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

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28 **Abstract**

29 A feeding trial was carried out to evaluate the utilization of residual nutrients from a shrimp
30 farming wastes as co-feed in different proportions in juvenile grey mullets. Four treatments
31 were designed offering shrimp farming wastes at 0, 33, 66, and 100%. The 4 % of feed
32 respecting the total biomass of each experimental tank was offered daily. The total feed ratio
33 was completed with commercial feed for marine fish in the treatments where it was
34 needed. At the end of the feeding trial, final weight, weight gain, relative weight gain, and
35 thermal growth coefficient were reduced in the fish when increasing the amount of SFW as
36 feed. However, the fish showed a digestive capacity to use residual nutrients up to 66 %,
37 increasing their initial weight by up to 25 %. The increase of shrimp farming waste as feed
38 negatively affected the whole-body proximal composition. The digestibility results showed that
39 the fish could digest up to 41 % of the shrimp farming waste protein. Results suggest that
40 juvenile grey mullets are capable of utilizing residual nutrients from shrimp
41 farming waste. However, it is necessary to use an alternative feed source to induce an optimal
42 growth performance for the juvenile grey mullets. It is also recommended that mullets be fed
43 with formulated feed to meet their nutritional requirements to maintain the protein and lipid
44 content of the whole-body under culture conditions.

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46

47 **Keywords:** *Mugil cephalus*, growth performance, apparent digestibility coefficients,
48 proximate whole-body composition, enzyme activity, residual nutrients, sustainability.

49

50 1. Introduction

51 World aquaculture production from 2000 to 2018 sustained annual growth of 5.3 %.
 52 Total production was reported at 82.1 million metric tonnes of aquatic animals (FAO,
 53 2020). Nevertheless, problems associated with the production increment have also
 54 arisen. The excessive supply of nutrients from the crop waste (i.e., ammonia and
 55 phosphorous), mainly formed by uneaten feed and feces, has caused eutrophication
 56 problems in the environment (Thomsen et al., 2020; Ottinger et al., 2016; Gowen, 1994;
 57 Talbot & Hole, 1994). Eutrophication can alter the aquatic ecosystem, inducing
 58 diseases, and cause mortalities in exposed organisms (Jasmin et al., 2020; Lananan et
 59 al., 2014). As an alternative, bioremediation has been used to eliminating or reducing
 60 harmful compounds through its use and biological processing (Jasmin et al., 2020;
 61 Divya et al., 2015).

62 In Mexico, Pacific white shrimp production (*Litopenaeus vannamei*) by its volume
 63 is situated in a second-place of the total aquaculture. Its average annual production
 64 growth rate in the last ten years has been around 1.67% (CONAPESCA, 2018).
 65 However, one of the major problems facing the Pacific white shrimp industry is the low
 66 utilization of nutrients supplied through the feed. Generally, only 30% of the nitrogen
 67 feed is used by farmed shrimp, while the remaining 70% is discarded or excreted as
 68 dissolved form or particles into the water (Troell et al., 1999). A viable strategy to
 69 reduce and reuse the discharge of nutrients from the Pacific white shrimp crop is a
 70 polyculture's practice through an Integrated Multi-Trophic Aquaculture (IMTA). The
 71 IMTA has the potential to reduce the environmental impact of cultivated species since
 72 the wastes are re-utilized as input for another species (Granada et al., 2018; Estim,
 73 2015; Ridler et al., 2007; Chopin et al., 2001). The IMTA systems benefit environments
 74 as a result of processes such as bioremediation, and combines economic viability (i.e.,
 75 cost reduction, diversified performance), social acceptability (i.e., environmental
 76 problems, organic quality), and provides a culture with feasible production (Estim,
 77 2015). Among the species of interest where the multitrophic systems have been
 78 evaluated are the Pacific white shrimp *Litopenaeus vannamei*, pacific oyster
 79 *Crassostrea gigas* and smooth clam *Chione fluctifraga* (Martinez-Cordova and
 80 Martinez-Porchas, 2006), seaweed *Gracilaria lemaneiformis*, and scallop *Chlamys*
 81 *farreri* (Mao et al., 2009), blue mussel *Mytilus edulis* and Atlantic salmon *Salmo salar*
 82 (Reid et al., 2010), rainbow trout *Oncorhynchus mykiss*, Nile tilapia *Oreochromis*

83 *niloticus* and seaweeds (*Porphyra*, *Ulva* and *Gracilaria*)(Pereira et al., 2012), *Eisenia*
84 *arborea* and red abalone *Haliotis rufescens* (Zertuche-González et al., 2014), Pacific
85 white shrimp *Litopenaeus vannamei* and grey mullet *Mugil cephalus* (Aghuzbeni et al.,
86 2016), Gilthead seabream *Sparus aurata*, grey mullet *Mugil cephalus*, sea urchin
87 *Paracentrotus lividus* and sea cucumber *Actinopyga bannwarthi* (Israel et al., 2019),
88 among others. The previous works have shown that IMTA is a feasible practice for
89 using waste nutrients to produce biomass of cultivated species. However, to achieve
90 maximum use of the feeds and reduce the excess of nutrients that are discharged into the
91 environment, it is necessary to keep evaluating cultured species' ability to harness the
92 remaining nutrients for its optimal growth.

93 The grey mullet (*Mugil cephalus*) is a marine fish species whose feeding habits
94 position it as a great candidate for aquaculture. One of the advantages of mullets is that
95 they can be fed from the unused nutrients from the shrimp farming waste (SFW). The
96 grey mullet is a low trophic species with omnivorous habits and can feed on detritus and
97 microflora, it is a euryhaline and eurytherm species. (Aghuzbeni et al., 2016; Moriarty,
98 1976). Because of those mentioned above, grey mullet is placed as an ideal candidate
99 for its polyculture. Also, grey mullet has a very acceptable marketing value and a great
100 potential for its production in crops (Biswas et al., 2012). In Mexico, there is still no
101 record of its aquaculture production in captivity (CONPESCA, 2018).

102 To date, most studies evaluating the viability of shrimp-mullet co-cultures have not
103 focused on separately evaluating the contribution of only shrimp farming waste to
104 mullet performance (Hoang et al., 2020; Hoang et al., 2020a; Legarda et al., 2019;
105 Hoang et al., 2018; Aghuzbeni et al., 2015). Most of the studies have evaluated the two
106 species growing them in the same tank, which becomes a real challenge to quantify how
107 much the mullet consumes from the shrimp wastes and how much it consumes from the
108 feed offered to shrimp. This information might be essential to evaluate the contribution
109 of the nutrients obtained from the waste to the mullet's performance. Therefore, the
110 present study evaluated the use of residual nutrients from Pacific white shrimp farming
111 wastes (i.e., uneaten feed and feces) on the growth performance, digestibility, whole-
112 body proximate composition, and enzymatic activity of juvenile mullet. It also evaluates
113 the incorporation of the Pacific white shrimp farming waste as a co-feed in the juvenile
114 mullet diet as a feeding strategy.

115 **2. Material and methods**

116 *2.1.Ethics statement*

117 All experimental fish used in the present study were handled under the procedures
118 that agreed with the State Committee of Bioethics in Nayarit (Number: 96/CEB/2017)
119 to cause minimal suffering to animals.

120 *2.2.Experimental treatments*

121 Four experimental treatments were designed to evaluate the biological performance,
122 feed utilization, apparent digestibility, whole-body proximate composition, and the
123 juvenile mullets' enzyme activity. The treatments were designed with a commercial feed
124 and a feed based on Pacific white shrimp farming wastes (SFW) offered in different
125 proportions. The proximate composition of the two experimental feeds is shown in
126 table1. The first treatment consisted of offering a generic commercial feed for marine
127 fish (Skretting, marine fish). The second treatment (SFW33) comprised offering 33 %
128 of SFW and 67 % the commercial feed, third treatment (SFW67) comprised offering 67
129 % of SWF and 33 % the commercial feed, finally, the fourth treatment (EC100)
130 comprised offering the total of the protion of SWF. Every treatment was evaluated in
131 triplicate. The bioassay ended until a significant difference was found in any of the
132 variables evaluated among the treatments, which resulted in 6 weeks.

133 *2.3.Collection of the Pacific white shrimp farming waste*

134 Shrimp waste was collected from the production Bioengineering Laboratory of the
135 Escuela Nacional de Ingeniería Pesquera from the Universidad Autónoma de Nayarit.
136 The production laboratory contained a culture module consisting of 9 circular
137 geomembrane tanks with a capacity of 80 cubic meters each (10 m diameter; 1 m
138 depth). Each tank contained a density of 100 shrimp per square meter. Shrimps that were
139 cultured in the laboratory were fed with a daily ration of 6% of their biomass. Likewise,
140 they were offered a commercial feed (Previtop Aquamar, Jal, Mexico) with a content of
141 35% crude protein, 6% lipids, 12% ash, and 31.5% NFE. At the time of waste
142 collection, the temperature was 28 °C, the salinity was 29 ppt, and the dissolved oxygen
143 was 6 mg L. The collection was performed in the morning before the first feeding to
144 avoid collecting fresh feed and to ensure collecting only feces and uneaten feed (i.e.,
145 farming wastes).

146 *2.4.Preparation of the Pacific white shrimp farming waste pellets*

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

155 *2.5.Fish and culture conditions*

156 The mullet's juveniles were captured from the wild in the estuary El Yugo in
157 Mazatlán, Sinaloa, Mexico. Then, fish were transported to the unit's laboratory
158 specialized in aquaculture management and innovation in the Centro Nayarita de
159 Innovación y Transferencia de Tecnología (CENITT) in Tepic, Nayarit, México. Before
160 performing the feeding trial, the mullets were kept for three weeks in laboratory
161 conditions for their acclimatization. A total of 120 juveniles of grey mullet weight of
162 1.51 g ± 0.0 an average length of 4.6 cm ± 0.2 were randomly distributed in 12 tanks (n
163 = 10 fish per tank).

164 Each experimental unit consisted of a 45-L tank adapted to a salt-water recirculating
165 system composed of a biofilter of polyurethane balls with a volume of 0.1 cubic meters,
166 a water pump (Lifeguard, Quiet One, 758 GPH, USA), and an air compressor (Boyu
167 Acq-007, China). Water temperature (°C) and dissolved oxygen (mg L⁻¹) were
168 measured every day in the early morning using a multi-parameter oxygen meter (YSI
169 model Pro 2030, USA). The concentration of ammonia, nitrites, and nitrates in the tanks
170 were monitored every week with an aquarium set kit (APA®; USA) to keep the values
171 of N- NH₃ < 1.0 mg L⁻¹, NO₂ < 0.3 mg L⁻¹, and NO₃ < 10.0 mg L⁻¹ respectively.

172 *2.6.Feeding protocol*

173 The feed was offered to the fish in each tank in a ratio of 4% of their wet weight (g)
174 in accordance with El Sayed (1994) and Wassef et al. (2001). Quantity (g) of the feed
175 ration offered was adjusted weekly after completing each biometry in order always to be
176 offering 4% of the biomass of the tank in the feed. The feed was offered in two portions,
177 the first at 9:00 am and the second 6 hours later.

178 2.7. Growth performance and feed utilization analysis

179 For monitoring the growth performance of juvenile mullets, fish weight (g) and
180 length (cm) were evaluated weekly using a scale (Adam Equipment, model HCB 1002,
181 USA) and an ichthyometer (Aquatic Eco - Systems, Inc. FL, USA), respectively. All fish
182 from each experimental tank were sampled and used for every morphometric
183 measurement. The feeding trial's total feed was determined by adding the daily feed
184 ration offered to each tank.

185 2.8. Calculations of growth performance and feed utilization

186 Growth performance and feed utilization were evaluated using the following
187 formulas:

$$188 \text{ Weight gain (WG)} = (\text{final weight g} - \text{initial weight g}). \quad (1)$$

$$189 \text{ Relative weight gain (RWG)} = [(\text{final weight (g)} - \text{initial weight (g)}) / \text{initial weight} \\ 190 \text{ (g)}] \times 100. \quad (2)$$

$$191 \text{ Thermal Growth Coefficient (TGC)} = [(\text{final weight})^{1/3} - (\text{initial weight})^{1/3}] / (\Sigma \\ 192 \text{ water temp} \times \text{number days}) \times 1000 \text{ (as presented by Jobling, 2003)}. \quad (3)$$

$$193 \text{ Survival (S)} = (\text{number of fish in each group remaining on day 84} / \text{initial number of} \\ 194 \text{ fish}) \times 100. \quad (4)$$

$$195 \text{ Fulton's factor (K)} = 100 [(\text{body weight (g)} / \text{length}^3 \text{ (cm)})]. \quad (5)$$

$$196 \text{ Feed conversion rate (FCR)} = \text{feed consumed (g)} / \text{weight gain (g)}. \quad (6)$$

$$197 \text{ Protein efficiency rate (PER)} = [\text{Final BW (g)} - \text{Initial BW (g)}] / \text{Weight of protein} \\ 198 \text{ consumed (g)}. \quad (7)$$

$$199 \text{ Nitrogen retention (NR)} = \text{nitrogen retained (g)} / \text{protein intake (g)} \times 100. \quad (8)$$

200 2.9. Proximate composition analysis

201 For the analysis of the whole-body proximate (%) composition, three fish at the
202 beginning of the feeding trial and three fish per tank at the end of the feeding trial were
203 collected by euthanizing them with a clove oil overdose. The samples were frozen and
204 kept at -20° C until evaluating the proximal body composition analysis, initial and final.
205 Five grams of each feed (i.e., commercial feed, shrimp farming waste) were stored in
206 conical tubes and kept at -20° C before analysis for evaluating the feed. The proximate

composition of all samples was determined using established methods, according to (AOAC, 1990). Crude protein (N x 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 nitrogen-free extract (NFE) was calculated by subtracting the added percentage of protein + fat + ash from 100%.

2.10. Apparent digestibility analysis

The fecal material for evaluating the apparent digestibility coefficients was collected daily. The collection started seven days after running the feeding trial until enough material was obtained to perform digestibility analysis. Feces were collected from the bottom of the tank. It used a 20-cm long and 2-mm wide PVC siphon immediately after the feces' release was observed (approximately 1 hour after the feed was offered) to avoid leaching. The collected samples were dry at 60 °C in an oven for 24 h to extract humidity. Then they were 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 were calculated using the insoluble ash in hydrochloric acid as an internal marker. The estimation of the values was assessed according to the method of Montaña-Vargas et al. (2002).

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

$$\text{AIA (\%)} = \text{insoluble ash (g)} \times 100 / \text{sample (dry weight) (g)}. \quad (9)$$

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

$$\text{ADC (\%)} \text{ of dry matter} = 100 - [(\% \text{ insoluble ash in diet} / \% \text{ insoluble ash in feces})]. \quad (10)$$

$$\text{ADC (\%)} \text{ of CP} = 100 - [(\% \text{ insoluble ash in diet} / \% \text{ insoluble ash in feces}) \times (\% \text{ CP in feces} / \% \text{ CP in diet})]. \quad (11)$$

2.11. 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

237 15 ml tube, previously cooled, to be homogenized with a tissue grinder (POLYTRON®
238 PT 1200, Kinematica AG, Switzerland), in 10 ml distilled water at 4 °C. Once the
239 extract was homogenized, it was centrifuged at 16000 g for 30 minutes, at 4 °C, and the
240 supernatant was collected and stored in 0.5 ml aliquots at -80 °C. Aliquots, once
241 thawed, were used within 48 h and kept refrigerated at 4 °C. Total activity per fish was
242 estimated as units of absorbance per mg (U mg⁻¹). The procedures were adapted to
243 perform the spectrophotometric measurements on a plate reader Varioskan Flash
244 (Thermo Scientific), and the data were processed in the software SkanIt RE 2.4.5. A
245 blank was included in each enzyme determination using distilled water instead of the
246 homogenate.

247 The trypsin activity was measured according to the method of Erlanger et al. (1961).
248 The procedure consisted of using one mM of BAPNA (N α -benzoyl-DL-arginine-p-
249 nitroanilide hydrochloride, Sigma B-4875) substrate dissolved 500 μ L of DMSO in 50
250 mM Tris-HCl buffer, 20 mM CaCl₂, pH 8.2 at 37 °C by 30 min. The reaction was
251 stopped with 30 % acetic acid, and the absorbance was recorded at 410 nm after 10
252 minutes of stabilization.

253 The chymotrypsin activity was according to the method of Hummel (1959), as
254 modified by Applebaum et al. (2001), using 0.56 mM of BTEE (N-Benzoyl-L-tyrosine
255 ethyl ester, Sigma 13110-F) as a substrate in 100 mM Tris-HCl buffer, 25 mM CaCl₂,
256 pH 7.8 and methanol 2.5 % (v/v) at 37 °C. The reaction was recorded every minute for
257 30 min at 256 nm in a 96-well quartz bottom plate.

258 The total alkaline protease activity was measured according to Sarath et al. (1989)
259 using 2 % of casein (Sigma, C5890) as a substrate in 50 mM HCl-Tris buffer, ten mM
260 CaCl₂, 9.0 pH at 37 °C. The reaction was incubated for 10 min at 37 °C and stopped
261 with 10 % trichloroacetic acid. Finally, the samples were centrifuged (21000 g, 5 min, 4
262 °C), and the supernatant's absorbance was recorded at 2800 nm.

263 According to Worthington Biochemical Corporation (1993), amylase activity was
264 measured using as substrate 1 % of starch (Sigma, S9765). The starch was mixed in 20
265 mM sodium phosphate buffer, six mM NaCl and 6.9 pH. The homogenate and the
266 buffer + substrate were mixed at intervals of time, incubate at 25°C, and at 3 min, 1 %
267 dinitrosalicylic acid was added as a colour reagent. After, all tubes were incubated in a
268 boiling water bath for 5 minutes. Afterward, tubes were cooled at ambient temperature

269 and mixed to read absorbance at 540 nm. A standard maltose curve was used to
270 calculate the micromoles of maltose released in each sample by the regression equation.
271 All the samples were diluted ten times in a final volume reaction of 1.2 ml to allow
272 readings within the standard range.

273 The lipase activity was estimated according to the method of Gjellesvik et al. (1992)
274 using 0.56 mM of 4- Nitrophenyl myristate (Sigma 70124) as substrate dissolved in 0.5
275 mL DMSO. The reaction conditions were 150 mM Tris-HCl buffer, 15 mM sodium
276 taurocholate, pH 8.5 at 37 °C. Also, the reaction was recorded every minute by 30 min
277 at 405 nm. Pepsin activity (i.e., acid proteolytic activity) was estimated according to
278 Sarath et al. (1989), using 1 % of hemoglobin as substrate (Spectrum Chemical, HE120)
279 in 200 mM, pH two at 37 °C. The reaction was incubated for 10 min and stopped with 5
280 % trichloroacetic acid. The samples were centrifuged (21000 g, 5 min, 4 °C), and the
281 absorbance of the supernatant was recorded at 280 nm. In all cases, one unit of enzyme
282 activity was defined as the amount of enzyme required to cause an increase of 1 unit of
283 absorbance (Lazo et al., 2000). Activities were expressed in units per gram of wet
284 tissue.

285 2.12. Statistics

286 All variables' values were analyzed using a completely randomized design and
287 presented as averages ($n = 3$) $\pm$ standard deviation (SD). All data were evaluated for
288 their normality with a Komologorov-Smirnov test and their homoscedasticity with a
289 Levene test. A one-way analysis of variance (ANOVA) test was applied to determine
290 possible statistical differences among treatments and a Tukey test when differences
291 were found ($P < 0.05$). Before analysis, all percentages (%) data were arcsine
292 transformed. A Student t-test with a significance level of 0.05 was used to find a
293 significant difference in the apparent digestibility coefficients between the commercial
294 feed and the shrimp farming waste. All the statistical tests were performed using the
295 STATISTICA® program, version 7.

296 3. Results

297 During the feeding trial, no signs of disease were observed in the juvenile mullets.
298 The temperature registered an average of 25.6 ± 0.4 °C regarding water quality
299 parameters, the salinity of 28.6 ± 1.1 , the pH of 7.8 ± 0.1 , and ammonia maintained an

average value of $0.1 \pm 0.1 \text{ mg L}^{-1}$, nitrites of $0.01 \pm 0.0 \text{ mg L}^{-1}$, nitrates of $0.01 \pm 0.0 \text{ mg L}^{-1}$, and dissolved oxygen at $5.7 \pm 0.24 \text{ mg L}^{-1}$.

3.1. 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. At the end of the feeding trial, there were no differences in the survival percentages among the treatments. Regarding the K values, the SFW0 and SFW100 treatments had the highest values, being different ($P < 0.05$) from the SFW33 and SFW67 treatments' values.

Experimental feed was never observed at the bottom of the tanks during feeding, indicating that it was always consumed (4% of biomass). However, 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 higher in treatment SFW0. However, the remained treatments did not show differences among 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.

3.2. Proximate composition of the whole-body

The proximate composition (%) values were different among treatments, as well as the initial sample. Mulletts of treatment SFW0 registered the lowest values of moisture. However, the rest of the treatments did not show statistical differences, 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, not with the initial sample. The rest of the proximate composition values are found in Table 3.

332 3.3. Apparent digestibility coefficients

333 In the present study, the apparent digestibility coefficients of commercial feed and
334 shrimp farming wastes in gray mullets were evaluated since the 4 treatments were
335 created from these two experimental feeds. The results showed that the highest ($P <$
336 0.05) values of the apparent digestibility coefficients with commercial feed were
337 observed, both for the feed's dry matter and protein. However, the juvenile mullets fed
338 with the shrimp farming wastes achieved a protein digestibility value of 41.29%. The
339 rest of the values of the digestibility coefficients are presented in table 4.

340 3.4. Enzyme activity

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

345 4. Discussion

346 As aquaculture grows, it is necessary to maintain a sustainable practice to reduce the
347 environment's negative impact (FAO, 2020; Granada et al., 2018). Information related
348 to the use of residual nutrients from aquaculture species is necessary to practice
349 sustainable aquaculture and reduce excess nutrients discharged into the environment. In
350 the present study, the use of residual nutrients by juvenile mullet for its growth
351 performance was evaluated. At the end of the feeding trial, a notable difference in the
352 growth variables among treatments was observed. The juvenile mullets of SFW0
353 treatment obtained higher ($P < 0.05$) values on the growth variables (i.e., FW, WG,
354 RWG, and TGC). Higher growth in juvenile mullets was expected due to the protein
355 and lipid content of the feed offered (100 % commercial feed) in the SFW0 treatment,
356 compared to the treatments that were offered different percentages (33, 66, 100%) of
357 SFW. In the present study, crude protein and lipid in the SFW resulted in 17.2 and 1.5
358 % content, respectively. In a similar study, Israel et al. (2019) collected the wastes from
359 a gilthead sea bream farm, *Sparus aurata*; the nutritional content of the waste they
360 collected resulted in 18.7% crude protein and 6% lipids. It is noteworthy that the
361 nutrient content of the farming wastes can vary depending on the digestibility of the
362 species cultivated (Galasso et al., 2017; Herath and Satoh, 2015). Digestibility depends
363 on biological, environmental, and dietary factors (Sugiura, 2000). Likewise, it was

noted to observe a decrease in the mullet's biological performance as the percentage of SFW when the percentage of 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 mullets' protein requirement (Talukdar et al., 2020) and a level of 6% lipids (De et al., 2011). However, Hoang et al. (2018) determined that a maximum of 10% biomass of mullets (*Mugil cephalus*) should be cultivated concerning Pacificshrimp's (*Litopenaeus vannamei*) cultivated biomass 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. (2020) observed in a shrimp-mullet (*Litopenaeus vannamei*-*Mugil cephalus*) co-culture that the shrimp was cultivated with the mullets kept in separate cages, the mullets' growth performance obtained low values. Similar results were reported by Borges et al. (2020) evaluating shrimp-mullet (*Litopenaeus vannamei*-*Mugil liza*) co-culture with biofloc technology. The authors reported that when mullets are grown in separate tanks from shrimp, the mullets' biological performance is considerably reduced compared to when they are grown in the same tank. The results mentioned above agree with the obtained in the present study, noticing that when the mullets were co-fed with SFW, a decrease in growth performance was observed. 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 amount of nutrients (i.e., protein, lipids) that SFW contained. Several studies that evaluated the mullets' biological performance fed with the shrimp waste did not report the mullets' FCR values (Hoang et al., 2020a; Hoang et al., 2020b; Borges et al., 2020). 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 utilized mullets to grow. A decrease in nitrogen retention (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

397 lack of feed nitrogen, and mullets had to catabolize nitrogen from the body to produce
398 their energy. Enzymes for catabolism and amino acid synthesis occur in each tissue.
399 The catabolism process involves the deamination resulting in a carbon skeleton that can
400 be channeled into the tricarboxylic acid cycle, where it is either oxidized that can be
401 oriented towards gluconeogenesis via pyruvate carboxylase (Bequette, 2003). Due to
402 catabolic reactions, energy is provided for different metabolic actions such as
403 mechanical work, transport, and anabolic activity such as the synthesis of
404 carbohydrates, proteins, and fats (McDonald et al., 2011).

405 The SFW percentage in the treatments affected the mullets' proximal whole-body
406 composition at the end of the feeding trial. The reduction of protein and lipids in the
407 whole-body was notable as the SFW percentage in the feed ration increased, showing
408 the lowest values with the SFW100 treatment mullets. This result can be explained due
409 to the low protein and lipid content in SFW. The lack of nutrients in the feed leads to a
410 nutritional deficiency, deteriorates the fish's health, and affects the composition of
411 nutrients in the tissues (Lall and Dumas, 2015; NRC, 2011). Likewise, protein
412 deficiency in feed reduces growth due to the extraction of proteins from less vital
413 tissues (i.e., muscle) to prioritize the most vital functions (Wilson, 2002). A similar
414 result was observed by Talukdar et al. (2020), whom found a higher protein accretion in
415 the mullets' carcass when they utilized 30 and 32% protein levels in the feed. Biswas et
416 al. (2012) also found differences in whole-body protein and lipid composition (%) of
417 the mullets. The proximal composition (% in dry matter) of the experimental feed was
418 27.5 CP and 5.2 L. They mention that the formulated feed is accepted rapidly and
419 contains a balance of nutrients, helping the mullets' performance. However, Gisbert et
420 al. (2016) found no differences in mullet fingerlings' proximate composition evaluating
421 different weaning diets. It is worth mentioning that although they used different protein
422 sources in the diets, they remained isoproteic and isolipidic, covering the nutritional
423 requirement of the mullets. Also, De et al. (2012) found no effect on carcass protein
424 composition in mullets fed with different protein levels (i.e., 20, 25, 30, and 35 % of
425 CP, in dry matter), which might be because the mullets had enough protein in the diet
426 and did not need to use tissue protein as an energy source.

427 Although mullets have a notable ability to feed on sediments or detritus (Whitfield
428 et al., 2012), in the present study, we found an evident low digestibility of the dry
429 matter and protein when feeding on SFW (i.e., below 50 %). Waste farms usually

decompose very fast (Galasso et al., 2017); the feed composition is one of the factors that affect organisms' 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 have the ability to 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 seabream (*Sparus aurata*) farm waste. The authors mention that for farm waste to have nutritional value for mullets, the waste must remain in the bottom sediment to accumulate nutrients and as well as 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 mullets' 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, to obtain crop production, it would be necessary to feed the mullets with feed formulated to satisfy the species' nutritional requirements.

Our results showed that there were no significant differences in the activity of digestive enzymes between the treatments. Generally, fish are able to 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 of the digestive enzymes and are able to use a wide range of food sources (NRC, 2011). It could have been that at the time of the collection of the fish sampled, and digestive enzymes were being secreted to be ready for feed consumption, regardless of the source, be it commercial feed or shrimp farming wastes, registered no significant difference among treatments. In subsequent studies, it is recommended to evaluate the enzymatic activity per organ of fish to eliminate the possible reading of non-digestive enzyme activity.

Conclusion

The present study suggests that mullets are capable of utilizing residual nutrients from shrimp farming waste. However, to have optimal growth performance, the addition of an alternative feed source is necessary. Utilizing residual nutrients from shrimp farm waste as a feed source for mullets will contribute to sustainable aquaculture and reduce

463 the environment's impact due to the excess of residual nutrients discharged. However, to
464 meet nutritional requirements, it is recommended that the mullets be fed with
465 formulated feed so that the protein and lipid content in the whole-body of the mullets do
466 not decrease and increase growth performance under growing conditions.

467 **Acknowledgments**

468 Thanks to the grant from Consejo Nacional de Ciencia y Tecnologia (CONACyT) for
469 the support of the postdoctoral stay (Convocatoria: Estancias Posdoctorales 1er Año
470 2019 – 1). Thanks to the State Council of Science and Technology (COCYTEN), of the
471 government of Nayarit, Mexico, for the partial financing of the experiments, though the
472 project “Ordenamiento y Tecnificación del Cultivo de Camaron en Nayarit: Primera
473 Fase.” This study was partially funded by the Consejo Nacional de Ciencia y
474 Tecnologia (CONACYT-SEP-CB-2016-284167) coordinated by Dr. Zohar-Zatarain.

475 Reference

- 476 Aghuzbeni, H., Hajirezaee, S., Khara, H., 2015. Polyculture of western white shrimp,
477 *Litopenaeus vannamei* Boone, 1931 with Grey mullet, *Mugil cephalus* Linnaeus,
478 1758 controls external parasites of western white shrimp. *Aquac. Res.* 47, 2983–
479 2988. <https://doi.org/10.1111/are.12751>
- 480 Aghuzbeni, S.H.H., Hajirezaee, S., Matinfar, A., Khara, H., Ghobadi, M., 2016. A
481 preliminary study on polyculture of western white shrimp (*Litopenaeus vannamei*)
482 with mullet (*Mugil cephalus*): An assessment of water quality, growth parameters,
483 feed intake efficiency and survival. *J. Appl. Anim. Res.* 45, 247–251.
484 <https://doi.org/10.1080/09712119.2016.1150845>
- 485 AOAC, 1990. Official methods of analysis, Fifteenth. ed, Association of Analytical
486 Communities. Association of Official Analytical Chemists, Inc., Virginia.
487 <https://doi.org/10.1007/978-3-642-31241-0>
- 488 Applebaum, S.L., Perez, R., Lazo, J.P., Holt, G.J., 2001. Characterization of
489 chymotrypsin activity during early ontogeny of larval red drum (*Sciaenops*
490 *ocellatus*). *Fish Physiol. Biochem.* 25, 291–300.
491 <https://doi.org/10.1023/A:1023202219919>
- 492 Bequette, B.J., 2003. Amino Acid Metabolism in Animals: an Overview, in: Amino
493 Acids in Animal Nutrition Second Edition. pp. 1689–1699.
- 494 Biswas, G., De, D., Thirunavukkarasu, A.R., Natarajan, M., Sundaray, J.K., Kailasam,
495 M., Kumar, P., Ghoshal, T.K., Ponniah, A.G., Sarkar, A., 2012. Effects of stocking
496 density, feeding, fertilization and combined fertilization-feeding on the
497 performances of striped grey mullet (*Mugil cephalus* L.) fingerlings in
498 brackishwater pond rearing systems. *Aquaculture* 338–341, 284–292.
499 <https://doi.org/10.1016/j.aquaculture.2012.02.004>
- 500 Borges, B.A.A., Rocha, J.L., Pinto, P.H.O., Zacheu, T., Chede, A.C., Magnotti, C.C.F.,
501 Cerqueira, V.R., Arana, L.A.V., 2020. Integrated culture of white shrimp
502 *Litopenaeus vannamei* and mullet *Mugil liza* on biofloc technology: Zootechnical
503 performance, sludge generation, and *Vibrio* spp. reduction. *Aquaculture* 524,
504 735234. <https://doi.org/10.1016/j.aquaculture.2020.735234>
- 505 Buddington, R.K., Krogdahl, A., Bakke-McKellep, A.M., 1997. The intestines of
506 carnivorous fish: Structure and functions and the relations with diet. *Acta Physiol.*
507 *Scand. Suppl.* 161, 67–80.
- 508 Chopin, T., Buschmann, A.H., Halling, C., Troell, M., Kautsky, N., Neori, A., Kraemer,
509 G.P., Zertuche-González, J.A., Yarish, C., Neefus, C., 2001. Integrating seaweeds
510 into marine aquaculture systems: A key toward sustainability. *J. Phycol.* 37, 975–
511 986. <https://doi.org/10.1046/j.1529-8817.2001.01137.x>
- 512 CONPESCA, 2018. Anuario Estadístico de Acuicultura y Pesca 2017. Mazatlan.
- 513 Worthington Biochemical Corporation, 1993. Amylase, Alpha, in: Worthington (Ed.),
514 Worthington Enzyme Manual: Enzymes and Related Biochemicals. Lakewood, NJ,
515 pp. 2–3.
- 516 De, D., Ghoshal, T., Kundu, J., Ali, S., 2011. Optimal Dietary Lipid Requirement for
517 Grey Mullet (*Mugil cephalus*). *Indian J. Anim. Nutr.* 28, 451–456.

518 De, D., TKGhoshal, Kundu, J., 2012. Effect of feeding different levels of protein on
519 growth performance, feed utilization and digestive enzyme of grey mullet (*Mugil*
520 *cephalus* L). *Anim. Nutr. Feed Technol.* 12, 179–186.

521 Divya, M., Aanand, S., Srinivasan, A., Ahilan, B., 2015. Bioremediation – An eco-
522 friendly tool for effluent treatment □: A Review. *Int. J. Appl. Res.* 1, 530–537.

523 El-Sayed, 1994. Mullet culture in Qatar: Effects of replacing fish meal with soybean
524 meal on growth rates and feed utilization efficiency of liza *Liza macrolepis* (pisces:
525 *Mugilidae*) By. *Qatar Univ. Sci. J.* 14, 207–211.

526 Erlanger, B.F., Kokowsky, N., Cohen, W., 1961. The preparation and properties of two
527 new chromogenic substrates of trypsin. *Arch. Biochem. Biophys.* 95, 271–278.
528 [https://doi.org/10.1016/0003-9861\(61\)90145-X](https://doi.org/10.1016/0003-9861(61)90145-X)

529 Estim, A., 2015. Integrated Multitrophic Aquaculture, in: *Aquaculture Ecosystems:*
530 *Adaptability and Sustainability.* pp. 164–181.
531 <https://doi.org/10.1787/9789264088726-15-en>

532 FAO, 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in
533 action. Rome. Rome. <https://doi.org/https://doi.org/10.4060/ca9229en>

534 Galasso, H.L., Callier, M.D., Bastianelli, D., Blancheton, J.P., Aliaume, C., 2017. The
535 potential of near infrared spectroscopy (NIRS) to measure the chemical
536 composition of aquaculture solid waste. *Aquaculture* 476, 134–140.
537 <https://doi.org/10.1016/j.aquaculture.2017.02.035>

538 Gisbert, E., Mozanzadeh, M.T., Kotzamanis, Y., Estévez, A., 2016. Weaning wild
539 flathead grey mullet (*Mugil cephalus*) fry with diets with different levels of fish
540 meal substitution. *Aquaculture* 462, 92–100.
541 <https://doi.org/10.1016/j.aquaculture.2016.04.035>

542 Gjellesvik, D.R., Lombardo, D., B.T., W., 1992. Pancreatic bile salt dependent lipase
543 from cod (*Gadus morhua*): Purification and properties. *Biochim. Biophys. Acta*
544 (BBA)/Lipids Lipid Metab. 1124, 123–134. [https://doi.org/10.1016/0005-](https://doi.org/10.1016/0005-2760(92)90088-D)
545 [2760\(92\)90088-D](https://doi.org/10.1016/0005-2760(92)90088-D)

546 Gowen, R.J., 1994. Managing eutrophication associated with aquaculture development.
547 *J. Appl. Ichthyol.* 10, 242–257. [https://doi.org/10.1111/j.1439-](https://doi.org/10.1111/j.1439-0426.1994.tb00164.x)
548 [0426.1994.tb00164.x](https://doi.org/10.1111/j.1439-0426.1994.tb00164.x)

549 Granada, L., Lopes, S., Novais, S.C., Lemos, M.F.L., 2018. Modelling integrated multi-
550 trophic aquaculture: Optimizing a three trophic level system. *Aquaculture* 495, 90–
551 97. <https://doi.org/10.1016/j.aquaculture.2018.05.029>

552 Herath, S.S., Satoh, S., 2015. Environmental impact of phosphorus and nitrogen from
553 aquaculture, in: Davis, D.A. (Ed.), *Feed and Feeding Practices in Aquaculture.*
554 Elsevier Ltd, pp. 369–386. <https://doi.org/10.1016/B978-0-08-100506-4.00015-5>

555 Hoang, M., Nguyen, P.N., Maria Vital Estrochio Martins Bossie, A., Bossier, P., 2020.
556 The effects of two fish species mullet, *Mugil cephalus*, and tilapia, *Oreochromis*
557 *niloticus*, in polyculture with white shrimp, *Litopenaeus vannamei*, on system
558 performances: A comparative study. *Aquac. Res.* <https://doi.org/10.1111/are.14602>

559 Hoang, M.N., Nguyen, P.N., Bossier, P., 2020. Water quality, animal performance,
560 nutrient budgets and microbial community in the biofloc-based polyculture system

561 of white shrimp, *Litopenaeus vannamei* and gray mullet, *Mugil cephalus*.
562 Aquaculture 515, 734610. <https://doi.org/10.1016/j.aquaculture.2019.734610>

563 Hoang, M.N., Nguyen, P.N., Le, D.V.B., Nguyen, D. V., Bossier, P., 2018. Effects of
564 stocking density of gray mullet *Mugil cephalus* on water quality, growth
565 performance, nutrient conversion rate, and microbial community structure in the
566 white shrimp *Litopenaeus vannamei* integrated system. Aquaculture 496, 123–133.
567 <https://doi.org/10.1016/j.aquaculture.2018.07.018>

568 Hummel, B.C.W., 1959. a Modified Spectrophotometric Determination of
569 Chymotrypsin, Trypsin, and Thrombin. Can. J. Biochem. Physiol. 37, 1393–1399.
570 <https://doi.org/10.1139/o59-157>

571 Israel, D., Lupatsch, I., Angel, D.L., 2019. Testing the digestibility of seabream wastes
572 in three candidates for integrated multi-trophic aquaculture: Grey mullet, sea
573 urchin and sea cucumber. Aquaculture 510, 364–370.
574 <https://doi.org/10.1016/j.aquaculture.2019.06.003>

575 Jasmin, M.Y., Syukri, F., Kamarudin, M.S., Karim, M., 2020. Potential of
576 bioremediation in treating aquaculture sludge: Review article. Aquaculture 519,
577 734905. <https://doi.org/10.1016/j.aquaculture.2019.734905>

578 Jobling, M., 2003. SHORT COMMUNICATION The thermal growth coefficient (TGC)
579 model of sh growth: a cautionary note. Aquac. Res. 581–584.

580 Lall, S.P., Dumas, A., 2015. Nutritional requirements of cultured fish, in: Davis, D.A.
581 (Ed.), Feed and Feeding Practices in Aquaculture. Elsevier Ltd, pp. 53–109.
582 <https://doi.org/10.1016/b978-0-08-100506-4.00003-9>

583 Lananan, F., Abdul Hamid, S.H., Din, W.N.S., Ali, N., Khatoon, H., Jusoh, A., Endut,
584 A., 2014. Symbiotic bioremediation of aquaculture wastewater in reducing
585 ammonia and phosphorus utilizing Effective Microorganism (EM-1) and
586 microalgae (*Chlorella* sp.). Int. Biodeterior. Biodegrad. 95, 127–134.
587 <https://doi.org/10.1016/j.ibiod.2014.06.013>

588 Lazo, J.P., Holt, G.J., Arnold, C.R., 2000. Ontogeny of pancreatic enzymes in larval red
589 drum *Sciaenops ocellatus*. Aquac. Nutr. 6, 183–192.
590 <https://doi.org/10.1046/j.1365-2095.2000.00124.x>

591 Legarda, E.C., Poli, M.A., Martins, M.A., Pereira, S.A., Martins, M.L., Machado, C., de
592 Lorenzo, M.A., do Nascimento Vieira, F., 2019a. Integrated recirculating
593 aquaculture system for mullet and shrimp using biofloc technology. Aquaculture
594 512, 734308. <https://doi.org/10.1016/j.aquaculture.2019.734308>

595 Lupatsch, I., Katz, T., Angel, D.L., 2003. Assessment of the removal efficiency of fish
596 farm effluents by grey mullets: A nutritional approach. Aquac. Res. 34, 1367–
597 1377. <https://doi.org/10.1111/j.1365-2109.2003.00954.x>

598 Mao, Y., Yang, H., Zhou, Y., Ye, N., Fang, J., 2009. Potential of the seaweed *Gracilaria*
599 *lemaneiformis* for integrated multi-trophic aquaculture with scallop *Chlamys*
600 *farreri* in North China. J. Appl. Phycol. 21, 649–656.
601 <https://doi.org/10.1007/s10811-008-9398-1>

602 Martinez-Cordova, L.R., Martinez-Porchas, M., 2006. Polyculture of Pacific white
603 shrimp, *Litopenaeus vannamei*, giant oyster, *Crassostrea gigas* and black clam,

604 Chione fluctifraga in ponds in Sonora, Mexico. *Aquaculture* 258, 321–326.
605 <https://doi.org/10.1016/j.aquaculture.2006.03.026>

606 McDonald, P., Edwards, R. a, Greenhalgh, J.F.D., Morgan, C. a, Sinclair, L. a,
607 Wilkinson, R.G., 2011. Animal nutrition. *Anim. Nutr.* 365.

608 Montaña-Vargas, J., Shimada, A., Vásquez, C., Viana, M.T., 2002. Methods of
609 measuring feed digestibility in the green abalone (*Haliotis fulgens*). *Aquaculture*
610 213, 339–346.

611 Moriarty, D.J.W., 1976. Quantitative studies on bacteria and algae in the food of the
612 mullet *Mugil cephalus* L. and the prawn *Metapenaeus bennettiae* (Racek & Dall).
613 *J.exp. mar. Biol. Ecol.* 22, 131–143.

614 NRC, 2011. Nutrient Requirements of Fish and Shrimp. National Academic Press,
615 Washington.

616 Ottinger, M., Clauss, K., Kuenzer, C., 2016. Aquaculture: Relevance, distribution,
617 impacts and spatial assessments - A review. *Ocean Coast. Manag.* 119, 244–266.
618 <https://doi.org/10.1016/j.ocecoaman.2015.10.015>

619 Pereira, R., Valente, L.M.P., Sousa-Pinto, I., Rema, P., 2012. Apparent nutrient
620 digestibility of seaweeds by rainbow trout (*Oncorhynchus mykiss*) and Nile tilapia
621 (*Oreochromis niloticus*). *Algal Res.* 1, 77–82.
622 <https://doi.org/10.1016/j.algal.2012.04.002>

623 Reid, G.K., Liutkus, M., Bennett, A., Robinson, S.M.C., MacDonald, B., Page, F.,
624 2010. Absorption efficiency of blue mussels (*Mytilus edulis* and *M. trossulus*)
625 feeding on Atlantic salmon (*Salmo salar*) feed and fecal particulates: Implications
626 for integrated multi-trophic aquaculture. *Aquaculture* 299, 165–169.
627 <https://doi.org/10.1016/j.aquaculture.2009.12.002>

628 Ridler, N., Wowchuk, M., Robinson, B., Barrington, K., Chopin, T., Robinson, S., Page,
629 F., Reid, G., Szemerda, M., Sewuster, J., Boyne-Travis, S., 2007. Integrated multi-
630 trophic aquaculture (IMTA): A potential strategic choice for farmers. *Aquac. Econ.*
631 *Manag.* 11, 99–110. <https://doi.org/10.1080/13657300701202767>

632 Sarath, G., DelaMonte, R.S., F.w., W., 1989. Protease assay methods, in: R.J. Beyon
633 and J.S., B. (Ed.), *Proteolytic Enzymes: A Practical Approach*. Oxford University
634 Press, New York, pp. 25–56. [https://doi.org/10.1016/0968-0004\(85\)90125-2](https://doi.org/10.1016/0968-0004(85)90125-2)

635 Silva, S.S. De, 1980. Biology of juvenile grey mullet: A short review. *Aquaculture* 19,
636 21–36. [https://doi.org/10.1016/0044-8486\(80\)90004-6](https://doi.org/10.1016/0044-8486(80)90004-6)

637 Sugiura, S.H., 2000. Digestibility, in: Stickney, R. (Ed.), *Encyclopedia of Aquaculture*.
638 John Wiley & Sons, Inc., New York, pp. 209–218.

639 Talbot, C., Hole, R., 1994. Fish diets and the control of eutrophication resulting from
640 aquaculture. *J. Appl. Ichthyol.* 10, 258–270. <https://doi.org/10.1111/j.1439-0426.1994.tb00165.x>

642 Talukdar, A., Dharmendra, A., Narrotam, D., Sahu, P., Sardar, P., Aklakur, M., Prakash,
643 S., Shamna, N., Kumar, S., 2020. Effects of dietary protein on growth performance
644 , nutrient utilization , digestive enzymes and physiological status of grey mullet ,
645 *Mugil cephalus* L. fingerlings reared in inland saline water. *Aquac. Nutr.* 1–15.
646 <https://doi.org/10.1111/anu.13050>

647 Thomsen, E., Herbeck, L.S., Jennerjahn, T.C., 2020. The end of resilience: Surpassed
648 nitrogen thresholds in coastal waters led to severe seagrass loss after decades of
649 exposure to aquaculture effluents: Long-term aquaculture effluents exposure
650 causes seagrass loss. *Mar. Environ. Res.* 160, 104986.
651 <https://doi.org/10.1016/j.marenvres.2020.104986>

652 Troell, M., Rönnbäck, P., Halling, C., Kautsky, N., Buschmann, A., 1999. Ecological
653 engineering in aquaculture: Use of seaweeds for removing nutrients from intensive
654 mariculture. *J. Appl. Phycol.* 11, 89–97. <https://doi.org/10.1023/A:1008070400208>

655 Wassef, E.A., H, E.M.M., R, M.F., 2001. Growth enhancement and muscle structure of
656 striped mullet, *Mugil cephalus* L., fingerlings by feeding algal meal-based diets.
657 *Aquac. Res.* 32, 315–322. <https://doi.org/10.1046/j.1355-557x.2001.00043.x>

658 Whitfield, A.K., Panfili, J., Durand, J.D., 2012. A global review of the cosmopolitan
659 flathead mullet *Mugil cephalus* Linnaeus 1758 (Teleostei: Mugilidae), with
660 emphasis on the biology, genetics, ecology and fisheries aspects of this apparent
661 species complex, *Reviews in Fish Biology and Fisheries*.
662 <https://doi.org/10.1007/s11160-012-9263-9>

663 Wilson, R.P., 2002. Amino Acids and Proteins, in: Halver, J., Hardy, R. (Eds.), *Fish*
664 *Nutrition*, Academic press., San Diego, pp. 143–179.

665 Zertuche-González, J.A., Sánchez-Barredo, M., Guzmán-Calderón, J.M., Altamirano-
666 Gómez, Z., 2014. *Eisenia arborea* J.E. Areschoug as abalone diet on an IMTA farm
667 in Baja California, México. *J. Appl. Phycol.* 26, 957–960.
668 <https://doi.org/10.1007/s10811-013-0138-9>

669

670

671 Table 1. Proximate composition of the commercial feed and the shrimp farming waste
672 used for the feeding trial (dry matter basis).

	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

673 Values are shown as mean (n = 3) ± Standard deviation (SD).

674

Table 2. Growth performance and feed utilization of juvenile grey mullet at the end of the feeding trial.

	SFW0	Experimental treatments			ANOVA
		SFW33	SFW67	SFW100	P-value
<i>Growth performance</i>					
IW (g)	1.51 ± 0.0	1.50 ± 0.1	1.50 ± 0.0	1.51 ± 0.0	
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
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
<i>Feed utilization</i>					
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

Values are shown as mean (n = 3) ± SD. Values in the same line with different superscripts are significantly different, determined by Tukey's test, P < 0.05.

680 Table 3. Proximate composition (wet weight) of the whole-body of juvenile grey mullet
681 at the end of the feeding trial among treatments and at the beginning of the feeding trial.

	Initial	Treatments				ANOVA
		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	

682 Values are shown as mean (n = 3) ± SD. Values in the same line with different
683 superscripts are significantly different, determined by Tukey's test, P < 0.05.

684 Table 4. Apparent digestibility coefficients of the dry matter and crude protein of the
685 commercial feed (CF) and the shrimp farming wastes (SFW) of juvenile grey mullet.

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

686 Values are shown as mean (n = 3) ± SD.

687

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689 Table 5. Digestive enzyme activity (U mg fish⁻¹) of alkaline proteases, trypsin,
690 chymotrypsin, amylases, and lipases of juvenile grey mullet fed with the experimental
691 treatments.

	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

692 Values are shown as mean (n = 3) ± SD.

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