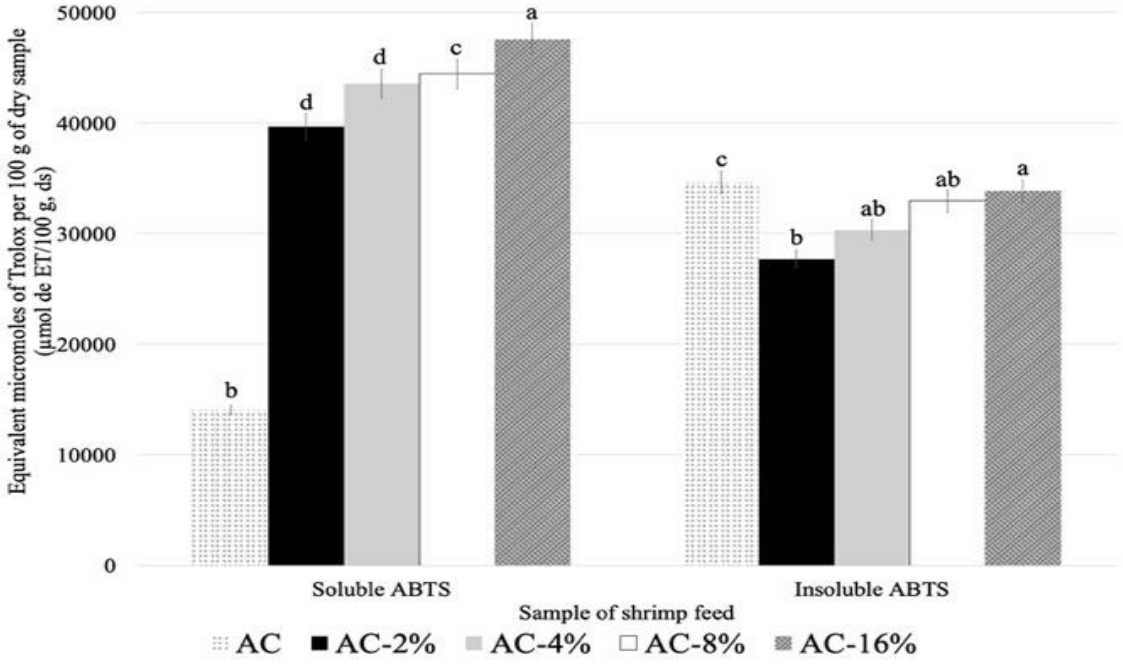

Figure 2. Antioxidant capacity by ABTS in shrimp feed formulations with nejayote. Literals in histogram bars represent significant differences ($p \leq 0.05$) between the means by one-factor analysis of variance (ANOVA) and the establishment of mean differences using the Tukey-Kramer test.

3.6 Bioassay I: Biological response of *Penaeus vannamei*

To carry out the bioassays, the AC-4% formulation was selected due to its optimal nutritional composition for adult *Penaeus vannamei* organisms. Additionally, this formulation had phenol content similar to AC but with antioxidant capacity higher than AC.

The values of weight gain, size and FCR of each of the fish tanks evaluated are found in Table 3. The biometry performed to the organisms fed with AC and AC-4% showed an increase in weight (1.61±1.87 and 1.06±0.66 g) and height (0.17±0.14 and 0.15±0.06 mm) in the organisms of the fish tanks compared to its starting weight and

height. It is worth mentioning that a survival of 100% was observed throughout the bioassay attributed to the adequate control of the physicochemical parameters (Table 4). There were no statistically significant differences ($p>0.05$) between the two-shrimp feed. However, the formulations used in the bioassay showed a better FCR ($p=0.0000$) on the organisms fed AC-4% (2.72±1.90) compared to AC.

The evaluation of biological parameters of shrimp during their growth allows to establish feed administration dynamics with optimal quantities to reach the desired weight and height, without affecting the chemical nature of the aquatic environment [53].

A previous study [54] in shrimp *Penaeus vannamei* with a starting weight of 5.94 g, obtained a FCR of 1.32±0.33 by replacing the fishmeal with fermented cottonseed meal during a period of 28 days. On the other hand, results reported in [55], organisms with a starting weight of 4.44 g showed a FCR of 1.36±0.04 during a period of 60 days.

Table 3. Biometric evaluation of *Penaeus vannamei* organisms exposed to concentrations of *Vibrio parahaemolyticus* and fed with a shrimp feed supplemented with nejayote.

Shrimp feed	Biometric parameter	Fish Tanks			
		I	II	III	IV
AC	Weight gain	1.25±1.12^{a}	0.43±0.25^{b}	0.50±0.08^{ab}	0.56±0.17^{ab}
	Height gain	0.32±0.35^{a}	1.03±0.73^{a}	0.85±0.61^{a}	0.24±0.12^{a}
	FCR	5.97±3.93^{c}	18.26±1.17^{a}	14.16±2.58^{ab}	12.63±1.28^{b}
AC-4%	Weight gain	0.85±0.77^{a}	3.00±0.99^{a}	0.70±0.14^{a}	1.13±1.21^{a}
	Height gain	0.30±0.29^{a}	0.07±0.08^{a}	0.39±0.23^{a}	0.10±0.03^{a}
	FCR	2.53±2.32^{a}	1.15±0.38^{a}	4.98±1.00^{a}	12.63±1.28^{a}

Fish tanks from I, II, III, IV represent the repetitions of the experiment. Row literals represent significant differences ($p\leq0.05$) between the means by one-factor analysis of variance (ANOVA) and the establishment of mean differences using the Tukey-Kramer test.

The use of adult-stage organisms with a starting weight of 11.00 g for the biological and cellular evaluation bioassays in this study justifies the difference and minimal gain in weight and height compared to the aforementioned studies. The increase in weight and height is reflected in the FCR values, since it is one of the variables to be considered as a whole of the food administered to obtain the value of the performance of the food in the biomass [33]. The FCR values of the organisms fed with shrimp feed formulation (AC) and the shrimp feed added with 4% nejayote (AC-4%) were higher than the reference values [33, 54, 55]. An adequate behavior of organisms in culture is demonstrated by obtaining greater weight and size gain, depending on the shrimp feed administered, the growth stage and the acquisition of biomass from the organism, with FCR values between 1.0 to 1.5.

3.7 Bioassay II: Cellular response of shrimp *Penaeus vannamei*

3.7.1 Determination of LD_{50}

The shrimps were challenged at a *V. parahaemolyticus* concentration of $1x10^{8}$ CFU/mL, $1x10^{7}$ CFU/mL and $1x10^{6}$ CFU/mL showed mortality of 100%, 80% and 60% within 3 h post-infection (p.i.), respectively. No mortality was observed in the control group. The regression analysis of accumulated mortality at 6 h (p.i.) revealed that the mean of lethal dose (LD_{50}) of the *V. parahaemolyticus* strain was $3.8x10^{5}$ CFU/mL. Regarding the bioassays performed, data reported suggest that the LD_{50} found for *V. parahaemolyticus* through the challenge by injection in *Penaeus vannamei* is $6.05x10^{5}$ CFU/mL [36].

3.7.2 Physiological evaluation of *Penaeus vannamei*

Regarding the histological analysis of the internal organs of the organisms infected with *V. parahaemolyticus* by injection at different concentrations, it revealed the existence of slight damage in the different monitored organs. A slight deformation in the tubules of the hepatopancreas and cellular hypertrophy were observed in fish tanks II and III with severity grade 1 on the scale of [37] in both tanks in which the AC-4% formulation was administered. In addition to this, a slight presence of tubules with apical strangulation was observed, without a decrease in lipids. In the case of fish tank I, the organisms fed with AC-4% did not show deformation in tubules of the hepatopancreas. On the other hand, gill necrosis was observed in fish tanks I and II that were fed with AC-4% and in fish tank IV fed with AC with severity grade 1 on the scale of [37]. A large presence of chromophore compounds was detected in the intestines of the organisms of all the fish tanks. Tubule deformations, inflammation, and increased cellular activity in *Penaeus vannamei* organisms can be caused by nutritional stress, presence of pathogens, or environmental disturbances [56, 57]. Atrophy that occurs in the tubules of the hepatopancreas is directly related to the presence of bacterial infections [58]. In our study, the degree of severity on the [37] scale helped us to elucidate that the manifestations in the tubules could be related to the LD_{50} to which the organisms were challenged, showing minimal levels of atrophy in the tubules in the first evaluation hours (pi). The *P. vannamei* organisms with lipid content in the hepatopancreas represent the storage of a sufficient supply of energy that can be used during exposure to external conditions such as hypoxia, heat stress, frequent molts or periods of inter-mutations and even when the supply of shrimp feed is interrupted [58].

3.7.3 Cellular response of *Penaeus vannamei*

The cellular response of the organisms exposed to *Vibrio parahaemolyticus* concentrations was determined by the hemocyte count (Figure 3b). The cellular response evaluated in shrimp by the hemocyte count was differentiated into 3 ($4.12x10^6$ cel/mL) and 6 ($1.08x10^{11}$ cel/mL) h (pi), with hemocyte counts higher than the initial count ($1.08x10^7$ cel/mL) before being infected (p=0.000), showing a higher response after the LD_{50} of *V. parahaemolyticus* administrated.

Formulations supplemented with natural compounds and administered to *Penaeus vannamei* organisms infected with *V. parahaemolyticus* have been evaluated, reporting the hemocyte content in response to infection and stimulated with natural ingredients is higher depending on the bioactive nature of the supplement [59]. On the other hand, the effect of the cellular response of the organisms against pathogenic bacteria that are inoculated at the muscular level, there is a decrease in hemocytes present in the hemolymph due to their migration to the point where the affectation was promoted. [59, 60]. In addition to physical parameters during organism infection, cellular parameters show a significant reduction when shrimp were subjected to the combined stress of *Vibrio spp* infections and low salinity [61, 62]. This justifies the behavior of the hemocyte cell count in our study, when the organisms were challenged with *V. parahaemolyticus*.

3.7.4 Physicochemical and microbiological monitoring

The monitoring of physicochemical parameters of seawater in fish tanks such as pH, temperature, dissolved oxygen and total ammonia nitrogen (NAT) remained within the optimal value [1, 63, 64] and in a stable

manner during the time of the bioassays (Table 4). On the other hand, the microbiological monitoring of the biota in each one of the fish tanks showed that the concentration of *Vibrio* spp was kept at minimum concentrations to be considered an infection factor of the organisms during the bioassay. The analysis of the bacterial concentration on fish tank I, which was fed with AC showed statistically different from the concentrations of fish tanks II, III and IV, fed with AC%4, which were similar to each other (p=0.000). All fish tanks were kept below the permissible concentration limits for *Vibrio parahaemolyticus* (10^4 MPN/g) [65, 65, NOM-242-SSA1-2009] (Figure 3a).

Water quality in ponds can severely affect shrimp health [66]. An important parameter is the nitrogen released into the environment through *P. vannamei* culture water, since it is considered among the main pollutants in water. Since only 20% is used, the remaining 80% remains immersed in the aquatic environment [67]. The high concentration of the ammonium ion is toxic and affects many physiological processes in shrimp, such as reproduction, growth and survival [68]. For this reason, constant water changes are recommended, in this way reducing the nitrogen concentration and avoiding toxic levels for the organisms in culture [67, 68].

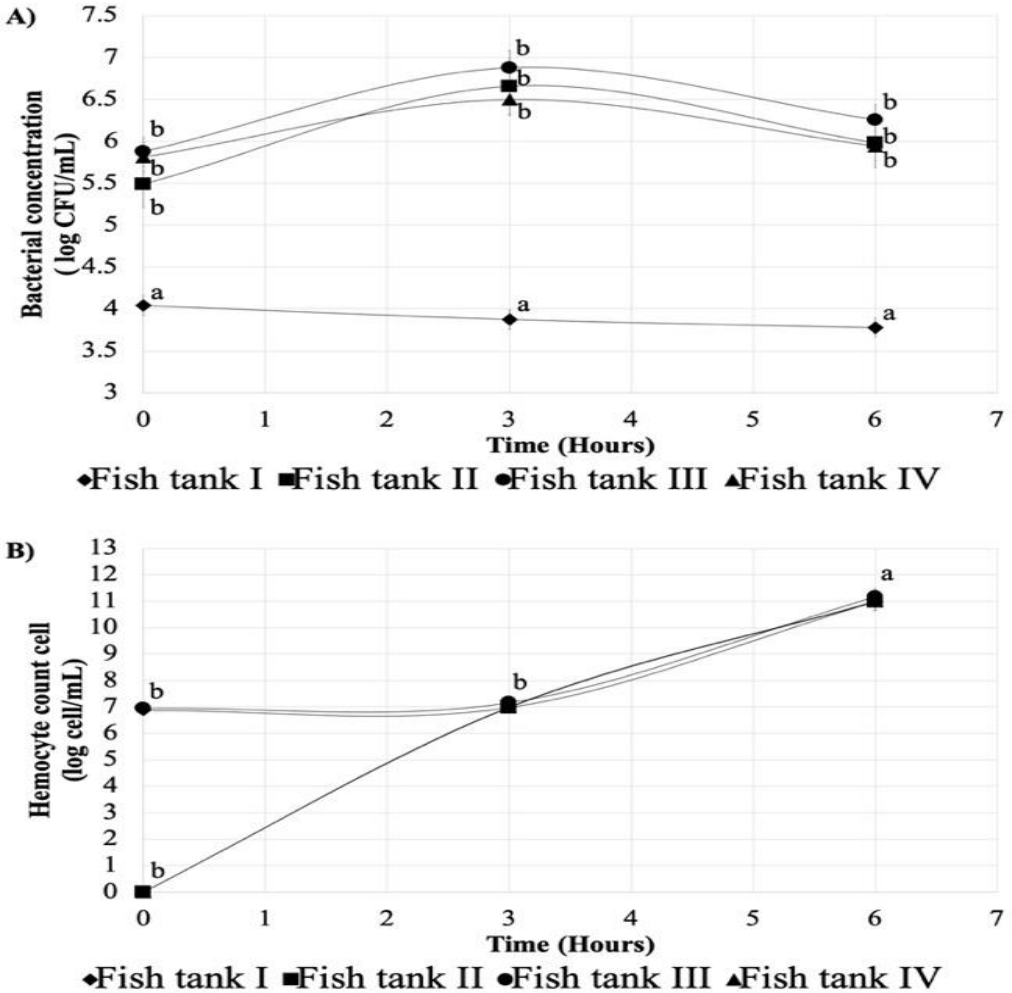

Figure 3. Microbiological and cellular monitoring during the *Penaeus vannamei* biological evaluation bioassay. A) Monitoring of the bacterial concentration in the fish tanks evaluated. B) Hemocyte cell count monitoring in *Penaeus vannamei* shrimp. Literals in intersection points represent significant differences ($p \leq 0.05$) between the means by one-factor analysis of variance (ANOVA) and the establishment of mean differences using the Tukey-Kramer test.

Monitoring of biota in fish tanks is a parameter that can influence the proliferation of bacterial diseases in organisms [69, 70]. In a previous report, it was pointed out that *Vibrio* spp concentrations of 10^5 CFU/mL in the water used for shrimp culture can cause mortality in the entire production [70]. Concentrations of 10^4 CFU/mL of *V. parahaemolyticus* can cause a mortality of 50% at 48 h (p.i.). The concentrations of the biota in each of the fish tanks evaluated in this study were lower than those reported by the authors, so the distribution of biota through the water during the bioassays did not influence the mortality of the organisms evaluated. A Pearson correlation showed that the values of the biota present in each of the tanks were not influenced by their own abiotic parameters ($p \geq 0.05$).

Table 4. Physicochemical monitoring during the *Penaeus vannamei* biological evaluation bioassay.

Evaluation	Parameter	Fish Tanks				Recommended value
		I	II	III	IV	
Physicochemical	pH	7.50±0.00[a]	7.50±0.00[a]	7.50±0.00[a]	7.50±0.00[a]	6.80-8.70 [1, 63, 64]
	Temperature (ºC)	27.00±0.00[a]	27.00±0.00[a]	27.00±0.00[a]	27.00±0.00[a]	27.00-31.00 [1, 63, 64]
	Dissolved oxygen (mgL^{-1})	6.00±0.00[a]	5.93±0.11[a]	6.00±0.20[a]	5.53±0.11[b]	3.00-6.00 [1, 63, 64]
	Total ammonia nitrogen (mgL^{-1})	2.50±0.00[a]	5.00±0.00[b]	0.25±0.00[c]	0.25±0.00[c]	1.20-6.50 [1, 63, 64]

Row literals represent significant differences ($p \leq 0.05$) between the means by one-factor analysis of variance (ANOVA) and the establishment of mean differences using the Tukey-Kramer test.

4. Conclusions

Shrimp feed supplemented with nejayote (AC 4%) can be considered as a source of nutrients with antioxidant properties for the cultivation and health of the *Penaeus vannamei* shrimp, as well as being a possible aid in diseases caused by *Vibrio parahaemolyticus* present during the production cycles. The shrimp feed formulations must have an adequate nutritional content for the shrimp growth stage, because the effect of the formulation is reflected in the biological development and in the reduction of physiological damage in the organs of the organisms in culture. As nejayote is used as an additive to the shrimp feed formulation, its biotechnological potential is used. However, the use of molecular techniques that quantify the response in the immune system of shrimp fed with a shrimp feed formulation supplemented with nejayote is suggested.

5. Acknowledgements

This research was supported by Universidad Autónoma de Sinaloa (Grant PROFAPI). Sergio Gamez-Bayardo's scholarship was provided by the Consejo Nacional de Ciencia y Tecnología (CONACYT). The authors would also like to thank QFB. Alejandra Daniela Jiménez Soberanes for her invaluable support on this research.

6. References

1. Food and Agriculture Organization of the United Nations (FAO). "The State of World Fisheries and Aquaculture". *Sustainability in action*. Rome. 2020 https://doi.org/10.4060/ca9229en

2. Food and Drug Administration (FAO). "The state of world fisheries and aquaculture 2018-Meeting the sustainable development goals". 2018. [Online:

http://www.fao.org/3/i9540en/i9540en.pdf] Last visited: July 25th, 2020.

3. Kumar V., Baruah K., Nguyen D. K., Smagghe D. V., Vossen G., Bossier P. Phloroglucinol mediated Hsp70 production in crustaceans: protection against *Vibrio parahaemolyticus* in *Artemia franciscana* and *Macrobrachium rosenbergii*. Frontiers in Immunology. 1091:9, 2018. https://doi.org/10.3389/fimmu.2018.01091.

4. Ayisi C. L., Hua X., Apraku A., Afriyie G., Kyei B. A. "Recent studies toward the development of practical diets for shrimp and their nutritional requirements". *HAYATI Journal of Biosciencies*. 24:3, 2017, 109-117. https://doi.org/10.1016/j.hjb.2017.09.004.

5. Rudtanatip T., Boonsri N., Asuvapongpatana S., Withyachumnarnkul B., Wongprasert K. "A Sulfated galactans supplemented diet enhances the expression of immune genes and protects against *Vibrio parahaemolyticus* infection in shrimp". *Fish and Shellfish Immunology*. 65, 2017, 186-197. https://doi.org/10.1016/j.fsi.2017.04.020

6. Prabu D. L. Chandrasekar S., Ambashankar K., Syama-Dayal J., Ebeneezar S., Ramachandran K., Kavitha M., Vijayagopal P. "Effect of dietary Syzygium cumini leaf powder on growth and non-specific immunity of *Litopenaeus vannamei* (Boone 1931) and defense against virulent strain of *Vibrio parahaemolyticus*". *Aquaculture*. 489, 2018, 9-20. https://doi.org/10.1016/j.aquaculture.2018.01.041

7. Trejo-Flores J.V., Luna-González A., Álvarez-Ruíz P., Escamilla-Montes R., Peraza-Gómez V., Diarte-Plata G., Esparza-Leal H. M., Campa-Córdova A. I., Gámez-Jiménez C., Rubio-Castro. "Protective effect of *Aloe vera* in *Litopenaeus vannamei* challenged with *Vibrio parahaemolyticus* and white spot syndrome virus". *Aquaculture*. 465:1, 2016, 60:64. https://doi.org/10.1016/j.aquaculture.2016.08.033

8. Wang Y. C., Chang P. S., Chen H Y. "Differential time-series expression of immune-related genes of Pacific white shrimp *Litopenaeus vannamei* in response to dietary inclusion of beta-1-3-glucan". *Fish and Shellfish Immunology*. 24:1, 2008, 113-121. https://doi.org/10.1016/j.fsi.2007.09.008.

9. Tseng D. Y., Ho P.L., Huang S. Y., Chen S. C., Shiu Y. L., Chiu C. S., y Liu C. H. "Enhancement of immunity and disease resistance in the white shrimp, *Litopenaeus vannamei* by the probiotic *Bacillus subtilis* E20". *Fish and Shellfish Immunology*. 26:2, 2009, 339-344. https://doi.org/10.1016/j.fsi.2008.12.003

10. Wang W., sun J., Liu C. y Xue Z. "Application of immunostimulants in aquaculture: current knowledge and future perspective". *Aquaculture research*. 48:1, 2016, 1-23. https://doi.org/10.1111/are.13161

11. Leyva-López N., Hernández-González C. "Compuestos bioactivos al servicio de la acuacultura". *Ciencia*. 70:4, 2019, 81-86.

12. Tacon A. G. J., Dominy W.G., Pruder G. D. "Tendencias y retos globales de los alimentos para camarón". La Paz, Baja California Sur, México. *Avances en Nutrición Acuícola IV. Memorías del IV Simposium Internacional de Nutrición Acuícola*. 2000, 1-27.

13. Rojas S., Lopera J. S., Uribe A., Correa S., Perilla N., Marín JS. "Consumo de nutracéuticos, una alternativa en la prevención de las enfermedades crónicas no

transmisibles". *Revista Biosalud*. 14:2, 2015, 91-103. http://doi.org/10.17151/biosa.2015.14.2.9

14. Reyes-Vidal, M. Y., Aceves Diez, Á., Martínez-Silva, A., Asaff, A. Investigación, desarrollo tecnológico e innovación para el cuidado y reuso del agua. Hermosillo, Sonora. *Estudios Sociales*. 2012. [Online: https://www.ciad.mx/archivos/revista-dr/RES_ESP2/RES_Especial_2_09_Asaff.pdf] Last visited: September 12th, 2020.

15. Díaz-Montes, E., Castro-Muñoz, R., Yáñez-Fernández, J. "An overview of nejayote, a nixtamalization by product". *Ingeniería Agrícola y Biosistemas*. 8:2, 2016, 41-60. http://dx.doi.org/10.5154/r.inagbi.2016.03.002

16. Velasco-Martinez, M., Angulo, O., Vázquez-Couturier, D. L., Arroyo-Lara, A., Monroy-Rivera, J. A. "Effect of dried solids of nejayote on broiler growth". *Poultry Science*. 76:11, 1997, 1531-1534. https://doi.org/10.1093/ps/76.11.1531

17. Téllez-Pérez, V., López-Olguín, J. F., Aragón, A., y Zayas-Pérez, M. T. "Lodos residuales de nejayote como sustratos para la germinación de semillas de maíz azul criollo". *Revista internacional de contaminación ambiental*. 34:3, 2018, 395-404. http://dx.doi.org/10.20937/RICA.2018.34.03.03

18. Castro-Muñoz, R., Fíla, V., Durán-Páramo, E. "A Review of the primary by-product (Nejayote) of the nixtamalization during maize processing: Potential reuses". *Waste and Biomass Valorization*. 10, 2019, 13-22. https://doi.org/10.1007/s12649-017-0029-4

19. Jing Pu., Bomser J. A., Schwartz S. J., He J., Magnuson B. A., Giusti M. M. "Structure-function relationships of anthocyanins from various anthocyanin-rich extracts on the inhibition of colon cancer cell growth". *Journal of Agriculture and Food Chemistry*. 56:20, 2008, 9391-9398. https://doi.org/10.1021/jf8005917

20. Mendoza-Díaz S., Ortiz-Valerio M. d. C., Castaño-Tostado E., Figueroa-Cárdenas J. d. D., Reynoso-Camacho R., Ramos-Gómez M., Campos-Vega R., Loarca-Piña G. "Antioxidant capacity and antimitagenic activitiy of anthocyanin and carotenoid extracts from nixtamalized pigmented creole maize races (*Zea mays* L.)". *Plants Foods for Human Nutrition*. 67, 2012, 442-449. https://doi.org/10.1007/s11130-012-0326-9

21. Acosta-Estrada, B. A., Lazo-Vélez, M. A., Nava-Valdez, Gutiérrez-Uribe, J. A., Serna-Saldívar, S. O. "Improvement of dietary fiber, ferulic acid and calcium contents in pan bread enriched with nejayote food additive from white maize (Zea mays)". *Journal of Cereal Science*, 60:1, 2014, 264-269. https://doi.org/10.1016/j.jcs.2014.04.006

22. Colín-Chávez C., Virgen-Ortiz J.J., Serrano-Rubio L. E., Martínez-Téllez M. A., Astier M. "Comparison of nutritional properties and bioactive compounds between industrial and artisan fresh tortillas from maize landraces". *Current Research in Food Science*. 3, 2020, 189-194. https://doi.org/10.1016/j.crfs.2020.05.004.

23. Serna-Saldivar S., Gutiérrez-Uribe J. A., Mora-Rochin S., García-Lara S. "Potencial nutracéutico de los maices criollos y cambios durante el procesamiento tradicional y con extrusion". *Revista Fitotecnica Mexicana*. 36:3a, 2013, 295-304.

24. Buitimea-Cantúa N. E., Antunes-Ricardo M., Gutiérrez-Uribe J. A., Rocha-Pizaña M. R., Rosa-Millán J. R., Torres-Chávez P. I. "Protein-phenolic aggregates with anti-inflammatory activity recovered from maize nixtamalization wastewaters (nejayote)." *LWT – Food Science and Technology*. 134, 2020, 109881. https://doi.org/j.lwt.2020.109881

25. Cruz-Suárez, L. E., Ricque-Marie, D., Tapia-Salazar, M., Marín-Zaldivar, L. F., Guajardo-Barbosa, C., Nieto-López, M., Salinas-Miller, A. "Historia y estatus actual de la digestibilidad y algunas características fisicoquímicas de los alimentos comerciales para camarón usados en México". *Avances en Nutrición Acuícola VI. Memorias del VI Simposium Internacional de Nutrición Acuícola.* 3, 2002, 1-22. [Online: http://nutricionacuicola.uanl.mx/index.php/acu/article/view/224/222.] Last visited: August 1st, 2020.

26. Adom K. F., Liu R. H. "Antioxidant activity of grains". *Journal of Agricultural and Food Chemistry*. 50:21, 2002, 6182-6187. https://doi.org/10.1021/jf0205099

27. Mora-Rochín S., Gutiérrez-Uribe J. A., Serna-Saldivar S.O., Sánchez-Peña P., Reyes-Moreno C., Milán-Carrillo J. "Phenolic content and antioxidant activity of tortillas produced from pigmented maize processed by conventional nixtamalization or extrusion cooking". *Journal of Cereal Science.* 52:3, 2010, 502-508. https://doi.org/10.1016/j.jcs.2010.08.010

28. Singleton V. L., Orthorfer R., Lamuela-Raventos R.M. 1999. "[14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent". *Methods in Enzymology*. 299, 1999, 152-178. https://doi.org/10.1016/S0076-6879(99)99017-1

29. Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C. "Antioxidant activity applying an improved ABTS radical cation decolorization assay". *Free Radical Biology and Medicine*. 26:9-10, 1999, 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3

30. Food and Drug Administration (FAO). "Manual para la cria de camarones peneidos, Capitulo 4: Operaciones en una granja camaronera". 1988, [Online: http://www.fao.org/3/ab466s/AB466S00.htm#TOC] Last visited: April 12th, 2021.

31. American Public Health Association (APHA). (2005). Standard Methods of Water and Waterwaste. American Health Association, American Water Works Association, Water Environment Federation Publication. Washington D.C. 1220 pp.

32. Arnold S.J., Coman F.E., Jackson C.J., Groves S.A. "High-intensity, zero water-exchange production of juvenile tiger shrimp, *Penaeus monodon*: an evaluation of artificial substrates and stocking density". *Aquaculture*. 293:1-2, 2009 42-48. https://doi.org/10.1016/j.aquaculture.2009.03.049

33. Xu L., Ji C., Zhao J., Wu H. "Metabolic responses to metal pollution in shrimp *Crangon affinis* from the sites along the Laizhou Bay in the Bohai Sea". *Marine pollution bulletin*. 113:1-2, 2016, 536-541. https://doi.org/10.1016/j.marpolbul.2016.08.052

34. Gámez-Bayardo S., Castañeda-Ruelas G.M., Espinosa-Plascencia A., Bermúdez-Almada M.C., Jiménez-Edeza M. "Characterization of *Vibrio parahaemolyticus* strains and evaluation of shrimp cultivation conditions in a farm at the northwestern of Mexico, as risk predictors for

its adaptation and dissemination." Latin American Journal of Aquatic Research. 49:1, 2021, 75-85. https://doi.org/10.3856/vol49-issue1-fulltext-2512

35. Loc N.H., MacRae T.H., Musa N., Bin-Abdullah M.D.D., Abdul-Wahid E.A., Sung Y.Y. "Non-lethal heat shock increased Hsp70 and immune protein transcripts but not *Vibrio* tolerance in the white leg shrimp." *PLOS ONE*. 8:9, 2013, e73199 https://doi.org/10.1371/journal.pone.0073199

36. Finney D.J. "Statistical method in biological assay". *Journal of the American Pharmaceutical Association.* 42,5, 1952. https://doi.org/10.1002/jps.3030420521

37. Lightner D.V. "A handbook of shrimp pathology and diagnostic procedures for diseases of cultured penaeid shrimp". *Baton Rouge (La.): World Aquaculture Society.* 1999. [Online: https://lib.ugent.be/catalog/rug01:002170818] Last visited: September 1st, 2020.

38. Hong X.P., Xu D., Zhuo Y., Liu H.Q., Lu L.Q. "Identification and pathogenicity of *Vibrio parahaemolyticus* isolates and immune responses of *Penaeus (Litopenaeus) vannamei* (Boone)". *Journal of fish diseases*. 39:9, 2016, 1085-1097. https://doi.org/10.1111/jfd.12441

39. Achupallas J. "Tecnología de alimentos para camarón. En Civera-Cerecedo R, Pérez-Estrada C. J., Ricque-Marie D., Cruz-Suarez L.E. (Eds.). *Avances en Nutrición Acuícola. IV Memorias del IV Simposium Internacional de Nutricion Acuícola.* La Paz, B.C.S. 1998, 520-525. [Online: https://www.uanl.mx/utilerias/nutricion_acuicola/IV/archivos/31achup.pdf] Last visited: September 19th, 2020.

40. Bortone, D. E. "Interacción de ingredientes y procesos en la producción de alimentos hidroestables para camarones. In: Cruz-Suarez L. E., Ricque-Marie D., Tapia-Salazar M., Simoes N. (Eds)." Cancún. México. *Avances en nutrición Acuícola. Memorias del VI Simposium Internacional de Nutrición Acuícola*. [Online: https://www.uanl.mx/utilerias/nutricion_acuicola/VI/archivos/A25.pdf] Last visited: July 13th, 2020.

41. Akiyama, D., Chwang, N. "Requerimientos nutricionales del camarón y manejo de cultivo". *Avances en Nutrición Acuícola III*. 1999, 479-491.

42. Ruiz-Díaz, P.P., Ricque-Marie, D.D., Guajardo-Barbosa, C., Villarreal-Cavazos, D., Nieto-López, M., Mendoza- Cano, J.F., Cruz-Suárez, L.E. "Características físico-químicas de alimentos comerciales para camarón utilizados en México en 2004". *Resumen de poster en Memorias del VII Simposio Internacional de Nutrición Acuícola*. 16-19 de Noviembre, 2004. Hermosillo, Sonora, México. 2004, 88.

43. Muñoz L., Marie, D., Nieto López, M.G., Villarreal, D., Scholz, U., González, M. "Comparación entre Extruído y Pelletizado en Alimentos de Camarones. In: Cruz Suárez, L.E., Ricque." Hermosillo, Sonora. *Avances en Nutrición Acuícola VII. Memorias del VII Simposium Internacional de Nutrición Acuícola*. [Online: http://eprints.uanl.mx/8392/1/22Osvaldo_Munoz.pdf] Last visited: 2nd August, 2020.

44. Lawrence, A., Castille, F., Samocha, T., Velasco, M. "Enviromentally friendly or Least polluting feed and feed management for aquaculture. In C. L. Browdy and Darryl E. Jory, Editors". Lousiana Orlando, USA. *The New Wave, Proceedings of the special session on sustainable Shrimp culture, Aquaculture*. 2002, 84-96.

45. Oberg E. Jones F. "27th Edition Machinery's Handbook. *Industrial Press Inc.* New York, New York. 2000, 1348-1349.

46. Torres, E. F. P. "Efecto de la suplementación de ácido ferúlico y ferulato de etilo en el comportamiento productivo y calidad de la carne de bovinos". Hermosillo, Sonora. *Master's thesis at the Centro de Investigación en Alimentación y Desarrollo*. 2014. [Online: https://ciad.repositorioinstitucional.mx/jspui/bitstream/1006/377/1/PE%c3%91A-TORRES-EF14.pdf] Last visited: August 1st, 2020.

47. Doss, H. M., Dey, C., Sudandiradoss, C., Rasool, M. K. "Targeting inflammatory mediators with ferulic acid, a dietary polyphenol, for the suppression of monosodium urate crystal-induced inflammation in rats". *Life Sciences*. 148, 2016, 201–210. https://doi.org/10.1016/j.lfs.2016.02.004

48. Gerin, F., Erman, H., Erboga, M., Sener, U., Yilmaz, A., Seyhan, H., "The effects of ferulic acid against oxidative stress and inflammation in formaldehydeinduced hepatotoxicity". *Inflammation*. 39:4, 2016, 1377–1386. https://doi.org/10.1007/s10753-016-0369-4

49. Lampiasi, N., Montana, G. "The molecular events behind ferulic acid mediated modulation of IL-6 expression in LPS-activated Raw 264.7 cells". *Immunobiology*. 221, 2016, 486–493. https://doi.org/10.1016/j.imbio.2015.11.001

50. Ghosh, S., Basak, P., Dutta, S., Chowdhury, S., Sil, P. C. "New insights into the ameliorative effects of ferulic acid in pathophysiological conditions". *Food and Chemical Toxicology*. 103, 2017, 41–55. https://doi.org/10.1016/j.fct.2017.02.028

51. Paz-Samaniego, R., Sotelo-Cruz, N., Marquez-Escalante, J., Rascon-Chu, A., Campa-Mada, A. C., Carvajal-Millan, E. (2019). "Nixtamalized Maize Flour By-product as a Source of Health-Promoting Ferulated Arabinoxylans (AX)". *Flour and Breads and Their Fortification in Health and Disease Prevention*. 2019, 225–235. https://doi.org/10.1016/b978-0-12-814639-2.00018-6

52. Velderrain-Rodríguez G. R., Álvarez M.V., Quirós-Sauceda A. E., Vallejo-Córdoba B., González-Coórdova A. F., Villegas-Ochoa M., Ayala-Zavala J. F. y González-Aguilar G. "Capitulo 3: Compuestos bioactivos como ingredientes en alimentos funcionales In: Los alimentos funcionales: Un nuevo reto para la industria de alimentos". Hermosillo, Sonora. Primera edición. Editorial AGT Editor S. A. 2014, 65-96. [Online: https://www.ciad.mx/archivos/dtaov/gustavo/Los_Alimentos_Funcionales.pdf] Last visited: September 12th, 2020.

53. Xu, W., Xu, Y., Su, H., Hu, X., Xu, Y., Li, Z., Wen, G., Cao, Y.. "Effects of feeding frequency on growth, feed utilization, digestive enzyme activity and body composition of *Litopenaeus vannamei* in biofloc-based zero-exchange intensive systems." *Aquaculture*. 522, 2020, 735079. https://doi.org/10.1016/j.aquaculture.2020.735079

54. Sun H., Tang J.W., Yao X.H., Wu Y.F., Wang X., Liu Y. "Effects of replacement of fish meal with fermented cottonseed meal on growth performance, body composition and haemolymph indexes of Pacific white shrimp, *Litopenaeus vannamei* Boone, 1931". *Aquaculture Research*. 47:8, 2016, 2623-2632. https://doi.org/10.1111/are.12711

55. Wangsoontorn S, Chuchird N, Wudtisin I, Crook A. "The effect of substituting fish meal with fermented soybean meal on the growth performance and immune parameters of Pacific white shrimp (Litopenaeus vannamei)". *Journal of Fisheries and Environment*. 42:2, 2018, 32-40. [Online: https://li01.tci-thaijo.org/index.php/JFE/article/view/122446/108476] Last visited: September 12th, 2020.

56. Gong H., Jiang D. H., Lawrence A., González-Félix M. y Pérez-Velázquez M. "Nuevos avances en el estudio de fosfolípidos nutricionales para camarón". *Avances en nutrición acuícola VII. Memorias del VII Simposium Internacional de Nutrición Acuícola*. Hermosillo, Sonora, México. 2004, 329-343.

57. Cuartas E. I., Díaz A. C. y Petriella A. M. "Estudio morfológico e histológico del hepatopáncreas del langostino Pleoticus muelleri (Bate) (*Crustacea, Penaeoidea*)". *Revista de Investigación y Desarrollo Pesquero*. 15, 2002, 5-13. [Online: http://hdl.handle.net/1834/1702] Last visited: September 20th, 2020.

58. Tapia-Salazar M., García-Pérez O. D., Velásquez-Soto R. A., Nieto-López, M. G., Villarreal-Cavazos D., Ricque-Marie D. y Cruz-Suárez L. E. "Growth, feed intake, survival, and histological response of White shrimp *Litopenaeus vannamei* fed diets containing grains naturally contaminated with aflatoxin". *Ciencias Marinas*. 38, 2012, 491-504. https://doi.org/10.7773/cm.v38i3.2094

59. Peña-Navarro N., Vargas-Cordero R., Varela-Mejías A. "Productos naturales como estimuladores del sistema inmunológico de *Litopenaeus vannamei*, infectado con *Vibrio parahaemolyticus*". *Agronomía Mesoamericana*. 24:1, 2013, 133-147. [Online: https://www.redalyc.org/pdf/437/43726204007.pdf] Last visited: September 12th, 2020.

60. Jiravanichpaisal, P., Lee, B.L., Söderhäll, K. "Cell mediated immunity in arthropods: Hematopoiesis, coagulation, melanization and opsonization". *Immunobiology*. 211, 2006, 213-236. https://doi.org/10.1016/j.imbio.2005.10.015

61. Lin Y.C., Chen J.C., Li C.C., Morni W.Z.W., Suhaili A.S.N., Kuo Y.H., Huang C.L. "Modulation of the innate immune system in white shrimp *Litopenaeus vannamei* following long-term low salinity exposure". *Fish & Shellfish Immunology*. 33:2, 2012, 324-331. https://doi.org/10.1016/j.fsi.2012.05.006

62. Li C.C., Yeh S.T., Chen J.C. "Innate immunity of the white shrimp *Litopenaeus vannamei* weakened by the combination of a *Vibrio alginolyticus* injection and low-salinity stress". *Fish & Shellfish Immunology*. 28:1, 2010, 121-127. https://doi.org/10.1016/j.fsi.2009.10.003

63. De la Mora G., Villarreal-Delgado E., Arredondo-Figueroa J., Ponce-Palafox J. y Barriga-Sosa I. "Evaluación de algunos parámetros de calidad del agua en un sistema cerrado de recirculación para la acuicultura, sometido a diferentes cargas de biomasa de peces". *Hidrobiológica*. 13:4, 2003, 247-253.

64. Franco M. A., Blancheton J. P., Deviller G., Charrier A. y Le J. Y. "Effect of fish size and hydraulic regime on particulate organic matter dynamics in a recirculation aquaculture system: elemental carbon and nitrogen approach". *Aquaculture*. 239:1-4, 2004, 179-198. https://doi.org/10.1016/j.aquaculture.2004.05.040

65. Boyd C. E. "Consideraciones sobre la calidad del agua y del suelo en cultivos de

camarón". *Métodos para mejorar la camaronicultura en Centroamérica*. 1, 2001, 1-30. [Online: http://www.cesasin.com.mx/CentroAmerica/1Calidad%20del%20agua.pdf] Last visited: July 25th, 2020.

66. Rojas A. A.A., M.C. Haws y J. A. Cabanillas (eds). "Buenas prácticas de manejo para el cultivo de camarón".United States of America. *The David and Lucile Packard Foundation. United States Agency for International Development* (Cooperative Agreement No. PCE-A-00-95-0030-05).

67. Frías-Espericueta M.G., Páez-Osuna F. "Toxicidad de los compuestos del nitrógeno en camarones". *Camaronicultura y medio ambiente*. 2001. 253-276. [Online: http://www.industriaacuicola.com/biblioteca/Camaron/Toxicidad%20de%20los%20compuestos%20del%20nitrogeno%20en%20camarones.pdf] Last visited: June 15th, 2020.

68. Jones A.B., O'donohue M.J., Udy J., Dennison W.C. "Assessing ecological impacts of shrimp and sewage effluent: biological indicators with standard water quality analyses". *Estuarine, Coastal and Shelf Science*. 52:1, 2001, 91-109. https://doi.org/10.1006/ecss.2000.0729

69. Kalburge S. S., Polson S. W., Boyd-Crotty K., Katz L., Turnsek M., Tarr C. L., Martinez-Urtaza J., Boyd E. F. "Complete genome sequence of *Vibrio parahaemolyticus* environmental strain UCM-V493." *Genome Announcements*. 2:2, 2014, e00159-14. https://doi.org/10.1128/genomeA.00159-14

70. Mizan M.F.R., Jahid I.K., Kim M.H., Kim, T.J., Ha. S.D. "Variability of biofilm formation correlates with hydrophobicity and quorum sensing among *Vibrio parahaemolyticus* isolates from food contact surfaces and distribution of genes involved in biofilm formation." *Biofouling*. 32:4, 2016, 497:509. https://doi.org/10.1080/08927014.2016.1149571

71. 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*. 81:5, 2015, 1689-1699. http://doi.org/10.1128/AEM.03610-14