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Research Article

Lipid requirement of bay snook (*Petenia splendida* Günther, 1862) juveniles

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ABSTRACT. This research study is focused on the lipid requirements of *Petenia splendida* (0.65 ± 0.19 g) juveniles. Fish were fed with trout commercial diet (Silver Cup™ - 45% protein and 16% lipids), as control diet - CD (which is usually used to feed this species at commercial scale), and four isoproteic (45% crude protein) diet formulated with different lipid levels (5, 10, 15 and 20%). Diets were administered for 60 days by triplicate per treatment. Our results showed that fish fed with 10, 15 and 20% lipid levels were significantly higher ($P < 0.05$) in average weight and total length. Same treatments were significantly lower ($P < 0.05$) in terms of FCR compared to those shown in 5% and CD. However, fish fed 20% lipid level, showed a significant decrease in WG and a significant ($P < 0.05$) increase in FCR compared to those shown in 15% lipid level. Fish fed with 15% showed the highest weight gain and the lowest FCR compared with the rest of the treatments. Lipid efficiency ratio (LER) showed significant ($P < 0.05$) differences among experimental groups. Compared to other diets containing lipid levels (5, 10, and 20%) fish fed 15% diet recorded the lowest protein and lipid content in whole body. Additionally, based on second order polynomial regression analysis of WG, it was demonstrated that 16.2% lipid provides an optimal growth for *P. splendida*.

Keywords: *Petenia splendida*; bay snook; diet; lipid requirement; aquaculture

INTRODUCTION

Current global aquaculture development is based on species diversification (Ahmed & Thompson, 2019). The worldwide freshwater fish farming industry is focused in exotic species such as Nile tilapia (*Oreochromis niloticus*), salmon (*Salmo salar*), and common carp (*Cyprinus carpio*) among others, which have achieved a steady production and a highly profitable economic return (Olaussen, 2018; Kim *et al.*, 2019). However, traditionally exploited species have faced aggressive international markets and high uncertainty (Anderson *et al.*, 2018). Furthermore, epizootic diseases, dramatic changes in environmental conditions, and massive pollution endanger the sustainability of current farmed species (Goldburg & Naylor, 2005; Shariff, 2007; Brander *et al*., 2017). Therefore, it is essential to reduce dependency/pressure in exploited animals utilizing culturing new species with high potential for aquaculture (namely: aquaculture diversification) (Bush *et al*., 2019).

Ichthyological biodiversity of Mexico possesses a significant number of native species with high potential to be exploited for aquaculture (Hulsey *et al.*, 2004;

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Chávez-López *et al.*, 2005; Abdo de la Parra *et al.*, 2015; Castillo-Torres *et al.*, 2017). Unfortunately, little attention has been focused on native species (Ross & Beveridge, 1995). Mexican cichlids have attracted attention as candidates for aquaculture (Chávez de Martínez, 1990; Deveze-Murillo *et al.*, 2004; Hernández *et al.*, 2014; Dávila-Camacho *et al.*, 2018).

Bay snook (*Petenia splendida* Günther, 1862), locally known as "tenhuayaca" (Pérez-Sánchez & Páramo-Delgadillo, 2008) is a highly valued native cichlid for human consumption, inhabiting freshwater bodies (sandy and moody bottoms) from southeast of Mexico (Tabasco, Chiapas, Campeche, & Quintana Roo states) down to Central America. It represents significant ecological importance and economic value (locally and regionally) for aquaculture (Méndez *et al*., 2011). *Petenia splendida* is a carnivorous fish (Álvarez-González *et al.*, 2008) that can be produced all year long. Experimental and semi-industrial production of *P. splendida* started 20 years ago at Universidada Juárez Autónoma de Tabasco (UJAT), Academic Division of Biological Sciences (DACBiol) southeast of Mexico, where production continues until now. This species' reproduction and growth have been conducted at experimental and pilot-commercial systems such as ponds (earthen & concrete) and geomembranes (Instituto Nacional de Pesca, 2019) semi-intensive and intensive levels. As in other cichlid species, such as *O. niloticus*, only male stocks are used for regular culture to obtain the fastest growth rates and control reproduction at early stages. In the south of Mexico, at commercial scale, *P. splendida* is growing up in all-male stocks to obtain efficient productivity in aquaculture systems. Sex reversal of tenguayaca is obtained using 17α-methyl-testosterone (MT) (Instituto Nacional de Pesca, 2019).

Petenia splendida has been subjected to several studies related to biology and physiology (Alvarez-González *et al.*, 2008; Jiménez-Martínez *et al.*, 2009), taxonomy and ecology (Méndez *et al.*, 2011), and aquaculture technology (Pérez-Sánchez & Páramo-Delgadillo, 2008; Jiménez-Martínez *et al.*, 2009; Vidal-López *et al.*, 2009) including embryonic development, optimal light, and temperature conditions, breeders management, larviculture, and stocking densities. Research in nutrition and feeding of *P. splendida* is not well developed. Therefore, bay snook is currently fed with rainbow trout (*Oncorhynchus mykiss*) diet obtaining acceptable parameters (Instituto Nacional de Pesca, 2019). However, an experimental formulation for this species (based in *in vitro* -pH stat- assays) developed at UJAT - DACBiol has obtained better results (Alvarez-González *et al.*, 2008). However, it is necessary to optimize *P. splendida* diet currently used in order to obtain improved outcomes. The efficiency of aquafeeds is achieved with balanced formulations with a proper nutrient level for each organism. It is important to know nutrient requirements (Sales & Janssens, 2003) and adequate feed management (Tacon & Foster, 2003) of each fish species to obtain desired outcomes. Lipid is one of the most fundamental nutrients providing an optimal growth performance of fish (Hua *et al*., 2019). These nutrients are the primary source of energy, have a structural function in cellular membranes, promote the protection of cells, recognize external elements, and absorbing liposoluble vitamins, among other functions (Ayisi *et al*., 2018). Lipid requirements studies have been reported in many species, spotted rose snapper *Lutjanus guttatus* (Abdo de la Parra *et al.*, 2010), Murray cod *Maccullochella peelii* (Turchini *et al*., 2011), grass carp *Ctenopharyngodon idella* (Jin *et al.*, 2013), and Florida pompano *Trachinotus carolinus* (Rombenso *et al*., 2016), among many others. However, knowledge of lipid requirement in certain species such as *P. splendida* is null. Thus, this research was aimed to assess the optimal lipid requirement of *P. splendida* juveniles, a highly valued native species of Mexico with a strong potential for aquaculture.

MATERIALS AND METHODS

Fish procurement

Broodstock of *Petenia splendida* (275 g average weight) was procured at facilities of the Tropical Aquaculture Laboratory of the Academic Division of Biological Sciences (DACBiol) at Universidad Juárez Autónoma de Tabasco (UJAT). Breeders were kept in circular tanks (2,000 L, 2 m diameter, 0.65 m depth of an open system with a 10% of daily water exchange. The average temperature was 30 ± 0.5ºC, and the pH was controlled at 7. Broodstock was fed three times per day at 10% of average body weight. Each tank was equipped with 6 PVC shelters functioning as nets. The sexual proportion was 1:2 (one ♂: two ♀). Once spawning was achieved, larvae were collected (4,500 fish) and stocked in 100 L tanks (27 ± 1ºC / constant aeration) for six days until the yolk sac was absorbed and external feeding was initiated. Larvae were fed with *Artemia* spp. nauplii. To obtain sexual reversion, *Artemia* was enriched with 20 mg of 17α-methyltestosterone (MT). *Artemia* spp. nauplii were procured following standard methods described by Vidal-López *et al.* (2009). After hatching (24 h), *Artemia* nauplii were enriched with MT for 2 h. MT enriched *Artemia*, was administered (at satiation level) to *P. splendida* larvae, four times a day (09:00, 12:00. 15:00 and 17:00 h) for 15 days. After this live-feed

period, to complete the masculinization process, fish was fed with commercial feed (Silver Cup®, Salt Lake, USA, 52% protein and 14% lipid) supplemented with 60 mg kg^{-1} of 17α-MT for 30 days.

Experimental diets

As noted above, the overall aim of this study was to simulate commercially relevant culture conditions for bay snook. Consequently, the commercial diet Silver Cup™ (45% protein and 16% lipids), commonly used to feed this species at commercial scale was a valid control diet. Four diets were isoproteic designed to contain 5, 10, 15, and 20% of lipid (Table 1). According to our laboratory working procedures, all raw materials used for diet preparation are subject to proximal composition analysis. Raw materials used in this research are themselves inferred from the analysis of the raw materials validated in numerous previous studies from our group (Uscanga-Martínez *et al.*, 2012; Montoya-Martínez *et al*., 2016; Trejo-Escamilla *et al.*, 2016). Casein was used as the main protein source, because of its valuable protein source in purified test diets in fish and its availability. Previous studies have used casein as the sole protein source in rainbow trout, *Oncorhynchus mykiss* (Morales *et al.*, 1994). Furthermore, casein is the protein source used in the original formulation prepared for bay snook, *P. splendida*, achieving optimal performance in this species fed with formulated diets with casein.

Fishmeal, sorghum flour, and casein were sieved (500 µm). Mineral premix, vitamin C, lysine, L-methionine, betaine, and gelatin were homogenized (15 min) in an industrial mixer (Bathamex, 178716, CDMX, Mexico). Soybean lecithin and fish oil were manually mixed until obtaining an orange color mixture. Other ingredients were added to this blend and mixed during 15 min with distilled water until obtaining a homogeneous mass. This mass was processed in a meat grinder (Torrey, M-22RI, 130 Monterrey, N.L., Mexico) to obtain the pellets. Pellets were manually cut and sieved to obtain different feed sizes: 300, 500, and 1,000 µm, to be used for different fish sizes during all experimental periods. Pellets were oven-dried (Coriat, HC-131 35-D, CDMX, Mexico) for 12 h at 35°C.

Feeding trial

The feeding trial was conducted at the wet lab of the Laboratory of Tropical Aquaculture of DACBiol - UJAT. At the beginning of the experiment, 30 fish (0.65 ± 0.19 g) were stocked for the experiment in a blue plastic tank (100 L each) set in a RAS (recirculating aquaculture system) equipped with a sedimenter, sand and biological filters (STA-RITE, S166T, Delavan, WI, USA). Water was propelled by a ¼ HP submersible pump (Jacuzzi Star-Rite, JWPA5D-230a Delavan, WI, USA) located in a reservoir (1,500 L). The temperature was controlled by two titanium thermostats (PSA, R - 9CE271, Delaware, USA). Water temperature (26.3 ± 0.51°C) was measured with a Brannan® thermometer (Salt Lake, Utah, USA). pH (7.1 ± 0.2) was estimated with a Hanna Instruments pH meter (HI 98311, Rhode Island, USA). Dissolved oxygen (5.3 ± 0.6 mg mL^{-1}) was monitored with a YSI® 55 oximeter (Springer, CA, USA). All parameters were daily measured in each experimental tank. Experimental diets were administered to triplicate tanks for 60 days. Daily feed consumption was measured using an analytical balance (Ohaus, NJ, USA, precision 0.0001 g). Initially, 10% of the total biomass was administered per tank. According to the daily consumption of feed, the amount was adjusted to keep a satiation level. During this period, fish were fed three times per day (09:00, 13:00 and 17:00 h).

Table 1. Experimental diets formulation (g kg^{-1}) for *Petenia splendida* juveniles, containing four levels of lipid (5, 10, 15 and 20%). [a]NZMP, Nueva Zelanda, [b]Pedregal, Toluca, Edo. Mex. México, [c]Sigma-Aldrich # catálogo F-8020, [d]Pronat Ultra. Mérida, Yucatán, México, [e]Sigma-Aldrich # catalogue C4888, [f]Research Organics # inventory 9086, [g]IU kg^{-1} or g kg^{-1} of premix: vitamin A, 1.0 MIU; vitamin D3, 0.5 MIU; vitamin E, 0.04 MIU; vitamin K, 3. 4 g; vitamin B1, 4 g; vitamin B2, 6 g; vitamin B5, 10 g; vitamin B6, 2 g; vitamin B9, 1.6 g; vitamin B12, 0.004 g; niacin, 40 g; biotin, 0.1 g; vitamin C, 100 g; choline, 200 g; inositol, 50 g, [h]per g mixture: mg; Cu: 8.3 mg; Mn: 67 mg; Co: 1.7 mg; y: 1.7; Zn: 200 mg, [i]Research Organics # catalogue 0122M. [j]ROVIMIX® C-EC (Roche), [k]Research Organics # catalogue B-2629. DM: Dry matter.

Ingredients	Lipids (%)			
	5	10	15	20
Casein[a]	44.57	45.13	45.69	46.25
Sorghum flour[b]	38.00	32.15	26.30	20.39
Fish oil[c]	2.02	5.81	9.60	12.95
Soy lecithin[d]	1.00	2.50	4.00	6.00
Fish meal[b]	10.00	10.00	10.00	10.00
Carboxymethyl cellulose[e]	2.96	2.96	2.96	2.96
Lysine[f]	0.50	0.50	0.50	0.50
Vitamin premix[g]	0.25	0.25	0.25	0.25
Mineral premix[h]	0.15	0.15	0.15	0.15
L-methionine[i]	0.50	0.50	0.50	0.50
Vitamin C[j]	0.05	0.05	0.05	0.05
Betain[k]	0.004	0.004	0.004	0.004
Proximate composition (g 100 g DM^{-1})				
Crude protein	45.0	45.0	45.0	45.0
Ether extract	5.0	10.0	15.0	20.0
Crude fiber	3.6	3.5	3.4	3.3
Ash	3.6	3.4	3.3	3.3
Nitrogen-free extract	43.0	38.1	33.3	28.4
Gross energy (kcal g)	3970	4224	4482	4732

Samplings

Total length and weight were assessed every 15 days by a biometry sampling. Fish were weighted with a precision balance 0.0001g (Ohaus, Atlantic City, NJ, USA). Before weighting each fish, excess of water was removed with absorbent paper. Total length was measured with a digital Vernier with a precision of 0.1 mm (Electronic Digital, 140677256, Madrid, Spain).

Chemical analysis

A proximal composition (crude protein, crude lipid, fiber, nitrogen-free extract, ash, and gross energy) of diets and whole body was conducted according to the Association of Official Analytical Chemistry (AOAC, 1995). Briefly, samples were dried to a constant weight at 105ºC for 2 h to determine their dry matter content. A muffle furnace determined ash at 550ºC for 3 h. Lipid content was calculated gravimetrically after hot extraction using Soxhlet equipment and petroleum ether. Total crude protein was analyzed by the Kjeldahl method (N × 6.25) after acid digestion, distillation, and titration. Fiber content was determined through acid digestion of defatted samples with H_2SO_4 (0.225 N), followed by alkaline digestion with NaOH (0.313 N). The residue was dried in an oven at 105ºC until constant weight and burned in a furnace at 550ºC for 30 min. Nitrogen free extract was calculated following standard formula (NFE = DM - (%EE + %CP + %ash + %CF), where DM = dry matter; E = ether extract; CP = crude protein; CF = crude fiber). Gross energy (for the whole body) content was calculated by a calorimeter (C2000, IKA). The analysis was conducted at Northwest Biological Research Center (CIBNOR), La Paz, Baja California Sur, Mexico.

Calculations

The following parameters were determined: weight gain: WG (%) = 100 × (FBW - IBW) / IBW; feed conversion ratio: FCR = weight of consumed feed / weight gain; daily feed intake: FI = (consumed feed) number of fish / number of days; daily lipid intake: DLI (g fish) = consumed lipid / time (d) × (final fish number); lipid efficiency rate: LER = fish weight gain / lipid weight in feed; condition factor: K= (final average weight / final total length) × 100; survival: S = (initial number of fish / final number of fish) × 100. NFE (%) = 100 - (protein% + lipid% + fibre% + ash%). Gross energy of experimental diets was calculated from the caloric content of protein, lipid and carbohydrates.

Statistical analysis

In order to determine the normality and homogeneity of obtained data, Kolmogorov-Smirnov and Levene tests were used. Differences between replicates were identified by ANOVA and later by the Tukey test. Regarding growth, whole body, and feed proximal composition, non-parametric tests (Kruskal-Wallis and later on Nemenyi) were applied. A second-order polynomial regression analysis (k = 2) with an interaction of a matrix analysis was applied ($y = y^0 + ax + bx^2$) to average weight concerning lipid content of diets. This model was obtained by a model structure built from regression coefficients to obtain a quadratic expression. Data were processed with Statistica 7.0, and the significance level for all tests was 0.05.

RESULTS

Proximal composition of diets did not show differences regarding crude protein, ether extract, crude fiber, ash, and nitrogen free extract. However, gross energy increased as dietary lipid inclusion increased in diets.

The growth of all experimental groups of *Petenia splendida*'s juveniles showed a steady and similar trend in the first 30 days of the experimental period. However, after this period, significant differences ($P < 0.05$) in weight gain and total length were recorded (Figs. 1a-b). A second-order polynomial regression analysis showed that an optimal level of lipids for *P. splendida* juveniles is 16.2% ($R^2 = 0.80$) for estimated parameters of the model (Fig. 2).

The full body composition analysis showed that fiber, ash, nitrogen-free extract, and gross energy of the whole body did not show significant ($P > 0.05$) differences among experimental groups. In contrast, protein content in the whole body of 5% group was significantly higher ($P < 0.05$) compared to that shown in control diet (CD) and 15% experimental groups. There were not significant ($P > 0.05$) differences among protein content of 5, 10, and 20% experimental groups. Similarly, lipid content in the whole body did not show significant ($P > 0.05$) differences between CD experimental group and fish consuming diets containing any supplementation levels of lipid. Lipid content in the whole body of fish fed 10 and 20% diets recorded higher ($P < 0.05$) values compared to those shown in 5 and 15% diets (Table 2). Feed intake (FI), daily lipid intake (DLI), condition factor (K), and survival did not show significant ($P > 0.05$) differences among experimental groups. Compared to the CD group, fish fed experimental diets containing any level of lipid (5, 10, 15, and 20%) resulted in a significantly higher ($P < 0.05$) weight gain (WG). Among lipid supplemented experimental diets, 15% showed the highest ($P < 0.05$) weight. Feed conversion ratio (FCR) showed a significantly higher ($P < 0.05$) value in CD compared to that shown in the rest of the experimental diets. Among lipid supplemented experimental diets, 15% showed the lowest ($P < 0.05$) FCR. Lipid efficien-

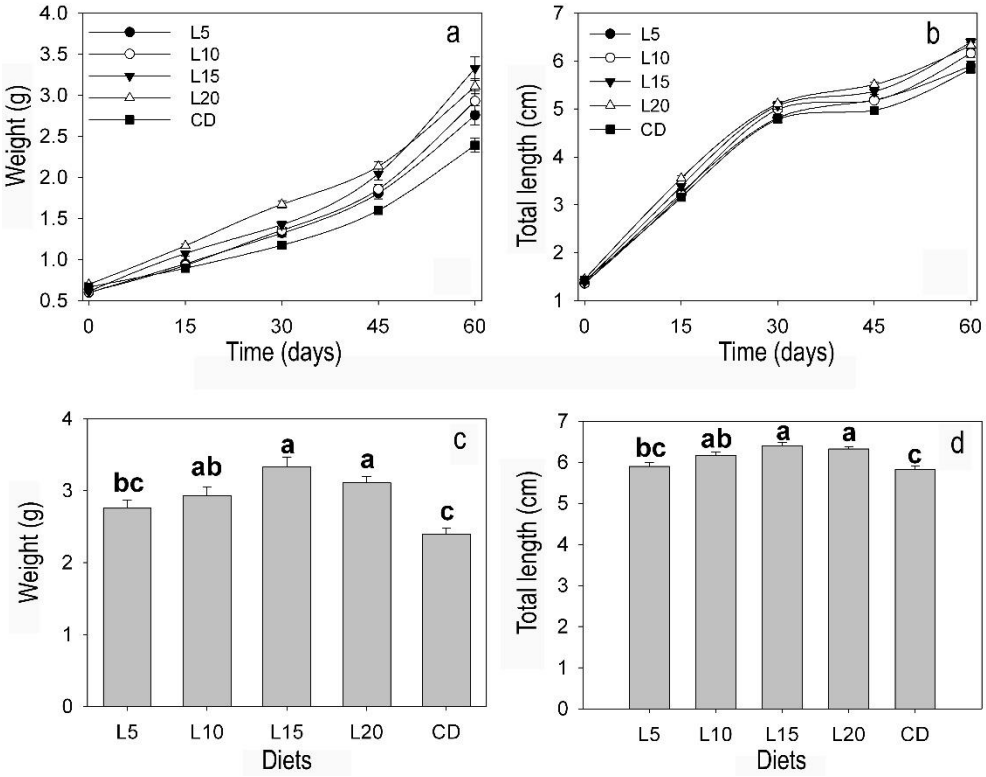

Figure 1. a) Weight-related to time, b) total length related to time, c) the average weight per experimental group, and d) total length average per experimental treatment, of *Petenia splendida* juveniles fed four diets formulated with different lipid levels (5, 10, 15 and 20%).

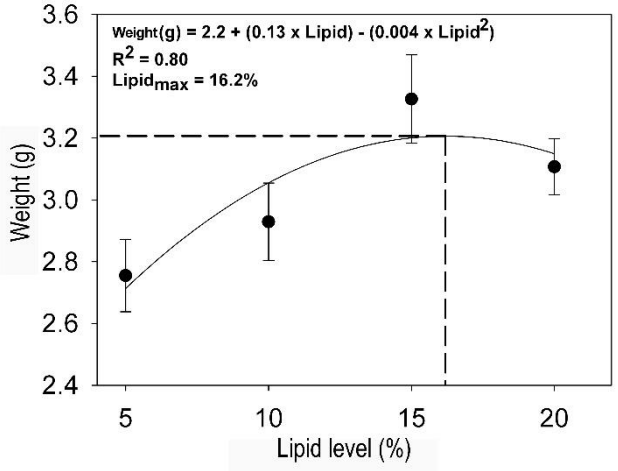

Figure 2. The second-order polynomial model concerning weight (mean ± standard deviation, g) of *Petenia splendida* juveniles fed four diets formulated with different lipid levels (5, 10, 15, and 20%) for 60 days.

cy ratio (LER) was significantly ($P < 0.05$) higher in 5 and 10% compared to that shown in CD, 15 and 20%. The highest ($P < 0.05$) LER was shown in 5% (Table 3).

DISCUSSION

Determination of nutritional requirements is a prerequisite in the formulation of feeds for newly cultured fish species (Toledo-Solís *et al.*, 2020). Ingredients in aquafeed formulations significantly affect certain ratios of diet proximate composition. Hence, it is important to accurately formulate diets when different lipid levels are supplemented in fish diets (Al-Thobaiti *et al.*, 2017). This study used formulations with a balanced profile intended to satisfy the nutritional requirements of *Petenia splendida.* Diets were designed to contain different levels of lipids. There were no differences in crude protein, ether extract, crude fiber, ash, and nitrogen-free extract of experimental diets. However, calculated energy, notably increased as dietary lipid level increased. Similar results were observed by Martino *et al.* (2005), who found that varying levels of dietary lipid (190, 210, 230, 250 and 270 g kg^{-1}) in diets for teleost carnivorous fish surubim *Pseudoplatystoma coruscans*, registered an increased gross energy (21.2, 21.6, 22.4, 22.8, 23.2 MJ kg^{-1}) content in experimental diets.

Table 2. Whole-body proximal composition (g kg^{-1}) (mean ± standard deviation) on a dry basis of *Petenia splendida* juveniles fed a commercial diet (as control) and four diets formulated with different lipid levels (5, 10, 15 and 20%). Values are means from triplicate groups of fish where the letters in each row with different superscripts are significantly different ($P < 0.05$).

Diet	Experimental groups (%)				
	C	5	10	15	20
Protein	50.9 ± 1.10^{b}	56.0 ± 0.71^{a}	54.0 ± 2.11^{ab}	51.9 ± 0.41^{b}	53.7 ± 1.60^{ab}
Lipids	17.4 ± 6.81^{abc}	13.9 ± 3.30^{bc}	20.2 ± 3.51^{ab}	12.6 ± 2.40^{c}	23.0 ± 1.72^{a}
Fibre	0.1 ± 0.10	0.1 ± 0.11	0.1 ± 0.14	0.1 ± 0.00	0.1 ± 0.01
Ash	10.6 ± 0.51	11.6 ± 0.50	8.3 ± 5.21	10.3 ± 0.71	10.7 ± 0.51
Nitrogen-free extract	21.1 ± 7.92	18.8 ± 2.91	17.5 ± 9.12	25.2 ± 2.21	12.5 ± 0.50
Gross energy (kcal g)	5681.5 ± 334.51	5549.0 ± 112.0	5406.5 ± 105.4	5735.8 ± 340.3	5598.9 ± 107.2

Table 3. Growth, feed, and lipid utilization and survival (mean ± standard deviation) of *Petenia splendida* juveniles fed a commercial diet (as control) and four diets formulated with different lipid levels (5, 10, 15, and 20%). Diferent superscripts letters in the same raw indicate significant differences ($P < 0.05$). WG: weight gain, FCR: feed conversion ratio, FI: feed intake, DLI: daily lipid intake, LER: lipid efficiency ratio, K: condition factor.

Indices	Experimental groups				
	CD	5	10	15	20
WG (%)	267.34 ± 10.90^{c}	361.12 ± 8.50^{b}	386.62 ± 38.90^{b}	444.42 ± 8.31^{a}	346.31 ± 26.30^{b}
FCR	0.52 ± 0.01^{c}	0.44 ± 0.10^{b}	0.41 ± 0.05^{b}	0.36 ± 0.05^{a}	0.41 ± 0.10^{b}
FI (g $fish^{-1}$)	0.039 ± 0.00	0.041 ± 0.00	0.040 ± 0.00	0.041 ± 0.00	0.042 ± 0.00
DLI (g fish)	0.006 ± 0.00	0.002 ± 0.00	0.004 ± 0.00	0.006 ± 0.00	0.008 ± 0.00
LER	12.10 ± 0.20^{c}	45.71 ± 5.84^{a}	24.80 ± 2.60^{b}	18.8 ± 2.40^{c}	12.4 ± 1.92^{c}
K factor	1.20 ± 0.00	1.35 ± 0.11	1.25 ± 0.03	1.27 ± 0.00	1.23 ± 0.01
Survival (%)	100.00 ± 0.00	88.90 ± 8.41	88.33 ± 10.00	84.42 ± 5.12	91.10 ± 6.90

No significant differences in whole-body proximate composition were detected among experimental diets, except in lipid content. However, there were no significant differences between control fish and those fed experimental diets, higher ($P < 0.05$) lipid values were detected in the whole body of fish fed diets containing 10 and 20% of lipid. Similar results were found by Han *et al.* (2014), who observed different crude lipids deposition in juvenile giant croaker, *Nivea japonica,* fed diets containing different lipid levels. The wholebody lipid composition was found to be the lowest in fish fed 15% (experimental group showing the highest growth in this study). Often, the proximate composition of the whole body will not indicate the real effects of diets in the lipid deposition in tissues (Denssen *et al.*, 2017). In this research, lipids could be stored differentially on the liver and muscle. Fish oil content in fish diets may affect body composition and its deposition in muscle and liver. In this respect, some authors have found significant or minor differences. For example, Niu *et al.* (2007) and Peng *et al.* (2008) observed that lipid deposition in dorsal fish muscle recorded changes by the use of different kind of oils in the diet, while Huang *et al.* (2016) and Yu *et al.* (2017) observed that lipid in liver suffered changes when experimental fish was fed diets supplemented with different lipid sources. Dietary lipid sources and levels in fish diets produce several changes in lipid deposition in tissues and activities related to lipid metabolism enzymes (Kim & Lee, 2004).

The present study showed that fish well-accepted experimental diets. Feed intake and survival were not significantly different among experimental groups. Our results agree with those found by Aliyu-Paiku *et al.* (2010), who observed that diets containing different lipid ratios did not compromise growth, survival, and feed intake of snakehead (*Channa striatus*). FCR recorded in all experimental fish was low, which can be explained by the small size of fish used in this study (0.65 ± 0.19 g). Physiologically speaking, FCR change as fish gets older. For example, FCR for larvae fish is generally lower than that of the same species of fish in juvenile or adult stages (Kolkovski, 2013).

It is well known that fish oil contains fatty acids necessary to cover the nutritional requirements of any carnivorous fish (Cowey & Sargent, 1977; Turchini *et al.*, 2009; Kabeya *et al.*, 2018; Wang *et al.*, 2018) like *P. splendida*. Lipids, together with cholesterol and

essential fatty acids provide energy and elements for building cellular membranes (Lee *et al*., 2002; Turchini *et al.*, 2011; Jin *et al.*, 2013). The importance of certain components of lipids such as n-3 highly unsaturated fatty acids, HUFA (present in fish oil), for carnivorous marine fish nutrition is evident. For carnivorous freshwater fish, HUFA requirements are lower and not essential as in marine fish because they can desaturate and elongate them (Sargent *et al.*, 1999). The present research used 5, 10, 15, and 20% of fish oil as a lipid source to assess the optimal requirement for *P. splendida*. In this study, it was demonstrated that *P. splendida* achieved an acceptable growth when fed experimental diets, compared with previous studies using with same specie (Alvarez-González *et al.*, 2008). Each species has different lipid level requirements to achieve desired growth. For example, Lazzari *et al.* (2016) demonstrated that South American catfish (*Ramdhia quelen*, Quoy & Gaimard, 1824) require a 10% lipid level in the diet to promote a correct WG and FCR. Han *et al.* (2014) reported that Japanese croaker (*Nibea japonica*) needs 8.22% of lipid in the diet to maintain an acceptable growth. Lipid requirement of fish has been reported to vary with species, size or age, water quality, presence of natural food, and feeding and culture management (NRC, 1993). In diet formulation and manufacturing, it is important to an optimized nutrient requirement to obtain in a short period, maximum growth, better nutrient retention, and an improved FCR (Tocher, 2010). Inadequate lipid levels could negatively affect the growth and health of fish (Arce & Luna, 2003; González-Félix *et al*., 2016). In contrast, an adequate level of lipids improves its utilization and the energetic use of other nutrients (Zhang *et al.*, 2018), hence providing improved fish growth. This study showed that diets containing a 15% level of lipid significantly improved experimental fish growth. This result agrees with dietary lipid requirements for other cichlid species, such as *Cichlasoma trimaculatum*, which reported a notably high (22%) lipid requirement. From a physiological point of view, high lipid requirement seems to be the result of the digestive enzyme activities of cichlid species (Toledo-Solís *et al*., 2020). In carnivorous fish, high lipid requirement has been reported. Ruey-Liang *et al*. (2001) observed that cobia, *Rachycentron canadum*, requires 18% of lipidic content. Huerta-Ortiz *et al*. (2009) reported that tropical gar (*Atractosteus tropicus*) has a lipid requirement of 15%. Buchet *et al*. (2000) reported 15% of lipid requirement for red drum (*Sciaenops ocellatus*). López *et al*. (2006) reported that between 15.5 and 18% of lipids for white seabass (*Atractoscion nobilis*). In comparison, other studies suggest that gilt-head sea bream (*Sparus aurata*) requires 26% of lipid (Moñino *et al.*, 2002) and Atlantic cod (*Gadus morhua*) requires 18% (Hansen *et al.*, 2008). Based on the second-order polynomial regression analysis of WG, we found that 16.2% lipid provides an optimal growth of *P. splendida*. This variability, between real lipid content in diet and optimal lipid requirement derived from a second-order polynomial regression analysis, has been previously reported. Mohanta *et al*. (2008) observed that based on the highest energy efficiency and proper balance between dietary protein and non-protein energy content, the optimal lipid level required by silver barb *Puntius gonionotus* is 8%. However, after further analysis by second-order polynomial regression analysis, it was found that the optimum level for this species is 9.6%.

In this study, a significant decrease in growth was recorded with 20% of dietary lipid levels. Previous studies have reported that a decrease in fish growth has been observed when dietary lipid levels exceed optimal requirements (Lv *et al.*, 2015; Chang *et al*., 2017; Zhang *et al.*, 2018). Wang *et al.* (2012) found that WG and specific growth rate of *Pseudobargrus ussuriensis* fingerlings decreased with increasing dietary lipid levels at the same dietary protein level. The authors concluded that extra lipid was not used as an energy source even if the protein level was appropriate for the species. Other authors as Sveier *et al*. (1999) and Sargent *et al*. (2002), suggested an excessive level of lipid reduced growth of fish due to the inhibition of fatty acid synthesis and the reduction of fish's ability to digest and assimilate it. Furthermore, it is well known that an elevated level of lipid in diets can exceed the energetic demand interrupting nutrient intake, which is reflected in reduced growth (Aliyu-Paiku *et al.*, 2010).

Lipid levels in the diet can affect lipid and protein content in whole-body proximate composition (Bolasina & Fenucci, 2007). Our study demonstrated that *P. splendida* showed different lipid deposition in the whole body with increasing levels of diet. Similar results were found by Han *et al.* (2014), who found that *N. japonica* increased lipid deposition in whole body and muscle as lipid level increased in diets with different lipid levels (5, 9, 13 and 17%). Other authors have concluded that high lipid levels in the diet can produce fatty fish farmed in controlled systems (Tan *et al*., 2019; Xu *et al*., 2019). In this study, 20% lipid level in diets for juveniles of *P. splendida* presented the highest percentage of lipids in the whole body, which can be explained by the deposition of lipids in the form of mesenteric fat. Similar results were found by Denssen *et al*. (2017), who observed an increased visceral mass and lipid levels in the whole-body proximate composition indicating a lipostatic regulation. Dietary lipids deposition is mainly carried out in both muscle and viscera, which are the major deposition sites of fats in fish, accounting for 60-65%

of body mass (Jobling *et al*., 2002). Excessive dietary lipids may lead to increased catabolism of the dietary nutrients compromising nutrient retention efficiency (Refstie *et al*., 2001) as demonstrated in this study where LER resulted significantly lower in fish fed 20 and 15% compared to those shown in fish fed 5 and 10% experimental diets.

Similarly, a lower protein and lipid levels in fish whole body fed 15% diet was observed, compared to those found in the whole body of experimental groups fed diets containing a lower, 5, and 10% lipid level. Variability of protein and lipid in fish carcass fed different levels of lipids in the diet is well documented. Guo *et al*. (2019) recorded different body protein levels in largemouth bass *Micropterus salmoides* fed five lipid levels (3.3, 8.2, 13.2, 18.1 and 23.3%). Similarly, Bolasina & Fenucci (2007), observed that Brazilian codling (*Urophycis brasiliensis*, Kaup, 1858) fed with a low level of dietary lipid (3%) showed 14.92% crude lipid and 80% of crude protein in muscle while fish fed with a higher level of dietary lipid (10%) recorded 16.43% crude lipid and 76.02% of crude protein in muscle.

CONCLUSIONS

In conclusion, this research suggests that *Petenia splendida* can grow properly with dietary levels of lipids ranging from 5 to 20%, being the optimal 15%. However, according to the second-order polynomial regression analysis of WG, 16.2% resulted in the optimal required for this species. Furthermore, it is concluded that a higher lipid level (20%) in *P. splendida* diets resulted in the growth reduction of fish.

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