*Research Article*



# **Preliminary study of diets supplemented with chitosan for white snook (***Centropomus viridis***) juveniles: effect on growth, survival, and nutritional efficiency**

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**ABSTRACT.** Due to its beneficial biological properties, chitosan extracted from crustacean byproducts emerges as a viable strategy to mitigate the anti-nutritional factors in vegetable-based diets for juvenile white snook (*Centropomus viridis*). The growth, survival, and nutritional efficiency of juvenile white snook-fed diets containing chitosan were evaluated. Chitosan was extracted chemically and enzymatically from shrimp byproducts (exoskeletons and heads). It was then included in a soybean meal-based diet (48% protein and 19% kJ g<sup>-1</sup> gross energy) at 0, 0.5, and 1.0% chitosan levels (D-Control, CHI-0.5%, and CHI-1.0%). Juvenile snook (initial weight:  $44.5 \pm 4.2$  g) were randomly distributed into 300 L tanks and fed the experimental diets three times daily for 45 days. Fish fed the CHI-0.5% and CHI-1.0% diets were significantly  $(P < 0.05)$  higher in growth rates than the D-Control diet. No significant differences in survival were observed. The FCR of the CHI-0.5% and CHI-1.0% diets were lower when compared to the D-Control  $(P < 0.05)$ . The results suggest the utilization of chitosan in diets with high soybean meal content for snook to obtain better growth and survival.

**Keywords:** *Centropomus viridis*; chitosan; shrimp byproducts; growth; functional food; body composition; soybeanmeal

## **INTRODUCTION**

In aquaculture, soybean meal (SBM) stands out as one of the primary ingredients due to its high protein content, replacing fish meals (FM) such as tuna, sardine, anchovy, and others. However, despite being a meal with high protein quality and balanced amino acid composition, it contains anti-nutritional factors (ANFs) such as phytic acid, saponins, lectins, phytoestrogens, proteolytic enzyme inhibitors, and immunogenic proteins such as glycinin and β-conglycinin (Krogdahl et al. 2015). Thus, the substitution of more than 30% of FM for SBM in diets may affect the growth and survival of several carnivores fish such as red seabream *Pagrus major* (Murashita et al. 2018), totoaba *Totoaba macdonaldi* (Fuentes-Quesada et al. 2018) and spotted rose snapper juvenile *Lutjanus guttatus* (Silva-Carrillo et al. 2012).

The white snook *Centropomus viridis* is a carnivorous marine species of high economic value, distributed along the tropical and subtropical coasts of the Pacific (Castro-Aguirre et al. 1999). Its main source of income is capture; however, overfishing and anthropogenic alterations have reduced natural populations. In Mexico, catches have decreased by 48% since 2018, increasing the demand and prices of this species in the

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market (Labastida-Che et al. 2013, CONAPESCA 2022). The advances in physiology and reproduction suggest that snook is an excellent option for controlled production (Ibarra-Castro et al. 2017, Baldini et al. 2022). However, the high protein (40%) and lipid (10%) requirements (Abdo-de la Parra et al. 2023) represent a significant challenge to designing specificspecies feeds, being key to developing diets with alternative sources to enhance productive performance. Recently, it has been possible to formulate diets for their growth and development under experimental conditions. Arriaga-Hernández et al. (2021) focused on snook growth and survival under a high FM replacement by SBM (15, 30, 45%), highlighting the adaptability of *C. viridis* to balanced diets. However, we are still seeking to achieve the highest productive potential of this species, improving diets with additives that promote the growth and survival of the species in aquaculture.

A strategy to mitigate the adverse effects on the growth performance of carnivorous species is the use of functional additives (Wang et al. 2022). Agricultural and fishery byproducts contain various bioactive components that can be incorporated as functional ingredients for producing more effective aquafeeds (Al Khawli et al. 2020). One of these bioactive molecules is chitosan, which has attracted much attention due to its unique properties and interesting applications that improve the growth and survival of cultured fish. It is a polysaccharide derived from the partial deacetylation of chitin extracted from crustacean byproducts. Its structure comprises linear chains of beta-1,4-linked Dglucosamine (deacetylated form) and N-acetyl-Dglucosamine (acetylated molecule). Chitosan presents bioactive characteristics such as biocompatibility, biodegradability, non-toxicity, mucoadhesive, and polycationic bioactivity (de Abram 2004). Chitosan is a mucoadhesive that acts as a glycosaminoglycan analog in the glycocalyx of the intestinal epithelium, binding to αvβ3 integrins in the intestinal epithelial cell membrane (Hsu et al. 2012) and enhancing paracellular transport of active molecules and increases nutrient permeability across the epithelial barrier (Bhattarai et al. 2010). Investigating whether chitosan supplemented in high soybean diets can influence the productive potential of a novel species such as snook is crucial. Therefore, this preliminary study aimed to evaluate the effects of chitosan supplemented in the SBM diet on the growth, survival, and body composition of Pacific white snook *C. viridis.*

## **MATERIALS AND METHODS**

#### **Obtention of chitosan**

A chemical-enzymatic method was used to extract chitosan from shrimp industry byproducts (exoskeleton and heads). The technique used by Hernández-Cocoletzi et al. (2009) and Cisneros-Pérez et al. (2019) was adopted to obtain chitin, starting with the deproteination of the byproduct, with pineapple stem bromelain (37189-34-7, Sigma-Aldrich Chemical, SA de CV). The chitin underwent deacetylation, and an alkaline bath with a concentrated solution of NaOH 12.5 M and a temperature of 140°C was conducted for 5 h. The resulting product (chitosan) was washed with potable water until reaching neutral pH and dried in an oven at 50°C for 12 h.

## **Deacetylation degree and molecular weight of chitosan**

The deacetylation degree of chitosan was analyzed using the potentiometric titration evaluation of amino groups method (Parada et al. 2004, Baltodano 2009). A solution of 0.08 g of chitosan in 70 mL of HCl 0.01 M was made and titrated with NaOH 0.1 M, measuring the pH with a potentiometer (ThermoScientific, Orion Star) and recording the expenditure until pH 2.0 and 6.0 was reached. The evaluation included measuring the pH change every 2 mL of added base, gradually adding it with constant agitation to homogenize the solution, making a pH curve, and calculating inflection points. The molecular weight was determined using chitosan viscosity. An Ostwald capillary viscometer equipped with a thermostatic bath at a temperature of 25°C was employed. The molecular weight was established based on chitosan viscosity using the Mark-Houwink-Sakurada equation (Parada et al. 2004)*.*

### **Formulation and experimental diets**

Three isonitrogenous (48.1-48.3%) and isocaloric  $(19.5-19.7 \text{ kJ g}^{-1})$  diets formulated from a base diet for juvenile white snook, according to Arriaga-Hernández et al. (2021). A basal diet was selected containing 33.8 and 24.6% of fish meal and soybean meal, respectively, and chitosan was added at proportions of 0% (D-Control), 0.5% (CHI-0.5%), and 1% (CHI-1.0%). All diets were formulated to contain identical proportions of macronutrients, vitamins, minerals, carotenoids, and antioxidants, and fish oil was used to adjust the lipid content. Sodium alginate was used as a binder, and dextrin was added to adjust the caloric content based on the fish's nutritional requirements. Dry ingredients were ground in a hammer mill (Micron) to a particle

size of  $250 \mu$ m. The ingredients were then mixed in the following order: macronutrients, followed by micronutrients and powdered additives, and finally, the wet ingredients (oils, soy lecithin, and water). The ingredients were continuously mixed for 30 min using a Hobart mixer (model AT-200, Troy, OH, USA). The mixture was pelletized using a Torrey meat grinder with a 2 mm opening die (Tor-Rey 22, model M-22 R2, Monterrey, Nuevo León, México). The pellets were placed in aluminum trays and dried in a convection oven at 40°C for 8 h. Subsequently, the obtained pellets were manually cut to an approximate size of  $1,300 \mu m$ (Sieves #14 and #16) and stored in airtight containers at 4°C until use.

#### **Experimental design and source of fish specimens**

The white snook juvenile was bred at the Laboratory of Reproduction and Marine Finfish Hatchery, from the Research Center for Food and Development CIAD (by its Spanish acronym), Mazatlán Unit, following the established protocols for spawning and larval rearing by Ibarra-Castro et al. (2011).

A complete randomized experimental design with three replicates per treatment was used. The fish (44.5  $\pm$  4.2 g average initial weight) were randomly placed (nine fish per tank) in nine black fiberglass tanks (0.6 m<sup>3</sup>). The center standpipe was covered with a mesh width of 0.5 cm, allowing tank cleaning to prevent fish escaping. The seawater system was flowed through at a rate of 7 L min<sup>-1</sup>, maintaining a constant aeration and a dissolved oxygen level of  $5.2 \pm 0.5$  mg O<sub>2</sub> mL<sup>-1</sup>, temperature of  $25 \pm 2$ °C, and salinity of  $34 \pm 0.7$ . Fish were fed thrice daily (09:00, 12:00, 16:00 h) to apparent satiation for 45 days. Food not consumed 1 h after the onset of feeding was removed from the tank using a siphon and dried in an oven at 60°C to quantify daily feed consumption.

#### **Sample collection**

Biometric parameters were performed every two weeks; all fish were individually harvested and anesthetized using  $0.2$  mL L<sup>-1</sup> clove oil. Each fish's total length and corporal weight were recorded to determine biometric parameters. At the end of the experiment, three fish per unit were randomly sampled and dissected to obtain liver, viscera, and intraperitoneal fat to determine biological indices. The growth performance, feed efficiency and biological indices of the fish were assessed by calculating weight gain (WG), specific growth rate (SGR), thermal growth coefficient (TGC), feed intake (FI), factor conversion rate (FCR), protein efficiency ratio (PER), survival (S), condition factor (CF), as follows:

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WG(\%) = \frac{\text{final weight (g)} - \text{initial weight (g)}}{\text{initial fish weight (g)}} \times 100
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SGR(\% d^{-1}) = \frac{\ln \text{ final weight (g)} - \ln \text{ initial weight (g)}}{\text{time (d)}} \times 100
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FI(\%BW d^{-1}) = \sum n \left[ \frac{\text{total feed consumption (g)}}{\text{No of fish} \times \text{days} \times \text{final weight}} \right] \times 100
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FCR = \frac{\text{FI}}{\text{final weight} - \sqrt[3]{\text{initial weight}}}
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TGC = \frac{\left(\sqrt[3]{\text{final weight}} - \sqrt[3]{\text{initial weight}}\right)}{\text{temperature (°C)} \times \text{time (d)}} \times 1000
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S(\%) = \frac{\text{final count}}{\text{initial count}} \times 100
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CF = \frac{\text{total individual weight}}{\text{length}^3} \times 100
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## **Chemical analysis of ingredients, diets, and whole body composition**

The proximal composition of chitosan (Table 1), the diets (Table 2), and the whole body of *C. viridis* (Table 3) were analyzed by methods described in the Association of Official Analytical Chemists (AOAC 2011). Ten fish were used at the beginning of the experiment to determine the initial body composition. At the end of the experiment, nine fish per treatment were randomly sampled to determine the final body composition. Clove oil  $(0.5 \text{ mL L}^{-1})$  was used to euthanize the organisms following the protocol of AVMA guidelines for animal euthanasia (Underwood & Anthony 2020). Dry matter was calculated by a gravimetric technique (method 4.1.06) using a drying oven (Heraeus, D-63450, Hanau, Germany) at 105°C for 12 h. The crude fat was estimated by the Soxhlet technique (method 4.5.05), with petroleum ether as a solvent in micro Foss Soxtec Avanti 2050 Automatic System (Foss Soxtec, Hoganäs, Sweden). The ash content was determined by calcinating the samples in a muffle furnace at 550°C for 6 h (Felisa®, model FE-363) for 5 h (method 32.1.05). The crude protein was determined by a Kjeldahl acid digestion technique (N×6.25; method 954.01, AOAC 2011) in a Micro-

**Table 1.** Chemical composition of chitosan. Values are means  $\pm$  standard deviation (n = 3).

Chemical composition	Results
Dry matter $(\%)$	$90.56 \pm 0.08$
Ash $(\%)$	$0.56 \pm 0.01$
Crude lipid (%)	$1.10 \pm 0.04$
Crude protein (%)	$6.81 \pm 0.03$
Molecular weight (kDa)	$75.70 \pm 0.70$
Deacetylation degree (%)	$88.77 \pm 0.69$
Particle size (µm)	250-300





Kjeldahl apparatus (LABCONCO, USA). The amino acid composition of experimental diets was determined by high-performance liquid chromatography (HPLC), following the protocol of Vázquez-Ortiz et al. (1995). This protocol consisted of derivatization of the samples with orthophthaldialdehyde in an HPLC system with a fluorescent detector (HPLC, Varian 9012, Walnut Creek, CA, USA) carried out on a  $4.5\times30$  mm Microsorb column with octadecylsilane packing (Microsorb Short C18). Excitation and emission wavelengths of 340-380 and 460 nm, respectively, were used, and flow rate conditions were maintained at  $1.5$  mL min<sup>-1</sup> at  $25\text{-}29^{\circ}\text{C}$ . Commercial standards of AA and α-aminobutyric acid (Sigma-Aldrich Chemical, SA de CV) were used as internal standards.

#### **Statistical analysis**

The data were subjected to normality tests (Kolmogorov-Smirnov test) and tests of homogeneity of variance (Levene's test) before further statistical analysis, such as one-way analysis of variance (ANOVA) (Zar 1999). Before statistical analysis, the percentage data (WG, SGR, FI, TGC, S) were arcsine transformed, but the results were reported as a percentage. The treatments that showed significant differences were analyzed by a mean multiple comparison test of Tukey,  $P < 0.05$ . All statistical procedures were conducted with Statistica software (TIBCO Software Inc. Palo Alto, CA, USA).

**Table 3.** Whole-body composition (g kg<sup>-1</sup> dry-weight) of juvenile white snook fed with experimental diets per 45 days. Values with different superscripts show significant differences between treatments *P* < 0.05. Values without superscripts do not have significant differences ( $P > 0.05$ ). Values are means  $\pm$  standard deviation (n = 3).

<b>Diets</b>	Moisture $(\%)$	Lipids $(\%)$	Ash $(\%)$	Protein $(\%)$
Initial $(\text{day } 0)$	$68.73 \pm 1.18^{\circ}$	$6.15 \pm 0.01^{\circ}$	$4.91 \pm 0.01$ °	$15.45 \pm 1.19$
D-Control	$66.72 \pm 1.15^{ab}$	$6.57 \pm 0.00^{\circ}$	$5.25 \pm 0.00^a$	$15.87 \pm 0.20$
$CHI-0.5%$	$66.48 \pm 0.53$ <sup>ab</sup>	$7.02 \pm 0.01^b$	$5.21 \pm 0.03^{\circ}$	$16.12 \pm 2.10$
$CHI-1.0\%$	$66.02 \pm 0.40^b$	$7.17 \pm 0.01^{\circ}$	$4.82 \pm 0.01^b$	$16.44 \pm 2.11$
P-value	0.026	<0.001	<0.001	0.131

#### **RESULTS**

#### **Characterization and overall production of chitosan**

The results of the physicochemical characterization of chitosan are presented in Table 1, which was used in the formulation of the experimental diets. In addition, this enzymatic process with bromelain achieved an average yield of  $18.7 \pm 2.5\%$  (mean  $\pm$  standard deviation, SD) of chitosan obtained per dry matter (chitin).

## **Experimental diets**

The experimental diets were formulated to be isonitrogenous (48.11-48.37%) and isocaloric (19.51- 19.74 kJ g-1 ) were shown (Table 2). Amino acid concentrations are detailed in Table 4, revealing a consistent balance across all experimental formulations.

#### **Environmental parameters**

According to the daily monitoring of the environmental parameters, the temperature was  $21.5 \pm 0.8$ °C, the average dissolved oxygen level was  $6.0 \pm 0.05$  mg L<sup>-1</sup>, and the salinity recorded was  $32 \pm 0.1$  during the experiment.

#### **Whole-body composition**

The results of the whole-body composition analysis of *C. viridis* at the beginning and end of the 45-day bioassay were shown (Table 3). There were no significant differences ( $P > 0.05$ ) in body crude protein content among the experimental groups. However, moisture, ash, and lipid levels varied among the three experimental groups. In particular, organisms fed CHI-1.0% showed elevated lipid contents after a 45-day feeding period.

#### **Growth performance**

The fish fed with CHI-0.5%, and CHI-1.0% presented significantly higher  $(P < 0.05)$  values in growth indexes

**Table 4.** Amino acids (AA) composition of experimental diets (g AA per 100 g protein).

Amino acid	Experimental diet				
	D-Control	CHI-0.5%	CHI-1.0%		
<b>Essential AA</b>					
Histidine	3.560	3.050	3.320		
Threonine	4.820	4.870	4.840		
Methionine	3.560	3.580			
Valine	6.020	6.890			
Phenylalanine	4.980	4.560	4.610		
Isoleucine	4.210	4.570			
Leucine	8.210	8.220	8.610		
Lysine	8.980	8.810	8.610		
Tryptophan	1.120	1.090	1.150		
Non-essential AA					
Aspartic acid	7.210	7.560	7.230		
glutamic acid	11.620	11.020	11.520		
Serine	4.600	4.230	4.230		
Glycine	3.330	3.290	3.540		
Arginine	4.910	7.920	7.530		
Alanine	5.630	5.210	5.320		
Tyrosine	4.912	4.830	4.820		

(FW, WG, and SGR) compared to the D-Control (Table 5). FCR is significantly lower in experimental diets with chitosan than D-Control  $(P < 0.05)$ . No significant differences are shown in the survival and condition factor of *C. viridis* when fed chitosan-added diets compared to the D-Control  $(P > 0.05)$ .

### **DISCUSSION**

The present study observed that 30% SBM can substitute FM and increase growth performance in *C. viridis* juveniles when supplemented with chitosan. One parameter was FCR, which decreased as chitosan was added to the diet. The D-Control showed an FCR value of 1.82, while the diet supplemented with  $1 \text{ g kg}^{-1}$ chitosan (CHI-1.0%) exhibited a significantly lower FCR of 1.13 ( $P < 0.05$ ). The result of CHI-1.0% has had a better performance compared to the study publi-

**Table 5.** Growth, survival, and nutritional efficiency of white snook juveniles *C. viridis* fed with diets added with different levels of chitosan inclusion for 45 days. IW: initial weight, FW: final weight, WG: weight gained, FI: feed intake, SGR: specific growth rate, FCR: feed conversion rate, GR: growth rate, TGC: coefficient of thermal growth, S: survival, CF: condition factor. Values with different superscripts show significant differences between treatments ( $P < 0.05$ ). Values without superscripts do not have significant differences  $(P > 0.05)$ . Values are means  $\pm$  standard deviation (n = 3).

Indices	Diet			P-value
	D-Control	CHI-0.5%	CHI-1.0%	
IW(g)	$44.5 \pm 0.07$	$44.60 \pm 0.11$	$44.5 \pm 2.04$	0.743
FW(g)	$69.47 \pm 2.25^b$	$82.6 \pm 5.98^{\text{a}}$	$83.12 \pm 2.04^{\circ}$	0.014
$WG(\%)$	$6847.26 \pm 225.42^b$	$8160.00 \pm 598.07^{\text{a}}$	$8212.37 \pm 393.17^a$	0.010
$SGR (\% d^{-1})$	$0.98 \pm 0.02^b$	$1.37 \pm 0.03^a$	$1.39 \pm 0.02^{\text{a}}$	0.010
$FI$ (%BW d <sup>-1</sup> )	$1.45 \pm 0.08^{\text{a}}$	$1.27 \pm 0.08^{ab}$	$1.17 \pm 0.07^b$	0.011
<b>FCR</b>	$1.82 \pm 0.00^{\text{a}}$	$1.25 \pm 0.03^b$	$1.13 \pm 0.01$ <sup>c</sup>	< 0.001
<b>TGC</b>	$1.31 \pm 0.1^b$	$1.86 \pm 0.24^{\text{a}}$	$1.90 \pm 0.18^{\text{a}}$	0.011
S(%)	100	100	100	>0.05
$CF (g cm-3)$	$0.81 \pm 0.10$	$0.86 \pm 0.11$	$0.83 \pm 0.07$	0.193

shed by Arriaga-Hernández et al. (2021), who conducted a study on the same species (*C. viridis*), where it was observed that in the SBM-30 diet (30% inclusion of SBM), obtained an FCR of 1.34. Also, it is important to highlight that the SGR was better in the diets supplemented with chitosan (1.37  $\pm$  0.03, 1.39  $\pm$ 0.02% d-1 , respectively) compared to the D-Control (*P* < 0.05). These results are also consistent with Geng et al. (2011), who observed that supplementation with chitosan has a significant effect on the growth performance (SGR 2.57  $\pm$  0.12% d<sup>-1</sup>) and survival (84%) of juvenile cobia (*Rachycentron canadum*), while Enciso-Contreras (2016) obtained a positive effect on the growth (SGR  $1.2 \pm 0.18\%$  d<sup>-1</sup>, survival 100% ) of totoaba (*T. macdonaldi*) in a diet with a 40% inclusion level of vegetable protein supplemented with chitosan. A similar effect of increased growth is also observed in non-tropical and carnivorous species when their diets were supplemented with chitosan, such as rainbow trout *Oncorhynchus mykiss* (Meshkini et al. 2012), gibel carp *Carassius auratus* (Chen et al. 2014) and sea bass *Dicentrarchus labrax* (Zaki et al. 2015). The temperature registered during the feeding trial was 21.5°C because the experiment was conducted in December and January. Despite this condition, the SGR of the organisms was not affected; they adapted to the temperature condition and maintained their SGR of  $1.37\%$  d<sup>-1</sup> during the experiment. The SGR of this experiment was superior compared to Baldini et al. (2022), who reported that all their groups of snook (*C. viridis*), cultured in floating cages, obtained an SGR of 0.77%  $d^{-1}$ , with temperatures <24 $\degree$ C.

On the other hand, Arriaga-Hernández et al. (2021) maintained conditions at 29°C, obtaining a higher SGR value of 2.86%  $d^{-1}$  in the D-Control and 3%  $d^{-1}$  in the SBM-30 diet. The present results are due to the wellbeing and health of the organisms promoted by the biological activity of chitosan. Several authors report positive effects of supplementing diets with chitosan, such as disease resistance observed in species such as the gibel carp *Carassius auratus gibelio* (Chen et al. 2014), *Misgurnus anguillicaudatus* (Chen & Chen 2019), and the golden pompano *Trachinotus ovatus* (Yu et al. 2023). Chitosan acts as a potent adhesive on intestinal mucins (Bravo-Osuna et al. 2007), thus protecting the gastrointestinal system and improving the health of the intestinal microanatomy as evidenced by an increase in the length of intestinal microvilli and a reduction in gastroenteritis (Chen et al. 2014, Zaki et al. 2015). Moreover, adding 1 g  $kg^{-1}$  of chitosan to diets increased the activity of digestive enzymes (proteases, lipases, and amylases) in the gastrointestinal system of carp *Barbonymus gonionotus* (Salam et al. 2021). Variation between moisture and ash is also observed in the present study. These differences could be due to the variation in fish weight when performing chemical body composition analysis.

However, fish's lipid content in body composition has been strongly related to chitosan supplementation. The lipid content in *C. viridis* is higher in the group fed with CHI-1.0%. Chen et al. (2014) assure that dietary chitosan in low or optimal doses improves lipid metabolism due to the intestinal mucoadhesive interaction. However, the excessive presence of chitosan in the diet (5 or 10%) impacts crude lipid

levels in the fish body through the adsorption effect of chitosan on dietary lipids, inhibiting their absorption in the intestine. This collection of effects on gastrointestinal health resulted in better utilization of nutrients from the provided feed, leading to enhanced species productivity and improved adaptation of organisms in captivity, and chitosan promises to be a versatile material with great potential for aquaculture applications (Abdel-Ghany & Salem 2020). This study remains inconclusive regarding the effects of chitosan on various factors that could promote the health, adaptation, and growth of snook. However, the growth and adaptation results of organisms cultured on chitosan-supplemented diets suggest the functional effects of this biopolymer. Further investigation is recommended to understand better the effects of chitosan on the health of snook when fed diets high in soybean.

In conclusion, adding 1% chitosan in diets with 30% SBM inclusion for *C. viridis* significantly enhanced their productivity, leading to increased growth and improved feed conversion ratio. Therefore, chitosan can be used in diets for *C. viridis* without adverse effects on survival. Further research into the physiological impact of chitosan on snook is required to elucidate its mechanisms of action.

## **Data availability**

The data presented in this study are available in the present article.

## **Credit author contribution**

Alondra Mesina-Peña: conceptualization, validation, methodology, data curation, formal analysis, writingoriginal draft; Roberto Tirado Osuna: conceptualization, data curation, methodology, formal analysis; A. Yeren Escobedo-Lozano: methodology, review, and editing; Crisantema Hernández: conceptualization, validation, funding acquisition, project administration, supervision, review, and editing. All authors have reviewed and approved the final manuscript.

# **Conflict of interest**

The authors declare no conflict of interest.

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