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Research Article

Effect of the shrimp farming wastes as co-feed on growth performance and digestibility of juvenile grey mullet, *Mugil cephalus*

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ABSTRACT. A feeding trial was carried out to evaluate the utilization of residual nutrients from shrimp farming wastes (SFW) as co-feed in juvenile grey mullets *Mugil cephalus*. Four treatments were designed to offer shrimp farming wastes in different proportions as feed. The first treatment (control) consisted of feeding the entire daily portion with only commercial feed for marine fish (SFW0). The remaining treatments replaced the daily feed with shrimp farming wastes in 33, 66, and 100%, respectively, treatments SWF33, SWF67, and SWF100. Feed was offered daily as 4% of the grey mullet total biomass at each experimental tank. Results showed that final weight, weight gain, specific growth rate, relative weight gain, and thermal growth coefficient were reduced in the fish when the proportion of SFW in their feed was increased. However, the fish showed a digestive capacity that allowed them to use residual nutrients in a ratio up to 66%, increasing their initial weight by up to 25%. The increase of shrimp farming waste as feed negatively affected the whole-body proximal composition. The digestibility results showed that the fish could digest up to 41% of the shrimp farming waste protein. Results suggest that juvenile grey mullets are capable of utilizing residual nutrients from shrimp farming waste. However, it is necessary to complement an alternative feed source to induce an optimal growth performance for the juvenile grey mullets. It is also recommended that mullets be fed with formulated feed to meet their nutritional requirements to maintain the protein and lipid content of the whole body under culture conditions.

Keywords: *Mugil cephalus*; growth; digestibility coefficients; proximate body composition; enzymatic activity; sustainability

INTRODUCTION

World aquaculture production from 2000 to 2018 sustained annual growth of 5.3%, and production reached a total of 82.1 million metric tons of aquatic animals (FAO 2020). Nevertheless, problems associated with the production increment have also arisen. The excessive supply of nutrients from the stock's waste (i.e. ammonia and phosphorous), mainly formed by uneaten feed and feces, has caused eutrophication problems in the environment (Gowen 1994, Talbot & Hole 1994, Ottinger et al. 2016, Thomsen et al. 2020). Eutrophication can alter the aquatic ecosystem, inducing diseases, and causing mortalities in exposed organisms (Lananan et al. 2014, Jasmin et al. 2020). As an alternative, bioremediation has been used to eliminate or reduce harmful compounds through their use and biological processing (Divya et al. 2015, Jasmin et al. 2020).

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In Mexico, Pacific white shrimp production (*Penaeus vannamei*) by its volume is situated in second place of the total aquaculture. Its average annual production growth rate has been around 1.67% (CONAPESCA 2018) for the last 10 years. However, one of the major problems facing the Pacific white shrimp industry is the low utilization of nutrients supplied through the feed. Generally, only 30% of the nitrogen feed is used by farmed shrimp, while the remaining 70% is discarded or excreted as dissolved form or particles into the water (Troell et al. 1999). Also, shrimp farms face problems associated with diseases and contamination resulting from effluents and the generation of eutrophication and nitrification of water (Martínez-Porchas et al. 2010). A viable strategy to reduce and reuse the discharge of nutrients from the Pacific white shrimp crop is by a polyculture practice through integrated multitrophic aquaculture (IMTA). IMTA can reduce the environmental impact of cultivated species since their wastes are re-utilized as input for another species (Chopin et al. 2001, Ridler et al. 2007, Estim 2015, Granada et al. 2018). IMTA creates a balanced system that benefits environments as a result of processes such as bioremediation (Barrington et al. 2009, FAO 2009) and combine economic viability (i.e. cost reduction, diversified performance), social acceptability (i.e. by reducing environmental problems or increasing organic quality), and provides a culture with feasible production (Estim 2015). Among the species of interest where the multitrophic systems have been evaluated are: Pacific white shrimp, Pacific oyster (*Crassostrea gigas*), and smooth clam (*Chione fluctifraga*) (Martinez-Cordova & Martinez-Porchas 2006); seaweed (*Gracilaria lemaniformis*), and scallop (*Chlamys farreri*) (Mao et al. 2009), blue mussel (*Mytilus edulis*), and Atlantic salmon (*Salmo salar*) (Reid et al. 2010), rainbow trout (*Oncorhynchus mykiss*), Nile tilapia (*Oreochromis niloticus*), and seaweeds (*Porphyra*, *Ulva*, and *Gracilaria*) (Pereira et al. 2012), *Eisenia arborea* and red abalone (*Haliotis rufescens*) (Zertuche-González et al. 2014), Pacific white shrimp and grey mullet (*Mugil cephalus*) (Aghuzbeni et al. 2016), gilthead seabream (*Sparus aurata*), sea urchin (*Paracentrotus lividus*) and sea cucumber (*Actinopyga bannwarthi*) (Israel et al. 2019), among others. The previous studies with the species described demonstrated that IMTA is a feasible practice for using waste nutrients to produce biomass of cultivated species. However, to achieve maximum use of the feeds and reduce the excess of nutrients discharged into the environment, it is necessary to continue evaluating cultured specie's ability to harness the remaining nutrients for optimal growth.

The grey mullet is a marine fish species whose feeding habits make it a great candidate for aquaculture (Abdel-Hakim et al. 2001, Whitfield et al. 2012). The grey mullet is a low trophic species with omnivorous habits and can feed on detritus and microflora, and it is a euryhaline and eurytherm species. (Moriarty 1976, Aghuzbeni et al. 2016). Due to the biological characteristics mentioned above, grey mullet is ideal for its polyculture. Also, grey mullet has a very acceptable marketing value and a great potential for its production in culture (Biswas et al. 2012). There is still no record of its aquaculture production in captivity in Mexico, and most of its consumption comes from fisheries (CONPESCA 2018).

To date, most of the studies have evaluated the two species growing them in the same tank, which becomes a real challenge to quantify how much the mullet consumes from the shrimp wastes and how much it consumes from the feed offered to shrimp (Aghuzbeni et al. 2015, Hoang et al. 2018, Legarda et al. 2019, Hoang et al. 2020a,b). This information might be essential to evaluate the contribution of the nutrients obtained from the waste to the mullet's performance. Therefore, the present study evaluated the use of residual nutrients from Pacific white shrimp farming wastes (i.e. uneaten feed and feces) on the growth performance, digestibility, whole-body proximate composition, and enzymatic activity of juvenile mullet. It also evaluates the incorporation of the Pacific white shrimp farming waste as a co-feed in the juvenile mullet diet as a feeding strategy.

MATERIALS AND METHODS

Ethics statement

All experimental fish used in the present study were handled under procedures that follow the State Committee of Bioethics in Nayarit (Number: 96/CEB/2017) to cause minimal animal suffering.

Experimental treatments

Four experimental treatments were designed to evaluate the biological performance, feed utilization, apparent digestibility, whole-body proximate composition, and the juvenile mullet's enzyme activity. The treatments were designed with a commercial feed for marine fish and a feed based on Pacific white shrimp (*Penaeus vannamei*) farming wastes (SFW) offered in different proportions. The proximate composition of the two experimental feeds is shown (Table 1). The first treatment (SFW0) offered a generic commercial feed for marine fish species (Skretting, Nutreco). The second treatment (SFW33) comprised offering 33% of

Table 1. Proximate composition of the commercial feed and the shrimp farming waste used for the feeding trial (dry matter basis). Values are shown as mean (n = 3) ± standard deviation.

	Experimental feeds	
	Commercial feed	Shrimp farming waste
Crude protein (%)	55.6 ± 0.4	17.2 ± 0.5
Ether extract (%)	15.1 ± 0.2	1.5 ± 0.3
Ash (%)	9.1 ± 0.4	35.5 ± 0.1
Nitrogen free extract + fiber (%)	20.4	45.8

SFW and 67% the commercial feed, the third treatment (SFW67) comprised offering 67% of SWF and 33% the commercial feed, finally, the fourth treatment (EC100) comprised offering the 100% of the portion of SWF. Every treatment was evaluated in triplicate. The bioassay test time ended until a significant difference was observed in one of the variables assessed among the treatments, resulting in six weeks.

Collection of the Pacific white shrimp farming waste

Shrimp farming waste was collected from the shrimp production facilities of the Bioengineering Laboratory of the National School of Fisheries Engineering from the Autonomous University of Nayarit. The production laboratory contained a culture module consisting of nine circular geomembrane tanks with a capacity of 80 m^3 each (10 m diameter; 1 m depth). Each tank contained a density of 100 shrimp per square meter. Shrimps cultured in the laboratory were fed with a daily ration of 6% of their biomass. Likewise, they were offered a commercial feed (Previtep Aquamar, Jal, Mexico) with a content of 35% crude protein, 6% lipids, 12% ash, and 31.5% nitrogen-free extract (NFE). At the time of waste collection, the water temperature was 28°C, the salinity was 29, and the dissolved oxygen was 6 mg L^{-1}. The sample collection was performed in the morning before the first feeding to avoid collecting fresh feed and ensure collecting only feces and uneaten feed (i.e. farming wastes).

Preparation of the Pacific white shrimp farming waste pellets

SFW was transformed into feed pellets by adhering and mixing 5% gelatinized starch as a binder. The mixing was performed for 15 min with a 7 L capacity food mixer (Torrey, model: B7, Mexico). Likewise, about 10% of water was poured into the mixing container to give the mixture's desired consistency. The mixture was then passed through a meat grinder (Rhino, 1HP model: MOCA-12, Mexico) with a 1/16″ diameter die to cold-extrude the pellets. The pellets were dried in an oven at 60°C for 24 h. Once the pellets were dried and cooled, they were placed in sealed plastic bags and stored at 4°C until the feeding trial.

Fish and culture conditions

Mullet (*Mugil cephalus*) juveniles were captured from the wild in the estuary El Yugo in Mazatlán, Sinaloa, Mexico. Then, fish were transported to the laboratory units specialized in aquaculture management and innovation in the Nayarit Center for Innovation and Technology Transfer (CENITT) in Tepic, México. Before the feeding trial was performed, the mullets were kept in laboratory conditions for their acclimatization for three weeks. A total of 120 juveniles of grey mullet with an average weight of 1.51 ± 0.0 g and length of 4.6 ± 0.2 cm, respectively, were randomly distributed in 12 tanks (n = 10 fish per tank).

Each experimental unit consisted of a 45-L tank adapted to a salt-water recirculating system composed of a biofilter of polyurethane balls with a volume of 0.1 m^3, a water pump (Lifeguard, Quiet One, 758 GPH, USA), and an air compressor (Boyu Acq-007, China). Water temperature (°C) and dissolved oxygen (mg L^{-1}) were measured every day in the early morning using a multi-parameter oxygen meter (YSI model Pro 2030, USA). The concentration of ammonia, nitrites, and nitrates in the tanks was monitored every week with an aquarium set kit (APA®; USA) to keep the values of N-NH_3 < 1.0 mg L^{-1}, NO_2 < 0.3 mg L^{-1}, and NO_3 < 10.0 mg L^{-1}, respectively.

Feeding protocol

The feed was offered to the fish in each tank in a ratio of 4% of their wet weight (g) following El Sayed (1994) and Wassef et al. (2001). Quantity (g) of the feed ration offered was adjusted weekly after completing each biometry always to offer 4% of the biomass of the tank in the feed. The feed was offered in two portions, the first at 09:00 h and the second 6 h later.

Growth performance and feed utilization analysis

Growth performance of juvenile mullets, fish weight (g), and length (cm) were evaluated weekly using a scale (Adam Equipment, model HCB 1002, USA) and an ichthyometer (Aquatic Eco-Sistems, FL, USA), respectively. All fish from each experimental tank were

sampled and used for every morphometric measurement. The feeding trial's total feed was determined by adding the daily feed ration offered to each tank.

Calculations of growth performance and feed utilization

Growth performance was evaluated with response variables of weight gain (WG), relative weight gain (RWG), specific growth rate (SGR), thermal growth coefficient (TGC) as presented by (Jobling 2003), survival (S), and Fulton's factor (K) using the following formulas:

$$\mathrm{WG} = \text{final body weight (g)} - \text{initial body weight (g)} \quad (1)$$

$$\mathrm{RWG} = \left[\frac{\text{final body weight (g)} - \text{initial body weight(g)}}{\text{initial weight(g)}}\right] \times 100 \quad (2)$$

$$TGC = \frac{\text{final body weight}^{1/3} - \text{initial body weight}^{1/3}}{\sum(\text{water temp} * \text{number days})} \times 1000 \quad (3)$$

$$\mathrm{SGR} = 100 \times ((\ln \mathrm{FW} - \ln \mathrm{IW}) / (\mathrm{T}) \quad (4)$$

$$S = (\text{final number of fish} - \text{initial number of fish}) \times 100 \quad (5)$$

$$K = \left(\frac{\text{body weight (g)}}{\text{body lenght}^{3}\text{ (cm)}}\right) \times 100 \quad (6)$$

Feed utilization was evaluated with the response variables of feed conversion rate (FCR), protein efficiency rate (PER), and nitrogen retention (NR) using the following formulas:

$$FCR = \frac{\text{feed consumed (g)}}{\text{body weight gain (g)}} \quad (7)$$

$$\mathrm{PER} = \frac{\text{final body weight (g)} - \text{initial body weight (g)}}{\text{protein consumed (g)}} \quad (8)$$

$$\mathrm{NR} = \left(\frac{\text{final body nitrogen (g)} - \text{initial body nitrogen (g)}}{\text{nitrogen consumed (g)}}\right) \times 100 \quad (9)$$

Proximate composition analysis

Three fish at the beginning of the feeding trial and three at the end of the feeding trial were collected per tank and sacrificed with a clove oil overdose (75 mg L^{-1}) to analyze the whole-body proximate (%) composition. Subsequently, the samples were frozen and kept at -20°C until evaluating the proximal body composition analysis, initial and final. Five grams of each feed (i.e. commercial feed, shrimp farming waste) were stored in conical tubes and kept at -20°C before evaluating the feed. The proximate composition of all samples was determined using established methods, according to (AOAC 1990). Crude protein (N × 6.25) was determined by the micro-Kjeldahl method. Lipids were determined by solvent extraction using petroleum ether in a Soxhlet extractor. Ash was estimated by incinerating the samples for eight hours in a muffle at 550°C. The NFE was calculated by subtracting the added percentage of protein + lipids + ash from 100%.

Apparent digestibility analysis (ADC)

The fecal material for evaluating the apparent digestibility coefficients was collected daily. The collection started seven days after the feeding trial until enough material was obtained to perform digestibility analysis. Feces were collected from the bottom of the tank, using a 20 cm long and 2 mm wide PVC siphon immediately after the feces release was observed (approximately 1 h after the feed was offered) to avoid leaching. The collected samples were dried at 60°C in an oven for 24 h to extract humidity and then stored at -4°C in conical-bottom polypropylene 50 mL capacity tubes for later analysis.

Determination of the ADC's of the dry matter and crude protein (CP) was calculated using the insoluble ash in hydrochloric acid as an internal marker. The estimation of the ADC values was assessed according to the method of Montaño-Vargas et al. (2002).

The acid-insoluble ash (AIA) was calculated as follows:

$$\text{AIA (\%)} = \frac{\text{insoluble ash in sample (g)} \times 100}{\text{sample weight (g)}} \quad (10)$$

The ADC's of the dry matter and the CP of the diets were estimated as follows:

$$\text{ADC (\%) of dry matter} = \frac{\text{insoluble ash in diet (\%)}}{\text{insoluble ash in feces (\%)}} \quad (11)$$

$$\text{ADC (\%) of CP} = \frac{\text{insoluble ash in diet (\%)}}{\text{insoluble ash in feces (\%)}} \times \frac{\text{Crude protein in feces (\%)}}{\text{Crude protein in diet (\%)}} \quad (12)$$

Enzyme activity

For enzymatic analyses, two juvenile mullets from each experimental tank were collected and lyophilized before analysis. The whole body of juvenile mullets was processed since they were too small to be dissected and obtain enough sample material for their analysis. The enzyme extract was obtained by placing the samples in a conical 15 mL tube, previously cooled, to be homogenized with a tissue grinder (Polytron® PT 1200, Kinematica AG, Switzerland), in 10 mL distilled water at 4°C. The extract was homogenized by centrifugation (16000 g for 30 min at 4°C). The supernatant of every sample was collected and stored at -80°C. Total enzyme activity per fish was estimated as absorbance per mg (U mg^{-1}) units, and a blank was included in each enzyme determination to adjust the spectrophotometer (Varioskan Flash, Thermo Scientific) measurements.

The evaluation of the enzymatic activities followed well-established protocols. The trypsin activity followed the method of Erlanger et al. (1961), using BAPNA as substrate, and reading at an absorbance of 410 nm. The chymotrypsin activity was measured following the method of Hummel (1959) and improved by Applebaum et al. (2001). The total alkaline protease

activity was measured according to Sarath et al. (1989), using casein (2%) as substrate, and reading at an absorbance of 280 nm in quartz well. According to WBC (1993), amylase activity was measured using Starch (Sigma, S9765) at 1% was as the substrate; absorbance was recorded at 540 nm. The lipase activity was estimated according to the method of Gjellesvik et al. (1992). The substrate was 4-nitrophenyl myristate (Sigma 70124). The reaction was recorded every minute for 30 min at 405 nm. Every unit of enzyme activity was defined as the amount of enzyme necessary to cause an increase of 1 unit of absorbance following what was previously established by Lazo et al. (2000). Activities were expressed in units per gram of wet tissue. Conditions of the protocols as described by Fuentes-Quesada et al. (2018).

Statistics

Before analysis, all percentages (%) data were arcsine transformed. All values were analyzed using a completely randomized design and presented as averages (n = 3) ± standard deviation, SD. The data were evaluated for normality and homoscedasticity with a Komologorov-Smirnov and Levene test, respectively. A one-way analysis of variance (ANOVA) test was applied to determine possible statistical differences among treatments and a Tukey test when differences were found ($P < 0.05$). A Student t-test with a significance level of 0.05 was applied to evaluate the difference in the apparent digestibility coefficients between commercial feed and shrimp farming waste. All the statistical tests were performed using the Statistica® program, version 7.

RESULTS

During the experiment, no signs of disease were observed in the juvenile mullets (*Mugil cephalus*). The temperature registered an average of 25.6 ± 0.4°C regarding water quality parameters, they were maintained within the optimal range for mullets: salinity of 28.6 ± 1.1, pH registered a range of 7.8-8.0, ammonia 0.1 ± 0.1 mg L^{-1}, nitrites of 0.01 ± 0.0 mg L^{-1}, nitrates of 0.01 + 0.0 mg L^{-1}, and dissolved oxygen at 5.7 ± 0.24 mg L^{-1}.

Growth performance and feed utilization

At the end of the feeding trial, the juvenile mullets of treatment SFW0 registered the highest value in FW, WG, RWG, and TGC, with a significant difference ($P < 0.05$) among the treatments (Table 2). However, although juvenile mullets from the SFW100 treatment recorded the lowest growth performance values, the feeding trial achieved an RWG value of 13.16 ± 5.3. Likewise, they had an increase of 0.2 g of the WG. Regarding the K values, the SFW0 and SFW100 treatments had the highest values, differing ($P = 0.024$) from the SFW33 and SFW67 treatment's values. No differences ($P = 0.512$) in the survival percentages among the treatments were detected.

Experimental feed was never observed at the bottom of the tanks during feeding, indicating that it was always thoroughly consumed (4% of biomass). Significant differences ($P < 0.05$) were observed among treatments on the feed utilization values. The juvenile mullets of treatment SFW100 registered the lowest value of FCR. However, although the feed offered to juvenile mullets of treatments SFW0, SFW33, and SFW67 contained different CP levels in the feed, the FCR values among treatments mentioned did not show a significant difference ($P < 0.05$). The PER values were significantly ($P = 0.009$) higher in treatment SFW0. However, the remaining treatments did not show differences ($P > 0.05$) between them. Although the feed received among them had different levels of crude protein. Juvenile mullets from the SFW100 treatment registered the lowest NR value, even with negative values.

Proximate composition of the whole-body

The proximate composition (%) values were different among treatments and the initial sample. Mullets of treatment SFW0 registered the lowest values of moisture. However, the rest of the treatments did not show statistical differences ($P < 0.05$), even with the initial sample. The CP presented the highest values in the initial sample and the juvenile mullets of the SFW0 treatment. Also, it was observed that as the SFW ratio increased in the treatments, the CP content decreased in the juvenile mullets, registering the lowest value in the juvenile mullets of the SFW100 treatment. Regarding the ash content, no significant differences ($P > 0.05$) were found among the treatments or the initial sample. The rest of the proximate composition values are shown (Table 3).

Apparent digestibility coefficients

The present study evaluated the apparent digestibility coefficients of commercial feed and shrimp farming wastes in gray mullets since the four treatments were created from these two experimental feeds. The results showed that the highest ($P < 0.05$) values of the apparent digestibility coefficients with commercial feed were observed, both for the feed's dry matter and protein. However, the juvenile mullets fed with the shrimp farming wastes achieved a protein digestibility value of 41.29%. The rest of the values of the digestibility coefficients are shown (Table 4).

Table 2. Growth performance and feed utilization of juvenile grey mullet (*Mugil cephalus*) at the end of the feeding trial. Values are shown as mean (n = 3) ± standard deviation. Values in the same line with different superscripts are significantly different, determined by Tukey's test, $P < 0.05$. IW: initial weight, FW: final weight, WG: weight gain, RWG: relative weight gain, SGR: specific growth rate, TGC: thermal growth coefficient, S: survival: Fulton's factor, FCR: feed conversion rate, PER: protein efficiency ratio, NR: nitrogen retention, SFW: shrimp farming wastes.

	Experimental treatments				ANOVA
	SFW0	SFW33	SFW67	SFW100	*P*-value
Growth performance					
IW (g)	1.51 ± 0.0	1.50 ± 0.1	1.50 ± 0.0	1.51 ± 0.0	0.000
FW (g)	3.0 ± 0.1^{a}	2.29 ± 0.1^{b}	1.89 ± 0.1^{c}	1.71 ± 0.1^{c}	0.000
WG (g)	1.51 ± 0.1^{a}	0.79 ± 0.1^{b}	0.39 ± 0.0^{c}	0.20 ± 0.1^{c}	0.000
RWG (%)	99.49 ± 13.8^{a}	52.58 ± 7.3^{b}	25.97 ± 3.2^{c}	13.16 ± 5.3^{c}	0.000
SGR	1.68 ± 0.0^{a}	1.03 ± 0.1^{b}	0.52 ± 0.0^{c}	0.3 ± 0.1^{d}	0.000
TGC	0.28 ± 0.2^{a}	0.16 ± 0.0^{b}	0.09 ± 0.0^{c}	0.04 ± 0.0^{c}	0.000
S %	93.3 ± 5.7	86.7 ± 15.2	90.0 ± 10.0	80.0 ± 10.0	0.512
K	1.77 ± 0.1^{a}	1.59 ± 0.0^{b}	1.60 ± 0.1^{b}	1.74 ± 0.0^{a}	0.024
Feed utilization					
FCR	2.11 ± 0.0^{a}	3.63 ± 0.4^{a}	6.32 ± 0.4^{a}	13.89 ± 5.3^{b}	0.002
PER	0.86 ± 0.0^{a}	0.65 ± 0.1^{ab}	0.53 ± 0.0^{b}	0.46 ± 0.2^{b}	0.009
NR (%)	19.1 ± 2.3^{a}	7.5 ± 2.7^{b}	0.2 ± 1.8^{b}	-12.5 ± 4.5^{c}	0.000

Table 3. Proximate composition (wet weight) of the whole-body of juvenile grey mullet (*Mugil cephalus*) at the end and the beginning of the feeding trial among treatments. Values are shown as mean (n = 3) ± standard deviation. Values in the same line with different superscripts are significantly different, determined by Tukey's test, $P < 0.05$. SFW: shrimp farming wastes.

	Treatments					ANOVA
	Initial	SFW0	SFW33	SFW67	SFW100	*P*-value
Moisture (%)	72.1 ± 1.06^{ab}	69.07 ± 1.68^{b}	73.05 ± 1.90^{ab}	74.51 ± 6.39^{ab}	78.07 ± 5.98^{a}	0.022
Crude protein (%)	18.04 ± 0.14^{ab}	20.66 ± 1.57^{a}	16.61 ± 2.2^{bc}	15.42 ± 0.18^{bc}	13.79 ± 0.29^{c}	0.000
Ether extract (%)	3.53 ± 0.31^{ab}	4.88 ± 0.62^{a}	3.26 ± 1.05^{ab}	2.75 ± 1.16^{bc}	1.05 ± 0.27^{c}	0.002
Ash (%)	5.26 ± 0.31	5.29 ± 0.22	5.99 ± 0.46	6.38 ± 0.63	5.77 ± 0.82	0.110
Nitrogen free extract + fiber	1.05	0.10	1.09	0.94	1.32	

Table 4. Apparent digestibility coefficients of the dry matter and crude protein of the commercial feed (CF) and the shrimp farming wastes (SFW) of juvenile grey mullet (*Mugil cephalus*). Values are shown as mean (n = 3) ± standard deviation.

	Experimental feeds		T-test
	CF	SFW	*P*-value
Dry matter	77.18 ± 6.08	35.42 ± 7.60	0.001
Protein	91.37 ± 1.98	41.28 ± 4.33	0.000

Enzyme activity

Although the feed offered in the experimental treatments had different levels of protein and lipids, the evaluated values of the activity of digestive enzymes (i.e. alkaline proteases, trypsin, chymotrypsin, amylases, and lipases) did not show significant differences among treatments ($P < 0.05$) (Table 5).

DISCUSSION

Information about aquaculture use of residual nutrients is necessary to practice sustainable aquaculture and reduce excess nutrients discharged into the environment. In the present study, this study's objective was to evaluate the digestive capacity of grey mullets (*Mugil cephalus*) juveniles using residual nutrients on their biological performance. At the end of the feeding trial, a notable difference in the growth variables among treatments was observed. The juvenile mullets of SFW0 treatment obtained higher ($P < 0.05$) values on the growth variables (i.e. FW, WG, RWG, SGR, and TGC). Higher growth in juvenile mullets was expected due to the protein and lipid content of the feed offered (100% commercial feed) in the SFW0 treatment, compared to the treatments offered different percentages (33, 66, 100%) SFW. However, the present study did not intend to completely replace commercial feed with shrimp farming waste but rather to evaluate the

Table 5. Digestive enzyme activity (U mg fish^{-1}) of alkaline proteases, trypsin, chymotrypsin, amylases, and lipases of juvenile grey mullet (*Mugil cephalus*) fed with the experimental treatments. SFW: shrimp farming wastes. Values are shown as mean (n = 3) ± standard deviation.

	Treatments				ANOVA
	SFW0	SFW33	SFW66	SFW100	*P*-value
Alkaline proteases	0.275 ± 0.05	0.243 ± 0.05	0.263 ± 0.04	0.256 ± 0.05	0.8949
Trypsin	0.376 ± 0.28	0.471 ± 0.26	0.257 ± 0.13	0.385 ± 0.25	0.7638
Chymotrypsin	1.388 ± 0.08	1.617 ± 0.19	1.606 ± 0.03	1.630 ± 0.01	0.0708
Amylases	0.011 ± 0.004	0.010 ± 0.003	0.007 ± 0.003	0.008 ± 0.002	0.6601
Lipases	0.251 ± 0.02	0.272 ± 0.11	0.232 ± 0.06	0.252 ± 0.101	0.9494

utilization of these compounds in mullets as supplementary feed.

Regarding the number of residual nutrients in waste used as experimental feed in the present study, crude protein and lipid in the SFW resulted in 17.2 and 1.5% content, respectively. In a similar study, Israel et al. (2019) collected the wastes from a gilthead sea bream *Sparus aurata* farm; the nutritional content of the waste they collected resulted in 18.7% crude protein and 6% lipids. It is noteworthy that the nutrient content of the farming wastes can vary depending on the digestibility of the species cultivated (Herath & Satoh 2015, Galasso et al. 2017). Digestibility depends on biological, environmental, and dietary factors (Sugiura 2000). Likewise, a decrease in the mullet's biological performance was observed as the percentage of SFW in feed increased. These results suggest that if the juvenile mullet feed only on SFW, they will not obtain enough nutrients to obtain optimal growth. Since a protein level of 30% has been determined in the feed to cover the mullet's protein requirement (Talukdar et al. 2020) and a level of 6% for lipids (De et al. 2011). However, Hoang et al. (2018) determined that a maximum of 10% biomass of mullets as related to that of Pacific shrimp's (*Penaeus vannamei*) cultivated biomass should be cultivated to obtain greater productivity and utilization of nutrients. These data were determined when the two species were kept in the same culture tank. Hoang et al. (2020b) observed in *P. vannamei-M. cephalus* co-culture that when the shrimp was cultivated with the mullets kept in separate cages, the mullet's growth performance obtained low values. Similar results were reported by Borges et al. (2020), evaluating *P. vannamei-Mugil liza* co-culture with biofloc technology. The authors reported that when mullets are grown in separate tanks from shrimp, their biological performance is considerably reduced compared to when they are grown in the same tank. The results mentioned above agree with those obtained in the present study, noticing that a decrease in growth performance was observed when the mullets were co-fed with SFW. The data suggest that it is not enough to feed them only with SFW for optimal mullet growth. It is necessary to supplement feeding with formulated feed or some source of extra nutrients like biofloc, as suggested by Legarda et al. (2019).

Regarding the feed utilization values, as the SFW increased as feed, the FCR values increased, and the PER and NR values decreased. These results are due to the lower nutrients (i.e. protein, lipids) that SFW contained. Several studies that evaluated the mullet's biological performance fed with the shrimp waste did not report the mullet's FCR values (Borges et al. 2020, Hoang et al. 2020a,b). Probably because of the difficulty of measuring the amount of waste that mullets consumed. Legarda et al. (2019) reported lower FCR values than the present study. However, that study used biofloc technology, which provides considerable nutrient content that mullets utilized to grow. A decrease in NR values was observed as the SFW was increased in the feed, registering significant differences among the treatments. At the end of the feeding trial, a more significant reduction in the NR value was observed in the SFW 100 mullets. This result could be because of the lack of nitrogen in their feed, and mullets had to catabolize nitrogen from the body to produce their energy. Enzymes for catabolism and amino acid synthesis occur in each tissue. The catabolism process involves the deamination resulting in a carbon skeleton that can be channeled into the tricarboxylic acid cycle, where it is either oxidized or can be oriented towards gluconeogenesis via pyruvate carboxylase (Bequette 2003). Due to catabolic reactions, energy is provided for different metabolic actions such as mechanical work, transport, and anabolic activity such as synthesizing carbohydrates, proteins, and fats (McDonald et al. 2011).

The SFW percentage in the treatments affected the mullet's proximal whole-body composition at the end of the feeding trial. The reduction of protein and lipids in the whole body was notable as the SFW percentage in the feed ration increased, showing the lowest values

with the SFW100 treatment mullets. This result can be explained due to the low protein and lipid content in SFW. The lack of nutrients in the feed leads to a nutritional deficiency, deteriorates the fish's health, and affects the composition of nutrients in the tissues (NRC 2011, Lall & Dumas 2015). Likewise, protein deficiency in feed reduces growth due to the extraction of proteins from less vital tissues (i.e. muscle) to prioritize the most vital functions (Wilson 2002). A similar result was observed by Talukdar et al. (2020), who found a higher protein accretion in the mullet's carcass when they utilized 30 and 32% protein levels in the feed. Biswas et al. (2012) also found differences in the mullets' whole-body protein and lipid composition (%). The experimental feed's proximal composition (% in dry matter) was 27.5 CP and 5.2 L. They mentioned that the formulated feed is accepted rapidly and contains a balance of nutrients, helping the mullet's performance. However, Gisbert et al. (2016) found no differences in mullet fingerling's proximate composition evaluating different weaning diets. It is worth mentioning that although they used different protein sources in the diets, they remained isoproteic and isolipidic, covering the nutritional requirement of the mullets. Also, De et al. (2012) found no effect on carcass protein composition in mullets fed with different protein levels (i.e. 20, 25, 30, and 35% of CP, in dry matter), which might be because the mullets had enough protein in the diet and did not need to use tissue protein as an energy source.

Although mullets have a remarkable ability to feed on sediments or detritus (Whitfield et al. 2012), in the present study, we found an apparent low digestibility of the dry matter and protein when feeding on SFW (i.e. below 50%). Farm waste usually decomposes very fast (Galasso et al. 2017); the feed composition is one of the factors that affect an organism's digestibility (Sugiura 2000), as well as the high amount of ash in the feed (NRC 2011). However, one of the essential nutritional characteristics of mullets is that they can feed on the organic matter of the sediment (Silva 1980), making the mullets an ideal species for polyculture and obtaining nutrients from farm waste (Lupatsch et al. 2003). Israel et al. (2019) found low coefficients of apparent digestibility of dry matter and protein in mullets, feeding them with *S. aurata* farm waste. The authors suggested that for farm waste to have nutritional value for mullets, the waste must remain in the bottom sediment to accumulate nutrients and produce microbial biomass and contribute to the nutritional value. The present study agrees with what was commented by Israel et al. (2019) since a lack of nutritional value was observed in the SFW, negatively affecting the mullet's growth performance. The result viewed from the sustainability of aquaculture and reduction of the environmental impact may be favorable. The reuse of shrimp farming wastes as feed for mullets may be a viable practice. However, feeding the mullets with feed formulated to satisfy the species' nutritional requirements to obtain stock production would be necessary, and exploring shrimp farming wastes as a food source for alternate low trophic level species.

Our results showed that there were no significant differences in the activity of digestive enzymes between the treatments. Generally, fish can adjust the secretion of pancreatic digestive enzymes concerning the level of feed and its quality (Buddington et al. 1997). Omnivorous fish, such as grey mullets, generally have a high activity of many digestive enzymes and can use a wide range of food sources (NRC 2011). It could have been that at the time of the collection of the fish sampled, digestive enzymes were already being secreted to be ready for feed consumption, regardless of the source, be it commercial feed or shrimp farming wastes, and this may explain why no significant difference among treatments was register. In subsequent studies, it is recommended to evaluate the enzymatic activity per fish organ to eliminate the possible reading of non-digestive enzyme activity.

CONCLUSION

The present study suggests that mullets can utilize residual nutrients from Pacific shrimp farming waste. Utilizing residual nutrients from shrimp farm waste as a feed source for mullets will contribute to sustainable aquaculture and reduce the environment's impact due to excess residual nutrients. However, to meet nutritional requirements, it is recommended that the mullet's feed be complemented with formulated feed so that the protein and lipid content in the whole body of the mullets do not decrease and increase growth performance under growing conditions.

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