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EFFECT OF THE DIETARY INCLUSION OF SHRIMP HYDROLYZATE ON GROWTH AND FEED EFFICIENCY OF WHITE SNOOK

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Abstract -- Shrimp waste hydrolysates (HRC) are used to improve the productive performance of different fish species. This study determined the effect of dietary replacement of fishmeal protein (FMP) with shrimp waste hydrolysate (HRC) on growth, feed efficiency and body composition of juvenile Pacific white sea bass, *Centropomus viridis*. Shrimp waste was enzymatically treated (bromelain) to obtain HRC. Three isonitrogenous (47-48% protein) and isoenergetic (18.81-19.25 kJ/ g) diets were formulated by replacing 0%, 4% and 6% of HP protein with HRC. Fish (average individual initial weight of 44 g) were fed the experimental diets (n = 24 fish per diet) for 45 days. Weight gained (WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PET), survival, and initial and final proximal whole body composition of snook were analyzed. Fish fed the control diet (0% HRC) presented the best results in growth (PG and TEC) and feed efficiency (TCA and TEP). TCA values did not show significant differences between the control treatment and the treatment with 4% HRC inclusion. PET was significantly higher in the control treatment than in the HRC-inclusive treatments. Overall, the HRC-inclusive diets negatively impacted ($p<0.05$) the growth (PG and TEC) of the organisms. Survival was not affected ($p>0.05$) by any of the treatments. Lipid and ash values in the whole body of snook were higher in the control treatment than in the HRC-inclusive treatments. In conclusion, the HRC obtained under these conditions and used in this study, it has no potential for use in the design snook feed. The residue of HRC procurement could work in future studies.

Keywords: shrimp peel and head, enzymatic hydrolysis, bromelain, *Centropomus viridis*

Abstract -- Shrimp waste hydrolysates are used to improve the growth performance of different fish species. This study determined the effect of dietary replacement of fishmeal protein (HP) with shrimp waste hydrolyzate (SWH) on the growth, feed efficiency, and body composition of juvenile white snook *Centropomus viridis*. Shrimp waste were treated enzymatically (bromelain) to obtain the SWH. Three isonitrogenous (47-48% protein) and isoenergetic (18.81-19.25 kJ/g) diets were formulated by replacing 0%, 4%, and 6% HP protein with SWH. Fish (initial weight 44 g) were fed the experimental diets (n = 24 fish per diet) for 45 days. Weight gained (WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), survival, and proximal whole-body composition of snook were analyzed. The fish fed the control diet (0% SWH) presented the best results in growth (WG and SGR) and feed efficiency (FCR and PER). The FCR values did not show significant differences between the control and treatment, including 4% SWH. The PER value was significantly higher in the control treatment than in the treatments, including SWH. In general, diets including SWH had a negative impact ($p<0.05$) on the growth (WG and SGR) of the organisms. Survival was not affected ($p>0.05$) by any treatments. Lipid, and ash values in the whole body of white snook were higher in the control treatment than in the treatments with SWH inclusion. In conclusion, the SWH obtained under these conditions and used in this study has no potential to be

used in the design of feed for white snook. The redesigning of the SWH acquisition could work in future studies.

Key words - Shrimp shell and head, enzymatic hydrolysis, bromelain, *Centropomus viridis*.

INTRODUCTION

The high demand for fish for human consumption in the world has forced the aquaculture industry to use new fish species with commercial potential. The Pacific white sea bass, *Centropomus viridis*, is a carnivorous fish of brackish waters, which demands high protein content in its diet and has potential intensive commercial aquaculture due to the quality and flavor of its meat [1]. The decrease in the supply of this species, due to the decline of the natural population due to anthropogenic pressures, has increased its demand and market price. For this reason, there is a growing interest in the controlled production of this species [2]. However, the carnivorous habits of *C. viridis* [3] pose challenges for the design of specific feeds for this species. Fish meal (FM) is the main source of dietary protein for carnivorous fish because it stimulates better growth [4]. However, HP could represent up to 50% of the total aquaculture feed production costs [5].

The use of less expensive protein sources that provide satisfactory growth is an advantage for both aquafeed manufacturers and aquaculture producers. Therefore, currently feeds for carnivorous fish are designed based on HP protein and vegetable protein such as soybean meal, which is relatively inexpensive, with high nutritional value and presents a good amino acid balance [6, 1]. The use of vegetable protein in the diet for carnivorous fish has shown favorable results in the growth of these organisms, therefore, its use is becoming more frequent. [1] reported that white sea bass are able to digest diets with high soybean meal content. However, due to the high demand for protein by aquaculture, it is convenient to explore sustainable additives that partially substitute the protein in HP, promote the growth of cultured carnivorous fish, reduce commercial production costs and allow reaching the aquaculture potential of this species.

The revalorization of waste from the processing of

The use of shrimp in fishery and aquaculture products, in additives with high quality protein for the development of more efficient aquaculture feeds, is becoming more and more important. Shrimp is widely consumed in Mexico and the world, so its demand increases every year. At the same time, the consumption of this crustacean generates a large amount of waste, including the head and shell, which normally end up in landfills, causing environmental contamination [7, 8].

These residues are rich in proteins, chitin and pigments [8]. Therefore, the transformation of these into protein hydrolysates can serve as a high quality dietary additive, mainly due to the presence of oligopeptides [7], free amino acids [9] and pigments with bioactive properties [10].

Multiple investigations have shown that dietary replacement of HP with shrimp waste hydrolysates HRC, improve the productive performance of different commercially important fish species. For example, [11, 12, 13, 14, 15] reported that dietary inclusion of HRC improves growth and feed efficiency of red sea bream, *Pagrus major*; cobia, *Rachycentron canadum* L.; catfish, *Pangasius hypophthalmus*; and rainbow trout, *Oncorhynchus mykiss*, respectively, so their use on a commercial scale would be promising for the aquaculture industry. Therefore, the objective of the present study was to evaluate the effect of dietary substitution of HP protein for HRC protein on growth, feed efficiency and body composition of juvenile Pacific white snook, *Centropomus viridis*.

DEVELOPMENT

Chemical analysis of ingredients

The dry matter, crude protein, crude lipid and ash contents of the ingredients used in the diets were analyzed by the methods described in the Official Association of Analytical Chemists [16]. Dry matter (method 4.1.06) was determined gravimetrically by drying the sample in an oven at 105°C for 12 h. Crude protein (method 954.03) was determined with a micro-Kjeldahl (N × 6.25). Crude lipids (method 4.5.05) were determined with petroleum ether using a Foss Soxtec Avanti 2050 automated extraction system (Foss Soxtec, Hogan¨as, Sweden). The ash content (method 32.1.05) was analyzed by calcination of the sample at 550°C for 6 h in a muffle (Fisher Scientific International, Inc. Pittsburgh, PA, USA). The high ash values observed (Table 1) are mainly attributed to the presence of potassium phosphate, which was used in the preparation of the HRC (see section "Preparation of the hydrolysate").

Table 1. Proximal composition (g/100 g DM) of HRC

Proximal composition	HRC
Protein	20.2±0.00
Lipids	3.15±0.03
Ash	51.23±0.59
ELN	11.43±0.00

Values are mean± standard deviation (n=5).

Hydrolysate preparation

Shrimp (*Penaeus vannamei*) shells and heads were washed with potable water, oven-dried at 65 ◦C for 6 h and ground to a particle size of 0.25 mm using an industrial blender (model T2L tapisa brand). The dried shrimp shells and heads were stored in airtight containers at 4°C**.**

The preparation of HRC was carried out following the methodology described by [17]. Fifty g of shrimp shell and 50 g of shrimp head were weighed and mixed with 56 g of bromelain enzyme and 500 mL of distilled water. The pH of the mixture was adjusted to 9 with a dibasic potassium phosphate buffer solution (pH 11). The reaction mixture was maintained at 50 °C for 3 h, and the enzyme was inactivated at 90 °C for 20 min. The HRC was separated from the deproteinized debris by centrifugation at 2500 rpm for 15 min. The proximate composition of the HRC was analyzed by standardized methods [16], described in the section "Chemical analysis of ingredients". The high ash content in HRC (Table 1) is mainly attributed to the presence of phosphorus and potassium, which were used to provide an optimum pH for the enzyme bromelain.

Experimental diets

Three isonitrogenic (47-48% crude protein) and isoenergetic (18.81-19.25 kJ/ g) diets were formulated by substituting 0% (D-Control), 4% (DHRC4) and 6% (DHRC6) fish meal protein for HRC protein. All diets were formulated with the same levels of macronutrients (soybean paste, krill meal, squid meal, corn gluten, soy lecithin and alginate) and micronutrients (vitamins and minerals, antioxidant, DL-methionine, phytase, vitamin C) (Table 2). Fish oil varied among diets according to the inclusion of HRC. The micronutrients phosphate and carotenoids were added only to the control diet, because the diets with the HRC contained phosphates (dibasic potassium phosphate buffer used in the hydrolysate process) and carotenoids, which are naturally present in shrimp shells and heads [10]. All diets were supplemented with dextrin until the caloric content was adjusted based on the nutritional requirements of the fish.

The dry matter, crude protein, crude lipid and ash contents of the experimental diets (Table 2) were determined as described in the section "Chemical analysis of ingredients". The crude energy of the diets was calculated according to the energy values of protein (23.4 kJ/g), lipids (39.8 kJ/g), and nitrogen free extract (17.2 kJ/g) [18].

Table 2. Formulation and proximate composition of experimental diets

Ingredients (g/kg)	D-Control	DHRC4	
Fish meal[a]	380	363	358
HRC[b]	0	40	60
Soybean paste[c]	300	300	300
Krill meal[d]	70	70	70
Squid meal[e]	70	70	70
Corn gluten[f]	80	80	80
Fish oil[g]	15	15	10
Dextrin[g]	40.05	18.35	8.35
Alginate[g]	20	20	20
Phosphate from dibasic calcium phosphate[h]	0.5	0	0
Mixture of vitamins and minerals[i]	4.5	4.5	4.5
DL-methionine[h]	2.6	2.6	2.6
Phytase[f]	0.05	0.05	0.05
Vitamin C[f]	1.0	1.0	1.0
Antioxidant[f]	0.5	0.5	0.5
Soy lecithin[f]	15	15	15
Carotenoids[f]	0.8	0	0
Composition (g/kg dry matter)			
Crude protein	47.0	48.65	48.4
Crude lipids	9.67	9.45	9.66
Ashes	9.59	10.81	11.51
ELN[j]	27.07	24.01	23.51
Energy (KJ/g)	19.25	18.97	18.81

[a] Selecta de Guaymas, S.A. de C.V. Guaymas, Sonora, Mexico.
[b] Hydrolyzed shrimp by-product hydrolysate
[c] Proteínas Marinas y Agropecuarias, S.A. de C.V., Guadalajara, Jalisco, Mexico.
[d] PROAQUA, S.A. de C.V. Mazatlán, Sinaloa, Mexico.
[e] Squid meal was made from fresh squid mantle.
[f] DSM Nutritional Products Mexico S.A. de C.V., El Salto, Jalisco, Mexico.
[g] Droguería Cosmopolita, S.A. de C.V. Mexico, D.F., Mexico.
[h] Sigma-Aldrich Chemical, S.A. de C.V. Toluca, Mexico State, Mexico.
[i] Trouw Nutrition Mexico S.A. de C.V. *Vitamin mix composition: Vitamin A, 10,000,000 IU or mg/g; Vitamin D3, 2,000,000 IU; Vitamin E, 100,000 g; Vitamin K3, 4.00 g; Thiamine B1, 8.00 g; Riboflavin B2, 8.70 g; Pyridoxine B6, 7.30; Vitamin B12, 20.00 mg; Niacin, 50.00 g; Pantothenic acid, 22.20 g; Inositol, 153.80 g; Nicotinic Acid, 160.00 g; Folic Acid, 4.00 g; 80 mg; Biotin, 500 mg; Vitamin C, 100.00 g; Choline 300.00 g, Excipient q.s. 2000.00 g. **Composition of

mineral premix: Manganese, 100 g; Magnesium, 45.00 g; Zinc, 160 g; Iron, 200 g; Copper, 20 g; Iodine, 5 g; Selenium, 400.00 mg; Cobalt, 600.00 mg. Excipient q.s. 1500.00 g.

[j] Nitrogen free extract= 1000− (g/kg crude protein + g/kg crude lipids + g/kg ash).

Fish culture and feeding conditions

Pacific white sea bass juveniles were obtained from the same spawning batch of wild parental broodstock, following the spawning and larval rearing protocols described by [19] at the Center Food and Development (CIAD), Mazatlan, Mexico. At the beginning of the experimental bioassay all fish were anesthetized in clove oil solution (0.5 mL/L water) for 10 min to determine their average initial weight.

A completely randomized experimental design with three replicates (8 fish per replicate) for each of the experimental diets was used to evaluate the growth and feed efficiency of white sea bass cultured for 45 days. A total of 72 fish (mean initial weight 44.51±0.08) were placed a cylindrical fiberglass tank (volume 300 L). Each tank was equipped with a 50 mm central drain covered with a 0.5 cm mesh net to prevent fish escape and allow fish cleaning. The tanks were supplied with a continuous seawater flow system of 6 L/min (equivalent to 28.8 daily refills), constant aeration and maintained on a natural photoperiod. Water temperature, dissolved oxygen and salinity were measured daily using a YSI 55-25 FT multiparameter oximeter (YSI Inc., Yellow Springs, Oh, USA) and maintained at 23± 1.2 ◦C,
5.99 ± 0.15 mg/L, and 34 ± 0.7 psu (Practical Salinity Unit) respectively throughout the experiment.

Fish were hand fed 3 times a day (8:30 h, 12:30 h and 16:30 h). Fish were fed to satiety. Feed not consumed after 1 h of each feeding was removed with a siphon and oven-dried at 60°C to calculate feed consumption. Dead fish were recorded to adjust the feed conversion ratio. All experimental procedures in this study complied with [20], technical specifications for the production, care and use of laboratory animals.

Growth and feed efficiency

Fish were weighed every two weeks and at the end of the experiment to determine feed conversion rate, weight and biomass. Fish were anesthetized in clove oil solution (0.5 mL/L water) for 10 min and weighed individually. Growth, feed efficiency and survival were evaluated in terms of: Weight Gain (WG), Specific Growth Rate (SGR), Feed Consumption. (CA), feed conversion ratio (FCR), protein efficiency ratio (PET) and survival (S). The formulas used are shown below:

PG (g)= *final average weight* (*g*) - *initial average weight* (*g*) Eq. 1

TEC (%/day)= 100 [((*ln average final weight*) - (*ln average initial weight*))/time (*days)*]. Eq. 2

CA (g/fish) = Σ_{i}^{45} [(*total feed consumption (g)*)/(*number of fish*)/number *of days*]. Eq. 3

TCA = *CA* /weight *gained (g)* Eq. 4

TEP = *weight gained (g)/protein intake (g)*

S (%)= 100 [(*final number of fish*)/(*initial number of fish*)]. Eq. 5

Proximal composition of fish

Three fish (one fish per tank) were randomly selected and euthanized in an overdose of clove oil solution. The proximal whole-body composition of the fish was analyzed by using standardized methods [16], as described in the section "Chemical analysis of ingredients".

Statistical analysis of data.

Normality of the data was determined by the Kolmogorov-Smirnov test, and homoscedasticity by the Levene test at a significance value greater than 5 %. Prior to statistical analysis, survival values (%) were transformed using arcsine, but the results were reported as percentages. The rest of the dependent variables were analyzed with a one-way analysis of variance and Tukey-Kramer multiple comparison test was used to determine differences between treatments. Differences were considered at a significance level of 0.05. SigmaPlot software, version 11.0 (Systat Software Inc.) was used for statistical analysis.

DISCUSSION AND ANALYSIS OF RESULTS

All HRC-inclusive diets significantly reduced the PG, TEC and TEP of white sea bass, with respect to the control treatment (Table 3). The CA values were significantly lower (31.61-33.19 g/fish) in fish fed the HRC-inclusive diets than in organisms fed the control diet (48.85 g/fish). The AQI value was significantly higher in the DHRC6 treatment than in the D-Control and DHRC4 treatments. Survival of organisms was not significantly affected by any of the dietary treatments.

The PG and TEC in the control treatment organisms are directly related to the highest values of CA, TEP and the lowest value of TCA. The results on PG, TEC, CA, TEP and TCA of the DHRC4 and DHRC6 treatments differ from other studies in which the growth and feed efficiency of fish fed diets were analyzed.

with HRC inclusion. For example, [21] reported that dietary inclusion of 6% HRC of *Penaeus vannamei* heads had no negative effects on PG, TEC, CA and TCA parameters of juvenile cobia, however, increasing HRC inclusion (12% and 18%) reduced growth and feed efficiency of this species. [22], observed that dietary supplementation of 2% HRC *Penaeus vannamei,* increased up to 184.35% the GP of juvenile catfish, *Pangasius hypophthalmus*. On the other hand, [23], reported that the dietary inclusion of the
5% of HRC increased PG, TEC, PET, AC, and
reduced the TCA of juvenile red sea bream, *Pagrus major* fed a diet with a high concentration of soybean meal. In addition, a recent study reported that dietary inclusion of shrimp head meal (212.7 g/kg feed) improved growth and feed efficiency of flamingo snapper *(Lutjanus guttatus*) [24]. In our study, the decreases observed in the PG and TEC of organisms fed with HRC, is probably due to the presence of high phosphorus concentrations in the HRC and consequently in the diets for juvenile snook. This is due to the fact that potassium phosphate was added as a buffer solution to activate the enzyme bromelain during the production of the HRC, and this solution was not removed. In addition, it is well known that the shrimp exoskeleton is composed of calcium carbonate [25], which explains the high ash content reported in the HRC (Table 1). According to the literature reviewed, there are no studies in fish that explain the negative effect of high phosphorus intake on growth. However, studies in mammals have reported that administration of a diet high in phosphorus causes disturbances in renal function, bone metabolism and vascular cell function [26], which directly affects growth, for example, [27] reported that a diet high in phosphorus (1.2% - 1.3%) reduces growth (lower body weight) of 1-month-old Wistar mice, as well as feed intake. On the other hand, another study reported that oral supplementation of 1.125 g/day of phosphorus reduces the body weight of obese adult rats, and food intake [28], the latter was attributed to the stimulation of hepatic ATP production, which transmits a neural afferent signal to the central nervous system resulting in decreased food intake through the stimulation of satiety [29, 30]. In this regard, [31] reported an increase in ATP production in the plasma of rainbow trout fed a diet supplemented with high potassium phosphate. Therefore, based on our results, we hypothesize that the mechanism by which phosphorus affected the growth (PG and TEC) of juvenile snook fed with DHRC4 and DHRC6 is related to its participation in the control of food consumption through the stimulation of ATP production, since that CA was reduced by up to 65%. Additionally, we related low CA to decreased PET and body protein retention (Table 4) in organisms fed the DHRC6 diet. However, further studies on the effect of excess dietary phosphorus in fish are necessary to confirm our findings.

Growth, feed efficiency and survival of Pacific white sea bass fed experimental diets for 45 days.

Parameter	Experimental diets		
	D-Control	DHRC4	DHRC6
PI (g)	44.51±0.08[a]	44.52±0.157[a]	44.51±0.165[a]
PF(g)	73.50±2.51[a]	62.30±1.59[b]	56.17±0.74[C]
PG (g)	28.99±2.51[a]	17.78±1.73[b]	11.75±0.75[C]
ECT (% d^{-1})	1.11±0.07[a]	0.74±0.05[b]	0.51±0.03[c]
CA (g/fish)	48.85±3.86[a]	31.61±2.84[b]	33.19±2.69[b]
TCA	1.68±0.01[b]	1.77±0.00[b]	2.85±0.0.09[a]
TEP	1.26±0.01[a]	1.15±0.01[b]	0.72±0.0.02[c]
S (%)	100[a]	100[a]	95.8[a]

Values are mean ± SD. Means with different superscript in each row are significantly different ($P < 0.05$), according to Tukey's test. PI= Initial weight; PF= Final weight; PG= Weight gained; TEC= Specific growth rate; CA= Feed intake; FCR= Feed conversion ratio; PET= Protein efficiency index; S = Survival.

Fish fed with the DHRC6 treatment presented a content content of protein content significantly lower than the D- Control and DHRC4 treatments (Table 4). The crude lipid content was significantly higher in fish fed the control diet (6.57%) than the rest of the treatments. With respect to moisture content, no significant differences were observed differences significant differences between treatments. The content content of ash content was significantly lower (4.88%) in fish fed DHRC6 than in fish fed the D-control y DHRC4 (5.25% y 5.01%, respectively).

[32] demonstrated that a high-phosphorus diet promotes energy expenditure in mice through the utilization of fatty acids in the liver. [33]. reported a decrease in visceral fat levels and inhibition of hepatic lipogenic gene expression in rats fed a high-phosphorus diet. Thus, our results on reduced body lipid content of DHRC4- and DHRC6-fed organisms are attributed to the high phosphorus content in the diets. By

On the other hand, the low levels of ash found in the body of organisms fed the DHRC4 and DHRC6 diets could be attributed to a low retention of the minerals phosphorus and calcium in the bone structure of the organisms, because a previous study reported an increase in the excretion of phosphorus in the feces and urine of rainbow trout fed a diet supplemented with potassium phosphate and calcium carbonate [31].

Whole body proximal composition of Pacific white sea bass fed experimental diets for 45 days.

Paramete	per diem		
	D-control	**DHRC4**	**DHRC6**
Humidity (%)	66.72±1.15[a]	68.47±1.46[a]	68.13±1.72[a]
Protein crude (%)	16.03±0.17[a]	16.19±0.05[a]	15.21±0.27[b]
crude (%) Lipids	6.58±0.009[a]	6.03±0.013[b]	6.20±0.007[c]
Ash (%)	5.25±0.003[a]	5.01±0.006[b]	4.88±0.005[c]

CONCLUSIONS

Dietary substitution of HP with HRC negatively affected growth and feed efficiency of juvenile white sea bass. Diets with HRC inclusion showed a decrease in whole body lipid content of *C. viridis*. Based on these findings, the HRC obtained (without removal of potassium phosphate) and used in this study, has no potential for use in aquafeed design. Future studies on the redesign of HRC procurement are necessary in order to rule out the use of this additive as a growth promoter in white sea bass. In addition, the evaluation of HRC in other species is indispensable in the development of efficient feeds for marine species.

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