Aquaculture Reports 30 (2023) 101599

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

Improvement in spotted rose snapper growth and skin coloration after incorporation of shrimp head meal in diet

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ARTICLE INFO

Keywords:
Carotenoids
Chitinolytic enzymatic activity
Growth promoter
Local feed resources
Shrimp byproduct
Skin-color

ABSTRACT

Four isonitrogenous (45% crude protein) and isoenergetic (20.29 – 20.47 kJ/g) experimental diets were formulated by replacing 0%, 10%, 20% and 30% of fish meal (FM) with shrimp head meal (SHM) as a source of protein and tested the rearing performance and skin coloration of spotted rose snapper *Lutjanus guttatus*. The SHM had a high content of protein and pigments (i.e., carotenes and xanthophylls), but also chitin which eventually could be an anti-nutritional constituent in the diet for some fish species. A total of 240 fish (initial average weight of 77 ± 0.5 g) were distributed in 12 fiberglass tanks (3000 L) (20 fish/tank) and were fed twice a day for 70 days. The fish feed diets containing SHM showed the highest growth rates (weight gained, WG; 82.8–88.2 g) and lowest feed conversion ratio values (FCR; 1.3 – 1.4) compared to the D-Control diet (WG; 62.1 g and FCR; 1.5). The chitinolytic enzymatic activity (CEA) significantly increased in the stomach of fish fed diets containing SHM. The fish fed diets containing 20% and 30% of SHM, showed higher redness and more reddish-orange tones than the fish fed with the D-Control diet. The FM replacement had improvements in final growth, chitinolytic enzymatic activity, and skin coloration of spotted rose snapper. Thus, the use of up to 30% SHM protein in practical diets for spotted rose snapper should be a good alternative to develop more efficient aquafeed production for these species, however, further research could be suggested to determine the effectiveness of SHM as a substitute for FM in diets for spotted rose snapper rearing at a commercial scale conditions.

1. Introduction

Fish meal (FM) is the main source of dietary protein for marine carnivorous species in captivity, due to that stimulate a better growth rates (NRC, 2011). However, FM could represent up to 50% of aquafeed production costs (Rana et al., 2009) encouraging the need to produce low-cost feeds using alternative protein sources. By-products from fisheries and aquaculture industries have great potential to meet the protein demand by the aquaculture market (Hua et al., 2019).

Shrimp heads (SH), are the principal by-product generated by fisheries and shrimp farms, which have a high feeding value (46% crude protein and 9.8% crude lipids). (Fox, 1993; Hertrampf and Piedad-Pascual, 2003) and represent an important source of lysine, methionine, polyunsaturated fatty acids, cholesterol, phospholipids (Liu et al., 2021) and free (< 10%) and esterified (>80%) astaxanthin (Quintana-López et al., 2021). Previous studies have shown that the high astaxanthin content in SH enhances the characteristic reddish pigmentation both freshwater fish (Choubert and Luquet, 1983; Sornsupharp et al., 2015; Liu et al., 2021), as in marine fish species, such as the Pacific and Atlantic salmon species (Stachowiak, Szulc, 2021).

On the other hand, SH present a high content of chitin, which is the major structural component of the exoskeleton of crustaceans (Borić et al., 2020). It is believed that chitin should be digested without negative effects on growth (Gutowska et al., 2004) due to marine fish have shown chitinolytic enzymatic activity since the chitin is part of their natural diet. However, this response could be species-specific,

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https://doi.org/10.1016/j.aqrep.2023.101599
Received 20 November 2022; Received in revised form 17 April 2023; Accepted 15 May 2023

therefore we hypothesize that in the case of spotted rose snapper they can digest diets with a high content of chitin in SH without a negative effect on the performance (Valle-Lopez et al., 2021). Mexican snapper farmers commonly fed their fish in net cages with SH because it represents an economical, sustainable, and responsible option to improve snapper-farmed production (personal communication).

Recently, the dietary substitution of protein FM by shrimp head meal (SHM) as a source of protein improved the growth of juvenile totoabas (*T. macdonaldi*) (Espinosa-Chaurand et al., 2015) and longfin yellowtail (*Seriola rivoliana*) (Benitez-Hernández et al., 2018). Lutjanid fish (or snappers) are a relevant fishery resource culture in Latin American countries and research on spotted rose snapper has led to protocols for juvenile finfish production under captivity conditions in Mexico and Costa Rica (Ibarra-Castro et al., 2020a; b; Chacón-Guzmán et al., 2021). Thus, the objective of this study was to evaluate the effect of the dietary replacement of FM protein by SHM protein on rearing performance, chitinolytic enzymatic activity, and skin coloration of spotted rose snapper.

2. Material and methods

2.1. Chemical analysis of ingredients

The dry matter, crude protein, lipids, and ash contents of the ingredients were assessed following the methods of the Association of Official Analytical Chemists (AOAC, 2011). The dry matter (method 4.1.06) was assessed by gravimetry by drying the sample in an oven at 105 °C for 12 h. The crude protein (method 990.03) was determined using a LECO FP-528 nitrogen analyzer (LECO Instrument Corporation, St. Joseph, MI, USA). The crude lipids (method 4.5.05) were extracted with petroleum ether using a micro Foss Soxtec Avanti 2050 Automatic System (Foss Soxtec, Hoganäs, Sweden). Finally, ash content (method 32.1.05) was analyzed by calcinating the samples at 550 °C for six h in a muffle furnace (Fisher Scientific International, Inc. Pittsburgh, PA, USA). Gross energy of FM and SHM was calculated according to the energy values of protein (23.4 kJ/g), lipid (39.8 kJ/g), and nitrogen-free extract (17.2 kJ/g) (Cho et al., 1982).

The amino acid composition of FM and SHM was quantified following (Vázquez-Ortiz et al., 1995) by high-performance liquid chromatography (HPLC, Varian 9012, Walnut Creek, CA, USA).

Table 1 shows the proximate composition, gross energy and essential amino acids (EAA) profile of FM and SHM.

Table 1
Proximate composition and essential amino acids (EAA) profile of the protein sources.

Proximate analysis (g/kg dry matter)	FM[a]	SHM[b]
Crude protein (N × 6.25)	678.3	495.1
Crude fat	88.0	61.0
Ash	124.5	236.0
NFE[c]	109.2	208.0
Total carotenoids	0.86	1.14
Gross energy[d] (kJ/g)	21.2	17.6
EAA profile (g/kg of crude protein)		
Arginine	53.2	35.2
Histidine	32.0	30.1
Isoleucine	45.2	39.0
Leucine	90.0	72.2
Lysine	45.1	41.1
Methionine	39.2	35.2
Phenylalanine	45.2	56.0
Threonine	28.0	50.2
Tyrosine	38.1	56.1
Valine	53.2	41.2

Values are means, (n = 3).
[a] sardine fish meal (FM)
[b] shrimp head meal (SHM)
[c] Nitrogen-free extract (including fiber) = 1000 - (g/kg crude protein + g/kg crude fat + g/kg ash).
[d] Gross energy was calculated according to the energy values of protein (23.4 kJ/g), lipid (39.8 kJ/g), and nitrogen-free extract (17.2 kJ/g) (Cho et al., 1982).

2.2. Experimental diets

White shrimp heads (*Penaeus vannamei*) were collected from the Gandhi S.A. shrimp freezer de C.V. (Mazatlán, Sinaloa, Mexico). Shrimp heads were cooked in boiling water for 10 min, dried in a forced-air oven at 65 °C for 6 h and ground to a particle size of 0.25 mm using a 50703 model hammer mill (California Pellet Mill laboratory Mill Champion, Waterloo, IA, USA). The generated SHM was stored at − 20 °C until analysis.

Four isonitrogenous (45% crude protein) and isoenergetic (20.4 kJ/g) practical diets were formulated by substituting 0% (D-Control), 10% (D-10SHM), 20% (D-20SHM) and 30% (D-30SHM) of FM protein with SHM protein. All diets were formulated to have the same levels of squid meal, corn gluten, krill meal, antioxidants, dextrin, vitamin and mineral premixes, antioxidant, alginate, and soy lecithin (Table 2). The fish oil varied slightly, while all diets were filled with dextrin until caloric content was adjusted. The macro ingredients FM, SHM, squid meal, corn

Table 2
Ingredients and proximate composition of the experimental diets for juvenile spotted rose snapper (*L. guttatus*).

Ingredients (g/kg dry matter)	Diets			
	D-Control	D-10SHM	D-20SHM	D-30SHM
Fish meal (sardine)[a]	517.3	465.6	413.8	362.1
Shrimp head meal	0.00	70.9	141.8	212.7
Squid meal[b]	60.0	60.0	60.0	60.0
Krill meal[c]	75.9	75.9	75.9	75.9
Corn gluten[d]	20.0	20.0	20.0	20.0
Fish oil[e]	91.7	92.4	93.0	93.7
Soybean lecithin[f]	15.0	15.0	15.0	15.0
Dextrin[e]	180.3	160.4	140.7	120.8
Vitamin premix[g]	6.0	6.0	6.0	6.0
Mineral premix[g]	2.3	2.3	2.3	2.3
Vitamin C[d]	1.0	1.0	1.0	1.0
BHT[h]	0.5	0.5	0.5	0.5
Alginate[e]	30.0	30.0	30.0	30.0
Proximate analysis (g/ kg dry matter)				
Crude protein	452.7	444.2	454.5	458.5
Crude lipid	151.6	158.3	154.1	152.9
Ash	113.8	122.2	125.1	133.6
NFE[i]	223.5	215.9	206.9	202.2
mg of total carotenoids/kg	42.4	49.76	56.12	61.46
Gross energy (kJ/g)[j]	20.5	20.4	20.3	20.3

[i]Mean, number of determinations = 3.
[a] Selecta de Guaymas, S.A de C.V, Guaymas, Sonora México
[b] Proteínas marinas y agropecuarias, S.A. de C.V., Guadalajara, Jalisco, México
[c] PROAQUA, S.A. de C.V., Mazatlán, Sinaloa, México
[d] DSM Nutritional Products Mexico S.A. de C.V., El Salto, Jalisco, México.
[e] Droguería cosmopolita, S.A. de C.V. México, D.F., México
[f] Sigma-Aldrich Chemical, Inc. de C.V., Toluca, México State, México
[g] Trout nutrition Mexico S.A de C.V. (by courtesy). Vitamin premix (mg/kg diet): riboflavin, 8.75; pantothenic acid, 150; niacin, 10; vitamin B12, 1; choline chloride, 1538.46; biotin, 10; thiamine, 1.08; pyridoxine, 7.31; inositol, 153.84; folic acid, 4.08; vitamin C 250, vitamin A, 0.75; vitamin E, 30; vitamin D, 0.06; vitamin K, 16.5. [f] Mineral premix (mg/kg diet): copper sulphate, 12; iodine, 11; iron sulphate, 375; manganese oxide, 20.96; zinc sulphate, 41.66; sodium selenite, 30.
[h] Butylated hydroxytoluene. Sigma-Aldrich Chemical, S.A. de C.V., Toluca, Mexico State, Mexico.
[i] Nitrogen-free extract (including fiber) = 1000 - (g/kg crude protein + g/kg crude fat + g/kg ash).
[j] Gross energy was calculated according to the energy values of protein (23.4 kJ/g), lipid (39.8 kJ/g), and nitrogen-free extract (17.2 kJ/g) (Cho et al., 1982).

gluten, and krill meal were ground to a particle size of 250 μm and homogenized in a Hobart mixer (model AT-200, Troy, OH, USA). Then, the micro-ingredients, soy lecithin, fish oil, and water were added and mixed until a homogeneous paste was obtained. The homogeneous paste was passed through a meat mill (Model 22, TorRey®, Monterrey, Mexico). The pellets obtained were dried in a forced-air oven at 39 °C for 12 h and cut manually to a size of 0.3–0.4 mm approximately. A sieve was used to remove the fine particles, and the solid pellets were stored in labeled containers at 4 °C until use. The dry matter, crude protein, crude lipids, and ash content of experimental diets (Table 2) were determined according to the described in the previous section, 2.1. Chemical analysis of ingredients. Finally, gross energy was calculated according to (Cho et al., 1982).

The total carotenoid content in the experimental diets and SHM was determined following the 43.015 method described in the (AOAC, 1984). Briefly, 3 g of sample (experimental diets and SHM) was homogenized in 100 mL of acetone-methanol (1:1, v/v) and 0.1 g $MgCO_3$. The mixture was filtered (Whatman No. 1) under vacuum, and the residue was mixed twice with acetone (25 mL) and once with methanol (25 mL) until the residue was colorless. The resulting extract was transferred to a separating funnel and mixed with distilled water (100 mL, five times) to remove acetone. The upper layer containing pigments (i.e., carotenoids and xanthophylls) was placed in a volumetric flask containing 9 mL acetone and diluted to volume with methanol. The absorbance of extracts samples was read at 470 nm, using the molar extinction coefficient ($E_{1\%,1\ cm} = 2100$), thus the concentration was expressed as mg of total carotenoids/kg diet (Table 2), and determined using the following equation:

$$mg \quad of \quad total \quad carotenoids/kg = 1000 * V * A/W * E1\%, 1cm$$

Where V is the volume of the stock solution, A is the absorbance at 470 nm and W is the weight of the sample.

2.3. Fish culture and feeding conditions

The spotted rose snapper used for the experiment were reared at the Finfish Marine hatchery, at the Center for Research in Food and Development (CIAD, for its acronym in Spanish), Mazatlán Unit, following the protocols for spawning and larvae rearing issued by (Ibarra-Castro and Alvarez-Lajonchere, 2011; Ibarra-Castro et al., 2020a; b).

At the beginning of the experiment, all fish were individually anesthetized using a clove oil solution (0.2 mL water) for 10 min to determine their initial length and body weight. Additionally, a sample of 10 fish were collected to analyze its proximate initial body composition following standard methods (AOAC, 2011).

The experimental trial to evaluate spotted rose snapper growth and feed efficiency was performed for 70 days. A completely randomized design with three replicates per treatment (20 fish per replicate) was used. A total of 240 fish (initial average weight 77 ± 0.5 g) were randomly distributed in 12 cylindrical fiberglass tanks with a working volume of 1500 L. The tanks were supplied with constant aeration and a seawater flow-through of 6.5 L/min, maintained under natural photoperiod. The temperature, dissolved oxygen, and salinity of the water was recorded daily using a YSI® 85–12FT multiparameter oximeter (YSI Inc., Yellow Springs, OH, USA) and maintained at 29 ± 1 °C, 5.4 ± 0.4 mg/L, and 34 ± 0.7 psu (Practical Salinity Unit) respectively throughout the experiment. The fish were manually fed twice a day (9:00 and 16:00 h) until satiation for 70 days. The feed not consumed after 45 min of each feeding was removed by siphoning and dried in an oven at 60 °C to determine the feed intake (FI). Dead fish were recorded and weighed to adjust the feed conversion ratio (FCR).

All the experimental procedures in this study followed the guidelines set by the Bioetic Committee at Centro de Investigación en Alimentación y Desarrollo (CIAD). CONBIOÉTICA-26-CEI-001–20200122. CEI/023–1/2021 "retrospectively registered".

2.4. Growth performance, nutrient utilization and zootechnical indices

Every 15 days and at the end of the experiment all fish were individually anesthetized in a clove oil solution (0.2 mL/L water) for 10 min. The total length and body weight of each fish was recorded to estimate the biometric indices. Growth, feed efficiency, weight gained (WG), specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER), survival (S), and condition factor (CF), were assessed following the next equations:

$$WG\ (g) = final\ mean\ weight\ (g) - initial\ mean\ weight\ (g)$$

$$SGR\ (\%/day) = 100\ [((ln\ final\ weight) - (ln\ initial\ weight))/time\ (days\ of\ experiment)]$$

$$FI\ (g/fish/day) = [(total\ feed\ consumption\ (g))/(number\ of\ fish)/number\ of\ days\ of\ experiment)]$$

$$FCR = FI\ /weight\ gain\ (g)$$

$$PER = weight\ gain\ (g)/protein\ intake\ (g)$$

$$S\ (\%) = 100\ [(final\ count)/(initial\ count)]$$

$$CF = 100\ [body\ weight\ (g)/length^3\ (cm^3)]$$

At the end of the experiment, nine fish from each treatment (three fish/tank) were fasted for 24 h and euthanized using an overdose of clove oil (0.5 mL/L) and dissected to obtain the liver, visceral mass, intraperitoneal fat and body weight to estimate the following biological indices: hepatosomatic index (HSI), viscerosomatic index (VSI) and intraperitoneal fat rate (IFR). The following equations were used:

$$HSI\ (\%) = 100\ [liver\ weight\ (g)/body\ weight\ (g)]$$

$$VSI\ (\%) = 100\ [viscera\ weight\ (g)/body\ weight\ (g)]$$

$$IFR\ (\%) = 100\ [intraperitoneal\ fat\ weight\ (g)/body\ weight\ (g)]$$

2.5. Proximate composition of fish

Nine fish per each treatment (three fish/tank) were selected and euthanized as previously described to assess their final proximate composition. The proximate composition of the whole fish was assessed following standard methods (AOAC, 2011) as described in the "2.1 Chemical analysis of ingredients and diets" section.

2.6. Chitinolytic enzymatic activity

At the end of the experiment, six fish were fasted (24 h) per treatment (three fish per tank) and euthanized as previously described and dissected to obtain the stomach and intestine, which were freeze-dried and stored at − 20 °C until the analysis. The chitinolytic enzymatic activity was determined using the procedure described by (Jeuniaux, 1966) with minor modifications. Briefly, the freeze-dried samples (50 mg) were homogenized (Ultra-Turrax D25 basic, IKA © -Werke, Germany) with 1.5 mL of cold distilled water (4 °C) and centrifuged at 44,900g for 20 min. The supernatant from the stomach (0.5 mL) was mixed with 0.5 mL of chitin suspension (0.5 mg/mL) and 0.5 mL 0.15 M citrate buffer (pH 5). The intestine supernatant (0.5 mL) was mixed with 0.5 mL of chitin suspension and 0.5 mL 0.3 M phosphate buffer (pH 7). The homogenates were incubated at 37 °C for 3 h in a shaking water bath and then boiled for 10 min to stop the reaction, cooled to room temperature, and centrifuged for 15 min at 13,600g. The supernatant (0.5 mL) was homogenized in 0.1 mL 0.8 M borate buffer (pH 9.3) and boiled for 3 min, then immediately cooled in tap water. A 3 mL aliquot

of *p*-dimethylamino-benzaldehyde solution was added, mixed, and the resulting solution was incubated at 37 °C for 20 min. The absorbance was read at 585 nm using an EPOCH 2NS microplate spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA), and the amount of formation of N-acetylglucosamine (NAG) was calculated using a standard curve of NAG with a concentration of 5–20 μg. The chitinolytic enzymatic activity was expressed in μg NAG/g wet tissue/h.

2.7. Analysis of reflective colors surfaces of juveniles skin

At the end of the feeding trial, nine fish from each treatment (three fish/tank) were randomly selected to assess the skin pigment pattern on the left side of the fish in the dorsal, caudal and pectoral regions. Fish were anesthetized in clove oil (0.2 mL/L water) for 10 min and subjected to pigment measurement with a CR-300 Chroma Meter (Konica Minolta Holdings, Inc.; Japan). The L*, a* and b* parameters were measured based on the CIELab scale (CIE, 1976). Chroma and Hue angle (°Hue) were calculated from a* and b* according with the following equations.

$$Chroma = \sqrt{(a*)^2 + (b*)^2}$$

$$^{\circ}Hue = \arctan\ [\![b*/a*]\!]$$

2.8. Statistical Analysis

Data was tested for normality using the Kolmogorov-Smirnoff test and homogeneity of variance (Levene's test) prior to statistical analysis. Percent data (SGR, S, VSI, HIS, IFR, and proximate composition) were arcsine transformed prior to analysis. One-way analyses of variance were performed to establish significant differences in growth, feed efficiency, and biological indices. When statistical differences were detected in One-way analyses of variance a Tukeý HSD test was applied. Pigments parameters and chitinolytic enzyme activity were analyzed using a two-way analysis of variance (ANOVA) using gastrointestinal tissue (GT) and diet as independent variables, followed by Tukey's HSD test. All data were analyzed using the software Minitab version 17.1 (Minitab Inc., State College, PA, USA). The results are expressed as means ± standard deviation (SD), and different letters show significant differences ($p < 0.05$).

3. Results

3.1. Growth, feed efficiency, and zootechnical indices

All diets with the inclusion of SHM significantly increased the WG and SGR of spotted rose snapper juveniles with respect to the D-Control diet group ($p < 0.001$) (Table 3). The FI value was significantly lower (96.7 g/fish) in the juveniles fed D-Control diet than in juveniles fed with SHM ($p = 0.004$). The FCR values observed in juveniles fed SHM-containing diets were (1.3 – 1.4) significantly lower than in the D-Control diet (1.55) ($p = 0.002$). The PER values were significantly higher in fish fed D-10SHM and D-20SHM diets (1.6 and 1.7, respectively) ($p = 0.003$) with respect to the D-Control and D-30SHM diets; while those PER values for D-30SHM and the D-Control diets were not different among them (Table 3). The juvenile's survival in all treatments was not significantly different among them. Finally, the biological indices such as VSI, HSI, and IFR were not significantly affected by the inclusion level of SHM in the diet.

3.2. Proximate composition of fish

The juveniles fed the D-Control and D-30SHM diets showed the lowest body protein content respecting juveniles fed D-10SHM and D-20SHM diets ($p < 0.001$) (Table 4). In relation to the crude lipid content, no significant differences were observed among treatments. The ash content was significantly lower (4.9%) in juveniles fed the D-Control diet than that the juveniles fed D-10SHM, D-20SHM, and D-30SHM (5.1%, 5.3% and 5.6% respectively) treatments. The moisture content in fish tissues increased proportionally with the increase in the inclusion level of SHM in the diet (Table 4).

Table 3
Growth performance and body indices of juvenile spotted rose snapper (*L. guttatus*) fed experimental diets for 70 days.

Variable	Diets				
	D-Control	D-10SHM	D-20SHM	D-30SHM	*p-value*[a]
IW (g)	77.80 ± 0.00	77.52 ± 0.25	77.36 ± 0.08	77.51 ± 0.28	ns
FW (g)	139.95 ± 2.33^{b}	161.16 ± 4.13^{a}	165.56 ± 6.21^{a}	160.37 ± 5.50^{a}	< 0.001
WG (g)	62.15 ± 2.33^{b}	83.64 ± 4.17^{a}	88.20 ± 6.18^{a}	82.80 ± 0.72^{a}	< 0.001
SGR (%/day)	0.84 ± 0.02^{b}	1.05 ± 0.04^{a}	1.09 ± 0.05^{a}	1.04 ± 0.01^{a}	< 0.001
FI (g/fish)	96.72 ± 6.38^{b}	116.56 ± 7.13^{a}	115.28 ± 4.81^{a}	115.63 ± 4.28^{a}	0.004
FCR	1.55 ± 0.03^{b}	1.39 ± 0.02^{a}	1.31 ± 0.09^{a}	1.40 ± 0.04^{a}	0.002
S (%)	96.67 ± 2.89	96.67 ± 5.77	95.00 ± 5.00	93.33 ± 2.89	ns
PER	1.42 ± 0.02^{b}	1.62 ± 0.01^{a}	1.69 ± 0.11^{a}	1.56 ± 0.05^{ab}	0.003
CF	1.75 ± 0.22	1.65 ± 0.10	1.70 ± 0.14	1.70 ± 0.16	ns
VSI (%)	9.41 ± 1.94	7.99 ± 1.14	8.38 ± 0.37	8.70 ± 1.51	ns
HSI (%)	1.28 ± 0.34	1.02 ± 0.19	1.02 ± 0.26	1.25 ± 0.30	ns
IFR (%)	5.65 ± 1.35	5.05 ± 1.36	5.28 ± 1.11	5.41 ± 1.61	ns

[a] Date represented the mean of three tank replicates± SD, n = 3. Means with different superscripts within each row are significantly ($p < 0.05$) different by Tukey's test; ns stands for "no significant difference". IW, initial weight; FW, final weight; WG, weight gained; SGR, specific growth rate; FI, feed intake; FCR, feed conversion ratio; S, survival; PER, protein efficiency ratio; CF, condition factor; VSI, viscerosomatic index; HSI, hepatosomatic index; IFR, intraperitoneal fat ratio.

Table 4
Whole-body composition of spotted rose snapper (*L. guttatus*) fed experimental diets for 70 days (wet basis).

Variable		Diets				
	Initial	D-Control	D-10SHM	D-20HSM	D-30HSM	*p-value*[a]
Crude protein (%)	17.57 ± 0.35	14.21 ± 0.22^{b}	15.71 ± 0.29^{a}	15.83 ± 0.29^{a}	14.67 ± 0.09^{b}	< 0.001
Crude lipids (%)	5.20 ± 0.04	9.53 ± 0.01	9.54 ± 0.05	9.40 ± 0.05	9.64 ± 0.19	ns
Ash (%)	5.03 ± 0.04	4.92 ± 0.14^{b}	5.12 ± 0.16^{a}	5.34 ± 0.10^{a}	5.59 ± 0.28^{a}	0.007
NFE (%)	6.52 ± 0.42	1.97 ± 0.12^{a}	0.41 ± 0.22^{b}	1.65 ± 0.40^{a}	2.10 ± 0.17^{a}	< 0.001
Moisture (%)	65.68 ± 0.84	69.37 ± 0.73^{a}	69.79 ± 0.41^{a}	66.82 ± 0.24^{c}	67.63 ± 0.65^{b}	< 0.001

NFE=Nitrogen Free Extract (NFE= 100- (%crude protein + %crude lipids + % ash + %moisture))

[a] Values are means± SD (n = 3). Means with different superscripts within each row are significantly ($p < 0.05$) different by Tukey's test; ns stands for "no significant difference".

3.3. Chitinolytic enzymatic activity

The chitinolytic enzymatic activity (CEA) was higher in the stomachs

of juveniles fed SHM compared to the D-Control diet. The CEA was significantly different between GT samples (Fig. 1). The stomach tissue showed the highest CEA about 3–3.5 times higher in comparison to intestine tissue, showing a significant difference between tissues ($p < 0.001$). A significant interaction between dietary inclusion levels of SHM and GT was observed ($p < 0.001$). The CEA was higher in the stomach tissue of juveniles fed diets containing SHM (26.42 – 26.70 μg NAG/g wet tissue/h), while the lowest value (22.85 μg NAG/g wet tissue/ h) was observed in the D-Control group.

3.4. Skin colors parameters

Skin coloration parameters in the dorsal, caudal and pectoral regions of juveniles fed experimental diets with different levels of SHM substitution are shown in Fig. 2. Lightness (Fig. 2A) in each region of the juvenile's body was not significantly influenced by the diets ($p > 0.05$). Regardless of the diet, the pectoral region of spotted rose snapper exhibited the highest ($p < 0.05$) values of L* , followed by the caudal and dorsal regions. On the other hand, the substitution levels of SHM in the diets significantly ($p < 0.05$) affected the a* , b* , chroma and angle °Hue values in the three measured regions of the juveniles.

The substitution levels of SHM in the diets significantly ($p < 0.05$) increased a* values in the pectoral, dorsal and caudal regions (Fig. 2B). The pectoral region showed lower redness levels than the caudal and dorsal regions in every diet group (Fig. 2B), whilst juveniles fed D-20SHM and D-30SHM diets reduced yellowness levels in the pectoral region when were compared with D-Control diet and D-10SHM diet, respectively (Fig. 2C). Overall, b* values in the pectoral and dorsal regions (Fig. 2C) were significantly ($p < 0.05$) reduced when juveniles were fed with experimental diets D-20SHM and D-30SHM. Chroma values (Fig. 2D) in the pectoral region were significantly reduced ($p < 0.05$) 25.56–27.38% and 22.45–24.36% when fish were fed with D-20SHM and D-30SHM diets, respectively, when they were compared with the D-Control and D-10SHM groups, while values of this same parameter augmented significantly ($p < 0.05$) in the caudal region when juveniles were fed diets containing SHM.

Moreover, chroma values in the dorsal region were 16% higher ($p < 0.05$) when juveniles were fed D-10SHM, though juveniles fed D-20SHM and D-30SHM did not alter the chroma values in this same region. The diets containing SHM significantly ($p < 0.05$) reduced the °Hue values (Fig. 2E) in the caudal and dorsal regions, while only D-20SHM and D-30SHM lowered the values of this parameter in the pectoral region. All the body regions studied in juveniles showed the lowest °Hue values in the D-20SHM and D-30SHM groups (Fig. 2E) compared to the D-Control and D-10SHM diets. In the three body regions chroma and °Hue values were similar between fish fed with D-20SHM and D-30SHM diets.

4. Discussion

This study showed that replacing up to 30% of FM protein with SHM protein in a practical diet for spotted rose snapper juveniles resulted in better growth, feed efficiency and skin coloration compared to the D-Control group. The aquaculture aquafeed industry needs to find low-cost sources of protein to replace fishmeal in aquafeeds. Currently, shrimp by-products generated from its processing for human consumption represents a problem for the environment. On the other hand, these by-products represents a rich source of high-quality protein, pigments such as carotenes and xanthophylls (i.e., astaxanthin), and chitin. Therefore, the valorization of shrimp heads (shrimp main by-product) through their use as an ingredient for elaborating diets for spotted rose snapper could be a sustainable alternative for the aquafeed industry.

On the other hand, the highest WG and SGR values for spotted rose snapper fed diets D-10SHM, D-20SHM and D-30SHM were directly related to higher FI and PER, and lower FCR values. Our results on WG, SGR, FI, PER and FCR were similar to those reported for totoaba (*Totoaba macdonaldi*) juveniles fed diets containing 15% or 30% of SHM protein (Espinosa-Chaurand et al., 2015). However, the replacement up to 25% of FM protein with shrimp waste meal (SWM) protein increased the WG of cobia (*Rachycentron canadum*), but adversely affected the FCR and PER compared to the D-Control group (Lu and Ku, 2013). Furthermore, the replacing up to 50% of FM with SWM increased the WG and SGR and improved the feed efficiency (FCR) of koi carp (*Cyprinus carpio haematopterus*). Nonetheless, this result was associated with the content of carotenoid pigments in SWM diets (Nandini et al., 2014). In this regard, previous studies have reported that carotenoids such as astaxanthin act as a growth promoter in fish. For example, the incorporation of dietary carotenoids (astaxanthin) incorporation improves the nutrient utilization and growth of European seabass (*Dicentrarchus labrax*) and rainbow trout (*Oncorhynchus mykiss*) (Goda et al., 2018; Lakeh et al., 2010), which is mainly attributed to the inclusion of astaxanthin in the intermediary metabolism of fish. It has been demonstrated that the dietary inclusion of shrimp shell waste-derived natural astaxanthin decreases the enzymes' aspartate aminotransferase (AST) and alanine

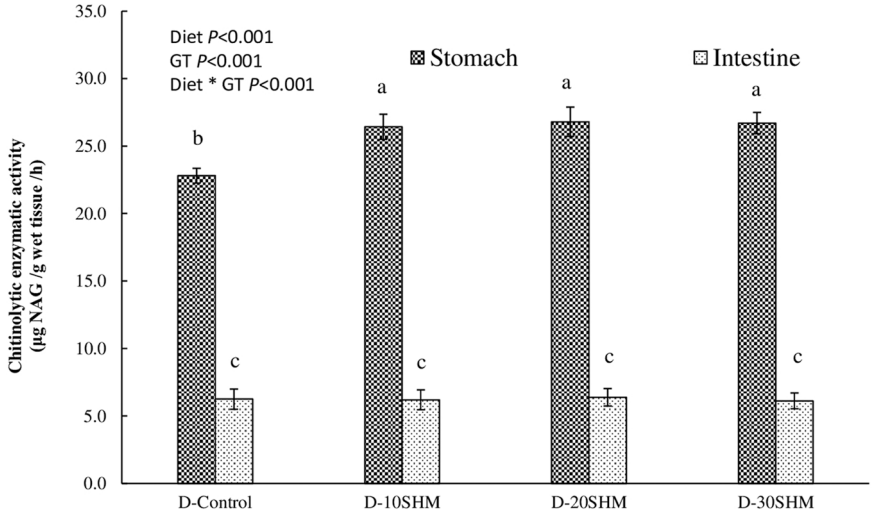

Fig. 1. Chitinolytic enzymatic activity in *L. guttatus* fed a D-Control diet and SHM diet was assessed as μg NAG/g wet tissue^{-} /h. Overall effects are indicated as text in the diagram. When the interaction between diet and gastrointestinal tissue (GT) was significant, the dataset was subjected to post-hoc using t Tukey's test ($p < 0.05$). Results are presented as mean ± SD, (n = 6 per treatment).

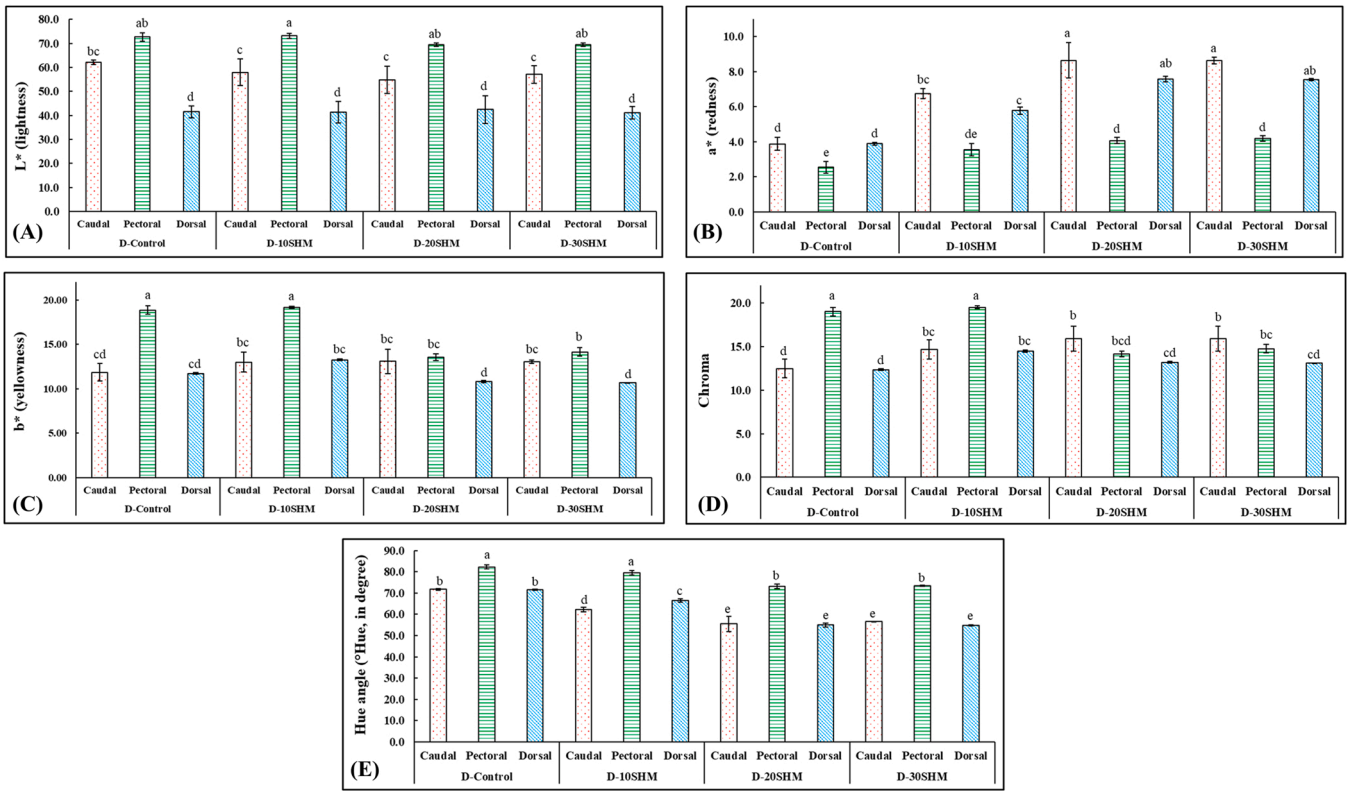

Fig. 2. Colour parameters **A** lightness L* , **B** redness a* , **C** yellowness b* , **D** chroma and **E** Hue angle of caudal, pectoral and dorsal regions of *L. guttatus* fed with shrimp head meal, as source of carotenoids, during 70 days. Different letters indicate significant differences among treatments and regions ($p < 0.05$) by Tukey's test. Results are presented as mean ± SD, (n = 9 per treatment).

aminotransferase (ALT) in discus *Symphysodon aequifasciatus*, (Haque et al., 2021). An increase in AST and ALT activities indicates that dietary protein is used for energy production; this is because both enzymes are associated with the synthesis and breakdown of dietary non-essential amino acids; thus, the decrease in its activities improves the use of dietary protein for the synthesis and accumulations of body proteins, which resulted in greater growth in *S. aequifasciatus* (Haque et al., 2021). In addition, a previous study demonstrated that dietary chitin accelerates protein breakdown and improves fish growth (Nakagawa, 2007). Therefore, our results suggest that the presence of both carotenoids, xanthophyll (i.e., astaxanthin), and chitin in SHM diets could be the promoter of the growth in spotted rose snapper juveniles by increasing the body protein synthesis and deposition.

In nature, crustaceans represent one of the main sources of food for marine fish in their natural habitat, such as spotted rose snapper (Valle-Lopez et al., 2021). Chitin is present in the exoskeleton of crustaceans (Kandra et al., 2012), which is considered a highly digestible material in the GT system of marine fish (Gutowska et al., 2004). This is due to chitinase enzymes ability to break down chitin into its amino sugars (i.e., β-N-acetyl-D-glucosamine) which are used as an energetic substrates, that spares the use of proteins contained in food and stored as muscular mass once proteins are digested by fish (Ringø et al., 2012). However, if dietary chitin is not utilized or used as a non-digestible energy source, it could limit or depress the growth of fish (Karlsen et al., 2015). In this study, we observed that the highest CEA was found in the stomach of fish-fed diets containing SHM, which agrees with Gutowska et al. (2004), who indicated that the stomach of marine fish is the GT with the highest CEA compared to the intestine. Although, we observed that the CEA in the different GT of the rose snapper was lower than that reported in other species such as cobia (*Rachycentron canadum*), arctic charr (*Salvelinus alpinus*) and sablefish (*Anopoploma fimbria*) (Abro et al., 2014; Gutowska et al., 2004; Lu and Ku, 2013). However, it is well known that the gastrointestinal tract of fish has variable enzymatic activity between species (Ikeda et al., 2017). Thus, based on our results, we hypothesize that the chitinases present in the stomach of fish fed diets containing SHM degraded the chitin until obtaining amino sugars β-N-acetyl-D--glucosamine for its later use as a source of energy, which may explain the increase in SGR, PER, and high retention of body protein observed in spotted rose snapper fed D-10SHM and D-20SHM. Additionally, this physiological advantage could increase the use of insect meal since their high level of chitin would not negatively impact the growth of this fish species, and therefore could represent an additional alternative to replace the use of FM (Mousavi et al., 2020).

Furthermore, vertebrates, such as fish, are unable of biosynthesize carotenoids *de novo* (Goodwin, 1984), therefore they need to obtain these pigments through their diet. After fish have consumed and digested the carotenoids in the diet, they are carried by lipoproteins through the bloodstream and stored mainly in the chromatophores of the skin and muscle, which can lead to the reddish colorations in fish (Chatzifotis et al., 2011; Torrissen, 1989). Consumers associate fish coloration with quality, so pigmentation in fish, is one of the most important parameters that determine their market value. Farm reared spotted rose snapper usually show reduced coloration (García-Ortega, 2009), it is thought to be due to the lack of carotenoids in the diet. In this sense, few researches have been carried out in order to determine the effect of diet on skin pigmentation in this species. It has been reported that astaxanthin and its esters are the predominant carotenoids present in the head and shells of different shrimp species such as *Penaeus monodon, Penaeus indicus, Metapenaeus dobsonii, Parapenaeopsis stylifera* and *Penaeus vannamei* (Sachindra et al., 2005; Quintana-López et al., 2021), demonstrating that shrimp by-products could be used as a viable source of carotenoids. Therefore, an important aim of this study was to evaluate the effect of the dietary inclusion of SHM on the colorimetric parameters L* , a* , b* , chroma and °Hue. Lightness (incident and reflected light)

was not affected by the different levels of inclusion of SHM in the diet of spotted rose snapper juveniles. Similarly, no significant differences were observed in the L* values of the pectoral and dorsal regions of Pacific red snapper (*Lutjanus peru*) fed with up to 0.1% of Carophyll Pink™, containing 10% of astaxanthin, when compared with the D-Control group (0% carotenoids) (Carvajal-García et al., 2018). Moreover, another study found that the carotenoids included in the diet did not modify the L* values of the skin of Australian snapper (*Pagrus aurata*), but significantly affected the a* and b* values (Booth et al., 2004). Additionally, Nogueira et al. (2021) reported that the a* values the in pectoral and dorsal regions of red porgy were significantly augmented when levels of carotenoids (Carophill®) were increased, but b* values remained the same among D-Control and experimental groups. In this study, the a* values (redness) increased with the inclusion of SHM in the diets, while the b* values (yellowness) decreased only in the pectoral region when compared with fish fed the D-Control diet. Furthermore, more reddish-orange tones (°Hue) were observed in the dorsal and caudal regions of fish fed with D-20SHM and D-30SHM, while the pectoral region exhibited slightly yellowish-orange tones. These results suggest positive effects in pigmentation of spotted rose snapper, as it is desirable for this species exhibit a reddish, pink-reddish or pink-yellowish skin color (Thomson et al., 2000). With respect to red porgy (*Pagrus pagrus*) fed with an experimental diet containing 16% of shrimp shell meal (21.2 mg astaxanthin/kg) significantly reduced the °Hue in the skin of the fish (Kalinowski et al., 2007). Moreover, a decrease in the °Hue values in the pectoral and dorsal regions of red porgy, from yellowish to reddish tones, was observed when synthetic carotenoid was added to the experimental diets (Nogueira et al., 2021). On the other hand, the °Hue value of the skin of flowerhorn cichlid (*Amphilophus citrinellus* x *Cichlasoma trimaculatum*) fed with dried fairy shrimp meal were significantly higher when compared with the D-Control group after 60 days of feeding (Sornsupharp et al., 2015). The results mentioned above, suggest that the deposition of carotenoids and skin coloration is species-specific (Yi et al., 2014), without ruling out other rearing factors, such as the background color of the tanks or cages that might exert an effect on coloration of fish flesh and skin (Doolan et al., 2008). Additionally, it has been reported that the increased carotenoid concentration might induce the proliferation of melanocytes (Liu et al., 2016; Poon et al., 2023) and that the quantity of melanocytes differs depending on the body zone of the fish (Pérez-Escalante et al., 2012; Poon et al., 2023). According with our findings it seems that carotenoids obtained from the SHM might are preferably be deposited in the caudal and dorsal region of spotted rose snapper.

5. Conclusion

SHM protein can suitably replace up to 30% of FM dietary protein by promoting growth and improving nutrient utilization in spotted rose snapper. The dietary replacement of 20% and 30% of FM protein by SHM protein increased redness and reddish-orange tones in *L. guttatus*. Nevertheless, further studies are needed to determine the effectiveness of SHM protein as a substitute for FM protein in diets for *L. guttatus* rearing at pilot scale conditions.

Funding

This study was supported by Fondo Institucional de Fomento Regional para el Desarrollo Científico, Tecnológico y de Innovacion (FORDECYT) through research grant Nº147325 "Development of technology for on-growing snapper in floating cages: A productive alternative to the shores of the Mexican northwest" and Comisión Nacional de Acuicultura y Pesca (CONAPESCA). Author C.H. has received research support from FORDECYT and CONAPESCA.

CRediT authorship contribution statement

Adriana Osuna-Salazar: Investigation, Data curation, Formal analysis, Methodology, Writing – original draft. **Crisantema Hernández:** Investigation, Data curation, Formal analysis, Resources, Supervision, Writing – review & editing. **Cynthia E. Lizárraga-Velázquez:** Methodology, Writing – review & editing. **Erika Y. Sánchez Gutiérrez:** Methodology, Writing – review & editing. **Miguel Ángel Hurtado-Oliva:** Writing – review & editing. **Asahel Benitez-Hernández:** Writing – review & editing. **Leonardo Ibarra-Castro:** Writing – review & editing All the authors have read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The authors confirm that data supporting findings of this study are available within the article. Additional data is available from the corresponding author [CH], upon reasonable request..

Acknowledgments

Adriana Osuna-Salazar is grateful to CONACYT for a master's fellowship. This manuscript is part of the activities of the Thematic Network 12.3: to reduce and valorize the losses and the waste of food systems, Project 294768. We are indebted to Samantha Koda and Mark Q. Martindale for final edit and the manuscript advice.

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