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Research Article

Optimum level of dietary lipids for growth, chemical composition and apparent digestibility of lipids for *Atractosteus tropicus*

Maricela Huerta-Ortiz[1], Carlos Alfonso Álvarez-González[1], Roberto Civera-Cerecedo[2] Rafael Martínez-García[1], Susana Camarillo-Coop[1], Ernesto Goytortúa-Bores[2] Emyr Peña[1,4] & Alfredo Pérez-Morales[3]

[1]Laboratorio de Acuicultura Tropical DACBIOL-UJAT, Villahermosa, Tabasco, México
[2]Centro de Investigaciones Biológicas del Noroeste, La Paz, Baja California Sur, México
[3]Centro Unversitario de Investigaciones Oceánológicas, Universidad de Colima, Colima, México
[4]Cátedra CONACyT
Corresponding author: Rafael Martínez García (biologomartinez@hotmail.com)

ABSTRACT. Lipids requirement of tropical gar *Atractosteus tropicus* prejuveniles and juveniles was evaluated on growth, survival, chemical composition and apparent digestibility coefficient (ADC). Four semi-purified experimental diets (5, 10, 15 and 20% of lipids) were obtained with a mix of menhaden oil and soybean lecithin (2:1 ratio). Two trials were carried out, first with 600 prejuveniles of 0.5 g (35 days post-hatching, dph) for 56 days, and the second with 160 juveniles of 15 g (two-months-old) for 42 days. In the first trial, 15 (40.8 g) and 20% (40.2 g) lipid diets showed the best weight. Significant differences were obtained in condition factor (CF), daily lipid intake (DLI), hepatosomatic index (HSI), crude protein, ashes, nitrogen-free extract, moisture and ether extract between treatments. In the second trial, fish fed with the 10% lipid diet obtained the highest weight (89.1 g) compared with the other treatments. Significant differences were recorded for weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), condition factor (CF), and lipid efficiency rate (LER). Also, significant differences were obtained in crude protein, ether extract, NFE, gross energy, apparent lipid ADC, protein ADC and dry matter ADC. The polynomial models applied to both trials indicated an optimum lipid requirement of 17% for 0.5 g juveniles and of 9% for 15.2 g juveniles.

Keywords: *Atractosteus tropicus*, Lepisosteidae, juveniles, tropical gar, lipids, polynomial models, digestibility.

INTRODUCTION

The tropical gar *Atractosteus tropicus* (Gill, 1863) is an endemic fish that inhabits swampy ecosystems in southeastern Mexico. It is a commercially important species, as it has a high consumption and constitutes an important fishery, as a consequence of which its natural populations have drastically decreased in the last years. It has been widely studied for several years as a potential aquaculture fish establishing its controlled reproduction for intensive seed production, although commercial trout feed is used as the only feed source (Márquez, 2004; Guerrero-Zárate *et al.*, 2014; Frías-Quintana *et al.*, 2017).

Lipid requirement has not been determined in *A. tropicus*, for this reason, this information is essential to develop a specific feed and also to optimize their culture. The use of balanced feeds that satisfy the nutritional requirements of a species could decrease feeding costs, which account for up to 60% of the total production cost (Heller, 2006; Asche *et al.*, 2016). The essential nutrients in the feed are proteins, which are used to acquire somatic tissue and, in the other hand, lipids that provide the energy necessary for fish metabolism (Luna-Figueroa *et al.*, 2001; Guzmán, 2003). An adequate interaction between proteins and lipids optimizes growth and thus, production at a commercial scale. Several studies have shown that a range of lipids between 10 and 20% could be optimum, for freshwater species such as: Nile tilapia *Oreochromis niloticus*, rainbow trout *Oncorhynchus mykiss* (Craig, 2004; Zhung, 2006), pangasius *Pangasianodon hypophthalmus* (Sivaramakrishnan *et al.*, 2017) and for the marine species white weakfish *Atractoscion nobilis* (Hillary,

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2005). Lipids requirements depend on the kind of species, the development stage, the culture conditions, the types of feed and the ingredients sources (Menoyo, 2004, 2006). This study was designed to determine the optimum level of dietary lipids for growth, chemical composition and apparent digestibility in prejuveniles and juveniles of *A. tropicus*.

MATERIALS AND METHODS

Two trials were carried out with two different sized groups (prejuveniles and juveniles) of *A. tropicus*, the first to evaluate growth and survival and the second to determine the apparent digestibility of nutrients. The fish used in both trials were obtained from the Laboratorio de Acuicultura Tropical, División Académica de Ciencias Biológicas, Universidad Juárez Autónoma de Tabasco, from two induced spawning using LHRH hormone (0.35 µg kg^{-1} of fish). Larvae culture developed following Márquez *et al.* (2006) using *Artemia* nauplii and biomass for 14 days, and a gradual adaptation to commercial feed diet (Silver Cup®) with 45% protein and 16% lipid for 21 days, up to the time the fish became prejuveniles. Once the larval period had concluded, 3000 fish were selected and fed until they attained the size required to carry out both trials. Both trials used a recirculating system with 12-70 L tanks connected to a sand filter (STA-RITE, RX-5034N3, Delavan, Wisconsin, USA), a 1500 L reservoir, and a pump for recirculating water (STA-RITE, JMPA5D-230-Washington, Washington, USA). Both trials were utterly random simple designs and used the concentration of lipids in the diet (5, 10, 15, and 20%) as a factor. Each level of lipids was evaluated in triplicate. The first trial used 600 pre-juveniles with an average weight and total length of 0.5 ± 0.1 g and 5.4 ± 0.1 cm, respectively. The fish were introduced randomly at a density of 25 individuals per tank (0.17 g L^{-1}). The second trial used 160 fish with an average weight and total length of 15.5 ± 1.4 g and 16 ± 0.6 cm, respectively. These were allocated randomly at a density of 10 fish per tank (2.21 g L^{-1}).

The fish in both trials were fed three times a day (8:00, 12:00 and 16:00 h), to complete satiation. Initial feeding included 10% of the biomass percentage, after an adaptation period to the experimental diets for three days. The feed consumption was calculated daily by siphoning out the feed remaining in each tank and subtracting it from the initial amount.

Diet preparation

Semi-purified diets were made for both experiments using the software Mixit win v.5 (Table 1), and the protocol of Álvarez-González *et al.* (2001) in which the macro-ingredients (casein, sorghum and sardine meal) were sifted through a 350-500 µm mesh, poured into a mixing machine (Bathamax PAT#178716, Monterrey, Nuevo León, México) and mixed for 20 min. The micro-ingredients (L-lysine HCl, vitamin premix, mineral premix, vitamin C and BHT) were simultaneously weighed, added to the macro-ingredients mixture and mixed for another 20 min. The oils were then weighed (menhaden oil and soybean lecithin) and added to the macro and micro ingredients mixture and mixed for another 20 min. Lastly, the binder carboxymethyl cellulose was added and mixed for another 20 min until the mixture was homogeneous. The mixture was passed through a meat grid (Tororey M-22 R1, Edomex, Naucalpan, México) to form pellets that were then cut, dried in an oven Coriat HG-25-A, D.F., Naucalpan, México) at 60ºC, and stored.

Water quality

Daily measurements were taken in both trials for temperature (29.8 ± 0.8°C (first trial) and 29.2 ± 0.2°C (second trial)), dissolved oxygen (6.2 ± 0.5 mg L^{-1} (first trial) and 5.1 ± 0.9 mg L^{-1} (second trial)) using a YSI 55 oxymeter (Springer, Ohio, USA), and pH (7.7 ± 0.9 (first trial) and 7.5 ± 1.3 (second trial)) with a Hanna ph-meter (Woonsocket, RI, USA). Weekly measurements were also taken in the second trial for nitrates (0.09 ± 0.01 mg L^{-1}), nitrites (0.08 ± 0.01 mg L^{-1}) and ammonium (0.08 ± 0.02 mg L^{-1}) following spectrophotometric techniques (APHA, 1995).

Growth

The first trial lasted 56 days and the second 42 days. Biometric measurements were taken in both trials every two weeks, to record individual weight with a digital scale (Denver Instrument XP-600, Denver, Colorado, USA) and total length using a digital caliper (Marathon C0-030300, Richmond Hill, Ontario, Canada).

Growth and feed quality indexes, survival and chemical composition

The following indexes were determined at the end of the experiments, including the calculation of the total feed consumed: Weight gain (WG%): [(final mean weight-initial mean body weight) / (final mean weight)] × 100, Feed conversion ratio (FCR): (feed intake, g dry matter) / (fish weight gain, (g)), Specific growth rate (SGR%): [(ln final weight - ln initial body weight) / days] × 100, Condition factor (CF): (final mean body weight / final mean body length^3) × 100, Daily feed intake (DFI): (total feed intake, g dry matter) / (number of fish / day), Daily lipid intake (DLI): (total lipid intake, g) / (number of fish / day) and Lipid efficiency rate (LER): (fish wet weight gain, g) / (total lipid intake,

Table 1. Experimental diets applied to both feeding trials of tropical gar *A. tropicus* juveniles. a) Casein [NZMP, New Zealand], b) integral sorghum meal [Pedregal, Toluca, Edo. Mex., México], c) fish oil from menhaden [Sigma-Aldrich # catalog F-8020], d) soybean lecithin [Pronat Ultra. Mérida, Yucatán, México], e) sardine meal [Pedregal Toluca, Edo. Mex., México], f) carboxymethyl cellulose [Sigma-Aldrich # catalog C4888], g) L-lysine HCl [Research Organics # inventory 9086], h) vitamin and mineral premix [Pedregal Toluca, Edo. Mex., México], i) L-methionine [Research Organics # catalog 0122M], j) ascorbic acid [ROVIMIX® C-EC Roche], k) chromic oxide [Probiotek, Toluca, Edo. Mex., México] (this ingredient was used only in the 2nd experiment to measure the apparent digestibility in *A. tropicus* juveniles), l) betaine [Research Organics # catalog B-2629]. Results expressed as dry base, except m) humidity: determination by the difference of weight to 105°C 4 h^{-1}, n) protein: Microkjeldahl method (%N × 6,25), o) ether extract: Soxtec-Avanti Method, TECATOR, p) crude fiber: successive hydrolysis method (acid/base), q) ash-gray: determination by the difference of weight, calcination at 600°C 5 h^{-1}, r) N.F.E: calculated by difference: 100-(%proteins + %lipids + % crude fiber + %ash), s) energy: determination by calorimetry.

Ingredients	Lipid (%)			
	5	10	15	20
Casein[a]	44.57	45.13	45.69	46.29
Integral sorghum meal[b]	33.85	28.00	22.15	16.20
Fish oil from menhaden[c]	2.02	5.81	9.60	12.95
Soybean lecithin[d]	1.00	2.50	4.00	6.00
Sardine meal[e]	10.00	10.00	10.00	10.00
Carboxymethyl cellulose[f]	3.00	3.00	3.00	3.00
L-lysine HCl[g]	0.50	0.50	0.50	0.50
Vitamin and mineral premix[h]	3.50	3.50	3.50	3.50
L-methionine[i]	0.50	0.50	0.50	0.50
Ascorbic acid[j]	0.05	0.05	0.05	0.05
Chromic oxide[k]	1.00	1.00	1.00	1.00
Betaine[l]	0.004	0.004	0.004	0.004
Proximate composition (g 100 g^{-1} dry matter, except moisture, mean ± SD)				
Crude protein[n]	56.7 ± 0.4	54.8 ± 0.1	54.3 ± 0.2	57.5 ± 0.1
Moisture[m]	6.9 ± 0.2	6.2 ± 0.2	5.9 ± 0.4	5.6 ± 0.9
Ether extract[o]	5.3 ± 0.1	10.4 ± 0.2	14.8 ± 0.1	19.7 ± 0.1
Crude fiber[p]	0.7 ± 0.5	0.5 ± 0.5	0.3 ± 0.5	0.3 ± 0.3
Ash[q]	4.2 ± 0.4	4.3 ± 0.7	4.4 ± 0.5	4.4 ± 0.7
Gross energy (cal g^{-1})[r]	5004.3 ± 1.0	5234.1 ± 1.2	5587.2 ± 1.1	5693.3 ± 1.6
Nitrogen-free extract[s]	33.1 ± 0.0	30.0 ± 0.0	26.2 ± 0.0	18.1 ± 0.0

g). Survival in both experiments was calculated considering the total count of organisms at the end of the trials. Ten and three fish were collected per tank for the first and second trial using an overdose of the anesthetic MS-222 (tricaine methanesulphonate). The liver was collected from each organism, and the hepatosomatic index (HSI) was calculated as: (wet weight of liver (g) / total body wet weight (g)). The remaining and 30 fish from the beginning of both experiments were sampled in the same way, frozen at -20ºC and freeze-dried to carry out a complete chemical analysis (AOAC, 1995).

Apparent digestibility

The apparent digestibility coefficient (ADC) of the nutrients was calculated exclusively for the fish in the second trial because prejuveniles were too small for feces recollection sample for ADC technique. The feces were siphoned out from these tanks one hour after feeding every day and frozen at -20ºC. At the end of the trial, all feces were freeze-dried for a complete chemical analysis to determine the apparent digestibility coefficient, measuring the concentration of chromic oxide following Furukawa & Tsukahara (1966) modified by Olvera-Novoa & Olivera-Castillo (1999) as follow: ADC dry matter (%) = 100 - [(Cr_2O_3 in feed / %Cr_2O_3 in feces) × 100]; ADC nutrients (%) = 100-100[(%Cr_2O_3 in feed / %Cr_2O_3 in feces) × (% nutrient in feces / % nutrient in feed)].

Statistical analyses

Homoscedasticity (Levine test) and normality (Kolmogorov test) were carried out to determine the parametric analysis to use. The weight and total length data of both experiments were analyzed with a one-way ANOVA, and the differences between treatments were

Table 2. Weight (g) and standard length (cm) in prejuveniles and juveniles fed different levels of dietary lipids (mean ± SD). Different superscripts between lines show significant differences. Means in the same row with different superscripts are significantly different ($P < 0.05$).

First trial								
Days	Weight (g) lipid (%)				Total length (cm) lipid (%)			
	5	10	15	20	5	10	15	20
0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	5.4 ± 0.7	5.4 ± 0.7	5.3 ± 0.9	5.4 ± 0.7
14	3.1 ± 0.5	3.8 ± 0.5	3.8 ± 0.6	3.7 ± 0.6	7.4 ± 0.8	7.8 ± 0.9	7.8 ± 0.8	7.5 ± 0.8
28	9.2 ± 1.4	9.4 ± 1.3	9.4 ± 1.7	9.4 ± 1.3	10.9 ± 1.5	10.5 ± 1.7	10.1 ± 1.4	10.5 ± 1.2
42	16.2 ± 3.5^{c}	21.2 ± 4.2^{a}	19.8 ± 3.9^{a}	20.3 ± 3.8^{a}	16.0 ± 1.6^{b}	17.6 ± 2.4^{a}	17.8 ± 3.8^{a}	17.6 ± 3.3^{a}
56	32.4 ± 3.9^{c}	36.8 ± 4.4^{b}	40.8 ± 5.4^{a}	40.2 ± 4.8^{a}	20.8 ± 3.9^{b}	21.3 ± 4.8^{b}	24.3 ± 5.2^{a}	23.7 ± 4.7^{a}
Second trial								
Days	Weight (g) lipid (%)				Total length (cm) lipid (%)			
	5	10	15	20	5	10	15	20
0	14.8 ± 1.4	15.2 ± 1.4	15.1 ± 1.3	15.5 ± 1.4	16.4 ± 0.6	16.6 ± 0.5	16.5 ± 0.7	16.5 ± 0.6
14	30.4 ± 6.8	34.7 ± 5.8	33.6 ± 4.3	34.8 ± 4.5	19.7 ± 1.5	20.2 ± 1.1	19.8 ± 1.0	20.0 ± 0.9
28	61.4 ± 14.2^{c}	81.2 ± 7.1^{a}	71.8 ± 12.9^{b}	71.2 ± 10.5^{b}	24.7 ± 2.0^{c}	26.6 ± 1.4^{a}	25.6 ± 1.4^{b}	25.4 ± 1.0^{b}
42	64.5 ± 13.1^{c}	89.1 ± 7.7^{a}	74.2 ± 12.0^{b}	73.7 ± 9.8^{b}	25.7 ± 1.8^{c}	33.6 ± 3.9^{a}	26.8 ± 1.3^{b}	26.5 ± 1.1^{b}

determined with a Tukey test. The survival, hepatosomatic index, growth index, food quality, proximal analysis of whole fish and apparent digestibility were analyzed with a nonparametric Kruskal-Wallis test, and the differences were determined with a Nemenyi test. The optimum level of lipids in both trials was determined using a third order polynomial model $Y = a+bX + cX^2 + dX^3$, and the weight was related to the level of lipids applying the iterative method of Levenberg-Marquardt. A significance value of 0.05 was used in all cases. All analyses were carried out with the software Statistica v.7.

RESULTS

First trial

Growth

No significant differences (ANOVA $P > 0.05$) were detected between treatments for weight and total length during the first 28 days of growth. However, significant differences ($P < 0.05$) were recorded for both biometrics on days 42 and 56 (last sampling). The growth showed at 15, and 20% lipid diets were not statistically different (40.8 and 40.2 g, 24.3 and 23.7 cm, respectively), but were different from the fish fed the 5 and 10% lipid diets (32.4 and 36.8 g, 20.8 and 21.3 cm, respectively) (Table 2).

Feed and growth indexes

Feed consumption, WG (%), survival (%), FCR, SGR (% day) and LER did not present significant differences (Kruskal-Wallis, $P > 0.05$) between treatments. However, significant differences were recorded for CF between 15 and 20% lipid diets (0.29 ± 0.07 and 0.39 ± 0.01, respectively). Significant differences were found for DLI among treatments (5% lipids 0.031 ± 0.01 and 10% lipids 0.081 ± 0.003). Significant differences ($P < 0.05$) were also recorded for the hepatosomatic index (HSI), with the fish fed with the 20% lipid diet presenting the greatest values (5.01 ± 1.48) in comparison with those in the other treatments (Table 3).

Proximate analysis

The humidity (6.5%) and crude protein (57%) in the fish fed with 20% lipid diet recorded the lowest significant values concerning other treatments ($P < 0.05$). The fish fed with the 20% lipids diet presented the greatest significant content of lipids (26.3%) compared with the fish at the beginning of the trial and those in other treatments. The content of ash was most significant for fish at the beginning of the trial (13.2%) ($P < 0.05$), compared with the fish fed with the experimental diets. Finally, the energy and crude fiber values did not show significant differences (Kruskal-Wallis $P > 0.05$) among treatments, and for the fish at the start of the experiment, it is observed an interaction between the increasing lipids in the diet and the energy (Table 4).

The optimum value of lipids calculated with the polynomial model for the best fish growth was 17%, with a statistical significance ($P < 0.05$) and a good correlation ($r = 0.61$) for the model, considering the weight data (Fig. 1a).

Table 3. Growth index and feed quality (mean ± SD) of prejuveniles and juveniles of tropical gar, fed semi-purified diets using four levels of lipids for 56 days. Each value represents the mean ± SD. Different superscripts between lines show significant differences. (abbreviations: consumption in grams of dried matter, WG: weight gain, FCR: feed conversion rate, SGR: specific growth rate, CF: condition factor, DFI: daily feed intake, DLI: daily lipid intake, LER: lipid efficiency rate, HIS: hepatosomatic index.

Indices	First trial lipids (%)			
	5	10	15	20
Consume feed (g)	451 ± 127	477 ± 41	481 ± 13	457 ± 108
WG (%)	5551 ± 1302	6325 ± 963	6980 ± 1358	6277 ± 779
Survival (%)	96 ± 7	99 ± 2	95 ± 6	87 ± 6
FCR	0.47 ± 0.21	0.51 ± 0.12	0.54 ± 0.14	0.52 ± 0.13
SGR (% d^{-1})	7.2 ± 0.3	7.4 ± 0.2	7.7 ± 0.5	7.7 ± 0.1
CF	0.35 ± 0.03^{ab}	0.38 ± 0.01^{ab}	0.29 ± 0.07^{b}	0.39 ± 0.01^{a}
DFI (g d^{-1})	0.61 ± 0.19	0.81 ± 0.03	0.86 ± 0.02	0.77 ± 0.14
DLI	0.031 ± 0.010^{b}	0.081 ± 0.003^{c}	0.128 ± 0.003^{a}	0.154 ± 0.29^{a}
LER	58.8 ± 4.4	20.6 ± 4.4	13.3 ± 2.5	10.5 ± 2.3
HSI	2.54 ± 0.85^{b}	2.55 ± 0.87^{b}	2.50 ± 0.86^{b}	4.91 ± 1.48^{a}
Indices	Second trial lipids (%)			
	5	10	15	20
Consume feed (g)	583 ± 24	619 ± 43	581 ± 36	586 ± 18
WG (%)	306 ± 20^{b}	484 ± 30^{a}	375 ± 83^{ab}	375 ± 57^{ab}
Survival (%)	93 ± 5	100 ± 0	97 ± 6	100 ± 0
FCR	1.22 ± 0.09^{a}	0.81 ± 0.01^{b}	1.00 ± 0.17^{a}	0.97 ± 0.13^{ab}
SGR (% d^{-1})	2.6 ± 0.2^{b}	3.2 ± 0.1^{a}	2.8 ± 0.3^{ab}	2.9 ± 0.3^{ab}
CF	0.38 ± 0.02^{a}	0.24 ± 0.05^{b}	0.38 ± 0.01^{a}	0.39 ± 0.01^{a}
DFI (g d^{-1})	1.10 ± 0.08	1.11 ± 0.15	0.99 ± 0.06	1.03 ± 0.08
DLI	0.055 ± 0.004^{d}	0.111 ± 0.015^{c}	0.148 ± 0.009^{b}	0.206 ± 0.017^{a}
LER	16.4 ± 1.2^{a}	12.4 ± 0.2^{b}	6.8 ± 1.2^{bc}	5.2 ± 0.7^{c}
HIS	4.02 ± 1.05^{b}	4.05 ± 1.02^{b}	4.09 ± 1.06^{b}	6.81 ± 0.99^{a}

Table 4. Biochemical composition (% dry matter) of whole fish prejuveniles and juveniles. Different superscripts between lines show significant differences. Values: mean ± SD of 30 fish per treatment (10 fish per replicate) for the first experiment, and 9 fish per treatment (three fish per replicate). Results expressed as dry base, except for humidity: determination by the difference of weight at 105°C 4 h^{-1}; protein: Microkjeldahl method (%N × 6,25); ether extract: Soxtec-Avanti Method, TECATOR, crude fiber: successive hydrolysis method (acid/base); ash-gray: determination by the difference of weight; calcinations at 600°C 5 h^{-1}; N.F.E: calculated by difference: 100-(%proteins + %lipids + % crude fiber + %ash); energy: determination by calorimetric technique.

Whole fish	First trial lipid (%)				
	5	10	15	20	Initial fish
Moisture (%)	7.3 ± 0.5^{a}	7.2 ± 2.3^{a}	7.0 ± 2.9^{ab}	6.5 ± 0.8^{b}	7.6 ± 0.0^{a}
Crude protein (%)	63.6 ± 1.3^{a}	64.6 ± 0.5^{a}	61.9 ± 2.7^{ab}	57.0 ± 0.7^{b}	62.6 ± 0.0^{ab}
Ether extract (%)	19.1 ± 0.2^{b}	18.6 ± 0.1^{b}	20.8 ± 0.2^{b}	26.3 ± 0.2^{a}	7.5 ± 0.1^{c}
Crude fiber (%)	0.3 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.21 ± 0.0	0.1 ± 0.0
Ash (%)	11.3 ± 0.1^{b}	11.3 ± 0.2^{b}	11.0 ± 0.0^{b}	10.3 ± 0.1^{b}	13.2 ± 0.1^{a}
N.F.E. (%)	5.7 ± 0.0^{b}	5.5 ± 0.0^{b}	6.3 ± 0.0^{b}	6.2 ± 0.0^{b}	16.5 ± 0.0^{a}
Gross energy (cal g^{-1})	5240.7 ± 11.6	5283.5 ± 10.0	5360.0 ± 10.2	5567.9 ± 11.3	4930.7 ± 10.2
Whole fish	Second trial lipid (%)				
	5	10	15	20	Initial fish
Moisture (%)	7.9 ± 0.1	7.5 ± 0.2	7.8 ± 0.1	7.5 ± 0.2	7.5 ± 0.2
Crude protein (%)	62.5 ± 0.3^{a}	57.1 ± 0.2^{b}	52.3 ± 0.2^{cd}	51.3 ± 0.2^{d}	65.1 ± 0.0^{a}
Ether extract (%)	14.8 ± 0.3^{b}	21.0 ± 0.6^{a}	25.4 ± 3.1^{a}	25.9 ± 1.1^{a}	5.0 ± 0.0^{c}
Crude fiber (%)	0.4 ± 0.2	0.2 ± 0.0	0.5 ± 0.4	0.2 ± 0.0	0.1 ± 0.0
Ash (%)	14.3 ± 0.3	14.4 ± 0.5	14.5 ± 0.7	14.5 ± 0.1	14.7 ± 0.0
N.F.E. (%)	8.0 ± 1.3^{b}	7.3 ± 0.7^{b}	7.3 ± 1.4^{b}	8.1 ± 0.8^{b}	15.1 ± 0.0^{a}
Gross energy (cal g^{-1})	5104.3 ± 64.8^{a}	5134.1 ± 128.2^{a}	5446.2 ± 86.0^{a}	5262.3 ± 193.7^{a}	4324.1 ± 0.0^{b}

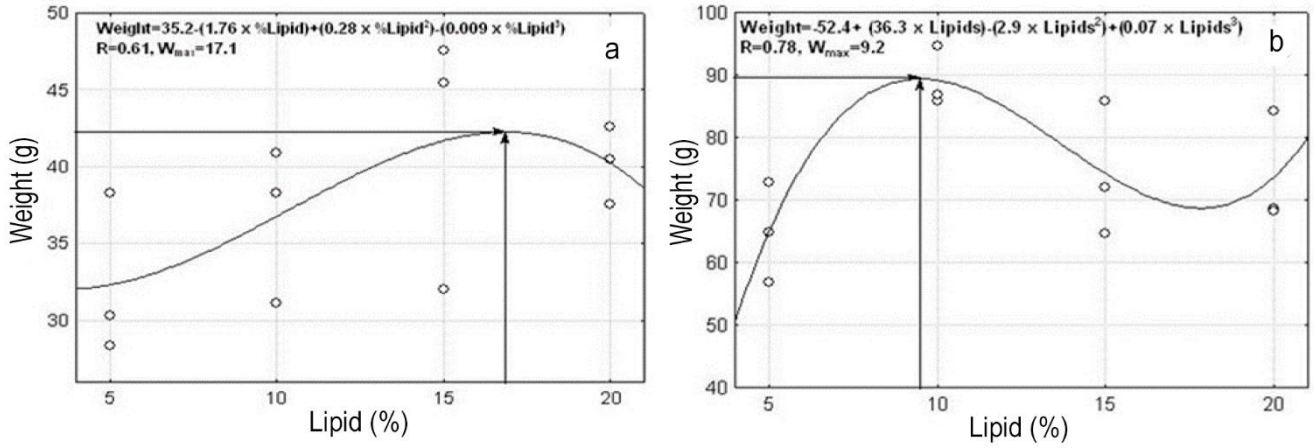

Figure 1. Polynomial models to determine the optimum lipids requirement for a) 0.5 g prejuveniles and b) 15.5 g juveniles of *A. tropicus*.

Second trial

Growth

No significant differences (ANOVA $P > 0.05$) were observed between treatments for the total length and weight values in the first sampling (day 14). However, significant differences ($P < 0.05$) for total length and weight were recorded for the samplings on days 28 and 42, where the fish fed with the 10% lipids diet were significantly higher (89.1 ± 7.7 g and 33.6 ± 3.9 cm) than those in the other treatments (Table 2).

Feed and growth indexes

No significant differences between the different treatments (Kruskal-Wallis, $P > 0.05$) were detected for the values of food consumption, survival (%), and DFI (g d^{-1}). The fish fed the 10% lipid diet showed the highest significant values ($P < 0.05$) of WG (%), FCR, SGR and CF (484%, 0.81, 3.2% d^{-1}, and 0.24, respectively) compared with the fish fed the other experimental diets. The highest significant values of DLI (0.206) and HIS (6.8) ($P < 0.05$) were recorded for the fish fed the 20% diet compared with the fish in the other treatments.

Proximate analysis

The values of humidity, crude fiber and ash did not show significant differences (Kruskal-Wallis $P > 0.05$) among treatments. The crude protein content of the fish fed with the 5% lipid diet (62.5%) and the fish at the beginning of the trial (65.1%) was not different ($P < 0.05$), but were different from the fish fed with the 10% lipid diet (57.1%). This treatment was statistically different ($P < 0.05$) from the fish fed with the 15% (52.3% crude protein) and 20% lipids (51.3% crude protein). The analyses of ether extract (5.0%), nitrogen-free extract (14.8%), and total energy 4324.1 cal g^{-1} indicated that the fish at the start of the trial were statistically different respectively, from the fish fed with the experimental diets ($P < 0.05$) (Table 4).

Apparent digestibility

The apparent digestibility of lipids (lipid ADC) of the fish fed with the 10, 15 and 20% lipid diets (95.5%, 93.6%, and 93.8%, respectively) did not show significant differences ($P > 0.05$), although a difference was detected in the case of the fish fed with 5% lipid diet (71.1%). The apparent digestibility of proteins (protein ADC) of the fish fed with 5, 10 and 15% lipid diets were not different (92.5%, 94.2%, and 92.2%, respectively), but significantly different ($P < 0.05$) from the fish fed the 20% lipid diet (89.8%). Finally, the apparent digestibility of dry matter (dry matter ADC) indicated that the fish fed with the 5 (85.5%), 15 (85.9%) and 20% (84.6%) lipid diets were not statistically different, but there was a statistical difference ($P < 0.05$) among the fish fed with the 10% lipid diet (78.93%) (Table 5).

The optimum value of lipids to obtain the greatest fish growth calculated with the polynomial model was 9%, with a statistical significance ($P < 0.05$) and a good correlation ($r = 0.78$) for the model, considering the weight data (Fig. 1b).

DISCUSSION

The best growth for *A. tropicus* prejuveniles (0.5 g mean weight) was recorded using lipid level of 15%, meanwhile, for 15 g juvenile the best growth was obtained with 10% lipids. The optimum requirements calculated for both sizes with the polynomial models

Table 5. Apparent digestibility coefficients of nutrients (mean ± SD, n = 36 fish) for tropical gar *Atractosteus tropicus*. Different superscripts between lines show significant differences. Formula used to calculate the value of the chromic oxide: X = ((Y–0.0032) / 0.2089) / 4). Where X = amount of chromic oxide present in the sample. Y = absorbance 0.0032, 0.2089 and 4 are constants and where % chromic oxide = 100 x (X / A), A = sample weight.

ADC (%)	Lipid (%)			
	5	10	15	20
Lipid	71.12 ± 0.20[b]	95.53 ± 0.80[a]	93.68 ± 0.24[a]	93.85 ± 0.08[a]
Protein	92.54 ± 0.54[a]	94.28 ± 0.53[a]	92.28 ± 0.20[a]	89.81 ± 1.46[b]
Dry matter	85.56 ± 0.31[a]	78.93 ± 0.34[b]	85.94 ± 1.16[a]	84.60 ± 0.57[a]

were established at 17.1% for the prejuveniles and 9.2% for the juvenile fish, with a difference of 8% between these two sizes. This agrees with several authors that have stated that the requirement of lipids decreases as fish increase in size, even within same species (Page & Andrews, 1973; Degani *et al.*, 1989; Parazo *et al.*, 1990; Ellis & Reigh, 1991; Bellastrazzi *et al.*, 1994; Chou & Shiau, 2001; Lee *et al.*, 2003; Sivaramakrishnan *et al.*, 2017). Another aspect that is attributed to the correct inclusion of lipids in the diet, is the possibility of reducing and saving the amount of protein that is used (protein sparing), and obtaining an increase in metabolism and an improved growth (Espinoza de los Monteros & Labarta, 1987; McGoogan & Gatlin, 1999; Thoman *et al.*, 1999; Vergara *et al.*, 1999). The adequate substitution of protein-energy by lipid energy could result in a decreased in feed cost because fishmeal (highly expensive) is commonly used as protein energy source (Heller, 2006).

Two important phenomena could run the relation between food quality index and apparent digestibility of nutrients. First, the excess of dietary lipids detected at a level of 20% in both trials, impacted SGR, CF, HSI and apparent digestibility coefficient of lipids (in the second trial) resulting poor growth. Our results agree with reports for American eel *Anguilla rostrata* (Rafinesque, 1820), European eel *Anguilla anguilla* (Linnaeus, 1758), Atlantic salmon *Salmo salar* (Linnaeus, 1758), European seabass *Dicentrarchus labrax* (Linnaeus, 1758), haddock *Melanogrammus aeglefinus* (Linnaeus, 1758) and barramundi *Lates calcarifer* (Bloch, 1790), which have noticed that the excess of dietary lipids provokes a high lipid accumulation in the body. In the case of fish consuming a lot of dietary lipids, the excess is deposited in the liver and tissues of the mesenteric cavity, and much of it goes to the dark muscles that are incapable of directly metabolize them to obtain energy that has been observed in *A. tropicus* feed 20% lipids. The catabolic pathway of CO_2 is slower when lipid concentration increases, and this results in lipid deposits increasing in the liver, consequential in an inefficiency of synthesize fatty acids and also causing liver damage (Otwell & Rickards, 1981; Gallagher *et al.*, 1984; García-Gallego & Ackman, 1993; Nanton *et al.*, 2001; Peres & Oliva-Teles, 2001; Williams *et al.*, 2003). Robaina (1998) mentioned that the liver is the organ most important in fat accumulation and the effect depends on species, age, size, development stage, and culture system. Thus, it is important to keep in mind the amount of dietary lipid provided to fish. From the commercial point of view, fish quality decrease with increasing lipids above its requirement, which can affect the energy budget increasing the CF, FCR, HSI, DLI and lipid content in fish that was detected in fish feed 20% in prejuveniles and 15-20% in juveniles *A. tropicus*. The accumulation of body lipids becomes rancidity of the flesh as a result of oxidation of fatty acids and of the liberation of radicals that accelerate oxidation when in contact with fish meat. It is thus necessary to deliver feed not only with an optimum lipids concentration but also with the best sources of lipids, to improve the quality of the product (Ackman, 1980).

An optimum amount of lipids in the diet allow fish to grow adequately, with the gain in weight determined by proteins and the energy provided by lipids. In this study, the optimum dietary lipids were found to depend directly on the size of the organisms, as FCR (0.54 and 0.81) and WG (6,980% and 484%) recorded their best values at 15% (first trial) and 10% (second trial) dietary lipids. These results agree with values obtained by Chou & Shiau (2001) for *O. niloticus* and *O. aureus* of FCR = 1.22 and WG = 390%, and by Lee *et al.* (2003) for the rockfish *Sebastes schlegeli* (Hilgendorf, 1880) of FCR = 1.03 and WG = 490%. These authors reported that the use of diets with adequate levels of lipids has a positive impact on growth index and allows fish to channel the energy that is necessary to satisfy metabolic demands, as well as protein catabolism to be used efficiently for somatic growth.

The proximal composition of the fish in both experiments showed a typical inversely proportional relationship between lipids and proteins, where at a

higher concentration of lipids the concentration of body protein decreases. Page & Andrews (1973), Reinitz *et al.* (1980), Williams *et al.* (1988) and Bjerkeng *et al.* (1997) mentioned that a decrease in proteins is associated with a high consumption of dietary lipids that affects growth and the efficiency of nutrient use and causes an imbalance in the concentration of proteins, this was observed in the fish with high levels of lipids in both trials. It agrees with several authors that have mentioned that it is not only the addition of high levels of dietary lipids that results in an accumulation of lipids and a decreased concentration of proteins in the muscle but also other factors such as fish size and inadequate feeding (Gomes *et al.*, 1993; Shearer, 1994; Glengross *et al*., 2002).

Another aspect that agrees with the above-stated is the high apparent digestibility coefficients for lipids (lipid ADC) and proteins (protein ADC) that were higher than 90% for the fish fed the 10% lipid diet (only in the second trial). We need to point out that the low ADC for the dry matter in fish feed 10% of lipids could be due to the best utilization of nutrients, which reduced the necessity of digest more food. An indication that when feed includes nutrients that are adequate in quantity and quality, high growths are obtained, and these are directly related to the dietary ingredients (NRC, 1993). Similar results to ours have been reported by Wilson & Poe (1985) for the channel catfish *Ictalurus puntactus* (Rafinesque, 1818), who recorded nutrient digestibility greater than 85% with the adequate use of lipids (10-15%) depending on the species, development stage, type of feed, etc. From the nutritional point of view, it is important to mention that, the quality of the ingredients used in these trials (menhaden oil and soybean lecithin) is adequate and may be used commercially. Although this also depends on aspects that are related to nutrition, feed technology, species, feeding habits, digestive capacity, development and, evidently, the cost-benefit relationship that is obtained with these dietary ingredients. It must also be noted that the concentrations of lipids observed in these trials correspond to those recorded for other carnivorous species as a result of the high-energy requirements (Hillestad *et al.*, 1999; Vandenrbeg & Nouee, 2001; Wedemeyer, 2001; Zhou *et al.*, 2004).

In conclusion, the optimum requirements of lipids are 15% (17% using the polynomial model) for 0.5 g prejuveniles (pre-growing stage) and 10% (9% using the polynomial model) for 15 g juveniles (growing stage) in agreement with the growth of the fish, feed quality indices, chemical composition, apparent digestibility of nutrients and polynomial models. It may be added that this information needs to be generated for bigger fish sizes and longer time spans, especially under commercial conditions and using practical feeds to develop a specific formula for the culture of *A. tropicus*.

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