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ORIGINAL ARTICLE

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Finishing plant diet supplemented with microalgae meal increases the docosahexaenoic acid content in *Colossoma macropomum* flesh

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Abstract

The need for a sustainable aquaculture is increasing the use of plant ingredients in replacement to fishmeal and fish oil in diets for tambaqui *Colossoma macropomum*, which is leading to not detectable levels of docosahexaenoic acid (DHA) in its flesh. We evaluated the effect of a finishing plant diet supplemented with 5% of microalgae meal from *Schizochytrium* sp. (MD) on tambaqui growth, on proximal composition and fatty acid content of its flesh, comparing it to a non-supplemented diet. One hundred and sixty-two fish (489.67 g) were distributed into six tanks (2,000 L) and fed the experimental diets for a 90-day period. Three fish per tank were euthanized for analyses every 15 days. The MD did not affect the growth and proximal composition of fish flesh. The MD increased the DHA content (from 14.81 to 38.60 mg/g of lipids) and the n-3:n-6 ratio (from 0.16 to 0.51) in the flesh of fish, beginning on the 15th day and reaching the highest DHA content on the 71st day (39.81 mg/g of lipids). We recommend *C. macropomum* to be fed with a finishing diet supplemented with microalgae meal for 71 days before slaughter to improve the DHA content and n-3:n-6 ratio in the flesh.

KEYWORDS
gamitana, n-3:n-6 ratio, nutraceutical fish, *Schizochytrium* sp., tambaqui

1 | INTRODUCTION

There is widespread concern about the benefits of promoting human health through highly unsaturated fatty acids (HUFA). The eicosapentaenoic acid (20:5n-3; EPA) contributes to the reduction of inflammatory processes, while the docosahexaenoic acid (22:6n-3; DHA) has been associated with the development and proper function of the brain and vision, Alzheimer prevention and the inhibition of degenerative processes of retinal photoreceptor cells (Judge, Harel, & Lammi-Keefe, 2007; Swanson, Block, & Mousa, 2012; Tully et al., 2003). Due to these benefits, the World Health Organization recommends the minimum consumption of 200 mg of EPA+DHA per day in adult diets and indicates foods with an n-3:n-6 ratio greater than 0.20 to provide the nutraceutical benefits related to HUFA (Simopoulos, 2008; WHO, 2015).

Fish have been considered the main HUFA source in human diet (Tocher, 2003) and aquaculture has contributed to the supply of fish with adequate nutritional profiles. Nowadays, the need for a more sustainable aquaculture has motivated the research and use of plant ingredients in replacement of fishmeal and fish oil to formulate aquafeeds, mainly to omnivorous fish (Li, Robinson, Tucker, Manning, & Khoo, 2009). However, these plant diets are rich in 18-carbon chain fatty acids (Barrows et al., 2008) influencing on the low content of HUFA in fish flesh and decreasing the nutraceutical and commercial value of fish.

Colossoma macropomum (Cuvier) is an Amazonian fish known as tambaqui in Brazil and gamitana in Peru. It is the main native fish produced by Brazilian aquaculture (IBGE, 2013) and the second native fish produced by continental aquaculture in Peru (PRODUCE, 2016). This is an omnivorous fish reaching sizes of 1 m in length and weighing up to 30 kg (Goulding & Carvalho, 1982). In aquaculture, it presents good performance weighing up to 1.80 kg in 8 months of farming from larva to juvenile (Izel & Melo, 2004), and tambaqui's market size are from 500 to 3,000 g. It has adaptation capacity to captivity, acceptance of commercial aquafeeds, laboratory-dominated reproduction and resistance to handling and adverse conditions like low concentrations of dissolved oxygen and acidic pH in water (Goulding & Carvalho, 1982; Saint-Paul, 1984; Wood et al., 1998).

Wild *C. macropomum* has presented EPA (9.3 mg/g of lipids) and DHA (40.2 mg/g of lipids) in its flesh, with highest values during the dry season due to the phytoplankton availability (Almeida & Bueno-Franco, 2006). Nevertheless, there are no detectable levels of EPA and DHA in *C. macropomum* flesh from aquaculture, and the n-3:n-6 ratio is lower than the minimum recommended by the World Health Organization (Melo-Filho, Oliveira, & Santos, 2013), since diets for this species are generally formulated using vegetable meals.

Schizochytrium sp. is a heterotrophic organism of the Thraustochytridea subclass (Chromista: Heterokonta) capable of producing high levels of DHA through a pathway called polyketide synthase (Metz et al., 2001). Its purified strains can contain up to 60% of DHA in relation to total lipid content (Jiang, Fan, Wong, & Chen, 2004). In addition, its industrialization could be less expensive when compared to photosynthetic algae, since it does not need light or oxygen for the metabolism of its nutrients (Lewis, Nichols, & McMeekin, 1999). The use of its meal in total or partial replacement of fishmeal and/or fish oil in aquafeeds has increased the DHA content in fish species such as Atlantic salmon (*Salmo salar* L.) (Carter, Bransden, Lewis, & Nichols, 2003; Miller, Nichols, & Carter, 2007; Sprague et al., 2015), Nile tilapia (*Oreochromis niloticus* L.) (Sarker et al., 2016) and seabream (*Sparus aurata* L.) (Ganuza et al., 2008). Thus, we considered that plant diets for *C. macropomum* could be supplemented with this microalgae meal just at the ending of its farming as an alternative for the promotion of *C. macropomum* farming using plant diets, but at the same time generating flesh with detectable levels of DHA and nutraceutical value.

We aimed to evaluate the effect of the supplementation of a finishing plant diet for *C. macropomum* with microalgae meal derived from *Schizochytrium* sp. on fish growth, on proximal composition and on fatty acid content in fish flesh, and to estimate the minimum time of supplementation to improve the DHA content and n-3:n-6 ratio in the flesh of fish before slaughtering.

2 | MATERIALS AND METHODS

This study has been approved by the Ethics Committee on Animal Experimentation and Research of the National Institute for Research in the Amazon (INPA), Manaus, Amazonas, Brazil (Protocol Number 028/2015).

2.1 | Experimental diets

Two isonitrogenous (301.84 g of crude protein/kg) and isocaloric (4,612.21 kcal of gross energy/kg) (Guimarães & Martins, 2015) all-plant diets were formulated and differing only in the lipid source: a control diet (CD) with soy oil as a lipid source and a diet supplemented with 5% of a DHA-rich microalgae meal (MD) derived from *Schizochytrium* sp. (All-G Rich™, Alltech®, USA) (Table 1). The ingredients were homogenized, hydrated and extruded in pellets (6 mm in diameter) (Inbramaq MX80, Brazil). After that, the diets were dried in a forced air oven (55°C, 24 hr, TE-394/3-MP), then packed into black polyethylene bags in order to avoid lipid photooxidation and stored at 16°C until use them.

2.2 | Experimental trial and sampling

The trial was performed at the Aquaculture Experimental Station of INPA, Manaus, Amazonas, Brazil (3°05'26.7"S and 59°59'41.1"W). Before initiation of the experiment, fish were fed with the control diet for 20 days so that they could adapt to the experimental conditions. The experiment followed a completely randomized factorial design with 2 diets and 7 sampling times, with three replicates for diet. Fish (489.67 g; 28.55 cm) were housed in six fiberglass tanks (2,000 L; 27 fish per tank) and were fed three times per day (8, 12 and 16 hr) at a feeding rate of 3% of their body weight for 90 days. All tanks had continuous aeration, constant water renovation (flow rate: 15 L/min) and they were cleaned every day. Fish were reared under natural photoperiod and water quality parameters, dissolved oxygen (5.23 mg/L), temperature (27.79°C) and pH (4.90), were monitored daily (YSI Professional Plus/Pro Plus, USA) and maintained within the comfort range for the species (Saint-Paul, 1984; Wood et al., 1998).

On the 0, 15th, 30th, 45th, 60th, 75th and 90th days of the experiment, three fish were euthanized per tank by lethal dose of anaesthetic (500 mg of benzocaine/L). Fish were measured in terms of total weight and length. Immediately after, fish were put into a 90 L thermal box with crushed ice and transported to the Food Technology Laboratory of INPA where right and left fillets were dissected out, ground (meat grinder CAF 5 Inox, Brazil), homogenized into a pool per tank and stored in a −80°C freezer. Afterwards, each sample was freeze-dried, vacuum packed and sent to the Chemistry Department of State University of Maringá for proximal and fatty acid content analyses. The specific growth rate (SGR% = 100 × [(ln final weight − ln initial weight)/days of feeding period]), the weight gain (WG = final weight − initial weight) and Fulton's allometric condition factor (K = weight/length^3) were estimated to evaluate the growth performance in the fish.

2.3 | Proximal and fatty acid analyses

Diets and fish flesh were analysed in terms of moisture, protein and ash according to the procedures described by Horwitz (2005). Total lipids of the microalgae meal, diets and flesh were

TABLE 1 Formulation, proximate composition and fatty acid content of the control and microalgae meal diets for *Colossoma macropomum*

		Diets[a]	
	Microalgae meal	Control (CD)	Microalgae meal (MD)
Ingredients (g/kg)			
Soybean meal	–	537.00	530.00
Wheat flour	–	59.00	50.00
Corn grain	–	360.00	350.00
Soy oil	–	24.00	00.00
Microalgae meal[b]	–	00.00	50.00
Premix[c]	–	20.00	20.00
Proximate composition on dry matter basis (g/kg)			
Dry matter	975.00	935.00	931.67
Crude protein	124.00	299.67	304.00
Total lipids	767.60	44.14	51.47
Carbohydrates[d]	62.40	606.19	590.20
Ash	46.00	50.00	54.33
Gross energy[e] (kcal/kg)	8,201.97	4,598.28	4,626.14
Fatty acids (mg/g of total lipids)			
SFA[f]	442.56	133.24	362.79
14:0	40.16	0.90	22.82
16:0	370.15	101.21	305.94
18:0	14.69	23.80	19.55
MUFA[g]	31.96	183.67	118.59
18:1n-9c	25.58	170.90	109.31
18:1n-7	1.86	8.68	4.85
PUFA[h]	309.16	380.64	359.87
18:2n-6	15.62	351.05	217.24
18:3n-6	0.57	0.16	0.28
18:3n-3	2.70	28.40	13.59
20:3n-6	1.41	0.18	0.70
20:4n-6	1.49	0.25	0.51
20:4n-3	n.d	0.15	0.07
20:5n-3	2.35	n.d	1.26
22:4n-6	0.58	n.d	n.d
22:5n-6	47.90	0.15	23.18
22:5n-3	0.56	n.d	0.26
22:6n-3	236.37	0.20	102.71
n-6[i]	67.18	351.89	241.99
n-3[j]	241.98	28.75	117.88
PUFA:SFA	0.70	2.86	0.99
n-3:n-6	3.60	0.08	0.49

Note. n.d = not detected.

[a]Means of triplicate analyses by sample are showed. [b]Microalgae meal derived from *Schizochytrium* sp. and produced by All-G Rich™ product/Alltech®. [c]Vitamin and mineral mix (Nutron©) per kg of product: folic acid (250 mg), pantothenic acid (5,000 mg), antioxidant (600 mg), biotin (125 mg), cobalt (25 mg), copper (2,000 mg), iron (13,820 mg), iodine (100 mg), manganese (3,750 mg), niacin (5,000 mg), selenium (75 mg), vitamin A (1,000,000 IU), vitamin B1 (1250 mg), vitamin B12 (3,750 mg), vitamin B2 (2,500 mg), vitamin B6 (2,485 mg), vitamin C (28,000 mg), vitamin D3 (500,000 IU), vitamin E (28,000 IU), vitamin K3 (500 mg), zinc (17,500 mg). [d]Carbohydrates (%) = 100 − (protein + lipids + ash). [e]Gross energy basis on values calculated for protein, 5.64 kcal/g; lipid, 9.44 kcal/g; carbohydrate, 4.11 kcal/g (NRC, 2011). [f]Total saturated fatty acids also included 12:0, 15:0, 17:0, 20:0, 21:0, 22:0, and 24:0. [g]Total monounsaturated fatty acids also included 14:1n-7, 15:1n-5, 16:1n-9, 16:1n-7, 17:1n-9 and 21:1n-9. [h]Total polyunsaturated fatty acids also included 22:2n-6. [i]Total n-6 fatty acids. [j]Total n-3 fatty acids.

TABLE 2 Weight gain (WG) and the Fulton's allometric condition factor (K) in *Colossoma macropomum* fed with plant diets without supplementation (CD) and supplemented (MD) with 5% of microalgae meal derived from *Schizochytrium* sp.

Growth parameters	Diets[a]	Time (days)						Pooled standard error	*p* values[b]		
		15	30	45	60	75	90		D	T	D × T
WG (g)	CD	112.00^{c}	123.11^{c}	212.22^{bc}	243.11^{bc}	366.22^{b}	336.44^{b}	2.69	n.s	*	n.s
	MD	132.22^{c}	154.67^{bc}	237.56^{ab}	314.89^{ab}	377.78^{a}	390.22^{a}				
K	CD	2.05	2.14	2.05	2.13	2.14	1.99	0.02	n.s	n.s	n.s
	MD	1.97	2.17	2.17	2.14	2.16	2.04				

Note. As the interaction diet ×sampling time was not significant ($p > 0.05$), factors were analysed separately; in those cases, different capital letters indicate that there is a statistically significant difference between the diets for each sampling time ($p < 0.05$), while different lower case letters indicate that there is a statistically significant difference between the sampling time for each type of diet ($p < 0.05$). No capital and/or lower case letters indicate that the values were similar ($p > 0.05$) between the diets for each sampling time and/or between the sampling time for each type of diet.

[a]Means of triplicate analyses by sample are showed. Microalgae meal produced by All-G Rich™ product/Alltech®. [b]*p* values from two-way ANOVA and Tukey's test, where D = diet, T = sampling time, D × T = interaction between diet ×sampling time, n.s = not significant ($p > 0.05$), *$p < 0.05$.

determined by the Bligh and Dryer (1959) method. For fatty acid analyses of the microalgae meal, diets and flesh, fatty acid methyl esters (FAME) were prepared by the method proposed by Santos-Júnior et al. (2014). Methyl esters were separated by gas chromatography using a Thermo Scientific Trace Ultra Gas Chromatographer (Thermo Scientific, Waltham, MA, USA) fitted with a flame ionization detector (FID) and fused-silica capillary column (100 m × 0.25 mm i.d., 0.25 µm cyanopropyl CP-7420 Select Fame). The operation parameters were as follows: temperature detector, 240°C; injection port temperature, 230°C; column temperature, 165°C for 18 min, programmed to increase at 4°C/min up to 235°C, with a final holding time of 14.5 min; carrier gas, hydrogen at 1.2 mL/min; nitrogen was used as the make-up gas at 30 mL/min; split injection at 1:80 ratio. For identification, the retention times of the fatty acids were compared to those of standard methyl esters (Sigma, St. Louis, MO, USA). Retention times and peak area percentages were automatically computed by the Software Chronquest 5.0. Quantification of fatty acids (mg/g of total lipids) was performed using tricosanoic acid (23:0) methyl ester (Sigma-Aldrich, USA) as an internal standard (Joseph & Ackman, 1992). Theoretical FID correction factor values were used to obtain concentration values of fatty acids in mg/g of total lipids (Visentainer, 2012) using the equation below:

FA = [(AX × WIS × CFX)/(AIS × CFAE × WX)]; in which FA is fatty acid in mg/g of total lipids, AX is the peak area (fatty acids), WIS is the standard weight (mg), CFX is the theoretical correction factor, AIS is the standard peak area (23:0), CFAE is the necessary conversion factor in order to express results in mg of fatty acid rather than as methyl ester, and WX is the sample weight (g).

2.4 | Statistical analysis

The initial homogeneity of fish weight was affirmed by Cochran's *Q* test ($p < 0.05$). Growth data, proximal and fatty acid analyses of fish were submitted to two-way ANOVA and, when the interaction (diet × sampling time) was significant, data were broken down and means were compared by Tukey's test ($p < 0.05$). To evaluate the minimum time to increase the DHA content in *C. macropomum* flesh, data were analysed using a non-linear polynomial regression analysis of quadratic function $y = ax^2+bx+c$. A Pearson correlation coefficient was performed to evaluate if there was an association between the DHA and the PUFA content ($p < 0.05$). The data were processed using the Statistic Software 10.0.

3 | RESULTS

3.1 | Fish growth

The inclusion of microalgae meal on *C. macropomum* diet did not affect the weight gain and the Fulton's allometric condition factor ($p > 0.05$) in all fish. The weight gain increased along the trial without being influenced by the type of diet, and the Fulton's allometric condition factor was always similar (Table 2).

3.2 | Proximal composition of flesh

The microalgae meal diet did not have any effect on moisture (76.54%), protein (18.59%), ash (1.38%) and lipid (2.57%) contents of fish flesh, since those values for fish fed with both diets were similar for the groups of fish at the same sampling time ($p > 0.05$) during all experimentation.

3.3 | Fatty acids content of flesh

Thirty fatty acids were quantified with predominance of saturated (SFA) followed by monounsaturated (MUFA) fatty acids in flesh of all *C. macropomum*. (Table 3). Regarding these fatty acid groups, the palmitic (16:0), stearic (18:0) and oleic (18:1n-9) acids were predominant along the trial. The diet ×sampling time interaction did not show any differential effect on the PUFA content in the flesh ($p > 0.05$). However, when analysing the factors separately, it was found that the PUFA content decreased along the trial in flesh of all fish, from 216.02 (day 0) to 129 mg of PUFA/g of total lipids (90th day) without being influenced by the type of diet. Similar to PUFA content, the linoleic (18:2n-6), linolenic (18:3n-3) and arachidonic (20:4n-6; ARA) acids exhibited this declining trend throughout the experiment. Although the microalgae meal diet did not have any effect on the PUFA content, it influenced the considerable increase of total n-3 content in the flesh of all fish, which increased from 30.19 (day 0) to 45.87 (90th day) mg of total n-3 per g of total lipids. This increase was directly related to the rise in DHA content in the flesh, since the Pearson correlation coefficient between the content of total n-3 and DHA showed a positive association ($R = 0.97$; $p < 0.05$).

TABLE 3 Fatty acid (mg/g of total lipids) content in flesh of *Colossoma macropomum* fed with plant diets without supplementation (CD) and supplemented (MD) with 5% of microalgae meal derived from *Schizochytrium* sp.

		Time (days)							*p* values[b]		
	Diets[a]	0	15	30	45	60	75	90	D	T	D × T
SFA[c]	CD	286.49^{de}	367.69^{ab}	339.61^{abcde}	368.98^{ab}	278.03^{e}	348.84^{abcd}	281.26^{de}	*	*	*
	MD	295.30^{cde}	329.32^{bcde}	358.13^{abc}	379.05^{ab}	384.46^{ab}	401.28^{a}	317.44^{bcde}			
16:0	CD	178.33^{c}	232.59^{abc}	216.45^{bc}	227.31^{abc}	187.27^{c}	229.18^{abc}	183.00^{c}	*	*	*
	MD	184.82^{c}	212.72^{bc}	233.59^{abc}	260.25^{ab}	263.89^{ab}	274.38^{a}	213.91^{bc}			
18:0	CD	90.83^{cd}	115.79^{ab}	107.04^{abc}	125.48^{ba}	78.51^{d}	105.67^{bc}	86.89^{cd}	*	*	*
	MD	93.35^{cd}	97.14^{cd}	103.43^{bc}	99.64^{bc}	100.48^{bc}	105.01^{abc}	87.13^{cd}			
MUFA[d]	CD	263.11^{cd}	357.27^{a}	322.04^{abcd}	352.11^{ab}	285.19^{bcd}	353.47^{a}	255.11^{d}	n.s	*	*
	MD	291.17^{abcd}	284.08^{cd}	312.64^{abcd}	326.90^{abc}	303.18^{abcd}	318.09^{abcd}	255.99^{d}			
18:1n-9c	CD	198.58^{c}	290.08^{a}	260.93^{ab}	290.94^{a}	237.44^{abc}	291.42^{a}	208.82^{bc}	*	*	*
	MD	227.23^{bc}	216.07^{bc}	246.95^{abc}	262.94^{ab}	247.19^{abc}	255.37^{ab}	208.45^{bc}			
PUFA[e]	CD	216.80^{Acd}	222.78^{Ac}	191.31^{Abd}	184.64^{Ab}	179.91^{Ab}	165.64^{Ab}	122.60^{Aa}	n.s	*	n.s
	MD	215.25^{Ad}	213.06^{Ad}	201.95^{Acd}	169.63^{Abc}	164.20^{Aab}	174.69^{Abc}	135.76^{Aa}			
18:2n-6	CD	138.80^{ab}	150.90^{a}	135.37^{ab}	138.07^{ab}	140.00^{ab}	120.55^{bc}	82.24^{de}	*	*	*
	MD	133.41^{ab}	118.34^{bc}	105.17^{cd}	99.79^{cd}	94.55^{cde}	94.86^{cd}	66.98^{e}			
18:3n-3	CD	8.10^{abc}	10.02^{a}	8.81^{ab}	9.27^{a}	6.91^{bcd}	7.25^{bcd}	4.96^{ef}	*	*	*
	MD	8.33^{abc}	7.14^{bcd}	7.07^{bcd}	6.57^{cdef}	6.89^{bcde}	5.86^{def}	4.77^{f}			
20:4n-6	CD	23.27^{Ad}	18.18^{Acd}	13.99^{Abc}	10.58^{Aab}	8.82^{Aa}	10.69^{Aab}	9.97^{Aab}	n.s	*	n.s
	MD	22.32^{Ad}	22.14^{Ad}	19.52^{Ad}	8.89^{Abc}	7.13^{Abc}	8.00^{Abc}	5.69^{Ab}			
20:5n-3	CD	3.05^{Ac}	2.25^{Abc}	1.09^{Aab}	0.85^{Aa}	0.71^{Aa}	0.78^{Aa}	0.70^{Aa}	n.s	*	n.s
	MD	2.72^{Abc}	2.98^{Ac}	2.56^{Abc}	1.18^{Abc}	1.08^{Ab}	1.40^{Abc}	1.06^{Ab}			
22:6n-3	CD	14.88^{e}	12.04^{ef}	8.73^{ef}	5.80^{f}	6.32^{f}	6.39^{f}	6.12^{f}	*	*	*
	MD	14.74^{e}	27.98^{d}	39.18^{ab}	32.18^{cd}	34.53^{bc}	42.69^{a}	38.60^{abc}			
n-3[f]	CD	28.43^{cd}	26.33^{cd}	20.14^{de}	17.17^{e}	15.10^{e}	15.62^{e}	12.88^{e}	*	*	*
	MD	30.19^{c}	40.33^{b}	50.94^{a}	41.04^{b}	43.63^{ab}	51.10^{a}	45.87^{ab}			

[a]Means of triplicate analyses by sample are showed. Microalgae meal produced by All-G Rich™ product/Alltech®. Only lower case letters indicate that the interaction, diet ×sampling time, was significant ($p < 0.05$). When the interaction diet ×sampling time was not significant ($p > 0.05$), factors were analysed separately; in those cases, different capital letters indicate that there is a statistically significant difference between the diets for each sampling time ($p < 0.05$), while different lower case letters indicate that there is a statistically significant difference between the sampling time for each type of diet ($p < 0.05$). [b]*p* values from two-way ANOVA and Tukey's test, where D = diet, T = sampling time, D × T = interaction between diet ×sampling time, n.s = not significant ($p > 0.05$), *$p < 0.05$. [c]Total saturated fatty acids also included 12:0, 14:0, 15:0, 17:0, 20:0, 21:0, 22:0, and 24:0. [d]Total monounsaturated fatty acids also included 14:1n-7, 15:1n-5, 16:1n-7, 16:1n-9, 17:1n-9, 18:1n-7 and 20:1n-9. [e]Total polyunsaturated fatty acids also included 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-3, 22:4n-6, 22:5n-3 and 22:5n-6. [f]Total n-3 fatty acid.

With regard to the DHA acid, there was a significant interaction between the diet and sampling time factors ($p < 0.05$), with a higher DHA content in flesh of fish fed with the microalgae meal diet (Table 3). This increase was observed since the second sampling time (15th day), being the DHA content from 14.74 (day 0) to 38.60 (90th day) mg of DHA/g of total lipids. The DHA content data in fish flesh fit into a quadratic equation represented by $y = -0.0044x^2+0.6257x+17.5648$ for fish fed with the microalgae meal diet. When deriving the equation, it is indicated that 71 days is the minimum period to maximize the DHA content (39.81 mg/g of lipids) in flesh of fish fed with the microalgae meal diet (Figure 1). In addition, the increase in DHA content promoted the increase of the n-3:n-6 ratio (Figure 2).

On the other hand, the interaction between diet ×sampling time did not cause any differential effect on EPA content in fish flesh ($p > 0.05$) (Table 3). However, the separate analysis of the factors allowed to identify that the low EPA content in flesh of all fish decreased along the experiment ($p < 0.05$).

It would be necessary to humans to consume 231.35 g of flesh of *C. macromopum* fed for at least 30 days with the microalgae meal diet to achieve the minimum of 200 mg EPA +DHA. However, under these same conditions, a range from 995.64 to 1,037.13 g of flesh of *C. macropomum* fed with the control diet should be consumed to achieve same quantity of EPA+DHA (Figure 3).

4 | DISCUSSION

4.1 | Fish growth

The microalgae meal diet was not enough to cause a differential effect on the weight gain between fish fed with both diets ($p > 0.05$) (Table 2). These results are in accordance with literature that reports no negative effects on fish performance when *Schizochytrium* sp. meal and/or *Schizochytrium* sp. oil replace, partially or completely, the fish oil in diets for the Atlantic salmon (Carter et al., 2003; Miller et al., 2007; Sprague et al., 2015), seabream (Ganuza et al., 2008) and the Nile tilapia (Sarker et al., 2016) or, when *Schizochytrium* sp. meal is included in plant diets for the channel catfish (*Ictalurus punctatus* Rafinesque) (Li et al., 2009).

The values of Fulton's allometric condition factor (Table 2), which estimate the overall condition of fish welfare (Lima-Júnior, Cardone, & Goiten, 2002), suggest that the microalgae meal diet does not

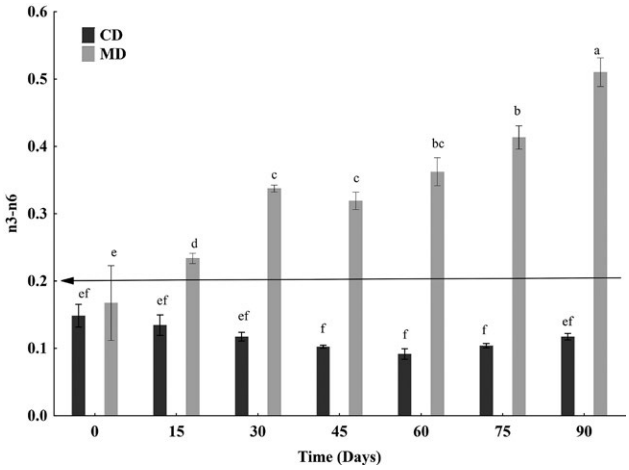

FIGURE 2 n-3:n-6 ratios in flesh of *Colossoma macropomum* fed with two plant diets formulated without supplementation or supplemented with 5% of a DHA-rich microalgae meal (MD) derived from *Schizochytrium* sp. during 90 days. Data represents values of three fish by treatment. Different letters indicate that there is a statistically significant difference between the interaction diet ×sampling time ($p < 0.05$) by two-way ANOVA and Tukey's test. Vertical bars denote confidence intervals at 0.95. Horizontal line represents the minimum n-3:n-6 ratio recommended in adult diets according to the World Health Organization in 2015 (WHO, 2015)

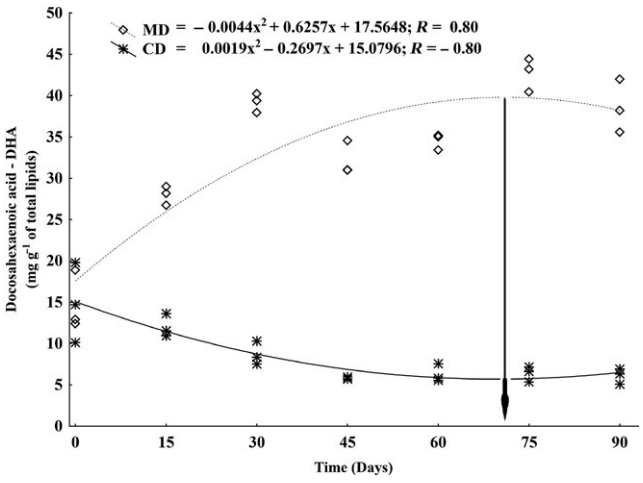

FIGURE 1 Effect of the farming time (days) on the DHA content analysed by a polynomial quadratic equation in flesh of *Colossoma macropomum* fed with two plant diets formulated without supplementation or supplemented with 5% of a DHA-rich microalgae meal (MD) derived from *Schizochytrium* sp. during 90 days. Data represents values of three fish by treatment

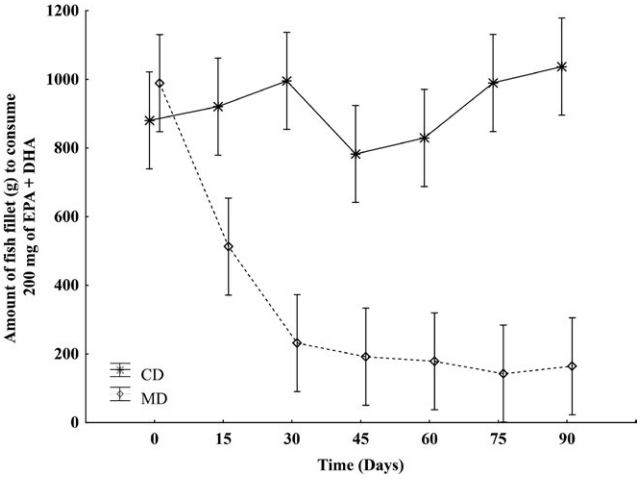

FIGURE 3 Amount of fish flesh to consume 200 mg of EPA+DHA in *Colossoma macropomum* fed with two plant diets formulated without supplementation or supplemented with 5% of a DHA-rich microalgae meal (MD) derived from *Schizochytrium* sp. during 90 days. Data represents values of three fish by treatment. Vertical bars denote confidence intervals at 0.95

affect the fish welfare ($p > 0.05$), as evidenced by the absence of mortality during the trial.

4.2 | Proximal composition of flesh

C. macropomum diet supplemented with 5% of microalgae meal derived from *Shizochytrium* sp. did not cause differential effect on proximal composition of flesh of all fish ($p > 0.05$), probably as a result of the isonitrogenous and isocaloric formulation of the diets. These values are expected for continental fish and close to those reported in flesh of farmed *C. macropomum* fed with commercial diets, which contain 77.49%, 19.63%, 1.14% and 1.59% of moisture, protein, ash and lipids respectively (Carbonilho & Jesus, 2011).

4.3 | Fatty acids content of flesh

Concerning the predominant fatty acid groups, SFA and MUFA, as well as the palmitic, stearic and oleic acids, they were also predominant in *C. macropomum* raised in ponds and fed with commercial diets (Almeida, Visentainer, & Bueno-Franco, 2008; Melo-Filho et al., 2013). Likewise, they were predominant in Atlantic salmon (Carter et al., 2003; Miller et al., 2007; Sprague et al., 2015) and Nile tilapia (Sarker et al., 2016) fed diets with *Schizochytrium* sp. meal and/or *Schizochytrium* sp. oil in replacement of fishmeal and/or fish oil. By the way, it is possible that the high content of palmitic and stearic acids in fish flesh obtained from the experiment is a product of the diet composition as well as the lipid biosynthesis in response to the possible excess of digestible carbohydrates in the diets. Concerning the high content of the oleic acid in flesh, it is probably derived from the deposition and/or from the carbon chain desaturation process of the stearic acid mediated by the Stearoyl-CoA-Δ9 desaturase enzyme, a common process in fish (Tocher, 2003).

The declining trends of linoleic, linolenic and arachidonic acids contents throughout the trial in all fish flesh are a result of the depletion of the PUFAs from the diet provided to fish prior to this experiment and/or the use of these fatty acids as energy sources. This study shows that a plant diet supplemented with 5% of microalgae meal derived from *Schizochytrium* sp. did not have any effects on the amount of PUFA content.

Concerning the increase of the DHA content in flesh of fish fed with the microalgae meal diet, this result is in accordance with other studies that evaluated the partial or complete replacement of fishmeal and/or fish oil by microalgae meal and/or microalgae oil derived from *Schizochytrium* sp. in diets for Atlantic salmon (Carter et al., 2003; Miller et al., 2007) and Nile tilapia (Sarker et al., 2016). Similarly, the DHA content in the flesh of the channel catfish is increased using plant diets supplemented with *Schizochytrium* sp. meal in levels of inclusion from 0.05% to 2.00% (Li et al., 2009). These results are therefore encouraging, since the analyses of the fatty acid content of *C. macropomum* from aquaculture systems in Brazil which are fed with commercial diets indicate that there are no detectable levels of DHA in its flesh (Melo-Filho et al., 2013). A factor that influenced the increase of the DHA content is the fact that it has the greatest retention capacity in fish tissues, since it tends to be conserved because it is a relatively poor substrate for mitochondrial β-oxidation and, consequently, for energy production (NRC, 2011). However, the decrease in DHA content from the 30th day to the 45th day in fish fed with the microalgae meal diet can be due to the mobilization of this fatty acid to other tissues, since DHA has important functions as a component of cellular membranes in the nervous system (Feller, 2008) and retina (NRC, 2011). The increase in DHA content promoted the increase of the n-3:n-6 ratio (Figure 2), that is important, since a dietary imbalance in the n-3:n-6 ratio is associated with an increased risk of cardiovascular diseases, pronounced inflammation processes and pathological deficiency (Simopoulos, 2008). In this study, the n-3:n-6 ratio that resulted from fish fed with the microalgae meal diet was higher (from the 15th day) than 0.20, which is the minimum ratio recommended by the World Health Organization (WHO, 2015) to provide the nutraceutical benefits related to these fatty acid groups (Simopoulos, 2008). By contrast, flesh of fish fed with the control diet presented n-3:n-6 ratios lower than 0.20 during the whole trial. Although 71 days is the minimum time to maximize the DHA content in flesh of fish fed with microalgae meal diet, we observed that only 15 days are required to make the n-3:n-6 ratio adequate according to the World Health Organization.

The low EPA content in the experimental diets that did not guarantee the deposition of this fatty acid in the fish muscle. Moreover, it could also be related to the low content of linolenic acid in the formulated diets, which might not be enough to promote the elongation and desaturation from linolenic acid to EPA, as verified in the low content of these two fatty acids in the flesh of fish analysed. Another possible cause is the high content of linoleic acid when compared to linolenic acid, since the processes of elongation and desaturation of these fatty acids are mediated by the same enzymes that compete with each other and preferentially act on the substrate of greater availability (Tocher, 2003). By the way, a higher deposition of ARA in comparison to EPA was observed in fish flesh, as a possible response to the substrate preference of enzymes on the ARA elongation and desaturation pathway of the linoleic acid in the diet.

The World Health Organization (WHO, 2015) also recommends the minimum consumption of 200 mg of EPA+DHA per day for humans in order to guarantee the nutraceutical effects in the prevention of cardiovascular, mental and retinal diseases, and to potentiate the cognitive capacity (Judge et al., 2007; Swanson et al., 2012; Tully et al., 2003). Thus, in order to achieve the minimum recommended by the World Health Organization, it would be necessary to consume 231.35 g of flesh of *C. macromopum* fed for at least 30 days with the microalgae meal diet. However, it is at least four times lower than the consumption of *C. macropomum* flesh fed only with a plant diet to achieve the same quantity of EPA+DHA.

In order to improve the DHA content and the n-3:n-6 ratio in the flesh of farmed *C. macropomum*, we recommend that the fish be fed with finishing plant diets supplemented with 5% of microalgae meal derived from *Schizochytrim* sp. for at least 15 days so as to improve the n-3:n-6 ratio or for 71 days so that the *C. macropomum* flesh is

produced with a high content of DHA. Thus, we are promoting the complete cycle farming of *C. macropomum* using plant diets.

We conclude that the supplementation of plant diets with microalgae meal derived from *Schizochytrium* sp. allows them to increase the DHA content, the n-3:n-6 ratio, and consequently, to improve the nutraceutical value of farmed *C. macropomum*, without detrimental effects on fish growth and welfare, and without influencing on the moisture, protein, ash and lipid content in fish flesh.

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